SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Fabrazyme. This scientific discussion has been updated until 1 September 2003. For information on changes after this date please refer to module 8B.

1. Introduction

Fabrazyme is a recombinant human α-galactosidase (r-hαGAL), INN: agalsidase beta, which is produced by genetically engineered Chinese Hamster Ovary (CHO) cells. Recombinant hαGAL is a highly purified recombinant form of the naturally occurring human lysosomal hydrolase enzyme responsible for the metabolism of globotriaosylceramide (ceramide trihexoside; CTH; GL-3). The rationale for enzyme replacement therapy is to restore a level of enzymatic activity sufficient to hydrolyse the accumulated substrate. After administration, agalsidase beta is rapidly removed from the circulation and taken up by vascular endothelial and parenchymal cells into lysosomes, likely through the mannose-6-phosphate, mannose and asialoglycoprotein receptors. The proposed indication of Fabrazyme is long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry disease.

Fabry Disease is a rare X-linked recessive glycosphingolipid storage disorder that is caused by deficient activity – subnormal or absent - of the lysosomal enzyme, α-galactosidase A. It is an extremely rare disorder, with an estimated prevalence of 500 to 1000 patients within the EU.

The absence of the enzyme leads to progressive accumulation of neutral glycosphingolipids, predominantly ceramide trihexoside (CTH), in most tissues and cell types, particularly the vascular endothelial and smooth-muscle cells leading to ischaemia and infarction. Fabry Disease is a heterogeneous multisystemic disorder with variable onset of symptoms affecting the nervous system, kidneys, heart, skin and gastrointestinal system. There are also atypical variants for which either renal or cardiac involvement is the only problem. It is not possible to predict the phenotype based on current knowledge of the different genotypes described for this disease. The presentation of the disease is highly heterogeneous. Early manifestations in childhood or adolescense may include crises of excruciating pain in the extremities, angiokeratoma, hypohydrosis and corneal and lenticular opacities. In the second or third decade of life, early renal accumulation of glycosphingolipids has been observed throughout the renal vessels and glomeruli, resulting on the onset of renal insufficiency. Premature death usually occurs in the fourth or fifth decade of life and results from renal, cardiac, or cerebrovascular complications. Approximately one-third of heterozygous female carriers of Fabry Disease ultimately become symptomatic. At present there is no specific curative treatment for the condition and patient management is limited to symptom control and supportive measures.

Fabrazyme treatment should be supervised by a physician experienced in the management of patients with Fabry disease or other inherited metabolic diseases. The recommended dose of Fabrazyme is 1 mg/kg body weight, administered once every 2 weeks, administered by intravenous infusion. Fabrazyme is for intravenous use in adults. No studies in special patient populations including children (0-16 years), in patients older than 65 years and those with hepatic impairment have been performed and no dosage regimen can presently be recommended in these patients. No dose adjustment is necessary for patients with renal insufficiency.

2. Chemical, pharmaceutical and biological aspects

Composition

Fabrazyme is a lyophilised sterile dosage form. The active substance agalsidase beta is produced by recombinant DNA technology. The finished product is supplied as powder for concentrate for solution for infusion that should be reconstituted and diluted prior to infusion.

One 20ml glass vial of Fabrazyme contains 35 mg of agalsidase beta as active ingredient (5 mg/ml after reconstitution with the appropriate volume of water for injections. The formulation used in the pivotal clinical trials is equivalent to the commercial formulation. Initial clinical trial material was

produced as a lower volume fill. In addition, the active ingredient of this clinical trial material was produced at a lower fermentation scale. Biochemical equivalence between the material produced at the different scales has been demonstrated.

Development studies with the excipients showed a stable formulation with good cake properties. The excipient mannitol is a typical lyoprotectant and tonicity agent. The lyophilisation cycle initially developed for the phase 1 / 2 clinical trial material was optimised for the commercial product.

The compatibility of r-hαGAL with the 0.9% NaCl infusion solution and typical infusion bags has been verified.

Active substance

Introduction

R-hαGAL is a non-covalently-linked homodimer with an approximate molecular weight of 100 kDa. The theoretical mass of the monomeric peptide is 45,349 Da (excluding the mass of the carbohydrate chains). The full-length cDNA for each subunit encodes a polypeptide of 429 amino acids. The mature r-hαGAL monomer is composed of 398 amino acids with three N-linked glycosylation sites at asparagines 108, 161 and 184, with the predominant carbohydrate structures at these sites being fucosylated biantennary bisialylated complex, monophosphorylated mannose[7] oligomannose, and biphosphorylated mannose[7] oligomannose, respectively.

Specifications and routine tests

Each batch of active ingredient is tested for identity, purity, and potency. The specifications according to which the tests are performed are justified by batch data as well as data obtained for batches used in preclinical studies and clinical trials.

All routine methods used as control or release tests of starting materials, process intermediate, drug product and stability samples were validated when appropriate.

No compendial reference standard of r-hαGAL is currently available. The company has developed its own reference standards for product testing purposes. The preparation, storage conditions, characterisation and periodic re-evaluation of the primary reference standard has sufficiently been described.

Development Genetics and Cell Bank System

Agalsidase beta is produced by mammalian cell culture using a Chinese Hamster Ovary (CHO)cell line co transfected with a recombinant plasmid containing DNA sequences encoding the α-galactosidase protein. A stable subcloned sell line expressing α-galactosidase was used to prepare a master cell bank (MCB). The MCB has been fully characterised, as were the WCB and EOP (end of production) cells. Results were typical for a cell substrate of Chinese Hamster origin. There was no evidence of fungal, bacterial or mycoplasmal contamination. No viral particles apart from A- and C-type particles were detected. A battery of in vitro and in vivo tests were negative for infective endogenous or adventitious viruses, including infective retroviruses.

Fermentation and Harvesting

Production of the active ingredient is performed by Genzyme, Framingham, Massachusetts, USA. The protein is produced by a continuous harvest fermentation using cells from the Working Cell Bank (WCB) which is derived from the MCB.

Comprehensive details on fermentation conditions, raw materials, growth media used in cell culture and in-process controls were provided. All materials of animal origin comply with the CPMP note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products. Validation data for fermentation lots were provided.

