Report on the Deliberation Results

March 6, 2007

Evaluation and Licensing Division,
Pharmaceutical and Food Safety Bureau,
Ministry of Health, Labour and Welfare

[Brand name]	Myozyme 50 mg for intravenous drip infusion
[Non-proprietary name]	Alglucosidase Alfa (Genetical Recombination) (JAN*)
[Applicant]	Genzyme Japan K.K.
[Date of application]	June 30, 2006

[Results of Deliberation]

In the meeting held on January 31, 2007, the First Committee on New Drugs concluded that the product may be approved and that this result was to be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

It was decided that the product is classified as a biological product, its re-examination period is 10 years, and both the drug substance and the drug product are classified as powerful drugs.

**Japanese Accepted Name (modified INN)*

Review Report

January 19, 2007

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.

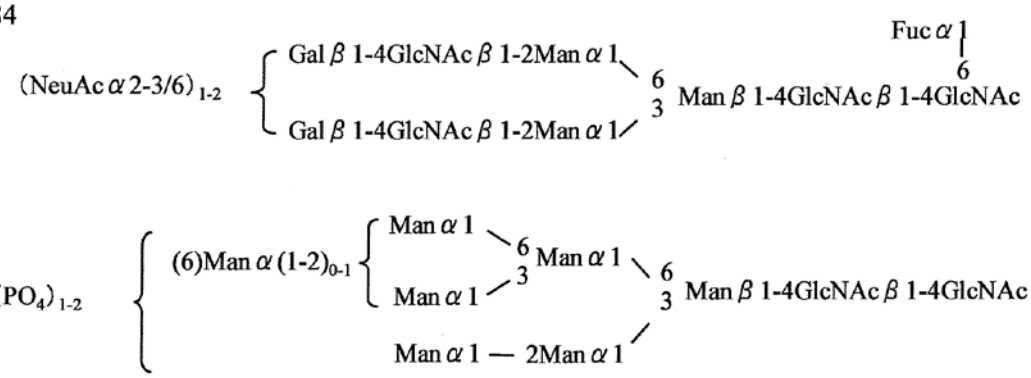
[Brand name]	Myozyme 50 mg for intravenous drip infusion
[Non-proprietary name]	Alglucosidase Alfa (Genetical Recombination)
[Applicant]	Genzyme Japan K.K.
[Date of application]	June 30, 2006
[Dosage form/Strength]	Freeze-dried formulation for injection containing 52.5 mg of Alglucosidase Alfa (Genetical Recombination) in a vial
[Application classification]	Prescription drug (1) Drug containing a new active ingredient
[Chemical structure]	
Structural formula:	See attachments
Chemical name:	Glycoprotein (molecular weight: ca. 110,000) consisting of 896 amino acid residues ($C_{4490}H_{6817}N_{1197}O_{1298}S_{32}$: molecular weight: 99358.49), produced in Chinese hamster ovary cells transfected with cDNA encoding human acid α-glucosidase

[Items warranting special mention]	Orphan drug
[Reviewing office]	Office of New Drug III

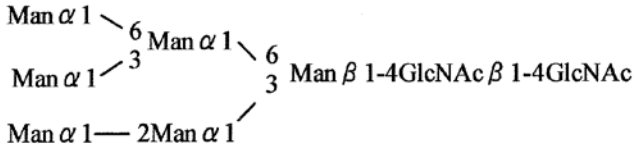
*1 pyroglutamic acid, *2 sugar-chain-binding site

Amino acid sequence

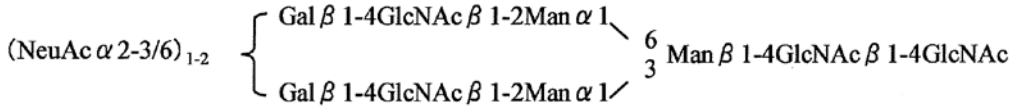
Asn84



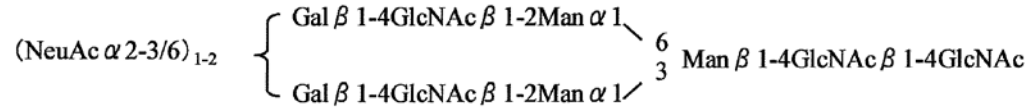
Asn177

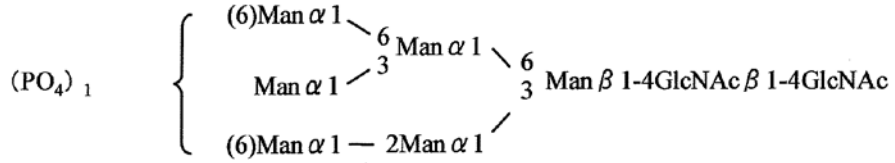


Asn334

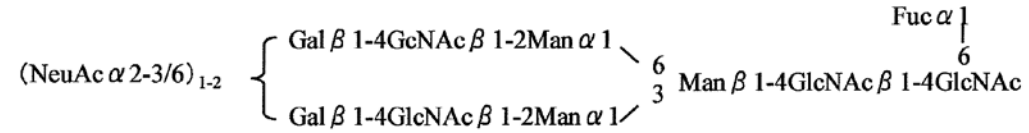


Asn414





Asn596, 826, 869



GlcNAc: N-acetylglucosamine
Fuc: fucose
NeuAc: N-acetylneuraminic acid
Man: mannose
Gal: galactose
PO_4: phosphate group

Major sugar chain structure

Review Results

January 19, 2007

[Brand name]	Myozyme 50 mg for intravenous drip infusion
[Non-proprietary name]	Alglucosidase Alfa (Genetical Recombination)
[Applicant]	Genzyme Japan K.K.
[Date of application]	June 30, 2006
[Items warranting special mention]	Orphan drug

[Review results]

It was judged that the results of survival rate, etc. obtained from studies AGLU01602 and AGLU01702 (including the one Japanese subject) suggest the efficacy of the product for type II glycogen storage disease. With respect to the safety, since the number of Japanese subjects examined in an expanded access program (AGLU02203-Japan, AGLU02603-Japan) was only 10 (5 each with infantile- and adult-onset form), the safety evaluation is considered to be limited. In particular, since the effects of antibody formation on the efficacy and safety of the product are uncertain at present and infusion-associated reaction occurs in many cases, the Pharmaceuticals and Medical Devices Agency (PMDA) considers it necessary that, measures such as pre-dosing with antipyretic analgesics and antihistamines, slowing the rate of infusion, and temporary dosing discontinuation be taken, as the need arises, and also that the therapy be performed by physicians who are knowledgeable about Alglucosidase Alfa (Genetical Recombination, hereafter referred to as rhGAA) therapy or under their guidance. In addition, PMDA considers it necessary that measures for the appropriate use of the product be implemented after launching rhGAA by examining the safety and efficacy of the product in all subjects treated with rhGAA and by collecting the relevant information, as experience with its use in Japanese patients is very limited.

As a result of its regulatory review, PMDA has concluded that the product may be approved for the following indications and dosage and administration.

[Indications]	Type II glycogen storage disease
[Dosage and administration]	The usual dosage for intravenous drip infusion is 20 mg/kg body weight of Alglucosidase Alfa (Genetical Recombination) once every other week.

[Conditions for approval]

As experience with using rhGAA in Japanese patients is very limited, a drug use investigation

should be conducted on an all-case basis until data from an adequate number of patients have been collected after launching rhGAA, in order to comprehend the background factors of patients on rhGAA and to collect data on the safety and efficacy of the product at an early postmarketing stage, and thereby measures required for appropriate use of the product should be taken.

Review Report (1)

December 27, 2006

I. Product Submitted for Registration

[Brand name]	Myozyme 50 mg for intravenous drip infusion
[Non-proprietary name]	Alglucosidase Alfa (Genetical Recombination)
[Applicant]	Genzyme Japan K.K.
[Date of application]	June 30, 2006
[Dosage form/Strength]	Freeze-dried formulation for injection containing 52.5 mg of Alglucosidase Alfa (Genetical Recombination) in a vial
[Proposed indications]	Type II glycogen storage disease
[Proposed dosage and administration]	The usual dosage for intravenous drip infusion is 20 mg/kg body weight of Alglucosidase Alfa (Genetical Recombination) once every other week. The dosage may be adjusted depending on the patient's symptoms.
[Items warranting special mention]	Orphan drug

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

Summary of the data submitted by the applicant for this application and the applicant's response to inquiries made by the Pharmaceuticals and Medical Devices Agency (PMDA) about the data are as follows.

1. Origin or background of discovery and usage conditions in foreign countries, etc.

Type II glycogen storage disease (GSD II [also known as, Type II glycogen storage disorder, Pompe's disease, or acid maltase deficiency]) is a fatal lysosomal disease characterized by progressive loss of muscle strength, including cardiomyopathy and respiratory dysfunction as a result of accumulation of glycogen in the tissues and cells caused by a congenital defect of the glycogen degradation enzyme, acid α-glucosidase (GAA; acid maltase) in lysosomes.

GSD II is an orphan disease, the worldwide incidence of which is estimated to be 1/40,000 (including all subtypes). The disease is classified into the following subtypes according to the age of onset, severity of involvement of the affected organs, and the survival time: the typical infantile-, atypical infantile-, childhood-, juvenile-, and adult-onset forms. In all the forms, the damage to the tissues arising from the defect of GAA is progressive, eventually leading to death. Among the various subtypes of the disease, the most severe subtype of the disease is the rapidly progressive infantile-onset form, which is defined in the literature as typical infantile-onset form GSD II. Patients with this form of the disease exhibit symptoms or signs within 12 months after birth.

Accumulation of a large amount of glycogen in the heart and skeletal muscles rapidly causes cardiomyopathy, generalized muscle weakness, and hypotonia, and almost all patients die of cardiac failure or respiratory failure within 1 year after birth. Some infants with a form of GSD II in whom the age of onset is somewhat delayed (although it is usually within 12 months after birth) and the rate of progression of cardiomyopathy is slow have been reported (Slonim AE et al., *J Pediatr* 2000; 137: 283-285). This type of GSD II is called atypical infantile-onset form GSD II. Some patients with atypical infantile-onset form GSD II may survive longer than 1 year, but most patients develop respiratory insufficiency within 1 to 2 years after birth. In patients with infantile-onset form GSD II, irrespective of typical or atypical, little or no GAA activity is detected. Patients with infantile-onset form GSD II have been treated by a combination of treatments, including therapeutic drugs for cardiac diseases, antibiotics, respiratory therapy and ventilatory support, nutritional supplementation, and physical therapy. However, since these treatments are only symptomatic therapies, most patients die within 1 year after birth and the mortality remains almost constant until the present time. Meanwhile, in adult-onset form GSD II, patients exhibit signs and symptoms of the disease from early childhood to the sixth decade of life, with gradual progression of skeletal muscle and respiratory muscle damage. In general, myopathy is observed in the pectoral girdle and pelvic girdle. Symptoms of the respiratory system vary among patients, and respiratory insufficiency is the most frequent cause of death. It has been reported that residual GAA activity tends to be higher in patients with adult-onset form GSD II as compared with that in patients with infantile-onset form GSD II.

This product is a freeze-dried formulation for injection containing Alglucosidase Alfa (Genetical Recombination, hereafter referred to as rhGAA) as the active ingredient. It was developed as a therapeutic drug for enzyme supplementation therapy in patients with a confirmed diagnosis of GSD II, and approved for use in the EU in March 2006 and in the US in April 2006.

In Japan, rhGAA was designated as an orphan drug in February 2006, and the 4th Investigational Committee for Usage of Unapproved Drugs (April 2006) decided that it might be appropriate to submit application for marketing approval of the drug based on the results of foreign clinical trials. For this reason, an application for marketing approval of rhGAA has been submitted.

2. Data relating to quality

Summary of the submitted data

rhGAA is a glycoprotein (molecular weight, ca. 110,000) consisting of 896 amino acid residues ($C_{4490}H_{6817}N_{1197}O_{1298}S_{32}$), produced in Chinese hamster ovary cells transfected with cDNA encoding human acid α-glucosidase. Seven regions (Asn-84, Asn-177, Asn-334, Asn-414, Asn-596,

Asn-826, and Asn-869) of the single peptide have an N-binding type sugar chain (complex-type sugar chain, high mannose-type sugar chain, and high mannose-type sugar chain bound to mannose-6-phosphate), 12 out of the 13 Cys residues possess disulfide bonds, and Cys-318 is in the reduced form.

After translation, GAA is converted into its mature form through processing by glycosylation and by protein degrading enzyme; however, the mature form lacks a sugar chain structure which is necessary for transportation of lysosome, although specific activity is higher than the precursor, and therefore, rhGAA was developed as the precursor form.

1) Manufacturing process of the drug substance

(1) Construction of cell bank system

cDNA of human GAA (hGAA) was synthesized using mRNA extracted from human liver cells, lymphocytes, and fibroblasts, and an rhGAA expression vector was constructed by inserting this into the vector, pCLH3AXSV2DHFR (Reddy VB et al., *DNA*, 1987; 6: 461-472). This was introduced by the lipofection method into a CHO cell line lacking the dihydrofolate reductase, and the transformed cells were selected using methotrexate (MTX). A clone which had stable rhGAA-producing ability and proliferative properties was selected from the obtained clones to establish the master cell bank (MCB), and working cell banks (WCB [two WCBs, the initial WCB and new WCB, were prepared]) were then prepared from the MCB.

(2) Characterization and control of the cell bank

Characterization and purity tests are performed on the MCB, WCB, and end of production (EOP) cells.

As for the tests for characterization of the MCB (at the time of establishment), WCB, and EOP (at the time of the 2000-L scale manufacturing), investigation of the genome DNA, number of DNA copies, hGAA cDNA sequence, mRNA analysis, and chromosome insertion site of the rhGAA plasmid (except for [redacted]) was performed. No differences in the characteristics of the gene construct in the WCB or EOP were observed, which suggested that it remained stable during the incubation period. Also, rhGAA mRNA analysis by northern blotting revealed the same single transcription product in the EOP as that detected in the WCB.

As for the purity tests of the MCB, WCB, and EOP, CHO cell identification tests (fluorescent antibody method, isoenzyme test), tests for virus particles (transmission electron microscopy), bacteria, fungi (sterile test method) and mycoplasma (agar and Vero cell inoculation), the *in vitro* foreign virus test (inoculation into MRC-5, Vero, A9, CHO-K1, and MDBK cells), *in vivo* foreign

virus test (inoculation into mature guinea pig, mature mouse, infant mouse, and chicken fertilized egg), endogenous virus test (after administration to a virus-free hamster, the antibody titers to HANT, LCMV, MVM, EV, PVM, REO-3, SEN, and SV-5 were measured), and *in vitro* bovine-derived virus test (inoculated into the bovine conchal bone cells to determine the CPE and HAD) were performed. Tests for BVDV, rabies, bluetongue, BP, reovirus, BRS, BA3, and BA5 (by the fluorescent antibody method) and retrovirus test (focus induction test by mink S^+L^- cell inoculation, XC plaque formation test by mouse SC-1 cell inoculation, co-incubation test with Mus dunni cells, and reverse-transcriptase activity test) were performed; all were identified as CHO cells. The results confirmed that there was no contamination by infectious agents (all of which can be detected under the range of testing).

For the control standard at the time of renewal of the WCB, the cell count, survival rate, *in vitro* foreign virus, *in vivo* foreign virus, and CHO cell identification tests are specified. Also, it is set forth that the cell density and probability of survival of both MCB and WCB should be measured at least [redacted] times every [redacted] years.

(3) Incubation process

Incubate using a growth broth ([redacted] medium, [redacted]% donor bovine serum [DBS], [redacted]% [redacted], [redacted] μmol/L [redacted]) starting from [redacted] vial of WCB until [redacted]L bioreactor by gradual scale-up. At the production incubation process, perform continuous agitation incubation by [redacted]L bioreactor using a production broth ([redacted] medium, [redacted]% DBS, [redacted]% [redacted]) [cell proliferation phase], change the broth to a [redacted] production broth [[redacted] elimination phase], add the [redacted] production broth at the rate of [redacted]fold bioreactor volume per day, and collect the broth (harvest solution) at [redacted] rate [harvest phase]. Store the harvest solution in the harvest collection container at [redacted]°C to [redacted]°C.

In relation to the incubation process, the production incubation process is defined as the critical process, and in-process control tests (mycoplasma, *in vitro* foreign virus test, microbial limit test, endotoxin, and rhGAA activity) are specified before collection of the harvest solution.

(4) Purification process

Filtrate the harvest solution using the [redacted] filtration system, perform condensation by [redacted] filtration, and exchange the buffer by diafiltration. Separate this solution by metal chelate affinity chromatography, hydrophobic interaction chromatography, and ion-exchange chromatography, concentrate the dissolution solution by ultrafiltration diafiltration, exchange the buffer solution, and separate by [redacted] ion-exchange chromatography. Concentrate the dissolution solution, exchange the buffer, filter via a PVDF filter of 20-nm pore size for virus removal, and store as drug

substance at [redacted]°C to [redacted]°C.

In relation to the purification process, the processes of metal chelate affinity chromatography, hydrophobic interaction chromatography, ion-exchange chromatography, [redacted] ion-exchange chromatography, and virus removal are defined as the critical processes, and each of the dissolution solutions, except for that subjected to the virus removal process, is defined as a key intermediate. For the in-process control tests, rhGAA [redacted] recovery and endotoxin test for the metal chelate affinity chromatography dissolution solution, protein content recovery, endotoxin and [redacted] test for the hydrophobic interaction chromatography dissolution solution, and protein content recovery and endotoxin test for the ion-exchange chromatography dissolution solution and the [redacted] ion-exchange chromatography dissolution solution are specified. Also, the specifications and test methods of the drug substance are specified for the virus removal process. For the purpose of setting the acceptable storage period of each key intermediate, stability testing was performed, and based on the results, the storage conditions and storage period has been established as follows: Harvest solution, [redacted]°C to [redacted]°C [stainless-steel container] [redacted] days; [redacted] chelate Sepharose column [metal chelate affinity chromatography] road solution, [redacted]°C to [redacted]°C [polyethylene bag/stainless-steel container] [redacted] weeks; [redacted] chelate Sepharose column dissolution solution, [redacted]°C to [redacted]°C [polyethylene bag/stainless-steel container] [redacted] months; [redacted] Sepharose column [hydrophobic interaction chromatography] dissolution solution, [redacted]°C to [redacted]°C [polyethylene bag/stainless-steel container] [redacted] weeks; [redacted] Sepharose column [ion-exchange chromatography] dissolution solution, [redacted]°C to [redacted]°C [polyethylene bag/stainless-steel container] [redacted] weeks; [redacted] column [[redacted] ion-exchange chromatography] dissolution solution, [redacted]°C to [redacted]°C [polyethylene bag] [redacted] weeks.

Process validation testing is performed for the production incubation process and purification process.

Purity of rhGAA at each step of the purification process was determined by SDS-PAGE, and it was confirmed that the purity was increased at each column chromatography process and purification was completed to quantitation limit already by the [redacted] chromatography process.

Tests for process-derived impurity protein and residual DNA were conducted by the ELISA method and it was confirmed that both were removed during the purification process. As for process-derived impurity proteins other than of CHO cell-derived protein, bovine serum protein-derived bovine IgG and BSA were selected and their removal during the purification process was determined. A bovine IgG-specific ELISA method was used and the result showed that bovine IgG was appropriately removed during the purification process. Also, measurement of BSA

in the drug substance by a BSA-specific ELISA method revealed the presence of BSA at below the lower limit of quantification, indicating that BSA was also appropriately removed during the purification process. It was also confirmed that [redacted] derived from [redacted] column and [redacted] (cell incubation process-derived) were appropriately removed. When the level of polymer at each step after [redacted] Sepharose was evaluated by HPLC-SEC (size-exclusion chromatography), the level of polymer in each column dissolution solution was found to be below the lower limit of quantification, and it was confirmed that the level of polymer was kept at a low level throughout the purification process.

The microbial limit test and endotoxin test were performed at each step of the purification process to evaluate the appropriate control of microorganisms during the purification process, and appropriate microorganism control was confirmed throughout the purification process.

(5) Safety evaluation against foreign infectious agents

The animal-derived ingredients used during the manufacturing process are bovine serum from New Zealand and porcine pancreas-derived trypsin/EDTA from the US and Canada, which are used for the MCB and WCB establishment and during the drug substance manufacturing incubation process. The bovine serum is manufactured at facilities approved by the European Directorate for the Quality of Medicines (EDQM), and the ingredient used is bovine blood, which conforms to the Standards for Ruminant Animal-derived Ingredients of the Standards for Biological Ingredients. Healthy animals conforming to the Standards for Animal-derived Ingredients of the Standards for Biological Ingredients are used for producing trypsin/EDTA, and purified trypsin is sterilized and virus-inactivated by γ-ray irradiation.

Purity tests were performed to rule out contamination of the established MCB, WCB, and EOP by external and internal infectious agents, and no contamination by external or internal infectious agents was confirmed [See “Characterization and control of the cell bank”].

Virus spike testing was performed during the chromatography processes of [redacted] chelate column, [redacted] Sepharose column, and [redacted] Sepharose column of the purification process and during the nanofiltration process, and the virus clearance ability was evaluated. Virus inactivation/removal efficiency was investigated using mouse micro virus (MMV), mouse leukemia virus (XMuLV), reo virus type 3 (Reo), and pseudo rabies virus (PsR), and the results were as follows.

Virus Clearance Efficiency (Log_{10}) in the Drug Substance Purification Process

	MMV		Reo		XMuLV		PsR	
Column resin	New	After reuse	New	After reuse	New	After reuse	New	After reuse
[redacted] column	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] Sepharose column	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] Sepharose column	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] Sepharose column (removing [redacted])[1)]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] Sepharose column PCR [2)]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] inactivation [3)]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	
Nanofiltration (UF/DF[redacted] concentrated solution)	[redacted]		[redacted]		[redacted]		[redacted]	
Accumulated clearance efficiency	13.25		≥12.03		≥19.35*		≥12.77	

nt: not tested

1) [redacted] buffer [redacted] from which [redacted] was removed was used.

2) Virus removal efficiency was measured by a quantitative PCR method for viral nucleic acid. It was not used for the calculation of the cumulative viral clearance efficiency.

3) Virus was added to [redacted] (including [redacted]% [redacted]).

*: For calculation of the cumulative virus clearance efficiency of XMuLV, [redacted] chelate column, [redacted] Sepharose column, nanofiltration, [redacted] Sepharose column ([redacted] removal), and clearance efficiency of [redacted] inactivation were used. Cumulative viral clearance of other viruses was calculated from [redacted] chelate column, [redacted] Sepharose column, nanofiltration, and [redacted] Sepharose column.

(6) History of development of the manufacturing process (equivalence/homogeneity)

In response to the demand expansion of the product, the incubation process was scaled-up from the initial scale (30-L/60-L) to the 160-L, and then to the 2000-L. The most important change in the scale-up from the 160-L to the 2000-L scale was the addition of [redacted] μmol/L [redacted] in the seed incubation process.

Likewise, a scale-up of the purification process was implemented in response to the increase in the handling volume with the scale-up of the incubation process. Although there were no changes in the major processes or the types of the columns at any scale-up, the [redacted] of filter and the [redacted] of column were amplified to increase the handling volume. Also, in accordance with the scale-up from the 30-L/60-L scale to the 160-L scale, the [redacted] of the harvest solution was changed from [redacted] filtration to [redacted] filtration, and the cutoff molecular weight in the ultrafiltration and diafiltration was changed from [redacted]D to [redacted]D. At the scale-up from the 160-L to the 2000-L, the nanofiltration which was performed before the [redacted] ion-exchange chromatography was performed instead after the [redacted] ion-exchange chromatography.

To examine the quality characteristics of the rhGAA manufactured at each scale, the drug substances produced at the 30-L/60-L and 160-L scales were compared, as also the drug substances produced at the 160-L and 2000-L scales. In addition to the specifications and test methods of the drug substance, investigations of the rhGAA structure ([redacted],

[redacted], [redacted]), degradation products ([redacted] etc.), and biological properties ([redacted] mannose-6-phosphate [M6P] receptor [sCIMPr] binding ability, enzyme reaction rate, uptake by GSD II fibroblasts etc.) were also undertaken. The results revealed no significant differences in the quality characteristics of the rhGAA manufactured at different scales.

Because a non-clinical pharmacokinetic study revealed differences in some of the pharmacokinetic parameters between the 2000-L formulation and the 160-L formulation, the pharmacokinetics of the preparations from multiple lots were analyzed. The results revealed a correlation between the AUC and the sialic acid content in the preparation, suggesting that the preparation with a lower content of sialic acid may be transferred more easily to the liver. The relationships of sialylation at the terminal of sugar chain of glycoprotein to the AUC and clearance are well known (Fukuda MN et al., *Blood* 1989; 73: 84-89, Webster R et al., *Xenobiotica* 1999; 29: 1141-1155). Also, to date, the sialic acid content in the lots manufactured at the 2000-L scale was in the same range as that in the lots manufactured at the 160-L scale, although there was a slight tendency for the lower sialic acid content in the 2000-L scale lots than in the 160-L scale lots. However, changes of the sialic acid content within the specification range appear to have no major impact on the efficacy of rhGAA, in view of the facts that i) a pharmacodynamic study revealed that similar glycogen-reducing effect was observed for all lots irrespective of the differences in the sialic acid content and ii) although the range of sialic acid content in the lots used for previous clinical studies was even wider than the one in the specification, there have been no reports as yet of any major differences in the efficacy and safety among the lots.

