SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Replagal. This scientific discussion has been updated until 15 October 2003. For information on changes after this date please refer to module 8B.

1. Introduction

REPLAGAL is a human α-galactosidase A (agalsidase alfa), which is produced in a continuous human cell line. Agalsidase alfa is a highly purified form of the naturally occurring human lysosomal hydrolase enzyme responsible for the metabolism of globotriaosylceramide (Gb3 or ceramide trihexoside; CTH). α-Galactosidase A is targeted to its lysosomal site of action by mannose-6-phosphate (M6P) residues on the α-galactosidase A molecule. The M6P moiety is recognised by specific M6P receptors on the cell surface. Inside the cell there are additional M6P receptors in the Golgi complex that direct the enzyme to the lysosomes. Replagal is indicated for use as long-term enzyme replacement therapy in patients with Fabry Disease (α-galactosidase A deficiency).
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. This leads to progressive accumulation of neutral glycosphingolipids, predominantly Gb3 in most tissues and cell types. Fabry Disease is a heterogeneous multisystem disorder with variable onset of symptoms affecting the nervous system, kidneys, heart, skin and gastrointestinal system. It is not possible to predict the phenotype based on current knowledge of the different genotypes described for this disease. It is an extremely rare disorder, with an estimated prevalence of 500 to 1000 patients within the EU. The most troublesome symptom in many of these patients is severe, debilitating neuropathic pain with onset usually from early childhood. Premature death usually occurs in the fourth or fifth decade of life and results from renal, cardiac, or cerebrovascular complications. Many 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.
Replagal treatment should be supervised by a physician experienced in the management of patients with Fabry Disease or other inherited metabolic diseases. Replagal is administered at a dose of 0.2 mg/kg body weight every other week by intravenous infusion over 40 minutes. Replagal is for intravenous use in adults. No studies in special patient populations including children (0-17 years), patients over the age of 65 and those with hepatic impairment have been performed and no dosage regimen can be recommended in these patients. No dose adjustment is necessary in patients with renal impairment.

2. Chemical, pharmaceutical and biological aspects

Composition

Replagal is supplied as a sterile concentrate for solution for infusion for dilution prior to administration presented in a 5 ml Type I glass (Ph.Eur.) vial. The vial is closed with a butyl rubber stopper that has a fluoro-resin coating on solution contact surfaces, a one-piece 20-mm aluminium seal and a white plastic flip-off cap. Each vial contains 3.5 mg of agalsidase alfa in 3.5 ml of concentrate, sodium phosphate monobasic, monohydrate, polysorbate 20, sodium chloride and water for injections.
Apart from the active substance, all other excipients used for formulation comply with specific monographs of the European Pharmacopoeia (Ph.Eur.) and /or the United States Pharmacopoeia (USP) monographs. No human or animal derived components are used as excipient.

Active substance

Description
Agalsidase alfa is a lysosomal acid hydrolase that specifically cleaves terminal α-linked galactose residues from the glycosphingolipid Gb3. Agalsidase alfa is the active ingredient in Replagal™ and is extensively purified from the cells and the culture medium by a five step chromatography process and

a viral filtration step. The mature enzyme is a glycoprotein and consists of a 100 kD homodimer of two approximately 50,000 Da subunits. Each subunit consists of 398 amino acids. The primary translation product is post-translationally modified by the cleavage of a signal peptide sequence and by the addition of the 3 N linked oligosaccharides. Alfa galactosidase activity is expressed in Units, where one unit is defined as the amount of enzyme required to hydrolyse 1 nanomole of 4-methylumbelliferyl-α-D-galactopyranoside substrate per hour at 37°C.
The identity of agalsidase alfa as well as product and process related impurities (including aggregates) are sufficiently verified by a broad range of tests. The applicant has committed to further revise the specifications post-authorisation based on further manufacturing experience.
Development genetics, Genetic stability and cell bank system
The assembly of the production strain has been comprehensively described. The production strain is derived from a human continuous cell line. The use of a human cell line and a human glycosylation pattern was considered necessary for an optimal efficacy. In view of the viral screening of the cell banks and the excessive and validated removal/ inactivation viral tests performed during production, the applicant has provided sufficient information to ensure that the viral safety is not a matter of concern. In addition, all vials tested from each cell bank were found to be free of microbial contamination.
To ensure a consistent productivity a well-characterised α-galactosidase A-producing continuous human cell line was developed, which has been comprehensively described in the application. The production strain was prepared by stable transfection with a fully characterised plasmid. The plasmid was assembled from well-known DNA sequences and synthetic DNA fragments. A Master Cell Bank (MCB) and Working Cell Bank (WCB) have been prepared from this agalsidase alfa producing continuous human cell line. The methods used to establish the MCB have been well described and involved standard techniques widely used in DNA recombinant technology. The preparation as well as maintenance (location and storage conditions) of the MCB and WCB is described in sufficient detail in the documentation. Approximately the same protocol was applied for both the MCB and the WCB expansion process.
All subsequent new WCB will be prepared using the same protocol. The stability program for both the MCB and WCB is considered to be sufficient and all relevant parameters are included in the testing program. However, the applicant post-authorisation will establish additional specifications. In addition, stability monitoring of MCB and WCB based on established protocols, will continue.
For determining the genetic stability at the nucleotide level, characterisation studies using classical tests were performed on material from the MCB and the WCB and end of production cells (EPC) from both the MCB and WCB. Additional tests to demonstrate genetic stability will be performed by the applicant post-authorisation.
Fermentation
Cell culture is performed in two phases: cell expansion and harvesting of conditioned medium. During the expansion phase, a culture is initiated by thawing a single vial of the WCB and expanded to a scale sufficient to produce approximately 330L of unprocessed bulk. In general, sufficient in-process controls are performed.

Purification
The purification process consists of 5 conventional chromatographic steps and a viral filtration step. For all column steps, sufficient details on operating conditions such as the size of the column, washing and elution conditions, flow rates, regeneration and in process controls are submitted. Several 0.2µm filtration steps are included in the purification process. The applicant has committed to introduce additional testing to further define column performance based on additional manufacturing experience.

Active substance characterisation
Characterisation studies have been performed by IEF, Western blot, N-terminal sequence analysis and the enzymatic activity was determined by a fluorometric assay. A cell based internalisation assay is performed to confirm that appropriate post-translational modifications are present for internalisation by human cells and localisation to lysosomes. Comparability between active substance manufactured at different sites was demonstrated by characterisation studies, pharmacokinetic studies in monkeys and clinical studies in man. The predicted molecular mass of the agalsidase alfa peptide monomer is 45400 Da. The agalsidase alfa mass spectrum of the reference standard as determined by MS covers

molecular masses from 46 to 55 kD, which is indicative for heterogeneous glycosylation. The dimeric nature of the native form of α-galactosidase-A is shown by SE-HPLC.
Routine procedures as well as characterisation studies for carbohydrate analysis are based on specific deglycosylation and dephosphorylation enzymes. Characterisation of all carbohydrates structures in the reference preparation is performed by anionic exchange HPLC. This glycosylation is different from CHO cell derived α-galactosidase A. The results of the ongoing characterisation studies will be submitted post-authorisation.