Harvests are stored in dedicated containers until further processing. The suitability of these holding conditions has been confirmed.

Purification

Multiple days of harvest meeting specifications are collected from the bioreactor and after filtration loaded onto the first column. Further purification by 4 column chromatography steps, utilising several

modes of separation, takes place before the product is being concentrated, diafiltered and diluted to a target concentration. Full details on reagents, column sanitisation and qualification have been provided. Storage times and conditions of the intermediates have been validated satisfactorily. In process control testing is conducted on all column eluates.

Characterisation

The company to investigate the primary, secondary and tertiary structure and post-translational modifications has performed comprehensive structural analysis of agalsidase beta. The amino acid sequence of agalsidase beta was proven to be identical to human α-galactosidase.

No significant differences were observed between the three r-hαGAL drug substance lots and the reference standard in any of the tests performed.

Process validation

Three consecutive cell culture runs including cell thawing, inoculum preparation and the subsequent culturing of cells using two bioreactors validated the cell culture process. Three consecutive runs including clarification, purification and formulation to produce the formulated drug substance validated the purification process.

Cleaning processes of the different columns and ultrafiltration/diafiltration system are appropriately validated. Validation of the nanofiltration step, which was added as an additional virus removal step, comprises verification of the physical parameters of the filtration process by measuring integrity before and after filter use, filtration pressure differentials throughout the process and flux rates throughout the process for three consecutive runs.

Other ingredients

Excipients

The excipients in the finished product are mannitol, sodium phosphate buffer, and water for injection and nitrogen gas. There are no Ph. Eur. monographs for the sodium salts used in the phosphate buffer. However, the company provides assurance that these salts are tested to meet Ph. Eur. specifications for comparable sodium phosphate salts with different degrees of hydration. The other excipients are subject to USP/Ph.Eur. test specifications.

Packaging material

Type I glass lyophilisation vials of 20 ml capacity manufactured from borosilicate glass tubing and sulphur treated are used to strengthen the glass and limit potential protein adsorption. Butyl lyophilisation stoppers that have been siliconised close the vials and the stoppers are crimped to the vials by an aluminium seal that has a plastic 'flip off' button. During the Pharmaceutical Development studies, bacteriological tests and sterility tests were carried out to confirm the integrity of the closure system. The packaging components comply with Ph. Eur. requirements.

Product development and finished product

Method of Preparation

r-hαGAL active substance is formulated to 5.0 mg r-hαGAL per ml by the addition of mannitol to a final concentration of 3% (w/w). This formulated drug substance is 0.22 µm filtered into a sterilised vessel. Sterile filtration, filling and lyophilisation takes place at Genzyme's Allston Landing Facility.Vials are aseptically filled with a target weight. After filling, the vials are partially stoppered and subsequently loaded to the lyophiliser. Filled vials are capped, stored under quarantine at 2-8 ^{o}C and subjected twice to a visual inspection. Finally, the vials are labelled and packaged.The manufacturing process has extensively been validated and comprised validation of the shipping vessel,

filters used, container closure and package integrity, sterilisation and depyrogenation processes, and validation of formulation, sterile filtration and lyophilisation.

Control tests on the intermediate and finished product

Acceptable tests and specifications have been defined for routine release of the intermediate and finished product.

The company has demonstrated that the assays validated for the active substance are also suitable for this testing.

Stability of the finished product

Three batches of phase 3 finished product were placed in stability at 2-8 °C. In addition, five lots manufactured using the proposed commercial manufacturing process were placed on stability. The stability studies for these lots are ongoing through 36 months. The results indicate that the r-hαGAL finished product is stable for 12- 18 months when stored at 2-8 °C.

Accelerated stability data did not show any significant decrease in purity or specific activity. All measured parameters remained within the specified limits.

The stability of the finished product is monitored in real-time studies, which are still ongoing. In the light of these results, a provisional shelf life of 18 months at 2-8°C is acceptable.

Reconstituted medicinal product

Three lots of Fabrazyme were evaluated for stability after reconstitution. Vials were reconstituted with 7.2 ml of water for injections and stored at 2-8°C or 25°C for 96 hours. All measured parameters remained within the specified limits at both storage conditions.

The proposed storage of reconstituted Fabrazyme for up to 24 hours at 2-8 °C or at 23-27 °C can be accepted.

Reconstituted and diluted medicinal product for infusion

Stability data for three lots show that reconstituted Fabrazyme, diluted for infusion, can be kept for 72 hours at 2-8°C, followed by a six-hour infusion period at room temperature. The proposed storage of Fabrazyme, diluted for infusion with 0.9% sodium chloride for up to 24 hours at 2-8 °C can be accepted.

Virological documentation

The cell bank system was determined to be free of microbial contamination. The company provided satisfactory information to demonstrate that the materials of bovine origin are compliant with the criteria of the TSE guideline. Virus clearance potential has been investigated of the chromatography steps and the nanofiltration step. Full details on the scaled down, viral spiking studies on the four relevant chromatographic steps and the nanofiltration have been provided. Each column chromatography step was evaluated separately with several model viruses. From these data it can be concluded that the combination of the column steps and the nanofiltration, which represent a broad spectrum of chromatographic separation, provides a considerable potential for virus removal. The viral safety of agalsidase beta is sufficiently demonstrated by the extensive test strategy (cell bank system, bioreactor harvest, and biological reagents) for viral contamination and the high capacity of the purification process for virus removal.

Conclusion

Appropriate commitments were made by the company to provide, after authorisation, further information on various parts of the documentation.

3. Toxico-pharmacological aspects

Pharmacodynamics

In vivo studies

Pharmacodynamic effects were evaluated in a mouse model of Fabry disease. These mice have considerable accumulation of globotriaosylceramide (GL-3) in the liver, skin, spleen, heart and kidneys. After treatment with a single low dose of agalsidase beta, the concentration of GL-3 was significantly reduced in liver, heart, spleen and plasma. Furthermore, multiple doses of 0.03 to 3.0 mg/kg provided significant progressive reduction of GL-3 in liver, heart, spleen, skin and kidney (98%, 99%, 83%, 77% and 24% respectively). Agalsidase beta reduced accumulation of GL-3 in a dose and time dependent manner. The reduction was maintained for several weeks post administration.

