

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125561Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: BLA 125561

Supporting document/s: 012, 036, 056

Applicant's letter date: SDN 012: January 8, 2015
SDN 036: April 14, 2015
SDN 056: June 2, 2015

CDER stamp date: SDN 012: January 8, 2015
SDN 036: April 14, 2015
SDN 056: June 2, 2015

Product: Sebelipase alfa/Kanuma™ (Recombinant Human Lysosomal Acid Lipase)

Indication: Treatment of Lysosomal Acid Lipase (LAL) Deficiency

Applicant: Synageva BioPharma Corporation

Review Division: Division of Gastroenterology and Inborn Errors Products (DGIEP)

Reviewer: Tamal Chakraborti, Ph.D.

Supervisor: Sushanta Chakder, Ph.D.

Division Director: Donna Griebel, MD

Project Manager: Kevin Bugin, MS, RAC

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125561 are owned by Synageva BioPharma Corporation or are data for which Synageva BioPharma Corporation has obtained a written right of reference. Any information or data necessary for approval of BLA 125561 that Synageva BioPharma Corporation does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125561.

TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	3
1.1	INTRODUCTION	3
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	3
1.3	RECOMMENDATIONS	4
2	DRUG INFORMATION	5
2.1	DRUG	5
2.2	RELEVANT INDs, NDAs, BLAs AND DMFs	6
2.3	DRUG FORMULATION	6
2.4	COMMENTS ON NOVEL EXCIPIENTS	7
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	8
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	25
2.7	REGULATORY BACKGROUND	25
3	STUDIES SUBMITTED.....	26
3.1	STUDIES REVIEWED.....	26
3.2	STUDIES NOT REVIEWED	26
3.3	PREVIOUS REVIEWS REFERENCED.....	26
4	PHARMACOLOGY	27
4.1	PRIMARY PHARMACOLOGY	27
4.2	SECONDARY PHARMACOLOGY	65
4.3	SAFETY PHARMACOLOGY	66
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	68
5.1	PK/ADME.....	68
5.2	TOXICOKINETICS	72
6	GENERAL TOXICOLOGY.....	73
6.1	SINGLE-DOSE TOXICITY	73
6.2	REPEAT-DOSE TOXICITY	73
7	GENETIC TOXICOLOGY	102
8	CARCINOGENICITY	102
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	102
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT	102
9.2	EMBRYONIC FETAL DEVELOPMENT	114
9.3	PRENATAL AND POSTNATAL DEVELOPMENT	131
10	SPECIAL TOXICOLOGY STUDIES.....	139
11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	139
12	APPENDIX/ATTACHMENTS.....	141

1 Executive Summary

1.1 Introduction

Sebelipase alfa (Kanuma™) is a recombinant human Lysosomal Acid Lipase (rhLAL) enzyme, purified from egg white of transgenic *Gallus*, with the same amino acid sequence as the native human enzyme. Under BLA 125561, the Applicant is seeking approval of sebelipase alfa for the treatment of Lysosomal Acid Lipase (LAL) deficiency. Sebelipase alfa has been granted Orphan Drug designation and Fast Track designation by the US Food and Drug Administration (FDA). The FDA has also granted sebelipase alfa Breakthrough Therapy designation for the treatment of infants with LAL deficiency.

In pediatric and adult patients with LAL deficiency, the recommended dose is 1 mg/kg administered as an intravenous (IV) infusion once every other week. In patients with rapidly progressive LAL deficiency within the first 6 months of life, the recommended dosage is 1 mg/kg as an IV infusion once weekly as an initial dose followed by escalation to 3 mg/kg once weekly.

1.2 Brief Discussion of Nonclinical Findings

In vivo primary pharmacology studies were conducted in the Donryu rat (“Yoshida”) model of LAL deficiency at IV doses ranging from 0.2 to 5 mg/kg. Sebelipase alfa caused improvements in several disease related parameters in this rat disease model, e.g., body weight gain, reduction in organomegaly, reduction in cholesteryl esters and triglycerides in the liver and spleen, and in serum transaminase levels. Results also indicated that the benefits of sebelipase alfa require maintenance of regular dosing, as the animals showed general decline in the health associated with a progressive decrease in growth velocity and subsequent body weight loss following cessation of sebelipase alfa treatment.

Intravenous repeated dose toxicology studies have been conducted with sebelipase alfa in rats (4-week) and in Cynomolgus monkeys (4-week and 6-month). The No Observed Adverse Effect Levels (NOAELs) in 4-week intravenous toxicology studies in rats and Cynomolgus monkeys were 50 mg/kg/day in both species. The NOAEL in the 6-month intravenous toxicity study in Cynomolgus monkeys was 30 mg/kg/day. No significant organ toxicities were identified in these studies.

Sebelipase alfa at intravenous doses up to 60 mg/kg did not cause any adverse effect on fertility and reproductive performance of male and female rats. In embryofetal development studies in rats and rabbits at intravenous doses up to 60 and 50 mg/kg, respectively, sebelipase alfa did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effect on pre and postnatal development at intravenous doses of sebelipase alfa up to 60 mg/kg.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, this BLA is recommended for approval for its proposed use as indicated in the label.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The proposed labeling of Kanuma™ appears to conform to the specific requirements on content and format of relevant nonclinical sections of the label for human prescription drugs under 21CFR 201.57. However, the following labeling changes are recommended.

8.1 Pregnancy

Animal Data

Sebelipase alfa administered during the period of organogenesis to rats (on gestation days 6, 9, 12, 15 and 17) and rabbits (on gestation days 7, 10, 13, 16 and 19) at intravenous doses up to 60 and 50 mg/kg, respectively, (approximately 164 and 526 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week, respectively) did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effects on pre and postnatal development at intravenous doses (administered on gestation days 6, 9, 12, 15, 18, and 20 and days 4, 7, 10, 14, and 17 postpartum) of sebelipase alfa up to 60 mg/kg/day (approximately 164 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week).

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate carcinogenic potential or studies to evaluate mutagenic potential have not been performed with sebelipase alfa.

Sebelipase alfa at intravenous doses up to 60 mg/kg administered twice weekly (approximately 164 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week) was found to have no adverse effect on fertility and reproductive performance of male and female rats.

13.2 Animal Toxicology and/or Pharmacology

In a rat disease model of LAL deficiency that exhibits several abnormalities analogous to the human disease, sebelipase alfa administered intravenously once weekly at 3 mg/kg showed improvements in survival, body weight gain, organ weight reduction, reduction in serum transaminases (ALT and AST), reduction in serum and hepatic lipids, and improvement in liver histopathology.

