SCIENTIFIC DISCUSSION

1. Introduction

Mucopolysaccharidosis VI (MPS VI) or Maroteaux-Lamy syndrome is a rare lysosomal storage disease (LSD) resulting from a deficiency in the enzyme N-acetylgalactosamine 4-sulfatase. There are an estimated 1100 cases in the developed world. Without sufficient enzyme activity, dermatan sulfate degradation is blocked, resulting in the intracellular accumulation of this substrate in the lysosomes. The accumulation causes a progressive disorder with multiple organ and tissue involvement. MPS VI is an inherited autosomal-recessive disorder, and carriers do not exhibit any biochemical or clinical evidence of disease.

As with all the MPS disorders, MPS VI is a clinically heterogeneous disease in terms of the extent of organ impairment and rate of disease progression in affected individuals. Case studies in the literature have identified subjects presenting with marked disease in the first year of life and those presenting with slowly advancing disease that progressed over many decades. The most rapidly advancing form of the disease presents with deceleration of growth, skeletal deformities, coarse facial features, upper airway obstruction, recurrent respiratory and ear infections, and joint abnormalities.

Ultimately, affected individuals become wheelchair bound or bedridden secondary to skeletal deformity, joint disease, cardiopulmonary disease, blindness and spinal cord compression. They eventually succumb to infection, the complications of surgery, or cardiorespiratory failure. MPS VI is not associated with impairment of cognitive function, although physical limitations may impact learning and development. Both slowly and rapidly progressive disease can lead to a significant loss of function for affected individuals.

The expression of functional ASB protein is a crucial determinant influencing the clinical phenotype of MPS VI. Although the key structural features of the ASB glycoprotein are known, the identification of over 45 mutations at the DNA level has hindered the ability to unequivocally predict phenotype from genotype .The majority of these mutant alleles are either unique or are present in a small number of subjects, and no predominant mutations have been described. Biochemical heterogeneity resulting from the combination of two different mutant alleles also contributes to clinical heterogeneity.

Subjects along the entire spectrum of slowly to rapidly advancing disease are profoundly deficient in endogenous enzyme activity, hindering predictions of clinical outcomes based on residual ASB activity levels. Despite the genotypic and phenotypic heterogeneity of MPS VI, the clinical outcomes resulting from chronic lysosomal storage can be traced to the characteristic anatomic, pathophysiological changes in affected organs in patients with this disease.

There is no satisfactory treatment for MPS VI. Many patients have benefited from bone marrow transplantation (BMT), and, in general, responses to engraftment in terms of biochemical and clinical parameters have been quite satisfactory, although corneal cloudiness and skeletal dysplasia are unaffected even with successful engraftment BMT is associated with substantial morbidity and mortality, and is limited by a lack of suitable donors. The European Group for Bone Marrow Transplantation reported transplant-related mortality of 10% (HLA identical) to 20–25% (HLA mismatched) for 63 transplantations for lysosomal disorders. As many as 50% of the surviving patients who engraft develop graft versus host disease. Finally, there is a relatively high rate of graft failure, even for patients undergoing a second transplant. The risk benefit profile of transplantation in LSDs has thus been complicated. More recently, reports of success in cord blood transplantation for patients with MPS VI and MPS I portend improvements in the risk/benefit profile for transplantation in these disorders.

In the absence of treatment with BMT, MPS VI patients typically receive only symptomatic care. In addition to medication for infections, cardiac failure, and other complications, MPS VI patients typically receive physical and occupational therapy for their stiff joints and other non-invasive nterventions such as positive airway pressure (CPAP or BiPAP) for sleep apnea.

Extensive surgical procedures are commonly undertaken to mitigate the progressive effects of MPS VI. Despite these interventions, MPS VI remains a serious and progressive disease producing profound disability that is fatal in most cases.

Galsulfase is a recombinant human N-acetylgalactosamine 4-sulfatase (rhASB, Arylsulfatase B), indicated for the long-term enzyme replacement therapy (ERT) for patients with mucopolysaccharidosis VI (MPS VI), also referred to as Maroteaux–Lamy Syndrome.

Recombinant human N-acetylgalactosamine 4-sulfatase is a soluble monomeric protein with a molecular weight of 56 kD; the glycosylated product has an apparent molecular weight of 66 kD on SDS–PAGE. The predicted amino acid sequence and the nucleotide sequence of the recombinant enzyme are identical to native human N–acetylgalactosamine 4–sulfatase (also known as arylsulfatase B, or ASB; E.C # 3.1.6.12). The enzyme is responsible for hydrolysis of the sulfate moiety of the glycosaminoglycan (GAG) dermatan sulfate during its stepwise degradation.

Galsulfase is indicated for long-term enzyme replacement therapy in patients with a confirmed diagnosis of Mucopolysaccharidosis VI (MPS VI; *N*-acetylgalactosamine 4-sulfatase deficiency; Maroteaux-Lamy syndrome) to treat the clinical manifestations of the disease.

The recommended dosage regimen for Naglazyme is 1 mg/kg body weight administered once every week as an intravenous infusion over 4 hours. The initial infusion rate is adjusted so that approximately 2.5% of the total solution is infused during the first hour, with infusion of the remaining volume (approximately 97.5%) over the next 3 hours. The safety and efficacy of Naglazyme in children below the age of 5 years and in patients older than 65 years have not been established and no dosage regimen can be recommended in these patients.

The safety and efficacy of Naglazyme in patients with renal or hepatic insufficiency have not been evaluated and no dosage regimen can be recommended in these patients.

2. Quality aspects

Introduction

Naglazyme is an orphan drug whose proposed therapeutic indication is the long-term enzyme replacement therapy in patients with a confirmed diagnosis of mucopolysaccharidosis VI (Matoteaux-Lamy syndrome), which is a rare storage disorder caused by a deficiency of the lysosomal hydrolase N-acetylgalactosamine 4-sulphatase (arylsulphatase B, ASB). Reduced or absent ASB activity results in an intracellular accumulation of the glycosaminoglycan dermatan sulphate, which causes a progressive and clinically heterogeneous disorder with multiple organ and tissue involvement.

Galsulfase, the active ingredient of Naglazyme, is a recombinant human N-acetylgalactosamine 4sulphatase (rhASB). It is a single-chain protein of 56 kDa heterogeneously glycosylated. Galsulfase uptake by cells into lysosomes is most likely mediated by the binding of its bisphosphorylated oligomannose₇ oligosaccharide chains to specific mannose-6-phosphate receptors.

Galsulfase is produced by recombinant DNA technology in a CHO-derived cell line, using a perfusion process, and purified by a series of concentration, column chromatography and filtration steps. The drug product is prepared by sterile filtration and aseptic filling into vials of the Formulated Bulk Drug Substance (FBDS).

Naglazyme is provided as a concentrate for solution for infusion in a single-use vial, which contains a nominal amount of 5 mg of galsulfase in 5 mL (concentration 1 mg/mL). Galsulfase is formulated with sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, sodium chloride, polysorbate 80 and water for injections. The drug product is a clear to slightly opalescent and colourless to pale yellow solution that has to be diluted in 0.9 % sodium chloride solution prior to administration.

The container closure system consists of a type I borosilicate glass vial (Ph. Eur.), a siliconized chlorobutyl rubber stopper (Ph. Eur.) and an aluminium seal with a polypropylene flip-off cap.

Active Substance

Nomenclature	
INN Name:	galsulfase
Compendial name:	not applicable
Chemical name: (rhASB)	recombinant human N-acetylgalactosamine 4-sulphatase, arylsulphatase B

Description of the drug substance

Galsulfase is a single-chain protein of 495 amino acids / 56 kDa after cleavage of the signal peptide. It contains six asparagine-linked glycosylation sites carrying a mixture of complex, high-mannose and phosphorylated high-mannose oligosaccharides. RhASB has eight cysteine residues, all of which are linked by intramolecular disulfide bridging. The cysteine residue in position 53 is post-translationally modified to formylglycine in endoplasmic reticulum. This modification, found in all sulphatases, is required for the enzymatic activity.

• Manufacture

The FBDS is produced by BioMarin, Galli, Novato, California, USA, with a manufacturing process similar to that used for the drug substance of the centrally authorised product Aldurazyme (recombinant human L-iduronidase) which is manufactured at the same facility. This manufacturing site was last inspected in June 2005 by the MHRA. The GMP issues raised during the evaluation procedure, such as definition of batch size and pooling strategy in the production of the FBDS, and the deficiencies observed during the previous inspection carried out in August 2002 are considered resolved. The facilities are operated in current GMP (cGMP) compliance, with standard operating procedures in place to describe all procedures and controls.

Development genetics

The expression plasmid pEF4S was constructed using the cDNA of rhASB and fused to the rat preproinsulin 5' UTR to provide strong translation initiation. This plasmid contains a neomycin phosphotransferase marker gene for the selection of transfected cell lines using geneticin, the SV40 origin of replication and the ampicillin resistance marker gene for the initial cloning in bacteria. This pFE4S plasmid was electroporated into CHO-K1 cells. After selection with geneticin, one stable clone was isolated and sub-cloned to obtain the cell line CSL-4S-342.

Cell bank system

A cell bank system has been developed and maintained in accordance to cGMP and ICH guidelines Q5A, Q5B and Q5D. For the construction of the Master Cell Bank (MCB), CSL-4S-342 cells underwent a series of amplifications and aliquots of cell suspension were processed for storage in cryovials in the vapour phase of liquid nitrogen. The MCB and the initial Working Cell Bank (WCB1) were prepared at Tektagen (now Charles River Laboratories), Malvern, Pennsylvania, USA. WCB1 was used to produce galsulfase for all preclinical studies, Phase 1 clinical trials and some Phase 2 clinical trials. Upon exhaustion of WCB1, a second Working Cell Bank WCB2 was prepared at BioReliance, Rockville, Maryland, USA, using a different serum-free media. Foetal calf serum (FCS) and trypsin were not used in the growth and passage of the cell line CSL-4S-342 but FCS was used in the culture of the originator cell line CHO-K1.

Procedures followed in the preparation of MCB and WCBs have been appropriately described. An appropriate range of tests has been performed for their characterisation. Cells banks were extensively examined for the presence of microbial and viral contaminants, including endogenous retroviruses known to be present in CHO cell lines. Genetic identity and stability studies, following ICH Guidelines Q5B and Q5D, confirmed the expected genetic characteristics of the cell line and the stability of cells over the course of the longest production run described below.

For commercial production, the MCB will only be used in case of emergency; otherwise the company will use WCB2, as this is presently the case. The current supply of WCB2 is expected to last for ten years. The applicant has taken measures to store some cell bank material offsite in the event of any unforeseen circumstance.

Fermentation process

The seed train starts by the thaw of one vial of WCB (or MCB) and inoculation of its content in a cell culture flask. When the target density is reached, a series of amplification steps is initiated. Bioreactors are inoculated according to a parallel or sequential seeding procedure. When the target density is reached, the harvested cell culture fluid (HCCF) is collected, sampled for analysis, and stored until the first step of the purification process is initiated.

The operation and control of the fermentation process from the cell culture inoculum expansion to the final cell harvest are adequately described and appropriate validation data were provided. Full details of the cell culture raw materials, their source and control were provided.

Purification process

Purification of rhASB from the HCCF involves the following steps: harvest filtration to remove cells and debris and concentration using an ultrafiltration system, pH adjustment and clarification, and column chromatography. One to three initial column chromatography cycles can be combined to form a single lot, which then passes through two additional orthogonal chromatography steps, andaccompanied by low pH viral inactivation. The product is then processed by ultrafiltration/dialfiltration for concentration and buffer exchange into the final formulation buffer5.. Following DNA removal filtration and 0.02 μ m viral removal filtration, formulation buffer is added to dilute the rhASB to a final protein concentration of 1 mg/mL and polysorbate 80 is added. The FBDS is then transferred through a 0.2 μ m filter into sterile storage containers and can be stored up to 12 months at 5 _ 3 C.

During the purification process, pooling can occur prior to three key stages: the initial ultrafiltration, pH adjustment (in conjunction with the initial column chromatography) and the second column chromatography step. One lot of FBDS is defined as the eluate from a single run of the second chromatography column, and then carried down through the process.

Appropriate information on the regeneration and sanitisation of the respective columns along with their storage conditions/solutions has been provided. Storage conditions for the process intermediates have been specified and validation of holding times for each intermediate has been performed.

Purification steps have been designed to remove process-related impurities, such as host cell proteins, retroviral particles and DNA, product-related impurities, such as aggregates and degraded products, and adventitious contaminants, such as viruses and mycoplasmas. Some of the methods used to determine the impurity profile have not been included in the release testing of the FBDS and their omission has been justified.

DNA content has been monitored during the purification of seven consecutive production lots manufactured at the Galli site using the current process. Conservative calculations in the worst-case scenario meet the WHO limit of <10 ng/dose. The justification for not including host cell DNA evaluation in the release testing was considered acceptable.

Residual host cell proteins (CHOP) have been determined at several points in the purification process of three consecutive production lots, using both qualitative and quantitative methods. The data support the capacity of the purification process to consistently remove host cell proteins and the fact that production lots will meet the release specification proposed.

Appropriate data were provided on the removal of glycerol (residual solvent used in the second column chromatography step) and potential column leachable materials.

With regard to product-related impurities, the three common degradation pathways for proteins such as rhASB, oxidation, aggregation, and deamidation have been considered. Forced degradation studies have been performed to show that rhASB is resistant to each of these pathways.

The viral safety of rhASB has been demonstrated following a series of studies to characterize the production process, raw materials and each lot of rhASB. The applicant is in compliance with the latest version of the CHMP/CVMP Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products. Cell banks have been satisfactorily demonstrated to be free from detectable viral contaminants.

No animal sourced materials are used in the commercial-scale rhASB manufacturing process.

Manufacturing process development and process validation

The drug substance is manufactured using a standard fermentation and purification process. A number of changes, however, have been made during product development, which can be grouped in four categories:

Cell culture: the initial process was conducted using a fed-batch process. Transition to a perfusion process was implemented to increase productivity for Phase 2 clinical studies and beyond.

Purification: modifications to the purification process, including the elimination of one chromatography step (DEAE Sepharose), optimization of the remaining chromatography steps, and increase of the diameters of all three remaining columns were made to improve the capacity of the process and the purity of the product.

Formulation: the addition of polysorbate 80 was implemented for Phase 2 studies and beyond to prevent particulate formation in the drug product that was observed during development.