General pharmacodynamics

Cardiovascular and respiratory toxicity were examined in a cardiac function study in dogs with no indications of any potential safety pharmacological problems. Any further safety pharmacology studies to assess the neurological effects of r-hαGAL were not performed, the argument being that r-hα-galactosidase is a protein and is unlikely to cross the blood-brain barrier. Neurological toxicity is therefore not expected.

Pharmacokinetics

After intravenous injection in mice and rats, r-hαGAL is rapidly cleared from the circulation uptake with distribution predominantly to the liver and to a lesser extent to the spleen and kidneys. Little or no r-hαGAL activity is recovered from the brain, heart, or lung. In rats and dogs clearance is rapid and serum half-lives are short. Elimination of r-hαGAL from serum in rats and dogs followed first order kinetics at lower doses. At higher doses, elimination was biphasic with a relatively slow initial phase and a faster terminal phase. After single dosing in rats and dogs, a less than dose-proportional increase in serum AUC levels was observed.

After repeat dosing for 27 weeks in rats, post-dose serum concentrations were 2 fold higher in week 26 compared to post-dose samples on Day 1. In addition, following 27 weekly doses levels of rh-αGAL in the liver were also increased (2-fold). This can be explained by the fact that at low doses r-hαGAL is eliminated by first order kinetics. At the higher doses however, the elimination follows non-linear kinetics, suggesting a saturable process. At higher doses each successive dose administration adds to the circulating plasma and/or liver concentration of enzyme remaining from the previous dose or doses, resulting in accumulation. While accumulation was observed following 27 weekly injections of high doses of r-hαGAL, there was no clinical or histopathological evidence of liver toxicity in the rats. When body weight and body surface area are taken into account, the high dose administered to rats is 5 times greater than the dose administered to humans. In addition, because the dosing regimen is every 2 weeks in humans as opposed to the every week schedule in the rat, the safety margin in humans for liver accumulation is higher. It is not known whether r-hαGAL distributes to the foetus nor whether it passes into milk and this is reflected in the SPC.

Toxicology

Single dose toxicity

In two acute toxicity studies in rats r-hαGAL was well tolerated at high doses.

In the cardiac function study in dogs, no acute cardiac effects were observed following a single administration at intermediate doses of r-hαGAL. A transient and immediate hypotension was observed in 5 of 6 dogs administered r-hαGAL at a high dose. Heart rate, respiration rate, and central venous pressure were not affected significantly or to the same degree as blood pressure. All parameters were considered normal 40 minutes post-dose. Moreover, cardiovascular effects were not observed in the clinical setting.

Repeated dose studies

There is only one repeated dose toxicity study in the dossier. In this study Sprague Dawley rats were intravenously (bolus) dosed with 3 different doses r-hαGAL weekly for 27 weeks. Severe hypoactivity, with associated cyanosis, laboured breathing and swelling of extremities were observed after dosing in Week 3. These symptoms were consistent with a hypersensitivity response and could be prevented in subsequent weeks by diphenhydramine pre-treatment. There was no mortality or treatment-related findings in clinical pathology parameters, macroscopic or microscopic observations or in organ weights. The antibody response in this study was evaluated using an ELISA method. As could be expected, the majority of animals developed antibodies to r-hαGAL after 12 weekly injections. After an initial increase in antibody titer some animals did not show a detectable antibody titre at Week 24, which is considered likely to be a result of the development of tolerance. There was no obvious relationship between dose and antibody titre or dose and development of tolerance. There was no obvious relationship between antibody titre and dose or antibody titre and sex. The no-observable-adverse-effect-level (NOAEL) for r-hαGAL following diphenhydramine hydrochloride pretreatment was the highest dose administered. Although there is no information on possible masking

of toxic effects by the neutralising capacity of the antibodies and by the pretreatment with diphenhydramine, this was considered acceptable since both factors occur also in the clinical application.

The highest administered dose was equivalent to 5 times (based upon equivalent body surface area) the highest dose of 1 mg/kg administered in the Phase III study and the maximum recommended therapeutic dose. Moreover, dosing in preclinical studies was performed every week and in the clinical situation dosing occurred only once in two weeks.

Genotoxicity

Mutagenicity studies were not performed. This is considered acceptable based on the nature of the compound.

Carcinogenicity

Carcinogenicity studies have not been conducted with r-hαGAL, although the compound is intended for long-term treatment. However, carcinogenic potential is not anticipated based on nature of the substance.

Reproductive and development toxicity studies

No studies on fertility, embryo-foetal development, and pre- and postnatal development were available.

The lack of information is adequately reflected in the SPC. In addition, the 26-week toxicity study in rats did not reveal any disturbance of the reproductive organs. It is also not known whether Fabrazyme is excreted in human milk.

Immunotoxicity studies

The presence and possible effects of antigen/antibody complexes were not directly investigated in the preclinical studies. However, immune complex formation may change renal function and there was no evidence of renal function changes in the repeat dose toxicity study in rats. Furthermore, an evaluation on kidney samples of 13 patients treated with r-hαGAL has been carried out in the pivotal study and there was no significant immune complex deposition present in the renal glomeruli of these patients. In addition, 12-month human safety data do not give raise to concerns with reference to immune deposits i.e. serum sickness and vasculitis.

Information on the long term monitoring for the presence of antibodies to Fabrazyme and their impact on clinical safety and efficacy, as well as any clinical evidence of immune complex disease will be reported as part of the Periodic Safety Update reports.

Local tolerance

No specific test of local irritancy to veins or the subcutaneous tissues has been performed. However, the findings in the repeated dose and other studies have not shown any signs of particular local irritancy to veins, nor has incompatibility with blood been indicated.

Ecotoxicity/Environmental risk assessment

Exposure to the environment is considered very limited and therefore no risk of concern would be expected.

Product interactions

Interaction studies have not been performed, but it was considered acceptable to address this issue in the clinical part. However, the theoretical risk of inhibition of intracellular α-galactosidase activity by chloroquine, amiodarone, benoquin or gentamicin is reflected in section 4.5 of the SPC. Any evidence of product interactions will be reported in the Periodic Safety Update Reports

Discussion on toxico-pharmacological aspects

Overall, pharmacodynamic and pharmacokinetic studies provided adequate evidence for efficacy of agalsidase beta. The repeat dose toxicity of agalsidase beta has been investigated only in the rat. However, given the availability of the 12-month human safety data, this was considered acceptable.