2 Drug Information

2.1 Drug

CAS Registry Number: 1276027-63-4

Generic Name: Sebelipase alfa

Code Name: SBC-102

Chemical Name: Recombinant human lysosomal acid lipase (rhLAL)

Molecular Formula/Molecular Weight: 55 kDa (b) (4)

Structure or Biochemical Description: Sebelipase alfa is a recombinant human lysosomal acid lipase (rhLAL) enzyme, purified from egg white of transgenic *Gallus*, with the same amino acid sequence as the native human enzyme. Purified sebelipase alfa is a glycoprotein with a molecular weight of approximately 55 kDa with 6 N-linked glycosylation sites. Structural and compositional analyses demonstrated that sebelipase alfa glycans consist of predominately N-acetylglucosamine (GlcNAc) and mannose terminated N-linked structures, as well as mannose-6-phosphate moieties. These glycans target uptake via receptors expressed on a number of cell types including Kupffer cells and hepatocytes in which substrate accumulation leads to disease pathogenesis. The described N-glycan structures are common to those found in human proteins and have been shown to facilitate protein uptake into cells via the macrophage mannose or mannose-6-phosphate receptors (Stahl, PD et al., 1978, Proc Natl Acad Sci USA, 75:1399-403; Coutinho MF et al., 2012, Mol Genet Metab, 105:542).

The primary amino acid sequence (Figure 1 from page 2 of Section 3.2.S.1.2 SDN 004 dated November 21, 2014) contains 378 amino acids and is identical to the Genbank reference sequence (Genbank Refseq, NM_000235.2) for human Lysosomal Acid Lipase (hLAL, EC 3.1.1.13) and is preceded by a 21 amino acid leader sequence. In the lysosomal compartment, LAL catalyzes the hydrolysis of cholesteryl esters and triglycerides to free cholesterol, glycerol and free fatty acids.

Figure 1: Amino Acid Sequence of sebelipase alfa

Pharmacologic Class: Hydrolytic lysosomal cholesteryl ester and triacylglycerol-specific enzyme

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 108460 (SBC-102, Synageva Biopharma Corp.)

2.3 Drug Formulation

Sebelipase alfa Drug Product (DP) is a sterile liquid concentrate for injection intended for single use by IV infusion. The DP is supplied in a clear, Type I (b) (4) glass vial containing 20 mg of sebelipase alfa. The sebelipase alfa DP is provided as an aqueous solution comprised of sebelipase alfa (2 mg/mL), formulated in trisodium citrate dihydrate (13.7 mg/mL), citric acid monohydrate (1.57 mg/mL), human serum albumin (HSA, 10 mg/mL) at pH 5.9. Sebelipase alfa formulation does not contain any preservative. The DP composition is shown below (from page 2 of Section 3.2.P.1).

Table 1: Dosage Form Composition

Component	Quality Standard	Function	Quantity per vial	Amount per mL
Sebelipase alfa	In-house standard	Active Ingredient	(b) (4)	2 mg
Trisodium Citrate Dihydrate	USP, PhEur, JP	(b) (4)	(b) (4)	13.7 mg
Citric Acid Monohydrate	USP, PhEur, JP		(b) (4)	1.57 mg
Human Serum Albumin	USP, PhEur, JP		(b) (4)	10. mg
				(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients used in the manufacturing of sebelipase alfa DP. Each of the excipients present in the sebelipase alfa DP is compendial and met the compendial specifications as shown in the following table (from page 2 of Section 3.2.P.4.1 of the submission).

Table 1: Excipients in sebelipase alfa Drug Product

Excipient	Functionality	Compendial Specification
Trisodium Citrate Dihydrate	(b) (4)	USP, PhEur, JP
Citric Acid Monohydrate	(b) (4)	USP, PhEur, JP
Human Serum Albumin (HSA)	(b) (4)	USP, PhEur, JP

The human serum albumin (HSA) used in the sebelipase alfa drug substance (DS) process (Albumin (Human) (b) (4)% solution) met multi-compendia requirements including USP, Ph Eur, and JP. The HSA is USP/EP/JP excipient grade, manufactured and processed for use in human pharmaceutical products. The following table (from page 2 of Section 3.2.P.4.5 of the submission) shows the composition of the HSA.

Table 1: Composition of Albumin (Human) ^{(b) (4)}% Solution

Names of Ingredients	Unit and/or Percentage Formula	Function	Reference
^{(b) (4)}			

2.5 Comments on Impurities/Degradants of Concern

The following tables (from pages 1 and 2 of Section 3.2.S.4.1 of the submission, SDN 003 dated November 21, 2014) show the specifications of the sebelipase alfa drug substance.

Table 1: Commercial Specifications for sebelipase alfa Drug Substance

Attribute	Method	Specification(s)
Appearance	Ph. Eur. 2.2.1, Ph. Eur. 2.2.2, and Ph. Eur. 2.9.20	Clear to slightly opalescent, colourless to slightly coloured liquid with no visible foreign particulate matter. Occasional translucent proteinaceous particles may be observed.
pH	USP <791>	(b) (4)
Concentration of sebelipase alfa	RP-HPLC	
Concentration of HSA	RP-HPLC	
Identity ^a	Western blot (Reduced and Non-Reduced)	
Enzyme Activity	(b) (4)	
Molecular Weight and Purity (b) (4)	SDS-PAGE Coomassie (Reduced and Non Reduced)	
Purity (SEC-HPLC) ^a	SEC-HPLC	
Purity (RP-HPLC)	RP-HPLC	
(b) (4)	(b) (4)	

Table 1: Commercial Specifications for sebelipase alfa Drug Substance

Attribute	Method	Specification(s)
Charge Variants ^a	Capillary isoelectric focusing (cIEF)	(b) (4)
(b) (4)		
Endotoxin ^a	ICH Q4B Annex 14	
Bioburden	ICH Q4B Annex 4A(R1)	

^a TAMC = total aerobic microbial count; TYMC = total yeast and mold count.

(b) (4) The proposed specification for (b) (4) in the drug substance (DS) was set at not more than (NMT) (b) (4) ppm (b) (4)%. For the lots that were used in clinical trials and nonclinical studies, the mean level of (b) (4) was (b) (4) ppm and the standard deviation was (b) (4) ppm. The acceptance limit of NMT (b) (4) ppm was aligned with the average plus three standard deviations [(b) (4) ppm] of the historical data. The level of (b) (4) (a process related impurity, (b) (4) in all lots of the DS was within the specification of NMT (b) (4) ppm, except lot 14MM55350006 (b) (4) ppm). The cause for the elevated level of (b) (4) in the above lot was (b) (4).

The following table (from page 3 of Section 1.11.1 of SDN 056) shows the levels of (b) (4) in the DS lots.