Specification of the active substance
The specifications and routine tests cover all tests commonly applied to purified protein solutions.
Specifications include physical tests for clarity, colour and pH. Identity is assured by several methods including Western blot, N terminal sequence, peptide map, IEF, SDS-PAGE .
Purity is controlled by SE-HPLC and RP-HPLC Tests for other impurities, which originate from the cell culture and purification process such as host cell DNA and host cell protein are also performed.
Potency is evaluated using a bioassay based on internalisation into human cells, protein content and a specific enzyme activity test.
Bioburden and endotoxins are also tested.
During the evaluation process a number of questions were raised regarding specifications and routine testing. Most of these have been adequately solved, while some remaining points will be addressed as post-authorisation follow up measures.

Analytical development
Assays used as release tests, tests on purified bulk and in process control tests have been extensively validated. The applicant has committed to further develop and validate the host cell protein assay. Furthermore the bioburden determination as well as the bacterial endotoxin assay has been validated.
Enzymatic activity is evaluated by use of a fluorometric assay. A qualified Internalisation bio-assay is also performed and the confidence limit set for agalsidase alfa activity will be re-evaluated on the basis of additional batch data.
The current reference standard is extensively characterised, compared with the original reference standard and has been sufficiently described.

Process validation
The manufacturing process has been validated with regard to growth and production media preparation, cell culturing and purification. For the validation of the media preparation the results of 5 lots of both growth and production are submitted. All acceptance criteria were met.
Validation of both the fermentation process and the purification process was accomplished through increased sampling and testing during the regular (full scale) manufacturing process.
The purification process is sufficiently validated by determination of the different parameters in the purified bulk.
Removal of DNA during purification was studied by spiking experiments using down scaled columns and by determination of DNA in the different fractions of the large scale production. The size and structure of host cell DNA isolated from the agalsidase alfa purified bulk was also determined. Given the additional commitments that have been made, along with the results of the used resin study, the applicant has demonstrated that the host cell DNA levels will be well controlled and that the theoretical risk presented by the use of a human cell line will be further minimised.
Chromatography column cleaning was monitored during washes for column preparation and regeneration by collecting samples of appropriate column effluents. It is not expected that the resins are damaged by the regeneration and storage conditions. The claimed life span of the different columns is sufficiently justified. The company will continue to monitor the performance of the different columns post-authorisation. The purification process is sufficiently monitored and controlled by the measures already taken and the commitments given by the applicant.

Impurities
To demonstrate consistency of impurity profiles, batch analysis results from eight lots used in clinical trials and process validation purified bulk lots were used.

Batch to batch consistency
Data are presented on all batches of α-galactosidase A products that have been used in preclinical and clinical studies described in the MAA stability studies. The data demonstrate that the ability of the manufacturing process to produce active substance that meets the specification in a reproducible manner.

Stability of the active ingredient
The Purified Bulk stability specification is in concordance with the specifications for release of the active substance and the finished product. Additional stability data will be submitted on an on-going basis.

Other ingredients
Excipients used to compose the finished product are polysorbate-20, sodium chloride, sodium hydroxide, sodium phosphate monobasic (monohydrate) and water for injections. Except for the sodium phosphate (monobasic monohydrate), which conforms to the USP, excipients used comply with Ph.Eur. The quality of the nitrogen (US National Formulary) used to fill the headspace of the vials is sufficiently guaranteed.

Packaging material
The primary packaging comprises two components, a 5-ml glass vial (Type I), and a grey serum stopper composed of butyl rubber laminated with fluoro-resin. The vial is closed with the rubber stopper, a one-piece 20-mm aluminium seal and a white plastic flip-off cap. Each final package contains 1 vial and it can be concluded that the packaging material used suits the intended purpose.

Product development and finished product

Method of preparation
The preparation of the formulated bulk and filling into the final container is performed at Chesapeake Biological Laboratories (CBL), Baltimore, USA. Measures to prevent contamination and sterilisation procedures are described. The thawed bulk is diluted with filtered (0.22µm) formulation diluent to the target protein concentration of 1mg/ml. An appropriate down scale procedure was applied for validation of the 0.22 µm membrane filters used for the filtration of the finished product. Polysorbate 20 is added, and, if necessary the pH is adjusted and either formulation diluent or Agalsidase alfa purified bulk is added to achieve the desired protein concentration. The filter units are sterilised and tested for filter integrity by bubble point method.
Sterile formulated bulk is filled by weight into the final container. Upper and lower action and alert limits are set in the batch record and limits for filling into the final container has been included in the application. Vial preparation includes dry heat sterilisation/depyrogenation and stoppers are autoclaved. Microbiological testing of raw materials used, and preparation of formulation buffer are described in sufficient detail. The process has been sufficiently validated (e.g. mixing, sterile filtration). The manufacturing process as defined in the current manufacturing batch record is considered capable of yielding a uniform product that meets specifications.

Product development
During development product derived from a different cell line as well as different formulations, manufacturing processes and manufacturing sites were used. Comparability between the different products used in clinical trials and that intended for commercial use was demonstrated by extensive characterisation studies and pharmacokinetic studies in animals.

Specifications of the finished product
Lot release and stability specifications for agalsidase alfa finished product have been developed to ensure the consistency and safety of the product. The list of tests performed on the finished product includes tests to address product identity (Western analysis), potency (specific activity and total protein), purity (Size Exclusion HPLC and RP-HPLC) and safety (endotoxin and sterility) in addition to standard quality assays (appearance, pH, particulates and volume of fill). The applicant will include determination of polysorbate 20 and IEF in the release testing of the Finished Product. The

manufacturing process as defined in the current manufacturing batch record is considered capable of yielding a uniform product that meets specifications.

Stability of the finished product
Studies were performed on GMP lots planned for clinical trial use and/or consistency support. All studies were performed using the proposed market formulation.
Agalsidase alfa Finished Products is stable over 12 months when stored at 2-8°C, the claimed shelf life. Ongoing stability studies will include assays to determine deamidation and oxidation of the finished product.

Viral safety
The cell line for the production of agalsidase alfa is derived from a human continuous cell line FBS was used in the production of the MCB and WCB and bovine calf serum, BSA and bovine transferrin are used during cell culture. Porcine trypsin is used during the propagation phase. Trypsin is not used during production phase. No other material of human or animal origin is used during production and purification except for immobilised heparin (porcine) linked to a column resin used for purification. A specific virus-reducing step is included in the purification process.

Microbiological quality of the starting material and reagents
The cell bank system was determined to be free of viral and microbial contamination by extensive testing. The applied test program is appropriate to sufficiently assure the microbiological quality of the cell bank system.