2) Drug substance

(1) Structure and composition

As for studies for characterization of the drug substance, peptide mapping, amino acid sequencing, N-terminal amino acid sequencing, C-terminal amino acid sequencing, absorption coefficient, disulfide binding, sugar chain structure (glycopeptides analysis, monosaccharide composition, oligosaccharide profile), [redacted], [redacted], [redacted], matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, isoelectric focusing (IEF), capillary isoelectric focusing (cIEF), rhGAA purity (SDS-PAGE), reduction and non-reduction SDS-PAGE, non-denatured PAGE, circular dichroism (CD) spectrometry, western blotting analysis, enzyme reaction rate, [redacted] M6P receptor (sCIMPr) binding, uptake by GSD II (type II glycogen storage disease) fibroblasts, comparison of hGAA and rhGAA, and forced degradation studies of rhGAA were performed.

- The mass of [redacted] degradation peptide by LC/MS and mass of the endoprotease [redacted]

degradation peptide were almost identical to the theoretical value estimated by cDNA sequencing. Also, the N-terminal was determined to be a pyroglutamate by LC/MS analysis of the endoprotease degradation peptide.

- There was no deficit at the C-terminal and it was confirmed that Cys-896 and Cys-882 at C-terminal were bound by a disulfide bond.
- Twelve out of 13 Cys residues contained disulfide bonds and Cys-318 was in the reduced form.
- [redacted] degradation peptide was analyzed by LC/MS and determination of the structure of the N-binding sugar chain of the glycosylpeptide revealed that sugar chains of the high mannose type, mannose-6-phosphate-binding high mannose type, complex type, and hybrid type were bound to rhGAA.
- Isoelectric focusing revealed about 11 isoforms between pI [redacted] and [redacted].
- SDS-PAGE revealed a single major band of 110 kDa by CBB staining, and a major band corresponding to the 110-kDa and minor bands of lower molecular weight were observed by silver staining. Western blotting analysis revealed not only a major rhGAA band corresponding to the 110-kDa, but also an rhGAA band of lower molecular weight, although in extremely small amounts, and the same band pattern as that of the reference standard was observed.
- As for the enzyme reaction rate, measurement of the amount of the generated product [redacted] by making [redacted] the substrate of rhGAA, the values of K_m and V_{max} for the 2000-L scale drug substance [redacted] lots were [redacted] to [redacted] U/mg and [redacted] to [redacted] mM, respectively, and there were no marked differences among the lots.
- The rhGAA amount dependent ([redacted]-[redacted] μg/mL) receptor binding curve for M6P receptor and the rhGAA amount dependent ([redacted]-[redacted] μg/mL) uptake curve for GSD II fibroblasts were shown.
- Information available in the literature was used to compare the characteristics of rhGAA with those of GAA purified from human placenta (human placental GAA). GAA is processed by glycosylation and a protein-degrading enzyme after translation (110-kD hGAA precursor → 95-kD type hGAA intermediate → 76-kD type hGAA intermediate → 70-kD type mature hGAA); however, human placental GAA is the processed low-molecular type (76 kD type and 70 kD type) and rhGAA is the 110 kD type, the precursor. rhGAA is glycosylated and bound by major sugar chains of the high mannose type, high mannone type to which the M6P receptor necessary for lysosome transportation is bound, and the complex type; however, human placental GAA has no mannose-6-phosphate or sialic acid at the terminal of sugar chain. Under the pH in lysosomes, the processed type of GAA (95 kD type, 76 kD type, and 70 kD type) is reported to show higher specific activity than the 110-kD type of GAA (Oude Elferink RP et al., *Eur J Biochem* 1984; 139: 489-495, Wisselaar et al., *J Biol Chem* 1993; 268: 2223-31). However, the processed type of GAA lacks the appropriate post-translational modification necessary for transportation to lysosomes.

- No degradation or denaturation of rhGAA was observed under the usual storage conditions (pH [redacted], [redacted]°C) and pH [redacted], [redacted]°C in the forced degradation studies. However, at pH [redacted], [redacted]°C and [redacted]°C, deamidation of rhGAA, protein degradation, decrease of activity, and change of secondary and tertiary structures were observed, as also insoluble particulate matter.
  At pH [redacted], degradation and denaturation of rhGAA were observed regardless of the temperature of exposure, and aggregation and degradation of the protein were especially significant. Also under storage at [redacted]°C and [redacted]°C, change of the secondary and tertiary structures, deamidation, and decrease of the contents of sialic acid and mannose-6-phosphate were detected, and insoluble particulate matter was also observed.
  At pH [redacted], degradation and denaturation of rhGAA were observed to some extent, regardless of the temperature of exposure. The major change at [redacted]°C was aggregation, and deamidation and change of the secondary and tertiary structures were observed at [redacted]°C and [redacted]°C. Also at [redacted]°C, the oxidation level of rhGAA was slightly increased.

As for impurities, desired substance-derived impurity and manufacturing process-derived impurity were investigated. In the harvest solution collected from the rhGAA-expressing CHO cells, not only the desired substance 110-kD type rhGAA (110 kD type), but also the processing-type rhGAA (processing type) were included. Because the processing type lacked the appropriate post-translational modification necessary for effective uptake by lysosomes, the purification process of rhGAA was designed for only the 110 kD type (the processing type is typically removed by [redacted] Sepharose column chromatography and [redacted] Sepharose column chromatography). Residual amounts of the processing type could be detected by 2 kinds of SDS-PAGE ([redacted] kD + [redacted] kD, [redacted] kD). Currentaly available data revealed that purity (relative value) of [redacted] kD rhGAA to [redacted] kD + [redacted] kD rhGAA is more than [redacted]% and purity (relative value) of [redacted] kD + [redacted] kD rhGAA to [redacted] kD + [redacted] kD + [redacted] is not less than [redacted]%. Both of the SDS-PAGE methods were defined as purity tests in the specifications and test methods for the drug substance.

The purification process of the drug substance was designed to remove the manufacturing process-derived impurities, such as process-derived impurity protein, DNA, [redacted], and [redacted], and based on the actual values of 6 lots of the drug substance, these impurities were confirmed to be removed. These tests are specified as the specifications and test methods for the drug substance.

(2) Specifications and test methods

The description, identification, peptide mapping, sugar chain structure identification test, isoelectric focusing, pH, purity tests (process-derived impurity protein, rhGAA purity test, [redacted], DNA, [redacted], [redacted], and polymer), [redacted], sialic acid, endotoxin, assay (protein content), and rhGAA specific activity are included in the specifications and test methods for the

drug substance.

(3) Stability of the drug substance

Stability of 3 drug substance lots (namely, A008, A009, and A010) manufactured at the 160-L scale was investigated under the condition of storage in a polyethylene bag at 8°C ± 2°C for [redacted] weeks, as also the stability of the 2000-L scale drug substance stored under conditions of the [redacted]L type stainless-steel container in the temperature range of 2°C to 10°C (2 lots [A019 and A026] at [redacted]°C ± [redacted]°C, 3 lots [A021, A023, and A024] at [redacted]°C ± [redacted]°C) for [redacted] weeks, and the activity, polymer, description, assay (protein content), pH, purity, specific activity, endotoxin, and microbial limit test were selected as test attributes. For 2 (A008 and A009) of the 3 lots of the 160-L scale drug substance, their activity fell by about [redacted]% at [redacted] weeks, compared with that at the beginning of test, but there was no change in the remaining one lot. No changes were observed in the endotoxin or microbial limit test in any lots during the storage period. As for the 2 lots of the 2000-L scale drug substance stored at 8°C ± 2°C for [redacted] weeks, no changes in any of the test attributes were observed during the storage period; however, an increase of polymer by up to [redacted]% was observed for 3 lots of the 2000-L scale drug substance stored at 4°C ± 2°C for [redacted] weeks. No changes were observed in the endotoxin or microbial limit test in any of the lots during the storage period.

In view of the facts that the activity of the 160-L scale drug substance fell at [redacted] weeks and at [redacted] weeks, and that the amount of polymer increased in the 2000-L scale drug substance stored under 4°C ± 2°C for [redacted] weeks, the storage period of the drug substance is set at 4 weeks for storage under 8°C ± 2°C in a polyethylene bag (160 L) or in a [redacted]L type stainless-steel container (2000 L).

3) Drug product

(1) Dosage form design

This product is a freeze-dried preparation of formulated drug substance which corresponds to 52.5 mg per vial (10.5 mL), so that 50 mg of rhGAA as the active ingredient can be collected with certainty, and it is formulated to be at 5 mg/mL when reconstituted in 10.3 mL of Water for Injection. As a result of several investigations, 25 mmol/L of sodium phosphate buffer, pH 6.2, under which rhGAA was stable, was selected as the buffer. Freeze-drying was chosen for the formulation, and D-mannitol and polysorbate 80 were used as the stabilizer/excipient and stabilizer, respectively.

(2) Formulation process

Formulation buffer was added to the drug substance, and after adjusting the final concentration of rhGAA protein to 5 mg/mL, of polysorbate 80 to 0.005% and of D-mannitol to 2%, it was filtered through the [redacted] filter of [redacted]μm pore size, stored as formulated drug substance in

[redacted], subjected to in-process control test, and pooled. Pooled formulated drug substance was filtered by the [redacted]μm [redacted] filter and stored in [redacted] This formulated drug substance was filtered by the PVDF filter of [redacted]μm pore size, sterile-filtered through a 0.2-μm PVDF filter, filled into a 20-mL washed and dry-sterilized glass vial, and partially stoppered using a siliconized butyl rubber stopper. After the freeze-drying, the vial was completely stoppered and sealed with an aluminum flip cap, in order to produce the filled drug product.

The formulation and concentration adjustment process, sterile filtered process, filling process, and freeze-drying process were defined as the critical processes of the drug product manufacturing process. Tests for specific activity, polymer, appearance, endotoxin, microbial limit, protein content, D-mannitol, osmotic pressure, pH, phosphate, polysorbate 80, and sodium salt of the formulated bulk, which was a key intermediate, are set as the in-process control tests of the formulation and concentration adjustment processes. Also, filter integrity for the sterile filtered process; fill amount for the filling process; identification test, isoelectric focusing, osmotic pressure, oxygen headspace, SDS-PAGE (purity test), and reconstitution time of the filled drug product for the freeze-drying process; and total vial number inspection and visual inspection of the label and packaging material for the packaging and labeling processes are specified as the in-process control tests.

(3) Specifications and test methods

The description (before reconstitution, after reconstitution), pH, polymer, water, insoluble particulate matter test, endotoxin, sterile test, assay (protein content), and specific activity are included in the specifications and test methods of the drug product. As for the identification test, enzyme activity test that is conducted in “specific activity” test is considered to be substituted; and as for content uniformity test, the filling into vial is performed by the validated filling machine, and the fill amount is controlled by weight in the process. Therefore, these are not included in the specifications.

(4) Stability of the drug product

Stability testing of the formulated drug substance (6 lots of the 160-L scale, 4 lots of the 2000-L scale), which is a key intermediate, was performed and the storage conditions for the actual manufacturing process at each scale were investigated. The formulated drug substance lots (stainless-steel container, 3 lots; polyethylene bag, 3 lots) of the 160-L scale and the first one lot (stainless-steel container) of the formulated drug substance of the 2000-L scale were stored at [redacted]°C to [redacted]°C, and the remaining 3 lots (both stored in a stainless-steel container and a polyethylene bag) of the 2000-L scale were each stored at 2°C to 10°C (setting temperature, [redacted]°C) for 6 months, and tests for the rhGAA activity, polymer, description (appearance), assay (protein content), pH, purity,

specific activity, the endotoxin, and microbial limit were chosen as the stability test attributes.

Although the amount of polymer increased by up to [redacted]% at [redacted] months, no changes of the other attributes were observed during the storage period at [redacted]°C to [redacted]°C. On the other hand, increases of polymer were observed (maximum [redacted]%) after storage of [redacted] months at [redacted]°C in all the lots, while the activity fell by [redacted]% and specific actity fell by [redacted]% for 1 lot. Slight changes in purity and pH were also observed.

From the above, when the formulated drug substance was stored in the [redacted]L stainless-steel container or polyethylene bag, the storage period was set at [redacted] months under storage at [redacted]°C to [redacted]°C and at [redacted] weeks under the setting of [redacted]°C (range, [redacted]°C to [redacted]°C). Because an increase of polymer was observed under storage at [redacted]°C, an additional stability testing for the drug substance stored at [redacted]°C to [redacted]°C is planned for the 2000-L scale formulated drug substance.

Long-term stability testing (at 2°C to 8°C for a storage period of [redacted] to [redacted] months depending on the lot) was performed for 6 lots of the drug product manufactured from the 160-L scale drug substance and 6 lots of the drug product manufactured from the 2000-L scale drug substance, and rhGAA activity, polymer, description (appearance), endotoxin, protein content, [redacted], isoelectric focusing, D-mannitol, water content, osmotic pressure, oxygen headspace, insoluble particulate matter, pH, phosphate, purity, reconstitution time, sodium, specific activity, polysorbate 80, container air-tightness, and sterility are specified as the test attributes. [redacted] was added to the stability test attributes, because the formation of [redacted] was speculated to be one of the possible degradation pathways following exposure to extreme pH/temperatures. The 160-L scale drug product ([redacted] lots) was shown to be stable at 2°C to 8°C for [redacted] month following a [redacted]-month storage. Also, based on the results of stability test for a maximum of [redacted] months and the results of the accelerated test, as described later, it seemed unlikely that there would be differences in the stability between the 160-L scale drug product and the 2000-L scale drug product.

Also, accelerated testing (25°C ± 2°C/60 ± 5% RH, 6-month storage) was performed, and rhGAA activity, polymer, description (appearance), protein content, [redacted], water content, oxygen headspace, pH, purity, reconstitution time, and specific activity are specified as the test attributes. The results revealed slight changes in some of the drug product characteristics (a slight increase in oxygen headspace, a change in water content, decreases in activity and specific activity, an increase in protein content, a decrease of purity, a decrease of pH, an extension of reconstitution time, and an increase of polymer).

From the above, the expiration date of drug product is set at 24 months for storage at 2°C to 8°C. As for long-term testing, it is ongoing.

Also, after the reconstitution of rhGAA, stability testing under storage at 2°C to 8°C and 25°C ± 2°C/60% ± 5% RH was performed, and rhGAA activity, polymer, description (after reconstitution), protein content, pH, purity, and specific activity are specified as the test attributes. The results revealed no change in any of the test attributes until 96 hours under storage at 2°℃ to 8°C, which conformed to the specifications. On the other hand, under the conditions of 25°C ± 2°C/60% ± 5% RH, an increase of the polymer to 0.7% was noted; however, the applicant explained that all of the test attributes conformed to the specifications during the storage period. From the above, the applicant concluded that it was possible to keep rhGAA until 96 hours after it was reconstituted in 10.3 mL of Water for Injection and kept in cold storage. However, considering the slight increase in the accumulation of particulate matter over time, it is specified that intravenous drip infusion be prepared by dilution with physiological saline immediately after the reconstitution of rhGAA.

Also, stability testing under storage at 2°C to 8°C or 25°C ± 2°C for 72 hours plus administration time (expected for 6 hours) was performed for the sample (protein concentration, 0.5 mg/mL and 4 mg/mL) prepared by dilution with physiological saline for intravenous drip infusion after the reconstitution of rhGAA, and the activity, polymer, description (infusion bag), protein content, purity, pH, and specific activity were measured. A decrease in the protein content by 0.05 mg/mL was observed after 72 hours plus 6 hours for one lot of 4 mg/mL under storage at 2°C to 8°C; however, no change was observed for 0.5 mg/mL. Also under the storage at 25°C ± 2°C, an increase up to about 0.4% after 72 hours plus 6 hours was observed for all the lots of 4 mg/mL in the polymer test; however, no change was observed for 0.5 mg/mL. From the above, it was concluded that storage until 24 hours at 2°C to 8°C and 25°C ± 2°C was possible when the sample was diluted to make the protein concentration of 0.5 to 4 mg/mL. However, from the microbiological viewpoint, immediate use after dilution was recommended.

4) Reference standard

Reference standard was used for the identification test, peptide mapping test, sugar chain structure identification test, and isoelectric focusing test for the specifications and test methods of the drug substance. It was also used for all of the tests which needed a comparison to the reference standard.

At the beginning of the development, the tentative reference standard B001 prepared from the drug substance manufactured at the 30-L/60-L scale was used, and it was confirmed to conform to the specifications and test methods (description, identification test, pH, rhGAA purity test, [redacted], DNA, [redacted], polymer, endotoxin, assay [protein content], rhGAA specific activity, and

mycoplasma) of the drug substance at that time, and at the same time, characterization analyses were performed.

The initial reference standard B002 was prepared from the drug substance manufactured at the 160-L scale and it was confirmed to conform to the specifications and test methods of the drug substance, and at the same time, characterization analyses were performed.

At the renewal of the reference standard, specifications and test methods (description, identification test, peptide mapping, sugar chain structure identification test, isoelectric focusing, pH, process-derived impurity protein, rhGAA purity test [SDS-PAGE], [redacted], DNA, [redacted], polymer, [redacted], sialic acid content, endotoxin, protein concentration, and rhGAA specific activity) of the drug substance and characterization analyses ([redacted], [redacted], hamster GAA, [redacted], SDS-PAGE [silver staining], monosaccharide composition, N-terminal sequence, circular dichroism [CD] spectrum, western blotting, molecular weight, enzyme reaction rate, non-denatured PAGE, [redacted] binding ability, [redacted] assay, [redacted] content, uptake by GSD II fibroblasts/ [redacted] modification, non-denatured PAGE, sugar chain structure analysis, and mass analysis) were performed and a reference standard was authorized. Also, the implementation of re-evaluation test [redacted] is specified, and therefore, no expiration date is set for the reference standard.

Outline of the review by PMDA

1) Process-derived impurity protein

As for the test method for process-derived impurity protein, a process-derived impurity protein reference standard (mainly containing CHO cell-derived protein), BSA, and bovine IgG, which were antigens, were detected by an ELISA method using antibody obtained by immunization of these antigens to [redacted]. However, before the development of this method, the testing had been performed individually by the ELISA method. Therefore, PMDA asked the applicant to explain the reason why the evaluation system was actively changed to the BSA and bovine IgG combination, and whether or not the sensitivity with the combination was reduced as compared with that of the individual evaluation.

The applicant responded as follows. A measurement method by which both cell-derived impurity protein and medium-derived impurity protein can be detected simultaneously was developed by the manufacturer to simplify the operation. Since the current test method for process-derived impurity protein was validated and it was also demonstrated that a very small amount of BSA and bovine IgG can be detected specifically in the presence of rhGAA, it was considered less necessary to

evaluate each impurity individually. The ELISA method specific to BSA or bovine IgG had been used for the characterization analysis etc. before the establishment of the current method, but its use was stopped because the reagent necessary for the measurement was unavailable.

- BSA and bovine IgG of known concentrations were diluted with [redacted] μg/mL of drug substance and buffer for dilution and the protein concentrations of BSA and IgG in the drug substance diluents were measured to estimate the recovery rate of these in the buffer-diluted sample. It was shown that the measurement of rhGAA was not interfered with, because each recovery rate was [redacted]% to [redacted]% (BSA) and [redacted]% to [redacted]% (IgG), and the detection of a very small amount of BSA and IgG was possible by the test method. Before the development of the current (proposed) test method, BSA- or bovine-IgG specific ELISA for research was used as a tentative test method, and the sensitivity was [redacted] ng/mL for both BSA and bovine IgG. On the other hand, the detection limit of BSA and IgG of the proposed test method was [redacted] ng/mL and the method showed higher sensitivity.
- Recovery was not measured using CHO cell-derived protein only; however, the recovery was measured as the validation test (accuracy test), when a mixed sample of BSA, bovine IgG, and CHO cell-derived protein was spiked to the purified drug substance. The recovery was [redacted]% to [redacted]%, detection limit was [redacted] ng/mL under the range of the spiked sample of [redacted] to [redacted] ng/mL, and it was confirmed that impurities in the spiked sample under the existence of rhGAA were detected specifically with higher sensitivity by this method. BSA and bovine IgG were both found to be very small in the order of ng/mL in the drug substance residual impurity protein, and most of the process-derived impurity protein detected by the specification and test methods was suggested to be CHO cell-derived protein.

PMDA accepted the above response because the current test method could detect BSA and bovine IgG specifically and the sensitivity was shown to be [redacted] times higher than that of the tentative method, and most of the process-derived impurity protein detected by this specifications and test methods was CHO cell-derived protein, although no measurement of recovery was performed using the CHO cell-derived protein only, and the addition of BSA and bovine IgG was unlikely to have a significant impact on the detection sensitivity and specificity of CHO cell-derived protein.

2) Manufacturing method

(1) Re-use of column resin

Because the use of resin, which had been repeatedly re-used, was investigated for the virus clearance study, PMDA asked the applicant to explain the criteria for re-use of each column resin, and the applicant specified the maximum number of re-use, and PMDA accepted the response.

(2) Fill amount of drug product

PMDA asked the applicant to explain why the fill amount of the drug product was defined by protein content, even though the specification of rhGAA specific activity was in the range of [redacted] to [redacted] U/mg.

The applicant responded as follows. rhGAA was administered at the dosage that was based on protein content (mg/kg) in all the past clinical studies, and the proposed dosage, which was established based on the results of the clinical studies, was also defined by protein content (20 mg/kg). In addition, the dosing solution can be prepared by diluting the product based on the protein concentration at the time of the actual administration. In considering the past clinical use results and convenience of the operation, the fill amount in commercial-scale was also defined by the protein content. It was also considered appropriate to define the fill amount by the protein content, because the accuracy of the test method was higher than that of the activity method. The uniformity of the active ingredient (rhGAA) amount in the final formulation is to be confirmed by implementation of the specifications and test methods for the protein content and rhGAA specific activity to see the conformity to the specifications.

PMDA considered that it is also possible to prepare the dosing solution by diluting rhGAA based on the activity at the time of the actual administration, and that it is not appropriate to justify the defining of the fill amount by protein content because of the higher accuracy of the test. However, PMDA accepted the applicant's response, because the fill amount is defined based on the past clinical use results, and the protein content and rhGAA specific activity are defined also for the final drug product.

3) Specifications

(1) Description

PMDA asked the applicant to explain the acceptable degree of slight particle accumulaton for the specifications of description of the drug substance and description of the drug product after reconstitution, which is defined as "clear solution or slightly particulate solution."

The applicant responded as follows. The establishment of specifications for the degree of particle formation was challenged; however, it was extremely difficult to set the quantitative and qualitative specifications, because i) a suspension in which particles were distributed homogeneously and uniformly was not obtained, and ii) the size and amount of the particles changed over time, depending on handling and so on. However, the particles in the drug product could be removed through a [redacted]μm filter without impact on the quality of the drug product, such as on the potency [See "*Outline of the review by PMDA*, 3) Stability, (2) Caution in handling" section], and the particles in the drug substance was also considered to be removed by the filtration through a

[redacted]μm filter during the drug product manufacturing process. Also, the results of specifications and test methods for the past drug product showed that no impact on the quality of drug product was observed from the existence of the particles even in the drug substance. Based on the above results, no concrete specifications for the degree of particle accumulation are established and the degree of particle accumulation is considered acceptable when it has no impact on the clarity defined as a specification of description.

PMDA accepted the above response under the consideration that these particles had no impact on the quality, such as potency of the drug product, and could be removed with a [redacted]μm filter, and that the observation of a slight amount of particles posed no major problem.

(2) Peptide mapping

PMDA asked the applicant to explain that the content actually confirmed for the equivalence should be defined, although the specification of peptide mapping, which is set as the specifications and test methods of drug substance, was "showing the same chromatography pattern as that of reference standard."

The applicant explained the acceptance criteria for the equivalence of the chromatography pattern as follows: (a) observing the same dissolution peak as that in the reference standard and observing no abnormal peak, (b) the retention time of each peak is the same as that in reference standard, (c) the correction peak area from [redacted] to [redacted] is [redacted]% to [redacted]% of that of reference standard and the correction peak area of [redacted] is [redacted]% to [redacted]%, which is specified as the acceptance criteria.

PMDA considered it possible to ensure the homeostasis of the primary structure because the above specification is established along with the specifications and test methods of activity, and a semi-quantitative analysis was performed based on the peak areas of both the peptide fragments and the reference standard, and accepted the above explanation by the applicant.

(3) Identification of the sugar chain structure

The specification of sugar chain structure is defined as observation of the [redacted] base peaks. PMDA asked the appilcant to investigate the definition of the peak areas of the reference standard and [redacted] to [redacted] to the extent possible.

The applicant responded as follows. The sugar chain structure identification test used the pulse ampere detection (PAD) method after separation of the sugar chain freed from rhGAA by high pH negative ion-exchange chromatography (HPAEC); however, in this method, variability of the peak area was observed, especially among the detector cells and among equipment (Ganesa C et al.,

BioPharm International 2003; June: 44-52). When the peak areas of sugar chains similar to the sugar chain structure of the standard peaks [redacted] to [redacted] of rhGAA were measured by the HPAEC-PAD method at the site of the manufacturer, the relative standard deviation of the peak areas among detector cells was about [redacted]% at the maximum, and the relative standard deviation of the percent peak areas among the detector cells was about [redacted]% at the maximum in the validation study of this method, in which a single rhGAA was treated as the sample. Therefore, given the characteristics of this measurement method, it was considered difficult to define the peak areas of the standard peaks [redacted] to [redacted] as specifications for equivalence of the chromatographic pattern among different samples.

PMDA asked the applicant to explain the reason why the peak area is defined in the US and Canada.