It can be concluded that the package on toxicity data as a whole suffices for this compound, provided that careful clinical observations are made and undertakings fulfilled post-marketing.

4. Clinical aspects

Fabrazyme is indicated for long-term enzyme replacement therapy in patients with Fabry disease, a disease caused by an inherited deficiency in the activity of the lysosomal enzyme α-galactosidase A. The recommended dose for Fabrazyme is 1 mg/kg body weight, to be administered by intravenous infusion over at least 2 hours every other week. Since Fabry disease is a genetic disorder, the replacement therapy is foreseen to be a life long therapy.

The clinical trials were performed according to Good Clinical Practise (GCP) standards and agreed international ethical principles.

Overview of Clinical Trials Programme

Protocol (Country)	Phase	Design	No Pts	IV Dose (mg/kg)	Duration	Status
FB9702-01 (US)	I/II	Supportive, dose-finding, single-centre, safety study	15	5 groups: 0.3, 1.0, 3.0 qow[2] 1.0, 3.0 qod[1]	5 inf.	Completed
AGAL-1-002-98 (US, Europe)	III	Pivotal randomised, double blind, placebo controlled multi-centre study	58	0 or 1 q2w[2]	20 wks	Completed
AGAL-005-99	III	Open label extension study	58	1 q2w[2]	18 months	Ongoing

1. qod = every other day (48 hours)
2. Q2w = every other week

Clinical pharmacology

Pharmacokinetic and pharmacodynamic studies were performed within all three clinical studies.

Pharmacodynamics

In the dose-finding study the effects of 0.3, 1.0 and 3.0 mg/kg every 14 days and 1.0 and 3.0 mg/kg every other day (48 hours) were evaluated. Each regimen was tested in three patients. In the placebo-controlled pivotal phase III study patients were treated with 1 mg/kg every 14 days. Pharmacodynamics was studied by monitoring the concentrations of GL-3 in plasma and urine and by biochemical and histological examinations of pre- and post-treatment biopsy samples of several organs. In addition, changes in physiological function and clinical parameters such as pain and quality of life were employed.

From the dose-finding study, it can be extrapolated that plasma concentrations of GL-3 were cleared rapidly and in a dose-dependent way in the 14 days dosing schedule. Patients showed a reduction in plasma GL-3 concentrations following the second infusion in the 1 mg/kg and 3 mg/kg dose groups, whereas in the 0.3 mg/kg dose group GL-3 did not substantially decrease until the third or fourth infusion. Patients receiving r-hαGAL every 48 hours showed a response from the fourth infusion - after 8 days of treatment.

In the phase I/II dose-finding study all three doses were effective in clearing GL-3 from endothelial vasculature, with the lower dose (0.3 mg/kg) cohort demonstrating less consistent clearance.

In the kidney samples of the Phase I/II study, there was also a general trend for reduction of GL-3 in glomerular mesangial cells and interstitial cells as well as in cells from the tubules and collecting

ducts, which held the greatest accumulation of GL-3. In cardiac tissue, the GL-3 clearance occurred predominantly from the endothelium and to a much lesser extent in cardiomyocytes. In the liver, histology samples demonstrated significant GL-3 clearance in endothelial cells and Kupffer cells.

Treatment with r-hαGAL significantly decreased GL-3 deposits in the vascular endothelium of capillary beds in heart, skin and kidney in the phase III pivotal study. In this study and its ongoing open label extension clearance of GL-3 from various other cell types in the kidney, pathologists in a blinded fashion assessed skin and heart.

Preliminary results from this study demonstrate that clearance occurred in the various parenchymal cells, however, in some cell types clearance was less complete than in the capillary endothelial cells. These data suggest that the effects seen on the deposits seen in the vascular endothelium can be extrapolated to the various parenchymal cells, although the clearance from some parenchymal cells seems slower and a complete clearance in these subtypes of parenchymal cells remains to be demonstrated.

Because the accumulation of sphingolipids is regarded as the cause for the disease and its clinical presentation, the pharmacodynamic results indicate that a clinical improvement or stabilisation among patients is to be expected. However, a concluding positive assessment of efficacy is not possible based on the pharmacodynamic results alone.

Pharmacokinetics

Following an intravenous administration of agalsidase beta at doses of 0.3 mg, 1 mg and 3 mg/kg body weight, the AUC values increased more than dose proportional, due to a decrease in clearance, indicating a saturated clearance. The elimination half-life was dose dependent and ranged from 45 to 100 minutes.

After intravenous administration of agalsidase beta with an infusion time of ca. 300 minutes and at a dose of 1 mg/kg body weight, biweekly, mean C_{max} plasma concentrations ranged from 2000 – 3500 ng/ml, while the AUC_{inf} ranged from 370-780 µg.min/ml. Vss ranged from 0.12-0.57 l/kg, plasma clearance from 1.7-4.9 ml/min/kg and the mean elimination half-life from 80-120 minutes.

Agalsidase beta is a protein and is expected to be metabolically degraded through peptide hydrolysis. Consequently, impaired liver function is not expected to affect the pharmacokinetics of agalsidase beta in a clinically significant way. Renal elimination of agalsidase beta is considered to be a minor pathway for clearance.

The inter-subject variability was explained by normal fluctuation and the small subject population size (n-11). No apparent correlation was found between the AUC and the antibody titre. Leukocytes appeared to accumulate enzyme activity with repeated administration and this might indicate that the uptake of enzyme at the cellular level in the target organs was unaffected by the antibody formation. In addition, the applicant has provided sufficient data to show that the presence of antibodies did not diminish efficacy in any of these subjects (see clinical part).

Interaction studies

Since agalsidase beta is a protein, it is not expected to bind to other proteins and metabolic degradation is expected to follow the pathways of other proteins, i.e. peptide hydrolysis. Agalsidase beta is unlikely to be a candidate for drug-drug interactions - due to the influence of cytochrome P450 enzymes - and neither in vitro interaction studies nor specific in vivo clinical drug interaction studies were therefore carried out. However, there is a theoretical risk of inhibition of intracellular α-galactosidase A activity by chloroquine, amiodarone, benoquin or gentamicin and this is appropriately reflected in section 4.5 of the SPC. Any evidence of product interactions will be reported in the Periodic Safety Update Reports.