Biological materials and reagents
Considering the limited use of this FBS (only MCB and WCB) the nature of the animal tissue (i.e. serum, category IV: no detectable infectivity) the information already supplied, the risk of TSE transmission through the FBS is considered negligible.
Concerning TSE risk assessment, in view of the country of origin, the nature of the animal tissue (i.e. serum, category IV: no detectable infectivity) used for the manufacturing of BSA, calf serum and bovine transferrin, the risk of TSE transmission is considered negligible. In conformity with the CPMP guideline on TSE (CPMP/BWP/1230/98, the applicant will audit the suppliers/manufacturers of FBS, BSA and bovine transferrin at regular intervals. Certificates of suitability for bovine transferrin and bovine serum albumin have been provided.
Porcine trypsin used during cell expansion phase has been tested for porcine parvovirus.
Several reports on virus reduction/inactivation during the process to prepare heparin from porcine source material are enclosed. The viral safety of the purification column is sufficiently guaranteed.

Virus validation studies of the production process
The agalsidase alfa purified bulk purification process consists of 5 chromatographic purification steps and a filtration step. All steps have been validated for the virus reducing capacity using scaled-down processes with intermediates and reagents taken from the full scale manufacturing process conducted at BSCP. Virus reduction factors were calculated in accordance with NfG on virus validation studies: the design, contribution and interpretation of the studies validating the inactivation and removal of viruses (CPMP/BWP/268/95), although the mechanism of clearance (inactivation or removal) was not determined.
The applicant provided data on the viral clearance by used resins. In principle, the reduction factors are comparable to the data obtained in the studies using new resins.
Viral safety of a product is based on; selection and testing of cell lines and raw materials, capacity of the production process to clear infectious viruses; and testing of product at different steps for absence of contaminating viruses. As can be concluded from the original application and the response of the applicant to several questions raised, cell lines and raw materials are extensively tested. In addition, during production, the product is also tested extensively for absence of contaminating viruses.
It is felt that viral safety of the product is sufficiently guaranteed by the total package of testing and validation data.
Reduction of viruses by column cleaning and sanitisation solutions were performed to evaluate the extent of virus reduction. Virus reducing capacity of the solutions used is sufficiently demonstrated.

Discussion on chemical, pharmaceutical and biological aspects

In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with the requirements in the Note for Guidance on Production and Quality Control of Medicinal Products Derived by Recombinant DNA Technology as well as other relevant guidelines. The information provided in the application demonstrated consistent production of agalsidase alfa achieving a well-defined quality for the active substance and the finished product. The fermentation, down-stream processes and purification of the active substance are adequately controlled. Agalsidase alfa has been well-characterised using state-of the-art methods with regard to its physicochemical characteristics. The microheterogenity has been sufficiently documented. The manufacturing process of the finished product, which complies with Good Manufacturing Practice (GMP), has been described in sufficient detail and product specifications are adequate. In general, methods to control the quality of the product are adequate. Moreover, this is a product of biological origin for which all the virological aspects have been satisfactorily addressed. Stability data support a shelf-life of 12 months for the finished product.
The quality of Replagal is considered to be acceptable when used in accordance with the conditions defined in the SPC. Some quality aspects will be further addressed as part of post-authorisation commitments. 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.

3. Toxico-pharmacological aspects

Bioequivalence
The initial agalsidase alfa drug product developed by TKT was produced from stably transfected human diploid fibroblast cells and designated DRX005A. Three studies in mice (single dose toxicity, pharmacokinetics, and biodistribution) were carried out with this formulation which was also used in the Phase I clinical study (TKT001).
For post-phase I studies, agalsidase alfa production was changed to a continuous human cell line**,** and the formulation was designated DRX005B. The site of manufacture also changed. Two single dose studies (one each in mice and rats) were performed to compare pharmacokinetics between DRX005A and DRX005B. DRX005B, was further characterised in the single and repeat dose toxicity-, the pharmacology, the biodistribution and the pivotal Phase II clinical (TKT003) studies and comparability of the enzyme produced at the two sites was adequately shown. The change in manufacturing site for agalsidase alfa did not have any impact on the quality of DRX005B that may effect the evaluation of safety or efficacy from clinical trials.
The manufacturer for bulk product for all subsequent clinical studies and proposed for release onto the European market is Bio Science Contract Production Corp (BSCP). Two pharmacokinetic studies have been carried out in rats and monkeys to compare products manufactured at different manufacturing sites. BSCP product was further characterised in reproductive and biodistribution studies in animals and in all subsequent clinical studies (TKT005, TKT006, and TKT007). The formulation aspects and characterisation studies are more fully discussed in Part II of this document.

Pharmacodynamics

In vivo studies
Pharmacodynamic effects were evaluated in an agalsidase alfa knock-out mouse model of Fabry disease. These mice have considerable accumulation of globotriaosylceramide (Gb3) in the liver, heart and kidneys. After treatment with a single dose of 0.2 mg/kg of DRX005B agalsidase alfa, the concentration of Gb3 was reduced in liver, heart, and kidney. Furthermore, the reduction was more evident at a higher dose.
Agalsidase alfa was effectively targeted in knockout mice to key tissues that show Gb3 induced pathology in Fabry Disease, indicating that the enzyme reaches the lysosomes in an active form, and catabolises Gb3 in those tissues. The fraction of agalsidase alfa that is taken up, however, and the duration of its activity, cannot be concluded from the pharmacodynamics/pharmacokinetics data. As

this is of importance for finding the proper dose interval, it is considered acceptable to address this issue in the clinical part. Reduced accumulation of Gb3 was demonstrated in endothelial cells and parenchymal cells. No severe general pharmacodynamic side effects were observed during the studies.

General pharmacodynamics
Safety pharmacology studies were not performed, the argument being that α-galactosidase A is a protein normally produced by humans. To the patients active α-galactosidase A is a foreign protein, possibly immunogenic, and the administration route causes abnormal exposure of the enzyme to various tissues. However, the lack of safety pharmacology studies is acceptable in light of the results of the repeat dose toxicity tests. In addition, α-galactosidase A is not highly active at plasma pH.

Pharmacokinetics
In the single-dose pharmacokinetic studies performed in mice, rats and monkeys, there was early distribution throughout the blood volume, followed by uptake into liver, spleen, lungs, heart, kidney and bone marrow within a few hours of injection. The administered enzyme had a biphasic distribution and elimination profile in serum with an elimination half-life of 2 hr or less in mice, rats, and monkeys, and clearance from the circulation within 3-6 hr in these species. Both AUC and C_{max} were proportional to dose.
Elimination of the enzyme from kidney and liver has been shown to follow first order kinetics. The enzyme, in an active form, was retained in those viscera for more than 24-48h after a single injection. Tissue half-lives was 28 h in liver and 1-2 days in kidney. Low enzyme was found in the brain. It is not known whether it penetrates to the foetus nor if it passes into milk, which has been reflected in the SPC. Sufficient information of tissue-bound enzyme activity has been provided.
After single and repeated weekly dosing, AUC levels generally increased dose-proportionally or slightly less than dose-proportional. Repeat, weekly dosing in rats and monkeys and repeat daily dosing in female rabbits showed no signs of accumulation. After repeated dosing in rats, total clearance was increased. The occurrence of antibodies to agalsidase alpha in this study suggested a role of immune-mediated alterations in pharmacokinetics. No antibodies were observed in monkeys. There were no differences in pharmacokinetics of different batches of agalsidase alfa.