Table 1: Levels of (b) (4) in sebelipase alfa DS Lots^a

Use	DS Lot Number	(b) (4) Results (ppm)
Clinical	12-0829	(b) (4)
Clinical	12-0945	(b) (4)
Clinical, Comparability	12-0981	(b) (4)
Clinical	13-0156	(b) (4)
Clinical	13-0503	(b) (4)
Clinical	13-0712	(b) (4)
Clinical	13-0785	(b) (4)
Clinical	13-0858	(b) (4)
Clinical	13-1065	(b) (4)
Clinical, Comparability	13-1202	(b) (4)
Nonclinical, Comparability, Stability	13-1276	(b) (4)
Clinical	14-0069	(b) (4)
Clinical	14-0150	(b) (4)
Clinical, Comparability, Stability	13MM5535003	(b) (4)
Clinical, Comparability, Stability	13MM5535004	(b) (4)
Clinical	13MM5535005	(b) (4)
Nonclinical	13MM5535006	(b) (4)
Clinical	13MM5535007	(b) (4)
Clinical	14MM5535001	(b) (4)
Clinical	14MM5535002	(b) (4)

^a Lots manufactured prior to process validation and used in clinical and nonclinical studies



The specification limit of (b) (4) at NMT (b) (4) ppm corresponds to approximately (b) (4) ng/mg of sebelipase alfa. The recommended dosage of sebelipase alfa in rapidly progressive LAL deficiency patients in the first 6 months of life is 1 mg/kg intravenous (IV) infusion once weekly as an initial dose followed by escalation to 3 mg/kg once weekly.

Considering a dose of 3 mg/kg/week, (b) (4) exposure would be equivalent to approximately (b) (4) µg/kg/week, which corresponds to a daily (b) (4) exposure of approximately (b) (4) µg/kg/day or (b) (4) µg/day (based on a 50 kg body weight, and once weekly administration). The acceptable daily intake (ADI) of (b) (4) as food additive is (b) (4) mg/kg (17th Report of the Joint FAO/WHO Expert Committee on Food

Additives, WHO Techn Rep Ser, 1974, No. 539; FAO Nutrition Meetings Report Series, 1974, No. 53). The above ADI of (b) (4) mg/kg/day is approximately (b) (4) times higher than the anticipated maximum daily (b) (4) exposure of (b) (4) µg/kg/day from sebelipase alfa. Per the above WHO report, the level causing no toxicological effect in the rat was (b) (4) ppm (b) (4) % in the diet equivalent to (b) (4) mg/kg (Nilson HW and JA Wagner, 1951, Feeding Tests with Some Algin Products, Proc Soc Exp Biol Med, 76:630-5). Based on this, Permitted Daily Exposure (PDE) was calculated per the ICHQ3C guidance as follows:

$$\begin{aligned}
 \text{PDE} &= (b) (4) \\
 \text{F2} &= (b) (4); \text{F3} = (b) (4) \text{ F4} = (b) (4); \\
 \text{F5} &= (b) (4)
 \end{aligned}$$

The PDE of (b) (4) mg/day is approximately (b) (4) times higher than the expected maximum daily (b) (4) exposure of (b) (4) µg/kg/day or (b) (4) µg/day from sebelipase alfa. Therefore, the specification of (b) (4) at NMT (b) (4) ppm in the DS does not raise a safety concern and is acceptable.

Extractables Study and Safety Assessment:

The Applicant provided a comprehensive safety assessment of all the extractables from the container closure system for the drug product (DP). These extractables may leach into the DP during its therapeutic use. The following table summarizes the Acceptable Daily Exposures (ADE) for each compound identified, compared to the highest concentration of that compound identified in the extractables study from all the following solvents studied: 1) (b) (4)

(b) (4)	(b) (4)
(b) (4)	(b) (4)

Table 2: Comparison of Extractable Compound Amounts in Adult, Child and Infant Dosing Regimens to Acceptable Daily Exposure Limits

(b) (4)



As shown in the above table, the ADE values for all extractables are higher than the daily exposures calculated for these extractables based on their concentrations determined in the final drug product and its expected use pattern.

The ADE values were derived based on the available toxicity data on each of the compounds detected. The ADE calculation for the organic compounds were based on current scientific approaches for establishing acceptable limits for non-carcinogens, using safety or uncertainty factors to a No Observed Adverse Effect Level (NOAEL), and taking into account of IV route of administration. For the risk assessment of elemental irons/metals, following references were consulted.

- US Department of Agriculture (USDA), 2010, Dietary Reference Intakes: RDA and AI for Vitamins and Elements, National Academy of Sciences, Institute of Medicine, Food and Nutrition Board)
- The International Conference on Harmonisation (ICH)Q3D Guideline for Elemental Impurities (ICHQ3D, 2014)

The derivation of ADE assumed daily exposure for a lifetime. If the intended use of the drug product is expected to be for a period significantly less than a lifetime, then the ADEs may be adjusted, based on intended use pattern, to higher values. The ADE values were used to determine whether the concentration of the extractable or potential leachable would be considered “acceptable” by comparing the ADE values against the concentration in the solution (expressed as mg/L) and based on the maximum amount of drug product administered to the patient averaged over the treatment period. The ADEs were compared to anticipated exposures at maximum daily doses of the drug product for an adult, child and infant using body weights of 50, 10, and 3 kg, respectively.

(b) (4)

7 Page(s) have been Withheld in Full as B4 (CCI/TS) immediately following this page

(b) (4)

Overall, the ADE values for extractables as described above are higher than the anticipated maximum daily exposures calculated for these extractables based on their expected concentrations in the final drug product and its expected use pattern.

Leachables Study and Safety Assessment:

The Applicant has provided the results from the leachables analysis on drug product (DP) samples held for 24 months and associated safety assessment. The end of shelf-life DP samples were evaluated using Inductively Coupled Plasma (ICP) for analysis of the metals as well as Gas Chromatography/Mass Spectrometry (GC/MS) for evaluation of volatile organic compounds (VOC) and semi-volatile compounds (SVOC) and Liquid Chromatography/Mass Spectrometry (LC/MS) for evaluation of non-volatile compounds (NVOC) and Liquid Chromatography/Ultra Violet-Visible Spectroscopy (LC/UV-Vis) for organic acids.

Overall, the results obtained in the leachables study were consistent with those obtained in the extractables study. (b) (4) were identified in DP samples. (b) (4) were also identified in DP samples. The leachables study also identified (b) (4) in one sample from one lot. (b) (4) were also detected in DP samples. (b) (4) was detected in one DP lot (2 out of 3 samples).

The highest concentration for each compound observed in the leachables study was compared to the highest concentration for each compound observed in the extractable study as shown in the following table (from page 2 of the leachables memorandum in Section 1.11.1 of SDN 056). (b) (4) were present in specific samples at higher levels in the leachables study than in the extractables study.

Extractable Compound	Highest Concentration in Extractable Study mg/L	Highest Concentration in Leachable Study mg/L
(b) (4)		

(b) (4)

The data obtained in the leachables study were analyzed in combination with the data obtained in the extractables study to perform a safety evaluation. For the chemicals whose concentrations were lower in the leachables study than in the extractables study, the above safety analysis performed with extractables demonstrated that the levels of these chemicals present in a single DP dose are below the ADE limits for parenteral delivery.