Special groups

The influence of renal function on the pharmacokinetics of α-galactosidase was not studied. Renal elimination of α-galactosidase is considered to be a minor pathway for α-galactosidase clearance.

The influence of hepatic function on the pharmacokinetics of α-galactosidase was not studied. As metabolism is expected to occur by peptide hydrolysis, an impaired liver function is not expected to affect the pharmacokinetics of α-galactosidase in a clinically significant way.

Pharmacokinetic measurements in children will be included as a part of a study in children. The lack of data in children is sufficiently reflected in the SPC.

Bioequivalence

Agalsidase beta was produced at various production scales. Comparison of the pharmacokinetics of agalsidase beta produced at two of these scales was performed within the phase I/II study and did not reveal significant differences.

Clinical Efficacy

The studies conducted involved a total of 73 patients with Fabry disease. Fifteen patients were enrolled in a Phase I/II open-label, dose-finding, single centre study and 58 patients in a Phase III, placebo-controlled, randomised, multicentre study and an open label extension study. The clinical trials were performed according to GCP standards and agreed international ethical principles.

Dose response study

The dose finding phase I/II study, FB9702-01, addressed mainly pharmacokinetics and initial safety and efficacy. Patients were enrolled to receive agalsidase beta in one of five treatment regimens (3 patients per group): either 0.3 mg/kg, 1.0 mg/kg or 3.0 mg/kg administered once every 14 days, or 1.0 mg/kg or 3.0 mg/kg administered once every 48 hours, for a total of five infusions. Plasma samples were taken at every infusion, and tissue (skin and liver) biopsy samples were taken at baseline and following the fifth infusion. Heart and kidney biopsies were considered as optional. Patients were exclusively male and aged 16 and older with a confirmed diagnosis of Fabry disease and with largely unaffected kidneys, as determined by clinical and laboratory kidney function parameters.

The study showed that enzyme replacement with agalsidase beta could be administered safely and that it cleared glycosphingolipids accumulated in the vascular endothelium in all organs studied.

Dose finding was based on plasma GL-3 levels. Plasma GL-3 levels decreased in a dose-dependent manner for all three dose levels when the 14 day dosing schedule was used. This is compatible with studies in the knock-out mice. With regard to the effect on the tissue deposit, however, the dose dependency was less clear due to the short duration of the study. The selection of the dose 1 mg/kg for the phase III study was based on the most favourable benefit-risk ratio for patients as assessed in the Phase I/II trial. The optimal dose in the long term, especially maintenance dosing – after clearance of the accumulated sphingolipids – will be further explored, as appropriate.

Main clinical studies

Description of the study

The second study (AGAL-1-002-98) was a double-blind, placebo-controlled, randomised multi-center study (US and Europe) enrolling 58 patients for evaluation of safety and efficacy of r-hαGAL administered at a dose of 1 mg/kg every 14 days for 20 weeks (11 doses). Pharmacokinetic parameters and enzyme leukocyte uptake were assessed at three infusions in a European sub-cohort of eleven r-hαGAL treated patients. Biopsies from kidney, heart and skin were planned for all patients at baseline and after 20 weeks of treatment. LM, TEM and biochemical GL-3 tissue content (ELISA) were used for GL-3 measurements. In addition, plasma samples for GL-3 (ELISA) were obtained at baseline and weeks 12 and 20.

Male or female patients (56 men and two women), aged 16 and older, without previous exposure to agalsidase beta and a confirmed diagnosis were included. Patients with kidney failure or renal insufficiency (defined as serum creatinine >2.2 mg/dl) or those who had undergone kidney transplantation or were on dialysis were not included.

Patients who successfully completed this double-blind study were eligible for entry into an open-label extension study (AGAL-005-99), during which all patients, including those previously receiving placebo, receive r-hαGAL and long-term safety and efficacy parameters are monitored for a duration of 18 months. All 58 patients from the phase III study participate in the extension, and patients have been on active treatment for a year or more. Assessments for efficacy and safety are being carried out as in the original study. A 6-month interim analysis was submitted during the review of this Marketing Authorisation Application.

Endpoints

The primary efficacy endpoint for this study was reduction of GL-3 accumulation from the capillary endothelium of the kidney to score 0 at week 20, as determined by LM. Three pathologists who measured the GL-3 inclusions in the capillary endothelium by semiquantitative light microscopy using a scale of 0-3 evaluated the tissue deposits. The reading was blinded for the treatment allocation and the sequence of the biopsies. The secondary efficacy parameters were defined as: the change in the composite score of GL-3 inclusions in the capillary endothelium of the kidney, skin and heart (assessed by LM); the change in composite score of kidney tissue and urinary GL-3 levels (measured by ELISA); and reduction in pain as assessed by the Short Form McGill Pain Questionnaire.

Several tertiary endpoints were evaluated: Quality of Life (SF-36), change of glomerular filtration rate, neuropathy impairment, and autonomic function status.

Statistical analysis

As noted in the final study report, a number of treatment dispensing errors occurred at two study sites. This resulted in two patient population groups: Intent-To-Treat (ITT) and As-Treated. Data were analysed for the groups separately and the major results were consistent in the two analyses. No statistically meaningful differences were observed among the treatment groups for demographic or for baseline disease characteristics. With the exception of the primary analysis, the data presented in this report reflect the data analysed by treatment actually received (As-Treated).

Results

Primary endpoint:

A highly statistically significant greater proportion of patients in the agalsidase beta group achieved a final kidney majority score of 0 at week 20 ($p<0.001$). This was achieved for both the ITT and the As-Treated analyses. In the ITT analysis, 18 of the 29 (62%) patients in the r-hαGAL treatment group compared to 2 of the 29 (7%) patients in the placebo group showed a final majority score of zero. In the As-Treated population, no patients in the placebo group demonstrated a final majority score of zero, as the two patients randomised to placebo actually had received r-hαGAL. Thus, 20 of the 29 (69%) r-hαGAL-treated patients had a final majority score of zero as compared to none in the placebo-treated group.