Toxicology
Dose extrapolation based on exposure (AUC) is hampered, because exposure was not determined in the key studies with rats, and there are indications for neutralising capacity of the antibodies formed in this species. Antibodies were not detected in the study with monkeys, and kinetic data revealed that exposure (AUC) at the highest dose level tested was approximately 4 times the exposure at human therapeutic dose levels.

Single dose toxicity
Single intravenous administration to rats and mice showed that treatment with agalsidase alfa was well tolerated up to the highest doses given, 10 mg/kg in rats and 2.3 mg/kg in mice, 50 and 11 times the biweekly clinical dose respectively.
The acute study with mice was performed with the initial product (see Bioequivalence), and therefore the dossier lacks an adequate study with a second species. However, a second species study is not strictly required according to ICH S6 and therefore this is acceptable.

Repeat dose toxicity
Intravenous dosing with agalsidase alfa caused no treatment related toxic effects in the 2 week dose finding study with rabbits (up to 1 mg/kg/day), in the 13 week studies with rats and Cynomolgus monkeys (up to 1 mg/kg/week) and in the 26 week study with rats (up to 1 mg/kg/week). The applicant, 13 weeks, has appropriately justified the duration of the repeated dose toxicity test in the monkey.
Antibodies to agalsidase alfa were detected in the majority of rats from the multiple dose studies, in several of the dogs dosed for 4 weeks, and in 9/10 rabbits dosed daily for 14 days. Antibodies were not detected in monkeys dosed for 13 weeks.
The beagle dog appeared not to be a good test species for evaluating multiple doses of agalsidase alfa due to the formation of antibodies to either human serum albumin (HSA) or agalsidase alfa or both

(HSA was not included in the final formulation proposed for marketing). This resulted in severe anaphylactic response after 3 or 4 weekly injections leading to several deaths and moribund sacrifices. All abnormal signs were related to the anaphylactic reaction.
Nephroblastomas were observed in 2 high dose females from the 26 week rat study. These were not considered related to treatment and it is likely they were pre-existing at initiation of the study.

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

Carcinogenicity
Formal carcinogenicity studies have not been conducted with agalsidase alfa, although the compound is intended for long-term treatment. However, carcinogenic potential is not anticipated based on the nature of the compound.
An *in vitro* study demonstrated that agalsidase alfa did not alter the *in vitro* tumourigenicity or growth rates of human kidney cells. The nephroblastomas observed in two female rats that received high dose treatment in the 26 week study are considered as not related to treatment. There are no other indications for carcinogenicity.

Reproduction Toxicity
A reproductive study in male rats was performed with maximum dose levels of 1 mg/kg and a dosing frequency of 3 times per week starting 4 weeks prior to mating. There were no adverse effects of intravenous dosing on any parameter of male reproduction. A combined fertility/teratogenicity study in female rats was performed with dose levels up to 1 mg/kg with daily dosing from pre-mating, during mating, and up to day 17 of gestation. There were no effects of agalsidase alfa on maternal reproductive performance or on foetal development. A teratology study in female rabbits was performed with daily intravenous dosing with levels up to 1 mg/kg from day 7 through day 19 of gestation. Replagal had no effect on female reproductive performance or on early embryo- or foetal development. Based on the Segment II studies in rats and rabbits, it is expected that Replagal will not cause adverse embryo/foetotoxic effects when exposed during organogenesis. However, peri- and postnatal studies are lacking. It is also not known whether Replagal is excreted in human milk. The absence of valid data on reproduction is adequately reflected in section 4.6 of the SPC**.**

Immunotoxicity
Immunotoxicological effects were not directly investigated in the preclinical studies and are not well characterised on tissue level (deposition of immune complexes). Tissues from antibody positive animals from the repeat dose toxicity studies were not investigated for the presence of immune-complexes. However, 18-month human safety data do not give raise to concerns with reference to immune deposits. In addition, tolerance seems to develop in humans after long-term exposure, and circulating immune complexes have not been observed.
The safety margin based on the results from the 26-week study in rats is 3 (based on the highest dose level tested), but might be lower as it cannot be excluded that toxicity was masked by the immunogenicity of the compound. The safety margin based on the results of the monkey study is 4 (based on the highest dose level tested) and is considered sufficient.
Information on the long term monitoring for the presence of antibodies to Replagal and their impact on clinical safety and efficacy, as well as any clinical evidence of immune complex disease will be submitted post-authorisation.

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
Product interactions were not studied, 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 post-authorisation.

Discussion on toxico-pharmacological aspects

Overall, pharmacodynamic and pharmacokinetic studies provided adequate evidence for efficacy of agalsidase alfa. Adverse effects were not observed in the toxicologic programme with rats, rabbits and monkeys. Although the incidence of Fabry disease is low in females, the lack of data on reproduction toxicity and lactation, is considered a drawback. Based on the nature of the product the lack of genotoxicity and carcinogenicity studies is considered acceptable. Having taken into account that the estimate of a safety margin from the study with rats is hampered, and that the study duration in the study with monkey is rather short given the life-long use of the product, it can never the less be concluded that the package on toxicity data as a whole suffices for this compound, provided that careful clinical observations are made post-marketing.

4. Clinical aspects

Replagal is indicated for use as 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. It is an extremely rare disorder, with an estimated prevalence of 1000 to 2000 patients within the EU. Due to lack of functioning α-galactosidase A, there is an abnormal accumulation and tissue deposition of the glycosphingolipid globotriaosylceramide (Gb3, or ceramide trihexoside; CTH) especially in the kidney, heart and nervous system. At present, there is no treatment available for the disease, other than palliative care.

α-Galactosidase A is a glycoprotein that consists of a homodimer of two approximately 50,000 kDa subunits, each consisting of 398 amino acids. Replagal contains the purified human enzyme α-galactosidase A (agalsidase alfa), produced by genetic engineering in a continuous human cell line. α-Galactosidase A is targeted to its lysosomal site of action by mannose-6-phosphate (M6P) residues on the α-galactosidase a molecule. The M6P moiety is recognised by specific M6P receptors on the cell surface. Inside the cell there are additional M6P receptors in the Golgi complex, which direct the enzyme to the lysosomes. Synthesized enzymes which escape this intracellular routing system are secreted by the cell via the constitutive secretory pathway and are often recaptured by cell surface M6P receptors that return α–galactosidase A to the lysosome via the endocytic pathway.