Facility: the process was moved from the BMK clinical facility to the Galli commercial facility to increase production capacity. The commercial (Galli facility) process represents a modified version of the BMK perfusion process in that longer cell culture duration and a different HCCF sublot pooling strategy were employed. The purification scheme remained unchanged except for larger column diameters for increased HCCF processing capacity.

Product manufactured by the commercial process has not been used in clinical trials. In this respect, an issue was raised during the assessment regarding the comparability of FBDS between lots manufactured at the Galli site using the current commercial process and those manufactured for the clinical studies at the BMK site prior to the final modifications to the manufacturing process (see below).

Satisfactory validation data have been provided for three qualification lots manufactured at the Galli site with the current commercial process. Validation studies were designed to demonstrate the acceptability, robustness and reproducibility of the defined manufacturing process.

Characterisation

- Physicochemical characterisation:

A series of standard techniques to study the protein and carbohydrate structure of rhASB has been employed.

The crystal structure of rhASB has been determined by X-ray diffraction; this and a variety of other biochemical studies have identified the amino acid residue formylglycine in position 53.

Peptide maps were produced from tryptic digests using standard methods. No significant structural differences were detected between the reference materials and materials from the production lots. Peptides were identified by mass spectrometry with the exception of several non-standard peptides that were identified by tandem mass spectrometry and were consistent with the rhASB sequence.

N-terminal amino acid characterisation was performed using standard methods (Edman degradation and mass spectrometry of tryptic peptides). All samples were found to be identical and consistent with the predicted nucleotide sequence of the rhASB cDNA used to prepare the production cell line. Two truncated N-terminal variants of rhASB have been identified. On the basis of the arguments provided by the applicant, it was considered unlikely that the observed degree of truncation would affect the clinical safety or efficacy of the product. C-terminal amino acid characterisation also detected smaller amounts of a variant resulting from the truncation at the C-terminus of rhASB. The applicant committed to continuing the investigation of these C-terminal truncated forms and to providing a formal review once sufficient data are available.

Determination of disulphide linkages demonstrated the presence of four disulphide bridges and no free sulphdryls. The results are consistent with crystal structure of rhASB.

Determination of protein concentration by UV absorbance and quantitative amino acid analysis have been performed. Amino acid composition coincided with that expected from the cDNA with

the exception of tryptophan composition which was consistently lower that expected in all lots tested.

IEF studies showed that the charge heterogeneity of rhASB is conferred by oligosaccharides and that the parent protein backbone is essentially homogeneous. In addition, it appeared that rhASB has a high phosphorylated oligomannose content compared to that of sialic acid.

Principal SDS-PAGE silver-stained bands observed have been categorised or specifically identified, including degradation products and host cell protein impurities, using multiple techniques.

FACE analysis was used for the characterisation and assignment of N-linked oligosaccharides. The most predominant class of oligosaccharides found on rhASB was phosphorylated high mannose, and the most abundant member of this class is bis-phosphorylated oligomannose₇, the oligosaccharide ligand for the target cell surface receptor. The other groups identified are high mannose (oligomannose_{5,6,7}) and complex (sialylated, core fucosylated) oligosaccharides. The site-specific glycosylation microheterogeneity of rhASB was studied by the analysis of tryptic peptide digests using RP–HPLC with electrospray mass spectrometry. The six consensus N–linked glycosylation sites on rhASB were confirmed, with an occupancy rate >99%.

With the sialic assay method, incorporating HPAEC and pulsed amperometric detection, the only species identified from the acidic hydrolysis of rhASB was NeuAc.

• - Biological characterisation:

Potency has been determined by 2 methods which are used in the release testing of the drug substance and drug product. The first one is the enzymatic activity assay that utilises 4-methylumbelliferyl sulphate (4-MUS), a small fluorigenic substrate. This assay has been modified and optimised during development. The second method is the fibroblast uptake assay, a cell-based assay that demonstrates the receptor-mediated endocytosis of rhASB into the cell in an active form.

Specifications

Release specifications for rhASB are based on historical manufacturing and stability data compiled on 21 batches of FBDS produced using the perfusion process. The proposed specifications reflect the variability of both the analytical methods and the manufacturing process and take into consideration the clinical experience gained during development of rhASB. The analytical methods included in the control system are used to evaluate the identity, quality, safety, purity, potency, strength and composition of the FBDS, and to assure the consistency of physical, chemical and biological attributes of rhASB. All assays used in the release and stability testing of rhASB have been validated according to ICH guidelines. The current FBDS release specifications were considered acceptable provided that the applicant commits to reviewing certain tests and limits as part of follow-up measures.

• Stability

The applicant has performed real-time and accelerated stability studies designed in accordance with ICH guidelines to monitor the time-temperature stability of cGMP lots.

The characteristics included in stability testing were chosen based on method validation results and characterisation studies. The appropriateness of the methods for indicating the stability of rhASB FBDS has been further supported by forced-degradation studies.

The applicant proposed a shelf life of 12 months at 5 3 C for the drug substance. This has been supported by 12 months of real-time data for five batches of FBDS manufactured using the current commercial process at the Galli site and 24 months of real-time data for two batches of FBDS manufactured using the clinical perfusion process at the BMK site.

The storage conditions and controls for the transportation of the FBDS from the Galli site to the site for manufacture of the drug product have been supported by appropriate data on stability and container systems.

Medicinal Product

• Pharmaceutical Development

Naglazyme is administered by intravenous infusion once weekly over 4 hours. The route and duration of administration were chosen to provide maximum bioavailability of the enzyme for lysosomal delivery. Oral administration was not considered due to concern for the stability of the protein within the digestive tract.

Acidic phosphate buffered saline was selected as the Naglazyme formulation buffer based on experience with a previous centrally authorised product (Aldurazyme) for which a similar buffering system had been successful. During pre-clinical production of the drug product, particulates were observed in vials of drug product. SDS–PAGE and Western blot analysis indicated that it was composed of aggregated galsulfase. After a series of formulation studies, polysorbate 80 was identified as stabiliser in a formulation composed of sodium phosphate, sodium chloride pH 5.8 (tonicity modifier) and water for injections. All the excipients comply with Ph. Eur. requirements.

In order to ensure that a 5 mL volume of solution can be extracted from the vial, an overage of 0.3 mL is filled into the vial (total fill volume of 5.3 mL corresponding to a 6% overfill).

• Manufacture of the product

The FBDS is shipped to Hollister-Stier Laboratories, Spokane, Washington, USA for manufacture of the drug product. This process involves final sterile filtration using 0.2 m filters, filling into vials and preliminary labelling. Satisfactory validation data has been provided to give assurance on the sterile filtration, filling and capping procedures. Media fill data are supplied to support the aseptic process. Media fills are performed routinely.

Hollister-Stier was last inspected in June 2005. The GMP issues raised during the evaluation procedure, such as definition of batch size, have been adequately addressed and it was confirmed that no pooling occurs at this site. The facilities are operated in current GMP compliance.

Specifications

Release testing is performed at NDA Analytics Ltd., Alconbury, Cambridgeshire, UK, and labelling, packaging and final QP release is performed at Cardinal Health, Westhoughton, Bolton UK.

The proposed specifications for the drug product have been justified based on historical manufacturing and stability data compiled on 17 batches of drug product using the perfusion process. Analytical procedures have been adequately described and validated. The current drug product specifications were considered acceptable provided that the applicant commits to reviewing certain limits as part of follow-up measures.

• Stability of the Product

Real-time and accelerated stability studies were initiated in accordance with ICH guidelines and per protocol to monitor the time-temperature stability of cGMP lots of rhASB drug product. 12 months of real-time data for four batches of drug product manufactured with the current commercial process at the Galli site were provided, as well as up to 30 months of real time data for 3 batches of drug product manufactured at BMK with the perfusion process. Based on the data provided, the approvable shelf life for the drug product is 12 months at 5 _ 3 C.

Discussion on chemical, pharmaceutical and biological aspects

In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines.

The cell bank system used for the manufacture of the drug substance is appropriately described and an appropriate range of tests has been performed. The fermentation and purification processes of the drug substance are adequately controlled and validated. The applicant has provided full details of the cell culture raw materials, their source and control.

An issue was raised during the assessment regarding the definition of batch size and the pooling strategy. Lots produced to date have varied widely in size dependent on manufacturing facility

and processing equipment. The applicant committed to reviewing the range of batch sizes of the FBDS once 20 lots will be manufactured. The applicant satisfactorily clarified the pooling procedures across the entire production of Naglazyme and the controls employed, including batch release testing of individual FBDS and pooled batches.

Issues regarding column sanitisation and viral validation studies raised during the assessment have been resolved. It was noted that only 60 cm columns for the orthogonal chromatography steps (the initial step uses an 80 cm column) could be approved at this stage for the current manufacturing process.

The drug substance has been well characterised with regard to its physicochemical and biological properties, using state-of-the-art methods. The current FBDS release specifications are considered acceptable but the applicant committed to reviewing certain tests and limits as part of the follow-up measures. This includes the development of a quantitative charge heterogeneity assay as a replacement for IEF, the development of an alternative assay to replace FACE analysis for the control of oligosaccharide consistency, the revision of the current specification limits for polysorbate 80, sialic acid levels and potency measured by the optimised 4-MUS activity assay once data from 20 batches manufactured using the current process will be available. The applicant also committed to continuing the investigation of C-terminal truncated forms and to provide a formal review of available data and specifications.

A number of changes have been made during product development including the manufacturing site, manufacturing process, procedures, test methods for the activity assay and reference standards. One major objection had been identified and concerned the comparability of FBDS between lots manufactured at the Galli site using the current commercial process and those manufactured for the clinical studies at the BMK site prior to the final modifications to the manufacturing process. Product produced by the commercial process has not been used in clinical trials. With the responses to the CHMP Day 120 list of questions and Day 180 list of outstanding issues, the applicant made progress towards demonstrating comparability. Given the impossibility to generate additional characterisation data due to age and availability of BMK material, the applicant provided a review of characterisation data previously submitted, raw data from release assays for five BMK batches of bulk drug substance used in Phase 3 and extension studies, and a side-by-side comparison of quantitative results at both manufacturing sites. The applicant confirmed that they will include additional glycan monitoring in their ongoing stability program. Given that the data currently available does not raise any significant concerns and that the applicant also provided justification of how their clinical post-marketing surveillance programme will evaluate comparability, this issue was considered resolved. However, it was recommended that the applicant should inform the CHMP promptly if post-marketing surveillance data indicates that a comparability concern regarding the product manufactured at the Galli site and the current clinical lots, such as immunogenicity, arises.

The manufacturing process of the drug product has been sufficiently described and validated. The quality of the drug product is controlled by adequate test methods and specifications. However, the applicant committed to revising the specification limits for the 4-MUS activity assay and polysorbate 80 once sufficient batch data are available, in line with reviewed FBDS specifications.

Based on the stability data provided, a shelf life of 12 months at 5 3 C has been granted for the drug product. An increase in shelf life may be considered post-approval by means of a variation once further real-time data on the product manufactured using the current commercial process has been obtained. The applicant agreed to introduce sialic acid testing and any other appropriate method of monitoring the oligosaccharide profile into the stability programme at suitable multiple time points for at least three lots of drug product manufactured from three different lots of FBDS. It was pointed out that any new oligosaccharide test introduced at release testing should also be evaluated with respect to stability testing. The requirement for such tests in the stability programme may be reviewed once sufficient batches have been tested to establish their stability indicating potential. The applicant committed to providing on an ongoing basis completed stability reports for the drug substance and drug product.

The viral safety and safety concerning other adventitious agents, including TSE, have been sufficiently assured.

Except for a number of quality points, which will be addressed as part of post-approval follow-up measures, the quality of Naglazyme has been adequately demonstrated.

3. Non-clinical aspects

Pharmacology

The lysosomal enzyme N-acetylgalactosamine 4-sulphatase (arylsulphatase B, ASB) is deficient in patients with MPS VI, resulting in an accumulation of glycosaminoglycans (GAGs) in tissues throughout the body that leads to widespread cellular, tissue and organ dysfunction. Recombinant human ASB (rhASB) is intended to replace ASB and reduce the manifestations of the disease by increasing the catabolism of GAGs.

• Primary Pharmacodynamics (In Vitro/In Vivo)

In vitro studies have shown that rhASB is taken up by normal and MPS VI patient fibroblasts via a mannose-6-phosphate receptor. The mannose-6- phosphate receptor is present on most cells. Once bound to the receptor, the enzyme is transported by endocytosis to the lysosomes, where it hydrolyses the sulphate ester from N-acetylgalactosamine 4-sulphate residues at the end of the GAGs such as dermatan sulphate.

The in vivo pharmacology studies were conducted in the feline model of MPS VI, which has similar aetiology and morphological characteristics/disease pathology to the human disease. Similarities include facial dysmorphia, corneal clouding, reduced body weight, bone abnormalities and reduced cervical spine flexibility, mild hepatosplenomegaly, thickened cardiac valves and the absence of CNS lesions, although there are also differences such as the lack of respiratory effects in cats, and differences in patterns of urinary GAG excretion.

Some of the studies also included an evaluation of safety and/or tissue distribution of rhASB. The main breeding colony of MPS VI cats in the world is at the Lysosomal Storage Disease Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, Australia. There is no commercial supplier of these cats, and the pharmacology studies were conducted under the supervision of Dr. J Hopwood, who maintains the colony at the Unit in Adelaide. The studies were designed as research studies rather than for regulatory submission and were non-GLP compliant. However this is acceptable as the studies have been conducted in a proper scientific manner, and despite the low numbers of animals used, the results appear consistent across studies.

The pharmacology studies ranged from 5 weeks to 20 months in duration. Doses and dose schedules were varied. Most of the cats received rhASB weekly; 3 cats in an early study received enzyme biweekly for part or all of their treatment. Doses ranged from 0.2 to 5.0 mg/kg rhASB but most of the cats received 1 mg/kg rhASB, although no rationale is presented as to why this dose was used more frequently. In the earlier studies, rhASB was administered by bolus IV injection, and in the later studies, the drug was administered by intravenous infusion over about two hours.

There were also changes in the production process and manufacturing site, as well as a change in formulation, during development: In the initial non-clinical pharmacology studies, rhASB was formulated in phosphate buffered saline (PBS). Due to the formation of aggregates of rhASB, the product was re-formulated. The addition of polysorbate 20 or polysorbate 80 (0.001%) did not appear to affect the pharmacological properties of rhASB in the feline MPS VI model, although only 4 cats were used. The formulation was subsequently changed to acidic phosphate buffered saline (10 mM sodium phosphate, 150 mM sodium chloride, pH 5.8) containing 0.005% polysorbate 80. This formulation was used in the remaining non-clinical studies and is the final formulation.

