CENTER FOR DRUG EVALUATION AND RESEARCH AND CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

APPLICATION NUMBER:

125117/0

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

STN BLA NUMBER:	**STN 125117/0**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**11/23/04**
DRUG NAME:	**Galsulfase (rhASB. —**
INDICATION:	**Treatment of Mucopolysaccharidosis VI (MPS VI)**
SPONSOR:	**BioMarin Pharmaceutical Inc.**
DOCUMENTS REVIEWED:	**E-BLA submission**
REVIEW DIVISION:	**Division of Biological Therapeutic Oncology Products (HFD-107)**
PHARM/TOX REVIEWER:	**Wen-Yi Gao, M.D., Ph.D.**
PHARM/TOX SUPERVISOR:	**M. David Green, Ph.D.**
DIVISION DIRECTOR:	**Patricia Keegan, M.D.**
PROJECT MANAGER:	**Katherine Needleman**

Date of review submission to Division File System (DFS):

4/7/05

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The Biologics Licensing Application STN BLA #125117/0 is approvable based on the data contained in the preclinical pharmacology and toxicology sections of the original submission.

B. Recommendation for nonclinical studies

We recommend conducting [redacted] developmental toxicity studies in a non-rodent species as a phase 4 commitment.

C. Recommendations on labeling

The sponsor claims a [redacted] safety margin based on the dosage used in the rat reproductive study. In the absence of comparative pharmacokinetic study between human and rat, such assertion is misleading. In addition, the negative results of the rat reproductive study have not been confirmed in a non-rodent species. Thus, the clinical correlation of the negative results is still unknown. The submitted labeling is as following:

"Pregnancy: Category B

[redacted]

Recommended changes to the section are as following:

"Pregnancy: Category B

[redacted]

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Recombinant human N–acetylgalactosamine 4–sulfatase (rhASB) is a lysosomal hydrolase that cleaves the sulfate ester at the end of the glycosaminoglycans.

rhASB is produced in a Chinese hamster ovary cell line, and the proposed use is for the [redacted]

The in vitro studies demonstrated that rhASB is efficiently taken up by normal and MPS VI patient fibroblasts via a high affinity mannose-6-phosphate receptor (1). The enzyme is internalized to the lysosomes, where it degrades glycosaminoglycan (GAG) rapidly (1). The feline MPS VI model is an animal disease model that shares the same etiology and similar pathology with the human MPS VI disease. It allows the pharmacological evaluation of the ability of rhASB to reduce tissue lysosomal GAG storage, and to monitor the bioactivity marker of rhASB and the urinary GAG (uGAGs) excretion. The results showed that rhASB treatment improved vertebral bone mineral volume, tibial cortical bone thickness and other histomorphometric parameters. The tissue and urinary levels of GAG were also decreased. These effects were dependent on the doses (1 mg/kg and 5 mg/kg) and the route (intravenous injection) of administration.

Safety pharmacology assessments of rhASB were conducted in conjunction with single-dose and repeated-dose toxicity studies in dogs and cynomolgus monkeys, respectively. There were no rhASB–related toxicities on clinical observations, body temperature, heart rate, respiration rate, blood pressure, blood oxygenation, ECG measurements, blood coagulation or urinalysis parameters. Interstitial pneumonia, pulmonary vasculitis and glomerulonephropathy were identified in rhASB treated MPS VI cats (Study Numbers ASB-PC-005, ASB-PC-006, ASB-PC-007, and ASB-PC-008). The possibility of immunologically mediated pathology can not be ruled out.

The pharmacokinetics was studied in dogs following a single intravenous infusion of rhASB. It was non-linear between 2 and 20 mg/kg. The mean C_{max} and AUC_{∞} values increased approximately 100-fold, when the dose was increased by 10-fold. The mean $T_{½}$ values were approximately 30 minutes.

The repeat-dose toxicity study was conducted in cynomolgus monkeys. The animals were received 1, 3, or 10 mg/kg of rhASB intravenous infusion weekly for 27 weeks. Histopathological examination was remarkable for bile duct hyperplasia, subacute hepatic periportal inflammation (moderate) and centrilobular necrosis (focal) at 3 and 10 mg/kg. Serocellular or pustular epidermatitis occurred at all dose levels in the female monkeys. Because of the epidermatitis, the NOAEL was not determined.

The reproductive performance and developmental toxicity study was conducted in rats. The study included estrous cycle, implantation, and development of the embryos and fetuses. No test article related changes were identified at the level of 3 mg/kg/day intravenous injection.

B. Pharmacologic activity

Treatment with rhASB IV infusion decreased the GAG levels in tissues, and increased the bone mineral volume resulting in greater mobility and flexibility in the MPS VI cats. The activities were dose- and administration route-dependent. The improvement was only observed when the therapy initiated before skeletal maturity.

Cellular uptake of rhASB involves the mechanism of receptor mediated pinocytosis. Lysosomal storage studies showed that treatment with rhASB was most effective in reduction of GAG storage in macrophage, Kupffer cells and other reticuloendothelial cells. These cells have high rates of pinocytosis/plasma membrane turn-over. In contrast, cells with low rates of membrane turn-over (chondrocytes in articular cartilage and cornea keratocytes) showed less reduction of the lysosomal GAG storage.

Safety pharmacology studies were conducted in conjunction with single-dose and repeated-dose toxicity studies in dogs and cynomolgus monkeys, respectively. There were no rhASB–related effects on clinical observations, body temperature, heart rate, respiration rate, blood pressure, blood oxygenation, ECG measurements, blood coagulation or urinalysis parameters.

The pharmacokinetic studies in dogs demonstrated a non-linear fashion between 2 and 20 mg/kg. When the dose increased 10-fold, the mean C_{max} and AUC_{∞} values increased approximately 100-fold. The mean $T_{½}$ values were approximately 30 minutes at the dose levels.

C. Nonclinical safety issues relevant to clinical use

The anticipated risks of rhASB IV infusion are primarily due to 1) hypersensitivity reaction to the formulation components, 2) administration of rhASB to patients with the preliminary sensitization, 3) immune complexes deposition mediated diseases, or 4) T-lymphocyte activation associated pathology.

In the acute single dose toxicity studies, rats and dogs had swelling of the mouth, nose or paws starting at 30 minutes after dosing with rhASB and persisted up to 8 hours post-treatment. The facial and paw edema may be attributed to one of the formulation components, polysorbate 80, which has been shown to have histamine-releasing properties (2). These observations indicate that anaphylactic or anaphylactoid reactions (such as hypotension or anaphylactic shock) may occur during the treatment. In the MPS VI-affected cat studies, interstitial pneumonia, pulmonary angiitis, and glomerulonephropathy were identified in the treated cats, suggesting that immunologically mediated pathology may occur. In the 27-week monkey study, rhASB-related toxicity was identified in the liver, adrenal and skin. Dose-dependent chronic hepatic periportal inflammation (moderate), centrilobular necrosis (focal), and bile duct hyperplasia were diagnosed in both male and female monkeys. These results indicate that rhASB treatment may

induce acute and chronic hepatitis. The adrenals of the male monkeys had mild atrophy (predominantly in zona reticularis). The clinical significance in the production of cortisol and dehydroepiandrosterone is unknown. Serocellular or pustular epidermatitis was reported at all dose levels in both sexes that received the test article. Microscopic findings included mild to moderate increases of eosinophils (perivascularly), lymphocytes, histiocytes, and acanthosis/hyperkeratosis. Based on the dermal inflammation pattern, we can not ruled out the possibility of immune complex deposition or T-lymphocyte activation associated epidermatitis.

Clinical toxicities of the product not predicted by the animal studies, but listed as historical adverse events, include secondary hypertension, neurological disorders (migraine) and autoimmune disorders (conjunctivitis). These historical adverse events occurred in the phase 3 clinical study have been provided in the draft of labeling.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: STN 125117/0
Sequence number/date/type of submission: Sequence number 0/11-23-2004/Original
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: BioMarin Pharmaceutical Inc.
Manufacturer for drug substance: BioMarin Pharmaceutical Inc., Galli Facility, 46 Galli Drive, Novato, California 94949

Reviewer name: Wen-Yi Gao, M.D., Ph.D.
Division name: Division of Therapeutic Biologic Oncology Products, ODE VI
HFD #: HFD-107
Review completion date: 01/18/05

Drug:

Trade name:

Generic name: Galsulfase

Code name: Galsulfase

Chemical name: Recombinant human N-acetylgalactosamine 4-sulfatase (rhASB), recombinant human N-acetylgalactosamine 4-sulfate sulfatase, arylsulfatase B

Molecular formula/molecular weight: rhASB is a polypeptide with molecular mass 55.9 kiloDaltons.

Structure: rhASB is a 495 amino acid single polypeptide. It contains six asparagine-linked glycosylation sites, and has lysosomal sulfatase activity. The crystal structure and biochemical studies show that the catalytic amino acid residue is located at the Cα-formylglycine, which is produced by post-translational modification of Cys53. This modification is required for enzyme activity and is conserved in all members of the sulfatase enzyme family.

Relevant INDs/NDAs/DMFs: BB-IND 9057

Drug class: Recombinant human N-acetylgalactosamine 4-sulfatase

Intended clinical population: patients with mucopolysaccharidosis VI

Clinical formulation: Each vial of rhASB is single use only and delivers 5.0 ml of a solution that includes rhASB at 1 mg/ml, — sodium phosphate, — sodium chloride, and — Polysorbate 80, with a pH of 5.8. The drug product is a solution that is clear to slightly opalescent. For administration to patients, rhASB is diluted with 0.9% sodium chloride to a volume of 250 ml. Whenever possible, the volume is adjusted to meet the fluid requirements of the patient. Patients receive rhASB at a nominal dose of 1 mg/kg. rhASB is administered intravenously once weekly.

Route of administration: intravenous infusion

Disclaimer: Tabular information is constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Pharmacology:

- BioMarin Study Report No. ASB-PC-004: Evaluation of Infusion Rate of Recombinant Human Arylsulfatase B in MPS VI Affected Cats
- BioMarin Study Report No. PC-BM 102-005: Nine Month Safety Evaluation of Recombinant Human N-acetylgalactosamine-4-sulfatase in MPS VI Affected Adult Cats: Final Report (pharmacology data)
- BioMarin Study Report No. PC-BM 102-006: Six Month Safety Evaluation of Recombinant Human N-acetylgalactosamine-4-sulfatase in MPS VI Affected Adult Cats from Birth: Final Report (pharmacology data)
- BioMarin Study Report No. ASB-PC-007: Six Month Efficacy and Safety Evaluation of Recombinant Human N-acetylgalactosamine-4-sulfatase (containing Tween = 80) in MPS VI Affected Adult Cats from Birth (pharmacology data)
- BioMarin Study Report No. ASB-PC-008: Long-term Combined Intravenous and Intra-articular Therapy with rh4S for the Prevention of Degenerative Joint Disease in MPS VI Cats

Pharmacokinetics:

- BioMarin Study Report No. ASB-AT-001: Acute Single Dose Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B (rhASB) in Dogs (Pharmacokinetic Analysis)
- BioMarin Study Report No. ASB-042-AT: 27-Week Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B (rhASB) in Cynomolgus Monkeys with a 2-Week Recovery (Toxicokinetic Analysis)
- BioMarin Study Report No. ASB-45-APK: Pharmacokinetic Study of Differing Production Lots of Recombinant Human Arylsulfatase B (rhASB) in Dogs
- BioMarin Study Report No. ASB-46-APK: A Pharmacokinetic Study of Differing Production Lots Manufactured at Two Different Sites of Recombinant Human Arylsulfatase B in Dogs Following Intravenous Administration
- BioMarin Study Report No. ASB-53-APK: A Pharmacokinetic Study of Two Production Lots Manufactured via the Same Process with Different — of Recombinant Human Arylsulfatase B (rhASB) in Dogs Following Intravenous Administration

Toxicology:

Single-Dose Toxicity:

- BioMarin Study Report No. ASB-AT-001: Acute Single Dose Intravenous Infusion Toxicity Study with Recombinant human Arylsulfatase B (rhASB) in Dogs
- BioMarin Study Report No. ASB-AT-002: Acute Single Dose Intravenous Injection Study with Recombinant human Arylsulfatase B in Rats

Repeat-Dose Toxicity:

- BioMarin Study Report No. ASB-042-AT: 27-Week Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B (rhASB) in Cynomologus Monkeys with a 2-Week Recovery

Reproductive and Developmental Toxicity:

- BioMarin Study Report No. ASB-043-AT: Combined Intravenous Fertility and Development Toxicity Study of Recombinant human Arylsulfatase B in Rats
- BioMarin Study Report No. ASB-044-AT: Intravenous Dosage Range Combined Fertility and Developmental Toxicity Study of Recombinant human Arylsulfatase B in Rats

Studies not reviewed within this submission:

All preclinical studies within this submission have been reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Previous studies by other investigators suggested that enzyme replacement therapy (ERT) with rhASB resulted in the improvement of bone mineral volume and other histomorphometric parameters in the MPS VI feline model(1;3-7).

The studies by BioMarine showed that rhASB treatment of MPS VI–affected cats lowered the GAG storage vacuoles in Kupffer cells, decreased urinary GAG excretion, increased bone mineral volume, and resulted in greater mobility and flexibility. The dose of 1 mg/kg rhASB was the lowest dose observed to produce clinical benefit. Early rhASB treatment had a better effect on bone growth and remodeling.

Safety pharmacology assessments of rhASB were performed in conjunction with single-dose and repeated-dose GLP toxicity studies in dogs and cynomolgus monkeys, respectively. There were no rhASB–related effects on clinical observations, body temperature, heart rate, respiration rate, blood pressure, blood oxygenation, ECG measurements, blood coagulation or urinalysis parameters.