The applicant responded as follows. For the above reason, the peak area in a single sample is specified as the relative peak area ratio (ratio of each base peak area to the total peak area) in the specification, not among different samples. Based on the results for past manufacturing lots of rhGAA, the percent peak area of each base peak was calculated, and the application was submitted to the US and Canadian regulatory authorities as the specification of sugar chain structure identification test for the product manuraftured at the 160-L scale. In Japan, the applicant intends to add a similar specification of the relative peak area ratio to the specification of this test after the specification for the product manufactured at the 2000-L scale is fixed in the US.

PMDA has directed the applicant to conduct an immediate investigation to define the peak area in a single sample as the relative peak area ratio, because the results for the product manufactured at the 2000-L scale has been accumulating. PMDA considers it important to change the specification as soon as possible in Japan as well, when the specification for the product manufactured at the 2000-L scale is defined in the US in the near future.

(4) Amount of mannose-6-phosphate

PMDA asked the applicant to explain a possible impact on the efficacy because mannose-6-phosphate was suggested to be involved in the uptake of rhGAA by the cells and lysosomes, but the specification range of mannose-6-phosphate defined in the drug substance varies about a [redacted]-fold.

The applicant provided the following explanation. The amount of mannose-6-phosphate in all the lots used for clinical studies in the past was in the range of [redacted] to [redacted] mol/mol, and this corresponded to the range of mannose-6-phosphate content in the [redacted] lots used in the setting of specifications. Although differences in the mannose-6-phosphate content were associated with

slight differences in the uptake of rhGAA by the cells, no direct impact of the defferences in the mannose-6 phosphate content within this range has been confirmed on the clinical efficacy. Also, there has been no report to date about any significant differences of the efficacy or safety for clinical use among the lots; therefore, the specification of [redacted] to [redacted] mol/mol is considered to be appropriate.

PMDA considers that it is unavoidable to use this specification in the immediate future because there is no report of any significant difference in the efficacy or safety on clinical use among the lots under the established specification, but recommends that an investigation be continued to re-establish the specification based on the actual values of lots manufactured in recent days because this is an index related to efficacy.

(5) Amount of sialic acid

PMDA asked the applicant to explain whether the efficacy of rhGAA would not be interfered with even when the specification range of the *N*-acetylneuramic acid (NANA) content is slightly less than about [redacted] fold of that established as the specification of sialic acid content in the drug substance, in view of the fact that the sialic acid content has been suggested to have an impact on the AUC and uptake by the liver in a non-clinical pharmacokinetic study.

The applicant gave the following explanation. The test results suggest a decrease in the uptake of rhGAA by the liver and increase in AUC associated with an increase in sialic acid content (NANA) in rhGAA; however, there has been no confirmation of the impact on the efficacy of the product resulting from the change in the AUC or liver uptake with a change in the sialic acid content in the specification. Also, when the efficacy (decrease in the tissue glycogen content) of the formulation ([redacted] lots) with sialic acid [redacted] to [redacted] mol/mol content was evaluated using GAA-knockout mouse, the decrease in the glycogen content of the heart and skeletal muscle were the same for all the lots. Furthermore, the sialic acid content in formulation lots used in the past clinical studies varied over a wider range of [redacted] to [redacted] mol/mol than the actual values in the [redacted] lots used to estsablish the specification of sialic acid; however, there have been no reports to date about any significant differences in the efficacy or safety among the lots. From the above, it is considerd that a sialic acid content ranging from [redacted] to [redacted] mol/mol, which was established based on the actual values, is appropriate, and that this have no impact on the efficacy of the product.

PMDA considers that it is unavoidable to use this specification in the immediate future because there is no report of any significant difference in the efficacy or safety on clinical use among the lots under the established specifications, but recommends that an investigation be continued to re-establish the specification based on the actual values of lots manufactured in recent days because

this is an index related to efficacy. Although the specification for the acceptable content of *N*-glycolylneuraminic acid (NGNA) has been considered in the US and Canada, there is no plan to specify such content of NGNA in Japan. Therefore, PMDA directed the applicant to perform the specification control, including that of NGNA in Japan as well, as it has been done overseas [See "3. Non-clinical data, (ii) Summary of pharmacokinetic studies, *Outline of the review by PMDA*, (1) Effects of sialic acid on the pharmacokinetics of rhGAA and assurance of post-marketing safety"].

(6) Differences from foreign specifications

PMDA asked the applicant to submit a list of specifications and test methods (at manufacturing and at storage) of the drug substance and drug product undertaken overseas. The applicant submitted the specifications and test attributes in the EU, US, and Canada, and PMDA performed the following review based on the information provided.

a. Since the specification of the rhGAA purity test (kD + kD) in Japan is less stringent than the one in the EU and US, PMDA asked the applicant to explain the difference and to revise the specifications in Japan to those in the EU and US.

The applicant responded as follows. The "not less than %" specification value submitted to the Japanese regulatory authority (at the end of June 2006) was the same as the contemporary value in the EU and US. However, the manufacturer subsequently specified a tighter "not less than %" value than the previous one in accordance with the overseas regulatory authorities. Since this specification value is proposed to be applied for the actual manufacturing in Japan as well, the specification value for the rhGAA purity test in Japan is going to be revised to the same "not less than %" value as in the EU and US.

PMDA accepted these responses; however, it underlined the necessity to take an appropriate action as soon as possible in Japan, as with the case overseas, if the specifications and test methods, etc. are revised overseas.

b. PMDA asked the applicant to explain why it was determined unnecessary to establish the specifications in Japan, even though the relevant test is, although tentatively, specified in the EU, along with showing the summary of the test method of the isoform assay of the drug substance, which is currently under validation.

The applicant responded as follows. The isoform assay is performed using capillary isoelectric focusing, major peaks observed between markers pI and are separated into 3 parts,

namely, [redacted] to [redacted], [redacted] to [redacted], and [redacted] to [redacted], and the isoforms are quantified by calculating the ratio of each peak to the total peak area.

The isoform assay has been developed as a method for research; however, during the review in the EU, the authority required establishment of a quantitative assay for the [redacted] rhGAA isoform as one of the specifications and test methods for the drug substance. According to this requirement, the manufacturer performed the validation study for this test method, and committed to establishing it as a specification and test method for the drug substance in the future, and further set tentative specifications for the test, although the validation study has not yet been completed. However, subsequent development revealed that the tentative specifications are not appropriate, and thus, more appropriate specifications would be established by obtaining data soon after completion of the validation study. Also, in Japan and the US, as in the EU, the application for this method is planned to be submitted as the specifications and test methods of the drug substance as a replacement for the current isoelectric focusing, after completion of the development.

PMDA accepted the above explanation.

c. PMDA asked the applicant to explain why equivalent quality to that overseas could be ensured in Japan without establishing the specifications defined overseas, and also PMDA asked the applicant to set the specifications, where appropriate.

The applicant gave the following explanation. The specifications and test attributes that are not established in Japan, as compared to overseas, are [redacted], isoelectric focusing, [redacted], [redacted], [redacted] purity test, and [redacted]. These tests are performed as in-process control tests after the freeze-drying process in Japan and the specifications were not established, considering that equivalent quality to that overseas could be ensured. Isoelectric focusing, [redacted], and [redacted] were not described in the application document as in-process control tests; therefore, these tests will be defined.

PMDA accepted these responses because the specifications and test methods, etc., at present, were established so as to ensure equivalent quality of the product to that overseas. However, in case changes are made, such as in the manufacturing methods, specifications and test methods, PMDA considers it necessary for the applicant to take an appropriate action as soon as possible, so that the quality of the product supplied in Japan would be at least not inferior to that of the product used for patients overseas.

3) Stability

(1) Storage temperature of the drug substance and formulated drug substance

PMDA sought the perspective of the applicant about the slightly unstable matter at 4°C ± 2°C as compared with that at 8°C ± 2°C, because in general, protein is more unstable at higher temperatures, although 8°C ± 2°C is set as the recommended storage temperature from the observation of the polymer increase at 4°C ± 2°C in the long-term testing of the drug substance and stability testing of formulated drug substance. Also, PMDA asked the applicant to submit comparative data, if any has existed, from examination of the same lot at 4°C ± 2°C and 8°C ± 2°C.

The applicant responded as follows. In general, protein has a tendency to be unstable at higher temperature, and rhGAA was also unstable at higher temperatures, as determined under the temperature conditions in the forced degradation studies. However, the polymer test under the long-term testing of the drug substance and stability testing of the formulated drug substance revealed that rhGAA was more unstable at 4°C ± 2°C as compared with that at 8°C ± 2°C. It was speculated that rhGAA had the characteristic of stabilizing thermodynamically at a slightly higher temperature than 4°C and of possible denaturation at the lower temperature condition of 4°C. Also all the stability test results, except that of polymer formation, of the same lot of drug substance (weeks) and formulated drug substance (weeks) at the temperatures of 4°C and 8°C, were the same, while, in regard to polymer formation, it was confirmed that storage at 8°C allowed greater stability, even for the same lot, with double the polymer formation noted at 4°C than at 8°C. Under all of the storage conditions, all the test results conformed to the specifications, and polymer formation was also in the specification range (not more than %) throughout the storage period. Based on the above results, it was determined that storage of the drug substance and formulated drug substance at 6°C to 10°C was the most appropriate.

PMDA determined that it is appropriate to set the storage temperature of the drug substance and formulated drug substance at 6°C to 10°C, as determined by the applicant, because of the reproducible result of the slight instability at 4°C ± 2°C as compared with that at 8°C ± 2°C for the drug substance and formulated drug substance of rhGAA, different from the case for proteins in general, and also because of the absence of lot-to-lot differences.

(2) Caution in handling

Formation of white or semi-transparent fiber-like particles (which was composed of rhGAA) was observed in rhGAA after reconstitution and after dilution with physiological saline, and several investigations have showed that the particle formation was accelerated by inappropriate handling (too much shaking, mixing, and so on). For this point, the applicant explained that the particle formation was almost entirely preventable by appropriate handling at the time of reconstitution and dilution of the drug product, and the particles could be removed from the drip infusion bag using a

0.2-μm protein low-affinity filter without significant impact on the potency and purity of the drug product. Therefore, PMDA sought the applicant's perspective on whether the caution of the current draft of the package insert is appropriate. Also, PMDA asked the applicant to submit concrete data about the removal of the particles without any impact on potency and purity of the drug product using a 0.2-μm filter.

The applicant gave the following explanation. To verify the removal of particles without impact on the potency and purity of the drug product using a 0.2-μm protein low-affinity filter, the particle was formed artificially and filtered through the 0.2-μm filter, and visual inspection and particulate measurement were performed. None of the samples showed clogging of the filter and the number of particles declined dramatically after the use of the filter. Also because no changes in activity, polymer, protein content, pH, specific activity, or purity were observed between before and after the filtration, it was determined that the particles could be removed without any impact on the potency and purity of the drug product using the 0.2-μm protein low-affinity filter. When actually using the in-line filter, there was a high possibility of clogging because the area of contact with the solution was small, and use of a larger membrane filter (hydrophilic polyethersulfone membrane filter) was considered to be preferable, with an area suitable for administration of the necessary volume in about 4 hours; therefore, it was determined to add the following description in the package insert.

"Be sure to use a transfusion set which contains a 0.2-μm hydrophilic polyethersulfone membrane filter with a flat housing form. Do not use a transfusion set equipped with a filtration net for foreign particulate matter removal."

PMDA accepted the above response.

3. Non-clinical data

(i) Summary of pharmacology studies

Summary of the submitted data

1) Primary pharmacodynamics

(1) Tissue glycogen-reducing effect

a. Dose-response relationship after repeated administration (Study SN-001, 4.2.1.1; SN-002, 4.2.1.2)

rhGAA was administered intravenously to GAA-knockout mice (GAA KO mice) at the dose of 0 (vehicle), 1, or 5 mg/kg once a week for 6 weeks (4 animals in each group) (4.2.1.1) or at the dose of 0 (vehicle), 20, or 100 mg/kg once a week for 4 weeks (5 to 6 animals in each group) (4.2.1.2). Because hypersensitivity reaction to a recombinant human protein was expected to occur with the administration of rhGAA, 5 mg of diphenhydramine (DPM) was administered intraperitoneally to animals 20 minutes before the 2nd and subsequent administrations of rhGAA. The results of a biochemical assay of glycogen levels in various tissues 24 hours after the administration indicated a decrease in the glycogen content in the cardiac muscle to a level below the detection limit at the dose of 100 mg/kg, and also about 25% decrease of the glycogen content of the skeletal muscles, while the tissue glycogen contents decreased to less than 2% in the vehicle group. When determined by a histomorphological assay method, decreases in the glycogen contents in the cardiac muscle and quadriceps muscle were also detected in the 100 mg/kg group.

b. Efficacy of long-term repeated administration (Study SN-007, 4.2.1.3)

After intravenous administration of rhGAA to GAA KO mice at the dose of 0 (vehicle), 10, or 20 mg/kg once a week for 16 weeks, the tissue glycogen content was determined by biochemical assay. A dose-dependent decrease in the glycogen content in the cardiac muscle was observed. The percent decrease at Week 16 relative to the tissue content in the control group was 73% in the 10 mg/kg group and 92% in the 20 mg/kg group. While a decrease in the glycogen content was also observed in the skeletal muscles, this decrease was not dose-dependent. Measurement by the histomorphological assay method revealed that the glycogen content in the cardiac muscle decreased in a dose-dependent and time-dependent manner up to Week 12, and the glycogen content in the quadriceps muscle also decreased in a time-dependent manner up to Week 12. However, in both the tissues, the glycogen content increased again by Week 16. In this study, 4 animals died after administration of rhGAA, possibly due to hypersensitivity reaction to the administration of foreign protein (2 animals in the 10 mg/kg group died within 24 hours after the 3rd administration, 1 animal in the 10 mg/kg group died within 15 minutes after the 4th administration, and 1 animal in the 20 mg/kg died about 24 hours after the 10th administration). All

the animals had received intraperitoneal administration of 5 mg/kg of DPH 20 minutes before each of the 4th to 9th administrations.

c. Decrease in glycogen content after single administration (Study SN-003, 4.2.1.4)

After single intravenous administration of rhGAA at the dose of 100 mg/kg to GAA KO mice (3 animals in each group), the glycogen content in each tissue was determined, the enzyme activity was assayed, and western blotting analysis was performed. The results indicated a greater degree of increase of the GAA activity in the cardiac muscle and diaphragm than in the quadriceps muscle, triceps muscle, and psoas muscle. The glycogen content in the cardiac muscle decreased with time to a level below the detection limit by Day 14, and no re-accumulation of glycogen was noted until Day 21. Measurement by the histomorphological assay method revealed similar results for the cardiac muscle and quadriceps muscle. The half-life of rhGAA in the cardiac muscle was about 6 to 8 days, being longer than that in the skeletal muscles, which was about 5 days. The results of the western blot analysis were consistent with the longer half-life in the cardiac muscle.

d. Changes with time in the tissue glycogen contents after repeated administration (Study SN-005, 4.2.1.5)

The tissue glycogen contents were determined after administration of rhGAA to GAA KO mice (3 animals in each group) at the dose of 100 mg/kg once a week for 4 weeks. The glycogen contents in all the skeletal muscles examined, except the triceps muscle, decreased to levels below the detection limit on the 3rd day after the final administration, and the decrease was maintained up to Day 21. Thereafter, the glycogen contents gradually increased but still remained under the baseline value up to Day 42. The glycogen content in the cardiac muscle and diaphragm remained below the detection limit from Day 1 to Day 42 after the final administration. Measurement by the histomorphological assay method revealed that the glycogen content in the cardiac muscle decreased to a level below the detection limit after the final administration and remained under the baseline value up to Day 42, although a slight re-accumulation of glycogen was noted. The glycogen content in the quadriceps muscle decreased to a level below the detection limit on Day 14, and re-accumulation was observed on Day 42. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 2nd and subsequent administrations.

Based on the above findings, the applicant explained that the effects of rhGAA, administered at intervals of 14 days or 21 days, in reducing the tissue glycogen content appears to be most pronounced in the cardiac muscle and skeletal muscles.

(2) Dosage and administration

a. Frequency of administration in repeated administration (Study SN-004, 4.2.1.6; SN-006, 4.2.1.11)

The tissue glycogen contents were measured after intravenous administration of 100 mg/kg of rhGAA or vehicle to GAA KO mice (8 to 12 animals in each group) once every other week × 2 times or once every other week × 4 times (4.2.1.6), or intravenous administration of 100 mg/kg to GAA KO mice (5 animals in each group) once a week for 4 weeks (4.2.1.11). Both administration methods resulted in reductions of glycogen content in the cardiac muscle to below the detection limit. Marked decrease of the glycogen content was also observed in the skeletal muscles in the group dosed with rhGAA as compared with that in the control group dosed with vehicle. The degree of decrease in the tissue glycogen content was similar when rhGAA was administered at the dose of 100 mg/kg once every other week × 4 times and once a week for 4 weeks. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 2nd and subsequent administrations.

b. Efficacy of repeated administration every other week (Study SN-009, 4.2.1.7)

After intravenous administration of vehicle or rhGAA at the dose of 10, 20, or 40 mg/kg to GAA KO mice (10 animals in each group) once every other week × 4 times, measurement by the biochemical assay method revealed a dose-dependent decrease in the cardiac muscle glycogen content in the group dosed with rhGAA. The glycogen content also tended to decrease in a dose-dependent manner in the skeletal muscles, including in the triceps and quadriceps muscles, but there was no apparent decrease of the glycogen content in other tissues. Comparison of the effect of rhGAA in reducing the tissue glycogen content observed in this study (78% decrease after administration of 20 mg/kg every other week × 4 times) with that in Study SN-007 (75% decrease after administration of 20 mg/kg once a week × 8 times) revealed the absence of any difference in effect between administration once every other week and once-a-week administration. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 3rd and subsequent administrations.

c. Administration method and dose for long-term administration (Study SN-012, 4.2.1.8)

After intravenous administration of rhGAA to GAA KO mice (5 animals in each group and 7 animals in control group) at the dose of 10, 20, or 40 mg/kg once every other week, or at the dose of 20 mg/kg once a week for 15 weeks, measurement by the biochemical assay method revealed that the cardiac muscle and quadriceps muscle glycogen contents decreased markedly along with increases in the dose of rhGAA in the groups dosed with 10, 20, and 40 mg/kg once every other week. Administration of 40 mg/kg of rhGAA once every other week and of 20 mg/kg once a week also showed similar effects in reducing the tissue glycogen contents. Similar results were obtained from the evaluation by the histomorphologic assay method. The serum IgG antibody titer increased in all the groups from Week 4 onwards, with the highest titers observed in the group dosed with 40 mg/kg once every other week and the lowest levels observed in the group dosed with 20 mg/kg once a week. The IgG antibodies were assumed to play no apparent role in the glycogen-reducing

effect of rhGAA. Seven of the 20 animals in the groups dosed with rhGAA died. Of these, 5 animals were considered to have died from hypersensitivity reaction to foreign protein. In the remaining 2 animals, the death was assumed to be unrelated to hypersensitivity, because these animals died 5 days after the administration of rhGAA; the cause of death in these 2 animals, however, remained unknown. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 2nd and subsequent administrations.

As shown by the results of the investigation on the dosage and administration method described above, if the total dose remained equal, the 2 administration regimens, i.e., administration once every other week and that once every week, resulted in almost the same degree of reduction of the tissue glycogen content. Based on this finding, the applicant explained that administration of rhGAA every other week might be more appropriate.

(3) Mode of administration

a. Investigation of the mode of administration (initial high dose + maintenance dose) (1) (Study SN-011, 4.2.1.9)

rhGAA was administered intravenously to GAA KO mice (9 animals in each group) at the dose of 100 mg/kg once a week for 2 weeks, once a week for 3 weeks, or once every other week × 2 times, and the tissue glycogen contents were determined 3, 7, and 14 days after the final administration. Marked decrease in the glycogen content was observed in all tissues, except the triceps muscle seen 3 days after the administration, 14 days after the final administration in the group dosed once every other week × 2 times and the group dosed once a week for 3 weeks. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 2nd or 3rd and subsequent administrations.

Based on the above findings, the applicant concluded that administration of rhGAA at 100 mg/kg once every other week × at least 2 times would be required for reducing the glycogen accumulation in the cardiac muscle and skeletal muscles.

b. Investigation of the mode of administration (initial high dose + maintenance dose) (2) (Study SN-014, 4.2.1.10)

rhGAA was administered intravenously to GAA KO mice (5-15 animals in each group) at a high dose of 100 mg/kg once every other week × 2 times. Then, to a group of animals, rhGAA was administered intravenously at the maintenance dose of 0 (vehicle), 5, 10, or 20 mg/kg for 16 weeks from Day 14 after the administration of the initial high dose, and to another group of animals, a maintenance dose of 20 mg/kg was administered intravenously once a month. Initial administration of the high dose once every other week × 2 times resulted in a decrease of the tissue glycogen

content, but after administration of 5 mg/kg once every other week or 10 mg/kg once every other week, in addition to the initial high dose once every other week × 2 times, re-accumulation of glycogen was observed. The addition of 20 mg/kg once every other week or once a month to the high dose once every other week × 2 times also resulted in an apparent re-accumulation of glycogen in the quadriceps, triceps, and psoas muscles, as also a slight re-accumulation in the cardiac muscle and diaphragm. Marked increase in the antibody titers was observed in all the animals (3,485 to 50,982 at Week 20), but there was no correlation between the doses and the antibody titers. Two mice (one in the group administered the maintenance dose of 5 mg/kg every other week and the other in the group administered the maintenance dose of 20 mg/kg every other week) died 2 days and 6 days, respectively, after the 4th administration of the maintenance dose; the cause of the deaths remained unknown. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 2nd and subsequent administrations.

The applicant explained that the above findings suggested that this mode of administration, that is, initial administration of a high dose followed by administration of a maintenance dose, would be inappropriate.

(4) Effects of age in months of the GAA KO mice (Study SN-006, 4.2.1.11; Study SN-017, 4.2.1.12)

rhGAA was administered intravenously at the dose of 100 mg/kg once a week for 4 weeks to GAA KO mice aged 3 months and 12 months old (5 animals in each group), and the tissue glycogen contents were determined 7 days after the final administration. The percent decreases in the quadriceps muscle, triceps muscle, and psoas muscle glycogen contents relative to the values in the vehicle-dosed animals were 93%, 77%, and 89%, respectively, in the 3-month-old animals, and 40%, 35%, and 45%, respectively, in the 12-month-old animals. Similar results were obtained using the biochemical assay method and the histomorphological assay method. After the administration, an increase in GAA activity was noted in all the tissues tested. The increase in activity was most pronounced in the cardiac muscle and diaphragm in the 12-month-old animals, while it was most pronounced in the quadriceps, triceps, and psoas muscles in the 3-month-old animals. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 3rd and subsequent administrations. Similar results were also obtained in Study SN-017 (4.2.1.12).

Based on the above findings, the applicant explained that the effect of rhGAA in reducing the skeletal muscle glycogen content was greater in the 3-month-old mice than in the 12-month-old mice. Since the increase in GAA activity was greater in the cardiac muscle and less in the skeletal muscles in the 12-month-old animals as compared with the corresponding changes in the

3-month-old animals, it was considered that there may also be an age (in months)-related difference in the tissue distribution of rhGAA.

(5) Comparative pharmacodynamic studies of various preparations

a. Efficacy of 2 lots manufactured at the 160-L scale (Study SN-016, 4.2.1.13)

Two lots manufactured at the 160-L scale (Lot Nos. A006 and A007), which were found to have different sialic acid contents by sialic acid analysis, and one intermediate standard lot B001 manufactured at the 30-/60-L scale (Lot No. A001) were compared. Each of these lots, at 100 mg/kg, or vehicle was administered intravenously once every other week × 2 times to GAA KO mice (aged 3 to 4 months, 6 animals in each group), and the tissue glycogen contents were determined 14 days after the final administration. Marked decreases in the cardiac muscle and diaphragm glycogen contents were observed in all the 3 rhGAA groups, but not in the group dosed with vehicle. There were no significant differences in the percent decreases of the cardiac muscle, triceps muscle, psoas muscle, or diaphragm glycogen contents between the 2 lots manufactured at the 160-L scale. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 2nd administration.

b. Efficacy of 2 lots manufactured at the 2000-L scale (Study SN-022, 4.2.1.14)

In order to compare the efficacy of rhGAA manufactured at the 2000-L scale in the initial phase and the final phase of cell culture, 100 mg/kg of rhGAA (2 lots manufactured at the 2000-L scale [Lot Nos. A017 and A018] and a lot manufactured at the 160-L scale [Lot No. A015]) or vehicle was administered intravenously once a week for 4 weeks to GAA KO mice (4 animals in each group), and the tissue glycogen contents were determined 6 days after the final administration. Similar percent decreases of the tissue glycogen contents were observed in all the 3 rhGAA lot groups, but not in the vehicle group, with especially marked decreases in the cardiac and other muscles. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 3rd and 4th administrations.

c. Efficacy of 2 lots manufactured at the 2000-L scale (Study SN-026, 4.2.1.15)

In order to assess the efficacy of rhGAA manufactured at the 2000-L scale in cell culture and using purification equipment, 100 mg/kg of rhGAA (2 lots manufactured at the 2000-L scale [Lot Nos. A022 and A025] and a lot manufactured at the 160-L scale [Lot No. A015]) or vehicle was administered intravenously once a week for 4 weeks to GAA KO mice (6 animals in each group), and the tissue glycogen contents were determined 7 days after the final administration. Similar percent decreases of the tissue glycogen contents were observed in all the 3 rhGAA groups, but not in the vehicle group. One animal in the group dosed with the lot manufactured at the 2000-L scale (Lot No. A022) died 5 days after the 3rd administration; the cause of death was unknown. In this

study, 5 mg/kg of DPH was administered intraperitoneally to the animals 20 minutes before the 3rd and 4th administrations

d. Comparison of the efficacy between rhGAA and its by-product (Study SN-023, 4.2.1.16)

In order to compare the efficacy between rhGAA and a preparation containing an rhGAA by-product, vehicle, 60 mg/kg of rhGAA (Lot No. A005), or the preparation containing the by-product (Lot No. B003) was administered intravenously once a week for 4 weeks to GAA KO mice (5 to 6 months old, 4 to 5 animals in each group), and the tissue glycogen contents were determined 7 days after the final administration. The percent decreases of the glycogen contents after intravenous administration of 60 mg/kg of rhGAA were 95% in the cardiac muscle, 54% in the quadriceps muscle, 65% in the triceps muscle, and 47% in the psoas muscle, indicating a marked decrease in all tissues. Meanwhile, the percent decreases after administration of the preparation containing the by-product (Lot No. B003) were 26% in the cardiac muscle, 27% in the quadriceps muscle, 38% in the triceps muscle, and 23% in the psoas muscle. rhGAA was more potent in reducing the cardiac muscle and skeletal muscle glycogen contents as compared with the preparation containing the by-product. In this study, 5 mg/kg of DPH was administered intraperitoneally to the animals 15 minutes before the 3rd or 4th administration.