Despite the low number and variability of the animals used in the studies and the changes in manufacture and formulation, treatment of MPS VI cats with rhASB resulted in a reduction in urinary GAG levels and reduction of lysosomal GAG storage in a number of tissues including liver, skin and kidney, although there was no effect in cornea or cartilage. In some studies,

skeletal pathology was reduced, producing more normalised bone dimensions and more uniform bone density. Greater mobility and flexibility were seen in some studies, and in others there was some improvement in the neurological symptoms caused by spinal cord compression.

Although the dose-ranging was restricted in the feline MPS VI model due to the limited number of available animals, the studies did show dose-dependent pharmacological effects in the reduction in MPS VI disease symptoms. There was minimal effect at 0.2mg/kg/week, with a clear improvement at 1 mg/kg/week and a slightly greater effect at 5.0 mg/kg/week. However, only one cat was treated at 0.2 mg/kg and there was no dose intermediate between 0.2 and 1 mg/kg administered weekly. The lowest dose to produce significant pharmacological effects in this study was 1 mg/kg/week. The pharmacological response to treatment with the homologous feline protein (rfASB) at 1 mg/kg/week was comparable to that of rhASB at 5.0 mg/kg in MPS VI-affected cats. This suggests that the lowest pharmacologically active dose of rhASB in cats (1 mg/kg/week) may be more active in the homologous (human) species.

Antibodies to rhASB were detected in MPS VI cats treated with rhASB, although there was much variability between animals with respect to the timing of development of antibodies and the levels produced. There was no correlation between the presence of antibodies and apparent clinical response. In some animals, the antibody level fell despite continuing treatment, suggesting tolerance. In one study (ASB-PC-001), the cat that developed high anti-rhASB antibody titres showed a reversal of clinical improvement; plasma from this cat inhibited rhASB activity in vitro confirming the neutralising property of these antibodies. However the same cat subsequently improved clinically once again, in the presence of fluctuating levels of antibodies. No other cases of neutralising antibodies were reported in the non-clinical pharmacology studies. In Study ASB-PC-005, elevated anti-rhASB antibody titres in some animals correlated with abnormal clinical signs during infusions, and may have been responsible for reductions in rhASB activity seen in a number of tissues. However, the titres did not appear to correlate with disease progression. Dialysed plasma from treated cats inhibited rhASB in vitro, although a similar effect was seen with dialysed plasma from untreated normal cats. Antibodies were not produced in studies where treatment was started in newborn cats, or where the homologous protein, rfASB, was administered.

There were some infusion-related mild to moderate anaphylactoid-type reactions to the heterologous protein in some studies. These reactions were managed in most cases by antihistamine treatment or by slowing the infusion rate. These reactions were not seen in the cats that were treated from birth. Treatment of the cats from birth also appeared to have more effect on reducing disease pathology, particularly skeletal pathology, than when treatment was started when the cats were at least three months old.

• Secondary Pharmacodynamics / Safety Pharmacology

Safety pharmacology parameters were monitored in the single-dose toxicity study in dogs and the repeated-dose toxicity study in cynomolgus monkeys. There were no adverse effects of rhASB on blood pressure, body temperature, respiration rate, ECG, blood coagulation or urinalysis parameters in dog or monkey, nor any effect on blood oxygenation in monkeys.

• Pharmacodynamic Drug Interactions

No studies were performed, and this was considered acceptable.

Pharmacokinetics

• Absorption- Bioavailability

Absorption and bioavailability are complete as the administration route is intravenous.

Three comparative pharmacokinetic studies in dogs showed that enzyme produced by different production runs, at different sites and from different run lengths of the same process showed that they were equivalent with respect to pharmacokinetic parameters, although the different runs from the same process were similar rather than strictly equivalent as the lower limit of the confidence interval fell slightly below the equivalence window.

• Distribution

Tissue distribution has been investigated in five of the pharmacology studies in MPS VI-affected cats. rhASB was widely distributed into tissues, with the largest proportion localised to the liver in all studies. There were also significant levels in the spleen, lung, kidney, heart, skin, aorta, cerebrum, cerebellum and lymph nodes in one study, compared to levels in a normal control cat. The tissue half-lives ranged from 2.4 to 4.2 days at 1 mg/kg. Low, but detectable levels were seen in these tissues (except heart) up to seven days following dosing. This suggests that the weekly clinical dosing frequency is reasonable.

In other studies, the levels found in heart, skin, mesenteric lymph nodes, aorta, cerebrum and cerebellum were lower or undetectable. rhASB was found in the cornea in only one study, and was not detected in the cartilage in any of the studies.

In one study, a reduced enzyme activity in a number of tissues in one cat and some tissues in the other three cats corresponded with increased antibody titres in these animals.

Two-hour infusions appeared to increase enzyme levels in some tissues and organs over those observed after the 15-minute infusion. The inclusion of polysorbate 20 or polysorbate 80 in the formulation did not appear to affect the pattern of distribution, pharmacokinetics or pharmacology of rhASB in MPS VI cats. The polysorbate was included at 0.001% in this study, as compared with the 0.005% polysorbate 80 that is included in the final formulation. However, the toxicity studies (and therefore toxicokinetics) were conducted with the final formulation containing 0.005% polysorbate 80.

• Metabolism And Excretion

No metabolism studies have been conducted as the product is eliminated via proteolysis.

In normal cats, rhASB is cleared rapidly from the plasma, with a half-life of about 15 minutes following an intravenous dose of 1 mg/kg. As mentioned previously, the tissue half-lives are approximately 2 to 4 days at 1 mg/kg.

Toxicology

The species used in the toxicology studies (rat, dog and cynomolgus monkey) are appropriate in that they all express the mannose-6-phosphate receptor that is required for the uptake of rhASB. The doses and dose regimens used in the studies were based on non-clinical pharmacology studies in the feline MPS VI model.

• Single Dose Toxicity

Acute toxicity studies in rats and dogs were conducted in compliance with GLP at doses up to 10 mg/kg and 20 mg/kg, respectively. The only findings were some transient infusion-associated swelling and reddening of the face and paws. In the dog study, this also occurred in some control animals and was attributed to the presence of polysorbate 80 in the formulation. Exposure in the dog study was greater than proportional to dose. At the high dose (males and females combined) exposure was 67 times that in man following a dose of 1mg/kg.

• Repeat Dose Toxicity (With Toxicokinetics)

A 27-week repeated-dose study was conducted in cynomolgus monkeys. Treatment-related findings were limited to microscopic effects in the liver, without correlating changes in clinical chemistry, at mid and high dose (subacute to chronic periportal inflammation with minimal bile duct hyperplasia), adrenal in high dose males (slight cortical atrophy) and skin in females at all doses. The skin findings in females (serocellular/pustular epidermitis) were often associated with minimal to moderately increased eosinophils. lymphocytes, histiocytes. and acanthosis/hyperkeratosis, findings that were also seen in males at all dose levels. These findings were reversible after a 2-week recovery period. Toxicokinetic analysis showed sex differences in exposure, with higher levels in males than females at the high dose and at all doses at the last time point (26 weeks). Increases in Cmax and AUC were generally greater than proportional to increase in dose. There was some evidence of accumulation in males at 3 and 10mg/kg, particularly over the first half of the study. Exposure at the high dose was 21 to 32 times (week 1) or 24 to 39 times (week 26) that in man following a dose of 1mg/kg. These safety margins are

reasonable, but no NOEL was established in this study given the findings in the skin at the low dose. At the mid-dose (3mg/kg), exposure was only 2 to 2.5 times that in man following a 1mg/kg dose, and at the low dose, $AUC_{0-\infty}$ was not calculable.

Anti-rhASB antibodies were detected in all treated monkeys by week 13. However, the antibody titre did not appear to correlate with plasma rhASB concentration or with the severity of the test article-related microscopic findings.

The applicant was asked to comment on the clinical relevance and possible cause of the skin findings. The applicant's suggestion that the findings were essentially an exaggerated form of the clinical findings (infusion-associated reactions) and that various inflammatory mediators, including histamine from mast cells, are presumed to participate in the pathology of the dermal lesions in monkeys was accepted by the CHMP.

• Genotoxicity And Carcinogenicity

Genotoxicity and carcinogenicity studies have not been conducted, which is in accordance with the Note for Guidance on Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals.

• Reproductive And Developmental Studies

A combined fertility and embryo-fetal developmental study has been conducted in rats. Doses up to 3 mg/kg/day had no effect on body weight, food consumption, fertility or mating parameters, male sex organ weights or sperm parameters, caesarian-sectioning or litter parameters. There were also no effects on gross external, visceral or skeletal observations. Toxicokinetic analysis was not conducted as part of the study. The applicant has committed to conduct a toxicokinetic study in pregnant rats as a post-marketing measure.

In the dose range-finding study that was conducted prior to the reproductive toxicity study, anaphylactoid-type reactions were seen that required treatment with diphenhydramine hydrochloride. Infusion-associated effects in the definitive study were not severe enough to require similar treatment. Anaphylactoid-type reactions were also seen in some of the early pharmacology studies in MPS VI cats. The effects were seen in the definitive study, but observation revealed that these were not life-threatening and decreased in severity on repeated administration of rhASB, therefore treatment with DPH was not initiated in the definitive study.

An embryo-fetal developmental study in rabbits, and a peri- and post-natal study have not been conducted. Mannose 6-phosphate receptors have been reported to be present in the placenta and developing fetus (Wenk et al, 1991), and therefore rhASB may cross the placenta and enter the fetus.

The applicant plans to conduct a dose-range finding study in rabbits as a post-authorisation commitment to assess the feasibility of conducting a definitive study in this species.

The applicant justified the absence of a pre- and post-natal study on the basis that the immunogenicity in rats would confound the interpretation of the results. Both the treatment of anaphylactoid-type reactions and the possibility of immune complex formation may have an impact on the physiological processes involved in pregnancy and lactation. The applicant has committed to collect information on human pregnancy in all subjects who become pregnant whilst on treatment, through the Clinical Surveillance Programme

Local Tolerance

Local tolerance of the product, as assessed during the toxicity studies, was acceptable.

Ecotoxicity/Environmental Risk Assessment

The product being a protein is not considered to pose a risk to the environment.

Discussion on the Non-Clinical Aspects

The feline MPS VI model has similar pathology to the human MPS VI disease and has been used to investigate the effects of rhASB on various manifestation of the disease such as lysosomal storage, urinary GAG excretion, body weight, physical appearance, mobility and flexibility, as

well as any possible immunological effects resulting from the infusion of a heterologous protein. These studies have shown that rhASB can reduce lysosomal GAG storage and improve some symptoms of the disease, depending on the dose and dose regimen and the age of the cat at start of treatment. In this model, rhASB had no effect on cartilage or cornea. Based on the pharmacological effects in the feline MPS VI model at a dose of 1mg/kg/week, and the tissue half-life of rhASB of 2 to 4 days, the clinical dose of 1 mg/kg/week appears reasonable. However, given that the human enzyme was less effective in the feline disease, a lower dose of rhASB may have been effective in patients. As some clinical benefits were observed at 0.2mg/kg/week in Phase 1, the minimally effective dose may indeed be between 0.2 and 1mg/kg/week , but this was not investigated. Based on the results of the Phase 1 study which showed more rapid and more pronounced uGAG reductions, the 1 mg/kg dose was selected for the clinical development by the applicant. The clinical dose will be kept under review as a post-marketing commitment.

In the chronic monkey study, liver and skin effects were observed. These findings were reversible after a 2-week recovery period, however, potential reversibility would not be relevant should the effects occur clinically, given the chronic nature of the treatment. Exposure at the high dose (10 mg/kg/week) was 21 to 39 times that in man following a dose of 1mg/kg. The lack of significant liver abnormalities in the clinical studies provides reassurance, however liver toxicity will be kept under review as a post-marketing commitment.

The fact that a full package of reproductive toxicity studies has not been conducted is mentioned in section 5.3 of the SPC. Post-authorisation commitments have been agreed for further reproductive toxicity studies with toxicokinetics and clinical monitoring of pregnant women.

4. Clinical Aspects

Introduction

The clinical development program for rhASB consisted of 2 controlled studies (ASB-00-01 and ASB-03-05), 1 uncontrolled study (ASB-01-04), and a long-term, open-label extension of the ASB-00-01 controlled study.

All were conducted to evaluate the efficacy and safety of weekly intravenous administration of rhASB. There were also 2 special studies conducted in support of rhASB development. One was a clinical survey study (ASB-00-02), an observational study designed to collect normative data in 121 MPS VI patients, and the other is an ongoing investigator-sponsored study of rhASB treatment of a newborn with MPS VI and a 3-year old MPS VI-affected sibling.

Multiple clinical aspects of this heterogeneous disease, reflective of the common clinical manifestations of MPS VI, were assessed. The primary endpoints of interest were endurance-based measures such as the 6- or 12-Minute Walk Test and Stair Climb Test.

These measures were considered appropriate given the very small patient population (estimated < 1100 patients in the developed world) with multiple functional deficits (cardiovascular, pulmonary, and joint mobility), each of which might improve only modestly during the course of a trial. Impaired endurance was documented in all patients assessed in a 121-patient Survey Study. In addition, the changes in lysosomal storage/accumulation, the underlying biochemical manifestation of the disease pathophysiology, were measured by following urinary GAG levels. Joint mobility, indirectly affected by the accumulation of GAGs in the joints and skeleton, was assessed by measuring by shoulder flexion, extension, and lateral rotation.

Details of studies are briefly outlined below.

Controlled Studies:

ASB-00-01, a 24 week, double-blind, two-dose level (0.2 mg/kg and 1 mg/kg) Phase 1 / 2 Study in 7 patients, with long term treatment at 1 mg/kg, for which efficacy and safety data up to Week 144 are reported

ASB-03-05, a 24 week, double-blind, placebo-controlled, level Phase 3 Study (19 patients randomized to rhASB and 20 patients randomized to placebo) for which efficacy and safety data up to Week 24 are reported in this application.

Uncontrolled Study:

ASB-01-04, an open-label, Phase 2 Study in 10 patients for whom efficacy and safety data up to Week 72 are reported.