2.6.2.2 Primary pharmacodynamics

I) In vitro Studies

rhASB is efficiently taken up by normal and MPS VI patient fibroblasts cultured in vitro via a high affinity mannose-6-phosphate receptor (1)that is expressed on most cells (3). Once bound, the enzyme is endocytosed and is transported through coated pits to the lysosomes, where it removes sulfate residues from accumulated dermatan sulfate. In MPS VI fibroblasts, the clearance of GAG storage is rapid following enzyme exposure (1). In vitro studies conducted at BioMarin have confirmed that rhASB is internalized by MPS VI fibroblasts and is enzymatically active.

II) In vivo Studies
There are five BioMarin sponsored pharmacological studies summarized as following:

Study title: Evaluation of Infusion Rate of rhASB in MPS VI Affected Cats (Study ASB-PC-004)

Key study findings: An extended 2-hour infusion of the enzyme was better tolerated as compared with a 10-15 minute infusion protocol. The complete clearance of lysosomal GAG storage was identified in reticuloendothelial cells.

Study No.: ABS-PC-004
Volume No., and page No.: CTD Section 4.2.1.1.
Conducting laboratory and location:

Date of study initiation: May 6, 1999
GLP compliance: No
QA reports: No
Drug, lot No., and % purity: rhASB BMP Lot Nos. 36A42699, 36B50799, 36C52199; information regarding the purity was not provided in the final study report.

Methods:
Dosing: 1 mg/kg intravenous infusion weekly for 5 weeks

Species/strain: MPS VI cats, 10 weeks old

Number/sex/group: 1 female and 3 males

Route, formulation, volume, and infusion rate: Intravenous infusion (cephalic vein); 0.125 mg/ml rhASB in phosphate-buffered saline, total volume 10 ml (2-hour infusion group); 0.25 mg/ml rhASB with total volume 5 ml (10-minute infusion group)

Study design: Two treatment groups: 2-hour IV infusion weekly for 5 weeks versus 10-minute IV infusion weekly for 5 weeks. Animals were euthanized two days after the last infusion. Selected tissues (liver, spleen, heart, lung, kidney, skin, aorta, cerebrum,

cerebellum, cartilage, cornea and lymph nodes) were collected for determination of ASB activity, and for histopathological evaluation.

Results:
Mortality: all cats survived until necropsy

Clinical signs: No abnormal observations in both groups except vomiting occurred in the 10-minite infusion group.

Clinical pathology: No significant abnormalities in clinical laboratory values were observed; urinary GAG levels decreased in all four treated MPS VI cats. Urinary GAG levels were reduced after the first or second infusion to levels below the range observed in untreated MPS VI-affected cats, indicating that this parameter may be used as a surrogate marker of treatment responsiveness.

Gross pathology: All thoracic and abdominal organs appeared normal in appearance.

Histopathology: Microscopic assessment of the major organs revealed no treatment-related abnormalities. In all ERT-treated MPS cats, the lysosomal GAG storage was completely eliminated in splenic macrophages, Kupffer cells lining the hepatic sinusoids, and in kidney interstitial cells (reticuloendothelial cells). No change was identified in the lysosomal storage of the smooth muscle cells (aorta) and fibroblasts (heart valve).

Tissue distribution of rhASB: rhASB tissue levels were measured 2 days after last infusion. The tissues were from liver, lung, spleen, kidney, heart bone marrow, mesenteric lymph nodes, aorta, cerebrum, cerebellum, skin, articular cartilage, and cornea. The highest enzyme concentration was found in the liver. No enzyme was found in the skin, articular cartilage and cornea in all cats. There were no controls of the untreated disease tissue and normal tissue.

Tissue distribution data revealed that one of the 2 cats that received 2–hour rhASB infusions had increased enzyme levels in some tissues compared to those observed after the 10–minute rhASB infusion. The other cat that received the 2–hour infusion had lower enzyme levels in all tissues, including the liver, possibly due to dislodging of the catheter during the final infusion.

Conclusions: The extended 2-hour infusion protocol is safe and better tolerated as compared to the 10-minute infusion protocol.

Comments: Clearance of cellular GAS by rhASB treatment was more effective in reticuloendothelial cells (splenic macrophages, Kupffer cells, and kidney interstitial cells) than in fibroblasts (heart valve) or smooth muscle cells (aorta). Explanations of the differential effects include that 1) reticuloendothelial cells had higher rates of pinocytosis. Internalization of rhASB was more effective in these cells than in fibroblasts or smooth muscle cells, and that 2) reticuloendothelial cells had better blood supply than

chondrocytes (articular cartilage). The role of cartilage changes in the clinical improvement is still unclear.

The sponsor claims that the 2-hour infusion protocol gives better tissue enzyme levels as compared with the 10-minute protocol. Their evidence is slim. The sample size is too small (2 cats per group), and the variation is too large (more than 10-fold of the test values).

Study title: Nine Month Safety Evaluation of rhASB in MPS VI Affected Adult Cats (Study ASB-PC-005)

Key study findings: Treatment of MPS VI-affected cats with rhASB decreased uGAG excretion to approximately 4– to 5-fold below the average value for age-matched, untreated historical control MPS VI–affected cats and to 2– to 3-fold above that of age–matched, historical control normal cats. However, there was no treatment-related radiologic and histopathological improvement.

Study No.: ABS-PC-005
Volume No., and page No.: CTD Section 4.2.1.1.
Conducting laboratory and location:

Date of study initiation: July 21, 1999
GLP compliance: No
QA reports: No
Drug, lot No., and % purity: rhASB BMP Lot Nos. 02-60-061799, 02-60-060799, 02-60-072799, 02-70-091699, 02-70-111599B, 02-70-092199, 02-70-121599, 02-70-020100B, 02-70-021500, 02-70-020100C, 02-70-022200; information regarding the purity was not provided in the final study report.

Methods:
Dosing: For the first 27 infusions, 1 mg/kg rhASB protein was administered weekly. Starting on infusion #28, the dose was increased from 1 mg/kg to 2 mg/kg rhASB protein until the conclusion of the study.

Species/strain: MPS VI cats (n = 5), 14 to 21 weeks old

Number/sex/group: 1 female and 4 males

Route, formulation, volume, and infusion rate: Intravenous infusion (cephalic vein); 1 mg/kg rhASB in 10 ml phosphate-buffered saline at the infusion rate 5 ml/hour for the first 27 infusion; From the infusion # 28, 2 mg/kg rhASB was administered.

Study design: Five MPS VI–affected cats (3–5 months old) were treated with rhASB for 27 weeks at a dose of 1 mg/kg/week followed by a dose of 2 mg/kg/week for 10 weeks

(total duration of treatment of 37 weeks). In an attempt to understand the etiology of infusion–associated reactions, serum complement levels were determined during some of those reactions or after large increases in antibody levels. Animals were euthanized two days after the last infusion and tissues were collected for determination of tissue ASB activity and for histopathological evaluation.

Results:
Mortality: all cats survived until necropsy

Clinical signs: Trembling and coughing were often detected during infusion. A number of episodes of vomiting, defecation, and fever were also observed. The degree of kyphosis remained. The flexibility of cervical spine of all cats was not improved.

Body weights: All of the treated cats had slow to steady weight gains throughout the study.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: not performed

Electrocardiography: not performed

Hematology: No significant treatment-related abnormalities were observed. All cats displayed evidence of MPS granulation of neutrophilis. Leucocytosis was observed during the first six months of ERT in all cats. This could be due to the immunostimulation by rhASB.

Clinical chemistry: No significant abnormalities were detected in the fortnightly or monthly examination. Alkaline phosphatase was moderately increased in all cats. Alanine aminotransferase (ALT) was increase 2-3 fold in 4 cats. Serum phosphate levels were slightly increased.

Serum anti-rhASB antibody: The antibody titers were elevated in 4 of the 5 cats after 4 months of ERT, however, the level of neutralizing antibodies observed was only slightly above that of the untreated normal cats.

Serum complement activity: Only 2 (40%) cats had a decrease of the complement activity as compared with the pre-infusion levels.

Urinary glycosaminoglycans: The overall urinary GAG levels in ERT treated cats decreased to a level approximately 4- to 5-fold lower than the levels observed in age-matched untreated MPS VI control cats, and 2- to 3-fold above that of age-matched, historical-control normal cats. No significant reduction in GAG levels were observed following increase in dose from 1 to 2 mg/kg of rhASB.

Radiology: The skeletal appearance of all 5 MPS VI cats (12 to 13.5 months of age) was not significantly different from historical age-matched untreated MPS VI control cats. Only one cat had an increased bone mineral volume up to the normal range.

Gross pathology: Gross examination only showed MPS VI disease related pathology in the major joints, thoracolumbar spinal cord, heart valve leaflets and tracheal cartilage. Spleen was moderate large to very large in 3 cats.

Histopathology: Microscopic assessment showed mild chronic interstitial pneumonia in 4 cats, and mild chronic glomerulonephropathy in all five cats. In the amendment (IMVS 1599/C), chronic pulmonary vasculitis was also reported. The pathology was characterized as frequent perivascular mononuclear cells (macrophages, lymphocytes, and plasma cells) infiltration, and mild increase in thickness of the alveolar septa by mononuclear cells and fewer neutrophils. The renal changes involved focal thickening of Bowman's capsule. It included basement membrane thickening, hyperplasia of parietal epithelial cells in crescents, and periglomerular fibrosis.

The clearance of lysosomal storage was observed in reticuloendothelial cells (liver and spleen). No clearance was identified in cartilage chondrocytes.

Tissue distribution of rhASB: rhASB tissue levels were measured 2 days after last infusion. Tissue samples (5 g or less) were from liver, lung, spleen, kidney, heart bone marrow, mesenteric lymph nodes, aorta, cerebrum, cerebellum, skin, articular cartilage, cornea, and muscle from injection site. The highest enzyme concentration was found in the liver. No enzyme was found in the skin, articular cartilage and cornea in all cats. There were no controls of untreated disease tissues and normal tissues.

Conclusions: Treatment of MPS VI-affected cats with rhASB decreased uGAG excretion to approximately 4– to 5-fold below the average value for age-matched, untreated historical control MPS VI–affected cats and to 2- to 3-fold above that of age–matched, historical control normal cats. This decrease was maintained despite the presence of antibodies towards rhASB. Weekly treatment with rhASB at 1-2 mg/kg for 9 months did not result in major alterations in clinical presentation, radiologic appearance and skeletal disease indicating that early treatment with rhASB (i.e. newborn animal) leads to better pharmacological response to MPS VI-associated skeletal disease.

Comments: Three types of microscopic findings were identified: 1) pulmonary perivascular inflammation, 2) interstitial pneumonia, and 3) glomerulonephropathy. Based on the present observations, we can not rule out the possibility that they are interrelated, and may have a common immunological etiology. Glycosaminoglycans are large negatively charged molecules associated with cell membrane proteins. They stabilize and support the surface structures. Hydrolyzing the GAG sulfate bond by rhASB may change the antigenicity of the target cells. In addition, anti-rhASB immune complex deposition may also lead to the above pulmonary and renal pathology.

In the tissue-distribution study, there were no controls of normal cat and untreated disease cat. The antibody F66 for detection of rhASB was not specific for rhASB. We do not know whether the distribution data represent rhASB, or the native ASB.

Study title: Six Month Safety Evaluation of rhASB in MPS VI Affected Cats from Birth (Study ASB-PC-006)

Key study findings: When MPS VI kittens (less than 27 hours old) were treated with ERT, their bone growth and remodeling were improved.
Study No.: ABS-PC-006
Volume No., and page No.: CTD Section 4.2.1.1.
Conducting laboratory and location: [redacted]

Date of study initiation: September 17, 1999
GLP compliance: No
QA reports: No
Drug, lot No., and % purity: rhASB BMP Lot Nos. 02-60-072799, 02-70-091699, 02-70-092199, 02-70-121599, 02-70-020100, and 02-70-021500; information regarding the purity was not provided in the final study report.

Methods:
Dosing: 1 mg/kg rhASB was administered weekly for 26 weeks.

Species/strain: MPS VI cats (n = 5), 1 day old

Number/sex/group: 3 females and 2 males

Route, formulation, volume, and infusion rate: Intravenous infusion (cephalic vein); 1 mg/ml rhASB at pH 5.8 containing 10 mM sodium phosphate, 150 mM sodium chloride; injection volumes for the first 6 bolus ranging 0.07 ml to 0.73 ml using undiluted enzyme due to low bodyweights (injection rate 2-10 minutes per injection); infusion volume for 2 hour infusion: 4 ml/kg/hour (maximum of 10 ml per infusion).

Study design: Five MPS VI–affected cats that were less than 27 hours old were given weekly two–hour intravenous infusions of rhASB at 1 mg/kg for six months. Four additional MPS VI–affected cats and one normal newborn cat were used as untreated controls. Animals were euthanized two days after the last infusion and tissues were collected for determination of ASB activity and for histopathological evaluation.

Results:
Mortality: all cats survived until necropsy

Clinical signs: The treated kittens were followed for gait, mobility, pelvic strength, coordination, and cervical spine flexibility. In the third month of therapy, the signs of

MPS VI in ERT treated kittens were milder than would be expected in age matched untreated MPS VI kittens. No significant abnormalities were observed during or after the infusions.

Body weights: In general, body weight increased for all cats throughout the study.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: not performed

Electrocardiography: not performed

Hematology: No significant treatment-related abnormalities were observed. All cats displayed evidence of MPS granulation of neutrophilis. Leucocytosis was observed during the whole trial in all cats.