2) Secondary pharmacodynamics

(1) Effects on the immune system (Study SN-019, 4.2.2.1)

The immune responses to administration of rhGAA were investigated. Vehicle (Group 1), 20 mg/kg of rhGAA once a week for 3 weeks (Group 2), or 20 mg/kg of rhGAA once a week for 8 weeks (Group 3) was administered to GAA KO mice (10 animals in each group), and the histamine, whole complement titer, C3a/C5a, rhGAA-specific IgG, IgG_1, and IgE antibody titers in the serum and plasma were measured. Slight increases of the histamine level were noted in 2 of the 4 animals after the 3rd administration, and in all of the animals after the 8th administration. In addition, the antibody titers of rhGAA-specific IgG and IgG_1 also increased, with the levels peaking after the 8th administration. The IgG antibody titers were determined to be 343 to 5,278 after the 3rd administration, and 9,515 to 30,179 after the 8th administration. Similarly, the antibody titers of IgG_1 increased from 5,000 to 20,000 after the 3rd administration to 1,280,000 after the 8th administration. In 1 animal in Group 3, IgG and IgG_1 were undetectable; the reason was unknown. The IgE antibody titer was below the detection limit in all the animals. Two animals in Group 3 died the day following the 4th administration, and somnolence and hypothermia were observed in several animals a few minutes after the administration of rhGAA. These symptoms were considered to be caused by hypersensitivity. The increases in the histamine level and IgG and IgG_1 antibody titers were also considered to be associated with hypersensitivity. In this study, 5 mg/kg of DPH was administered to the animals in Group 1 and Group 3 15 to 20 minutes before the 3rd to

7th administrations.

3) Safety pharmacology

After single administration of rhGAA into the cephalic vein of dogs (3 male and female each, total 6 animals in each group) at the dose of 0 (control), 1, 10, or 100 mg/kg, dead or moribund animals were checked twice a day, general conditions were observed before and 10, 15, 30, and 60 minutes after the administration and once a day up to Day 15, and the blood pressure, heart rate, respiratory rate, and body temperature (rectal temperature) were measured before and 2 and 24 hours after the administration and once a week thereafter. Tremor was observed 60 minutes after the administration in 2 animals (1 male and 1 female) in the 10 mg/kg group and 3 animals (2 male and 1 female) in the 100 mg/kg group, but none of the animals died. Administration of rhGAA caused no changes in general conditions or behavior.

To monkeys (5 male), vehicle was administered intravenously at a single dose, and 3 days later, rhGAA was administered intravenously at a single dose of 100 mg/kg, and the effect of rhGAA on the cardiovascular system was investigated. There were no abnormal changes of the blood pressure, heart rate, or electrocardiogram after administration of rhGAA.

Overview of the review by PMDA

(1) Mode of action

PMDA asked the applicant to explain the mechanism by which rhGAA is incorporated into muscle cells and then reaches the lysosomes.

The applicant explained it as follows. This product is a recombinant acid α-glucosidase (rhGAA) with a molecular weight of 110 kDa containing mannose-6-phosphate (M6P) in its sugar chain. After intravenous administration, rhGAA reaches the target tissues and is incorporated into the cells by binding to the M6P receptor (MPR) expressed on the surface of the target tissue cells (N. Raben et al., *Mol Genet Metab* 2003; 80: 159-169). Then, rhGAA undergoes enzymatic degradation in the cells with the formation of a multi-subunit complex which is the active form with a molecular weight 95 kDa, which degrades glycogen under the low-pH condition in lysosomes (Van der Ploeg et al., *Pediatr Res* 1988; 24: 90-94, Moreland et al., *J Biol Chem* 2005; 280: 6780-6791). Because rhGAA is a lysosomal enzyme, it is assumed to be incorporated into lysosomes through the same route as other common lysosomal enzymes. Acid α-glucosidase (GAA) is formed in the rough endoplasmic reticulum, and after reaching the Golgi body from the intra-reticulum space, an M6P-recognition marker becomes attached to it, then, it binds to a cation-dependent M6P receptor (CDMPR) to form the CDM6P-GAA-MPR complex. Clathrin-coated vesicles containing the CDM6P-GAA-MPR complex bud from the Golgi body, then these clathrin-coated vesicles are

transported to the early endosomes. The CDM6P-GAA-MPR complex dissociates under the low-pH condition, thus, GAA reaches the lysosomes. MPR is recycled by its return to the trans-Golgi network (TGN) or the cell membrane. A very small amount of M6P-GAA may be released outside the cells by exocytosis. The released M6P-GAA binds to the cation-independent MPR (CIMPR) in clathrin-coated pits on the cell surface, and incorporated into the cells. Lysosomal enzymes not binding to MPR in the TNG are excreted without undergoing any change. rhGAA is also assumed to bind to CIMPR, incorporated into the cells by endocytosis, transported to the lysosomes, to degrade the excess glycogen that accumulates in the cells, thereby preventing damage to the cells (Storch S & Braulke T, [2005] "Lysosomes, Chapter 2 Transport of Lysosomal Enzymes," Edited by Safitg. P, p. 17-23, Eurekah.com and Springer Science + Business Media).

PMDA asked the applicant to explain the reason why the decrease in glycogen content and increase in GAA activity after administration of rhGAA were more prominent in the heart, and the reason why there was an inter-tissue difference in the activity at different ages (in months) of the GAA KO mice.

The applicant responded as follows.
Regarding the reason why the decrease in glycogen content and increase in GAA activity associated with the administration of rhGAA were observed prominently in the heart:
After single intravenous administration of rhGAA, the GAA activity increased more prominently in the heart as compared with that in the skeletal muscles (the ratio of the activity to the dose in the heart and quadriceps muscle were 0.19% to 0.48% and 0.05% to 0.17%, respectively). In Study SN-003, the tissue half-life of rhGAA was found to be longer in the heart than in the skeletal muscles (6 to 8 days in the heart and about 5 days in the skeletal muscles). This finding suggests that a larger amount of rhGAA is distributed in the heart as compared with that in the skeletal muscles. In Study SN-003, after single administration of 20 mg/kg of rhGAA, the values of the AUC in the heart, quadriceps muscle, triceps muscle, psoas muscle, and diaphragm were 713.2, 153.9, 155.5, 125.3, and 477.8 nmol/hr/mg, respectively. As stated above, it is assumed that because a larger amount of rhGAA is distributed in the heart, the glycogen content decrease is also more prominent in the heart. It is considered that such difference in tissue distribution may be caused by differences in the expression levels of the M6P receptor. It has been reported that the M6P receptor is expressed more abundantly in the heart as compared with that in the skeletal muscles in GAA KO mice (N. Raben et al., *Mol Genet Metab* 2003; 80: 159-169). Another possible reason is that the much larger blood flow volume in the heart than in the skeletal muscles causes the enzyme to be transported more efficiently to the cardiac muscle.

Regarding the reason why there was an inter-tissue difference in the activity by age in month of

GAA KO mice:

It is unknown why the tissue distribution of GAA activity after administration of rhGAA differed between 3-month-old and 12-month-old mice. However, it has been shown that the amount of glycogen accumulated in the cellular cytoplasm is larger in 12-month-old mice than in 3-month-old mice, suggesting that the damage of skeletal muscles may proceed with advancing age. Raben et al reported (N. Raben et al., *Mol Ther* 2006; 14: 831-839) that since autophagy (autophagocytosis) builds up in skeletal muscle, especially in Type II muscle fibers, in GAA KO mice with advancing age, a part of the rhGAA incorporated into the cells by endocytic pathway is possibly transferred to the autophagic areas instead of reaching the lysosomes. For this reason, it is likely that the incorporation and trafficking of rhGAA into the skeletal muscle will be inhibited with age-dependent disease progression.

PMDA concluded that the decrease in tissue glycogen content after administration of rhGAA has been confirmed in pharmacological studies and that the applicant offered an appropriate discussion based on the latest data available at the time, although the mode of action may not yet be fully elucidated, and thus accepted the applicant's above response.

(2) Safety

In view of the finding that tremor was observed in a safety pharmacology study 60 minutes after the administration of rhGAA in 2 animals (1 male and 1 female) in the 10 mg/kg group and in 3 animals (2 male and 1 female) in the 100 mg/kg group, PMDA asked the applicant to explain whether there are safety concerns about the use of rhGAA in humans.

The applicant responded as follows. Regarding the tremor observed after administration of rhGAA in the safety pharmacology study, although causal relationship with rhGAA cannot be ruled out, it was considered that a definite judgment about the causality might be impossible due to the small number of animals. This issue was, therefore, investigated in a repeated-dose toxicity study in monkeys. When rhGAA was administered to monkeys at the dose of 4, 20, or 100 mg/kg for 26 weeks (4.3.2.5 [4.4.2.4], 6354-152), and at doses of up to 200 mg/kg (4.3.2.6 [4.4.2.5], 6354-157), no tremor was observed. On the other hand, in the foreign clinical studies AGLU01602 and AGLU01702, 3 episodes of tremor were reported in 2 subjects (5.1%). In Study AGLU01702, 1 patient (subject No. 201405) dosed with 20 mg/kg of rhGAA once every other week experienced mild tremor 14 weeks after the administration, but the patient recovered after discontinuation of rhGAA. This event occurred on the administration day and the causal relationship with rhGAA could not be ruled out. In Study AGLU01602 also, 1 patient (subject No. 203318) dosed with 40 mg/kg of rhGAA once every other week experienced moderate tremor on the administration days at 27.9 and 53.9 weeks after the drug administration, and it was determined that the causal

relationship with rhGAA could not be ruled out for either event. Because no investigation was performed on the blood pharmacokinetics of rhGAA at the time that the tremor occurred, the relationship of this event to the blood drug concentration remains unknown. As described above, it is uncertain at present whether or not the tremor was related to the pharmacological effect of rhGAA. However, since tremor was reported in 2 subjects in the 2 foreign clinical studies and its causal relationship with rhGAA cannot be ruled out, caution about possible occurrence of tremor is included in the section "3. Adverse reactions, (2) Other adverse reactions" of the package insert (draft).

PMDA concluded that although the mechanism of occurrence of tremor observed after administration of rhGAA remains unknown, the information has properly been provided, and PMDA accepted the applicant's above response.

Regarding rationale for the dosage and administration, see "4. Clinical data, 3) *Outline of the review by PMDA*, (3) Rationale for dosage and administration".

(ii) Summary of pharmacokinetic studies

Summary of the submitted data

In order to investigate pharmacokinetics, including absorption, distribution, and placental transfer, of rhGAA in mice, rats, dogs, and monkeys, preparations of different formulation and in different manufacturing scale were compared, and the results obtained have been submitted.

The GAA activity in biological samples was measured by a method using the fluorescent compound, 4-methyl umbelliferone (4-MU) (4-MUG method). Tissue glycogen was determined by the biochemical assay method using amyloglucosidase and a histomorphological assay method using the image analysis system, and the serum anti-rhGAA IgG antibody, anti-rhGAA IgG_1 antibody and non-specific IgG antibody titers were measured by enzyme immunoassay (ELISA). In exploratory studies using ^{125}I-labeled compounds, the GAA concentrations in biological samples were determined by a γ-ray measuring system (not yet validated). Pharmacokinetic parameters were expressed as means or means ± standard deviations, unless otherwise noted.

1) Absorption

(1) Single administration

a. Single-dose study in mice (4.3.2.1, SN-020)

After single intravenous administration of rhGAA at the doses of 10, 20, and 40 mg/kg to mice (4 animals/group), a linear relationship was noted between the AUC and the doses. The elimination

half-life ($t_{1/2}$) was 81.1 to 84.3 minutes and the clearance rate (CL) was 0.64 to 0.83 mL/min/kg, and elimination of the serum GAA activity in the primary elimination phase was observed.

b. Single-dose toxicity study in rats (4.3.2.2 [4.4.1.1], 6354-134)

After single intravenous administration of rhGAA at the doses of 1, 10, and 100 mg/kg to rats (5 animals/group), a linear relationship was observed between the AUC and the doses. The $t_{1/2}$ value was 80.1 to 129.7 minutes and the CL was 0.51 to 0.94 mL/min/kg, showing elimination of the drug in the primary elimination phase. There were no gender differences in the pharmacokinetics.

c. Single-dose toxicity study in dogs (4.3.2.3 [4.4.1.2], 6354-132)

After single intravenous administration of rhGAA at the doses of 1, 10, and 100 mg/kg to dogs (3 animals/group), a linear relationship was observed between the AUC and doses. The $t_{1/2}$ was 81.9 to 181.6 minutes and the CL was 0.27 to 0.41 mL/min/kg, showing elimination of the drug in the primary elimination phase. There were no gender differences in the pharmacokinetics.

(2) Repeated administration

a. Four-week repeated-dose toxicity study in rats (4.3.2.4 [4.4.2.1], 6354-133)

When rhGAA was given intravenously at the doses of 1, 10, and 100 mg/kg once a week for 4 weeks to rats (5 male and female animals each/group), the plasma $t_{1/2}$ at Weeks 1 and 4 were 123.8 ± 22.5 and 64.7 ± 35.0 minutes, respectively, in the male animals in the 1 mg/kg group. The $t_{1/2}$ was significantly lower at Week 4 as compared with that at Week 1. In the female animals of the 1 mg/kg group, GAA activity was detectable in all the animals at Week 1 and the $t_{1/2}$ was 114.7 ± 4.5 minutes, but at Week 4, the GAA activity was around or below the detection limit ([redacted] ng/mL) in 4 of the 5 animals, and 133.0 minutes in the remaining 1 animal. The AUC at Week 4 was significantly smaller as compared with that at Week 1 in 1 male animal of the 1 mg/kg group, and the AUC at Week 4 was significantly larger as compared with that at Week 1 in the female animals of the 10 mg/kg group. As a cause of these changes, the applicant mentioned the possible effects of antibodies, but that since the changes were not consistent across the dose groups, the biological significance is unclear.

b. Twenty-six-week repeated-dose toxicity study in monkeys (4.3.2.5 [4.4.2.4], 6354-152)

When rhGAA was given at the doses of 4, 20, and 100 mg/kg by continuous intravenous infusion (3.3 mL/kg/h for 6 hours) once every other week for 26 weeks to monkeys (3 male and female animals each/group), the serum AUC in the female animals was smaller as compared with that in the male animals. In the male animals of the 100 mg/kg group, the $t_{1/2}$ values on Day 85 and Day 169 after the first administration were significantly higher as compared with the value on Day 1 (the first day of administration), and in the female animals of the 100 mg/kg group, the $t_{1/2}$ on Day

85 was significantly higher as compared with that on Day 1. In both the male and female animals of the 20 mg/kg group, the $t_{1/2}$ values were higher on Day 85 and Day 169 as compared with that on Day 1, but the differences among the dose groups were not significant. No prolongation of $t_{1/2}$ was noted in the 4 mg/kg group. The prolongation of $t_{1/2}$ by repeated administration in the 20 and 100 mg/kg groups was considered to be caused by saturation of the antibody or receptor (M6P receptor) in the blood by rhGAA. In the female animals of the 100 mg/kg group, the total body clearance (CL) and distribution volume at steady state (V_{ss}) were significantly increased and the AUC significantly decreased on Day 169 as compared with the corresponding values on Day 1. The reason why the $t_{1/2}$, CL, and AUC/dose in the male and female animals of the 4 mg/kg group were different from those in the animals of the 20 and 100 mg/kg groups was that the data of the terminal phase (1080 and 1440 minutes, respectively) in the 4 mg/kg group were excluded from the analysis, as the values were around or below the detection limit (4.41 ng/mL). Therefore, according to the applicant's comment, these differences were not caused by biological differences between the dose groups.

c. Thirteen-week repeated-dose toxicity study in monkeys (4.3.2.6 [4.4.2.5], 6354-157)

When 200 mg/kg of rhGAA was given by continuous intravenous infusion (3.3 mL/kg/h for 12 hours) once every other week for 13 weeks to monkeys (4 male and 4 female animals/group), the AUC on Day 1 in the female animals was smaller as compared with that in the male animals, but the difference was not statistically significant, and no difference in the AUC between the male and female animals was noted on Day 85. The $t_{1/2}$ values on Day 1 and Day 85 were similar in the male and female animals, but the $t_{1/2}$ on Day 85 was higher than that on Day 1, and the difference in the values between Day 1 and Day 85 was statistically significant in the male animals. The values of CL in both the male and female animals were similar on Day 1 and Day 85, but were higher in female than male animals. The prolongation of $t_{1/2}$ observed on Day 85 in both the male and female animals was assumed to be due to antibody formation.

2) Distribution

Preparations from the following lots were used: a 30-L/60-L scale lot (A001) prepared by reconstituting rhGAA containing 2% mannitol, 0.5% sucrose, and 20 mmol/L sodium phosphate in polysorbate 80, and a 30-L/60-L scale lot (A003) and a 160-L scale lot (A015) prepared by reconstituting rhGAA containing 2% mannitol, 25 mmol/L sodium phosphate, and 0.005% polysorbate in Water for Injection. Each of these preparations was given intravenously at a single dose of 20 mg/kg to GAA KO mice (7 male and 5 female animals/group). One hour after the administration, the distribution rates of rhGAA were 14.8% to 23.2% in the liver, 0.24% to 0.31% in the heart, and 0.05% to 0.07% in the quadriceps muscle, showing that the major part of the drug was distributed in the liver. The results indicated that there were no differences in the tissue

distributions or the changes of distribution with time among the different formulations and lots from different manufacturing scales (4.3.3.1 [4.3.2.7], SN-008).

Preparations from two 160-L scale lots with different contents of sialic acid (A006, *X* N-acetylneuraminic acid [NANA] mol/mol; A007, 1.446*X* NANA mol/mol) and a standard preparation from a 30-L/60-L scale lot (B001) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (6 male and 6 female animals/group). One hour after the administration, the distribution rates of rhGAA were 29.5% to 46.5% in the liver, 0.19% to 0.28% in the heart, 0.050% to 0.055% in the quadriceps muscle, 0.49% to 1.19% in the kidney, 0.83% to 0.91% in the spleen, and 0.016% to 0.022% in the triceps muscle, showing that the major part of the drug was distributed to the liver. The tissue distribution of rhGAA was similar for the preparations from the two 160-L scale lots and the 30-L/60-L scale lot (4.3.3.2 [4.3.2.9], SN-015).

Two preparations from 2000-L scale lots (A017 and A018) and a preparation from a 160-L scale lot (A015) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (male and female animals, 9 in total /group). One hour after the administration, the distribution rates of rhGAA were 33.8% to 40.7% in the liver, 0.38% to 0.48% in the heart, 0.06% to 0.08% in the quadriceps muscle, 0.91% to 1.30% in the spleen, and 0.02% in the triceps muscle, showing that the major part of the drug was distributed to the liver. The tissue distribution of rhGAA was similar for the preparations from the two 2000-L scale lots and the 30-L/60-L scale lot [4.3.3.3, SN-021].

Two preparations from 2000-L scale lots (A022 and A025) and a preparation from the 160-L scale lot (A015) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (male and female animals, 12 in total/group). For the preparation from Lot A015, the distribution rates of rhGAA in the liver 1 to 8 hours after the administration were 40.0% to 48.7%, while the corresponding values for the preparations from Lot A022 and A025 were 51.4% to 72.2% and 67.2% to 76.4%, respectively, higher than the values for the preparation from Lot A015. Although slight differences were observed in the distribution to the heart, spleen, quadriceps muscle, and triceps muscle depending on measurement time-points, these changes were not consistent for either the measurement time-points or the tissues of distribution (4.3.3.4, SN-025).

Preparations from a 160-L scale lot (A012, 1.383*X* NANA mol/mol) and a 2000-L scale lot (A020, 1.362*X* NANA mol/mol), both containing similar levels of sialic acid, were given intravenously at a single dose of 20 mg/kg to GAA KO mice (14 male and 4 female animals/group). For the preparation from Lot A012, the distribution rates of rhGAA in the liver 1 to 8 hours after the administration were 28.4% to 58.2%, while the corresponding values for the preparations from Lot A020 were 40.8% to 71.2%, which were significantly higher than the values for the preparation

from Lot A012. The distribution rates to the heart were 0.33% to 0.60% for the preparation from Lot A012 and 0.24% to 0.37% for that from Lot A020. The values for the preparation from Lot A020 (0.24%-0.37%) were significantly lower as compared with those for the preparation from Lot A012 (0.33%-0.60%), indicating that there was a significant difference in tissue distribution between the preparation from the 160-L scale lot and that from the 2000-L scale lot. There was no difference in tissue distribution to the quadriceps muscle (4.3.3.5, SN-029).

3) Other pharmacokinetic studies

The results of the following pharmacokinetic studies have been submitted. Pharmacokinetics were compared among preparations of different formulations and in different manufacturing scales (4.3.2.7 [4.3.3.1], SN-008; 4.3.2.8, SN-010; 4.3.2.9 [4.3.3.2], SN-015; 4.3.2.10, SN-024; 4.3.2.11, SN-027; 4.3.2.12, SN-028; 4.3.3.3, SN-021; 4.3.3.4, SN-025; 4.3.3.5, SN-029). Pharmacokinetic studies were also conducted in pregnant mice (4.3.2.13 [4.4.5.2.1], 6354-153) and in the presence of antibody (4.3.7.1, SN-013 [Reference] and 4.3.7.2, SN-018). The results of studies on plasma protein binding, transfer to blood cells, metabolism, excretion, and drug-drug interactions of rhGAA have not yet been submitted.

(1) Pharmacokinetics of preparations of different formulations and in different manufacturing scales

Preparations from 2 lots manufactured at the 30-L/60-L scale (A001 and A003) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (male and female animals, 4 in total/group). The $t_{1/2}$ value for the preparation from Lot A001 (337.0 ± 26.4 minutes) was significantly higher than that for the preparation from Lot A003 (247.8 ± 13.1 minutes), but there were no significant differences in the other pharmacokinetic parameters, including the AUC (4.3.2.7, SN-008).

A preparation each from the 30-L/60-L scale lot (A001) and 160-L scale lot (A015) was given intravenously at a single dose of 20 mg/kg to GAA KO mice (4 male and female animals each/group). There were no differences in the pharmacokinetic parameters obtained, indicating that the preparation from the 30-L/60-L scale lot and that from the 160-L scale lot were similar in terms of the pharmacokinetic profile (4.3.2.8, SN-010).

Preparations from 2 lots, manufactured at the 160-L scale, with different contents of sialic acid (A006 and A007) and a standard preparation from the 30-L/60-L scale lot (B001) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (2 male and female animals each/group). The MRT value of the preparation from Lot A006 (97.8 ± 16.2 minutes) and that from

Lot A007 (105.0 ± 7.7 minutes) were significantly lower as compared with that of the preparation from Lot B001 (160.2 ± 42.1 minutes), but there were no significant differences in the other pharmacokinetic parameters, including the AUC, among these preparations (4.3.2.9, SN-015). There was also no difference in the glycogen-reducing effect among these preparations (4.2.1.13, SN-016).

Preparations from 2 lots manufactured at the 2000-L scale (A017 and A018) and a preparation from a 160-L scale lot (A015) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (10 male and female animals each/group). The $AUC_{0\sim\infty}$ of the preparation from Lot A015 (45724 ± 4334 μg min/mL) was significantly greater than that of the preparation from Lot A017 (37973 ± 3589 μg min/mL) and significantly smaller than that of the preparation from Lot A018 (53374 ± 5768 μg min/mL) (4.3.2.10, SN-024). However, there was no difference in the pharmacodynamic glycogen-reducing effect among these preparations (4.2.1.14, SN-022). The applicant concluded that the difference in the drug exposure levels of rhGAA among the preparations might be related to the differences in the sialic acid content (2.4.3.2.1).

Preparations from 2 lots manufactured at the 2000-L scale (A022 and A025) and a preparation from a 160-L scale lot (A015) were given intravenously at a single dose of 20 mg/kg to GAA KO mice (12 male and female animals each/group). The $AUC_{0\sim\infty}$ of the preparation from Lot A015 (38531 ± 13144 μg min/mL) was significantly larger than those of the preparations from Lot A022 (24165 ± 5956 μg min/mL) and Lot A025 (27633 ± 3169 μg min/mL). The $t_{1/2}$ of the preparation from Lot A015 (136.8 ± 39.3 minutes) was significantly longer than that of the preparation from Lot A022 (107.9 ± 17.5 minutes) (4.3.2.11, SN-027). However, there were no differences in the pharmacodynamic glycogen-reducing effect among these preparations (4.2.1.15, SN-026).