With the exceptions of (b) (4), for those compounds found in both the extractable and leachable studies, the highest concentration of these chemicals was in the extractable study. As discussed above under extractables, the levels of each of these compounds, if present at the levels determined in the extractable study, then the levels of these chemicals from the leachables study would also be acceptable. For (b) (4), the levels are well below the respective ADEs discussed above for the extractables and therefore, offer adequate margins of safety.

(b) (4) was detected in the leachable study but was not detected in the extractables study. Limited toxicological data is available for (b) (4). However, there are data available on the same chemical class of compounds: (b) (4)

(b) (4) was not mutagenic (b) (4)
A study was conducted to determine the oral toxicity of structurally similar (b) (4) in rats after 28 days of exposure. The NOAEL for rats was considered to be greater than (b) (4) mg/kg/day (b) (4). The ADE for (b) (4) was determined using the following (from page 4 of the leachables memorandum in Section 1.11.1 of SDN 056) equation as discussed before.

Where: (b) (4)

(b) (4)

(b) (4)

The following table (from page 3 of the leachables memorandum in Section 1.11.1 of SDN 056) shows the ADE for (b) (4)

Leachable Compound	Highest Concentration mg/L	Daily Adult Exposure ^a	Adult ADE mg/day	Daily Child Exposure ^b	Child ADE mg/day	Daily Infant Exposure ^c	Infant ADE mg/day
(b) (4)							

As shown in the above table, the anticipated maximum daily exposure values for (b) (4) are less than ADEs for adults, children or infants determined from the leachable study and the maximum amounts of (b) (4) as leachable is acceptable for the drug product.

Overall, the levels of leachables from the container closure system in the drug product at 24 months are acceptable based on the available data and conduct of the leachables study.

2.6 Proposed Clinical Population and Dosing Regimen

Sebelipase alfa is indicated for the treatment of lysosomal acid lipase (LAL) deficiency. In pediatric and adult patients with LAL deficiency, the recommended dose is 1 mg/kg administered as an intravenous infusion once every other week. In patients with rapidly progressive LAL deficiency with growth failure in the first 6 months of life, the recommended dosage is 1 mg/kg as an intravenous infusion once weekly as an initial dose followed by escalation to 3 mg/kg once weekly.

2.7 Regulatory Background

The following are the major regulatory milestones:

- PIND meeting held on July 29, 2010
- Initial IND 108460 was submitted in December, 2010 and received Orphan Drug designation in July 2010
- Fast Track designation was granted on June 14, 2011
- End of Phase 1 (EOP1) meeting was held on June 12, 2012
- Breakthrough Therapy designation for the treatment of LAL deficiency (b) (4) (b) (4) (b) (4) Wolman disease (b) (4) was requested on March 14, 2013 and was granted the designation on May 13, 2013
- Proprietary Name “Kanuma” was requested on May 15, 2014 and the proprietary name was accepted by the Agency on August 01, 2014
- Type B Pre-BLA meeting was held on August 19, 2014 under IND 108460

3 Studies Submitted

3.1 Studies Reviewed

The following table shows the list of studies reviewed.

STUDY TITLE	REPORT NO/STUDY NO	PAGE
PHARMACOLOGY		27
PHARMACOKINETICS		68
Single Dose Intravenous Pharmacokinetics of SBC-102 in Sprague-Dawley Rats	SBC-102-POO2	69
TOXICOLOGY		73
Acute		73
<i>Cynomolgus Monkey</i>		73
Intravenous	SBC-102-T003/8234874	73
Subacute/Subchronic/Chronic		73
<i>Rat</i>		74
Four-Week Toxicity and Toxicokinetic Intravenous Infusion Study in Rats with SBC-102 with a 2-Week Recovery Phase	SBC-102-T002/8232384	74
<i>Monkey</i>		85
Four-Week Toxicity and Toxicokinetic Intravenous Infusion Study in Cynomolgus Monkeys with SBC-102 with a 2-Week Recovery Phase	SBC-102-T001/8232455	85
Six-Month Toxicity and Toxicokinetic Study of SBC-102 by Intravenous Infusion in Cynomolgus Monkeys with a 2-Week Recovery Period	SBC-102-T006/20006580	94
REPRODUCTIVE TOXICOLOGY		102
<i>Rat</i>		102
Fertility and Early Embryonic Development to Implantation of SBC-102 by Intravenous Infusion in Male Rats	SBC-102-T009/902526	102
Fertility and Early Embryonic Development Study of SBC-102 in the Female Rat by Intravenous Infusion	SBC-102-T010/902528	108
Range-Finding Embryo-Fetal Development Study in the Albino Rat by Intravenous Infusion	SBC102-T007/902522	114
Embryo-Fetal Development Study in the Albino Rat by Intravenous Infusion	SBC102-T011/902524	115
Pre and Postnatal Study of SBC-102 in the Rat by Intravenous Infusion	SBC102-T013/902527	131
<i>Rabbit</i>		121
Embryo-Fetal Development Dose Range-finding Study in the Rabbit by Intravenous Infusion	SBC102-T008/902523	121
An Embryo-fetal Development Study of SBC-102 in the Rabbit by Intravenous Infusion	SBC102-T012/902525	125

3.2 Studies Not Reviewed

- Analytical method validation study reports submitted in Section 4.2.2.1 are not reviewed.
- The study report entitled “Description of the LAL-Deficient and Wild Type Phenotypes-Overt Clinical Observations (SBC-102-P006)” is not reviewed.

3.3 Previous Reviews Referenced

- Pharmacology review of IND 108460 dated April 17, 2012 by Dr. Babatunde E Akinshola

4 Pharmacology

4.1 Primary Pharmacology

The following review is incorporated below from the pharmacology review of IND 108460 dated April 17, 2012.

In vitro pharmacodynamics

SBC-102 Uptake and lysosomal localization in NR8383 macrophage cells (Study No. SBC-102-P001)

Methods: SBC-102 in PBS was incubated with Oregon Green (488) fluorescent dye. The reaction mixture was dialyzed and concentrated against PBS. The fluorescently-labeled SBC-102 at 5 µg/ml (91 nM) and the lysosomal marker "LysoTracker red" were incubated with NR8383 cells for 2 hrs. The co-localization of SBC-102 and the lysosomal marker in the cells was examined by confocal fluorescence microscopy. The binding specificity of SBC-102 to the macrophage mannose receptor (MMR) was also assessed by competitive binding assays using the MMR-expressing rat alveolar macrophage (RAM) cell line, NR8383.

Results: SBC-102 localization into lysosomes was captured by the confocal microscope as overlapped images different from the green or red fluorescence. A dose-dependent inhibition in SBC-102 binding-uptake by mannose in MR8383 macrophage cells was observed, which is consistent with a SBC-102: MMR interaction.