Clinical chemistry: No significant abnormalities were detected in the monthly examination. Alkaline phosphatase was moderately increased in all cats. Alanine aminotransferase (ALT) was mild increased in 5 cats. Creatine phosphokinase (CK) was mild elevated post-infusion in 3 cats.

Serum anti-rhASB antibody: Low levels of antibody titers were detected 5 cats from week 7 to week 25.

Urinary glycosaminoglycans: A significant decrease in urinary GAG concentration compared with untreated MPS VI cats was observed after infusion #8 in 4 out of 5 kittens (~50 days of age) and after infusion #10 and #12 in all kittens. The GAG levels were situated midway between the range observed in age-matched untreated MPS VI and normal control cats.

Radiology: No skeletal disease features were normalized. Improvement in lumbar vertebrae and femoral head was observed as compared with untreated MPS VI cats. Bone histomorphometry showed that 4 of the treated cats had increased bone volume up to the levels between untreated MPS VI and normal cats, and values for one cat fell well within the normal range.

Gross pathology: All gross abnormalities observed were due to MPS VI disease. The gross appearances of abdominal and thoracic organs were within normal range.

Histopathology: A mild chronic interstitial pneumonia, pulmonary vasculitis and focal glomerulonephritis were found in both untreated and ERT treated MPS VI cats. The degrees of inflammation in the rhASB treated animals were higher than the untreated cats.

The clearance of lysosomal storage was observed in all 5 treated cats. Liver and spleen exhibited no residual stored material, while the degree of storage in chondrocytes and corneal keratocytes was unchanged from historical untreated MPS VI cats.

Tissue distribution of rhASB: Two days after the last infusion, rhASB was detected in the liver, lung, bone marrow, spleen, kidney, heart, and mesenteric lymph node. The highest rhASB activity was detected in the liver of all cats. No activity was detected in articular cartilage. There was no untreated disease tissue control and normal tissue control.

Conclusions: rhASB treatment of MPS VI–affected cats from birth improved bone growth and remodeling, suggesting that the initiation of treatment before significant bone pathology developed may have better therapeutic effects. No infusion–associated reactions were observed and no significant antibody response to rhASB developed, suggesting that early initiation of therapy may have induced some immunological tolerance against the heterologous protein.

Comments: Chronic interstitial pneumonia, pulmonary perivascular inflammation, and focal glomerulonephropathy occurred in rhASB treated MPS VI cats. The etiology is unknown (see comments to Study ABS-PC-005).

Moderate leucocytosis (< 2-fold of the upper limit of the reference) was observed during the trial in all cats. It could be due to the immune stimulation by rhASB and the response to the infusion stress.

Study title: Six Month Efficacy and Safety Evaluation of rhASB (containing Tween — 80) in MPS VI Cats from Birth (Study ASB-PC-007)

Key study findings: No significant differences were noted between the results of using rhASB formulated with — Polysorbate 80 (both at — and those of Study ASB-PC-006 in which the rhASB formulation did not contain any polysorbates.
Study No.: ABS-PC-007
Volume No., and page No.: CTD Section 4.2.1.1.
Conducting laboratory and location: —

Date of study initiation: February 12, 2000
GLP compliance: No
QA reports: No
Drug, lot No., and % purity: rhASB BMP Lot Nos. 02-70-020100A, 02-70-030700, 02-70-032100B, AP-600-30-T, 02-70-061900, and AP600-29-T; information regarding the purity was not provided in the final study report.

Methods:

Dosing: 2 mg/kg rhASB protein was administered weekly for 26 weeks. The transition from [redacted] Tween 80-containing enzyme occurred at infusion #9 for cat 356m, infusion #11 for cats 353m and 354m, and infusion #12 for cat 349m.

Species/strain: MPS VI cats (n = 4), approximately 1 day old

Number/sex/group: 1 female and 3 males

Route, formulation, volume, and infusion rate: Intravenous infusion (cephalic vein); 2 mg/kg rhASB in 10 ml phosphate-buffered saline at the infusion rate 2-10 minutes for the first six treatments, then 2 hours for all the other treatments.

Study design: Four MPS VI–affected cats less than 46 hours old were given 2–hour intravenous infusions of the rhASB formulations at a dose of 2 mg/kg for 6 months; one MPS VI–affected cat and one normal newborn cat were used as untreated controls. rhASB formulated drug product containing [redacted] was infused for the first weeks 9–12 (depending on the cat), while the rhASB formulated drug product containing [redacted] of Polysorbate 80 was infused weekly for the remaining weeks of the study. Animals were euthanized two days after the last infusion and tissues were collected for determination of ASB activity and for histopathological evaluation.

Results:

Mortality: all cats survived until necropsy

Clinical signs: The change from [redacted] to Tween 80 in the formulation was not associated with any change in clinical signs. The treated kittens were followed for gait, mobility, pelvic strength, coordination, and cervical spine flexibility. In the third month of therapy, the signs of MPS VI in 3 out of 4 ERT treated kittens were milder than would be expected in age matched untreated MPS VI kittens. No significant abnormalities were observed during or after the infusions.

Body weights: In general, body weight was steady increased for all cats from birth until 5 months of age. During the last month, weight gains were generally reduced.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: not performed

Electrocardiography: not performed

Hematology: No significant treatment-related abnormalities were observed. All MPS cats displayed evidence of granulation of neutrophilis. Leucocytosis was observed during the whole trial in all MPS cats.

Clinical chemistry: No significant abnormalities were detected in the monthly examination. Alkaline phosphatase was moderately increased in all cats. Creatine phosphokinase (CK) was mild elevated post-infusion in 2 cats.

Serum anti-rhASB antibody: Low levels (<0.9 OD/μl) of antibody titers were detected throughout the 6 month trial.

Urinary glycosaminoglycans: A decrease in urinary GAG concentration compared with untreated MPS VI cats was observed at 23 to 50 days of age in all MPS VI cats. The GAG levels were between the range observed in age-matched untreated MPS VI and normal control cats.

Radiology: No skeletal disease features were normalized. Improvement in lumbar vertebrae and femoral head was observed as compared with untreated MPS VI cats. Bone histomorphometry showed that 3 ERT cats had increased bone volume up to the levels between untreated MPS VI and normal cats, and values for one cat fell well within the normal range.

Gross pathology: The only gross pathology observed was due to MPS VI disease.

Histopathology: A mild chronic interstitial pneumonia, pulmonary vasculitis, and focal glomerulonepritis were found in both untreated and ERT treated MPS VI cats. The degree of inflammation in the treated group was higher than in the untreated group.

The clearance of lysosomal storage was observed in all 4 treated cats. Liver and spleen exhibited no residual stored material, while the degree of storage in chondrocytes and corneal keratocytes was unchanged from untreated MPS VI cats.

Tissue distribution of rhASB: Two days after the last infusion, rhASB was detected in the liver, lung, bone marrow, spleen, kidney, heart, and mesenteric lymph node. The highest rhASB activity was detected in the liver of all cats. No activity was detected in articular cartilage. There was no untreated disease tissue control and normal tissue control.

Conclusions: rhASB containing Polysorbate 80 (—) did not have significant different efficacy and safety profiles as compared with those containing — or those did not containing any polysorbates (Study ASB-PC-006).

Study title: Long-term Combined Intravenous and Intra-articular Therapy with rh4S (rhASB) for the Prevention of Degenerative Joint Disease in MPS VI Cats (Study ASB-PC-008)

Key study findings: Combined intra-articular and intravenous administration of rhASB attenuates joint pathology associated with MPS VI disease as compared with intravenous rhASB therapy alone.

Study No.: ABS-PC-008
Volume No., and page No.: CTD Section 4.2.1.1.
Conducting laboratory and location: —

Date of study initiation: December 6, 2001
GLP compliance: No
QA reports: No
Drug, lot No., and % purity: rhASB BMP Lot Nos. AV60003, 102PD0089-01B, AV60103, and AP60206; information regarding the purity was not provided in the final study report.

Methods:
Dosing: 1 mg/kg rhASB was administered intravenously weekly for 42 weeks.

Species/strain: MPS VI cats (n = 7), 1 day old at onset of intravenous therapy and 24-36 days old at onset of intra-articular therapy; Normal control cats (n = 2), 1 day old, no intravenous therapy, no intra-articular therapy.

Number/sex/group: 4 females and 3 males

Route, formulation, volume, and infusion rate: Intravenous infusion (cephalic vein); 1 mg/kg rhASB in 10 ml phosphate-buffered saline at the infusion rate 2-10 minutes for the first six treatments, then 2 hours for all the other treatments.

Study design: The preliminary studies were designed to determine the dose of rhASB required to clear GAG storage from lysosomes within cartilage cells in young MPS VI-affected cats. Initially, joint cavities of the right shoulders, elbows and knees were injected with rhASB at doses levels of 1, 10, 100 or 500 μg per joint. The cavities of contralateral joints (left shoulders, elbows and knees) were injected with vehicle. One week after the injections, the changes observed within the cartilage were recorded. Subsequently, the changes 1, 2, or 3 months following a single intra-articular injection of 500 μg of rhASB were recorded. Results of these preliminary studies indicated that, while the effect of rhASB treatment was noticeable at doses of 1, 10, 100 μg, a dose of 500 μg produced greater depth of clearance of lysosomal storage within the articular cartilage. Therefore, a 500 μg intra-articular dose of rhASB was selected for the long-term combined therapy study.

In the long-term study, seven MPS VI-affected cats received intra-articular treatment. The joint cavities of the right shoulders, elbows and knees were injected monthly (n=5) or once every third month (n=2) with rhASB (500 μg per joint) and with vehicle (n = 5) or rhASB (500 μg per joint) (n = 2) in the contralateral joints. Four of these MPS VI-affected cats also received weekly intravenous infusions of rhASB, 1mg/kg/week. Treatments were continued for 42 or 43 weeks. The MPS VI-affected cats were observed for changes in gait or sign of inflammation around the treated joints. Two days following the last rhASB intravenous administration animals were euthanized and changes observed

within the cartilage were recorded (one week after the last intra-articular injection). Animals were approximately ten months of age at euthanasia. At postmortem, the appearance of the cartilage and synovial fluid, and signs of inflammation or other abnormalities were recorded for each treated or control joint. Synovium and cartilage were processed for histological evaluation. A smear of the synovial fluid was prepared for cytological evaluation. Evaluation of pharmacological effect was primarily based on histological evaluation of the degree GAG clearance within lysosomes of articular chondrocytes. The effects of intravenous rhASB administration in the MPS VI-affected cats were monitored as in previous nonclinical pharmacology studies.

Results:

Mortality: all cats survived until necropsy

Clinical signs: The treated kittens were followed for gait, mobility, pelvic strength, coordination, and cervical spine flexibility. In the third month of therapy, the signs of MPS VI in ERT treated kittens became milder than would be expected in age matched untreated MPS VI kittens. No significant abnormalities were observed during or after the infusions.

Body weights: In general, body weight increased for all cats throughout the study.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: not performed

Electrocardiography: not performed

Hematology: No significant treatment-related abnormalities were observed. All cats displayed evidence of MPS granulation of neutrophilis. Leucocytosis was observed during the whole trial in all cats.

Clinical chemistry: No significant abnormalities were detected in the monthly examination. Alkaline phosphatase was moderately increased in all cats. Alanine aminotransferase (ALT) was mild increased. Creatine phosphokinase (CK) was mild elevated post-infusion.

Serum anti-rhASB antibody: No significant antibody titers against rhASB developed in the 4 MPS VI cats that received combined therapy.

Urinary glycosaminoglycans: A decrease in urinary GAG concentration was observed in all MPS VI cats treated weekly with ERT compared with the MPS VI cats treated with IA injection only. The urine samples of ERT-treated MPS VI cats had slightly higher GAG concentrations compared with that of untreated normal cats. Urinalysis was within the normal range.

Radiology: No skeletal disease features were normalized. Compared with the 3 MPS VI cats that received IA injection only, the most obvious changes were 1) larger epiphyses with more uniform trabecular pattern, 2) improvement in shape of the shoulders, elbows and knees, and 3) mild improvements in shape and epiphyseal outline of cervical vertebrae (still short in size).

Gross pathology: All gross abnormalities observed were due to MPS VI disease. The gross appearance of abdominal and thoracic organs was well within normal range.

Histopathology: In all cats (including the normal controls), marked splenic congestion, marked pulmonary congestion and pulmonary edema were observed. Mild diffuse fatty changes were found in most animals. Mild (patchy) chronic interstitial pneumonia and perivascular inflammation was present in most cats. Moderate to severe chronic tracheitis with lymphoplasmacytic infiltration of lamina propria was detected in 2 cats. Mild focal glomerular atrophy and capsular basement membrane thickening were identified in 2 cats. Glomerular atrophy occurred in one cat. Vacuolation of smooth myocytes (aorta) and fibroblasts (trachea) was seen in all MPS VI cats.

In the rhASB treated joints of MPS VI cats, the level of synovium hyperplasia was reduced and the synovial cells looked less foamy than the control. Mild to moderate signs of inflammation were observed in 3 treated cats.

In general, the types of cells in synovial fluid in rhASB treated joints were similar to that in the buffer-treated control and in the normal joints.

The cartilage in all joints of normal cats was thinner than in MPS VI cats. The overall cartilage thickness measured histologically was similar between rhASB-treated and buffer-treated joints.

In all rhASB-treated joints, decreased distension of articular chondrocytes was observed, indicating the reduction of lysosomal storage of GAG (the primary data are not submitted).

Uronic acid content of cartilage in rhASB-treated joints was lower than the buffer-treated control, but is higher than the normal untreated cats.

Conclusions: Combination of intra-articular and intravenous rhASB therapy attenuated the joint pathology of MPS VI cats as compared to intravenous rhASB therapy alone.