Preparations from a 160-L scale lot (A012) and a 2000-L scale lot (A020), both containing similar levels of sialic acid, were given intravenously at a single dose of 20 mg/kg to GAA KO mice (10 male and female animals each/group). The $AUC_{0\sim\infty}$ of the preparation from Lot A012 (44649 ± 10645 μg min/mL) was significantly larger than that of the preparation from Lot A020 (32776 ± 4810 μg min/mL). For other parameters, including the $AUC_{0\sim last}$, C_{max}, MRT, and $t_{1/2}$, significantly higher values were obtained for the preparation from Lot A012 as compared with those for the preparations from Lot A020 (4.3.2.12, SN-028).

(2) Pharmacokinetics in pregnant mice

After single intravenous administration of rhGAA at the doses of 10, 20, and 40 mg/kg to pregnant GAA KO mice (6 animals/group), linearity was noted between the AUC and the doses. The $t_{1/2}$ was

71.9 to 78.8 minutes and the CL was 0.60 to 0.65 mL/min/kg, suggesting elimination in the primary elimination phase. Since the pharmacokinetic parameters obtained in this study were similar to those in a study in non-pregnant mice (4.3.2.1, SN-020), it appeared that the pharmacokinetic parameters after administration of rhGAA would not be affected by pregnancy. Detection of a low level of GAA activity in the fetuses suggested that rhGAA might possibly pass through the placenta, even if in very small quantities (4.3.2.13 [4.4.5.2.1], 6354-153).

(3) Pharmacokinetics in the presence of antibody

After repeated intravenous administration of vehicle (non-sensitized group, 8 male and female animals each) or 40 mg/kg of rhGAA (sensitized group, 10 male and female animals each) once every other week for 10 weeks to GAA KO mice, the antibody titers in the sensitized animals were 1:36,000 to 1:120,000, but no hypersensitivity reactions were observed. To animals in the sensitized group (16 animals had high antibody titers) and the non-sensitized group, radiolabeled rhGAA was given intravenously at a single dose of 10 mg/kg. The mean $t_{1/2}$, MRT, and V_{ss} in the sensitized group (male and female animals, 4 in total) were 84.6 ± 16.0 minutes, 75.0 ± 30.7 minutes, and 50.0 ± 25.5 mL/kg, respectively, while those in the non-sensitized group (male and female animals, 4 in total) were 144.4 ± 11.8 minutes, 150.2 ± 20.0 minutes, and 95.5 ± 20.3 mL/kg, respectively. The mean $t_{1/2}$, MRT, and V_{ss} in the sensitized group were significantly lower than those in the non-sensitized group, indicating that rhGAA was more quickly eliminated in the animals in the sensitized group. However, since there was no difference in the AUC between the 2 groups, the presence of antibody was considered to have no effect on the exposure levels of rhGAA. Animals in the sensitized group (male and female animals, 12 in total) had higher distribution levels of rhGAA in the quadriceps muscle, triceps muscle, and psoas muscle, but lower distribution levels in the heart and the spleen as compared with the values in the animals of the non-sensitized group (male and female animals, 12 in total). The effects of these differences on the glycogen-reducing effect of rhGAA are unknown (4.3.7.2, SN-018).

After repeated intraperitoneal administration of vehicle (non-sensitized group, 11 male and 5 female animals) or 1 mg/kg of rhGAA (sensitized group, 13 male and 7 female animals) once a week for 16 weeks to GAA KO mice, 10 mg of radiolabeled rhGAA was given intravenously at a single dose (16 animals had high antibody titers). Investigation of the pharmacokinetics and drug distribution could not be performed, because the animals in the sensitized group had cold skin, developed labored respiration, or died (within 1 hour of administration in 8 animals), which seemed to be attributable to severe hypersensitivity reactions. The antibody titers after repeated intraperitoneal administration for 16 weeks were 1:162,000 to 1: 7,768,000 (4.3.7.1, SN-013 [Reference]).

Outline of the review by PMDA

(1) Effect of sialic acid on the pharmacokinetics of rhGAA and assurance of post-marketing safety

Based on the finding that preparations from the 2000-L scale lots, being planned for the Japanese market, produced a somewhat greater increase in the GAA activity in the liver as compared with those from the 160-L scale lots being used in many clinical studies, PMDA sought the applicant's view on the effect of this difference on the post-marketing safety of the preparations from the 2000-L scale lots.

The applicant gave the following explanation. In general, in patients with infantile-onset form or adult-onset form GSD II, elevated ALT and AST possibly caused by myopathy associated with the underlying disease are observed. In Studies AGLU01602 and 01702, in which preparations from the 160-L scale lots were used, although abnormal changes in the ALT and AST due to the underlying disease were noted, there was no change suspected to have causal relationship with rhGAA, except in 1 case. According to an interim report of a foreign post-marketing clinical study (AGLU2804) in which a preparation from a 2000-L scale lot was used, the levels of both ALT and AST were similarly high at the baseline and at Week 26 after administration of the product for 26 weeks in 5 patients. Changes in hepatic function parameters caused by rhGAA have not been confirmed. In addition, in Periodical Safety Update Report (PSUR) submitted 1 year 8 months after the start of a clinical study, no abnormal changes in ALT or AST related to rhGAA were reported. When the pharmacokinetics of a preparation from the 2000-L scale lot used in Study AGLU02804 were compared with those of preparations from the 160-L scale lots used in Studies AGLU01602 and 01702, the only factor affecting the pharmacokinetics was the body weight, and it was considered that there were no apparent differences in the pharmacokinetics between the preparation from the 2000-L scale lot and those from the 160-L scale lots. However, taking into account the small number of subjects (5 patients) in Study AGLU2804, the investigation on the effect of the different manufacturing scale on the pharmacokinetics might have been inadequate.

PMDA asked the applicant to explain consultation with the foreign regulatory authorities regarding the specification control of the content of sialic acid and the applicant's policy for the future on this issue in Japan.

The applicant responded as follows. The proposed specifications for the content of sialic acid (N-acetylneuraminic acid [NANA]) in rhGAA drug substance are [redacted] to [redacted] NANA mol/mol, which is in the same specification range as that approved in the UE, US, and Canada. Regarding the content of N-glycosylneuraminic acid (NGNA), the content specifications will be established

after validation tests in accordance with the request of the regulatory authorities in the US and Canada, but the regulatory authorities in the EU have decided that setting up of the content specification is unnecessary, because the content of NGNA is very small. In Japan, the applicant considers that specifications for the content of NGNA are unnecessary at present, but even so, if the manufacturer establishes specifications for the content of NGNA, products for the Japanese market will conform to the specifications. The proposed specifications for the content of NANA, [redacted] to [redacted] NANA mol/mol, were established based on the means ± [redacted] standard deviations of the actual measurement values ([redacted] to [redacted] NANA mol/mol, [redacted] lot) for the 160-L scale lots used in clinical studies. Since these specifications are more stringent than the means ± [redacted] standard deviations of the actual measurement values ([redacted] to [redacted] NANA mol/mol, [redacted] lot) for the 30/60-L, 160-L, and 2000-L scale lots in the specification tests, it is considered that the proposed specifications reflect the experience in manufacturing the drug for clinical use more properly.

PMDA made the following comments to the applicant's answer. The effect of the sialic acid content on the pharmacokinetics of rhGAA is unknown at present, and it could not be expected to collect enough data to clarify the relationship between the sialic acid content and the pharmacokinetics. For products controlled by the specifications for the NANA content in the drug substances, the difference of the sialic acid content may cause no safety concerns. However, it will be necessary to fully observe the safety in ongoing clinical studies and after the release of rhGAA on market. PMDA directed that, in order to make it clear that the product for the Japanese market conforms to the foreign specifications, if specifications are newly established in foreign countries, similar specifications should be established also in Japan as quickly as possible, and the content of NGNA should be controlled by specifications as done in the US and Canada.

(iii) Summary of toxicology studies

Summary of the submitted data

1) Single-dose toxicity (4.4.1.1, 6354-134; 4.4.1.2, 6354-132)

Single-dose intravenous toxicity studies of rhGAA were performed in rats and dogs at the doses of 0, 1, 10, and 100 mg/kg. No deaths were observed. Tremor was noted in dogs at ≥10 mg/kg. No other changes were detected in the general conditions or autopsy findings. The approximate intravenous lethal dose was estimated to be over 100 mg/kg.

2) Repeated-dose toxicity

(1) Four-week intravenous study in rats (4.4.2.1, 6354-133)

Doses of 0, 1, 10, and 100 mg/kg of rhGAA were given once weekly (1st, 8th, 15th, and 22nd days) and the animals were autopsied on the day following the final administration. After the 3rd

administration, anaphylaxis was observed at the highest dose; therefore, 5 mg/kg of DPH was administered to all the groups, including the control group, at the 3rd and 4th administration. Decrease in body weight gain was noted in the males of the high-dose group; therefore, the no-observed-adverse-effect-level (NOAEL) was estimated to be 10 mg/kg/week. In all the dose groups, reactions to foreign protein were noted, such as erythema in both limbs, erythema of the external ears, and swelling in the forelimbs. These findings were mild and were not regarded as toxicity findings.

(2) Four-week intravenous study in rats (4.4.2.2, 6354-140)

Doses of 0, 1, 5, 10, and 50 mg/kg of rhGAA were given once weekly (1st, 8th, 15th, and 22nd days) and the animals were autopsied on the day following the final administration. In order to prevent anaphylactic reactions, 5 mg/kg of DPH was administered to all the animals, including those of the control group, before the 3rd and 4th administration. Furthermore, an additional 2.5 mg/kg of DPH was given to animals that showed dyspnea after the 3rd administration. At 10 and 50 mg/kg, 1 animal each out of 8 female animals died or was killed in moribund state after the administration in the 3rd week, which was attributed to allergic reaction. In both animals, red-colored lesions were noted in the stomach and lungs, but no dose-dependency in the seriousness of the symptoms was noted. The lesion in the stomach was also observed in the vehicle control group and was not considered to be drug-related toxicity. Decrease in locomotor activity, lateral position, dilatation of the pupil, transparent eye discharge, irregular respiration, pale skin, cold skin, etc. were noted in all the dose groups, and the NOAEL could not be determined.

(3) Four-week intravenous study in mice (4.4.2.3, 02009)

Doses of 0, 1, 10, and 100mg/kg of rhGAA were given once weekly (1st, 8th, 15th, and 22nd days) and the animals were autopsied on the day following the final administration. In order to prevent anaphylactic reactions, 10 mg/kg of DPH was given to all the animals, including those of the control group, before the 3rd and 4th administration. Decreases of the leukocyte count, MCV, eosinophil count, neutrophil count, and basophil count, and an increase in the serum globulin were observed, but the changes were mild and were not considered to be of toxicological significance. The NOAEL was considered to be 100 mg/kg/week.

(4) Twenty-six-week continuous intravenous infusion study in monkeys (4.4.2.4, 6354-152)

Doses of 0, 4, 20, and 100 mg/kg of rhGAA were given by 6-hr continuous infusion every other week. Namely, rhGAA was given every 14 days, or on 1st, 15th, 29th days and so on. The animals were autopsied on the day (on the 170th day) following the 13th administration on the 169th day, except those of the control group and 100 mg/kg group, which were allowed a 2-week recovery period before being autopsied on the 184th day. In all the groups, including the control group,

inflammation, fibrous thickening, etc. were observed at the injection site. Increases in the lung weight, leukocyte count, neutrophil count, lymphocyte count and blood urea, and a decrease in the serum ALT were observed, but all the changes were mild and no dose-dependence was recognized. Metastatic thrombi were observed in the heart and lungs, but these were similar to the changes usually observed following intravenous administration using a catheter, and were not considered to be directly related to the drug. The NOAEL was considered to be 100 mg/kg given every other week.

(5) Thirteen-week continuous intravenous infusion study in monkeys (4.4.2.5, 6354-157)

Doses of 0 and 200 mg/kg of rhGAA were given by 12-hr continuous infusion every other week. One control animal and 3 dosed animals were autopsied 8 days after the 7th administration (93rd day), and 1 control animal and 1 dosed animal were autopsied 22 days after the 7th administration (107th day). One out of 4 female mice in the dosed group died on the 5th day. This animal adopted a crouching position on the day before death, and suppression of locomotor activity with complete absence of food intake, etc. was noted. Based on the findings at necropsy, the cause of death was considered to be a combination of renal insufficiency and cardiovascular collapse, caused by multiple-organ embolic septicemia. On histopathological examination, periportal inflammation in the liver, skeletal myositis, myocarditis, renal infarction, pulmonary abscess, angiitis accompanied by fibrin thrombus, and sinus histiocytosis in tracheal and bronchial lymph nodes were observed. The rectal temperature of this animal was slightly higher than that of the other animals before the 1st administration; therefore, bacterial infection was suspected. In the surviving animals, fibrosis at the injection site, mononuclear cell infiltration in the liver, and granulomatous cell infiltration in the cecum were observed, but these were also observed sporadically in control animals, and were, therefore, considered to be unrelated to the drug. The NOAEL was estimated to be 200 mg/kg given every other week.

3) Reproductive and developmental toxicity

(1) Fertility and early embryonic development study in mice (4.4.5.1.1, 6354-155)

Doses of 0, 10, 20, and 40 mg/kg of rhGAA were intravenously given every other day. Males received rhGAA drug for 9 weeks, starting 28 days before mating. Females received rhGAA from 14 days before mating until the 7th day of gestation. On the 12th day (7th administration), anaphylactic reactions, such as decrease in locomotor activity and labored respiration, were observed in some males after the administration of rhGAA, and DPH was additionally administered to the mice later. Other mice were pretreated with 5 mg/kg of DPH before the administration of rhGAA. After the 12th day (7th administration), all the mice received 5 mg/kg of DPH before the administration. In the moderate-dose group, 1 of 22 males died within 10 minutes of the 7th administration and 1 of 22 females died on the 1st day of gestation immediately after the

administration of rhGAA. In the high-dose group, 1 of 22 males died on the 53rd day. In this animal, forward bending, decrease in locomotor activity, and unkempt fur were noted before death and a decrease in the body weight was observed for a few days before death (49th to 53rd day). On necropsy, dilatation of the ureter, a patchy and rough pattern of the kidney surface, and enlarged bladder were recognized, which were considered to be unrelated to anaphylactic reactions. The fertility index was low in all the groups, including the control group, which was considered to be due to the stress caused by intraperitoneal administration of DPH or the method of handling of the animals. The pre-implantation death rate was high in the high-dose group, but the number of corpora lutea was large in some animals in this group and, even though there was no difference in the number of implantations from that in the control group, there was apparently an increase in the pre-implantation death rate. Therefore, this was not considered to be directly related to rhGAA. There was some effect on the sperm, such as decrease of the sperm motility in the epididymis; however, based on the results of examination of the relationship with the fertility index, the changes in the sperm were considered to be unrelated to fertility or pregnancy. The NOAEL for general toxicity in the male and female parental mice was considered to be less than 10 mg/kg for rhGAA given every other day, as allergic reactions such as crouching and lateral position were observed in this study. In regard to reproductive performance of the male and female parental animals, the NOAEL could not be determined, because a decrease of the fertility index was observed in all the groups, including the control group.

(2) Fertility study in mice (4.4.5.1.2, 6354-163)

A no-treatment group, physiological-saline group, vehicle group, and DPH group were set up as control groups. Doses of 10, 20, and 40 mg/kg of rhGAA were given intravenously to females every other day, starting 14 days before mating until the 7th or 8th day of gestation. From the 7th administration, DPH was given at the dose of 5 mg/kg before the administration of rhGAA. In the high-dose group, 1 of 22 animals died on the 10th day (6th administration) immediately after the administration of rhGAA. No abnormal findings were noted on necropsy and it was considered highly likely that the death was caused by anaphylaxis. No difference was noted between the control groups and the rhGAA groups in terms of the pregnancy rate or the pre-implantation death rate. The NOAEL was considered to be 40 mg/kg given every other day.

(3) Embryonic and fetal development study in mice (4.4.5.2.1, 6354-153)

Doses of 0, 10, 20, and 40 mg/kg of rhGAA were given intravenously to females from day 6 to day 15 of gestation. In the high-dose group, an increase in the post-implantation death rate was observed and no live fetuses were noted in 1 of 25 dams. The number of corpora lutea in this dam was 7 and the number of implantation traces was 4. This animal was considered to have abnormal pregnancy occurring before the administration of rhGAA. When this animal was excluded, the

post-implantation death rate was nearly equal to that in the control group. The incidence of the 14th rib increased in the low-dose group, but this was not observed in the moderate-dose or high-dose group and the possibility of this being caused by rhGAA was considered to be small. The NOAEL was estimated to be 40 mg/kg/day. In order to examine the transferability of rhGAA to the fetuses, the GAA activity was measured in the placenta and liver of the dams and fetuses. Dose-dependent increase of the activity was noted in the liver and placenta of the dams, and trace activity was detected in the liver of the fetuses. It was considered likely that rhGAA passes through the placenta, although to a very limited extent.

4) Local irritation

No study specifically designed to examine local irritation was performed. In repeated-dose toxicity studies, inflammation and induration were observed at the injection site, but the extent did not differ from that in the control group, and it was considered unlikely that rhGAA induces local irritation.

Outline of the review by PMDA

(1) Allergic reaction observed in toxicity studies

PMDA asked for the applicant's view on the possibility of the allergic reactions observed in the toxicity studies posing problems in humans.

The applicant responded as follows. The allergic reactions observed in the rodents were concluded to represent non-specific reactions to foreign proteins. In a pharmacological study performed in mice to examine the effects of rhGAA on the immunological system (SN-019), increase in the serum histamine level and increase in the serum IgG and IgG1 antibody titers were observed following intravenous administration of rhGAA, which is in agreement with the immunogenicity study data. However, these reactions cannot predict the potential antibody production or hypersensitivity reactions in humans, and extrapolation of the immunological results to humans would be difficult. On the other hand, no allergic reactions were noted in the repeated-dose toxicity studies conducted in monkeys, in which rhGAA was given by continuous intravenous infusion, even though specific anti-rhGAA antibody was detected. However, when rhGAA was given in repeated bolus doses in rodents because continuous intravenous infusion is technically difficult, the allergic reactions were noted. At present, adequate data do not exist to conclude that the allergic reactions were induced by rapid bolus administration of rhGAA, but the fact that no allergic reactions were observed in the monkeys given rhGAA by continuous intravenous infusion even though specific anti-rhGAA IgG was detected, suggests that the method of administration (rate of infusion) may also be an important factor as to whether allergic reactions of rhGAA are induced in humans.

In relation to the allergic reactions observed in rodents, PMDA is of the opinion that adequate data do not exist for reasoning that the possibility of allergic reactions in humans might be small based on the data on the repeated, continuous intravenous infusion studies in monkeys, in which allergic reactions did not occur even though antibody formation was recognized. Although the occurrence of allergic reactions in rodents cannot be directly extrapolated to humans, it is necessary to pay due attention to the possibility of occurrence of allergy reactions, including the need for adjustment of the injection rate or other precautionary pretreatments, because adverse reactions related to allergy occurred in patients who were anti-rhGAA antibody positive in clinical studies.

(2) Longest administration period in toxicity studies

PMDA asked the applicant to present its view regarding the reason why the longest period of rhGAA administration in the repeated-dose toxicity studies was set at 6 months and why carcinogenicity was not examined.

The applicant responded as follows. Following long-term administration of rhGAA, formation of antibodies was observed; therefore, it was considered difficult to perform a 6-month or longer study in animals. Regarding the study of carcinogenicity, carcinogenicity was considered to be a highly unlikely problem with rhGAA, because rhGAA is a glycogen-decomposing enzyme localized in lysosomes, no activity of inducing cell proliferation has been reported so far, no histopathological findings suggestive of proliferative changes were obtained in the 26-week continuous intravenous infusion study in monkeys, and no clinical findings have been reported so far suggesting tumor induced by rhGAA.

PMDA considers that the potential for carcinogenicity of rhGAA has not been fully examined, as the longest duration of the repeated-dose toxicity studies was 26 weeks. On the other hand, it is considered unlikely that this would pose a serious problem to clinical use of rhGAA. However, in view of the fact that rhGAA is expected to be administered for a long time as a supplemental therapy for a missing enzyme, it will be necessary to carefully monitor the safety information for long-time use, including the possibility of carcinogenicity.

(3) Animal species used in reproductive and developmental toxicity studies

PMDA asked the applicant to explain the rationale for the only use of mice as animal species in reproductive and developmental toxicity studies.

The applicant responded as follows. Rats developed severe hypersensitive reactions in repeated-dose toxicity studies and were, therefore, considered to be unsuitable for use in the

reproductive studies. On the other hand, mice were used for the repeated-dose toxicity studies and pharmacokinetic studies, and the GAA KO mice, a model of human GSD II, were used for the pharmacological efficacy studies. Pharmacological efficacy similar to that observed in humans has been demonstrated in GAA KO mice, and the product showed the same pharmacokinetic profile in normal mice as that in the GAA KO mice. Therefore, it was considered possible to use only mouse species in reproductive and developmental toxicity studies. In view of the fact that the NOAEL in the mice obtained from reproductive and developmental toxicity studies was slightly higher than the human exposure level, etc., it was unlikely that serious reproductive toxicity would be exhibited in GSD II patients. However, rhGAA has not yet been used in pregnant women or lactating women, and safety in humans is not yet considered to be established. Attention will be drawn to these facts in the package insert and other documents. In the US, a study of the effects of rhGAA on pre-natal and post-natal development in mice, a study on the reproductive performance of maternal mice, and a developmental toxicity study in rabbit embryo/fetus are required as pre-conditions for approval, and these studies are currently in progress.

PMDA is of the opinion that in the reproductive and developmental toxicity studies submitted, the optimal dosing period or the effects of DPH has not been adequately examined. However, as overt reproductive toxicity was not observed in the studies performed and the applicant expresses intent to draw due attention to the package insert and other documents, it is unlikely that significant problems would arise immediately in clinical use. Furthermore, PMDA considers it is necessary for the applicant to report the data on the reproductive and developmental toxicity studies currently in progress soon after they are completed, and present the information in an appropriate manner, such as reflecting the findings in the package insert or other documents.

PMDA understands that there are limitations in detecting drug-specific toxicity by administering human protein preparations to animals. However, in the data submitted, some inadequacies are found in a few sections, such as purpose of the study, basis for setting of the dose range, and evaluation of findings. It cannot necessarily be assumed that toxicity evaluation has been carried out in an appropriate manner. However, in view of the efficacy of rhGAA and target patient population, additional studies may not be required immediately, but the safety of rhGAA should be confirmed by information on the clinical use of the drug, such as that from the post-marketing surveillance of all patients treated with the drug.

4. Clinical data

1) Summary of biopharmaceutics and related analytical methods

Summary of the submitted data

Bioequivalence and bioavailability studies have not been performed in human subjects. With the respective validated methods, GAA activities in human plasma and skeletal muscle were measured by the 4-MUG method, skeletal muscle glycogen content by the biochemical method (lower limit of quantification, [redacted] mg/dL or [redacted] mg/g wet tissue) using amyloglucosidase and by the histomorphological measurement method with a graphic analytical system, and the concentrations of oligosaccharides in plasma and urine by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (lower limit of quantification: Glc_4, [redacted] μmol/L; M_4, [redacted] μmol/L; Hex_4 [in urine], [redacted] μmol/L). Plasma anti-rhGAA antibody was evaluated and measured using ELISA (lower limit of quantification, [redacted]μg/mL), and confirmed by the radio-immunoprecipitation method (RIP). Since a 2000L-scale product is to be used as the commercial product in Japan, the clinical pharmacokinetic data for rhGAA were obtained from the results of Study AGLU02804 in patients with adult-onset GSD II.

2) Summary of results of clinical pharmacokinetic and pharmacodynamic studies

Summary of the submitted data

Foreign clinical studies, Study AGLU01602 (5.3.5.1.2) and Study AGLU01702 (5.3.5.2.1), were submitted as the results for infantile-onset patients. In addition, a foreign clinical study, Study AGLU02804 (5.3.5.2.7), was submitted as the results for adult-onset patients. However, no data have been submitted concerning the pharmacokinetics in Japanese patients.