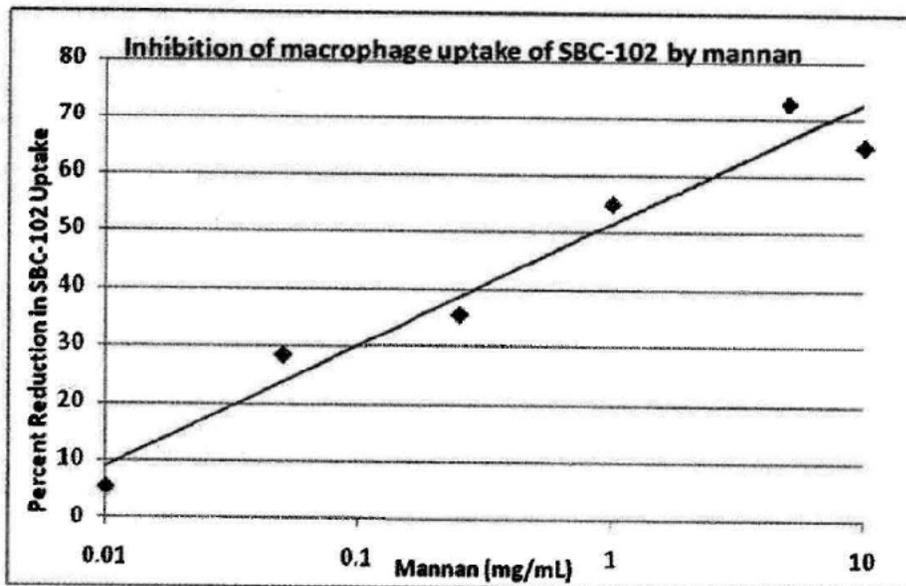


Figure 2.6.2-2. Uptake inhibition of SBC-102 by mannose. Fluorescently-labeled SBC-102 and mannose, at the indicated concentrations, were incubated with NR8383 cells for 2 hours. SBC-102 uptake was determined by fluorescence-activated cell sorting using median fluorescence intensity as the endpoint.

Increase in LAL activity in SBC-102 treated fibroblast cells. (Study No. SBC-102-P001)

Methods: The ability of SBC-102 to increase LAL activity in normal and LAL-deficient fibroblast cells was examined in vitro in this study.

Untransformed fibroblasts isolated from a patient with Wolman disease and normal human fibroblasts were incubated with SBC-102 at concentrations up to 5 µg/ml for 4 hr at 37 °C. Cells were washed to remove non-specific signal, and cell lysates were assayed for LAL activity using 4-methylumbelliferyl oleate as the enzymatic substrate.

Results: Endogenous cell-associated LAL activity was substantially lower in Wolman disease fibroblasts compared to normal human fibroblasts and dose-dependent increases in enzymatic activities were observed in the Wolman disease fibroblasts after incubation with increasing concentrations of SBC-102 as shown in the figure below.

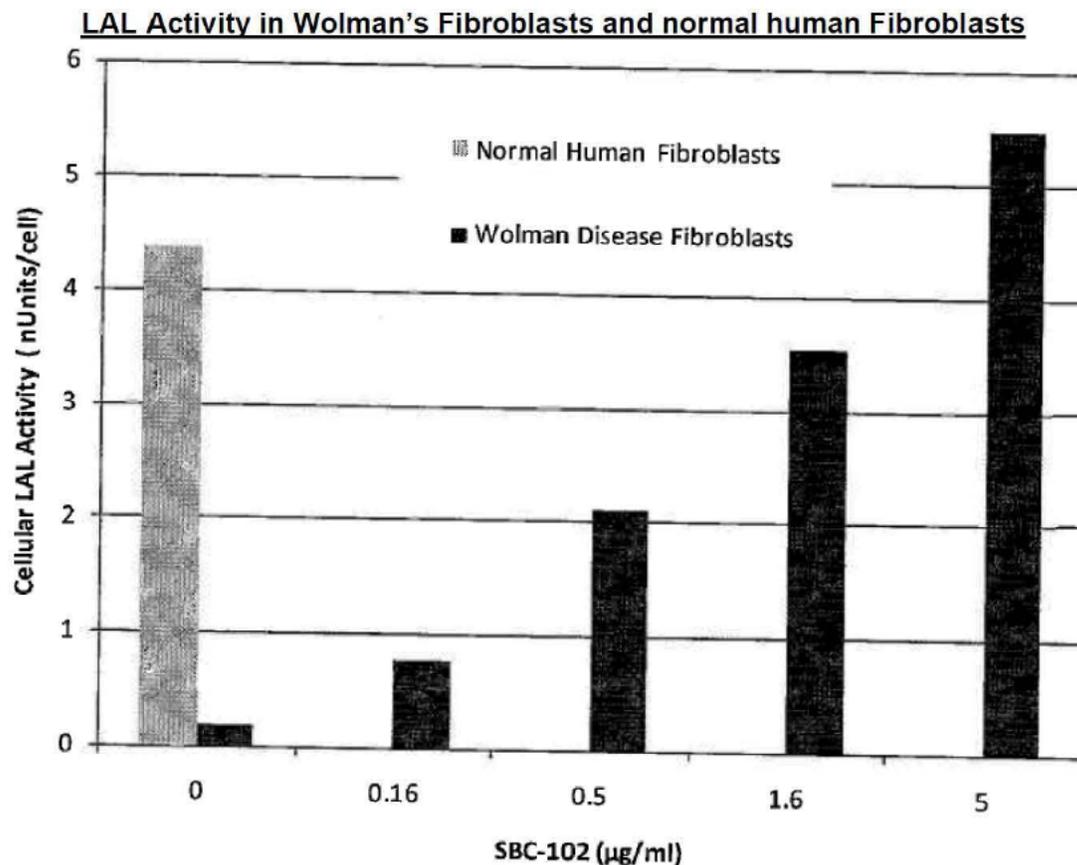


Figure 2.6.2-3. LAL activity in Wolman's Fibroblasts and normal human fibroblasts after incubation with SBC-102.

SBC-102 was incubated at the indicated concentrations with fibroblast cells for 4 hours. Cells were washed with PBS and lysed with lysis buffer. LAL activity analysis was performed on the cell lysates.

Summary: The ability of SBC-102, a recombinant human lysosomal acid lipase (rhLAL), to bind to cell and to be internalized to the lysosomal compartment, was examined in vitro, using rat macrophages. When incubated with macrophage cells, fluorescently-labeled SBC-102 localized to the lysosome. This effect could be attenuated by using a mannose polysaccharide competitor, implicating the MMR receptor as a mechanism of recognition and uptake by these cells. SBC-102 caused an increase in the cell-associated LAL activity in LAL deficient human fibroblasts after incubation in vitro, indicating SBC-102 endocytosis by M6P expressing cells and showing that SBC-102 corrects the intracellular enzyme deficiency in the cells.