MPS VI cats undergoing combined therapy:

- Weekly infusions of 1 mg/kg rh4S into four MPS VI cats over 42-43 weeks were well tolerated and resulted in no significant abnormalities in body weight, regular serum biochemistry and hematology profiles.

- Reduction in urinary GAG levels to concentrations approaching that found in normal cats indicated that early response to ERT occurred and that reductions were

sustained by the treatment dose.

- No skeletal disease features were normalized. Improved radiographic skeletal appearance in all cats with combined therapy. Compared with the three MPS VI cats that received IA injection only, the most obvious changes observed were listed as following:
    - Generally larger epiphyses with more uniform trabecular pattern (proximal tibia, distal femur, proximal radius, proximal ulna, proximal and distal humerus)
    - Improvement in shape of the shoulders, elbows and knees
    - Mild improvements in shape and epiphyseal outline of cervical vertebra (still short in size)

- No significant antibody titers against rhASB developed in the 4 MPS VI cats that received combined therapy, suggesting that early initiation of ERT (at ~ 1 day of age) may have induced some immunological tolerance against the foreign protein (rhASB).

MPS VI cats treated with intra-articular injections only:

- The radiological appearance of the control and treated joints were similar in the degree of degenerative joint disease, suggesting that the bone had not benefited from localized therapy with rhASB.

- In the rhASB treated joints, the level of synovial cell hyperplasia was generally reduced and the cells looked less foamy.

- The percentage of cartilage thickness appeared to be decreased. However, because of the large variation, it was not conclusive.

- Mild to moderate signs of synovium inflammation (focal lymphocytic infiltration) in the treated joints.

Comments: The acute pulmonary congestion and edema found in both control and treated animals were judged to be the result of barbiturate euthanasia. Patchy interstitial pneumonia, perivascular inflammation, and glomerulonephropathy were found (see comments to Study ABS-PC-005).

The sponsor claims that intra-articular injection of rhASB decreased the percentage of cartilage thickness at the joints. However, the final report (Table 7A and 7B, Study ASB-PC-008) did not provide the convincing results. The variation of cartilage thickness was as large as 250%, whereas the claimed depth of improvement was as small as 20%. In addition, the cartilage thickness of some normal cats was actually in the similar range of the disease cats.

The combination therapy may improve the radiographic skeletal appearance that is largely due to ERT, not the intra-articular injection. The injection of rhASB may reduce the cellular GAG storage in synovium and improves the hyperplasia condition. This therapeutic effect appears not reach the articular cartilage layer.

2.6.2.3 Secondary pharmacodynamics

No studies of this type are included in the present submission.

2.6.2.4 Safety pharmacology

No studies of this type are included in the present submission.

2.6.2.5 Pharmacodynamic drug interactions

No studies of this type are included in the present submission.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.3.1 Pharmacology Overview

Primary Pharmacodynamics

- ASB-PC-004: Evaluation of Infusion Rate of Recombinant Human Arysulfatase B (rh4S) in MPS VI Affected Cats
- ASB-PC-005: Nine Month Safety Evaluation of Recombinant Human *N*-acetylgalactosamine-4-sulfatase in MPS VI Affected Adult Cats: Final Report
- ASB-PC-006: Six Month Safety Evaluation of Recombinant Human *N*-acetylgalactosamine-4-sulfatase in MPS VI Affected Cats from Birth: Final Report
- ASB-PC-007: Six Month Efficacy and Safety Evaluation of Recombinant Human *N*-acetylgalactosamine-4-sulfatase (containing Tween — 80) in MPS VI Cats from Birth
- ASB-PC-008: Long-term Combined Intravenous and Intra-articular Therapy with rh4S for the Prevention of Degenerative Joint Disease in MPS VI Cats.

Secondary Pharmacodynamics (data not provided)

Safety Pharmacology (data not provided)

Pharmacodynamic Drug Interactions (data not provided)

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Preclinical pharmacokinetics was studied in Beagle dogs after a single intravenous infusion and in cynomolgus monkeys before and during a 26-week treatment. rhASB disappeared rapidly from the blood after administration to dogs and monkeys with plasma half-lives of approximately 30 minutes. Pharmacokinetic parameters were linear between the low and mid doses in both studies, but non-linear between the mid and high doses in both studies. The reasons for this are unknown, but may be due to the presence of a high affinity mannose-6-phosphate cellular receptor that removes rhASB from circulation and becomes saturated at the high dose. rhASB pharmacokinetics was similar for males and females in dogs and at the low dose at Weeks 1 and 13 and at the mid dose at Week 1 in monkeys. At all remaining sampling times in the monkey study, pharmacokinetic parameters were higher in the male monkeys than in the female monkeys. There was no plasma accumulation of rhASB from Day 1 through Week 26 at the low dose in both sexes, or at any dose in female monkeys. However, there was apparent accumulation in the male monkeys at the mid and high doses, primarily between Day 1 and Week 13. The reasons for this apparent gender associated accumulation are unknown.

2.6.4.2 Absorption: No studies of this type are included in the present submission.

2.6.4.3 Distribution: Tissue distribution of rhASB was studied in MPS VI cats in Studies ASB-PC-004, ASB-PC-005, ASB-PC-006, and ASB-PC-007.

2.6.4.4 Metabolism: No studies of this type are included in the present submission.

2.6.4.5 Excretion: No studies of this type are included in the present submission.

2.6.4.6 Pharmacokinetic drug interactions: No studies of this type are included in the present submission.

2.6.4.7 Other Pharmacokinetic Studies

Study title: Acute Single Dose Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B in Dogs (Study ABS-AT-001)

Key study findings: The pharmacokinetics of rhASB is non-linear between 2 mg/kg and 20 mg/kg after IV administration to dogs.
Study No.: ABS-AT-001
Volume No., and page No.: CTD Section 4.2.2.5
Conducting laboratory and location: —

—

Date of study initiation: September 18, 2001
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. AP60108; information regarding the purity was not provided in the final study report.

Methods:

Dosing: A single dose of 0, 0.2, 2, or 20 mg/kg rhASB was administered intravenously for 4 hours.

Species/strain: Beagle dogs (n = 24), approximately 9-month old

Number/sex/group: 12 females and 12 males; 3 dogs/sex/dose

Route, formulation, volume, and infusion rate: Dogs were dosed by a slow continuous intravenous infusion over a single 4-hour period via a jugular indwelling catheter. The rhASB vehicle contained 10 mM NaPO4, 150 mM NaCl, and — polysorbate 80 at pH 5.8. The infusion rate is at 5 ml/kg/hour.

Study design: Male and female Beagle dogs were assigned to four groups (three/sex/group). On Day 1 each group received a single intravenous infusion containing rhASB Vehicle (10 mM NaPO4, 150 mM NaCl. — polysorbate 80, pH 5.8, Lot No. 010925) or 0.2, 2.0, or 20.0 mg of rhASB/kg of body weight (mg/kg) (Lot No. AP60108). Dogs were dosed by continuous intravenous infusion (5.0 ml/kg/hour) over a single 4-hour period via an indwelling jugular catheter. Blood samples were collected prior to infusion, at 2, 4 (end of infusion), 4.1, 4.3, 5, 6 and 8 hours after the start of the infusion. rhASB was measured in canine plasma by ELISA. The LOQ for the plasma rhASB assay was — ng/ml. The maximum rhASB plasma concentration (Cmax) and time to Cmax (Tmax) were taken directly from the data. The rhASB terminal elimination rate constant, λz, was calculated as the negative of the slope of the terminal log-linear portion of the plasma concentration time curve. The area under the plasma concentration-time curve to infinity (AUC_∞) was calculated using the linear trapezoidal method to the last time point (tf) with a concentration above the validated limit of quantitation (AUC_{0-t}), with extrapolation to infinity.

Results:

rhASB plasma concentrations were below the detection limit for the majority of the plasma samples from the 0.2 mg/kg dose group. Therefore, pharmacokinetic analysis was limited to the higher dose groups. The results are shown in Table 3.

Table 3: Pharmacokinetic Parameters of rhASB after a Single 4-hour Infusion in Dogs

PK Parameter	Units	Dose (mg/kg)			
		2		20	
		Male (n = 2)	Female (n = 3)	Male (n = 2)	Female (n = 2)
AUC_∞	h.ng/ml	2346 (±545)	2254 (±692)	253,928 (±33182)	209106 (±63,390)
CL	ml/h/kg	876 (±204)	950 (±313)	79.4 (±10.4)	100.3 (±30.4)
C_{max}	ng/ml	736	781	80,832	71,406

		(±166)	(±177)	(±4964)	(±15030)
T½	h	0.504 (±0.130)	0.431 (±0.283)	0.516 (±0.024)	0.501 (±0.056)

The pharmacokinetics of rhASB was similar for males and females, and non–linear between 2.0 and 20.0 mg/kg. Although the dose increased by 10–fold, the mean C_{max} and AUC_{∞} values increased approximately 100 (± 10)–fold between the doses. The mean plasma $t_{½}$ values were approximately 0.5 hour at both dose levels. The AUC_{∞} value for 20.0 mg/kg rhASB administered via a four–hour intravenous infusion in dogs was 231,517 ± 48,745 hours·ng/ml. The AUC_{∞} value for rhASB administered via a four–hour intravenous infusion at a dose of 1.0 mg/kg to humans was 207,810 ± 74,587 minutes·ng/ml (3463 ± 1243 hours·ng/ml), indicating an approximate 66-fold safety factor between humans treated at 1.0 mg/kg and dogs treated at 20.0 mg/kg.

Conclusion
rhASB pharmacokinetics was non–linear between 2 and 20 mg/kg via a 4–hour infusion. rhASB has an approximate plasma half–life of 30 minutes. An approximate 66-fold safety factor between humans treated at 1 mg/kg and dogs treated at 20 mg/kg based on AUC_{∞} values.

Study title: 27-Week Intravenous Infusion Toxicity Study with rhASB in Cynomolgus Monkeys with a 2-Week Recovery (Study ABS-042-AT)

Key study findings: Based on AUC_{0-t} values following 4-hour infusion, the exposure of monkeys to rhASB at a dose of 10 mg/kg was 21 to 32-fold higher than humans given 1 mg/kg.
Study No.: ABS-042-AT
Volume No., and page No.: CTD Section 4.2.2.5
Conducting laboratory and location: ——

Date of study initiation: July 31, 2003
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. AP60109-A; information regarding the purity was not provided in the final study report.

Methods:
Dosing: 1.0, 3.0, or 10 mg rhASB/kg/week IV for 27 weeks

Species/strain: Cynomolgus monkeys (n = 22), approximately 2-3 years of age

Number/sex/group: 11 females and 11 males; 3 monkeys/sex/dose in the low and mid dose groups, 5 monkeys/sex/dose in the high dose group

Route, formulation, volume, and infusion rate: Monkeys were dosed by intravenous infusion using an infusion jacket and tether system and a subcutaneous — to provide vascular access via the femoral vein. Doses were given by slow continuous intravenous infusion (5 ml/kg/hour) for approximately 4 hours once weekly for 27 weeks. The control article (rhASB Vehicle) contained 10 mM $NaPO_4$, 150 mM NaCl, polysorbate 80, pH 5.8 (Lot No. AP120305). Line integrity was lost in two animals. However, these animals remained on study, with dosing via the leg (saphenous) vein using an indwelling catheter and restraint chair.

Study design: Male and female cynomolgus monkeys (*Macaca fascicularis*), approximately 2–3 years of age and 2.2–2.9 kg in weight, were assigned to four groups. On Day 1 each group received a single intravenous infusion containing 1.0, 3.0, or 10.0 mg of rhASB/kg of body weight (mg/kg) (Lot No. AP 601019–A). Monkeys were dosed by intravenous infusion using an infusion jacket and tether system and a subcutaneous — to provide vascular access via the femoral vein. Doses were given by slow continuous intravenous infusion (5 ml/kg/hour) for approximately 4 hours once weekly for 27 weeks. Blood samples for the measurement of rhASB plasma concentrations were collected before and at 1.5, 3, 4 (end of infusion), 4.25, 4.5, 5, 5.5, 6, 8, and 24 hours after the beginning of the infusion on Day 1 and at Weeks 13 and 26. The concentration of rhASB in the plasma samples was measured by ELISA. The lower limit of quantitation (LOQ) for the rhASB in monkey plasma i[illegible] — ng/ml. Pharmacokinetic parameters for rhASB were calculated using noncompartmental methods. The maximum rhASB plasma concentration (C_{max}) and time to C_{max} (T_{max}) were taken directly from the data. The terminal elimination rate constant, λ_z, was calculated as the negative of the slope of the terminal log–linear portion of the plasma concentration time curve. The area under the plasma concentration-time curve to infinity (AUC_∞) was calculated using the linear trapezoidal method to the last time point (tf) with a concentration above the validated limit of quantitation (AUC_{0-t}), with extrapolation to infinity.

Results:

A summary of the toxicokinetic parameters is shown in Table 5.