α–Galactosidase A administered intravenously to animals or humans is internalized by cell surface M6P receptors and ultimately enters the cell's lysosome via the endocytic pathway. This aspect of cellular uptake of lysosomal enzymes makes α–galactosidase A enzyme replacement therapy a feasible therapeutic strategy for patients with Fabry Disease.

The recommended dose for Replagal is 0.2 mg/kg body weight, to be administered by intravenous infusion over 40 minutes every other week. Since Fabry disease is a genetic disorder, the replacement therapy is foreseen to be a life long therapy.

Overview of Clinical Trials Programme

Protocol	Phase	Design	No Pts	IV Dose (mg/kg)	Duration
TKT001[1]	I	Open label, dose escalation safety study	10	0.007 – 0.11	Single dose
TKT003	II	Randomised, double blind, placebo controlled trial	26	0, 0.2 eow[2]	24 wks
TKT005	II	Randomised, double blind, placebo controlled trial	15	0, 0.2 eow	24 wks
TKT006	Ext 003	Open label extension study	25	0.2 eow	1 year+
TKT007	Ext 005	Open label extension study	15	0.2 eow	1 year +
TKT014		Open label, PK and safety study	15	0.2 eow	4-12 months+

1. Study used α-galactosidase A produced from stably transfected human fibroblasts (DRX005A); all other studies utilise DRX005B (α-galactosidase produced from a human cell line)
2. eow = every other week

In the clinical programme, two formulations of human α-galactosidase A were used. The first designated as DRX005A contained α-galactosidase A derived from stably transfected human diploid fibroblast cells formulated in a buffer containing human serum albumin. This was used in the Phase I study TKT001. The second formulation designated as DRX005B, was used in all subsequent clinical studies as well as supporting preclinical studies. This formulation contained agalsidase alfa produced in a continuous human cell line in a phosphate buffer formulation identical to that intended for marketing. The establishment of bioequivalence for the different agalsidase alfa formulations are discussed in more detail in Part III of this document. The clinical trials were performed according to Good Clinical Practise (GCP) standards and agreed international ethical principles.

Clinical Pharmacology

The pharmacodynamics of α-galactosidase A was assessed in study TKT001 and the pharmacokinetics in studies TKT001 and TKT006, both in a limited number of patients due to the rarity of the disease.

Pharmacodynamics

In one faze I clinical trial in patients with Fabry disease (TKT001), following a single intravenous infusion no correlation was observed between the dose of DRX005A administered (0.007-0.11 mg/kg) and pharmacodynamic effects on liver (decrease of Gb3 accumulation), plasma or 24 hour urine sediment Gb3 levels. Accumulation of sphingolipids in cardiac (TKT005), liver (TKT001) and renal parenchymal cells (TKT003) was established at baseline.

Liver Gb3 content was measured in pre-dose and 44 hr post-dose biopsy samples and Gb3 content in 24 hr urine sediment was measured at pre-dose, 1, 7, and 28 days post administration. Blood samples were taken for plasma Gb3 analysis just prior to dosing and at scheduled intervals up to 28 days post-dose.

Plasma Gb3 concentrations were variable between patients, and showed no specific trends or changes after drug administration. Liver Gb3 levels decreased in 9 of the 10 patients with a mean decrease in liver Gb3 of 31% ($P<0.05$). 24 hour urine sediment Gb3 levels demonstrated a decrease in 9 of the 10 subjects, with a mean of 38 % at 28 days post-dose ($p<0.01$). No decrease was seen in the samples taken 1 and 7 days post-infusion.

These data indicate that agalsidase alfa is capable of decreasing the sphingolipid accumulation in the tissue. Because the accumulation of sphingolipids is regarded as the cause for the disease and its clinical presentation, the pharmacodynamic results indicate that a possible 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

Pharmacokinetic data in male Fabry Disease patients were derived from two studies. In study TKT001, pharmacokinetics of α-galactosidase A was evaluated following single dose escalation over the range of 0.007 – 0.110 mg/kg and in study TKT006 after multiple dose administration (0.2 mg/kg every 14 days). Pharmacokinetic data in females was derived from Study TKT014 after a single dose of 0.2 mg/kg. One analytical method was used for the determination of α-galactosidase A activity in plasma. The method was an enzyme activity assay, which utilises α-galactosidase A to hydrolyse 4-methylumbelliferyl-α-D-galactopyranoside. The amount of 4-methylumbelliferyl end products present in the sample following hydrolysis is measured with fluorescence detection.
Pharmacokinetic parameters (C_{max}, T_{max}, $T_{1/2}$, AUC_{∞}, MRT, V_{ss}, CL, dose proportionality) were estimated by both non-compartmental and compartmental analyses and were summarised using descriptive statistics. As α-galactosidase A is a protein itself, protein-binding studies were not carried out. All male subjects included were , 19 to 48 years old and female patients were 20 to 66 years old. The SPC reflects the lack of pharmacokinetic data in children.Data from an ongoing study in children, in which the pharmacokinetics of agalsidase alfa will be investigated, will be submitted post authorisation.
In study TKT001, patients received a single dose of α-galactosidase A as an intravenous infusion (25 ml over 20 min) at a dose of 0.007, 0.014, 0.028, 0.056 or 0.110 mg/kg. At each dose level 2 patients were included (male, aged 21 - 46 years). Blood samples were collected pre-dose and at frequent intervals after dosing. In addition, a liver biopsy was taken pre-dose and 44 hours post-dose.
After single dose administration, C_{max} and AUC of α-galactosidase A increased dose proportionally over the dose range 0.007 – 0.11 mg/kg. This indicates that α-galactosidase A clearance is not saturated at these dose levels. Following a single intravenous dose of 0.2 mg/kg, agalsidase alfa had a biphasic distribution and elimination profile from the circulation. Pharmacokinetic parameters were not significantly different between male and female patients. Elimination half-lives were 108 ± 17 minutes in males compared to 89 ± 28 minutes in females and volume of distribution was approximately 17% body weight in both sexes. Clearance normalised for body weight was 2.66 and 2.10 ml/min/kg for males and females, respectively. Based on the similarity of pharmacokinetic properties of agalsidase alfa in both males and females, tissue distribution in major tissues and organs is also expected to be comparable in male and female patients.

Data from liver biopsies showed a high uptake in liver tissue and a significantly longer half-life (greater than 24 hours) compared with plasma. The estimations of the in vivo tissue half-life are based on limited data (single liver biopsy specimens taken 44 hours post-infusion). These *in vivo* half life data were used to justify the dosing interval and the choice of dosing interval should therefore be supported by additional data.

The applicant has committed to performing a Phase IV clinical study post-authorisation to evaluate alternative initial and maintenance dosing schedules of agalsidase alfa and to identify the optimal dose and dosing interval. No anti-human α-galactosidase A antibodies were detected in any of the patients following single dose administration in Protocol TKT001.
In study TKT006, male Fabry patients were enrolled which completed the clinical trial TKT003. The subjects received a single dose of 0.2 mg/kg as a 40 min intravenous infusion every other week. Twenty-three patients could be evaluated. Ten of them received placebo in study TKT003 and 13 of them received 12 biweekly doses of 0.2-mg/kg agalsidase alfa. At the start of TKT006, antibodies were detected in 10 of the 14 patients from the active treatment arm of Study TKT003 by at least 1 of the 4 assays utilised (ELISA, immunoprecipitation assay, in vitro neutralisation assay, internalisation assay).