In vivo pharmacodynamics

Effects of SBC-102 in a rat model of lysosomal acid lipase deficiency. (Study No. SBC-102-P002)

Methods:

Homozygous (LAL^{-/-}), hemizygous (LAL^{+/-}), and wild-type (LAL^{+/+}) Yoshida rats were identified by PCR analysis of DNA isolated from tail-clip tissues.

The LAL deficiency (LAL^{-/-}) phenotype manifestation were examined from the age of 3 weeks to 12 weeks.

Prior to SBC-102 administration, rats were injected intraperitoneally (IP) with diphenhydramine (5 mg/kg, to reduce the hypersensitivity response to the recombinant human protein), and 20 min later, were anesthetized with isoflurane, and SBC-102 or vehicle (saline) was injected via tail vein. Beginning at 4 weeks of age, rats were either given 4 doses (at 1 dose/week) for 4 weeks or 2 doses (at 1 dose every other week) for 4 weeks, with dose termination at 8 weeks of age. For animals injected with less than 3 mg/kg test article, the entire dose was administered at once, and for animals dosed with 3 mg/kg and above, doses were divided into 3 separate injections approximately 10 mins apart (to eliminate the transient hind limb swelling observed in some rats dosed with SBC-102).

After euthanasia by carbon dioxide inhalation, animals were weighed and blood collected via cardiac puncture. Organs (spleen, mesenteric lymph node, liver, duodenum, jejunum, ileum, gastric lymph node, adrenal gland, pancreas, and brain) were harvested, weighed, and tissue samples retained for histological evaluation.

Results: Of the first 100 offspring genetically characterized, 25 % were LAL^{-/-}, 31 % were LAL^{+/+} wild-type (WT), and 44 % were LAL^{+/-}. The genotyping result is close to the expected Mendelian ratio of 1:1:2, indicating that there is no significant embryonic lethality associated with the LAL-deficiency genotype. Within the age of 3 weeks to 14 weeks, the phenotypic manifestation of LAL-deficiency in Yoshida rats resulted in severe moribundity or death. The life-span of the LAL^{-/-} rat is dramatically shortened relative to the WT rat due to the disease manifestations which progressed over time.

The hemizygous rats do not display an LAL-deficiency phenotype and are used to generate LAL^{-/-} and LAL^{+/+} offsprings.

The body weights of the WT rats continue to increase from week 3 through week 12 whereas, the LAL^{-/-} rats are at their maximum weight at approximately 7 weeks of age. By 12 weeks, the body weights of the WT males and females were 3.04 and 2.76 times the body weights of the LAL^{-/-} males and females respectively.

Body Weight (g) of LAL-Deficient and WT Rats

Table 1. Body weight (g) of LAL-deficient and WT rats from 3 to 12 weeks of age

Age (weeks)	LAL ^{+/+}						LAL ^{-/-}					
	Males			Females			Males			Females		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
3	41	39.9	5.8	38	40.2	6.2	17	37.9	5.7	20	32.3	5.3
4	38	68.7	8.4	31	69.0	8.3	17	53.3	8.5	20	48.9	8.4
5	23	111.5	13.5	25	106.6	12.4	14	72.8	13.3	17	64.5	11.0
6	21	158.1	16.2	25	140.1	13.8	12	91.0	16.4	16	77.5	15.1
7	20	199.7	18.4	23	164.7	12.7	12	99.0	17.7	16	82.9	17.3
8	25	237.1	18.0	26	182.8	14.9	15	97.4	17.3	18	84.8	17.2
9	12	255.0	11.2	5	195.0	11.4	8	93.3	15.3	8	79.8	20.3
10	12	274.1	14.5	5	206.7	12.5	7	96.6	16.8	8	80.9	19.2
11	11	289.5	13.0	4	213.1	7.6	6	100.5	14.1	8	79.7	18.5
12	11	301.8	13.6	4	218.0	7.5	6	99.4	13.5	7	78.9	19.3

Disproportionate increases in organ weight (relative to body weight) were noted in key tissues in the LAL^{-/-} rats. Differences between wild type and LAL^{-/-} rats in relative organ size were present by week 4 and in most tissues showed progression over time. Organ weights in LAL^{-/-} rats were expressed as a percent of total body weight as shown in the table below.

Organ Weight as Percent of Body Weight in LAL-Deficient and WT Rats

Table 2. Organ Weight as percent of body weight in LAL-deficient and WT rats at 4 and 10 weeks of age (% of body weight)^a

Age (weeks)	genotype	Liver			Spleen			Mesenteric LN			Duodenum			Jejunum			Ileum		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
4	LAL ^{-/-}	6	7.4	0.9	4	0.63	0.1	4	0.61	0.09	4	0.6	0.06	4	3.4	0.68	4	2.1	0.3
	LAL ^{+/+}	4	5.2	0.5	4	0.36	0.1	4	0.16	0.02	4	0.3	0.04	4	1.6	0.23	4	1.3	0.1
8	LAL ^{-/-}	9	11.50	1.62	8	0.86	0.16	5	0.62	0.08	6	0.90	0.29	5	3.67	0.35	5	2.75	0.54
	LAL ^{+/+}	11	4.78	0.26	11	0.29	0.05	11	0.14	0.09	11	0.34	0.10	11	1.33	0.22	11	1.18	0.22
10	LAL ^{-/-}	3	14.7	0.6	3	1.00	0.1	3	0.74	0.06	3	0.9	0.11	3	4.2	0.46	3	3.2	0.1
	LAL ^{+/+}	2	4.6	0.2	2	0.26	0.02	2	0.25	0.08	2	0.2	0.11	2	1.0	0.16	2	1.2	0.1

^a Organ weight as a percent of total body weight
SD = standard deviation

At 4 weeks of age, gross pathological findings in animals included moderate hepatomegaly (with a distinct yellow-orange color), splenomegaly, enlargement of the mesenteric and gastric lymph nodes, and moderate thickening of the small intestinal walls. Histological findings included; multifocal collections of foamy macrophages/Kupffer cells in the liver and foamy macrophages in the spleen, expansion of the lamina propria in the small intestine with infiltration of foamy macrophages, diffuse medullary histiocytosis and multifocal expansion of the cortex due to foamy macrophages in the affected lymph nodes and atrophy of mesenteric fat, often with transformation to brown fat.