Special Studies:

ASB-00-02, a completed Survey study of 121 MPS VI subjects characterizing clinical, genetic and biochemical aspects of the disease;

An investigator-sponsored study of the rhASB treatment of 2 siblings, a female who was 3 years and 7 months at enrolment, and her 2–month old brother, was initiated in October 7, 2003. This study constitutes the first attempt to assess the benefits of initiating treatment with rhASB close to the time of birth, and of comparing the progression of disease in the infant to the progression of disease in the older affected sibling prior to rhASB treatment.

A table of all clinical studies is given below:

Protocol and Clinical Study Report No	Study Design	Study Center Location/No . of Patients	Completion Status/Stud y Dates	Study Objectives	Treatment Doses (IV infusion)	Duration of Treatmen t (weeks)	No. of Patients Entered/ Evaluable
ASB-03- 05	Phase 3 Double Blind, Placebo- Controlled, Randomized Study	USA:6 Germany:8 England:6 Brazil:8 France:5 Portugal:6	Completed 21Jul03 to 8APR04	Safety and efficacy	1 mg/kg once weekly	24	39/39 19 to rhASB 20 to placebo
ASB-00- 01	Phase 1/2 Double- Blind Randomized , Dose Comparison Study, followed by an Open- Label Extension Study	USAA6 Austria:1	Double- Blind study complete. Open-Label Extension Study is ongoing 26SEP00 to 16OCT03	Comparison of safety and efficacy for 2 dosages of rhASB; Long-term safety and efficacy	0.2mg/kg vs. 1 mg/kg once weekly for 24 weeks. After 24- week analysis, patients assigned to the 0.2 mg/kg dose were transitione d to the 1 mg/kg dose	144 (this report)	7/6 4to 0.2 mg,kg 3 to 1 mg/kg
ASB-01- 04	Phase 2 Open-Label, Non- randomized Study	USA;5 Australia:5	Ongoing 29 MAR 02 through 20 DEC 03	Safety and efficacy, Long- term safety and efficacy	1 mg/kg once weekly	72 (this report)	10/10
ASB-00- 02 and ASB-03- 05	Survey Study	USA:27 Germany:29 England:15 Australia:11 Brazil:28 France:7 Portugal:6	Completed	Establish range and diversity of clinical symptomatology in patients with MPS VI	None	None	123/121*

Table: Summary of all Clinical Studies

Protocol and Clinical Study Report No.	Study Design	Study Center Location/No . of Patients	Completion Status/Stud y Dates	Study Objectives	Treatment Doses (IV infusion)	Duration of Treatmen t (weeks)	No. of Patients Entered/ Evaluable
None	Sibling Study	Australia:2	Ongoing Investigator- sponspored Study	Safety and efficacy in 2 siblings with MPS VI, a newborn and a 3-year 7-month old	1 mg/kg once weekly	32	2

*2 patients were found to have a disease other than MPS VI.

Pharmacokinetics

The pharmacokinetics of rhASB have been characterized in 30 patients with MPS VI from PK studies nested in the three clinical studies of safety and efficacy: ASB–00-01-PK (Phase1/2, 6 patients), ASB–01–04-PK (Phase 2, 10 patients), and ASB-03-05-PK (Phase 3, 14 patients). PK data have not been collected in normal volunteers. This is acceptable given the rarity of disease and the inappropriateness of using rhASB in healthy volunteers.

In Study ASB-00-01 (Phase 1/2), rhASB was administered at doses of 0.2 or 1 mg/kg/week and the dose in the Phase 2 Study ASB-01-04 and Phase 3 (ASB-03-05) studies was 1 mg/kg/week. All three studies included pharmacokinetic assessments at various intervals through 24 weeks of double-blind treatment.

In addition, a comparison of the commercial and the clinical processes of rhASB were done in study ASB-XO-001 which is a pharmacokinetic analysis of the open-label extension of studies ASB-00-01 (Phase 1/2) and ASB-01-04 (Phase 2). The 4 patients in the Phase 1/2 study were switched to the commercial process between 179-188 weeks and the 10 patients of the Phase 2 study were switched to the commercial process between 98-177 weeks respectively.

Analytical Methods

The following bioanalytical methods were developed and qualified/validated during the rhASB clinical development program:

Plasma concentrations of rhASB, were measured using a sandwich ELISA method. Reported values are rhASB concentrations (ng/mL) in undiluted plasma. The lower limit of quantitation for this assay is 33.75 ng/mL.

Anti–ASB (IgG) antibodies using a sandwich ELISA method. The lower limit of quantification for the assay was $0.2 \text{ OD}/\mu\text{L}$ serum.

Total urinary GAG (sulfated glycosaminoglycans) excretion were measured by an automated system based on a spectrophotometric detection (A592 nm) of metachromatic changes in 1,9-dimethylmethylene-blue (DMMB) that occur after the formation of the GAG-DMMB complexes. The results were normalised with the concentration of creatinine in the urine.

A Neutralizing Antibody Assay was developed using a sensitive and specific rhASB activityinhibition assay. However, due to a lack of a positive control from a patient sample, the assay is not fully validated. The applicant committed to developing and validating an alternative assay in post-authorisation.

A direct immunoassay (ASB IgE ELISA) was developed for use in detecting the presence of rhASB-specific IgE antibodies in human serum. The assay involves the binding of the rhASB drug substance to an ELISA multiwell plate, followed by a serum binding step and an antibody detection step with an HRP-conjugated affinity-purified goat antibody specific for human IgE.

• Absorption and Bioavailability

Galsulfase being administered intravenously, a 100% bioavailability can be assumed

• Bioequivalence

Based on C_{max} , AUC_{0-t}, and urinary GAGs, the clinical and commercial materials appear to be equivalent. However, there was a possible 9% increase in AUC_{0-∞} with the commercial material. The individual patient anti-rhASB antibodies measured at the time of the last administration of the clinical material and after the administrations of the commercial material showed no change in antibody level.

As for the change in the manufacturing process due to polysorbate 80 with respect to PK parameters from week 84, although mean values for AUC appears to increase and those for CL decrease by approximately 25%, there is substantial overlap of the individual values and the small number of patients precludes a firm conclusion to be drawn regarding the equivalence of pharmacokinetic parameters for rhASB produced by the original and modified process (see Quality section).

• Distribution and Elimination

The main pharmacokinetic characteristics in patients with MPS VI (n=13) receiving 1 mg/kg of galsulfase as a 4 hour infusion/week at 24 weeks are shown in the table below.

T _{1/2}	$T_{\frac{1}{2}} = 22.8 \ (\pm 10.7) \ min$
Cmax - AUC	$C_{max} = 2,357 (\pm 1,560) \text{ ng/ml}$; AUC _{0-t} = 5,860 (± 4,184) h × ng/ml
Distribution	Vz=316 (± 752) ml/kg
Metabolism	Peptide hydrolysis
Elimination	CL=7.9 (± 14.7) ml/min/kg

• Elimination

The metabolic degradation of rhASB occurs through peptide hydrolysis.

• Dose Proportionality and Time Dependencies

The pharmacokinetics of rhASB were not linear between 0.2 and 1 mg/kg/week as demonstrated by an increase in AUC0-t far in excess of the 5–fold increase in dose in the Phase 1/2 Study. Nevertheless no PK parameter other than AUC could be estimated for the 0.2mg/kg dose.

In the phase 1/2 study, the absence of values for AUC ∞ , CL, Vz and $t_{1/2}$ in the lower dose group, together with the variable effect of antibodies on the plasma concentration and ELISA and the small number of patients in both groups, complicated a definitive assessment of the PK parameters relating to a dose effect or indeed a single dose. Accordingly, no firm conclusions could be drawn.

The AUC_{o-t} and AUC_{∞} between weeks 1 and subsequent weeks in the phase 2 study are significantly different. Although a specific mechanism for this difference is not elucidated, the hypothesis is that clearance is more rapid and AUC is reduced at week 1 relative to subsequent dose intervals and are likely related to the rapid uptake by cells deficient in the enzyme. Following 24 weeks of rhASB treatment, steady-state conditions are presumably in place and are reflected in Week 24 PK parameters that are comparable to those from Week 2.

• Special Populations

No studies have been performed with rhASB in patients with renal or hepatic impairment since neither of these conditions are associated *per se* with MPS VI, and the expected metabolism of the drug is through peptide hydrolysis.

The effects of age and gender on the PK of rhASB was examined by graphically comparing the individual patient values for AUC, CL Vz, and t_2^{\prime} at Week 24, the end of double–blind treatment in the three studies. No apparent differences were observed, although the number of patients is small.

• Immunogenicity

The development of anti-ASB IgG antibodies was assessed, at a minimum, at 6-week intervals during each of the clinical studies.

Initial evidence of antibody development typically appeared following 4–8 weeks of treatment. Antibody levels in 14 of 34 patients did not exceed 2.0 OD/ μ L at any time during the course of their treatment with rhASB. Among patients completing at least 24 weeks of rhASB treatment, 1 of 6 (16.7%) from Study ASB–00–01, 3 of 10 (30%) from Study ASB–01-04, and 6 of 19 (31.6%) from Study ASB–03–05 developed antibody levels 10 OD/ μ L. For patients developing these higher antibody levels, an initial increase to 2.0 OD/ μ L typically occurred between Weeks 6 and 12 of treatment.

In the Phase 3 study, with respect to the overall relationships between CL, Vz and antibody levels in those patients with high antibody levels, it was not entirely clear if this is an effect of the antibody on the clearance and distribution of rhASB or interference of the antibody with the ELISA, resulting in apparently lower plasma concentrations and thus higher CL. The effect of outliers on the statistical analysis of the results, is further complicated by the small number of patients. Two of the 3 patients with high antibody levels had significant reduction in urinary GAG levels, while the remaining patient did show a smaller reduction in GAG level. The exact mechanism for the variability of the effects of antibody on the ELISA, AUC and urinary GAG levels is not fully understood, but could be supported by the high genetic variability of the patients (> 40 different genetic mutations in the clinical trials). However, the occurrence of neutralizing antibodies cannot be excluded. Therefore, high antibody levels will be monitored as part of the clinical surveillance programme with a view to determining effects on efficacy and urinary GAG levels.

• Pharmacokinetic Interaction Studies

No specific drug-interaction studies have been performed. The metabolic degradation of rhASB through peptide hydrolysis makes it an unlikely candidate for drug–drug interactions.

Inhibition of intracellular ASB activity through interference of the delivery of receptor-bound enzyme to the lysosome by chloroquine and related amines has been reported in the literature. However, the risk extrapolated from these *in vitro* effects appears theoretical as the levels of inhibitors required are far greater than those expected at the recommended doses of these compounds for humans.

Pharmacodynamics

• Mechanism of Action

The lysosomal enzyme N-acetylgalactosamine 4-sulphatase (arylsulphatase B, ASB) is deficient in patients with MPS VI, resulting in an accumulation of glycosaminoglycans (GAGs) in tissues throughout the body that leads to widespread cellular, tissue and organ dysfunction. Recombinant human ASB (rhASB) is intended to replace ASB and reduce the manifestations of the disease by increasing the catabolism of GAGs.

• Primary and Secondary Pharmacology

The pharmacodynamic endpoint reflecting the removal of excess dermatan sulphate from vascularized tissues measured the level of urinary GAGs. Excessive lysosomal storage of dermatan sulfate within the kidney, particularly in the collecting tubules, leads to the excretion of excessive amounts of GAGs in the urine.

Urinary GAG levels thus provide a gauge of total body GAG storage. When GAG storage product in the kidney is reduced by treatment with exogenous rhASB, urinary GAG levels fall. Preclinical studies in the feline MPS VI model have documented that reduction in lysosomal GAG storage reflects a decline in storage in other well-vascularised tissues.

The PK/PD findings in some patients in the clinical trials suggest that the measurement of urinary GAG levels may be more appropriate for assessing the activity of rhASB than AUC and the possible impact of an anti–ASB antibody on this activity (see discussion on clinical efficacy)

Clinical Efficacy

Dose Response Study

Study No. ASB-00-01: A Phase 1/2 Randomized, Double-Blind, Two Dose Group Study of Recombinant Human *N*-acetylgalactosamine 4-sulfatase (rhASB) Enzyme Replacement Therapy in Patients with Mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome)

METHODS

Objectives: To evaluate the safety (primary objective), efficacy and pharmacokinetics (PK) of 2 dose levels of weekly intravenous (IV) infusions of recombinant human *N*-acetylgalactosamine 4-sulfatase (rhASB) for a minimum of 24 weeks in patients diagnosed with MPS VI.

Measures of safety included clinical chemistry, hematology and tracking of adverse events (AEs). Specific to enzyme replacement therapy, the study gauged the immune response and anaphylactoid reactions (including complement activation and antibody formation) of patients receiving therapy.

Efficacy parameters included exercise tolerance, respiratory capacity, joint range of motion (ROM), functional status, levels of urinary GAGs, hepatomegaly, visual acuity, cardiac function, and sleep apnea.

Design: This was a double-blind, randomized, two dose level study (0.2 mg/kg and 1 mg/kg). An interim analysis of unblinded safety and efficacy data of treatment for each patient was performed after the sixth patient enrolled completed 24 weeks of treatment.

After results of the interim analysis were available and reviewed with the U.S. Food and Drug Administration (FDA), all patients randomized to the lower dosage (0.2 mg/kg) were transitioned to the higher dosage (1 mg/kg) for the remainder of the study. Patients originally randomized to the higher dosage level remained at that level.

Study Participants

7 patients enrolled; 6 completed 32 weeks of treatment, and 5 completed 144 weeks of treatment.

Treatments

All patients were premedicated with 0.5 mg/kg of diphenhydramine IV.

rhASB (0.2 mg/kg or 1 mg/kg) was diluted into 100 to 250 mL of 0.9% Sodium Chloride Injection, and infused over a 4-hour period. Cardiorespiratory and pulse oximeter monitoring was conducted throughout the infusion. The infusion rate was adjusted so that approximately 2.5% of the total volume of study drug solution was infused during the first hour, with infusion of the remaining volume (approximately 97.5%) over the next 3 hours. Per Protocol Amendment, study drug manufactured by a modified process was administered to all on-study patients starting at Week 84.

Statistical Methods

All demographic and safety data were summarized using the safety population, defined as all patients who enrolled in the study and had at least one infusion of rhASB. Efficacy data were analyzed using descriptive statistics, frequency tabulations, and graphical displays over time, as appropriate. No inferential testing was performed.