(1) Pharmacokinetics

a. Foreign phase II/III dose-finding study (5.3.5.1.1, AGLU01602)

rhGAA 20 or 40 mg/kg was administered intravensously in multiple doses every other week in 18 foreign subjects with infantile-onset GSD II, who were 6 months or younger at the time of initial dosing. Interim analyses were performed 26 weeks after the start of administration to the last-registered subject, and pharmacokinetics was assessed at the initial dosing and at Week 12 (10 male and 5 female subjects for pharmacokinetic evaluation). For this study, the 160-L scale preparations（A011, A012, A013, A014, A015, and A016） were used. After the initial and Week 12 administrations, pharmacokinetic parameters at respective times were: C_{max}, 161 ± 28 and 196 ± 73 μg/mL for 20mg/kg, and 271 ± 61 and 256 ± 51 μg/mL for 40mg/kg; while AUC were 938 ± 199 and 1017 ± 262 μg･hr/mL for 20 mg/kg, and 1884 ± 407 and 1861 ± 407 μg･hr/mL for 40mg/kg; thereby, C_{max} and AUC increased in proportion to the dosage and did not show a tendency for accumulation with multiple doses. rhGAA, depending on the individual subjects, disappeared in a

single- or bi-phasic manner, and its $t_{1/2\,\alpha}$ (0.57 to 0.59 hours) and $t_{1/2\,\beta}$ (2.7 to 2.8 hours) were adequately brief as compared to the treatment interval (2 weeks). The multiple doses did not affect $t_{1/2}$, CL, central compartment distribution volume (V_1), or V_{ss}. Body weight was the only covariate in the pharmacokinetics of rhGAA. Body weight affected CL, V_1, and V_{ss}, and variances (%) between subjects after correction for body weight were 15%, 19%, and 11 %, respectively. Also, 16 of 18 subjects were anti-rhGAA antibody positive, and the pharmacokinetics of rhGAA was not affected by their anti-rhGAA antibody titers. The PK analyses in this study were not corrected for gestational period.

b. Foreign phase I/II study (5.3.5.2.1, AGLU01702 [interim data])

rhGAA 20mg/kg was administered intravenously in multiple doses every other week to 21 foreign infantile-onset GSD II subjects older than 6 months but younger than 36 months of age at the first dosing. Interim data were assessed at the completion of the 52-week treatment in the initially registered 15 subjects, and pharmacokinetics was evaluated at the initial dosing and at Week 12 (pharmacokinetic evaluation for 9 males and 5 females after initial dosing, 8 males and 4 females after dosing at Week 12). Both 30-L/60-L scale preparations (A002, A003, and A004) and 160-L scale preparations (A012, A013, and A015) were used. After the initial and Week 12 administrations, C_{max} were 188 ± 83 and 208 ± 59 μg/mL, respectively, and $AUC_{0-\infty}$ were 901 ± 314 and 1103 ± 278 μg･hr/mL, respectively, both showing slight increases after Week 12 dosing but without statistical significance. After the initial and Week 12 administrations, nearly the same values were obtained for CL (19.5-26.1 mL/hr/kg), terminal phase distribution volume (V_z) (70.6-74.1 mL/kg), and V_{ss} (75.8-84.8 mL/kg). Of the 20 subjects tested, 19 showed positive antibody expression, and 1 was positive at baseline. The pharmacokinetics of rhGAA was not affected by anti-rhGAA antibody. The pharmacokinetic analyses in this study were corrected for gestational period. It is noteworthy that 1 Japanese subject in this study was excluded from the pharmacokinetic assessment because cannulation for blood sampling was not possible.

(2) Pharmacodynamic studies

In Study AGLU1602, the quadriceps muscle was collected as a tissue biopsy sample of skeletal muscle at baseline, Week 12, and Week 52, and GAA activity and glycogen content were measured. Concentrations of oligosaccharides in plasma and urine were also measured at baseline, and at Weeks 4, 12, 26, 38, and 52. At baseline, skeletal muscle GAA activity was below the lower limit of quantification ([redacted] nmol/hr/g) in 15 of the 18 subjects, and in the remaining 3 subjects, the maximum was 8.8 nmol/hr/g; an extremely low skeletal muscle GAA activity characteristic of GSD II. At Weeks 12 and 52, respective GAA activities in the skeletal muscle samples were 101.1 ± 125.3 and 100.3 ± 50.8 nmol/hr/g in the 20 mg/kg group, and 342.1 ± 232.9 and 310.1 ± 431.2 nmol/hr/g in the 40 mg/kg group, indicating an elevation in skeletal muscle GAA activity and the

effects were greater in the 40 mg/kg group. Furthermore, median values (minimum to maximum) of skeletal muscle GAA activity in the 20 and 40 mg/kg groups were, respectively, 65.2 (22.3-428.9) and 228.8 (88.6-799.9) nmol/hr/g at Week 12, and 70.1 (49.0-185.8) and 137.1 (55.4-1298.3) nmol/hr/g at Week 52. Skeletal muscle glycogen contents were measured by the biochemical assay method at baseline, Week 12, and Week 52; respective values were 50.9 ± 28.9, 48.5 ± 28.6, and 40.8 ± 28.2 mg glycogen/g in the 20 mg/kg group, and were 62.3 ± 27.0, 46.0 ± 33.0, and 34.3 ± 37.3 mg glycogen/g in the 40mg/kg group. At Week 52, 12 of the 18 subjects showed a decrease (over 20% reductions from baseline), 3 remained unchanged (changes within 20% of baseline), 2 showed an increase (over 20% increases from baseline), and 1 was without measurement. On the other hand, respective results from the histomorphometoric assay method for the 20mg/kg group were 25.6 ± 12.1%, 27.9 ± 18.0%, and 28.1 ± 25.4%; and for the 40mg/kg group were 30.6 ± 13.1%, 26.4 ± 20.1%, and 23.4 ± 28.4%. At Week 52, 6 subjects showed a decrease, 8 remained unchanged, 3 showed an increase, and 1 was without measurement. The 2 methods yielded the same results for 8 of the 17 subjects, with data measured at Week 52. While the biochemical assay showed a greater glycogen content decrease in the 40 mg/kg group as compared to the 20 mg/kg group, the histomorphometoric assay did not show such a marked difference between the 2 groups. However, no correlation was recognized between the increase in skeletal muscle GAA activity and the decrease in skeletal muscle glycogen content, both being caused by the administration of rhGAA. For glucose tetrasaccharide (Hex_4) concentrations in urine, all 16 subjects, with available baseline data, showed extremely high values (normal upper limit, 18.8 for those below 0.5 years of age; 14.0 for those 0.5 to 1 years; and 5.0 mmol/mol creatinine [C_r] for 1 to 5 years of age; normal reference values from the [redacted] University Medical Center). In the 20 mg/kg group, the urinary Hex_4 concentration was 35.0 ± 11.4 mmol/mol C_r at baseline, and decreased to 17.5 ± 9.8 mmol/mol C_r at Week 26, and in the 40 mg/kg group, the baseline concentration of 41.2 ± 11.3 mmol/mol C_r decreased to 15.9 ± 11.3 mmol/mol C_r at Week 26. Urinary Hex_4 concentrations were decreased in 14 of 15 subjects with available baseline and Week 26 data, and those in 7 subjects decreased to the normal value range. Thereafter, at Week 52, the concentrations in the 20 and 40 mg/kg groups were 39.2 ± 31.5 and 25.6 ± 18.9 mmol/mol C_r, respectively. These values represented increases from Week 26. Urinary Hex_4 concentrations decreased but remained stable in subjects with sustained motor function. However, in subjects without improvement in motor function, they increased at Week 52 as compared to the baseline values, showing great individual variations in urinary Hex_4 concentrations. At baseline, Week 26 and Week 52, respective plasma Hex_4 concentrations were 2.34 ± 0.96, 1.12 ± 1.05, and 1.27 ± 1.09 mmol/mol C_r in the 20 mg/kg group, and 2.70 ± 1.20, 0.86 ± 0.47, and 0.95 ± 0.79 mmol/mol C_r in the 40 mg/kg group; showing reductions in both dosage groups at Week 26 and Week 52 as compared to the baseline values. As to reductions in urinary and plasma Hex_4 concentrations, no major differences were observed between the two dosage groups (5.3.5.1.1, AGLU01602).

In Study AGLU01702, quadriceps muscle tissue was collected as a skeletal muscle tissue biopsy sample at baseline, Week 12, and Week 52, and GAA activity and glycogen content were measured. In 5 of 14 subjects, skeletal muscle GAA activity at baseline was below the lower limit of quantification (nmol/hr/g), and the maximum value was 28.0 nmol/hr/g. At Week 12 and Week 52, GAA activities were 33.4 to 129.0 nmol/h/g (12 subjects) and 44.6 to 153.9 nmol/h/g (9 subjects), respectively; showing increases from baseline values. At Week 52, skeletal muscle glycogen contents in 9 subjects, as determined by the biochemical assay method, were decreased in 5 subjects, unchanged in 3, and increased in 1. On the other hand, the histomorphometoric assay for 10 subjects showed a decrease in 1, no change in 6, and an increase in 3 subjects. The two assay methods yielded the same results in 3 of 9 subjects. In regard to the intra- and inter-subject variations observed in the results obtained by the biochemical assay method and the histomorphometoric assay method, it is inferred that the variations are probably attributable to: tissue inhomogeneity due to sampling from different skeletal sites, different degrees of aridity in processing frozen tissue samples and also to differences between the two methodologies. In the Japanese subjects in this study, skeletal muscle GAA activity increased from 28.0 nmol/h/g at baseline to 86.5 nmol/h/g at Week 52, and glycogen content measured by the biochemical asay method decreased from 40.1 mg glycogen/g to below the lower limit of quantification. Also, glycogen content measured by the histomorphometoric assay method decreased from 37.0% to 2.6 % (5.3.5.2.1, AGLU01702).

Outline of the review by PMDA

(1) Antibody effects on pharmacokinetics

PMDA asked the applicant to explain the correlations between antibody titers in antibody positive subjects in Study AGLU01602 and Study AGLU01702, and pharmacokinetic parameters.

The applicant responded as follows. Using the pooled data in antibody positive subjects in Study AGLU01602 (15 subjects) and Study AGLU01702 (18 subjects), correlations between antibody titers (expressed as a common logarithm) and each pharmacokinetic parameter at Week 12 were assessed. Pearson's coefficients of correlation were -0.30 (P = 0.12) for total body CL, -0.21 (P = 0.28) for V_1, 0.33 (P = 0.08) for $AUC_{(0\text{-inf})}$, -0.26 (P = 0.18) for V_{ss}, -0.53 (P = 0.004) for intercompartment CL, -0.51 (P = 0.006) for terminal compartment distribution volume, and -0.04 (P = 0.86) for C_{max}, which, along with increases in antibody titers, show significant decreases in intercompartment CL and terminal compartment distribution volume. However, $AUC_{(0\text{-inf})}$, C_{max}, and other parameters did not correlate significantly with antibody titers. Thus, changes in intercompartment CL and terminal compartment distribution volume are regarded as exerting no effect on total drug exposures. In addition, the review of the correlation between antibody titers and

changes in each pharmacokinetic parameter from baseline to Week 12 did not reveal significant correlations in Pearson's correlation coefficients for any pharmacokinetic parameter.

PMDA asked the applicant to explain the background of the US package insert that includes a note concerning antibody effects on pharmacokinetics, namely that "Five patients with antibody titers ≥ 12,800 at Week 12 had an average increase in clearance of 50% from Week 1 to Week 12."

The applicant responded as follows. At a regulatory review by the FDA, the outcomes from the meta-analysis of Study AGLU01602 and Study AGLU01702 had not been submitted. The FDA requested the applicant, based on each of the clinical summary reports, to describe, in the package insert, the outcomes of the 5 subjects with pharmacokinetic parameters differing from those of other subjects, and with antibody titers exceeding 12,800. In the future, Genzyme in the US is to submit the meta-analysis outcomes to the FDA, followed by consultation regarding the revision of the aforementioned package insert.

PMDA infers that the inter-subject variations in the pharmacokinetics of rhGAA are a manifestation of the consequence of complex mutually influencing factors, such as differences in skeletal muscle lesions, expression of the mannose-6-phosphate receptor that is possibly involved in glycogen intake into muscle, effects of antibodies, inhomogeneity in biopsy tissue samples, sialic acid content in rhGAA, and variations among assay systems. PMDA also offered the following considerations. Judging from the outcomes of the correlation analysis between antibody titers and pharmacokinetic parameters, antibody is not regarded as a major cause of inter-subject variations. However, not enough data have been collected from subjects with extremely high antibody titers and other influencing factors have not yet been examined. Since, at present, factors responsible for the inter-subject variations are uncertain, it would be appropriate to provide as much information as possible from an individual case analysis, and, henceforth, it will be necessary to further address this issue carefully in line with the status in the US.

(2) Pharmacokinetics excluding infants

PMDA asked the applicant to explain the differences in pharmacokinetics between adult-onset and infantile-onset patients.

The applicant responded as follows. In Studies AGLU01602, AGLU01702, and AGLU02804, when pharmacokinetic parameters were adjusted to the patient's body weight, inter-subject variations decreased markedly, to a coefficient of variation approaching zero for terminal compartment distribution volume. As for the adult-onset form, pharmacokinetic parameters were estimated from a model which was developed based on data for the infantile-onset form. The fixed

estimated parameters of infantile-onset subjects were applied to adult-onset subjects after taking body weight differences into account. The results showed that, although a slight bow-shaped tendency was discerned, the estimated values were comparable to the measured values. Subsequently, when a patient's body weight was taken into account, the pharmacokinetics of the adult-onset and infantile-onset forms was regarded as being analogous. The same conclusion was derived from pharmacokinetic parameters corrected by body weight.

PMDA offered the following considerations. At present, the pharmacokinetic data on adult-onset GSD II subjects in Study AGLU02804 are available for only 5 subjects and no comparable data have been collected to allow adequate comparison of pharmacokinetics between adult-onset and infantile-onset subjects. Despite this, based on the analytical outcomes from available data, given that body weights are taken into account, the pharmacokinetics in adult-onset subjects do not differ markedly from those in infantile-onset subjects.

3) Summary of clinical efficacy and safety studies

Summary of the submitted data

For the data for this application, study results were submitted as follows: 1 foreign epidemiological survey (5.3.5.4, AGLU-004-00), five foreign clinical studies (Phase I/II non-blinded studies [5.3.5.2.1, AGLU01702], Phase II non-blinded extension studies [5.3.5.2.3, AGLU02003; 5.3.5.2.6, AGLU02103; 5.3.5.2.4, AGLU01205-02 revisions 4 and 5; 5.3.5.2.7, AGLU02804]), Phase II/III non-blinded dose-finding studies (5.3.5.1.1, AGLU01602), and two expanded access studies for severe patients (5.3.5.2.2, AGLU02203; 5.3.5.2.5, AGLU02503). In addition, the data from Japanese patients were submitted from an expanded access program (5.3.5.2.8, AGLU02203-Japan; 5.3.5.2.9, AGLU02603-Japan).

(1) Epidemiological survey of natural history in foreign infantile-onset GSD II patients (5.3.5.4.1, AGLU-004-00)

Targeting infantile-onset GSD II patients who had a symptom onset by age 12 months, and in whom GAA deficiency or a GAA gene mutation had been confirmed, with the aims of clarifying the natural history of infantile-onset GSD II and to use them as a historical cohort in clinical studies of rhGAA, a retrospective review study on the patient's medical history was conducted during a 10-month study period at 32 institutions in 9 countries.

Of all 300 registered patients, 172 met the inclusion criteria, 168 were assigned as subjects for analysis, and 4 were excluded because they had been treated with enzyme supplementation therapy for GAA or it was uncertain whether they had been treated with the same (stratification between

typical and atypical infantile-onset subjects was not possible). Ages at the first symptom onset were 2.0 ± 2.48 (median ± standard deviation, the same applies hereinafter) months, and 89.9% of subjects were under 6 months of age. Also, month-ages when the diagnosis of GSD II was confirmed were 4.7 ± 8.83 months after birth (5.1 prenatal to 84.2 postnatal months).

In this survey, patients surviving till 12 months of age accounted for 22.1% (36 of 163 subjects[1]), whereas 12.3% (20 of 163 subjects) survived until 18 months. Additionally, subjects who survived 12 months without artificial ventilatory support accounted for 14.5% (24 of 165 subjects[2]), whereas 6.7% (11 of 165 subjects) survived 18 months, and the 50% survival period without ventulatory support was 5.9 months. Furthermore, in 49 patients with a history of artificial ventilatory support, the age at which they first required ventilatory support was 5.9 months (0.1 to 39.1 postnatal months) for 50% of patients.

The most frequently observed symptoms/signs accompanying this disease were cardiomegaly (91.7%), hypotonia (88.1%), cardiomyopathy (87.5%), respiratory distress (78.0%), muscular weakness (62.5%), eating disorder (57.1%), failure to thrive (53.0%), and cardiac failure congestive (50.0%). The median age at first symptom onset was approximately 4 to 4.5 months.

Table　Age at death in each patient group classified by time of death

Category	before 1985	1985-1989	1990-1994	1995-1999	2000-present
Number of deceased patients	15	12	27	52	33
Average month-age at death	9.4	7.1	9.4	10.6	10.9
50% survival time ［95%CI］	9.8 ［7.1, 11.3］	5.9 ［4.3, 7.0］	6.9 ［5.6, 9.9］	8.6 ［8.2, 9.0］	8.9 ［7.5, 11.6］
Maximum, Minimum	3.6, 16.6	1.7, 23.9	2.8, 47.9	1.6, 56.9	2.6, 28.0

With the aforementioned discussions, the applicant articulated the opinion that despite various symptomatic therapies for infantile-onset GSD II patients, many of these infants die within 12 months after birth, and median age at death has remained unchanged during the past several decades, such that infantile-onset GSD II is regarded as a rapidly progressive and fatal disease.

Infantile-onset GSD II study

(2) Foreign phase I/II study (5.3.5.2.1, AGLU01702 [Under treatment 3 years 10 months after the start of the study; Interim data])

To evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of rhGAA in patients

[1] Excluded patients with unknown death time

[2] Excluded cases of which the period until the death and the need of ventilator support is unknown

with a confirmed diagnosis of infantile-onset GSD II with ages ranging from 6 to 36 months (target number, 20 patients), a non-blinded and uncontrolled sudy was conducted. The study data obtained up to 1 year 6 months after the start of the study were submitted [See "2) Summary of results of clinical pharmacokinetic and pharmacodynamic studies" for pharmacokinetics and pharmacodynamics].

rhGAA was to be administered at a dose of 20 mg/kg every other week by intravenous infusion, and dose increase up to 40 mg/kg after 26 weeks was allowed. rhGAA infusions were administered, beginning with an initial rate of 1 mg/kg/hr, if no infusion associated reaction (IAR) signs were present, with gradual increments of 2 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 7 mg/kg/hr. Although the treatment period was 52 weeks, for patients who showed efficacy at the time of completion and in whom further treatment was desired, extension of rhGAA therapy was possible with a cycle of 52 weeks.

All 21 treated subjects (including 1 Japanese subject) were included in the safety analysis, and 15 subjects (including those who died before Week 52) who completed 52 weeks of administration by the cut-off date (1 year and 6 months after the start of the study) were designated as suitable subjects for the efficacy analysis. Specifically, 11 of 15 subjects completed 52 week courses, but, among them, 1 (the Japanese subject) terminated this study after the dose administration at Week 52 due to an intent to return to Japan. In two subjects, the dosage was increased to 40 mg/kg after Week 26 and this dosage was maintained through Week 52, but no dose-reduction cases were reported.

The survival rate at Week 52, the primary endpoint, was 73.3% (11 of 15) of subjects. Furthermore, the survival rate at Week 52 without artificial ventilatory support, a secondary endpoint, was 50.0% (5 of 10 subjects[3]; the Japanese subject temporarily used artificial ventilatory support for 13 days at Week 13).

On the other hand, as to cardiovascular function (left ventricular mass index [LVMI]), 13 of 15 subjects had decreased LVMI and decreased left ventricular mass (LVM) at Week 52 or through the final assessment time point. In 10 (including the 1 Japanese subject) of the 13 subjects, who were assessed using the Peabody Developmental Motor Scale-2 (PDMS-2), an improvement in age-equivalent scores was observed in 2 subtests.

A total of 636 adverse events (including results of clinical laboratory tests, physical examinations,

[3] The number of subjects who did not require invasive artificial ventilatory support at baseline

electrocardiogram, vital signs, etc.) were reported for all 21 subjects. Six deaths (of which 1 occurred at baseline before treatment) were noted, and there were 5 deaths (cardio-respiratory arrest; cardiac arrest and respiratory arrest; arrhythmia, acute pulmonary edema, and cardiac failure acute; respiratory arrest; cardiac arrest in 1 subject each) from the initial dosing through Week 52, for which causal relationship to rhGAA was ruled out. A total of 76 serious adverse events were noted in 71.4% (15 of 21) of subjects, and mainly consisted of respiratory failure, respiratory distress (5 subjects each), pneumonia, pyrexia (3 subjects each), acute bronchitis, and catheter related infection (2 subjects each). Causal relationship between all the events, excluding 6 events of IAR in 1 subject, and rhGAA was ruled out.

Adverse events whose causal relationship to rhGAA could not be ruled out (adverse drug reactions) were noted on 33 occasions, 38.1% (8 of 21 subjects), of which IAR occurred in 7 subjects. Major events were flushing (3 subjects), blood pressure increased, oxygen saturation decreased and tachypnoea (2 subjects each). All were mild or moderate in severity.

Among abnormal changes in clinical laboratory tests, 42.9% (9 of 21 subjects) were on biochemical tests, namely, CK-MB increased, AST increased (5 subjects each), ALT increased, creatinine kinase elevated (4 subjects each); and 14.3%（3 of 21 subjects）were noted on hematology tests, namely, leucopenia (all recovered), anemia in 1 subject at Week 52; and urinalysis revealed mild transient proteinuria in 3 subjects (causal relationships to rhGAA were ruled out).

As to immunogenicity, excluding 1 death 4 days after the first dosing, anti-rhGAA IgG antibody was detected in 19 of 20 subjects, and positive conversion was noted in most subjects during the period from Week 4 through Week 12, of which 6 subjects showed antibody titers exceeding 1:10000 during the study period.

With the aforementioned discussions, the applicant articulated the opinion that the administration of rhGAA prolonged the survival period, and is thus a safe and effective therapy.

(3) Foreign phase II/III dose-finding study (5.3.5.1.1, 2; AGLU01602 [Under treatment 2 years 1 month after the start of the study])

To evaluate the efficacy, safety, pharmacokinetics, and pharmacodynamics of rhGAA in ventilation-free patients with a confirmed diagnosis of infantile-onset GSD II and ages below 26 months, a randomized non-blinded study was conducted [See “2) Summary of results of clinical pharmacokinetic and pharmacodynamic studies” for pharmacokinetics and pharmacodynamics].

rhGAA was to be administered at a dose of 40 mg/kg every other week by intravenous infusion. rhGAA infusions were administered, beginning with an initial rate of 1 mg/kg/hr, if no IAR signs were present, with gradual increments of 2 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 7 mg/kg/hr. The minimum treatment period was to be 52 weeks.

All 18 treated subjects (20 mg/kg, 9 subjects; 40 mg/kg, 9 subjects) were included in the efficacy and safety analyses, and all completed the 52-week treatment. Additionally, for all 18 subjects, mean GAA activity in skin fibroblast cells was less than 1% of the normal mean value.

The Kaplan-Meier estimates of survival rates in subjects without invasive artificial ventilatory support at 18 months of age, the primary endpoint, were 88.9% for the 20 mg/kg group, 77.8% for the 40 mg/kg group, and 83.3% as a whole (95% CI. [66.1%, 100.0%]), and 1 subject survived untill age 18 months (estimated survival rate 1.9%) among 61 subjects[4] in the historical cohort.

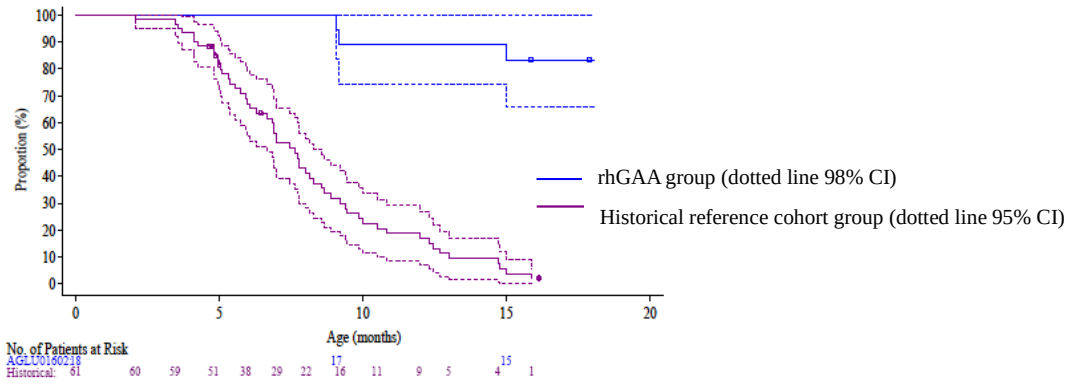


Figure Survival periods in rhGAA-treated patients and a historical cohort without (invasive) ventilatory support

LVMI, a secondary endpoint, was measured in 15 subjects, and the mean LVMI decreased from 193.4 g/m^2 (range, 59.3-301.8 g/m^2) at baseline to 86.8 g/m^2 (range, 44.9 to 157.3 g/m^2) at Week 52. Functional status (Pompe PEDI) at Week 52 were also assessed as: capable of independent ambulation 38.9% (7 of 18 subjects), capable of independent standing-up/supported walking 16.7% (3 of 18 subjects), capable of rolling over and maintaining a sitting position (standing position not possible) 16.7% (3 of 18 subjects), and cognitive function (BSID-Ⅱ) 52.9% (9 of 17 subjects) within a normal range. At Week 18, 33.3% (6 of 18 subjects) required artificial ventilatory support (either invasive or non-invasive). At Months 7.8, 12, and 13.7, artificial ventilatory support was needed for 3 subjects in the 20 mg/kg group, and at Months 9.1, 12.0, and 14.8, such support was

[4] Extracted population from infantile-onset GSDII subjects in AGLU-004-00

needed for 3 subjects in the 40 mg/kg group.