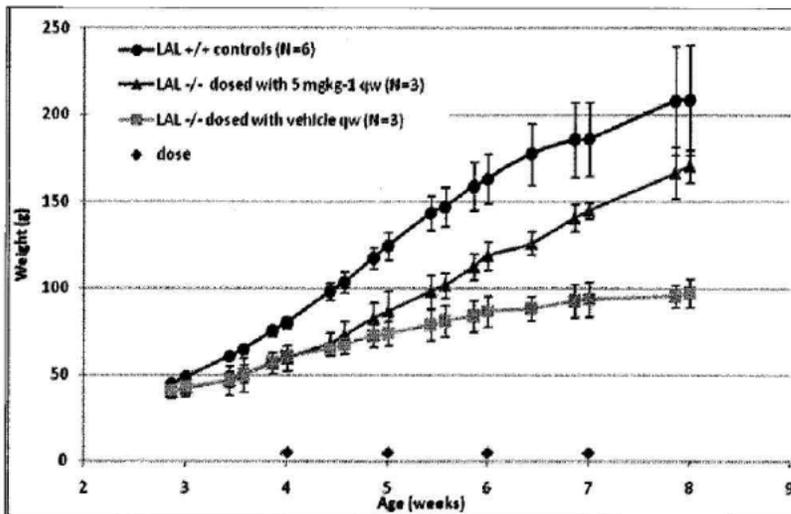
At 8 weeks of age, gross findings were similar to 4 weeks findings, but with more severity. Histological findings at 8 weeks of age included; severe sinus histiocytosis and multifocal expansion of the cortex due to foamy macrophages with secondary severe atrophy of the lymphoid elements in the affected lymph nodes, severe histiocytosis of the lamina propria of the small intestine, multifocal large collections of foamy histiocytes in the liver and spleen, serous atrophy of mesenteric fat, and mild multifocal cortical histiocytosis of the adrenal gland.

Histological staining of liver hepatocytes by oil red-O positive pale stain indicated the presence of neutral triglycerides and lipids, while H & E-stained sections revealed acicular (needle-like) clefts compatible with cholesterol crystals. The presence of unilateral or bilateral microphthalmia was observed in some LAL^{-/-} and LAL^{+/+} rats. These histological findings in the rat model of LAL deficiency are reportedly similar to those seen in patients with LAL deficiency.

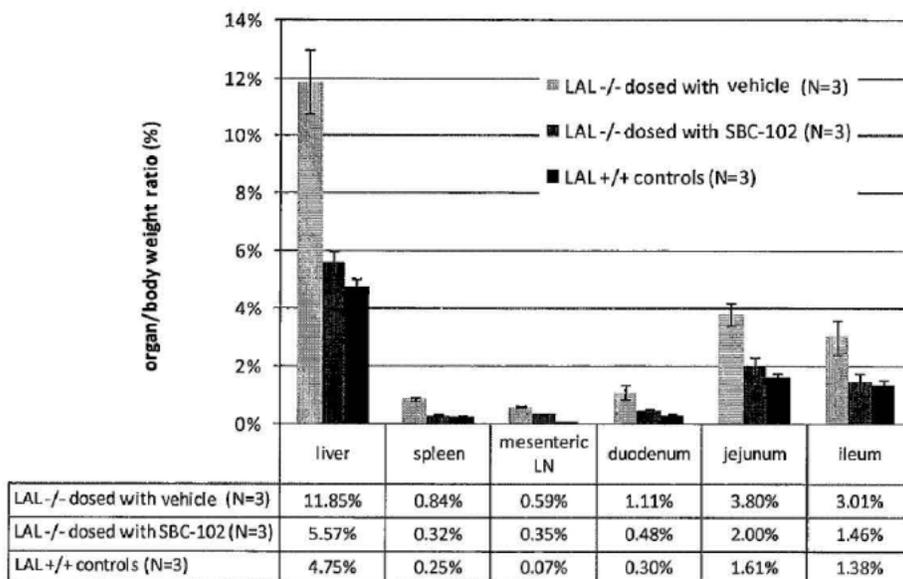
Effects of SBC-102 in the LAL^{-/-} rat following administration for One Month

After 4 weekly doses of SBC-102 at 5 mg/kg, the LAL^{-/-} rats demonstrated significant improvements in body weight gain, and reduction in organ weights and tissue total cholesterol, cholesteryl ester and triglyceride contents, compared to vehicle treated LAL-deficient rats, as shown in the figures below. The SBC-102 treated rats showed normal liver histology, color and reduction in liver size in contrast to the vehicle-treated rats. The characteristically elevated serum ALT and AST levels in LAL-deficient rats were reduced. The body weight gain in vehicle-treated LAL^{-/-} rats were markedly reduced relative to WT controls, as shown in the figures below.

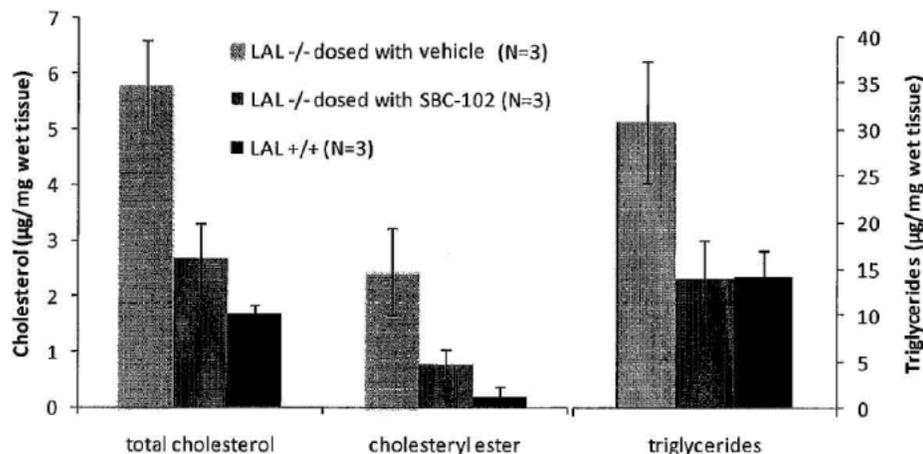
Effects of SBC-102 on Body Weight in Wild-Type and LAL-Deficient Rats



Effects of SBC-102 on Organ Size as Percent of Body Weight in Wild-Type and LAL-Deficient Rats



Effects of SBC-102 on Liver Lipids in Wild-Type and LAL-Deficient Rats



Dose-Dependent Effects of SBC-102 in the LAL^{-/-} Rat Following Administration for One Month

Based on the effects seen with weekly administration of SBC-102 (5 mg/kg, weekly) for 4 weeks, the effects of a range of doses and dose schedules were studied in LAL^{-/-} rats weekly and every other week (qw and qow). Prior to SBC-102 administration, rats were injected intraperitoneally (IP) with diphenhydramine (5 mg/kg, to reduce rat hypersensitivity response to recombinant human protein), and 20 mins later, were anesthetized with isoflurane. SBC-102 was administered at dosages of 0.2, 1, 3 and 5 mg/kg qow, or 0.35, 1, and 5 mg/kg qw or vehicle (saline) was injected via tail vein beginning at 4 weeks of age through study termination at 8 weeks of age.