Summary tables were produced. All patients who were on study for at least 12 weeks were included in the analysis. Analyses, when appropriate, include evaluations of changes in efficacy measures from screening for each patient to determine whether any enzyme effect was observed.

RESULTS

Week 24 Double-Blind Dosage Comparison Analysis:

Seven patients were enrolled and randomized to 0.2 mg/kg rhASB (n=4) or to 1 mg/kg rhASB (n=3). In order to be included in the efficacy analysis, patients had to complete at least 12 weeks of treatment. One patient withdrew from the study after receiving 3 infusions; therefore, the 24-week analysis was based on 3 patients in each dose group. An interim analysis performed after all

patients completed 24 weeks of treatment indicated that the higher dose produced a larger mean decrease in urinary GAG and appeared to result in greater clinical benefit.

Six-Minute Walk Test

In the 6-Minute Walk Test, patients receiving the 1 mg/kg dose of rhASB had a mean increase of 65 m at Week 24 compared to baseline (83% increase); the patients who received 0.2 mg/kg had a mean increase of 23 m (10% increase).

Urinary GAG Levels

The patients in the 1 mg/kg group had a 70% mean reduction from baseline in urinary GAGs to a mean level of 100.0 μ g/mg creatinine; the patients in the 0.2 mg/kg group had a mean reduction from baseline of 55% to a mean level of 144 μ g/mg creatinine.

Shoulder ROM

All patients showed some improvement for flexion or extension in at least 1 shoulder at Week 24.

Other Endpoints:

Liver size as a percent of body weight generally decreased, particularly for the 2 patients, one in each dosage group, with the largest livers at baseline .

There were no clinically meaningful changes in bone mineral density, height, weight, chest and cervical spine X–rays, ECG, Echocardiogram, FVC, FEV1, other CHAQ variables, and the pinch and grip strength results. There were no clinically meaningful changes in the other efficacy assessments.

Discussion:

The small number of patients in this phase 1/2 study complicates assessment of the dose response using the 2 doses, which is further compounded by the variability seen in individual patient responses Nevertheless the data are supportive of the superiority of the 1 mg/kg dose in terms of urinary GAG reduction and clinical improvement.

However, it should be noted that there are no data for intermediate doses between 0.2 and 1 mg/kg, to determine an optimal dose.

• Main Study

Phase 3 Trial

Protocol No. ASB-03-05: A Phase 3, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Multinational Clinical Study of Recombinant Human N-acetylgalactosamine 4-sulfatase (rhASB) in Patients with Mucopolysaccharidosis VI

Objectives:

Primary Efficacy Objective

The primary objective was to evaluate the ability of rhASB versus placebo to enhance endurance in patients with Mucopolysaccharidosis VI (MPS VI), as evidenced by an increase in the number of meters walked in the 12-Minute Walk Test at Week 24 compared with baseline.

Secondary Efficacy Objectives:

The secondary efficacy objectives were as follows:

To evaluate the ability of rhASB versus placebo to enhance endurance and respiratory capacity in patients with MPS VI, as evidenced by an increase in number of stairs climbed in the 3–Minute Stair Climb Test at Week 24 compared with baseline

. To evaluate the ability of rhASB to reduce GAG excretion in the urine in patients with MPS VI at Week 24 compared with baseline Tertiary Efficacy Objectives

The tertiary efficacy objectives were as follows:

. To evaluate the ability of rhASB to decrease joint pain in patients with MPS VI as evidenced by a decrease in joint pain scores on an analogue scale at Week 24 compared with baseline.

- To evaluate the ability of rhASB to decrease joint stiffness in patients with MPS VI as videnced by a decrease in joint stiffness scores on an analogue scale at Week 24 compared with baseline.

- To evaluate the ability of rhASB to increase physical energy in patients with MPS VI as evidenced by an increase in physical energy score on an analogue scale at Week 24 compared with baseline.
- To evaluate the ability of rhASB to improve joint ROM as evidenced by an increase in shoulder ROM at Week 24 compared with baseline in a subset of patients with MPS VI who have a pre-existing limitation of shoulder ROM (baseline average of $\leq 90^{\circ}$ for range of active flexion at the shoulders).
- To evaluate the ability of rhASB to improve dexterity and sensation in patients with MPS VI as evidenced by an increase in the number of coins picked up in 1 minute at Week 24 compared with baseline.

Design and Methods:

Patients underwent eligibility assessments during a 1-2 weeks screening period. Patients had to be at least 7 years of age. To be eligible for the study a patient must have been able to walk independently at least 5 metres and no more than 270 metres in the first 6 minutes, or no more than 400 metres in 12 minutes in a 12-minute walk test. Eligible patients underwent baseline assessments during a 2-week baseline period and were then randomised to receive either rhASB or placebo. Patients received weekly double-blind infusions of either 1 mg/kg rhASB or placebo solution for 24 consecutive weeks. After 24 weeks, all patients were eligible to receive rhASB in a separate open-label extension study.

Study Participants

There were 39 patients randomised into the trial, 20 received placebo and 19 received rhASB. Thirty-six of these patients had previously participated in study ASB-00-02. There is a concern that this creates a population enriched in some way for response to treatment.

All 39 patients completed the study, and there was no missing data except for one subject on rhASB who missed the week 18 visit, and one subject on placebo who withdrew consent before the week 6 visit and provided no data after baseline.

There were 11 patients, 8 on placebo and 3 on rhASB who did not fulfil the eligibility requirements but were granted exceptions and randomised. There were 3 patients, all on placebo, who were under 7 years of age. One of the patients randomised to rhASB had a bone marrow transplant at age 7 (about 11 years previously); the exclusion criteria prohibited the inclusion of patients that had previously undergone hematopoietic stem cell transplantation.

The main group of ineligible patients were 7 patients (5 on placebo, 2 on rhASB) who exceeded the walk test criteria at screening. Following completion of the screening of all known potential study candidates, the applicant considered that the required number of eligible patients could not be identified. Therefore the population was expanded by enrolling patients who met all other study criteria but could walk further than the study criteria permitted. All the additional patients walked between 400 and 600m in 12 minutes at screening.

Statistical Methods:

Patients were randomized to either the rhASB or placebo group, stratified by primary site of treatment. Within each site, randomized blocks governed the allocation to treatment group.

Demographic and background information were summarized and presented by the means, standard deviations, medians, and ranges for continuous variables and as counts and percentages for categorical variables.

The efficacy analyses included all randomized patients. The safety analyses included all patients who received at least one dose of study drug.

Primary Efficacy Endpoint Analyses:

The primary efficacy assessment was a 12-Minute Walk test. This was conducted at screening (to determine eligibility for the study) then twice (on separate days) at each of baseline, Week 6, Week 12, Week 18 and Week 24.

This was analysed using a repeated measures linear analysis stratified by site with the baseline walk distance as a continuous covariate, and time and treatment by time interaction as covariates.

The applicant used three analysis populations. The ITT population included all randomised patients; the walk eligible subset includes only patients who fulfilled the walk test criteria at screening; the \leq 400 m subset includes only patients who walked \leq 400 m (average of the two assessments) at baseline. Note that the patient who provided no data after baseline is not in either of the analysis sub-groups, as he/she did not fulfil the walk test criteria at either screening or baseline.

Secondary Efficacy Endpoint Analyses:

Stair Climb Test: The analyses for the 3–Minute Stair-Climb Test was similar to that used for the primary endpoint; i.e., a repeated measures linear model. Sensitivity analyses explored the robustness of the results. This analysis used rate of climb, defined by the (number of stairs climbed)/(number of minutes in the climb), as the outcome variable.

Urinary GAG Measurements: An analysis of variance of Week 24 urinary GAG level was used to compare the rhASB and placebo groups. The model was stratified by site and used baseline urinary GAG level as a continuous covariate. A "responder" was defined as a patient with at least 50 percent reduction in urinary GAG from baseline to Week 24. A supportive analysis compared the proportion of responders in the two groups using a Mantel–Haenszel test stratified by clinical site.

Tertiary Efficacy Endpoint Analyses: The primary analyses used repeated measures linear models on the endpoints of interest to estimate the treatment effect comparing the differences between Week 24 and baseline. For shoulder joint range of motion, the primary endpoint was a binary variable: improvement by at least 10 _ analyzed using a Mantel–Haenszel test stratified by site.

RESULTS:

Primary Efficacy Endpoints:

12–Minute Walk Test. The primary efficacy endpoint showed a statistically significant difference in mean distance walked in 12 minutes between the rhASB and placebo Group. The rhASB group walked a mean \pm SE of 92 \pm 40 m further than the placebo group at Week 24 (p-value \pm 0.025).

Baseline imbalances

At baseline, the observed mean \pm SD 12–Minute Walk Distance for the rhASB group was 227 ± 170 m; the comparable value for the placebo group was 381 ± 202 m. This imbalance was in part due to a disproportionate number of the walk–ineligible patients being randomly assigned to the placebo group (see table below).

	rhAS	rhASB			bo	Difference		
Population	n	Mean	sd	n	Mean	sd	Mean	SE
ITT	19	227	170	20	381	202	-154	60
Walk eligible	17	197	146	15	329	199	-131	62
≤400m subset	16	170	110	12	243	125	-73	45

Baseline 12-Minute Walk Test (Metres)

Week 24 12-Minute Walk Test (Metres)

	rhASI	rhASB			00	Difference		
Population	n	Mean	sd	n	Mean	sd	Mean	SE

ITT	19	336	227	19	399	217	-63	72
Walk eligible	17	311	199	15	347	212	-36	77
≤400m subset	16	298	225	12	283	160	15	73

Because of an imbalance in baseline walk distances between the rhASB and placebo groups and because 7 exceptions were made to the walk distance inclusion criteria (these patients exceeded the criteria of not more than 270 m in 6 minutes OR no more than 400 m in 12 minutes for the screening walk test), additional analyses were performed to demonstrate the robustness of the data.

There were 2 major subset analyses performed for the Phase 3 study following the observation of a baseline imbalance between treatment groups for results of the 12-Minute Walk Test and 3–Minute Stair Climb test.

One subset analysis excluded patients who did not meet the eligibility criteria using their screening walk test data; the remaining 32 patients constituted the "Walk-Eligible Subset." The second subset applied even stricter criteria relative to the inclusion criteria, and analyzed the data from 28 patients who walked ≤ 400 m at baseline, comprising the " ≤ 400 m Subset."

Using a longitudinal model, the estimated differences adjusted for baseline from placebo at Week 24 for the "Walk-Eligible Subset" and the "< 400 m Subset" were 115 m and 118 m respectively; the corresponding p-values are 0.016 and 0.024 (see table below).

	n Baseline (Mean ± SD)		Estimated Mean±SE of			
	rhASB	Placebo	rhASB	Placebo	difference between rhASB and Placebo	p- value
All patients randomized	19	20	227±170	381±202	92±40	0.025
Walk-Eligible Subset	17	15	197±146	329±199	115±47	0.016
≤400 m Subset	16	12	170±110	243±125	118±51	0.024

Table 11-7: 12–Minute Walk Tests for Walk-Eligible and ≤400 m Subsets

Similar findings were seen for the subset analyses of the 3-Minute Stair Climb results.

Despite the apparent statistically significant difference in the primary endpoint of the phase 3 study, the substantial imbalance in treatment groups at baseline, makes interpretation of the data difficult. This is particularly important as patients on placebo were walking substantially longer distances compared to those randomised to active treatment. This could be a matter for concern as it would appear that healthier patients have been allocated to placebo while those with more advanced disease have been allocated to active treatment. In addition, the distances walked at week 24 by both groups were similar, but due to the imbalance larger improvements were seen in the rhASB treatment group.

The CHMP requested further analyses without any adjustment for baseline but by making comparison of patients with similar baselines. This was possible due to the large overlap of baseline values between treatments.

The table below shows the results from an analysis made by the assessor, excluding the patients with the 5 highest, and the 5 lowest baseline scores.

12-Minute Walk Test – Excluding 10 Baseline Extremes

	rhASB		Plac	cebo	
	n	Mean (sd)	n	Mean (sd)	Difference
Baseline	1	255 (122)	1	322 (158)	-67

	4		4		
Week 24	1	375 (205)	1	355 (174)	+20
	4		4		
Change from BL	1	120 (162)	1	33 (114)	+86
	4		4		
Adjusted change*	1	117	1	36	+81
	4		4		

*adjusted for baseline

This can be compared to the all-patient analysis shown below:

12-Minute Walk Test – All Patients

	rhA	SB	Plac	cebo	
	n	Mean (sd)	n	Mean (sd)	Difference
Baseline	1 9	227 (170)	1 9	373 (204)	-147
Week 24	1 9	336 (227)	1 9	399 (217)	-63
Change from BL	1 9	109 (154)	1 9	26 (122)	+83
Adjusted change*	1 9	103	1 9	31	+72

*adjusted for baseline

The results from this sub-group analysis, combined with those from the company supplied analyses using different subgroups, suggest that the treatment advantage for rhASB is not dependent upon the baseline imbalance.

There is further supportive evidence from the open-label extension trial where the placebo patients who had only minimally improved over the first 24 weeks made substantial improvements over the next 24 weeks when switched to active treatment. Although this data are open-label they still serve to provide some reassurance

The reasons for this baseline imbalance were also investigated at the request of the CHMP. The result is that the randomisation scheme seemed robust and appropriate. It seems that the imbalance is most likely to have been a chance occurrence. Perhaps this was a function of the small size of the study which makes chance imbalances more likely.

Secondary Efficacy Endpoints:

In the analysis, the rate of stairs climbed per minute was used because the percent of stair climbs in which the patient reached the top of the stairs exceeded the predefined limit of 10% in the data analysis plan.

3–Minute Stair Climb. The primary analysis of rate of stair climb (stairs/minute) in all randomized patients showed a difference between the mean change in the rates between rhASB and placebo of 5.7 ± 2.9 stairs/minute (p=0.053).

For both the Walk-Eligible and ≤ 400 m subsets, the rhASB group climbed a mean of approximately 21 more stairs than the placebo group, p= 0.019 and p= 0.048, respectively.

However these results need to be interpreted with caution because of baseline imbalances

Urinary GAG. Seventeen of 19 rhASB patients and no placebo patients had a \geq 50% percent reduction in urinary GAG levels between baseline and Week 24 (p = 0.001). This decline suggests an affect on both lysosomal storage and accumulation of GAG.