Table 5: Toxicokinetic Parameters of rhASB after IV Administration of 1, 3, and 10 mg/kg/week in Cynomolgus Monkeys

Parameter	1 mg/kg/week		3 mg/kg/week		10 mg/kg/week	
	Female	Male	Female	Male	Female	Male
Week 1						
C_{max} (ng/ml)	575±106	696±283	2,325±704	2,732±704	24,381±8,238	32,560±8,692
AUC_{0-t} (h•ng/ml)	1,679±255	1,726±586	7,443±1,109	8,668±2,032	73,825±34,348	110,802±33,510
T½ (h)	ND	ND	0.196±0.110	0.223±0.067	0.525±0.169	0.729±0.483
CL (ml/min/kg)	ND	ND	6.80±1.06	5.97±1.41	2.76±1.53	1.62±0.51
Week 13						
C_{max} (ng/ml)	714±102	736±225	2,097±1,909	3,467±1,442	25,761±16,227	47,078±10,833
AUC_{0-t} (h•ng/ml)	1,983±76	2,101±529	6,302±5,663	10,542±3,637	80,763±47,357	148,801±31,422
T½ (h)	ND	ND	0.107	0.150	0.310±0.119	0.306±0.062

CL (ml/min/kg)	ND	ND	4.56	4.80	2.97±2.31	1.15±0.23
Week 26						
C_{max} (ng/ml)	569±146	880±143	2,548±1,179	5,019±2,088	25,942±21,612	43,332±6,116
AUC_{0-t} (h·ng/ml)	1,750±543	2,647±343	6,159±2,016	14,336±5,994	82,461±66,705	133,976±19,460
T½ (h)	[1]ND	ND	0.160	0.143	0.372±0.253	0.359±0.118
CL (ml/min/kg)	ND	ND	6.52	4.77	4.23±4.62	1.27±0.20

[1]ND = not determined
[2]Mean ± standard deviation. If N = 1, then the individual values is reported.

Mean plasma concentrations of rhASB were comparable in male and female monkeys at Weeks 1 and 13 for the 1 mg/kg/week and at Week 1 for the 3 mg/kg/week–treated animals, as were mean values for C_{max} and AUC_{0-t} (refer to Table 5). However, for all remaining doses and sampling times, the mean plasma concentrations and associated toxicokinetics parameters (C_{max} and AUC_{0-t}) were higher in the male monkeys than in the female monkeys. Comparison of C_{max} and AUC_{0-t} across doses demonstrates a reasonably proportional increase between 1 and 3 mg/kg/week in the females and a greater than proportional increase in males. For both genders, the increases between 3 and 10 mg/kg/week were greater than the 3.3-fold increase in dose, indicating nonlinearity. There was apparent accumulation in the male monkeys at the 3 and 10 mg/kg/week doses, primarily between Day 1 and Week 13. The AUC_{0-t} values for 10 mg/kg rhASB administered via a four–hour intravenous infusion in monkeys 73,825 and 110,802 hours·ng/ml, for female and male animals, respectively. The AUC_{0-t} value for 1 mg/kg rhASB administered via a four–hour intravenous infusion in humans was 207810 ± 74587 minutes·ng/ml (3463 ± 1243 hours·ng/ml), indicating an approximate 21–32-fold safety factor between humans treated at 1 mg/kg and monkeys treated at 10 mg/kg.

Conclusion:
Based on AUC_{0-t} values following 4–hour infusions, the exposure of monkeys to rhASB at a dose of 10 mg/kg was 21–32-fold higher than humans given 1 mg/kg.

Study title: Pharmacokinetic Study of Differing Production Lots of Recombinant Human Arylsulfatase B in Dogs (Study ABS-45-APK)

Key study findings: The pharmacokinetics of rhASB test lot (Lot No. AP60307-1) — was comparable to the reference lot (Lot No. AP60109A) — in male beagle dogs, after single 4-hour intravenous infusions.
Study No.: ABS-45-APK
Volume No., and page No.: CTD Section 4.2.2.5
Conducting laboratory and location: —

Date of study initiation: November 12, 2003
GLP compliance: Yes
QA reports: Yes

Drug, lot No., and % purity: rhASB BMP Lot No. AP60307-1 (test lot) and Lot No. AP60109A (reference lot); information regarding the purity was not provided in the final study report.

Methods:

Study design: rhASB was administered by a 4-hour intravenous infusion to 10 adult male purebred Beagle dogs at a dose of 2.0 mg/kg. Dogs were randomized to one of two sequences: test then reference or reference then test. Lot No. AP60307–1 was the test lot of rhASB while Lot No. AP60109A was designated the reference lot, for purposes of this study. Treatments were separated by a 2–day washout period. The study design is presented in Table 6.

Table 6: Design of a Pharmacokinetic Study (ASB-45-APK) of Differing Production Lots of rhASB in Dogs

Group/Phase[1]	Number of Males	Test Article Formulation Lot Number	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (ml/kg)
1/1	5	AP60109A	Intravenous	2	20
1/2	5	AP60307-1	Intravenous	2	20
2/1	5	AP60307-1	Intravenous	2	20
2/2	5	AP60109A	Intravenous	2	20

Note: Doses administered as a 4-hour intravenous infusion.
[1]There was a washout period of at least 48 hours between the dosing of each phase.

Blood samples for measurement of rhASB plasma concentrations were collected before and at 2, 4 (end of infusion), 4.1, 4.3, 4.5, 5, 6, and 8 hours after the beginning of the infusion. Plasma concentrations of rhASB were measured at BioMarin. (Novato, CA) using a qualified ELISA method. The lower limit of quantitation (LOQ) was — ng/ml. Only those plasma rhASB concentrations that were equal to or greater than the LOQ were used in the pharmacokinetic analyses. Concentrations less than LOQ prior to C_{max} were set equal to 0 and those after C_{max} were set to missing. Pharmacokinetic parameters for rhASB were calculated using noncompartmental methods. The pharmacokinetic parameters C_{max}, AUC_{0-t}, and AUC_{∞} for the test and reference lots of rhASB were compared using an analysis of variance statistical model with sequence, animal within sequence, treatment, and period as the classification variables, using the natural logarithms of the data. Confidence intervals (90%) were constructed for the ratios (test–to–reference) of the three parameters using the log–transformed data and the two one–sided t–tests procedure. The point estimates and confidence limits were exponentiated back to the original scale.

Results

Plasma rhASB concentrations were below LOQ after 4 h in the majority of animals.

Consequently, it was not possible to estimate λz and, thus, AUC_∞ in a sufficient number of animals to analyze statistically. Therefore, comparisons between formulations are based on C_{max} and AUC_{0-t}. Mean plasma concentrations were comparable for the two formulations, as were mean values for C_{max}, and AUC_{0-t} (Table 7). The 90% confidence intervals for the geometric mean ratios for both parameters were well within the 80%→125% window indicating that the two formulations were equivalent (Table 8).

Table 7: Pharmacokinetic Parameters for Lots AP60307–1 and AP60109A of rhASB after Administration of a Dose of 2.0 mg/kg to Male Beagle Dogs

Parameter	Lot AP60307-1	Lot AP60109A
C_{max} (ng/ml)	375 ± 69.4	361 ± 62.3
AUC_{0-t} (h • ng/ml)	1,110 ± 222	1,045 ± 172
AUC_∞ (h • ng/mL)	1,390 ± 220	1,231 ± 297
T½ (h)	0.080 ± 0.002	0.102 ± 0.032

[1]Mean value ± SD

Table 8: Statistical Analysis of Pharmacokinetic Parameters for Lots AP60307–1 and AP60109A of rhASB after Administration of a Dose of 2.0 mg/kg to Male Beagle Dogs

Parameter	Ratio (%)	
	Estimate	90% confidence Interval
C_{max}	103.5	95.35→112.27
AUC_{0-t}	105.6	96.03→116.09

[1]The table was abstracted from the sponsor's final study report; Mean value ± SD

Conclusion
The pharmacokinetic profiles of two different production lots of rhASB derived from different — were comparable in male Beagle dogs, after single 4–hour intravenous infusions.

Study title: Pharmacokinetic Study of Differing Production Lots Manufactured at Two Different Sites of rhASB in Dogs Following Intravenous Administration (Study ABS-46-APK)

Key study findings: The pharmacokinetics of rhASB produced at BioMarin's clinical trials manufacturing site (BMK) (test Lot No. P60401) was comparable to the reference lot (Lot No. AS60309) produced at BioMarin's commercial manufacturing site (Galli) in male beagle dogs, after single 4-hour intravenous infusions.
Study No.: ABS-46-APK
Volume No., and page No.: CTD Section 4.2.2.5
Conducting laboratory and location: —

Date of study initiation: February 25, 2004
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. P60401 (test lot) and Lot No. AS60309 (reference lot); information regarding the purity was not provided in the final study report.

Methods:
Study design: rhASB was administered by a 4-hour intravenous infusion to 10 adult male purebred Beagle dogs at a dose of 2.0 mg/kg. Dogs were randomized to one of two sequences: test then reference or reference then test. Lot No. P60401 was the test lot of rhASB while Lot No. AS60309 was designated the reference lot, for purposes of this study. Treatments were separated by a 2–day washout period. The study design is presented in

Table 9: Design of a Pharmacokinetic Study (ASB–46–APK) of Differing Production Lots of rhASB in Dogs

Group/Phase[1]	Number of Males	Test Article Formulation Lot Number	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (ml/kg)
1/1	5	AS60309	Intravenous	2	20
1/2	5	P60401	Intravenous	2	20
2/1	5	P60401	Intravenous	2	20
2/2	5	AS60309	Intravenous	2	20

Note: Doses administered as a 4-hour intravenous infusion.
[1]There was a washout period of at least 48 hours between the dosing of each phase.

Blood samples for measurement of rhASB plasma concentrations were collected before and at 2, 4 (end of infusion), 4.03, 4.08, 4.17, 4.25, 4.33, 4.5, 4.75, 5, 6, and 8 hours after the beginning of the infusion. Plasma concentrations of rhASB were measured at BioMarin. (Novato, CA) using a qualified ELISA method. The lower limit of quantitation (LOQ) was — ng/ml. Only those plasma rhASB concentrations that were equal to or greater than the LOQ were used in the pharmacokinetic analyses. Concentrations less than LOQ prior to C_{max} were set equal to 0 and those after C_{max} were set to missing. Pharmacokinetic parameters for rhASB were calculated using noncompartmental methods.
The pharmacokinetic parameters C_{max}, AUC_{0-t}, and AUC_{∞} for the test and reference lots of rhASB were compared using an analysis of variance statistical model with sequence, animal within sequence, treatment, and period as the classification variables, using the natural logarithms of the data. Confidence intervals (90%) were constructed for the ratios (test–to–reference) of the three parameters using the log–transformed data and the two

one-sided t–tests procedure. The point estimates and confidence limits were exponentiated back to the original scale.

Results

Mean plasma concentrations were comparable for the two formulations as were mean values for C_{max}, AUC_{0-t}, and AUC_{∞} (Table 10). The 90% confidence intervals for the geometric mean ratios for both parameters were well within the 80%-125% window indicating that the two formulations were equivalent (Table 11).

Table 10: Pharmacokinetic Parameters for Lots AS60309 and P60401 of rhASB after Administration of 2.0 mg/kg to Male Beagle Dogs

Parameter	Lot AS60309	Lot P60401
C_{max} (ng/ml)	491 ± 77.5	451 ± 96.7
AUC_{0-t} (h • ng/ml)	1,402 ± 214	1,343 ± 283
AUC_{∞} (h • ng/mL)	1,434 ± 262	1,405 ± 239
T½ (h)	0.138 ± 0.070	0.118 ± 0.034

[1]Mean value ± SD

Table 11: Statistical Analysis of Pharmacokinetic Parameters for Lots AS60309 and P60401 of rhASB After Administration of 2.0 mg/kg to Male Beagle Dogs

Parameter Compared	Ratio (%)[1]	
	Estimate Value	90% Confidence Interval
C_{max}	109.8	99.54→121.09
AUC_{0-t}	105.5	97.23→114.39
AUC_{∞}	102.0	88.62→117.47

[1]Geometric mean ration of the test to the reference. Based on natural log-transformed data.

Conclusion

The pharmacokinetic profiles of two different production lots of rhASB manufactured at different production sites were comparable in male beagle dogs, after single 4–hour intravenous infusions.

Study title: Pharmacokinetic Study of Two Production Lots Manufactured via the Same Process Differing with Different — of rhASB in Dogs Following Intravenous Administration (Study ABS-53-APK)

Key study findings: The pharmacokinetics of rhASB harvested from — (test Lot No. P60406) was comparable to the reference lot (Lot No. AV60307)

Study No.: ABS-53-APK

Volume No., and page No.: CTD Section 4.2.2.5

Conducting laboratory and location:

Date of study initiation: May 18, 2004
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. P60406 (test lot) and Lot No. AV60307 (reference lot); information regarding the purity was not provided in the final study report.

Methods:
Study design: rhASB was administered by a 4-hour intravenous infusion to 10 adult male purebred Beagle dogs at a dose of 2.0 mg/kg. Dogs were randomized to one of two sequences: test then reference or reference then test. Lot No. P60406 was the test lot and Lot No. AV60307 was the reference lot, for the purposes of this study. Treatments were separated by a 2–day washout period.

Table 12: Design of a Pharmacokinetic Study (ASB–53–APK) of Differing Production Lots of rhASB in Dogs

Group/Phase[1]	Number of Males	Test Article Formulation Lot Number	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (ml/kg)
1/1	5	P60406	Intravenous	2	20
1/2	5	AV60307	Intravenous	2	20
2/1	5	AV60307	Intravenous	2	20
2/2	5	P60406	Intravenous	2	20

Note: Doses administered as a 4-hour intravenous infusion.
[1]There was a washout period of at least 48 hours between the dosing of each phase.