At baseline, 72% of patients in the r-hαGAL group and 86% of the placebo group had scores of 2 or 3, thus indicating a high level of GL-3 clearance, as reflected by a 2 or 3-point reduction. Each of the three pathologists independently achieved a statistical difference between treatment groups for the primary endpoint ($p<0.001$).

These results were confirmed by an interim analysis of an open label extension of the placebo controlled trial in which patients from both randomisation groups are scheduled to receive Fabrazyme for an additional 18 months. Placebo patients analysed after receiving 6 months of treatment, achieved clearance of GL-3 in the vascular endothelium of the kidney. Patients continuing to receive Fabrazyme maintained or further improved clearance of GL-3 after 1 year of treatment.

Secondary endpoints:

Reductions in GL-3 inclusions by LM were observed in the vascular endothelium of the kidney, skin and heart tissue using a combined severity score ranging from 0 to 3 for each organ. A score reduction of 86% was found in the r-hαGAL treated patients compared to no change in the placebo patients ($p<0.001$). The significant decrease in GL-3 deposits observed in the tissues combined was also noted for each individual organ (67% score reduction in heart and 100% score reduction in skin for r-hαGAL group, both $p<0.001$). These results were confirmed by an interim analysis of the open label extension of the placebo-controlled trial.

Another secondary endpoint assessed the change in urinary and kidney GL-3 as measured by ELISA. Despite a large variation in urinary GL-3 levels at baseline and at week 20 (most likely explained by a sporadic, rather than constant, clearing of GL-3 or GL-3-containing cells into the urine), the r-hαGAL treated patients showed a median decrease in urinary GL-3 of 23% compared to an increase of 43% in the placebo-treated patients ($p=0.005$). GL-3 levels in the kidney decreased by 34% with r-hαGAL , compared to a decrease of 6% with placebo, but this was not statistically significant.

Normalisation of plasma GL-3 was found in the r-hαGAL treated patients compared to a 17% decrease in the placebo patients, a statistically significant difference ($p<0.001$). In the r-hαGAL - treated group, almost all patients had values below the detection level of 1.2 ng/µl.

The secondary efficacy analysis also included an assessment of change in pain using the Short Form McGill Pain Questionnaire. In many of the pain score categories statistically significant improvements from baseline were observed, but this occurred in both treatment groups. Pain was not a selection criterion and many of the older patients were at a stage of the disease where pain is minimal, and almost three quarters of patients assessed their pain at baseline as none or mild. In addition, pain medication was allowed, but no treatment algorithm had been pre-defined. However, in the active treated group the improved pain score, although still non-significant, stabilised during the first 6 months of the open extension study, thus suggesting an effect.

Tertiary endpoints:

Overall in the pivotal phase III study, no statistically significant differences between the treatment groups were observed –including quality of life - when analysing the tertiary endpoints. However, patients showed little baseline pathology and the study duration of 20 weeks might not be sufficient to detect significant changes.

When analysing 6-month interim data from the ongoing phase III open-label extension study, results indicate that the renal function remained stable during the first year of the treatment thus suggesting a clinical effect.

Clinical studies in special populations

No studies in special patient groups were performed although the applicant is currently undertaking a study to evaluate the effect of Fabrazyme in patients with a more extensive form of Fabry disease, including renal impairment.

No studies have been performed in children, which is adequately reflected in the SmPC. A study in children will be performed post-authorisation.

Impact of Antibody Development on the clinical efficacy results

In an attempt to assess whether the formation of IgG antibodies against agalsidase beta could interfere with enzyme activity, an analysis was performed to compare the primary efficacy endpoint between agalsidase beta treated patients who seroconverted and those who did not develop antibodies. There was no difference in the proportion of patients achieving a final kidney score of 0 ($p=1.000$). These results are in line with the observation that cellular uptake of agalsidase beta, as measured by uptake of enzyme into leukocytes, appeared not to be affected by the formation of IgG antibodies.

<u>Clinical Safety</u>

Patient exposure

Safety data were reported on a total of 73 patients from 3 clinical studies. One of the studies is an ongoing open label extension of the placebo controlled trial and only interim safety data from the first 6 months of this trial (for a total exposure of 12 months) were provided.

Adverse events and serious adverse event/deaths

In clinical trials it has been demonstrated that r-hαGAL can be administered safely at a dose of 1 mg/kg every 2 weeks to patients with Fabry disease. Safety evaluations included adverse event monitoring, physical examinations, standard laboratory testing, vital signs, ECG, echocardiograms and IgG antibody to r-hαGAL.

The majority of adverse events reported in the pivotal study were assessed as mild or moderate in severity. The most common adverse event was pain associated with the biopsies that were part of the study procedures. Postoperative pain was therefore the most frequently reported adverse event in both the agalsidase beta (76%) and the placebo (55%) groups. A statistically significant difference was observed for three adverse events, which were reported more frequently in patients treated with r-hαGAL compared to placebo. These adverse events included chills (rigors), fever and skeletal pain. Both rigors and, in the majority of cases, fever were associated with infusion-related reactions. Skeletal pain presented primarily as neck and shoulder pain/discomfort.

Twenty (69%) patients in the r-hαGAL treatment group had at least one adverse experience that was thought related to their study medication versus 11 (38%) in the placebo group. Combining the safety data from the placebo controlled study and the open label extension phase thereof, 74% of patients treated with r-hαGAL had at least one adverse experience that was thought related to their study medication. In the latter analysis, the adverse events related to the treatment reported in at least 10% were, rigors, sensation of temperature change (verbatim: feeling cold or warm), fever, headache, tremor, nausea, rhinitis, pain at the extremities, oedema at the extremities, hypertension, vomiting, myalgia, and dyspnoea. Those reported in 5 to 10% were chest pain, somnolence, anaemia, flushing, fatigue, paraesthesia, tachycardia, bronchospasm, abdominal pain, pain, pallor, back pain, throat tightness, pruritus, albuminuria and abnormal vision. Almost all these events were reported as mild to moderate in severity.