A majority of the patients who had received agalsidase alfa in TKT003 showed lower AUC values as compared to those who received placebo, something that may be due to an increase in clearance. The inter-individual variability in clearance was very large however. In all subjects with altered pharmacokinetics, antibodies against agalsidase alfa, which could serve as an additional clearance route, were detected. Overall, these results indicate that the forming of antibodies can alter the pharmacokinetics of α-galactosidase, causing a higher clearance of agalsidase alfa and a lower systemic exposure. However, the apparent change in clearance does not result in a change in the elimination half-life. 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
No specific in vivo clinical drug interaction studies have been performed. Drug-drug interactions arising from displacement of α-galactosidase and co-administered drugs - due to the influence of the cytochrome P450 enzymes - are not expected. Therefore it is acceptable, that no in vitro interaction studies are carried out and that no specific in vivo clinical drug interaction studies have been performed. However, there is a theoretical risk of inhibition of intracellular α-galactosidase activity by chloroquine, amiodarone, benoquin or gentamicin and this is appropriately reflected in section 4.5 of the SPC. In addition, any evidence of potential for drug interactions will be reported post-authorisation.

Special groups
The pharmacokinetics of agalsidase alfa was not altered in patients with end stage renal failure and dosage adjustment in renal impairment is not necessary. Renal elimination of agalsidase alfa is considered to be a minor pathway for clearance.
The influence of hepatic function on the pharmacokinetics of agalsidase alfa was not studied. As metabolic degradation is expected to follow the pathways of other proteins, i.e. peptide hydrolysis, an impaired liver function is not expected to affect the pharmacokinetics of agalsidase alfa in a clinically significant way.
The lack of data in children is sufficiently reflected in the SPC.

Clinical Efficacy

Dose response study

TKT001 was an unblinded, single dose, dose escalation, safety study of 10 patients. A single infusion ranging from 0.007 to 0.11 mg/kg was shown to be safe and well tolerated in this study.
The two major factors that determine the delivery of the enzyme to target tissues are hepatic clearance and plasma concentration. Data suggest that hepatic uptake of the enzyme can be saturated as proportionally less enzyme is taken up by the liver with increasing dose. A dose of 0.2 mg/kg was chosen for the pivotal studies so that, according to the applicant, a larger fraction of the dose would potentially be available to other target organs. An alternate week schedule was chosen for patients' convenience.

Although the clinical data from the two pivotal clinical studies (TKT003 and TKT005) have shown that the recommended dose is efficacious and safe, efficacy of other doses over a longer time of treatment should be explored. The applicant has committed to performing a Phase IV clinical study post-authorisation to evaluate alternative initial and maintenance dosing schedules of agalsidase alfa - after clearance of the accumulation of sphingolipids - and to identify the optimal dose and dosing interval.

Main clinical studies

Description of the Studies
Both pivotal phase II studies, TKT003 and TKT005, were randomised, double blind, placebo controlled, single centre, parallel group studies designed to evaluate the safety and efficacy of multiple intravenous doses of agalsidase alfa in male patients with confirmed Fabry Disease. Patients received agalsidase alfa at doses of 0 or 0.2 mg/kg every other week for a total of 24 weeks (12 doses).

TKT006 is a single centre, open-label maintenance study in patients who had completed TKT003. 12-month data from TKT006 were submitted. All patients from TKT005 have been entered into open-label study TKT007; 6-month interim data from TKT007 are available. The inclusion criteria in both studies were comparable in that only patients with clinical and biochemical evidence of Fabry Disease were eligible with the additional requirement of severe neuropathic pain in TKT003 and left ventricular enlargement in TKT005. Patients in TKT003 have mild cardiac involvement and patients in TKT005 have little if any pain.
The clinical trials were performed according to GCP standards and agreed international ethical principles.

Primary endpoints
The primary endpoint in study TKT003 was the effect of the enzyme replacement on serious debilitating pain, as measured by the brief pain inventory BPI, a quantitative, validated pain assessment scale. Measuring the effect of enzyme replacement therapy on medication use for chronic neuropathic pain, renal function, kidney pathology, plasma, urine sediment and kidney Gb3 content and cardiac structure and function also assessed efficacy.
The primary end point in study TKT005 was the effect of enzyme replacement therapy on cardiac Gb3 levels as determined from cardiac biopsy samples. Secondary endpoints were cardiac mass (MRI based assessment), renal function and Gb3 measurements in plasma and urine.

Statistical analysis
All statistical analyses of the efficacy data were carried out on all randomised patients (intent-to-treat analyses) and ANCOVA analyses were performed using the baseline value as the covariate for each analysis unless otherwise stated. Missing data was handled by the last observation carried forward method.

RESULTS
Pain:
During the first 24 weeks (Study TKT003) there was a progressive effect of Replagal on pain compared to placebo (p=0.021). In the extension phase (TKT006), the patients in the original Replagal treatment group of Study TKT003 demonstrated a 1.2 unit decline in their level of pain (p=0.063). Placebo patients switched to active treatment had a statistically significant decline in pain (p=0.020). After 12 to 18 months of maintenance therapy for all patients combined, Replagal significantly reduced pain by 1.9 units (p=0.003).
Pain medication was evaluated in two ways: a time-to-event analysis where the event was a permanent discontinuation of neuropathic pain medications and the number of days off pain medication.
There was a statistically significant effect of Replagal therapy on pain medication use in TKT003. Furthermore, four patients in the active treatment group were able to discontinue pain medication permanently as compared to none in the placebo group (p=0.031). The total number of days that patients were able to remain off pain medications in the Replagal treatment group was 93.5 days compared to 25.4 days in the placebo group (p=0.013). Further reductions in the use of pain medication were seen in the extension phase (TKT006).
Renal function, renal histology and metabolic correction:
The effect of Replagal on renal function was measured using creatinine clearance and GFR (glomerular filtration rate, using inulin clearance). GFR and creatinine clearance measured at baseline were 77.2 ml/min and 101.3 ml/min respectively for the agalsidase group, compared to 90.9 ml/min and 111.6 ml/min in the placebo group. By the end of the study, patients in the Replagal group had a higher creatinine clearance at 104.7 ml/min versus 93.0 ml/min in the placebo group (p=0.016). In the placebo group, on average, patients suffered a 20 ml/min reduction of their GFR. The stabilisation of renal function for Replagal patients was maintained during the extension phase in TKT006. In the placebo-active treatment cross-over group, the renal function improved after the introduction of Replagal. After 12 to 18 months of maintenance therapy, Replagal improved renal function as measured by inulin based glomerular filtration rate by 8.7 ± 3.7 ml/min (p=0.030).
The fraction of normal glomeruli at baseline as determined by histopathology was approximately 40% in the Replagal group compared to 60% in the placebo group. In line with the improved functional parameters, there was a 21% increase in the fraction of normal glomeruli and 33% decrease in mesangial widening following therapy with Replagal. In the placebo group, there was a 27% decrease