Blood samples for measurement of rhASB plasma concentrations were collected before and at 2, 4 (end of infusion), 4.03, 4.08, 4.16, 4.25, 4.33, 4.5, 4.75, 5, 6, and 8 hours after the beginning of the infusion. Plasma concentrations of rhASB were measured at BioMarin. (Novato, CA) using a qualified ELISA method. The lower limit of quantitation (LOQ) was — ng/ml. Only those plasma rhASB concentrations that were equal to or greater than the LOQ were used in the pharmacokinetic analyses. Concentrations less than LOQ prior to C_{max} were set equal to 0 and those after C_{max} were set to missing. Pharmacokinetic parameters for rhASB were calculated using noncompartmental methods.
The pharmacokinetic parameters C_{max}, AUC_{0-t}, and AUC_{∞} for the test and reference lots of rhASB were compared using an analysis of variance statistical model with sequence, animal within sequence, treatment, and period as the classification variables, using the natural logarithms of the data. Confidence intervals (90%) were constructed for the ratios (test–to–reference) of the three parameters using the log–transformed data and the two one-sided t–tests procedure. The point estimates and confidence limits were exponentiated back to the original scale.

Results

Mean plasma concentrations were somewhat lower for the test lot as were mean values for C_{max}, AUC_{0-t}, and AUC_{∞} (Table 13). Although the geometric means ratios (test-to-reference) for all three parameters were within the 80% - 125% equivalence window, the lower limits of the respective 90% confidence intervals were slightly below 80% (Table 14).

Table 13: Pharmacokinetic Parameters for Lots AS60309 and P60401 of rhASB after Administration of 2.0 mg/kg to Male Beagle Dogs

Parameter	Lot P60406	Lot AV60307
C_{max} (ng/ml)	368 ± 71.8	439 ± 104
AUC_{0-t} (h • ng/ml)	1,016 ± 183	1,251 ± 261
AUC_{∞} (h • ng/mL)	1,019 ± 184	1,260 ± 264
T½ (h)	0.049 ± 0.014	0.139 ± 0.025

[1]Mean value ± SD

Table 14: Statistical Analysis of Pharmacokinetic Parameters for Lots P60406 and AV60307 of rhASB After Administration of 2.0 mg/kg to Male Beagle Dogs

Parameter Compared	Ratio (%)[1]	
	Estimate Value	90% Confidence Interval
C_{max}	81.28	77.87→84.83.09
AUC_{0-t}	81.60	78.21→85.14
AUC_{∞}	84.39	79.81→89.24

[1]Geometric mean ration of the test to the reference. Based on natural log-transformed data.

Conclusion

The pharmacokinetics of two production lots manufactured via the same process with different run lengths was comparable in male Beagle dogs after single 4–hour intravenous infusions. Bioequivalence was demonstrated based on geometric mean ratios for C_{max}, AUC_{0-t}, and AUC_{∞}; however the lower limits of the 90% confidence intervals were slightly below the equivalence window.

2.6.5 TOXICOLOGY

2.6.5.1 Overall toxicology summary

The nonclinical toxicology of intravenously administered rhASB was characterized in vivo in five GLP studies. All test species used to conduct the toxicological assessment express the mannose–6–phosphate receptor, the major uptake receptor for rhASB. In the acute rat and canine rhASB toxicology studies, with dose levels up to 10 and 20 mg/kg, respectively, the only findings were transient swelling of the face and paws.

In Beagle dogs, these changes are attributed to polysorbate in the rhASB formulation. In the cynomolgus monkey 27–week repeat–dose toxicology study rhASB was administered at 1, 3 and 10 mg/kg/week. Moderate serocellular and pustular epidermatitis were observed in several animals in all treatment groups. Microscopically, minimal bile duct hyperplasia and periportal inflammation was noted in some 3 and 10 mg/kg-treated animals. All rhASB–treated animals were seropositive towards rhASB. However, no infusion-related reactions were noted.

Based on AUC0-t values from these studies, rhASB has a 66-fold safety factor between humans treated with 1 mg/kg and dogs treated with 20 mg/kg via a four–hour infusion. Similarly, rhASB has an approximate 21-32-fold safety factor between humans treated at 1 mg/kg and monkeys treated at 10 mg/kg via a four hour infusion (see Section 2.6.4.7 for details)

The effects of rhASB on fertility and embryo fetal development were evaluated in two studies in rats. Male and female animals received daily bolus intravenous rhASB administration before and during the cohabitation period, and through the period of organogenesis in females. Body weights, food consumption, fertility and mating parameters, male sexual organ weights and sperm evaluation parameters were unaffected by rhASB treatment at dose levels up to 3 mg/kg/day. Regarding embryo–fetal development through the period of organogenesis, there was no effect of rhASB on the gross external pathology and skeletal observations at dose levels up to 3 mg/kg/day of rhASB.

2.6.5.2 Single-dose toxicity

Study title: Acute Single Dose Intravenous Injection Study with Recombinant Human Arylsulfatase B in Rats (ASB-AT-002)

Key study findings: Administration of 0.1 and 1 mg/kg rhASB IV was well tolerated in rats. A transient swelling of the face and/or paws was observed at 10 mg/kg. The no observed adverse effect level (NOAEL) of rhASB in this study was 1 mg/kg.
Study No.: ABS-AT-002
Volume No., and page No.: CTD Section 4.2.3.1
Conducting laboratory and location: [redacted]

Date of study initiation: September 7, 2001
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. 102PD0089-01; information regarding the purity was not provided in the final study report.

Methods:
Dosing: A single dose of 0, 0.1, 1, or 10 mg/kg rhASB was administered intravenously.
Species/strain: [redacted] CD IGS BR rats (n = 40), approximately 7 weeks old

Number/sex/group: 20 females and 20 males; 5 rats/sex/dose
Route, formulation, volume, and infusion rate: Rats were dosed on Day 1 intravenous administration in a tail vein at a dose volume of 10 ml/kg for approximately 1 minute. The rhASB vehicle contained 10 mM NaPO4, 150 mM NaCl, and — , polysorbate 80 at pH 5.8.
Study design: Male and female CD(SD)IGS BR rats were assigned to 0, 0.1, 1.0, and 10 mg/kg four groups (five animals/sex/group). On Day 1, each animal received a single intravenous injection containing the control article (rhASB Vehicle) or 0.1, 1.0, or 10.0 mg of rhASB/kg of body weight (mg/kg) (Lot No. 102PD0089–1). The rats were dosed on Day 1 by intravenous administration in a tail vein for approximately 1 minute. Clinical observations were recorded prior to dosing, and at 10, 30, 60 and 120 minutes after administration. From Day 2 and daily thereafter, cage side observations were made for each animal twice daily. Individual body weights were recorded prior to treatment (at randomization) and on Days 1, 8, and 15. All animals were sampled for hematology, coagulation and clinical chemistry parameters on Day 15. On Day 17, all the rats were weighed, anesthetized with sodium pentobarbital, and exsanguinated. The gross pathologic examination was performed.

Results:
Mortality: all rats survived until necropsy

Clinical signs: Treatment-related clinical observations were initially noted at 30 minutes after dosing (Day 1) in the 10 mg/kg rats (four males and five females) and were limited to swelling of the mouth, nose, and /or paws. Signs were still present at 2 hours and were resolved by 4 hours post injection.

Body weights: All rats treated with rhASB gained weight during the course of the study and weight gains were comparable to controls.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: not performed

Electrocardiography: not performed

Hematology: The mean values for total leukocyte, neutrophil, and lymphocyte counts were slightly increased in 1 and 10 mg/kg males at Week 3. The relationship to treatment is unclear, as the females were not affected. There were no effects on erythrocyte counts, platelet counts, and other hematology values. There were no effects on coagulation parameters and cellular morphology.

Clinical chemistry: No significant abnormalities were detected in serum chemistry.

Gross pathology: There were no treatment-related macroscopic pathology findings.

Histopathology: not performed

Conclusions: Single intravenous injection of 0.1 and 1 mg rhASB per kg body weight to rats was well tolerated. A transient swelling of the face and/or paws at 10 mg/kg was observed. The no observed adverse effect level (NOAEL) of rhASB in this study was 1 mg/kg.

Study title: Acute Single Dose Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B (rhASB) in Dogs (ASB-AT-001)

Key study findings: There was no toxicologically significant observation, when rhASB was administered to Beagle dogs as a single intravenous infusion at doses of 2.0 and 20.0 mg/kg. Dogs from all groups, including controls, had reactions (reddening of the skin and facial edema) after dosing; which were disappeared within 4 hours of the completion of dosing. This reaction has been seen previously in dogs administered formulations that contain polysorbate (Masini 1985, Agents Actions). Because the severity of the reactions was comparable across the control and treated groups, a NOAEL was not determined in this study. An approximate 66-fold safety margin between humans treated at 1.0 mg/kg and dogs treated at 20.0 mg/kg based on AUC_∞ values (also see Section 2.6.4.7).
Study No.: ABS-AT-001
Volume No., and page No.: CTD Section 4.2.3.1
Conducting laboratory and location: —

Date of study initiation: October 9, 2001
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. AP60108; information regarding the purity was not provided in the final study report.

Methods:
Dosing: A single dose of 0, 0.2, 2, or 20 mg/kg rhASB was administered intravenously.

Species/strain: Beagle dogs (n = 28), approximately 9 month old

Number/sex/group: 14 females and 14 males; 3/sex/dose

Route, formulation, volume, and infusion rate: Dogs were dosed by a slow continuous intravenous infusion over a 4-hour period via a jugular indwelling catheter. The IV infusion rate was 5 ml/kg/hour. The rhASB vehicle contained 10 mM NaPO4, 150 mM NaCl, and — , polysorbate 80 at pH 5.8.

Study design: Male and female Beagle dogs were assigned to four groups (three/sex/group). On Day 1, each group received a single intravenous infusion containing the control article or 0.2, 2.0, or 20.0 mg of rhASB/kg of body weight (mg/kg) (Lot No. AP60108). Dogs were dosed by continuous intravenous infusion (5 ml/kg/hour)

over a 4–hour period via an indwelling jugular catheter. Clinical observations were performed twice daily during the acclimation period, immediately prior to dosing, once hourly during the dose administration period and twice daily, to Day 15, thereafter. Vital sign measurements were performed on all dogs at the following intervals: prior to initiation of dosing on Day 1, midway through the infusion (2 hours), 24 hours after the end of infusion, and on Day 15. Measurements included blood pressure, respiration rate, rectal body temperature, and heart rate. Electrocardiographic (ECG) examinations were performed on all dogs at the following intervals: during acclimation, prior to initiation of dosing on Day 1, and at 1 and 24 hours after the end of infusion. Body weights were measured at least once during acclimation, prior to initiation of dosing on Day 1 and once weekly thereafter. All dogs were sampled for hematology, coagulation and clinical chemistry parameters prior to the initiation of treatment and approximately 24–hours after the start of the infusion. On Day 15, all dogs were fasted overnight, weighed, anesthetized with sodium pentobarbital, and exsanguinated. The gross pathologic examination was performed.

Results:

Mortality: All dogs were survived until necropsy.

Clinical signs: Reddening of the skin inside the ears and/or facial edema (characterized by swelling of the lips, mouth, muzzle, ears and/or periorbital region) developed during dosing in all dogs, except in 20 mg/kg male H39061. The severity of the reactions was comparable across groups. In the males, the time of onset appeared to be dose-related; however, there were no apparent differences in the reactions in the females. The reddening of skin and facial edema are attributed to one of the vehicle components, polysorbate 80, which has been shown to have histamine-releasing properties (2). After the reactions developed, the signs persisted through the infusion period (4 hours) and were not evident at the p.m. room check (8 to 10 and 8 to 12 hours after dose initiation in males and females, respectively).

Body weights: There was no effect of rhASB on body weights.

Body temperature: The body temperatures were within normal limits across groups.

Respiration rates: The respiratory rates were within normal limits across groups.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: not performed

Blood pressures: The blood pressure data were within normal ranges across groups on Days 1 and 15. In the 20 mg/kg male group, the blood pressures decreased 24 hours after dosing relative to the other groups; however, the dogs were clinically normal and active. It is unclear whether this change is treatment-related.

Electrocardiography: All EKG data were within normal limits across groups.

Hematology: The hematology data, coagulation values, and cellular morphology results were within normal limits across groups.

Clinical chemistry: No significant abnormalities were detected in serum chemistry.

Gross pathology: There were no treatment-related macroscopic pathology findings.

Histopathology: not performed

Toxicokinetic evaluation: See Section 2.6.4.7 for details

Conclusions: There were no toxicologically significant findings when rhASB was administered to Beagle dogs as a single intravenous infusion dose up to 20 mg/kg. This dose level is approximate 20-fold greater than the maximum administered human dose. Dogs from all groups, including controls, had reactions (reddening of the skin and facial edema) after dosing which are attributed to the histamine-releasing properties of the polysorbate 80 in the vehicle. The reddening of the skin and facial edema had resolved within approximately 5 to 6 hours after the completion of dosing. The toxicokinetic data indicated that the plasma concentrations were similar for males and females during and following intravenous infusion (see Section 2.6.4.7 for details). The mean Cmax and AUC_∞ values at 20 mg/kg were approximately 100-fold greater than at 2 mg/kg. The mean $t_{1/2}$ values were approximately 0.5-hour at both dose levels. A NOAEL was not determined in this study because the severity of the reactions was comparable across groups.

2.6.5.3 Repeat-dose toxicity

Study title: 27-Week Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B (rhASB) in Cynomolgus Monkeys with a 2-Week Recovery (ASB-042-AT)

Key study findings: Serocellular or pustular epidermatitis was noted in female animals at all dose levels and males at high dose levels. Histopathological examination showed minimal to moderately infiltrations of eosinophils (perivascularly), lymphocytes, and histiocytes. Chronic hepatic periportal inflammation (moderate to marked) and necrosis (focal) were identified at the 3 mg/kg level. Based on these findings, a NOAEL was not determined. No other toxicologically significant observations were identified.
Study No.: ABS-042-AT
Volume No., and page No.: CTD Section 4.2.3.2
Conducting laboratory and location: [redacted]

Date of study initiation: August 27, 2003
GLP compliance: Yes
QA reports: Yes

Drug, lot No., and % purity: rhASB BMP Lot No. AP60109-A; information regarding the purity was not provided in the final study report.