Tertiary Efficacy Endpoints:

Tertiary endpoint variables that assessed joint pain, joint stiffness, physical energy, shoulder joint range of motion, and dexterity as measured by the coin pick–up test showed little change from baseline over time, however tertiary variables were not statistically powered to show a difference between the groups. No improvement in cardiac valvular disease was observed, although the majority of patients enrolled had only mild disease at baseline.

Long-Term Assessments–Pulmonary Function:

The more rapid changes in maximum voluntary ventilation (MVV) and best Forced Inspiratory Rate (FIR) measures suggest improvements in ribcage excursion due to improved strength or flexibility and possibly imply that improved respiratory function rather than enhanced pulmonary function may be an important contributor to early improvements in endurance.

Uncontrolled Studies

Phase 2 trial

Protocol No. ASB-01-04: A Phase 2 Open-Label Clinical Study of the Efficacy and Safety of Recombinant Human *N*–acetylgalactosamine 4–sulfatase (rhASB) Enzyme Replacement Therapy in Patients with Mucopolysaccharidosis VI (Maroteaux–Lamy Syndrome)

Objectives: To evaluate the efficacy, safety, and pharmacokinetics of weekly intravenous infusions of 1 mg/kg recombinant human *N*-acetylgalactosamine 4-sulfatase (rhASB) in patients diagnosed with mucopolysaccharidosis VI (MPS VI). Study ongoing.

Patient Population: Ten patients with MPS VI, 7 females and 3 males, were enrolled in the study. Ages ranged from 6 to 21 years and urinary GAG levels ranged from 138.4 to 518.5 at enrollment. All 10 patients were on study through Week 72.

Primary Efficacy Endpoints: The main primary efficacy variables consisted of shoulder range of motion (ROM), stair climb test, 12-Minute Walk Test and Urinary GAG levels

Results:

Ten patients were enrolled in the Phase 2 Study and all 10 patients remained on study at Week 24.

Primary Efficacy Endpoints

12-Minute Walk Test: All 10 patients had improvements in the distance walked at 6 (9/10) and 12 minutes at Week 24 compared to baseline. The mean increase was 64 ± 62 m in 6 minutes and 155 ± 146 m in 12 minutes.

Stair Climb Test: All 10 patients had an increase in the number of stairs climbed in three minutes between baseline and Week 24. The mean increase was 48 ± 48 stairs

Urinary GAG Levels: Urinary GAG levels showed a rapid decline from baseline, with 71% reductions at Week 6 and at Week 24. Mean levels declined from $336 \pm 116 \ \mu\text{g/mg}$ creatinine at baseline to $103 \pm 50 \ \mu\text{g/mg}$ creatinine at Week 24.

Shoulder ROM: At Week 24, 7 patients had an improvement in active shoulder flexion, 9 had improvement in active shoulder extension, and 8 had improvements in active lateral rotation. However, none of these results were clinically significant being <10 degrees. Similar results were seen with passive ROM

Other Efficacy Endpoints

The secondary efficacy variables measured at Week 24 were pulmonary function, physical activity, oxygenation during sleep, bone density, and ECG and echocardiogram. The majority of patients had decreases in liver and spleen size, but there were no clinically meaningful changes in the other efficacy variables.

Discussion

The results of this open label phase 2 study are supportive of efficacy and appear to suggest that at a 1 mg/Kg dose, all patients showed improvement in the 6 and 12 Minute Walk test, as well as showing an increase in the number of stairs climbed. Consistent with these changes, there was a 70% reduction in the level of urinary GAG levels.

• Supportive study

Open label extension of the Phase 1/2 Study (ASB-00-01)

Long-Term Efficacy Results (Up to Week 144):

At the end of 144 weeks of treatment, all patients showed substantial reduction in urinary GAG levels, indicative of improvement in the biochemical manifestation of MPS VI. Urinary GAG levels at Week 144 were reduced to 56.5 - 101.4 ug/mg creatinine, reflecting percent reductions of 60% to 86% from screening levels. These levels approached the normal range for age for these patients. All patients experienced improvement in one or more clinical endpoints. No changes were seen in cardiac function, sleep disturbances, bone density, and chest and cervical spine X-rays results.

Survey Study

The first special study, "A Survey Study of Subjects with Mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome)" was a multicenter, multinational study covering a 14-month period. The objective of the study was to establish the range and diversity of clinical symptomatology in selected subjects diagnosed with MPS VI. Clinical and biochemical parameters known to affect MPS VI individuals were evaluated at a single time-point including height, weight, baseline urinary GAG levels, and clinical presentation The study was conducted in 7 countries at centers with expertise in evaluating and treating individuals with MPS VI (n=121 MPS VI patients surveyed). Endpoints of specific interest in the survey study were distance walked in the 6-Minute Walk Test, respiratory function, joint ROM, and urinary GAG levels. The results showed a general classification of patients as those with rapidly advancing or slowly advancing phenotypes. This study also revealed that impaired endurance affected the entire spectrum of slowly to rapidly advancing disease and documented that urinary GAG levels were an important indicator of morbidity and potentially, mortality.

Sibling Study

The second special study is the Sibling Study, "An Investigator-Sponsored Study Comparing Treatment of Newborn and Sibling Mucopolysaccharidosis VI Patients with Recombinant Human N-acetylgalactosamine 4-sulphatase (rhASB)". The primary objective of this study, which is underway in Australia, is to compare the safety profile of 1 mg/kg rhASB treatment in a newborn, enrolled at 2 months of age, and a sibling, enrolled at 3 years 7 months of age, who are both affected by MPS VI.

The 2 siblings enrolled in this study have been treated for approximately 10 months. The older sibling has shown improvements in distance walked in the 6–Minute Walk Test and in joint and spine mobility. Growth rate is normal. Corneal clouding has changed from mild at baseline to moderate at Week 24. The younger sibling has developed significant rib cage flaring and pectus excavatum and mild corneal clouding at Week 24. Weight and length continue to be above the 97th percentile, joint range of motion appears normal, and organ systems appear normal.

• Discussion on Clinical Efficacy

The three primary clinical studies included 56 individuals with MPS VI (20 males, 36 females) between the ages of 5-29 years representing the entire spectrum of disease manifestation. The majority of patients enrolled in the clinical studies presented with profound skeletal dysplasia and heights of 100 cm or less, consistent with the rapidly advancing phenotype. However, 10 of the 56 patients enrolled in the 3 studies were considered to be of the slowly progressing phenotype. Two populations were excluded from the clinical studies, subjects 5 years of age or younger and patients who had received BMT.

While there are no published studies employing the clinical endpoint variable of stair climbing to establish a treatment effect, results of the stair climb have substantially correlated with pulmonary function tests, including FVC and FEV1. The Stair Climb Test is also an indicator of other parameters, including cardiovascular status, patient cooperation, and determination.

Despite the apparent statistically significant difference in the primary endpoint of the phase 3 study, the substantial imbalance in treatment groups at baseline, makes interpretation of the data difficult.

At the request of CHMP, the applicant has provided a re-analysis of the efficacy data which confirms the trend in efficacy in the pivotal trial despite the observation of baseline imbalance between the active and placebo groups. It appears that the imbalance is most likely to have been a chance occurrence. Perhaps this was a function of the small size of the study which makes chance imbalances more likely. Importantly however, the reanalysis suggests that the treatment advantage for galsulfase is not dependent upon the baseline imbalance. In addition results from the open label extension of the pivotal trial have provided reassuring supportive data which help to remove the concern that differences between treatments in the randomised phase were only seen because the placebo patients started with long walk distances and had no room for improvement.

The improvement in urinary GAG excretion was rapid, consistent, and reproducible across all 3 studies for patients receiving the 1 mg/kg dose. There was a dose-related effect on results for this variable. Increased shoulder joint ROM and associated improvements in joint pain and arthritis scores were observed in all 3 clinical studies. Nevertheless, the results of the Phase 1/2 and Phase 2 Studies produced conflicting results for the expected gain in joint function. The Phase 1/2 data showed 8-10-degree gains at 24 weeks in the 3 patients with shoulder flexion < 90 degrees at baseline, and, with extended treatment on the higher dose out to 144 weeks, all patients improved 10 degrees or more. The Phase 2 Study failed to show a similar gain at Week 24, and results at Week 48 were variable.

There was no evidence of a significant change in functional lung capacity across the MPS VI studies after 24 weeks of treatment. Small improvements did emerge in several patients during longer treatment, but increased growth or improvement in existing hepatosplenomegaly could possibly have been a contributing factor to these changes.

Seven of the 8 pre-pubertal patients (< 11 years old) enrolled in the Phase 1/2 and Phase 2 studies experienced 5% or greater increase in height. These changes did not occur prior to 48 weeks of treatment. The one pre-pubertal patient who did not experienced this degree of growth is a genetic "null" who has received chronic steroid treatment. No significant growth has been seen in patients 12 years of age or older in these studies. Decreases in hepatosplenomegaly were observed in Phase 1/2 and Phase 2 patients with enlarged livers and/or spleens, and sleep apnea improved in some patients. No improvements in bone density were observed in the Phase 1/2 and Phase 2 studies.

It should be noted that disease in relatively avascular tissues appears unaffected by rhASB treatment, as reflected by development of both corneal clouding and skeletal dysplasia of the ribs in the 2–month old patient in the sibling study after 10 months of treatment.

Reduction in urinary GAG levels was achieved in the majority of patients in all 3 studies.

However, the relationship between AUC, antibody level and clinical response was not entirely clear in patients who were deemed to be outliers. Although one patient had no measurable plasma levels of rhASB during the first 24 weeks of treatment in the Phase 1/2 study and another patient had a relatively large AUC relative to others in the higher dose group, both patients had a > 70% reduction in urinary GAG levels from baseline. This does suggest that the enzyme was available in target tissues. However, both patients had elevated antibodies. The former patient had antibodies that appeared to block the ELISA assay, but apparently did not impair peripheral uptake of ASB from the circulation. The latter patient had elevated antibody levels, which appeared to extend the plasma circulating time, but not eventual uptake, of rhASB. Nevertheless by Week 84, the PK and PD for these two patients were similar to group means, while antibody levels had fallen and urinary GAG levels were low during the entire period.

In contrast, a patient in the Phase 3 study had reduced plasma AUC and high antibody levels at Week 24, but the urinary GAG level reduction was < 50%. Nevertheless, despite a lesser

reduction in urinary GAG levels, this patient demonstrated improvement in both measures of endurance during the 24-week study period.

The PK/PD findings in these patients would therefore appear to suggest that the measurement of urinary GAG levels may be more appropriate for assessing the activity of rhASB than AUC and the possible impact of an anti–ASB antibody on this activity

The possible relationship between antibody development and clinical efficacy/GAG urinary excretion was re-evaluated by the applicant at the request of CHMP.

The applicant has presented a reasoned argument which suggests no obvious relationship between antibody level either with physical endurance or urinary GAG levels in the majority of patients. However, in view of the variability of response in some patients, this will be kept under review as part of the clinical surveillance programme.

The rationale for the selection of the proposed dose was not entirely clear, since there were no data on intermediate doses, which would have further clarified the non-linearity of the pharmacokinetics of ASB. The selection of the 1 mg dose appears to have been significantly influenced from the pre-clinical results of the feline study which had one feline treated at a dose of 0.2mg/kg. It was therefore unfortunate that due to a paucity of patients, the dose finding phase 1/2 study had very small numbers of evaluable patients, to firmly draw conclusions regarding optimal dose levels.

At the request of CHMP, the applicant has provided a reasoned argument for the justification of the 1 milligram dose as a result of the reduction in GAG levels by 70-75%, independently of the rate or stage of disease progression or sex and age

The applicant has undertaken to further define an optimal maintenance dose in the proposed clinical surveillance programme. Additionally, the applicant intends to monitor dose and frequency implemented by individual physicians, given the extremely limited number of patients.

Clinical Safety

The adverse event (AE) and laboratory data for the first 24 weeks of treatment from the 2 controlled studies were pooled by combining numerators and denominators; no weighting was used. Data from the first 24 weeks from the open-label Phase 2 Study are shown separately. Long-term safety data (post-week 24) from the 2 ongoing studies (Phase 1/2 extension through Week 144 and Phase 2 through Week 72) are also shown separately.

Patient Exposure

A total of 56 patients have been treated in the 3 clinical studies; 20 of these patients

received placebo in the Phase 3 Study. Patients received their assigned dose of rhASB as a 4-hour IV infusion once weekly (every 7 ± 3 days) for the duration of each clinical study. All patients were treated with diphenhydramine 0.5-1.25 mg/kg (up to 50 mg), p.o. or IV, or an approved alternative antihistamine 30-60 minutes prior to infusion in an effort to prevent anaphylactoid reactions to the study drug.

In the Phase 1/2 Study, 4 patients were randomly assigned to receive the 0.2 mg/kg dose.

Two of these patients withdrew from the study before they could transition to the 1 mg/kg dose 6 of the 7 patients enrolled completed the Week 24 assessment double-blind dose comparison part of the study. Compliance with the rhASB treatment regimen was high, with few infusions missed in the first 24 weeks of treatment and overall.

In the Phase 3 Study,18 of the 19 patients in the rhASB group received 24 infusions and 1 patient received 23 infusions over the 24–week study period. In the placebo group, 1 patient received 4 infusions before withdrawing from the study after the Week 5 infusion, 1 patient received 22 of 24 infusions, and 2 patients received 23 of 24 infusions. The remaining 16 placebo patients received all 24 infusions.

Adverse events:

Common adverse events up to week 24 for the phase 1/2 and phase 3 studies are shown in tables 14 and 15.