in the fraction of normal glomeruli and a 69% increase in mesangial widening. These effects were statistically significant thus demonstrating an improvement in renal pathology in the Replagal treated patients and a deterioration of renal pathology in the placebo treated patients.
Compared with placebo, treatment with Replagal resulted in a metabolic correction of glycosphingolipid levels in plasma, urine sediment and in kidney biopsy specimens with a mean decrease within the range of 20-50%. After 12 to 18 months treatment further metabolic correction of glycosphingolipid levels was observed with 50 – 80% reduction in plasma and urine sediment
Effects on cardiac Gb3, cardiac structure and function (TKT005):
Compared with placebo, treatment with Replagal resulted in a reduction of cardiac Gb3 storage. Cardiac Gb3 decreased by 19% in the active treatment group compared to a 9% increase in the placebo group (p=0.42). Compared with placebo, enzyme replacement induced a statistically significant (p=0.041) reduction in mean cardiac mass (4% decrease) on MRI compared with placebo (9% increase) after 6 months of Replagal treatment. Consistent with the effect on cardiac mass, there was a decrease in left ventricular end diastolic volume, however not statistically significant. In TKT006 Replagal effected a significant reduction in cardiac mass after 12 – 18 months of maintenance therapy ($p<0.001$).

Clinical studies in special populations

At the time of initial authorisation no studies in special patient groups had been performed and this was sufficiently reflected in the SPC. It is anticipated that children will be treated with agalsidase alfa and the applicant is currently undertaking studies in children in order to make an assessment of the benefit-risk ratio in this patient group possible.

In view of the X-linked nature of Fabry Disease, textbook descriptions of female heterozygous carrier patients have minimized the burden of disease compared with male patients**.** However, current evidence indicates that whilst the expression of the disease is variable, disease manifestations in female heterozygotes, in terms of symptoms and severity of disease, are very similar to male patients. To study the effects of Repagal in females an open label, safety and pharmacokinetic study in 15 patients (TKT014) treated for approximately 4 to 12 months with the recommended dose of 0.2 mg/kg every other week, has been completed post-authorisation and the SPC updated accordingly. Secondary efficacy endpoints were more or less the same as used in the pivotal studies conducted in males and included assessment of effects on the heart, kidney, metabolism and quality of life.

Results
The pharmacokinetics of Replagal in female Fabry patients was comparable with those in male patients. Similarly, the clinical effects seen in women are more or less comparable with those seen in men, suggesting comparable clinical efficacy.
Eleven female patients received at least 6 months of therapy with Replagal, which is the same duration as the efficacy studies performed in male patients. After six months of therapy, there were statistically significant declines in left ventricular mass (p=0.003), left ventricular mass index (p=0.003), interventricular septal thickness ($p < 0.001$), and left ventricular posterior wall thickness (p=0.028). A significant increase in the internal diameter of the left ventricle in diastole translated into a slight, but not statistically significant, improvement in ejection fraction. After six to nine months of Replagal, the mean cardiac mass index in these patients fell from 148.1 to 123.8 and 122.5 g/m2 Weeks 27 and 41 respectively. These changes represented an approximately 15% decline in cardiac mass in these patients over six months. Improvements in cardiac conduction system function were consistent with the echocardiographic results. After six months of treatment, there was an 8.7 msec decline in QRS complex duration in the 11 female patients who completed Week 27. This was statistically significant ($p = 0.007$).
Renal function remained stable in all 15 female patients throughout the course of therapy. The effects of Replagal on measured glycolipid levels over time were variable, making assessment of an overall effect in female patients treated for varying lengths of time difficult to interpret. In some patients there were demonstrated improvements in plasma and urine sediment Gb3 levels, consistent with metabolic correction of the underlying enzymatic defect of Fabry Disease with Replagal therapy. However, the value of glycolipid levels as potential surrogate markers of clinical efficacy has not been determined, and glycolipid levels may in fact be a poor predictor of clinical efficacy.

Improvement in several measures of quality of life as measured by the SF-36 health survey was reported, including statistically significant improvements in physical function, role-physical, role-emotional, and general health scales. The improvement in general health scale was noted as early as Week 13 and persisted at Week 27. These results suggest that the improvements in cardiac and metabolic function were translated into overall improvements in general health, which were reflected in statistically significant improvements in quality of life.

Clinical Safety

Patient exposure

Safety data were reported on a total of 40 patients from 4 clinical studies. Study TKT007 was ongoing and interim safety data from the first 6 months of the trial were provided.

Adverse events and serious adverse event/deaths

The most commonly reported adverse events were headache, influenza like symptoms, back pain, paraesthesia, pain, neuralgia, nausea, hyperkinesia, abdominal pain, diarrhoea, increased sweating and pharyngitis. Most AEs occurred in a similar number in both treatment arms, with the exception of infusion reactions and skeletal pain, which occurred more frequently in patients who received the active treatment. Infusion reactions, dizziness, fever, flushing and nausea were assessed as probably or possibly related to the study drug.

There were no treatment related deaths reported in any of the studies. In TKT003 a total of 7 serious adverse events were reported for four patients on agalsidase alfa: two infusion reactions, two cases of fever and one injection site bleeding related to the renal biopsy procedure, one chest pain, one decreased hearing and one case of anemia. The infusion reactions and one of the two fever reports were classified as possibly or probably related to the study drug.

All serious adverse events resolved except for hearing and vestibular disturbances in 2 patients and these are known complications of Fabry Disease. Among the placebo treated patients, two patients had serious adverse events reported; one patient developed renal failure and required peritoneal dialysis and one case of constipation and abdominal pain. No serious adverse events considered related to treatment were reported in TKT005 or in the extension phase of the studies.

Infusion reactions

With the recommended 40-minute infusion, the incidence of the infusion reactions was less than 10%. The reactions were mild – symptoms have included rigors (chills), facial flushing and back pain- and controllable by administration of low dose anti-histamines and low dose corticosteroids. The infusion reactions were not accompanied by respiratory symptoms, changes in vital signs, or urticaria. All patients were able to continue the infusion, despite the infusion reactions.