No patients died during these studies, and no patients discontinued treatment because of adverse events. In the placebo controlled trial the number of patients experiencing an SAE was similar between the two treatment groups (five patients in each group) and none of the events were assessed as being related to the study medication. SAEs reported were pain related to the protocol-required biopsies, hypotension, chest pain and haemopericardium. All patients experiencing SAEs recovered, with the exception of one placebo patient who continued to have sequelae due to head trauma at study end. In the extension study 13 patients experienced one or more SAEs, however only the investigators as being related judged 4. These four were associated with an infusion.

The most commonly reported AE in the phase I/II study was a mild transient elevation in blood pressure in 14 patients (93%), among which four had a prior history of hypertension. Other related adverse events were allergic reaction, pain, headache, fever and abdominal pain, all seen in three or four patients.

No patients died during this study. Two patients experienced SAEs; one patient experienced symptoms suggesting a possible allergic reaction. The symptoms included abdominal discomfort, nausea, diaphoresis, and pruritus, vomiting and bradycardia. The symptoms resolved with discontinuation of infusion and treatment. A second patient who discontinued anticoagulation therapy prior to study participation developed pulmonary emboli; the investigator could not rule out a relationship between study drug and this event.

Infusion reactions

The majority of patients developed IgG antibodies specific to r-hαGAL with approximately half of the patients developing adverse events on the day of the infusion, which is suggestive of a hypersensitivity-type reaction. The events reported included rigors, fever, headache, temperature changed sensation, Fabry pain, hypertension and were successfully managed with a reduction in infusion rate and with conventional treatment including antipyretics (i.e. ibuprofen), antihistamines and/or corticosteroids. None of these reactions resulted in discontinuation of scheduled infusions, although 2 patients in the Phase I/II open-label, dose ranging study did not complete their fifth and final infusion.

Antibody formation

All patients enrolled in the Fabry studies were evaluated for the development of antibodies specific to agalsidase beta. Monitoring included a two-step procedure; patients' blood samples were screened for the presence of antibodies against agalsidase beta using an ELISA and the results were confirmed by using radio-immunoprecipitation or Western blot analysis.

In the pivotal study, a total 24 (83%) of 29 patients treated with agalsidase beta developed specific IgG antibodies. Seroconversion was found up to the last infusion, with the first conversion seen at the third infusion. In patients tested, no IgE antibodies were detected. Fifteen of the 24 patients who developed IgG antibodies experienced infusion-related adverse events. Eleven of these patients were tested for complement activation using a blood sample collected within one hour of the event, with ten patients testing positive. The majority of the events consisted of fever/chills. Other events included hypersensitivity-like reactions, gastrointestinal, cardiovascular and/or pain symptoms and headache. All these reactions were mild to moderate in severity and were most often managed by reducing the infusion rate or by administration of antihistamines, antipyretics (e.g. ibuprofen) and, in three patients,

the addition of corticosteroids. All 15 patients continued to receive treatment until study completion without additional problems.

Similar results were obtained in the extension phase of the placebo-controlled study. In patients treated over 12 months the antibody response was found in general to plateau or even decrease over time. One patient with a low titer became negative.

In the dose-ranging, open-label study, eight of 15 patients developed IgG antibodies. Of the eight patients who seroconverted, four patients developed symptoms suggestive of a hypersensitivity-type reaction. All four were negative for IgE.

These infusion reactions were thus not problematic since they either subsided or were manageable with premedications. There is no evidence of IgE-mediated hypersensitivity. In addition, the available human safety data and the immunohistological analysis of tissue samples in a subset of patients do not raise concerns with reference to immune deposits, resulting in serum sickness or vasculitis. The company will continue to evaluate any clinical evidence of immune complex disease and results will be reported as part of the PSURs.

Laboratory findings

No unexpected laboratory findings were reported. The various abnormal findings could be explained as being manifestations of Fabry disease.

Safety in special populations

No studies in special patient populations were submitted and this has been sufficiently reflected in the SPC. A study in children will be initiated.

Discussion on Clinical aspects

Discussion on Clinical Efficacy

Depletion of accumulated sphingolipids from cells in key target tissues could be of major advantage for the patients, for whom previously only symptomatic therapy has been available. It is evident from the studies performed that enzyme replacement therapy with agalsidase beta for Fabry disease resulted in a significant and almost complete clearance of GL-3 in the vascular endothelial cells. Results of histological evaluation performed in various other cell types demonstrated that GL-3 is cleared, although longer treatment periods may be required to obtain complete clearance in some cell types. Also plasma GL-3 levels were reduced in patients receiving agalsidase beta.

The characteristic histopathological finding in Fabry disease is the accumulation of sphingolipids, ultimately leading to the morbidity associated with this disorder. The demonstrated reduction of these tissue deposits may be indicative for a clinical improvement or a stabilisation of the clinical condition.

The 12-month data available through the interim report from the uncontrolled extension study suggest a clinical improvement and/or stabilisation of the disease course. Some improvement in the pain score was maintained through one year of treatment, quality of life scores slightly improved and kidney function remained stable. However, none of these clinical parameters reached a statistical significant improvement. The lack of clear clinical benefit may be due to the fact that the patients were not selected on the basis of a particular symptom. Thus, the variability of the clinical presentation makes it difficult to demonstrate clinical benefits in the short term. Longer follow-up is needed and special patient groups need to be studied for an accurate assessment of the clinical benefit, including the reversibility of organ dysfunction. Because severe end organ damage may not be expected to be fully reversible, starting treatment before symptoms develop should be considered.

Critical manifestations of the disease including significant changes in renal, cardiac and cerebrovascular status and/or death can be considered important endpoints. The applicant is currently undertaking a study to evaluate the effect of Fabrazyme in patients, both male and female, with a more extensive form of Fabry disease, including renal impairment. Annual updates on the status of this study will be provided. The Market Authorisation Holder will extend the ongoing open label extension study with an additional follow-up period. Data from historical controls will be collected for comparison.

Furthermore, potential surrogate markers for the assessment of progress of disease should be explored further and validated.

The dose advised is sufficiently studied and can be considered the optimal dose. The optimal dose in the long term, however, will be further studied. The applicant has committed to performing a clinical study post-authorisation to evaluate maintenance dosing regimens of agalsidase beta - after clearance of the accumulation of sphingolipids- and to explore, if appropriate, alternative dosing regimens.