Controlled studies (Phase 3and phase 1/2)

	No. of	Patients/ No. of I	Events
-	0.2 mg/kg (n = 4)	1.0 mg/kg (n = 22)	Placebo (n = 20)
Any adverse event	4/36	22 / 422	20/344
Deaths	1 / 1	0/0	0 / 0
Discontinuations due to adverse events	0 / 0	0/0	0 / 0
Serious adverse events	1/3	4/5	4 / 12
Severe adverse events	1/2	4/7	4/9
Study drug-related adverse events	2/4	13 / 100	6 / 14
Adverse events during infusion	1/2	13 / 80	8 / 13
IARs (study drug-related adverse events during infusion)	1 / 2	11 / 61	4 / 6

Table 14: Incidence and Frequency of Adverse Events at 24 Weeks in StudiesASB-00-01 and ASB-03-05

Table 15: Incidence and Frequency of Adverse Events at 24 Weeks by System OrganClass in Studies ASB-00-01 and ASB-03-05

	No. (%) of Patients / No. of Events		
System Organ Class	0.2 mg/kg (n = 4)	1.0 mg/kg (n = 22)	Placebo (n = 20)
Blood and Lymphatic System Disorders	1 (25.0%) / 1	1 (4.5%) / 1	5 (25.0%) / 6
Cardiac Disorders	0 (0%) / 0	3 (13.6%) / 4	4 (20.0%) / 7
Ear and Labyrinth Disorders	1 (25.0%) / 1	9 (40.9%) / 25	6 (30.0%) / 8
Eye Disorders	1 (25.0%) / 1	10 (45.5%) / 24	7 (35.0%) / 13
Gastrointestinal Disorders	2 (50.0%) / 2	19 (86.4%) / 51	13 (65.0%) / 58
General Disorders and Administration Site Conditions	3 (75.0%) / 7	17 (77.3%) / 73	13 (65.0%) / 48
Hepatobiliary Disorders	0 (0%) / 0	0 (0%) / 0	4 (20.0%) / 4
Immune System Disorders	0 (0%) / 0	0 (0%) / 0	0 (0%) / 0
Infections and Infestations	1 (25.0%) / 3	18 (81.8%) / 44	19 (95.0%) / 55
Injury, Poisoning, and Procedural Complications	0 (0%) / 0	2 (9.1%) / 2	2 (10.0%) / 5
Investigations	0 (0%) / 0	3 (13.6%) / 3	3 (15.0%) / 7
Metabolism and Nutrition Disorders	0 (0%) / 0	0 (0%) / 0	1 (5.0%) / 2
Musculoskeletal and Connective Tissue Disorders	1 (25.0%) / 2	12 (54.5%) / 56	12 (60.0%) / 37
Neoplasms (Benign, Malignant, and Unspecified)	1 (25.0%) / 2	0 (0%) / 0	0 (0%) / 0
Nervous System Disorders	2 (50.0%) / 4	13 (59.1%) / 46	14 (70.0%) / 34
Psychiatric Disorders	1 (25.0%) / 1	1 (4.5%) / 3	1 (5.0%) / 3
Renal and Urinary Disorders	0 (0%) / 0	3 (13.6%) / 3	1 (5.0%) / 1

Reproductive System and Breast Disorders	0 (0%) / 0	0 (0%) / 0	1 (5.0%) / 1
Respiratory, Thoracic, and Mediastinal Disorders	3 (75.0%) / 8	15 (68.2%) / 44	14 (70.0%) / 36
Skin and Subcutaneous Tissue Disorders	2 (50.0%) / 2	9 (40.9%) / 32	7 (35.0%) / 12
Surgical and Medical Procedures	0 (0%) / 0	0 (0%) / 0	0 (0%) / 0
Vascular Disorders	1 (25.0%) / 2	7 (31.8%) / 11	6 (30.0%) / 7

The specific AEs that occurred with the highest incidence ($\geq 15\%$ of patients) through Week 24 among patients receiving rhASB at any dose in the Phase 1/2 and Phase 3 Studies respectively, (n = 26) were pyrexia; (12 patients), arthralgia and headache (10 patients each); abdominal pain (9 patients); ear pain and vomiting (8 patients each); cough (7 patients) otitis media, upper respiratory infection, and pain (6 patients each), diarrhea, chest pain, and back pain (5 patients each); and conjunctivitis, nausea, influenza-like illness, rigors, dyspnea, nasal congestion, pruritus, and rash (4 patients each). This pattern of events was similar for the placebo group (n = 20)

The AEs with the highest frequency of occurrence (> 2.5% of all events) among rhASB-treated patients in the Phase 1/2 and Phase 3 Studies were headache, pyrexia, arthralgia, myalgia, abdominal pain, URI, cough, ear pain, and rigors. Arthralgia, myalgia, and rigors, were seen more frequently among rhASB. The pattern of AEs for patients treated with 0.2 mg/kg rhASB was similar to that observed for patients treated with 1 mg/kg rhASB.

Of the 802 AEs reported for 46 patients treated in the 2 controlled clinical studies, 684 events (85.3%) were judged by the investigator to be unrelated to study drug administration.

The remaining 118 AEs (14.7%) were considered related to study drug administration. Of these 118 AEs, 49 did not occur during infusion. Of the related events that did not occur during infusion, 41 were reported for rhASB-treated patients and 8 for placebo-treated patients. Among rhASB-treated patients, these events occurred most commonly in the Musculoskeletal Disorders and in the Skin and Subcutaneous Tissue Disorders system organ classes, and the most frequently reported event was myalgia.

Uncontrolled Study: Phase 2 Study ASB-01-04

Common adverse events up to week 24 for the phase 2 studies are shown in tables 16 and 17

Table 16 Incidence and Frequency of Adverse Events at 24 Weeks inStudy ASB-01-04

	No. of Patients/ No. of Events	
System Organ Class	1.0 mg/kg (n = 10)	
Any adverse event	10 / 147	
Deaths	0 / 0	
Discontinuations due to adverse events	0 / 0	
Serious adverse events	2/5	
Severe adverse events	3 / 3	
Study drug-related adverse events	4 / 12	
Adverse events during infusion	4 / 12	
IARs (study drug-related adverse events during infusion)	1 / 6	

	No. (%) of Patients / No. of Events
System Organ Class	1.0 mg/kg (n = 10)
Blood and Lymphatic System Disorders	0 (0%) / 0
Cardiac Disorders	2 (20.0%) / 2
Ear and Labyrinth Disorders	5 (50.0%) / 12
Eye Disorders	2 (20.0%) / 10
Gastrointestinal Disorders	7 (70.0%) / 21
General Disorders and Administration Site Conditions	6 (60.0%) / 17
Hepatobiliary Disorders	0 (0%) / 0
Immune System Disorders	0 (0%) / 0
Infections and Infestations	7 (70.0%) / 20
Injury, Poisoning, and Procedural Complications	5 (50.0%) / 7
Investigations	2 (20.0%) / 4
Metabolism and Nutrition Disorders	1 (10.0%) / 1
Musculoskeletal and Connective Tissue Disorders	7 (70.0%) / 15
Neoplasms (Benign, Malignant, and Unspecified)	0 (0%) / 0
Nervous System Disorders	6 (60.0%) / 13
Psychiatric Disorders	2 (20.0%) / 3
Renal and Urinary Disorders	0 (0%) / 0
Reproductive System and Breast Disorders	0 (0%) / 0
Respiratory, Thoracic, and Mediastinal Disorders	6 (60.0%) / 13
Skin and Subcutaneous Tissue Disorders	4 (40.0%) / 6
Surgical and Medical Procedures	0 (0%) / 0
Vascular Disorders	3 (30.0%) / 3

 Table 17: Incidence and Frequency of Adverse Events at 24 Weeks by System Organ

 Class in Phase 2 Study ASB-01-04

In the Phase 2 Study, the AEs with the highest incidence ($\geq 20\%$ of patients) were headache (5 patients); vomiting, URI, pain in extremity, and pyrexia (4 patients each); and abdominal pain, nausea, pneumonia, infusion site pain, and otorrhea (3 patient each).

The most frequently reported AEs ($\geq 2.5\%$ of all events) were headache, pyrexia, vomiting, pain in extremity, otorrhea, nausea, pneumonia, and infusion site pain.

Of the 147 AEs reported for the 10 patients treated in this study, 135 events (91.8%) were judged by the investigator to be unrelated to study drug administration.

The remaining 12 AEs (8.2%) were considered related to study drug administration.

(Of these 12 AEs, 6 did not occur during infusion). Of the related events that did not occur during infusion, events occurred most commonly in the Skin and Subcutaneous Tissue Disorders system organ class; these events included abdominal pain, pyrexia, complement factor decreased, asthma, eczema, and pruritus.

Common Adverse Events from Weeks 25 to 144

Among the Common Adverse Events from Weeks 25 to 144, the specific AEs with the highest incidence ($\geq 15\%$ of patients) were URI (10 patients); diarrhea, pyrexia, arthralgia, and headache (9 patients each); vomiting (8 patients); otorrhea (7 patients); ear pain, infusion site pain, otitis media, contusion, and cough (5 patients each); rash, urticaria, chest pain, viral URI, and ear

infection (4 patients each); and abdominal pain, nausea, herpes simplex, post-procedural pain, sunburn, thermal burn, back pain, myalgia, pain in extremity, eye infection, nasal congestion, rhinorrhea, and poor venous access (3 patients each).

The most frequently reported AEs ($\geq 2.5\%$ of all events) were pyrexia, headache, rash, URI, otorrhea, diarrhea, and pain in extremity.

Of the 562 AEs reported for the 16 patients treated beyond 24 weeks, 488 events (86.8%) were judged by the investigator to be unrelated to study drug administration.

A total of 74 adverse events (13.2%) were considered to be related to study drug administration. Of these, 6 did not occur during study drug infusion. The remaining 68 study drug–related events did occur during infusion and were therefore considered IARs.

Serious Adverse Events And Deaths

A total of 39 SAEs (2.6% of all events) were reported overall for the 3 studies; 27 in rhASBtreated patients and 12 in placebo-treated patients. Three SAEs were considered to be related to study drug. Two related SAEs, apnea and urticaria, occurred during study drug infusion, both in patients treated with rhASB. The apnoeic event, although judged to be possibly related to study drug, may have been precipitated by antihistamine use in the setting of severe upper airway obstruction. Moderate urticaria requiring slowing of the rhASB infusion occurred at Week 76. The third related SAE, asthma, occurred in an rhASB-treated patient with a long history of steroid-dependent asthma several hours after study drug infusion. A total of 36 SAEs were considered unrelated to study drug administration; none of these occurred during infusion. Twenty-four of these events occurred in patients receiving rhASB. Pneumonia was the most frequently reported unrelated SAE (3 events each for rhASB and placebo)

Deaths

One death was reported among patients treated with rhASB. Patient 40, Phase 1/2 Study elected to withdraw from study drug treatment after 3 weeks. He subsequently developed a malignant brain glioma (10 months after withdrawal) and carcinoma of the colon (14 months after withdrawal). He died of complications of the brain glioma approximately 20 months following his final study drug infusion. This patient was determined to have a germline mutation of the MSH2 gene that was believed to be the underlying cause of both the glioma and colorectal cancer.

Laboratory Findings

Anemia was the most common event reported; a total of 4 episodes were reported for 3 of 36 rhASB-treated patients and 9 episodes were reported for 8 of 19 placebo-treated patients. Two episodes of increased INR (blood clotting time) were reported for 1 patient. Single instances of decreased serum albumin, increased alkaline phosphatase, increased phosphorus, decreased potassium, decreased hemoglobin, decreased complement factor, abnormal INR, hyponatremia, hematuria, and proteinuria were also reported.

One event each of anemia and decreased complement factor was considered to be related to study drug administration. None of these events were severe or serious adverse events.

• Safety in Special Populations

Safety Results from the Sibling Study

Because of the age of these patients at enrolment (2 months, 3 years, 7 months), the data from this study are not included in any of the integrated tables. There have been no adverse events, no anaphylactoid reactions during infusion, and no clinically significant changes in laboratory test values during the 8-month period of this report. None of the siblings has developed antibody to rhASB.

• Immunological Events

Infusion-Associated Reactions (IARs)

Infusion-Associated Reactions up to Week 24

Controlled Studies:

In the 2 controlled clinical studies, 95 of the 802 AEs (11.8%) occurred during infusion. Of these 95 AEs, 69 (72.6%) were considered to be related to study drug and thus met the definition for IAR. Sixty-three of these events were reported for rhASB-treated patients, while the remaining 6 events were reported for placebo-treated patients.

IARs were most frequently reported for the General Disorders and Administration Site Conditions and Respiratory Disorders system organ classes. The most frequently reported IARs included rigors (15.0% of events), dyspnea (7.5%), and pyrexia (6.3%). In 5 rhASB-patients, IARs recurred and were considered to represent an anaphylactoid reaction to the study drug.

Uncontrolled Study:

In the initial 24 weeks of this Phase 2 Study, 12 AEs occurred during infusion. Of these 12 AEs, 6 events were considered to be related to study drug and thus met the definition for IAR.

ARs included pyrexia (3 events), and dyspnea, chest pain and headache (1 event each). None of these IARs recurred with a pattern suggestive of an anaphylactoid reaction.

Infusion-Associated Reactions Weeks 25 Through 144

Of 562 adverse events reported during long-term treatment, 112 events (19.9%) occurred during study drug infusion. Of those, 68 events were considered to be related to study drug administration and thus met the definition for IAR .

The most frequently reported IARs were rash (30.8% of events), urticaria (27.9%), hypotension (14.7%), and pyrexia (11.7%). Rash and urticaria were components of anaphylactoid reactions that were observed in 3 patients.

Anaphylactoid Reactions

Anaphylactoid reactions to study drug infusion occurred in 8 (22.2%) of the patients treated with rhASB. Reactions began as early as Week 6 and as late as Week 55 of study drug treatment, and occurred during multiple infusions, though not always in consecutive weeks. The most common symptoms included fever, chills/rigors, rash, and urticaria, although hypotension, nausea, vomiting, dyspnea, bronchospasm, retrosternal pain, abdominal pain, headache, malaise, and joint pain were also reported. Symptoms typically abated with interruption of the infusion and administration of additional antihistamines, antipyretics, and occasionally steroids. Incomplete infusions were uncommon. Subsequent infusions were managed with a slower rate of drug administration, treatment with additional prophylactic antihistamines, and, infrequently, in the event of a more severe reaction, treatment with prophylactic steroids. Corticosteroid use was limited to 6 of the patients with infusion-associated reactions (IARs) across studies.

A number of patient characteristics were examined in an effort to identify factors that might put patients at risk for anaphylactoid reactions. No relationship was observed between the development of anaphylactoid reactions and anti–ASB antibody level, pre-medications, concomitant medications, concurrent illnesses, missed infusions, or manifestations of MPS VI.

Complement Depletion

Decreases in complement parameters were observed intermittently, primarily in the first 24 weeks of treatment, in all three clinical studies, in both dose groups and placebo. Decreases in many cases involved a single complement measure and occurred only sporadically. In approximately one-half of the patients with decreases in more than one complement parameter, antibody levels rose above 10 OD/ μ L at some time during the course of treatment, not always in a timeframe consistent with complement decline. There was no clear correlation between decreases in complement parameters and the development of anaphylactoid reactions during infusion.