A total of 1,150 adverse events (including results of clinical laboratory tests, physical examinations, electrocardiogram, vital signs, etc.) were noted in all 18 subjects (480 events in the 20 mg/kg group, 670 events in the 40 mg/kg group). There was one death in the 20 mg/kg group (after completing 52 weeks of treatment, this subject, while hospitalized for respiratory distress and pneumonia, died at 19.8 months of age [61 weeks of administration] of oxygen saturation decreased and bradycardia) and there was one death in the 40 mg/kg group (this subject suffered cardiac arrest at Week 108, and was resuscitated but died at 31.9 months of age of multi-organ failure due to sepsis), but their causal relationships to rhGAA was ruled out. A total of 174 serious adverse events was noted in 17 of 18 subjects (88 events [8 of 9 subjects] in the 20 mg/kg group, and 86 events [all 9 subjects] in the 40 mg/kg group) and were mainly respiratory failure (7 subjects), pneumonia, bronchopneumonia, catheter-related infection, RS virus infection (5 subjects each), bronchiolitis, viral infection, respiratory distress, and aspiration pneumonia (4 subjects each). Causal relationship between all the events, excluding moderate urticaria and rales in 1 subject in the 40 mg/kg group, and rhGAA was ruled out.

Adverse drug reactions were noted on 170 occasions in 12 of 18 subjects (43 events [6 of 9 subjects] in the 20 mg/kg group and 127 events [6 of 9 subjects] in the 40 mg/kg group), of which IARs accounted for 164 of 170 events (96.5%: 41 events [5 of 9 subjects] in the 20 mg/kg group, 123 events [6 of 9 subjects] in the 40 mg/kg group). The major adverse drug reactions were pyrexia (7 subjects), urticaria (5 subjects), and oxygen saturation decreased (4 subjects).

Clinically significant laboratory abnormalities were noted in 55.6 % (10 of 18 subejcts), namely, in the 20 mg/kg group, 7 events involving urinary occult blood, 5 events involving plasma CK variations, and 5 events involving ALT variations. Causal relationship between all the events, excluding 1 episode of blood calcium increased and 1 episode of blood CK-MB increased in the 20 mg/kg group, and rhGAA was ruled out.

As to immunogenicity, anti-rhGAA IgG antibody was positive in 16 of 18 subjects (88.9%: 8 subjects in each group, measured by the ELISA method and confirmed by the RIP method). For confirmation, 1 subject in the 40 mg/kg group (subject No. 1602-303) was demonstrated to be positive at baseline and showed cross-reactivity.

As to vital signs, clinically significant changes were noted in tachycardia (3 subjects), hypertension (2 subjects), and hypotension (1 subject), and all of these events were attributed to IARs. There were no clinically significant changes in electrocardiogram.

With the aforementioned discussions, the applicant articulated the opinion that rhGAA was effective in prohibiting or delaying the onset of severe symptoms of GSD II.

(4) Phase II extended study (5.3.5.2.3, AGLU02003 [Under treatment 3 years 9 months after the start of the study])

Targeting infantile-onset GSD II patients, who had participated in a clinical study using a CHO cell-derived rhGAA formulation (Synpac rhGAA) developed by Synpac, a non-blinded uncontrolled clinical study was conducted to assess the efficacy and safety of rhGAA for long-term treatment, and study data obtained 1 year after the start of the study* were submitted.

rhGAA was to be intravenously administered at a dose of 10 mg/kg/week, or 20, 30, or 40 mg/kg/2 weeks, with an allowed increase and decrease between 10 mg/kg/week and 40 mg/kg/2 weeks.

All 7 treated subjects (5 infantile-onset and 2 adult-onset subjects) were included in the safety analysis, and, 6 subjects were targets for efficacy analysis, with exclusion of the 1 subject who died at Week 16. Five subjects completed the 52-week treatment.

Three of 5 typical infantile-onset GSD II subjects were alive at Week 52, and did not need artificial ventilatory support. As to cardiac function, in 5 of 6 subjects (83.3%), LVMI and left ventricular posterior wall thickness（LVPWT）were within normal range or decreased (in only 1 subject [subject No. 206], LVMI and LVPWT increases were noted at Week 34). Moreover, for functional assessments, score improvements were observed in motor function (PDMS-2) in 3 of 5 subjects, muscle assessment (MRC) in 4 of 4 subjects, functional skills (PEDI) in 6 of 6 subjects, and cognitive function (BSID-II) in 3 of 3 subjects.

A total of 151adverse events (including results of clinical laboratory tests, physical examination, electrocardiogram, vital signs, etc.) were noted in all 7 subjects. There were 2 deaths (respiratory failure in 1 subject, Week 16 [33.8 months of age]; cardiac arrest in 1 subject, Week 36 [32.1 months of age]), but all causal relationships to rhGAA were ruled out. Eighteen serious adverse events were observed in 3 of 7 subjects (42.9%), and all events were mild or moderate in severity; and all causal relationships to rhGAA were ruled out. Major events (noted in at least 2 subjects) were pneumonia and bacteraemia (2 subjects each).

Adverse drug reactions were noted on 49 occasions in 2 of 7 subjects, and major adverse reactions were 48 IAR events in 2 subjects. All events were mild in severity and could be addressed by decreasing the rate of infusion or discontinuing administration. Although 1 subject (subject No.

206) experienced IAR as many as 47 times, during the entire 52-week treatment period, his/her IgE antibody was negative, and complement activity and serum tryptase levels were within their normal ranges.

No clinically significant problems were seen on clinical laboratory tests, vital signs, physical tests, or twelve-lead electrocardiograms. Although all 7 subjects were positive for IgG antibody at baseline as to immunogenicity tests, the change from Synpac rhGAA to rhGAA did not accompany an increase in antibody titers and rhGAA inhibitory activity caused by IgG antibodies.

With the aforementioned discussions, the applicant articulated the opinion that rhGAA, when used for long-term treatment, showed its efficacy and generally acceptable tolerability.

(5) Phase II extended study (5.3.5.2.4, AGLU1205-02 [revisions 4 and 5] [Under treatment 2 years 8 months after the start of the study])

The clinical data through 52 weeks were submitted based on the outcome of a non-blinded study with an intravenous 40 mg/kg dose of rhGAA (25 administrations every other week, followed by 28 administrations every week) in 1 infantile-onset GSD II patient (infant girl, 41.0 months of age at the start of this study), who had been treated in past clinical studies with the Pharming rhGAA formulation (prepared from transgenic rabbit milk) and the Synpac rhGAA formulation.

Echocardiography showed LVMI to be decreased from 175 g/m^2 (the normal upper limit, 98.2 g/m^2) at baseline to 160 g/m^2 (the normal upper limit, 102.5 g/m^2) at Week 48, and LVPWT was maintained at a value of 6 mm, which is within the normal range, (the normal upper limit, 7.4 mm) at both baseline and Week 48.

Adverse events (including results of clinical laboratory tests, physical examinations, electrocardiogram, vital signs, etc.) were noted on 6 occasions, of which 5 (aspiration pneumonia [2 cases], rhinitis, urinary tract infection, pneumonia [1 each]) were regarded as serious adverse events, but all were mild in severity and all causal relationships to rhGAA was ruled out. In addition, the anti-rhGAA IgG anyibody was positive at baseline (not measured thereafter). As to abnormal clinical laboratory changes, although high values were reported for CK, CK-MB, ALT, AST, and LDH, no clinically significant problems were observed on hematological tests or urinalysis. Vital signs remained stable throughout this study, and physical examination showed that systemic hypotonia. On electrocardiography, except for the abnormal findings at baseline, no new abnormalities were noted.

With the aforementioned discussions, the applicant articulated the opinion that rhGAA could

maintain the improvement in cardiac myopathy, and showed good tolerability.

Study on adult-onset GSD II

(6) Foreign phase II study（5.3.5.2.7, AGLU02804 [Under treatment 1 year 11 months after the start of the study])

Targeting adult-onset GSDⅡ subjects (more than 5 years but less than 18 years of age), a non-blinded uncontrolled clinical study was conducted to assess the efficacy, safety, and pharmacokinetics of rhGAA, and the data from the interim analysis obtained 6 months after the start of this study were submitted.

rhGAA was to be intravenously administered at a dose of 20 mg/kg every other week, beginning with an initial rate of 0.2 mg/kg/hr, if no IAR signs were present, with gradual increments of 0.8 mg/kg/hr, then 3.5 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 10 mg/kg/hr. The administration period was to be 26 weeks.

All 5 treated subjects were included in the efficacy and safety analyses and at baseline all showed deficiency in GAA activity in leukocytes.

Improvements were observed in 3 subjects in the standing (sitting) position forced vital capacity (FVC), in 4 subjects in the manual muscle test (MMT), and in 3 subjects in the hand-held dynamometer. Also, in the 6-minute walking test (6MWT), despite the median (range) of the distance walked at a moderate speed decreasing from 360 m (333-412 m) at baseline to 345 m (216-457 m) after 26 weeks, the distance walked at a fast speed increased from 468 m (344-546 m) at baseline to 520 m (368-664 m).

A total of 33 adverse events (including results of clinical laboratory tests, physical examinations, electrocardiogram, vital signs, etc.) were noted in all 5 subjects, but were mild in severity. There were no deaths or treatment discontinuations. Although 1 traffic accident was reported as a serious adverse event, a causal relationship to rhGAA was ruled out. No adverse drug reactions were noted. Although, on clinical laboratory tests, no clinically abnormal changes were observed on hematological tests or urinalysis, all subjects showed high CK, CK-MB, AST, and ALT values on the biochemical tests. As to immunogenicity, 4 subjects treated with rhGAA showed anti-rhGAA IgG antibody, but none showed inhibitory activity exceeding 10%. As to vital signs (body temperature, heart rate, respiratory rate, and blood pressure), no clinically significant variations were noted. On electrocardiography, 1 subject, having already shown abnormal transmission (delta waves and a non-specific interventricular conduction block) at baseline, experienced it again at Week 26, but a causal relationship to rhGAA was ruled out.

With the aforementioned discussions, the applicant articulated the opinion that although the number of treated patients was small, the efficacy and safety of rhGAA in adult-onset GSD II patients were both confirmed.

(7) Phase II extended study (5.3.5.2.6, AGLU02103 [Under treatment 3 years 9 months after the start of the study])

The clinical data through 52 weeks were submitted based on the outcome of a non-blinded study with an intravenous rhGAA dose of 30 mg/kg every other week in 1 GSD II patient (female, 20 years old), who had been treated in past clinical studies with the Pharming rhGAA formulation and the Synpac rhGAA formulation

No changes of clinical significance were observed in pulmonary functions (% FVC, FEV1/FVC, etc.), motor development (muscle strength, Gross Motor Function Manual [GMFM], and the Walton & Gardner-Medwin Scale) or physical growth (body weight and height).

Adverse events (including results of clinical laboratory tests, physical examinations, electrocardiogram, vital signs, etc.) were noted on 285 occasions. Serious adverse events were nausea, chest discomfort, flushing, paresthesia, and dyspnoea (1 each), all being moderate in severity, and all causal relationships to rhGAA was ruled out. Adverse drug reactions were noted in 5 IARs (flushing, injection site pain, injection site reaction, blood pressure increased, and dizziness, 1 each); all were mild in severity and disappered within 1 day of onset without treatment. As to clinical laboratory tests, elevated CK, CK-MB, ALT, AST and LDH values were noted, but ALT and LDH had normalized by Week 52. In addition, anti-rhGAA IgG antibody was positive but symptoms of immune complex disease and moderate or greater IAR were not observed. Vital signs showed mild body temperature elevation at Week 10 (causal relationship to rhGAA was ruled out), and blood pressure increased (IAR) at Week 40; all events resolved without treatment. On physical examination, muscular weakness appeared at Week 48 and persisted through Week 52, but a causal relationship to rhGAA was ruled out.

With the aforementioned discussions, the applicant articulated the opinion that rhGAA effectively inhibits disease progression in adult-onset GSD II patients and shows good tolerablity.

Expanded access study

(8) Expanded access study in severely affected infantile-onset patients (5.3.5.2.2, AGLU02203 [Under treatment 3 years 1 month after the start of the study])

The outcomes through Week 26 were submitted based on an expanded access study in severely

affected infantile-onset GSD II patients who failed to meet the inclusion criteria for clinical studies of rhGAA and could not be treated with any drug other than rhGAA.

rhGAA was to be intravenously administered at the dose of 20 mg/kg every other week (if not tolerated, a change to 10 mg/kg every week would be allowed), beginning at an initial rate of 1 mg/kg/hr, if no IAR signs were present, with gradual increases in the drip infusion rate of 2 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 7 mg/kg/hr.

In all 5 treated subjects, the status of artificial ventilatory support use (at baseline, all used 24-hour invasive artificial ventilatory support) was not changed. Furthermore, the 2 subjects with cardiomyopathy at baseline showed a tendency for improvement (remarkable LVM and LVMI decreases in 1 subject at Week 26; LVM and LVMI decreases in the other subject at Week 12), and there were no new cardiomyopathy cases. Moreover, improvement in functional skills (PEDI) was seen in 3 subjects.

Although adverse events were reported for 4 of 5 subjects (59 events), there were no deaths, serious adverse events, or rhGAA discontinuations. Although adverse drug reactions were noted in 2 subjects (26 events), both experienced IAR (24 events). For IAR, all subjects recovered and were free of after-effects with appropriate measures, taken as necessary, such as the administration of antipyretics or antihistamines during infusion or before rhGAA dosing, slowing the rate of infusion, and discontinuing rhGAA temporarily.

As for immunogenicity, all 5 subjects showed positive conversions of anti-rhGAA IgG antibody in 4 to 16 weeks, but no rhGAA inhibitory activity was noted.

With the aforementioned discussions, the applicant articulated the opinion that the efficacy and safety of rhGAA was confirmed in severely affected infantile-onset GSD II patients

(9) Expanded access study in severely affected adult-onset patients (5.3.5.2.5, AGLU02503 [Under treatment 3 years 2 months after the start of the study])

The outcomes through 26 weeks were submitted based on the expanded access study in severely affected adult-onset GSD II patients.

rhGAA was to be intravenously administered at a dose of 20 mg/kg every other week (if not tolerated, change to 10 mg/kg every week is allowed), beginning at an initial rate of 1 mg/kg/hr, if no IAR signs were present, with gradual increases in the drip infusion rate of 2 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 7 mg/kg/hr.

The total number of subjects was 3; one (subject No. 9301) died of cardio-respiratory arrest at Week 22, but a causal relationship to rhGAA was ruled out. In the other 2 subjects, improvements were noted in pulmonary functions, muscle strength, and motor function.

Adverse events were reported for 2 (29 events) of 3 subjects, but not in 1 (subject No. 9401). One subject showed 2 serious adverse events (subject No. 9301, respiratory distress, cardio-respiratory arrest), but the causual relationships to rhGAA were ruled out. As adverse drug reactions, 16 events of IAR were noted in in 1 subject (subject No. 9201), but each was mild in severity. After the 6th treatment, measures such as pre-dosing with antihistamines, slowing the rate of infusion, and temporary dosing discontinuation were employed, and the patient subsequently recovered without after-effects. As to immunogenicity, all subjects became positive for anti-rhGAA IgG antibody, but did not show formation of the inhibitory antibody.

With the aforementioned discussions, the applicant articulated the opinion that the efficacy and safety of rhGAA were confirmed in severely affected adult-onset GSD II patients.

Expanded Access Program in Japan

(10) Expanded access program in infantile-onset GSD II patients (5.3.5.2.8, AGLU02203-Japan [Under treatment 2 years 8 months after the start of the study])

To provide enzyme supplementation therapy with rhGAA, an expanded access program (EAP) was conducted in severe infantile-onset GSD II patients, and the study data from 5 Japanese patients[5] (treatment period: 38 to 90 weeks) were submitted based on outcomes obtained 1 year and 11 months after the start of this study. This program is not, however, a study in conformity with GCP standards.

rhGAA was to be intravenously administered at a dose of 20 mg/kg every other week (if not tolerated, change to 10 mg/kg every week is allowed), beginning at an initial rate of 1 mg/kg/hr, if no IAR signs were present, with gradual increases in the drip infusion rate of 2 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 7 mg/kg/hr.

Clinical conditions of the 5 subjects at enrollment are presented in the Table below. In addition, hypotonia and limb muscle weakness had been observed in all subjects, as well as scoliosis in 2 (subject Nos. 58718 and 74735) and joint contracture in 1 (subject No. 74735).

[5] One subject (No.111727), who had completed the 52-week foreign Study AGLU01702, participated in this program after returning to Japan.

Table Clinical conditions of GSD II subjects

Subject No.	Gender	Age at initial treatment	Macro-glossia	Respiratory distress	Venti-latory support	Cardio megaly	Cardiom yopathy	Congestive cardiac failure	Hepato-megaly	Spleno-megaly
58718	Female	3-15 years	No	Yes	Yes	Yes	No	No	No	No
74735	Male	1-3 years	Yes	No	No	Yes	Yes	Yes	Yes	No
111727	Female		No	Yes	No	Yes	Yes	No	Yes	No
144757	Male	0-1 year	Yes	Yes	No	Yes	Yes	Yes	Yes	No
178778	Female		Yes	Yes	No	Yes	Yes	Yes	Yes	Yes

During rhGAA treatment, duration of ventilatory support shortened in 1 subject (subject No. 58718), while ventilation support became necessary in 2 (subject No. 111727, pneumonia and respiratory failure at Week 90; subject No. 178778, respiratory failure after Week 12), of whom 1 (subject No. 178778) showed progression of the respiratory disorder. All 4 subjects with cardiac myopathy showed LVM and LVMI decreases, and ejection fraction increases. Of the 4 subjects with severe delays in motor development (2 of whom evaluation was impossible), one (subject No. 74735) showed improvement in motor capability, but one (subject No. 111727) with standard motor capability showed a decrease in this capability.

Adverse events were reported for 4 subjects (45 events), but there were no deaths or rhGAA discontinuations. Serious adverse events were reported for 3 subjects (subject No. 58718, pneumothorax and bronchial asthma; subject No. 111727, pneumonia, atelectasis, and respiratory failure; subject No. 178778, respiratory failure and cyanosis); all the events excluding bronchial asthma were assessed as causally unrelated to the drug. Adverse drug reactions were reported for 2 subjects (8 events in 1 subject, i.e. malaise [3 events], asthma, nausea, headache, urticaria papular, flushing; 2 events in 1 subject, i.e. oxygen saturation decreased, pyrexia), all were mild in severity and were followed by full recovery. As to immunogenicity, all 5 subjects became anti-rhGAA IgG antigen positive by Week 12. No clinically significant changes were observed on clinical laboratory tests, vital signs, physical examination, or 12-lead electrocardiography

With the aforementioned discussions, the applicant articulated the opinion that the tolerability of rhGAA treatment was good in these 5 Japanese patients with severe infantile-onset GSD II disease.

(11) EAP in adult-onset GSD II patients (5.3.5.2.9, AGLU02603-Japan [Under treatment 1 year 5 months after the start of the study])

To provide enzyme supplementation therapy, an EAP was conducted in severe adult-onset GSD II patients, and the study data from 5 Japanese patients (treatment period: 12 to 26 weeks) were submitted based on the outcomes obtained 8 months after the start of the study. This program is not, however, a study in conformity with GCP standards.

rhGAA was to be intravenously administered at a dose of 20 mg/kg every other week (if not tolerated, change to 10 mg/kg every week is allowed), beginning at an initial rate of 1 mg/kg/hr, if no IAR signs were present, with gradual increases in the drip infusion rate of 2 mg/kg/hr every 30 minutes to an allowed maximum infusion rate of 7 mg/kg/hr.

Clinical symptoms of the 5 subjects at enrollment are presented in the Table below. Although all subjects showed hypotonia and muscular weakness in the limbs, no macroglossia, otitis media chronic, deafness, congestive cardiac failure, or splenomegaly was recognized.

Table Clinical status of GSD II subjects

Subject No.	Age at initial treatment	Gender	Respiratory distress	Ventilatory support	Hepatomegaly	Scoliosis	Joint contractures
174833	20-35 years	Female	Yes	Yes	Yes	Yes	No
174834		Female	Yes	Yes	No	Yes	Yes
177830		Male	Yes	Yes	No	Yes	No
203844		Male	Yes	Yes	No	Yes	Yes
203845	18-20 years	Female	Yes	Yes	No	No	Yes

During rhGAA treatment, none of the 5 subjects showed changes in manual muscle tests, limb functional assessment, height or body weight, but one subject (subject No. 174834) showed worsening of motor function (Walton & Gardner-Medwin Scale).

In 3 subjects, 3 adverse events were observed, and all were IARs, whose causal relationships to rhGAA could not be ruled out. There were no deaths, serious adverse events, or rhGAA discontinuations. Furthermore, the physical examination, vital signs and electrocardiogram (taken in 2 subjects) showed no changes. Assessment of clinical laboratory data has not yet been performed. As to immunogenicity, 3 subjects became anti-rhGAA IgG antigen positive during Weeks 4 to 8.

With the aforementioned discussions, the applicant articulated the opinion that the tolerability of rhGAA was good in these 5 Japanese patients with severe adult-onset GSD II disease.

Outline of the review by PMDA

(1) Target patients

PMDA asked the applicant to explain the clinical positioning of rhGAA in relation to the current therapy for GSD II patients, referring to differences in clinical symptoms between infantile- and adult-onset GSD II patients.

The applicant responded as follows. In GSD II, the lysosomal GAA deficiency induces accumulation of glycogen in cells and tissues (muscle, in particular), which lead to the clinical

symptoms (mainly impairments in cardiovascular, respiratory, and motor functions). The rapidity of disease progression correlates with residual GAA activity; rapid progression in the infantile-onset form that develops before 12 months of age, and mild progression in the adult-onset form that manifests between the early infancy and the sixth decade of life. No curative therapy is presently available for GSD II but there is symptomatic treatment, that is, the GAA supplementation therapy provided by rhGAA only reduces accumulated glycogen or suppresses its accumulation, thereby possibly leading to improvements in body functions and the suppression of disease progression. Based on the clinical experience with rhGAA use in the infantile-onset GSD II patients, the maximum effect can be expected when this therapy is initiated at an early stage of disease progression. Also, based on experience with its use in a limited number of patients, rhGAA is expected to be effective even in those in the terminal stage of adult-onset GSD II.

PMDA asked the applicant to explain the types of target patients for rhGAA in light of the fact that a foreign phase II study (AGLU02804, non-blinded study, 5 patients) is the only study to date involving adult-onset GSD II patients, that a post-marketing placebo-control study (AGLU02704) is currently ongoing, and that in both the EU and the US, the efficacy and safety of rhGAA in adult-onset GSD II patients are considered to not yet be established.

The applicant responded as follows. In adult-onset GSD II patients, clinical symptoms progress at a relatively slow rate, and therefore earlier initiation of enzyme supplementation therapy is considered to bring about greater clinical improvement. At the time of regulatory submissions in the EU and the US, a clinical study was still underway in patients with adult-onset GSD II, and therefore the package insert describes the efficacy and safety of rhGAA as not being established in adult-onset GSD II. However, based on the outcomes of the currently ongoing study, Study AGLU02704, this description is subject to change. In this Japanese application, the interim report from Study ALGU02804 was submitted as evaluation data and the study data from the Japanese EAP (AGLU02603-Japan) was submitted as reference data. However, such study results cannot be taken as definitive evidence of the efficacy and safety of rhGAA in adult-onset GSD patients. Therefore, to call attention, the package insert will carry a statement that the efficacy and safety of rhGAA have not yet been established in adult-onset GSD II patients.

Considering that neither the efficacy nor the safety of rhGAA was confirmed in GSD II patients (patients with cardiac failure with ejection fractions below 40%, serious organic disease, and severe congenital anomaly), who meet study exclusion criteria in clinical studies, PMDA asked the applicant to explain the usefulness of rhGAA in these patient populations.

The applicant responded as follows. Such exclusion criteria were established in order to exclude

any confounders that might affect outcomes of the clinical study. However, in GSD II, induction of glycogen accumulation due to GAA deficiency is a direct cause of the disease, such that, even in those patients excluded from clinical studies, to replenish the missing enzyme is expected to inhibit progression or improve disease conditions in GSD II. Accordingly, although the efficacy and safety of rhGAA in GSD II patients have not as yet been confirmed, a careful judgment can be made as to treatment with rhGAA in light of adequate observation of patients' disease status.

PMDA considers it appropriate that the applicant presented the intention to call attention regarding the efficacy and safety of rhGAA not yet being established. Although PMDA considers that, in patients with serious disease symptoms, it may be reasonable to expect some improvement with rhGAA treatment, the risk-benefit profile has not been fully assessed at present. Thus, PMDA advocates that whether to treat patients with serious disease symptoms with rhGAA be judged based on the risk-benefit assessment by patient. Since, except for enzyme supplementation therapy, only symptomatic therapy is available (Kishnani PS et al., Pompe disease diagnosis and management guideline. *Genetics in Medicine* 2006;8: 267-288), and guidelines for GSD II therapy are not available in Japan, PMDA considers it to be desirable, in the future, to develop a system for implementing appropriate therapies for GSD II patients, and also to establish treatment guidelines in collaboration with the academic sector.

(2) Efficacy

a. Differences between domestic and overseas disease status

PMDA asked the applicant to explain the possible differences in the efficacy and safety of rhGAA between Japan and other countries in light of differences between domestic and overseas GSD II therapies, because the applicant submitted this application with only the data from foreign clinical studies, instead of conducting domestic studies.