Methods:
Dosing: Repeat dose of 0, 1, 3, or 10 mg/kg rhASB was administered intravenously once a week for 27 weeks.

Species/strain: Cynomolgus monkeys (n = 32), approximately 2-3 years old

Number/sex/group: 16 females and 16 males; 3/sex/dose for low dose (1 mg/kg) and mid dose (3 mg/kg), respectively; 5/sex/dose for control and high dose (10 mg/kg), respectively.

Route, formulation, volume, and infusion rate: Monkeys were dosed by slow continuous intravenous infusion over a 4-hour period using an infusion jacket via the femoral vein. The IV infusion rate was 5 ml/kg/hour. The rhASB vehicle contained 10 mM NaPO4, 150 mM NaCl, and — polysorbate 80 at pH 5.8.

Study design: Male and female cynomolgus monkeys, approximately 2–3 years of age and 2.2–2.9 kg in weight, were assigned to four groups. On Day 1, each group received a single intravenous infusion containing the control article or 1, 3, or 10 mg of rhASB/kg of body weight (mg/kg) (Lot No. AP 601019–A). Monkeys were dosed by intravenous infusion using an infusion jacket and a subcutaneous — to provide vascular access via the femoral vein. Doses were given by slow continuous intravenous infusion (5 ml/kg/hour) for approximately 4 hours once weekly for 27 weeks. Each animal was observed twice daily (a.m. and p.m., at least 6 hours apart) for mortality and signs of pain or distress. Physical examinations including body temperature, respiration rates and ophthalmic examinations were performed on each animal once prior to the initiation of treatment and during weeks 13, 26 and 29 (recovery animals). Body weights were recorded prior to the initiation of treatment on Day 1 and weekly thereafter. Blood pressure, blood oxygenation and ECG measurements were performed prior to the initiation of treatment and during weeks 13, 21, 26 (24 to 30 hours after dosing) and 29 (recovery animals). Blood samples were collected twice prior to initiation of treatment and during weeks 6, 13, 26 and 29 (recovery animals) for hematology, coagulation, and clinical chemistry tests. Urine samples were taken once prior to initiation of treatment and during weeks 13, 26, and 29 (recovery animals) for urinalysis tests. Blood samples were collected twice prior to treatment and during weeks 2, 6, 13, 26 and 29 (recovery animals) for serum rhASB antibody analysis. After 27 weeks of treatment, three animals/sex/group were euthanized. Following two weeks of recovery, all remaining animals were euthanized. The gross and microscopic pathologic examinations were performed.

Results:
Mortality: One of the five male monkeys (Animal No. I00701) treated with 10 mg/kg rhASB died at Week 2 during removal of the infusion jacket. The cause of death was not determined. The rest were survived until necropsy.

Clinical signs: Scabs was noted in one female receiving 10 mg/kg/week. The microscopic findings were consistent with epidermatitis. No other clinical signs associated with the treatment.

Body weights: There was no effect of rhASB on body weights.

Body temperature: The body temperatures were within normal limits across groups.

Respiration rates: The respiratory rates were within normal limits across groups.

Food consumption: Information regarding the food consumption was not provided in the final study report.

Ophthalmoscopy: The ophthalmoscopic examinations were within normal limits across groups.

Blood pressures: The blood pressure data were within normal limits across groups.

Electrocardiography: The blood pressure data were within normal ranges across groups.

Hematology: The hematology data, coagulation values, and cellular morphology results were within normal limits across groups.

Clinical chemistry: No significant abnormalities were detected in serum chemistry.

Histopathology: Test article-related microscopic pathology was identified in the liver, adrenal, and skin. In the liver, bile duct hyperplasia (mild) and chronic periportal inflammation occurred in one animal given 3 mg/kg and in five animals given 10 mg/kg. In the adrenal of males, mild cortical atrophy (predominantly the inner cortex, *zona reticularis*) occurred in three animals given 10 mg/kg. In the skin, minimal to moderate serocellular/pustular epidermatitis occurred at all dose levels in several female animals treated with rhASB. Mild to moderate increases of eosinophils (perivascularly), lymphocytes and histiocytes infiltrations along with acanthosis/hyperkeratosis at all dose levels in both sexes that received the test article.

Immunogenicity: All animals given the test article developed antibody titers against rhASB. The response appeared to be dose dependent. All animals in each group except three animals given 1 mg/kg (two males, Animal Nos. I00700 and I00704, and one female, Animal No. I00732) developed antibody titers by Week 6. At Week 13 antibody titers were present in all animals given the test article. Animal No. I00704 also had the lowest final titer of 2.6 at Week 26, where the final titers ranged for all groups from 2.6 to 5.3. The highest titer was noted for Animal No. I00705, given 3 mg/kg. The range of titers at Week 26 for animals given 1 mg/kg was 2.6 to 4.4 (mean of 3.8), for animals given 3 mg/kg/day was 3.5 to 5.0 (mean of 4.0), and for animals given 10 mg/kg was 4.0 to 5.3 (mean of 4.5). There was no apparent correlation between antibody titer and

plasma rhASB concentration, or in general, the severity of test article-related microscopic findings.

Toxicokinetic evaluation: See Section 2.6.4.7 for details

Conclusions: Long term treatment with rhASB may cause pustular epidermatitis and chronic hepatic portal inflammation. No other toxicologically significant findings were identified. Based on the epidermatitis and rhASB-induced hepatitis, a NOAEL was not determined in this study. The cause of the death (Animal No. I00701) is not determined.

Comments: The monkey (male, high dose group) died at week 2 after the dosing when removal of the infusion jacket. No abnormal clinical signs were observed. Histopathological findings were non-specific that included chronic active pulmonary inflammation, renal lymphohistocytic infiltration, hepatic lymphohistocytic infiltration, bile duct hyperplasia, and splenic lymphocytic depletion. Based on these findings, the causes of death that can not be ruled out include: 1) anaphylactic shock and hypotension, and 2) injection volume overload or protein overload leads to functional pulmonary venous obstruction and hypertension, resulting in pulmonary edema and cardiac failure. The monkey weighed approximately 2.5 kg. The total IV volume was 50 ml/4 hours.

2.6.5.4 Genetic toxicology: No studies of this type are included in the present submission.

2.6.5.5 Carcinogenicity: No studies of this type are included in the present submission.

2.6.5.6 Reproductive and developmental toxicity

Study title: Intravenous Dosage-Range Combined Fertility and Developmental Toxicity Study of Recombinant Human Arylsulfatase B in Rats (ASB-044-AT)

Key study findings: The results of this study suggested that dosages of 0 (rhASB Vehicle), 0.3, 1 and 3 mg/kg/day of rhASB were reasonable for the fertility and developmental toxicity study in rats.
Study No.: ABS-044-AT
Volume No., and page No.: CTD Section 4.2.3.5.1
Conducting laboratory and location: [redacted]

Date of study initiation: November 3, 2003
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. AP60109-A; information regarding the purity was not provided in the final study report.

Methods:

Dosing: Rats were dosed by intravenous injection once daily beginning 14 days before cohabitation, continuing through the cohabitation period (maximum 12 days) until, in males, the day before euthanasia (total of 29 doses) and, in females, day 17 of presumed gestation (range of 32 to 42 doses). The dosages were 0, 0.1, 0.3, 1, or 3 mg/kg/day, respectively.

Species/strain: —.CD®(SD)IGS BR rats (n = 96)

Number/sex/group: 48 females and 48 males; 8/sex/dose

Route, formulation, volume, and infusion rate: Rats were dosed by intravenous injection of rhASB in 150 mM NaCl, 10 mM $NaPO_4$ and — , polysorbate 80. The dosage volume was 3 ml/kg. The injection rate was not provided in the final study report.

Study design: A total of 48 rats of each sex were assigned to six dosage groups, eight rats per sex per group. rhASB (Lot No.: AP60109-A) or the rhASB Vehicle (Lot No.: AP120305) were administered via intravenous injection once daily to the male and female rats beginning 14 days before cohabitation, continuing through the cohabitation period (maximum 12 days) until, in males, the day before euthanasia (total of 29 doses) and, in females, day 17 of presumed gestation (range of 32 to 43 doses). The dosages were 0, 0.1, 0.3, 1.0 and 3.0 mg/kg/day for Groups 1 and 3 through 6, respectively, and the dosage volume was 3 ml/kg. Following the appearance of anaphylactoid-type responses in male rats at 0.3 mg/kg and higher dosages on day 9 of the study, those rats were administered 5.0 mg/kg/day of diphenhydramine hydrochloride (DPH) via bolus intravenous (tail vein) injection as soon as possible after the rhASB dose to relieve symptoms. The remaining male rats at 3.0 mg/kg that had not yet been given the test article on that day, and all female rats, were administered DPH prophylactically (i.e., approximately 20 minutes before dosage with the test article) to prevent possible anaphylactoid-type reactions. To ensure that all rats were treated identically, all male rats that had already been given the test article on that day, but did not show these responses, were administered DPH, and dosing was initiated in the animals assigned to Group 2 (DPH alone). For the remainder of the study, all rats in Groups 3 through 6 were given a prophylactic dosage of DPH before each dosage of test article, and rats in Group II were given DPH alone. The DPH was administered at a dosage of 5 mg/kg/day and dose volume of 0.4 ml/kg, with the dosages adjusted daily based on the individual body weights recorded immediately before injection of the test article. Clinical observations, body weights and feed consumption values were recorded during the study for all rats. Estrous cycling was evaluated in female rats by examination of vaginal cytology after the first administration and continuing until mating was confirmed. All male rats were euthanized and necropsied after completion of the cohabitation period; testes and epididymides were excised and paired organ weights were recorded. All surviving female rats were euthanized and necropsied on gestational day 21 (DG 21) and examined for the number and distribution of corpora lutea, implantation sites and uterine contents. Fetuses were weighed and examined for gross external alterations and sex.

Results:

Mortality: All male rats survived to scheduled euthanasia. Two female rats in the 0.1 mg/kg/day dosage group were found dead during the gestation period of the study. Observations for these rats are described below.

> Female rat 871 was found dead at the postdosage observation 50 minutes after dosage on day 13 of gestation. This rat received 29 dosages of the test article and had no remarkable clinical observations prior to death. Body weight gains and feed consumption values for this rat were comparable to other rats in its dosage group throughout the premating and gestation periods. All tissues appeared normal at necropsy. The uterus had 13 implantation sites: one conceptus had been resorbed and twelve were dead (viability at time of maternal death could not be determined).
>
> Female rat 873 died on DG 16 immediately after dosage with rhASB. This rat received 33 dosages of the test article, and just before dying it had decreased motor activity, ataxia, and labored breathing/gasping. The rat also had missing or broken upper incisors from predosage day 16 through DG 16. Body weight gains and feed consumption values for this rat were comparable to other rats in its dosage group throughout the premating and gestation periods. At necropsy all lobes of the lungs were bright red. The uterus had 17 implantation sites: all conceptuses were dead (viability at time of maternal death could not be determined).

All other female rats survived to scheduled euthanasia. The deaths at 0.1 mg/kg were considered unrelated to the test-article's biochemical activity because no deaths occurred at dosages up to 30 times higher. The proximity between dosage and death in each case and the clinical and necropsy observations in one of the animals suggested that these deaths were due to a possible dosing accident or failure of the prior DPH injection to protect against an anaphylactoid-type response.

Clinical observations: The incidences of decreased motor activity and ptosis were increased in a dose-dependent manner, and occurred at 0.3, 1 and 3 mg/kg/day in both male and female rats. In addition, male rats had excess lacrimation at 1 and 3 mg/kg/day, and labored breathing, body jerks (two males and a female), muscle twitches, hyper-reactivity, and soft or liquid feces (a male and a female) occurred at 3 mg/kg/day. These observations were considered components of an anaphylactoid-like response to the test article on the basis of their occurrence on days 9 and/or 10 of the study and then disappearance following one or more prophylactic dosages of DPH. Chromorhinorrhea occurred in one to four rats in the groups of 0.1, 1 and 3 mg/kg/day dose in disparate patterns over the dosage period, and for this reason was considered unrelated to the test or treatment articles. During the gestation period, the incidences of scabs and/or discoloration at the injection site were increased in the 3 mg/kg/day female rats.

Body weights: Body weights of the male rats given rhASB were slightly reduced (6% to 9% as compared to the control group on DS 29), though not in a dosage-dependent pattern. Male rats given the DPH treatment alone had a body-weight gain for the study

period between that for the control group and those given the test article. In the period prior to cohabitation, female rats given the test article also had slightly reduced body weights (2% to 5% as compared with the control group on day 14 of dosage) that were comparable to the rats given DPH (2% reduction). During gestation, slight reductions in maternal body-weight gain (6% to 11% for DG 0-18) occurred at 0.3, 1 and 3 mg/kg/day, with the largest reduction at the high dosage.

Food consumption: Absolute food consumption (g/day) was slightly reduced in the groups of male rats given rhASB (6 to 11%, as compared with the control group and the group given DPH alone) during the dosage period (DS 1-29, excluding the cohabitation period), but the pattern was not dosage-dependent. Relative food consumption values (g/kg/day) were comparable among the dosage groups. Among the female rats both absolute and relative food consumptions values were slightly reduced in the groups given rhASB (6 to 9% for absolute food consumption on days 1 to 14 of dosage, as compared with the control group). Values for the group given DPH were similar to the control. During gestation, maternal food consumption, both absolute and relative, was comparable among the dosage groups with the exception of a slight reduction at 3 mg/kg (6% for DG 0-17).