Immunogenicity

Ninety-four percent (34/36) of all patients treated with rhASB developed anti-ASB IgG antibodies. Initial evidence of antibody development typically appeared following 4–8 weeks of treatment. Ten patients developed antibody levels > 10 OD/ μ L. The incidence of levels > 10 OD/ μ L was 16.7% (1/6), 30% (3/10), and 31.6% (6/19) for rhASB-treated patients receiving at least 24 weeks of treatment in the Phase 1/2, Phase 2, and Phase 3 studies, respectively. Analysis of the course of antibody levels over the longer term (in the Phase 1/2 and Phase 2 studies

revealed a steady increase in mean antibody level through Week 48 of treatment, with a gradual decline beginning at Week 72 to levels $< 2.0 \text{ OD}/\mu L$ by Week 144 in the 5 patients treated for the longest duration.

Four patients with high antibody levels had observable differences in pharmacokinetic parameters relative to the overall study population. One patient had a low plasma concentration of ASB coincident with high antibody levels, and 3 additional patients showed an increase in CL and Vz. These changes were considered to be related either to the effects of the antibody on rhASB clearance and distribution or to the antibody's interference with the ELISA assay for ASB. In 1 patient, reduction in urinary GAG was less than the median for the group overall, suggesting an impact of antibody on rhASB uptake and distribution.

The risk of antibody development was associated with very low levels of endogenous ASB protein. Higher antibody levels were more often seen in younger patients, but the majority of these individuals had low endogenous ASB relative to the older age groups.

Higher antibody levels appeared to correlate with decreases in complement parameters in some patients; however, higher antibody levels were not associated with anaphylactoid reactions, and no apparent increase in adverse events or alteration in the adverse event profile was seen in patients with high antibody levels.

• Safety Related to Drug-Drug Interactions and Other Interactions

No formal drug interaction studies have been conducted.

Discontinuation Due to AES

There were no discontinuations due to AEs

• Post Marketing Experience/Risk Management

Not applicable

• Proposals for Post Authorisation Follow Up (Post Marketing Surveillance)

Two core risk management programs proposed for safety data collection for rhASB treatment in the post-marketing setting are Pharmacovigilance/Safety Reporting and the Clinical Surveillance Program. The Clinical Surveillance Program (CSP) will involve the collection and analysis of the maximum amount of data regarding MPS VI and rhASB treatment deemed to be reasonably collected in a voluntary, registry like, setting and is intended to be an ongoing, observational database that tracks the specific clinical outcomes, including safety outcomes of patients with MPS VI disease, in particular those who receive rhASB treatment.

Discussion on Clinical Safety

Adverse events were generally mild to moderate and the majority judged to be unrelated to study drug administration. Severe and serious adverse events were uncommon and were comparable in incidence between rhASB– and placebo–treated patients. The majority of SAEs appeared to be unrelated to ASB administration. The apnoeic event which occurred during ASB infusion was judged to be possibly related to study drug. There is a possibility however, that it may have been precipitated by antihistamine use in a patient with severe upper airway obstruction.

One death occurred in the Phase 1/2 Study after a patient elected to withdraw from study drug treatment after 3 weeks. He subsequently developed a malignant brain glioma (10 months after withdrawal) and carcinoma of the colon (14 months after withdrawal) and died of complications of the brain glioma approximately 20 months following his final study drug infusion. This patient was determined to have a germline mutation of the MSH2 gene that was believed to be the underlying cause of both the glioma and colorectal cancer.

Anti–ASB IgG antibodies were detected in the majority of rhASB–treated patients. Four patients with high antibody levels had observable differences in pharmacokinetic parameters relative to the overall study population. One patient had a low plasma concentration of ASB coincident with high antibody levels, and 3 additional patients showed an increase in CL and Vz. It was not entirely clear if these changes were related to the effects of the antibody on rhASB clearance and distribution or to the antibody's interference with the ELISA assay for ASB. In 1 patient,

reduction in urinary GAG was less than the median for the group overall, suggesting an affect of antibody on rhASB uptake and distribution.

However, the clinical relevance to safety of even higher antibody levels (> 10 OD/ μ L) was unclear, as there was no clear correlation between elevated antibody levels and anaphylactoid reactions or clinical symptoms. Anaphylactoid reactions occurred in approximately 22% of rhASB-treated patients; however, these were generally readily managed with interruption and/or slowing of the rate of infusion, and the administration of antihistamines, antipyretics, or steroids.

There were no consistent or significant abnormalities, either in the laboratory data to suggest tissue toxicity or immune complex disease, or in vital signs among the treatment groups.

At the request of the CHMP, the applicant has presented data which suggests that there is no consistent correlation between antibody levels and anaphylactoid reactions, infusion associated reactions and complement depletion. However, in order to understand the immunogenicity profiles with galsulfase, the applicant intends to conduct several analyses of serum samples obtained in the clinical surveillance programme, including subtyping of IgG to ascertain possible relationship with overall antibody response and complement fixation.

In order to address related adverse events the applicant has presented safety data tables of adverse events for the pivotal trial, together with a reasoned argument underlining similarities across clinical studies, consistent with the judgment of the investigators with regard to likely relationship.

To address the concern regarding causal and causal/temporal adverse events in the pivotal trial, the applicant has presented safety data tables for related and unrelated events in the galsulfase and control groups for all patients. The majority of adverse event were considered unrelated to study drug and represented common manifestations of MPS VI disease. However, while there was a clear tendency for increased infusion associated reactions which were possibly related in the active group; few were considered probably or definitely related.

The applicant has also provided a 120 day safety update for each individual study as well as a combined analysis of all studies.

The applicant has proposed a comprehensive prospective pharmacovigilance plan as part of the clinical surveillance programme to follow the long term safety and efficacy of galsulfase treatment.

The applicant will monitor particularly immunogenicity with respect to the effect on PK/PD parameters and relationship to infusion associated reactions, anaphylactoid reactions and complement depletion. In addition, further long term efficacy data should be submitted. Safety data will especially include adverse events in patients with substantially compromised airways.

Overall Conclusions, Benefit/Risk Assessment and Recommendation

Quality

In general, the different aspects of the chemical, pharmaceutical and biological documentation comply with existing guidelines. The fermentation and purification of the drug substance, have been adequately described, controlled and validated. The drug substance has been well characterised with regard to its physicochemical and biological characteristics, using state-of the-art methods, and appropriate specifications have been set. The manufacturing process of the drug product has been satisfactorily described and validated. The quality of the drug product is controlled by adequate test methods and specifications. The viral safety and the safety concerning other adventitious agents including TSE have been sufficiently assured. Except for a number of quality points, which will be addressed as part of post-approval follow-up measures, the overall quality of Naglazyme is considered acceptable.

Non-Clinical Pharmacology and Toxicology

The feline MPS VI model has similar pathology to the human MPS VI disease and has been used to investigate the effects of rhASB on various manifestation of the disease such as lysosomal storage, urinary GAG excretion, body weight, physical appearance, mobility and flexibility, as well as any possible immunological effects resulting from the infusion of a heterologous protein.

These studies have shown that rhASB can reduce lysosomal GAG storage and improve some symptoms of the disease, depending on the dose and dose regimen and the age of the cat at start of treatment. In this model, rhASB had no effect on cartilage or cornea. Based on the pharmacological effects in the feline MPS VI model at a dose of 1mg/kg/week, and the tissue half-life of rhASB of 2 to 4 days, the clinical dose of 1 mg/kg/week appears reasonable. As some clinical benefits were observed at 0.2mg/kg/week in Phase 1, the minimally effective dose may indeed be between 0.2 and 1mg/kg/week, but this was not investigated. Based on the results of the Phase 1 study which showed more rapid and more pronounced uGAG reductions, the 1 mg/kg dose was selected for the clinical development by the applicant. The clinical dose will be kept under review as a post-marketing commitment.

In the chronic monkey study, liver and skin effects were observed. These findings were reversible after a 2-week recovery period, however, potential reversibility would not be relevant should the effects occur clinically, given the chronic nature of the treatment. Exposure at the high dose (10 mg/kg/week) was 21 to 39 times that in man following a dose of 1mg/kg. The lack of significant liver abnormalities in the clinical studies provides reassurance, however liver toxicity will be kept under review as a post-marketing commitment.

The fact that a full package of reproductive toxicity studies has not been conducted is mentioned in section 5.3 of the SPC. Post-authorisation commitments have been agreed for further reproductive toxicity studies with toxicokinetics and clinical monitoring of pregnant women.

Efficacy

It is important to note that galsulfase is an enzyme replacement therapy, for MPS VI disease, and fulfils an unmet medical need, since there were no satisfactory treatment options for the majority of patients having this rare disease. It is to the applicant's credit that despite a global paucity of patients, data from a well-conducted 24-week double-blind, placebo-controlled, randomised clinical trial has been submitted.

In the clinical studies, the data are supportive of efficacy for the 1 mg/kg dose in comparison to the 0.2 mg/kg weekly dose in terms of providing a rapid reduction in urinary GAG excretion to levels approaching normal. However, assessment of the evidence of clinical benefit in terms of endurance, based on the 12–Minute Walk Test, was complicated by substantial baseline imbalances in the main trial and further confounded by the variability in individual patient responses. Clarification of this issue was provided by the reanalysis of the efficacy data in the pivotal trial, which demonstrated that the treatment advantage for galsulfase is not dependent upon the baseline imbalance. In addition, results from the open label extension study of the pivotal trial provided supportive corroborative data from improvements in the 12 minute walk test as well as reduction in urinary GAG levels in patients who were previously on placebo and were switched to galsulfase after 24 weeks.

It remains unclear whether intermediate dosage schedules would have shown sufficient efficacy, since none were clinically evaluated. In addition, individual PK data from the ten patients in the phase 2 uncontrolled study would appear to indicate that a maintenance dose may need to be determined separately as GAG levels reduce over time. Both an initial as well as a maintenance dose would need to be further defined through the clinical surveillance program. Furthermore, the period of treatment in non-responders also needs to be defined, since a proportion of patients did not appear to respond satisfactorily even after six months of treatment.

The studies did not include patients under the age of 5 or over the age of 29. It should be noted that, disease in relatively avascular tissues appears unaffected by rhASB treatment, as reflected by development of both corneal clouding and skeletal dysplasia of the ribs in the 2–month old patient in the sibling study, after 10 months of treatment and the non-regression of these parameter in older patients.

Safety

The overall limited safety profile of rhASB does not raise major clinical issues for this focused group of patients. However, an important primary safety concern with rhASB treatment appears to be the management and treatment of patients with highly compromised airways by careful

monitoring of antihistamine and other sedative medication use. Accordingly, the institution of positive–airway pressure during sleep and even tracheostomy in clinically appropriate situations is an important consideration and needs to be clearly communicated. In addition, long term safety will be addressed as proposed in the clinical surveillance program.

From an immunological perspective, four patients with high antibody levels had observable differences in pharmacokinetic parameters relative to the overall study population. One patient had a low plasma concentration of ASB coincident with high antibody levels, and three additional patients showed an increase in CL and Vz. It is not entirely clear if these changes were related to the effects of the antibody on rhASB clearance and distribution or to the antibody's interference with the ELISA assay for ASB. In one patient, reduction in urinary GAG was less than the median for the group overall, suggesting an affect of antibody on rhASB uptake and distribution. Higher antibody levels appeared to correlate with decreases in complement parameters in some patients; however, higher antibody levels were not consistently associated with anaphylactoid reactions, and no significant increase in adverse events or alteration in the adverse event profile was seen in patients with high antibody levels. For the majority of patients who showed generation of IgG antibodies, no significant impact on the pharmacodynamic properties of the drug over the long-term was observed. Nor were there consistent or significant abnormalities in the laboratory data to suggest tissue toxicity or immune complex disease. However, the variability seen with immunological events needs to be further defined in a larger patient population and will be addressed in the Clinical Surveillance Program proposed by the applicant.

Risk management plan

The Applicant has provided a Clinical Surveillance Program (CSP) that will involve the collection and analysis of the maximum amount of data regarding MPS VI and rhASB treatment deemed to be reasonably collected in a voluntary, registry like, setting and is intended to be an ongoing, observational database that tracks the specific clinical outcomes, including safety outcomes of patients with MPS VI disease, in particular those who receive rhASB treatment. (specific obligations and follow-up measures). The following commitments are included:

The long term safety and efficacy of galsulfase treatment will be implemented by the time of launch of the product (follow-up measure.).

The level of antibodies will be monitored in relation to safety and efficacy parameters

Serious and severe adverse events related to liver function will be monitored as part of the Pharmacovigilance activities.

Data collected will be examined to determine if a suitable Naglazyme maintenance dose can be recommended relative to the efficacy endpoints used in the clinical studies, and will be discussed with the Rapporteur on an ongoing basis.

No additional risk minimisation measures are deemed necessary other than those stated in the SPC/PL recommendations, as Naglazyme treatment will be supervised by a physician experienced in the management of patients with MPS VI or other inherited metabolic diseases. Administration of Naglazyme will also be carried out in an appropriate clinical setting where resuscitation equipment to manage medical emergencies would be readily available.

The applicant committed to submit an update of the RMP with the first PSUR and further updates in line with the CHMP "Guideline on Risk Management Systems for Medicinal Products for Human use", i.e. at the same time as PSURs, when new information is received, within 60 days of an important milestones being reached or the results of a study becoming available (specific obligations/FUM timelines), and upon request of a Competent authority.

Benefit/Risk Assessment

Considering the progressive nature of MPS VI disease and the implications for prognosis, together with the heterogeneity of patients in terms of disease manifestation and age, as well as the problems of reversing the chronic effects caused by long term accumulation of lysosomal storage, the improvements demonstrated in the clinical studies, some albeit limited, appear to

represent clinical benefits for MPS VI patients. The safety profile from the data submitted does not raise major clinical concerns.

However, in view of the rarity of the disease, comprehensive safety and efficacy data, such as possible effects of antibodies on the efficacy and safety, the determination of the optimal maintenance dose, the safety in children below 5 year old, or the long-term safety and efficacy cannot be obtained in a reasonable time.

The risks will be kept under control with the RMP proposed by the applicant

Accordingly, the benefit-risk is considered to be positive under the condition that a number follow-up measures and specific obligations are performed in the specified timeframe.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk profile of Naglazyme was favourable in the treatment of "long-term enzyme replacement therapy in patients with a confirmed diagnosis of Mucopolysaccharidosis VI".