The applicant responded as follows. In accordance with the diagnostic criteria for lysosomal diseases as defined by the task force for the specified disease study by the Ministry of Health, Labour and Welfare, the diagnostic criteria for GSD II are: i) to observe a remarkable decrease in enzyme activity or to detect deficiency of proteins, which is etiologically responsible for the desease, by the biochemical test, or to recognize mutations in the genes responsible for the disease by genetic tests, ii) to present the symptoms specific to this disease and, for reference purposes, to confirm accumulation in biopsy tissue using biochemical or morphological testing. It is conceivable that these diagnostic criteria do not differ between Japan and other countries. As to clinical symptoms, in Japan, the EU, and the US, most patients follow a terminal course, with death due to cardiac or respiratory failure, after developing cardiomyopathy and systemic muscular weakness. Thus, there are no reports to date that have suggested any racial differences in disease

status. With regard to gene mutations, in the EU and the US, 3 mutations, IVS1-13T→G, 525T deletion and exon 18 deletion, occur at a high frequency; however, in Japan, 5 mutations, S529V, R600C, D645E, S619R, and R672Q, have been confirmed (Naomi Kanazawa, Seiichi Tujino, *Seitai No Kagaku* 2005; 56: 386-387); and as such, racial differences cannot necessarily be ruled out from a genetic perspective. As the disease emerges and progresses owing to the glycogen accumulation caused by GAA deficiency, despite the possible differences in gene mutations according to ethnic origin, any serious clinical issues are unlikely to arise regarding the efficacy and safety in GSD II patients who receive replenishment of enzyme deficiencies with rhGAA. With the aforementioned discussions, there are considered to be no racial differences in the diagnosis and clinical symptoms of GSD II, and rhGAA can be expected to exert the same therapeutic effects. In addition, since there are no known examples of racial differences in enzyme supplementation therapy in other disease areas, the safety of rhGAA in Japanese patients is considered to be assessable based on the study data from foreign clinical studies. Although a strict comparison of the study data is not possible, the results were compared, to the extent possible, between AGLU01702 and AGLU02203-Japan, both of which have relatively comparable patient backgrounds. The results showed that, in AGLU01702, the number of patients free of non-invasive or invasive ventilatory support was 9 at the start of treatment, and 5 at Week 52. Furthermore, in AGLU02203-Japan, 4 of 5 patients were free of non-invasive or invasive ventilatory support at the start of treatment, and 2 of 5 patients were free of ventilatory support at the time of interim reporting. Regarding the assessment of left ventricular hypertrophy, in AGLU01702, 87% of patients experienced LVMI decreases and, at Week 52, showed a mean decrease of 48% from the baseline. In AGLU02203-Japan, LVMI was decreased in 4 patients, excluding 1 with no pre-treatment test data. When evaluated using the Pompe PEDI, in AGLU01702, most patients showed improvement in functional skills during the treatment, and, in AGLU02203-Japan, 3 showed significant improvements in functional skills, while the other 2 showed no improvement. As to physical development (height and body weight), in AGLU01702, most patients showed sustained or improved development. In AGLU02203-Japan, most patients were noted for height and body weight increases although the improvements were not significant except 1 (subject No. 178778) whose height increased. Furthermore, in terms of safety, there were no major differences in the incidences of adverse events. In AGLU02203-Japan, 10 adverse drug reactions were noted (malaise in 3; oxygen saturation decreased, asthma, nausea, pyrexia, headache, urticaria papular, flushing in 1 each), and, with the exclusion of malaise, asthma and nausea, the other adverse drug reactions were also observed in AGLU01702. In nearly all patients who showed IgG antibody positivity, this manifestation was seen by Week 12. With the aforementioned discussions, there are considered to be no significant differences between Japan and other countries in the efficacy or safety of this treatment in infantile-onset GSD II patients.

PMDA consideres that, although there may be differences in the types of gene mutations between Japan and other countries, a definitive conclusion is still down the road when taking into account the different research circumstances regarding this disease. However, since GAA deficiency causes GSD II, PMDA accepted the applicant's response that the disease conditions of GSD II are similar between Japan and other countries. PMDA has determined that it is possible to assess the efficacy of rhGAA based on the foreign clinical data because this drug is used for enzyme supplementation therapy, and, in this sense, its action to replenish insufficient enzyme levels should not differ between Japanese and other populations.

b. Efficacy endpoint

PMDA asked the applicant to explain the relationship between the efficacy endpoints used in the clinical studies and the true endpoint, i.e., life prognosis, since foreign clinical studies used different efficacy assessment parameters.

The applicant responded as follows. Although no publications are available on the relationship between efficacy endpoints and survival rates, the analytical results from the plural covariances and death risk in the Natural History Study (AGLU-004-00), comparing the death rate in patients up to 6 months old who had documented cardiac hypertrophy, muscular weakness (or hypotonia), respiratory distress or failure to thrive, with the death rate in patients more than 6 months old (including unknown cases), showed the relative risk of death rates to have increased from 1.45 to 2.53 times. It was thus inferred that, given the development of these symptoms being delayed by 1 month, the relative risk of death rate decreased by 0.84 to 0.95 fold. Accordingly, to estimate the life prognosis in GSD II patients, the following parameters on cardiac hypertrophy, muscular weakness, respiratory distress, or failure to thrive are considered important: LVM and LVMI (assessment factors for cardiac hypertrophy), the manual muscle, the hand-held dynamometer, and the 6-minute walking tests (muscular weakness or hypotonia), the survival rate without ventilatory support and the forced vital capacity (respiratory function), and changes in height and body weight (failure to thrive).

As glycogen accumulation displays a variety of clinical symptoms in GSD II, and severity differs among patients, PMDA recognizes that, with universal efficacy endpoints being difficult to establish, there are no established assessment methods at present. Despite the problems with the study design, such as in regard to setting up a control group and employing a non-blinding method, and the limitation in the number of subjects, PMDA considers that the efficacy of rhGAA was suggested by the survival rate results obtained from Study AGLU01602.

(3) Rationale for dosage and administration

PMDA asked the applicant to explain the reason and rationale for the Japanese recommended dosage, i.e., 20 mg/kg, since the dosage regimen differs among clinical studies.

The applicant responded as follows. (a) In two non-clinical studies with GAA KO mice designed to evaluate repeated administration dosages, Studies SN-009 and SN-012 (4.2.1.7 and 4.2.1.8), comparing the dose increase between the 20 mg/kg group and the 40 mg/kg group, a decrease in glycogen was observed in the heart, hindpaws, and diaphragm, and, in Study SN-012 (4.2.1.8), the 2 results from administering identical total doses every week and every other week showed the same effects. (b) In Study AGLU01602, making a comparison between the 20 mg/kg and 40 mg/kg groups, there were no differences in efficacy or serious adverse events, but the number of adverse events was greater in the 40 mg/kg group. (c) When infantile-onset GSD II patients, and adult-onset GSD II patients, though few in number, were treated with rhGAA 20 mg/kg every other week, the efficacy and safety of rhGAA were confirmed. In accordance with these results, a dosage of 20 mg/kg every other week was established.

Although, at submission, increasing the dosage in accordance with patient symptoms was specified, the discription was removed because setting clear criteria for dose increase was not possible, judging from the clinical study data. Also, since there are no clinical study results reported as overdosage even in subjects with consistent and continuous administration of a fixed dose, such a fixed dose regimen is presently regarded as appropriate. Moreover, the rate of intravenous infusion is specified based on the experience with the use of gene recombinant human protein products approved in Japan (Cerezyme®, Aldurazyme®, and Fabrazyme®).

PMDA considers as follows. Clear criteria for dose increase were not predetermined even in the study that allowed a dose increase in accordance with patient symptoms, the rationale and significance of the dose increase were not evident even in Study AGLU1702 with only 2 patients, for whom the dose was increased from 20 mg/kg to 40 mg/kg, and dose increase is not permitted in the EU or the US. Therefore, the applicant's response is appropriate that the dose increase in accordance with patient symptoms should not be specified for dosage and administration. On the other hand, the applicant asserts that the different dosing interval with identical overall total dose resulted in the same decrease in glycogen content in a target tissue (a comparison between the 40 mg/kg every other week and 20 mg/kg every week dosages [Study SN-012, 4.2.1.8)), while an identical glycogen-reducing effect was noted in cardiac muscle even with different dosing intervals and total doses (a comparison between the 20 mg/kg every week × 4 group [Study SN-009, 4.2.1.7] and the 20 mg/kg every week × 8 group [Study SN-007, 4.2.1.3]). Furthermore, the applicant comparatively discusses results from different studies as if they had been obtained from the same study. Therefore, the applicant's discussion to define the dosage regimen lacks consistency.

However, taking into account that GSD II is a fatal disease, the number of patients is very limited in Japan, and that conducting domestic clinical studies is difficult, the applicant's proposal to specify the optimal dose as 20 mg/kg from the data of foreign clinical studies is reasonable to a certain extent.

(4) Safety

a. Effect of specific antibody

PMDA asked the applicant to explain the effects of the frequently observed specific antibody (anti-rhGAA IgG antibody) on the efficacy and safety of rhGAA.

The applicant responded as follows. As formation of anti-rhGAA IgG antibody was observed in almost all patients in Study AGLU01602 and Study AGLU01702, it is difficult to investigate the presence of the anti-rhGAA IgG antibody and its effect on efficacy and safety, and to investigate their relationships with the background factors of patients. In a clinical study, anti-rhGAA IgG antibody was generated within 3 months after treatment, and, after reaching a maximum titer, showed a decreasing tendency. In Study AGLU01602, as compared to patients in the 20 mg/kg group, patients with high antibody titers (6 of 7 patients had antibody titers exceeding 25,600 by Week 24) were observed in the 40 mg/kg group. Gaucher's disease and Fabry's disease patients who are receiving enzyme supplementation therapy reportedly have natural immunologic tolerance (Goodnow CC et al., *Science* 1990; 248: 1373-9, Richards SM *Clin Appl Immun Rev* 2002; 2: 241, Richards SM et al., *Blood* 1993; 82: 1402-9). This suggests that, with continuous exposure to an antigen, the immune system ceases producing the antibody. Therefore, the US Genzyme plans a clinical study to examine the characteristics of the patient group exhibiting immunologic tolerance, the patients' genotypes versus α-glucosidase protein content, and the presence and the level of the antibody.

PMDA considers the effects of antibody formation on the efficacy and safety of rhGAA to await sufficient study and this is not yet evident. PMDA considers it necessary, when new information is obtained from the foreign study now being planned, that it be promptly forwarded to healthcare professionals. Furthermore, such feedback information must be confirmed in a post-marketing surveillance.

b. Infusion-Associated Reaction (IAR)

PMDA asked the applicant to explain the risk factors for IAR and the pertinent preventive measures, as IAR occurred frequently as adverse events during rhGAA treatment. PMDA also asked for the applicant's view on the risks and benefits of rhGAA treatment as related to IAR, as there were patients who experienced IAR repeatedly despite prophylactic measures being taken.

The applicant responded as follows. According to the clinical data obtained 2 years 1 month and 2 years 3 months after the start of the study from the 2 foreign studies of infantile-onset GSD II patients (AGLU01602, 18 patients; AGLU01702, 21 patients), as at least 1 IAR was reported in 20 of 39 patients (51.3 %), and the first onset in half of these patients was within 15 weeks (0 to 66.7 weeks), IAR is considered to develop at an early time after the initiation of rhGAA treatment. In addition, patients with concurrent cardiac hypertrophy have the high risk of developing acute cardiopulmonary failure that is probably associated with fluid overload. Furthermore, patients with acute diseases at the time of rhGAA treatment are likely to be more susceptible to IAR due to the increased immune system response. For this reason, sufficient attention should be paid to the clinical status of these patients. In order to avoid risks, it is necessary to fully observe the clinical status of patients, and, starting with a slow rate of intravenous infusion of rhGAA, paying careful attention to the possible onset of IAR to raise the rate of intravenous infusion. Once a patient shows IAR, the rate of drip infusion should be slowed, or treatment should be discontinued temporarily until the symptoms disappear, and, if necessary, steroids should be administered. In resuming treatment after the cessation of symptoms, it is necessary to begin injection with a rate reduced to one half of that at the occurrence of IAR, and, after administration for 30 minutes, to increase the injection rate by 50% every 15 to 30 minutes. In the next administration and thereafter, it is also necessary to administer antihistamines, or antipyretics and analgesics, etc. as premedication 30 to 60 minutes before intravenous infusion of rhGAA. Also, in order to avoid the risks of IAR, the applicant plans to improve descriptions in the package insert of rhGAA to call attention by, for example, defining patients with high risk of IAR, approaches to patients developing IAR and prophylactic procedures, as well as providing emergency measures for the occurrence of anaphylactic shock. Moreover, rhGAA induces enzyme activity to a certain level in patients with any form of GSD II disease, and, therefore, some improvement in clinical conditions such as heart, respiratory and skeletal muscle functions, or some prevention of disease progression would be expected, which is considered to prolong survival time. Therefore, given such measures as prophylaxis using DPH as premedication, and, in the case of IAR, slowing of the drip infusion rate, temporary discontinuation of rhGAA, administration of steroids, etc., patients who experience IAR repeatedly can be treated with rhGAA with the benefit of rhGAA therapy outweighing the risk of IAR.

PMDA advocates that rhGAA be administered under the strict management of a physician, because, in the event of IAR, depending on the clinical situation, it may become necessary to predose with antipyretics and analgesics as well as antihistamines to reduce the injection rate, and to discontinue treatment temporarily, and so forth. As a great number of serious adverse events are reported in clinical studies, although causal relationships to rhGAA have been ruled out, rhGAA is a drug

intended for long-term therapy, and the number of patients in clinical studies is extremely limited, PMDA considers it necessary to confirm, in a post-marketing surveillance, risk management of IAR, cases of discontinuation and resumption of the use of rhGAA, and the incidence of overdoses.

(5) Post-marketing surveillance

PMDA requested the applicant's view on the possibility of participating in the registration system for foreign studies in GSD II patients, thus allowing the planned post-marketing surveillance in Japan to be conducted as part of the international study.

The applicant responded as follows. Currently, under the international program "POMPE Registry," that includes all GSD II patients regardless of the use of rhGAA, a survey is underway to collect data on disease status, progression, and therapeutic effects of all treatments given for GSD II. Although Japanese physicians can participate in registration for the "POMPE Registry," the targets and purposes are different from those of post-marketing surveillance, such that, it would be difficult to perform a post-marketing surveillance as part of the international survey. Accordingly, as post-marketing surveillance, it is considered necessary to collect all information on patients who are treated with rhGAA in Japan and to appropriately feedback the results.

PMDA asked for the applicant's view on the necessity of making rhGAA available only to physicians who are knowledgeable about GSD II, because experience with rhGAA use in Japanese patients is very limited.

The applicant responded as follows. Most patients who developed the disease in infancy or childhood are cared for at pediatric facilities. Furthermore, although patients who developed the disease after childhood are also cared for, depending on their predominant symptoms, at cardiovascular or respiratory medicine department, while some are treated, because of the associated motor dysfunction, at neurology and internal medicine departments. rhGAA will be used by physicians experienced with rhGAA treatment, or under their guidance, and in pediatric departments, physicians or medical institutions treating many lysosomal disease patients will play a central role in the treatment of this disease. In the case of adult-onset patients, with progression of the disease, many patients lose the ability to walk on their own, and depending on their circumstances, it is not always possible to continue therapy under the supervision of experienced physicians and institutions. Therefore, after launching rhGAA, it will be necessary to develop a network with a core of experienced physicians to establish a system that provides appropriate advice on the use of rhGAA and suitable medications while minimizing the burden on patients.

Because, at this time, basic information on GSD II itself and differences between Japan and other

countries are poorly understood, and the number of domestic patients is very limited, PMDA considers it necessary to collect more information by participating in the international program, aiming to collect basic information on this disease and seek appropriate therapy. Considering the necessity of establishing a network as proposed by the applicant, with physicians experienced in managing this disease serving as a core, in collaboration with academic societies, companies, and patient groups, and thereby developing a system that can provide appropriate advice on the therapy for this disease and rhGAA treatment, PMDA accepted the applicant's above response.

III. Results of Compliance Review Concerning the Documents Appended to the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion of the results of document compliance review and GCP on-site inspection

A document compliance review was conducted, and as there were no major problems that may have affected the reliability or evaluation of the data, PMDA concluded that there should be no problem with conducting a regulatory review based on the application dossier.

In addition, no on-site GCP inspection is conducted.

IV. Overall Evaluation

PMDA considers that the efficacy of rhGAA for GSD II is suggested by the assessed survival rate data in Study AGLU01602. However, this safety evaluation has its own limits because the total number of patients treated with rhGAA in the EAP in Japan was only 10 (5 infantile-onset and 5 adult-onset patients). The influence of antibody formation on the efficacy and safety of rhGAA is unclear at present, and IAR reportedly occurs in a large number of patients. Therefore, as the need arises, it is necessary to predose patients with antipyretics, analgesics, and antihistamines, to reduce the infusion rate, and to discontinue treatment temporarily. Thus, PMDA advocates rhGAA therapy being conducted under physicians' strict management. PMDA considers it necessary to confirm the effect and safety of rhGAA in post-marketing surveillance with registration of all patients treated with rhGAA.

Among orphan diseases, the number of GSD II patients is extremely limited, and, due to the fatal nature, the seriousness, etc. of this disease, a new drug application based on foreign clinical studies was regarded as appropriate by the 4th Investigational Committee for Usage of Unapproved Drugs (April 2006). PMDA considers it significant to approve rhGAA, because, at present, the only

available treatment for GSD II is symptomatic therapy and treatment aimed at replenishing a deficient enzyme in patients is awaited as the only therapy in line with disease status. Since the number of patients receiving rhGAA is very small, information on its efficacy and safety is presently limited. Therefore, PMDA considers that rhGAA may be approved with the assumption that, taking into account the evaluation of the risk-benefit profile on each patient, rhGAA should be made available only under the management of physicians who are knowledgeable about GSD II. However, the final decision should be made after receiving the comments made during the Expert Discussion.

Review Report (2)

January 19, 2007

1. Product Submitted for Registration

［Brand name］	Myozyme 50 mg for intravenous drip infusion
［Non-proprietary name］	Alglucosidase Alfa (Genetical Recombination)
［Applicant］	Genzyme Japan K.K.
［Date of application］	June 30, 2006

2. Content of the Review

The members of the Expert Discussion supported the judgment from the Pharmaceuticals and Medical Devices Agency (PMDA) that rhGAA may be approved on the grounds that type II glycogen storage disease (GSD II) is a progressive disease; at present, no effective therapy is available and the introduction of rhGAA is thus a pressing need as it provides a fundamental therapy; and rhGAA should be made available only under the management of physicians who are knowledgeable about this disease, based on a risk-benefit evaluation for each patient.

PMDA, based on discussions by the Expert Discussion, took necessary actions including other additional issues.

(1) Specifications

PMDA had requested that, as to identification of the sugar chain structure, the peak area in a single sample be specified urgently as the relative peak area ratio, the same specification employed in the US and Canada [See Review Report (1) "2. Quality data, *Outline of the review by PMDA,* 3) Specifications"].

The applicant responded that the relative peak area ratio specification is to be established based on the allowance calculated from the analytical results of all available manufactured samples to date (160-L scale drug substance [redacted] lots, 2000-L scale drug substance [redacted] lots).

Also, PMDA requested that, as the specification for the content of *N*-glycolylneuraminic acid (NGNA) is being considered in the US and Canada, the same measures also be taken in Japan [See the above].

The applicant responded that, once the specification for the content of NGNA is established in the US and Canada, the same measure will be taken in Japan. PMDA accepted this response.

(2) Post-marketing surveillance

Regarding Infusion-Associated Reaction (IAR), it must be taken into account that a large number of events are reported in clinical studies, rhGAA is a drug for long-term use, and the number of patients in these clinical studies is extremely limited. Therefore, PMDA considers it necessary in a post-marketing surveillance to confirm the risk management of IAR and cases of discontinuation and resumption of the use of rhGAA after the occurrence of IAR. Because the effects of antibody formation on the efficacy and safety of rhGAA are uncertain at present, PMDA considers it necessary that, when additional information is obtained from the foreign study (currently in the planning stage), the feedback should be promptly provided to healthcare professionals, and such information should be confirmed in the post-marketing surveillance. This determination of PMDA was supported by members of the Expert Discussion. Moreover, the Expert Discussion pointed out that rhGAA has the potential to induce an allergic reaction when administered repeatedly, and GSD II is categorized into three forms: infant-, adult-onset forms and, as an intermediate type, a juvenile-onset form, so that, in performing post-marketing surveillance, this classification must be taken into account. Based on the above, PMDA instructed the applicant to submit a draft plan to conduct the post-marketing surveillance, reflecting the aforementioned contents.

The applicant, presenting the draft plan for the post-marketing surveillance, replied as follows. The information on “risk management of IAR, discontinuation and resumption of administration of rhGAA after IAR occurrence” is to be provided as “Guidance for the Management of IAR” in the written procedures for rhGAA preparation to be distributed to medical institutions. As to post-marketing surveillance, a Specified Drug Use Investigation is to be conducted to evaluate the safety and efficacy in all patients receiving long-term administration. Assuming the observation period to be 1 to 9 years for each patient, background factors and clinical symptoms of each patient are to be surveyed. Regarding the safety, the following are to be assessed: (a) occurrence status of adverse drug reactions and infections, (b) possible factors impacting the safety (kinds and incidence of adverse drug reactions according to factors, such as gender, disease type, genotype, GAA activity, age at onset, age at the first treatment, dose at the first treatment, concomitant therapy), (c) relationship between the treatment period (dosage) and the incidence of adverse drug reactions, (d) relationship between antibody formation and the occurrence of IAR. Also, in terms of the efficacy, overall improvement with long-term use of rhGAA shall be assessed based on clinical symptoms and test results. With regard to “effects of antibody formation on the efficacy and safety,” a survey will be performed, with maximum effort, by measuring the specific IgG antibody to rhGAA every three months. The survey will be performed based on the 3 disease categories (infantile-, juvenile- and adult-onset) in the clinical questionnaire for the specified disease (lysosomal disease).

Since there are many uncertain points at present regarding basic information on this disease itself,

and the number of patients in Japan is extremely limited, PMDA, in the hope of exploring appropriate therapy, accepted the above applicant's response with instructions requiring information collection, albeit minimal, from the post-marketing surveillance and the international program "POMPE Registry" led by the US Genzyme. Upon obtaining new information, prompt feedback is to be provided to healthcare professionals.

(3) Establishment of an appropriate therapeutic system

The Expert Discussion supported the necessity of developing a network consisting of a core of physicians who are highly knowledgeable about the disease, and to establish a system that can provide suitable advice. Moreover, the members of the Expert Discussion raised the following issues: as periodic injections are necessary to continue the therapy, it is desirable to provide treatments under the guidance of physicians well versed in this disease without limiting this therapy to the accredited institutions, and it is necessary to collect as much domestic information as possible and exchange the information with counterparts overseas.

PMDA asked the applicant to explain its assertions as to "the establishment of a network with core physicians experienced in GSD II therapy, which can advise appropriately about the therapy including rhGAA treatment."

The applicant responded as follows. By organizing the Pompe Expert Panel, a group of physicians who have a thorough knowledge and treatment experience of GSD II, a system with the Pompe Expert Panel as the core will be established that can advise appropriately about the treatment of this disease including administration of rhGAA. To physicians in the Pompe Expert Panel, the data from the phase IV study conducted in the EU and the US, the report of the "Pompe Registry," and data from domestic post-marketing surveillance, etc. will be submitted, and, at the same time, a study concerning therapeutic guidance for GSD II and appropriate use of rhGAA will be contracted out to the Panel. The applicant will periodically set aside time ([redacted] in approximately 1 year) to report the study results to physicians who are conducting this therapy. The applicant is developing a plan, in that, in the future, its Web site will carry information relating to the Pompe Expert Panel, safety information necessary for treatment, and information on testing laboratories that can measure enzyme activity and specific antibody levels and on how to request the procedures from the laboratories, etc. For medical institutions, updated safety information will be delivered by a medical representative (MR), and the following documents will also be developed and provided: a product information summary, an interview form, an instruction manual providing PRECAUTIONS, a manual specific to this disease (including a list of testing laboratories for enzyme activity measurement and contactable physicians) and a written procedure for preparation (including a "Management Manual of IAR"). Furthermore, information will be provided to GSD II

patients in the form of a patient pamphlet (including a list of available physicians).

Compared to other approved enzyme products, larger protein amounts are administered in rhGAA therapy, such that sufficient attention to safety should be paid. Therefore, PMDA considers it necessary that the therapy be performed by physicians experienced in the use of rhGAA or under their guidance. PMDA instructed the applicant to establish a suitable system as quickly as possible, since the proposed system and data collection from the post-marketing surveillance are extremely important, and accepted the above response.

III. Overall Evaluation

As a result of the above review, PMDA has concluded that rhGAA may be approved with the conditions for approval shown below, and after modifying the indications and dosage and administration. As rhGAA is an orphan disease drug, the 10-year re-examination period is appropriate.

rhGAA is regarded as a biological product, and both the drug substance and the product are classified as powerful drugs.

[Indications] Type II glycogen storage disease

Precautions for Indications:
The efficacy and safety of rhGAA have not been established in patients with adult-onset type II glycogen storage disease.

[Dosage and administration] The usual dosage for intravenous drip infusion is 20 mg/kg body weight of Alglucosidase Alfa (Genetical Recombination) once every other week.

[Conditions for approval]
As experience with using rhGAA in Japanese patients is very limited, a drug use investigation should be conducted on an all-case basis until data from an adequate number of patients have been collected after launching rhGAA, in order to comprehend the background factors of patients on rhGAA and to collect data on the safety and efficacy of the product at an early postmarketing stage, and thereby measures required for appropriate use of the product should be taken.