Antibody formation

In total, 22 of the 40 male patients who received agalsidase alfa in the 4 multidose studies have developed a low titer IgG antibody response. These IgG antibodies were measured by at least one of the four assays utilised (ELISA, immunoprecipitation, *in vitro* neutralisation of enzyme activity, or internalisation into normal human fibroblasts). There is evidence for development of immunological tolerance to agalsidase alfa, in that these antibodies became unmeasurable or antibody titres fell in 18 patients who developed antibodies. The presence of serum antibodies to agalsidase alfa has not been associated with clinically significant adverse events or any effect on clinical efficacy. In the studies conducted to date there has been no correlation between antibody status and infusion reactions; this will continue to be monitored post-authorisation. These infusion reactions were not problematic since they either subsided or were manageable with premedications. There is no evidence of IgE-mediated hypersensitivity of immune complex disease. In addition, 18-month human safety data do not give rise to concerns with reference to immune deposits such as serum sickness or vasculitis. No evidence of

immune complex deposition – based on electron microscopy - was found in the kidney following enzyme replacement in a limited study of antibody positive patients. No IgE, IgA or IgM mediated antibody responses were observed.

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 populations such as children or patients over the age of 65 were submitted and this has been sufficiently reflected in the SPC. Because it is to be expected that children will be treated, this should be considered an omission in the dossier. Studies in children have been initiated in order to make an assessment of the benefit-risk ratio possible for children.

Study TKT014 in female patients showed there were no significant gender related safety differences and the adverse events profile emerging from the submitted data was more or less comparable with that seen in males, with a higher reported incidence of headache in females compared to males. The SPC has been updated to include the safety experience in females accordingly. No infusion reactions were reported in females and there was no evidence of the development an antibody reponse. The comparative lack of immunogenicity in female patients compared to males suggests that Replagal is recognised by the immune system as a native protein. This may reflect that the presence of a small amount of residual enzyme in female patients infers some degree of immunological protection. There were no treatment related deaths reported during the study.

Discussion on Clinical aspects

Discussion on Clinical Efficacy

The results show that neuropathic pain in Fabry Disease is reduced but not abolished by Replagal treatment after 12 – 18 months of treatment. The benefit is maintained while on treatment.
The trial data showed that accumulation of sphingolipids could be found in both cardiac and renal parenchymal cells at baseline.
Reduced levels of Gb3 were shown in plasma, urine sediment and kidney compared with baseline. It is reasonable to conclude that the results of the primary and secondary end points in both trials consistently demonstrate that there is a benefit from treatment. The exact magnitude of pain reduction and the effect on reversibility in renal pathology are more difficult to estimate due to methodological limitations. There is an initial effect in stabilising renal function followed by improvement with long-term therapy. There is also an effect on reducing the mean cardiac mass (on MRI) and LVEDV but how that translates to improvement in function and morbidity remains to be defined. Any conclusion on metabolic corrections can only be drawn in comparison with the "normal" ranges when steady state levels are attained.
The overall conclusion that can be drawn is that all the chosen assessment parameters consistently show the following differences in the effects of Replagal compared with placebo, namely reduction in pain, improvement in cardiomyopathy, initial stabilisation followed by improvement in renal function, and some metabolic correction. It is difficult, given the limited data available, to quantify the precise magnitude of effect of agalsidase alfa and to estimate the relative amounts of glycosphingolipid deposits cleared from different cell types.
Treatment before symptoms develop should be considered because severe end organ damage may not be expected to be fully reversible. Although renal and cardiac function can be considered clinically important endpoints, death - the most important clinical endpoint – should be considered, since patients with Fabry disease are known to die prematurely from cardiac disease, renal insufficiency or cerebrovascular disease. Furthermore, potential surrogate markers for the assessment of progress of disease should be explored further and validated.

Discussion on Clinical Safety

The applicant has submitted data with a maximum follow-up of 18 months. The majority of adverse events were considered mild to moderate in severity in both treatment groups in the controlled trials. The majority of adverse events were considered unlikely to be treatment related except for infusion reactions. A greater number of patients experienced severe AEs in the placebo group compared with the agalsidase alfa treatment groups in the two controlled trials.
Safety data show an acceptable adverse event profile and tolerability to Replagal. However, although not problematic the infusion reactions and anti-agalsidase antibody formation give rise to some concern and should be paid special attention in post-authorisation studies. Mild infusion reactions accounted for the commonest treatment related serious adverse events. Infusion reactions are easily managed and may be prevented with appropriate oral medications such as anti-histamines prior to infusion.
Antibodies to α-galactosidase A developed in over 50% of patients but immunological tolerance could be demonstrated in over 80% affected within one year. No alteration to the treatment schedule was required in the studies. The presence of these antibodies was not associated with clinically significant adverse events or any effect on clinical efficacy. The applicant will provide information on the long term monitoring for the presence of antibodies to agalsidase alfa and their impact on safety and efficacy , as well as any clinical evidence of immune complex disease post-authorisation. In addition, any evidence of potential for drug interactions will be reported.
Safety data in patients without sphingolipid accumulation are lacking. These data should be submitted in order to assess safety after longer treatment.

5. Overall conclusions and benefit/risk assessment

Quality

The quality of Replagal is considered to be acceptable when used in accordance with the conditions defined in the SPC. Some additional points will be addressed as part of post authorisation commitments. 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 Replagal in decreasing the sphingolipid accumulation in the key target organs. Because the accumulation of sphingolipids is the cause for the disease and its clinical presentation, the pharmacodynamic results indicate that a possible clinical improvement or stabilisation among patients is to be expected. Results from the toxicology programme did not raise particular concerns for the safe use of agalsidase alfa. The lack of data on reproduction toxicity and lactation is considered a drawback. This has been adequately reflected in the SPC.

Efficacy

The results from clinical studies support the use of Replagal in the approved indication long-term treatment of Fabry disease (α-galactosidase A deficiency). The clinical data from the two pivotal clinical studies have shown that the recommended dose is effective and safe. The applicant has committed to evaluate alternative initial and maintenance dosing schedules. 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 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

The safety data demonstrates that Replagal is safe. Safety data show an acceptable adverse event profile and reasonable tolerability to Replagal. However, although not problematic the infusion reactions and anti-agalsidase alfa antibody formation give rise to some concern and should be paid special attention in post-authorisation studies. Safety data in patients without sphingolipid accumulation are lacking. The period of agalsidase alfa treatment in the clinical trials does not reflect the life-long treatment for necessary for Fabry disease. The number of patients who have received agalsidase alfa is relatively small, and thus the safety database is not as large as is often the case for new medicinal products. However, since the structure of agalsidase alfa is very similar to the enzyme produced naturally in humans, agalsidase alfa is not likely to cause unexpected adverse events. In order to collect additional long-term 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 supplementary documentation provided by the applicant, it was concluded that further data was needed to support the quality of the product. Although the applicant could address the majority of these questions as post-authorisation commitments, a number of issues were identified that needed further clarification. At an oral explanation before the CPMP, the applicant focused on the outstanding chemical, pharmaceutical and biological issues.
On the basis of the available information from on-going studies and on relevant commitments from the applicant, these issues were considered resolved.

A marketing authorisation for Replagal has been granted under exceptional circumstances, subject to fulfilling the chemical, pharmaceutical and biological follow-up measures and clinical obligations undertaken by the applicant. 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 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 Replagal.

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Replagal for use as long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry Disease (α-galactosidase A deficiency) was favourable.