Antibody formation seems to be unrelated to clinical efficacy as showed when looking at the primary endpoint as well as uptake of agalsidase beta on a cellular level. It therefore appears that seroconversion is not associated with in vivo neutralising activity although this possibility cannot be completely ruled out.

Discussion on Clinical Safety

In clinical trials it has been demonstrated that agalsidase beta can be administered safely at a dose of 1 mg/kg every 2 weeks to patients with Fabry disease. No deaths occurred during the clinical studies. The majority of adverse events reported were assessed as mild or moderate in severity. The most common adverse event was pain associated with the biopsies that were part of the study procedures. Laboratory studies, electrocardiogram and echocardiogram showed no direct toxic effect suggesting that agalsidase beta has no intrinsic toxic effect.

The majority of patients developed IgG antibodies specific to agalsidase beta with approximately half of the patients developing adverse events on the day of the infusion, which is suggestive of a hypersensitivity-type reaction.

The events reported included rigors, fever, headache, temperature changed sensation, Fabry related pain, hypertension and were successfully managed with conventional anti-hypersensitivity medical treatment. However, all patients were able to continue treatment with a combination of infusion rate reduction and administration of standard anti-allergic treatment prior to infusion. In addition, neither the incidence nor the severity of the infusion-associated reactions reported in patients previously sensitised and re-challenged with r-hαGAL after discontinuation of treatment for up to 2 years warrant any additional concern at this time. The activation of complement noted in a number of patients warrants careful examination, as this event suggests the formation of circulating immune-complexes, which could potentially induce immune complex disease. It is reassuring that none of the three pathologists who scored the renal biopsies detected signs of inflammation, which could be suggestive of immune-complex mediated glomerulonephritis. In addition, an immunohistological analysis of renal tissue samples in a subset of patients did not demonstrate presence of immune complex deposition. It is therefore likely that complement activation is a transient phenomenon linked with the development of hypersensitivity reactions, with no direct impact on the renal structure and function, and that immune-complexes, if formed, are rapidly cleared from the kidney. There is currently insufficient information to determine whether patients will develop tolerance over time although data provided with the 6-month interim report from the extension study suggest that this may be the case.

The applicant has indicated that any evidence of the presence of tissue bound-immune complexes will be monitored. Results will be reported as part of the Periodic Safety Update Reports together with information on the long term monitoring for the presence of antibodies to Fabrazyme and their impact on safety and efficacy. In addition, any evidence of potential for drug interactions will be reported in PSURs.

The Marketing Authorisation Holder has committed to performing a clinical study post-authorisation to evaluate maintenance dosing regimens of agalsidase beta - after clearance of the accumulation of sphingolipids- and to explore, if appropriate, alternative dosing regimens.

5. Overall conclusions and benefit/risk assessment

Quality

Apart from a number of points, which can all be addressed as part of post-authorisation commitments, the quality of Fabrazyme is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Viral safety and batch-to-batch consistency has been documented and the relevant tests will be performed according to the agreed specifications.

Preclinical pharmacology and toxicology

Overall, the primary pharmacodynamic and pharmacokinetic studies provided adequate evidence for efficacy of agalsidase beta since significant clearance of the tissue deposits of GL-3 in the key target organs was observed. Because the accumulation of sphingolipids is ultimately leading to the morbidity associated with the disease and its clinical presentation, the pharmacodynamic results are indicative for clinical efficacy. Although repeated dose toxicity testing was only performed in one species, the overall results from the toxicology programme did not raise particular concerns for the safe use of agalsidase beta. Adverse effects were not observed in the toxicity studies with rats. The lack of data on reproduction toxicity and consequently the recommendation to stop the medication during pregnancy and lactation is adequately reflected in the SPC.

Efficacy

The results from clinical studies support the use of agalsidase beta in the approved indication long-term treatment of Fabry disease (α-galactosidase A deficiency). The demonstrated reduction of sphingolipids in the target organs is encouraging and may be indicative for a clinical improvement or a stabilisation of the clinical condition. However, although positive trends were observed for the clinical parameters investigated as secondary endpoints, none of these parameters reached a statistically significant improvement. The indication for which the medical product in question is intended is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence/data on the safety and efficacy of the medicinal product. In order to collect additional long-term efficacy and safety data, the applicant has committed to complete a programme of clinical studies post-authorisation, the results of which shall form the basis of the annual reassessment of the benefit/risk profile.

Safety

Safety data show a mild adverse event profile and reasonable tolerability to Fabrazyme. However, the infusion reactions and anti-agalsidase antibody formation warrant special attention post-authorisation. The period of agalsidase beta treatment in the clinical trials does not reflect the life-long treatment necessary for Fabry disease. The safety database is not as large as is often the case for new medicinal products. However, one should realise that Fabry Disease is rare with an estimated prevalence of 500-1000 patients in the EU. Furthermore, since the structure of agalsidase beta is very similar to the enzyme produced naturally in humans, Fabrazyme is not likely to cause unexpected adverse events. In order to collect additional long-term efficacy and safety data, the applicant has committed to complete a programme of clinical studies post-authorisation, the results of which shall form the basis of the annual reassessment of the benefit/risk profile.

Benefit/risk assessment

Following the assessment of the documentation provided by the applicant, it was concluded that further data was needed to support the quality of the product. After discussing a number of issues at the CPMP's Biotechnology Working Party, the BWP considered that Fabrazyme is approvable at present from a quality point of view.

On the basis of the available information from on-going studies and on relevant commitments from the applicant, the CPMP did not consider that further preclinical and/or clinical data were needed prior to opinion.

At a clarification before the CPMP, the applicant focused on the post-authorisation commitments, as previously defined by the CPMP.

A marketing authorisation for Fabrazyme will be granted under exceptional circumstances, subject to fulfilling the chemical and pharmaceutical, as well as the clinical follow-up measures and clinical specific obligations undertaken by the applicant. The applicant has committed to complete the clinical studies within specified time frames and the results shall form the basis of the annual reassessment of the benefit/risk profile.

In view of the limited clinical data available, section 5.1 of the SPC has been expanded in order to give the treating physician a clearer overview of the anticipated clinical effects of Fabrazyme.

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Fabrazyme, as long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry disease, was favourable and therefore recommended the granting of the marketing authorisation.