Estrous cycling, mating and fertility: Estrous cycling was unaffected in the female rats given rhASB across groups. All rats had mated within 5 days and were pregnant.

Gross pathology: All male and female rats that survived to scheduled necropsy appeared normal. Slight reductions in the weights of the testes and epididymides (up to 9%, as compared with the control group) were not apparent when expressed as a percentage of terminal body weight.

Cesarean section observations: All female rats that survived to scheduled termination. Corpora lutea, implantations, live fetus size, resorptions, percentage of male fetuses, and fetal body weights were comparable among the groups. No dam had a fetus with all conceptuses resorbed and there were no late resorptions or dead fetuses. No malformed fetuses were found in the gross external examination.

Conclusions: Based on the results of this study, dosages of 0.3, 1 and 3 mg/kg/day of rhASB were selected for the fertility and developmental toxicity study in rats.

Study title: Combined Intravenous Fertility and Development Toxicity Study of Recombinant Human Arysulfatase B in Rats (ASB-043-AT)

Key study findings: The results of this study showed that the parental no–observable–effect-level (NOEL) for intravenous administration of rhASB is 1 mg/kg/day. An increased number of rats at 3 mg/kg/day had swollen snouts. The NOELs for fertility, reproduction, and development are 3 mg/kg/day.
Study No.: ABS-043-AT
Volume No., and page No.: CTD Section 4.2.3.5.1

Conducting laboratory and location: —

Date of study initiation: January 19, 2004
GLP compliance: Yes
QA reports: Yes
Drug, lot No., and % purity: rhASB BMP Lot No. AP60109-A; information regarding the purity was not provided in the final study report.

Methods:
Dosing: Rats were dosed via intravenous injection once daily beginning 28 days before cohabitation for the male rats and 15 days before cohabitation for the female rats, continuing through the cohabitation period (maximum 18 days) until, in males, the day before euthanasia (total of 42 to 45 doses) and, in females, day 17 of presumed gestation (range of 33 to 45 doses). The dosages were 0, 0.3, 1, or 3 mg/kg/day, respectively.

Species/strain: — .CD®(SD)IGS BR VAF/Plus rats (n = 250)

Number/sex/group: 125 females and 125 males; 25/sex/dose

Route, formulation, volume, and infusion rate: Rats were dosed by intravenous injection of rhASB in 150 mM NaCl, 10 mM $NaPO_4$ and — , polysorbate 80. The dosage volume was 3 ml/kg. The injection rate was not provided in the final study report.

Study design: A total of 125 rats of each sex were assigned to five dosage groups, 25 rats per sex per group. rhASB or vehicle, were administered via intravenous injection once daily to the male and female rats beginning 28 days before cohabitation for the male rats and 15 days before cohabitation for the female rats, continuing through the cohabitation period (maximum of 18 days) until, in males, the day before euthanasia (total of 42 to 45 doses) and, in females, day 17 of presumed gestation (DG 17 [DG 0 = day mating detected]; range of 33 to 45 doses). The dosages were 0 (rhASB Vehicle; Lot No.: AP120305), 0.3, 1 and 3 mg/kg/day rhASB (Lot No.: AP60109-A) for Groups 1, 3, 4 and 5, respectively, and the dosage volume was 3 ml/kg. The dosage volume was adjusted daily for individual body weight changes and given at approximately the same time each day. Male and female rats in Group 2 would have been administered diphenhydramine hydrochloride (DPH) as a control if anaphylactoid–type responses had been observed in the other dosage groups which required treatment with this antihistamine. Because this treatment was not required during this study the Group 2 male rats were sacrificed and the carcasses discarded without further evaluation, and the female rats in this group were sacrificed on DG 21, examined for pregnancy status, and the carcasses of the these rats and their fetuses discarded without further evaluation. Viability, clinical signs, body weights and feed consumption values were recorded during the study for all rats. Estrous cycling was evaluated in female rats by examination of vaginal cytology beginning 14 days before initiation of dosage and continuing until mating was confirmed. Male rats in the 0 (Vehicle), 0.3, 1, and 3 mg/kg/day groups were euthanized after the cohabitation period, a necropsy was performed, sexual organs were weighed and sperm concentration and motility were evaluated. Female rats in these groups were sacrificed on day 21 of

presumed gestation, Caesarean–sectioned and a gross necropsy performed. Fetuses were weighed and examined for sex, gross external, soft tissue and/or skeletal alterations.

Results:
Male rats:
Mortality: All male rats survived until scheduled necropsy.

Clinical observations: The number of rats with swollen snouts was significantly increased ($p \leq 0.01$) at 3 mg/kg/day in both male and female rats. This clinical sign was observed on day 2 of the study (DS 2) in one rat, but first occurred in the other affected rats on DSs 27 to 33. This swelling may have been an indication of an anaphylactoid-like response; however, treatment with diphenhydramine hydrochloride was never initiated in the groups given rhASB or the group (II) designated for treatment with DPH alone because the responses were brief and their severity never escalated to a level that required treatment. No other clinical sign was associated with rhASB treatment.

Body weights: Body weights and body weight changes were unaffected by dosages of rhASB as high as 3 mg/kg/day. Values for the groups given rhASB were comparable to the control group with the exception of a significant decrease ($p \leq 0.01$) in body weight gain at 1 mg/kg/day on DSs 8 to 15 and a significant decrease ($p \leq 0.05$) in body weight in this group on DS 19. These differences were considered unrelated to the test article because they occurred in only one among several intervals and were not dosage dependent.

Food consumption: Absolute and relative food consumption values were unaffected by dosages of rhASB as high as 3 mg/kg/day. Rats at 3 mg/kg/day had significantly increased ($p \leq 0.05$) absolute food consumption values on DSs 15 to 22 and significantly increased ($p \leq 0.05$ to $p \leq 0.01$) relative food consumption on DSs 8 to 15 and 15 to 22 as well as the cumulative precohabitation period (DSs 1 to 28); however, these differences were considered incidental events because all were within 7% of the control group values.

Mating and fertility: All male rats in the control group and the groups given rhASB mated; only Group II had rats (2x) that did not mate. Values for the number of days in cohabitation prior to mating and other fertility parameters were unaffected by dosages of rhASB as high as 3 mg/kg/day.

Gross pathology: Small seminal vesicles, a small prostate, and slight dilation of the renal pelvis were observed in only one or two rats in each of the groups and were considered unrelated to rhASB.

Terminal body weights, organ weights and the ratios of organ weight to terminal body weight were unaffected by dosages of rhASB as high as 3 mg/kg/day. Rats at 1 mg/kg/day had significant decreases ($p \leq 0.05$ to $p \leq 0.01$) in the absolute weights of the left and right epididymides, left cauda epididymis and right testis and in the relative weight of

the right epididymis. These differences were considered incidental because all values were within 10% of the control group values and were not dosage dependent.

Sperm evaluation: Sperm parameters were unaffected by dosages of rhASB as high as 3 mg/kg/day. At 1 mg/kg the average percentage of motile sperm was decreased, the average static count was increased, and the cauda epididymal sperm count was significantly decreased ($p \leq 0.05$). These differences were considered incidental because they were not dosage dependent.

Female rats:

Mortality: All female rats survived until scheduled Caesarean sectioning on day 21 of presumed gestation (DG 21) with the exception of dam 2614 at 1 mg/kg/day that delivered on DG 21 and was sacrificed. This dam had no gross lesions at necropsy; its entire litter of 15 pups was delivered, and these pups appeared normal at the gross external, soft tissue, and skeletal examinations. This single event is considered unrelated to rhASB because DG21 is still a frequent day of delivery for this strain of rats.

Clinical observations: The number of rats with swollen snouts was increased ($p \leq 0.01$) at 3 mg/kg/day during both the precohabitation and gestation periods. This clinical sign was first observed between DS 13 and DG 13. This swelling may be an indication of an anaphylactoid-like response.

Body weights: Body weights and body weight gains were unaffected by dosages of rhASB as high as 3 mg/kg/day. Rats at 1 mg/kg/day had a significant decreases ($p \leq 0.05$) in body weight gain on DGs 0 to 6; this difference was considered incidental because it occurred in only one among several intervals and was not dosage dependent. Body weights and body weight changes at other intervals during gestation and throughout the precohabitation period were comparable among the groups.

Food consumptions: Absolute and relative food consumption values were unaffected by dosages of rhASB as high as 3 mg/kg/day. During the precohabitation period values were comparable among the groups and did not significantly differ. During gestation rats at 1 mg/kg/day had significant decreases ($p \leq 0.05$ to $p \leq 0.01$) in absolute and relative feed consumption values on DGs 0 to 6 of gestation. These differences were considered incidental because all values were within 10% of the control group values, they occurred in only one among several intervals, and the differences were not dosage dependent. Food consumption values at other intervals during gestation were comparable among the groups.

Estrous cycling, mating and fertility: These parameters were unaffected by dosages of rhASB as high as 3.0 mg/kg/day. The number of estrous stages per 14 days did not differ significantly among the groups and no rat had 6 or more days of estrus. One rat in the control group had 6 or more days of diestrus during the predosage period, and this pattern was also observed one rat in each of the 1 and 3 mg/kg/day groups in the precohabitation period. All rats in the control group and groups given rhASB mated, the averages for days in cohabitation were comparable, and pregnancy occurred in 24 (96%) or 25 (100%)

of the rats in these groups; there were no significant differences among the groups. Pregnancy occurred in 23 (90%) of the rats in Group II, which was not dosed.

Cesarean section observations: Caesarean-sectioning observations were based on 96 pregnant rats in the 0 (Vehicle), 0.3, 1, and 3 mg/kg/day dosage groups that had confirmed dates of mating and did not deliver before scheduled necropsy. The Caesarean-sectioning and implantation observations were unaffected by dosages of rhASB as high as 3 mg/kg/day. The number of dams at 1 mg/kg/day with resorptions was significantly increased ($p \leq 0.01$). These differences were considered incidental because the values were within the ranges observed historically and were not dosage dependent. All other parameters, including the corpora lutea, implantations, implantation sizes, percent live male fetuses, and fetal body weights were comparable among the control group and groups given rhASB. All placentas appeared normal with the exception of the occurrence of a fused placenta in one dam at 1 mg/kg/day; this observation was considered incidental because it occurred in only litter and was not dosage dependent.

Gross pathology: No gross lesions were observed at necropsy of the female rats in this study.

Fetal alterations:
No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of rhASB as high as 3 mg/kg/day. Incidences were comparable among the groups and did not significantly differ.

Conclusion: The parental no-observable-effect-level (NOEL) for intravenous administration of rhASB is 1.0 mg/kg/day. Rats treated with 3 mg/kg/day had swollen snouts. The NOELs for fertility, reproduction, and development, may be greater than 3.0 mg/kg/day (no higher levels studied). No test article related changes of the parameters were observed at 3.0 mg/kg/day.

Comments: 1) The negative results of the rat reproductive study should be confirmed in a non-rodent species. 2) The commitment of reproductive study in non-rodent species is necessary, because of the pregnancy potential and the variability of symptoms of the disease. Mucopolysaccharidosis VI (Maroteaux-Lamy disease) is an autosomal recessive disorder with variable symptoms. The patients have normal intelligence and the life span can extend beyond three decades. In the phase 3 study, 19 patients aged from 5 to 29 years (mean 13.7 year old) were treated. Two-thirds of the patients were female.

2.6.5.7 Local tolerance
No study of this type are included in the present submission.

2.6.5.8 Special toxicology studies
No study of this type are included in the present submission.

2.6.6 TOXICOLOGY TABULATED SUMMARY

2.6.6.1 Toxicology Overview

- Acute Single Dose Intravenous Injection Study with Recombinant Human Arylsulfatase B in Rats (ASB-AT-002)
- Acute Single Dose Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B in Dogs (ASB-AT-001)
- 27-Week Intravenous Infusion Toxicity Study with Recombinant Human Arylsulfatase B in Cynomologus Monkeys with a 2-Week Recovery (ASB-042-AT)
- Intravenous Dosage Range Combined Fertility and Developmental Toxicity Study of Recombinant Human Arysulfatase B in Rats (ASB-044-AT)
- Combined Intravenous Fertility and Development Toxicity Study of Recombinant Human Arysulfatase B in Rats (ASB-043-AT)

2.7 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The Biologics License Application for rhASB (Galsulfase) is approvable based on the preclinical pharmacology and toxicology submission. Toxicities demonstrated in the preclinical studies may be monitored or be superseded by the benefit/risk ratio of clinical concerns.

Unresolved toxicology issues (if any): Interstitial pneumonia, pulmonary angiitis and glomerulonephropathy in rhASB treated MPS VI cats were reported in the General Pathology Sections of the safety evaluations (Study Numbers ASB-PC-005, ASB-PC-006, ASB-PC-007, and ASB-PC-008). The etiology is not entirely unclear at the present time.

In the 27-week monkey study (Study No. ASB-042-AT), dose-dependent chronic hepatic periportal inflammation (moderate), centrilobular necrosis (focal), and bile duct hyperplasia were identified in both male and female animals. The mechanism of actions is unknown.

In the same study, a male monkey (Animal No. I00701) in high dose group died at week 2 after the dosing when removal of the infusion jacket. The cause of death is unclear (see comments to Study ASB-042-AT).

Recommendations: The preclinical pharmacology, pharmacokinetics, and toxicology data for rhASB support the safety and biologic activity of this product for use in the treatment of mucopolysaccharidosis VI. Recommendation is for approval.

Suggested labeling: Recommended changes to the Precautions section are as following:

"Pregnancy: Category B

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Signatures (optional):

Reviewer Signature Wenyi Gao 4/7/2005

Supervisor Signature Martin D. Green concurrence Yes ✓ No ___