There were improvements in body weight gain, organomegaly, tissue substrate levels and serum transaminases. Anti-SBC-102 antibodies were not detected in any of the serum samples analyzed from a total of 36 animals.

LAL^{-/-} rats treated with SBC-102 showed greater body weight at 8 weeks of age compared to the vehicle-treated rats, and were comparable to the wildtype (WT) rats. The difference in body weights (BW) was dose-dependent with the greatest effects observed in rats treated with (highest dose) SBC-102 at 5 mg/kg weekly which exhibited a 1.74-fold increase in body weight, or at the (intermediate dose) 3 mg/kg every other week, which exhibited a 1.5-fold increase in body weight over the vehicle control animals. There were no differences in absolute BW or BW gains in LAL^{-/-} rats treated with 0.2 mg/kg SBC-102 every other week, when compared to the vehicle control-treated rats. The percentage increase in body weight and body weight changes observed in the animals with SBC-102 treatment are shown in the tables below.

A dose-dependent reduction in organ weights (as percent BW) was observed in male and female LAL^{-/-} rats after 4 weeks of SBC-102 treatment, with maximum effect observed at 5 mg/kg dose, and the measured organ weights (as % BW) coming close to

those of the LAL+/+ rats. A dose dependent trend in the decrease in organ weight observed at the mid dose (3 mg/kg qow) was absent at the 0.2 mg/kg qow dose.

The administration of SBC-102 at 5 mg/kg/week for 4 weeks to LAL-/- rats reduced the liver and tissue concentrations of cholesterol, cholesteryl ester and triglyceride to similar levels measured in the male and female WT rats. Although the reduction of the LAL substrates in the rats were dose-dependent, the cholesteryl ester concentrations in the liver and spleen of rats treated with 0.2 mg/kg qow, were comparable to LAL-/- control values, whereas triglyceride levels in the liver and spleen were apparently reduced at the low dose level of 0.2 mg/kg qow.

The elevated serum AST levels in the LAL-/- rats administered a dose of 5 mg/kg/week SBC-102 for 4 weeks were reduced to levels seen in (age matched) wildtype rats, whereas no effects were observed with lower SBC-102 doses.

Effects of SBC-102 on Body Weight and Percent Body Weight Change in LAL-/- (Deficient) Rats

Table 2.6.2-3 Body weight (g) in LAL-/- rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age^a

Dose ^b (mg·kg ⁻¹)	Male			Female			All		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vehicle	5	111.5	15.6	6	92.1	14.8	11	100.9	17.6
0.2 qow	3	101.0	25.1	4	79.1	12.1	7	88.5	20.5
0.35 qw	3	111.2	9.5	2	117.3	20.4	5	113.6	12.6
1 qow	4	112.3	7.7	3	101.3	4.5	7	107.6	8.4
1 qw	2	162.3	32.2	3	141.7	15.3	5	149.9	22.4
3 qow	4	154.0	24.0	3	143.9	14.9	7	150.3	17.4
5 qow	1	199.2		1	116.1		2	157.8	58.5
5 qw	2	181.8	16.5	2	166.3	5.9	4	175.6	14.8
LAL +/+	15	237.4	22.1	20	184.6	13.6	35	207.2	31.8

SD=standard deviation

^aBody weight (g) at 8 weeks of age.

Table 2.6.2-4. Percent (%) Increase in body weight in LAL-/- rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age^a

Dose (mg·kg ⁻¹)	Male			Female			All		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vehicle	5	85.0	40	6	62.0	18	11	72.4	31
0.2 qow	3	83.6	33	4	63.3	21	7	72.0	26
0.35 qw	3	92.5	7	2	116.3	24	5	102.0	18
1 qow	4	107.5	8	3	93.4	10	7	101.4	11
1 qw	2	190.2	80	3	154.4	59	5	168.7	61
3 qow	4	169.1	52	3	148.6	12	7	160.3	39
5 qow	1	230.9		1	133.7		2	182.3	69
5 qw	2	210.7	15	2	171.8	31	4	191.2	30
LAL +/+	15	252.8	24	20	168.5	23	35	204.6	48

^aIncrease in weight (g) at 8 weeks of age as a percent of pre-dose (4 week) weight.

SD=standard deviation

Effects of SBC-102 on Organ Weight Change in LAL-/- (Deficient) Rats

Table 2.6.2-5. Organ weight as percent of body weight in LAL-/- rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age^a

Dose (mg·kg ⁻¹)	Sex	Liver			Spleen			Mesenteric LN			Duodenum			Jejunum			Ileum		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vehicle	M	4	10.88	0.25	3	0.80	0.25	2	0.66	0.14	2	0.71	0.22	2	3.49	0.32	2	2.37	0.10
	F	5	12.01	0.83	5	0.90	0.10	3	0.59	0.59	4	1.00	0.29	3	3.80	0.37	3	3.01	0.58
	All	9	11.50	1.62	8	0.86	0.16	5	0.62	0.08	6	0.90	0.29	5	3.67	0.35	5	2.75	0.54
0.2 qow	M	3	9.30	1.66	3	0.49	0.13	3	0.57	0.06	3	0.85	0.20	3	3.49	0.47	3	2.45	0.16
	F	4	10.94	0.56	4	0.78	0.16	4	0.80	0.21	4	0.87	0.12	4	3.44	0.61	4	2.83	0.34
	All	7	10.24	1.36	7	0.65	0.21	7	0.70	0.19	7	0.86	0.14	7	3.44	0.51	7	2.67	0.33
1 qow	M	4	7.29	0.26	4	0.47	0.12	4	0.53	0.08	4	0.64	0.10	4	3.11	0.32	4	1.95	0.20
	F	3	8.06	1.24	3	0.49	0.12	3	0.58	0.03	3	0.72	0.07	3	3.29	0.28	3	2.57	0.31
	All	7	7.62	0.85	7	0.48	0.10	7	0.55	0.06	7	0.67	0.09	7	3.19	0.29	7	2.21	0.40
3 qow	M	4	6.16	0.41	4	0.41	0.04	4	0.59	0.13	4	0.51	0.10	4	2.73	0.40	4	1.88	0.32
	F	3	6.57	0.46	3	0.45	0.04	3	0.53	0.15	3	0.63	0.19	3	3.08	0.43	3	2.02	0.32
	All	7	6.34	0.45	7	0.43	0.04	7	0.57	0.13	7	0.56	0.14	7	2.88	0.42	7	1.94	0.30
5 qow	M	1	5.44		1	0.36		1	0.36		1	0.40		1	1.84		1	1.73	
	F	1	6.57		1	0.63		1	0.80		1	0.43		1	3.66		1	2.11	
	All	2	6.01	0.80	2	0.50	0.19	2	0.58	0.31	2	0.42	0.02	2	2.75	1.29	2	1.92	0.27
LAL +/+	M	5	4.87	0.24	5	0.31	0.04	5	0.18	0.10	5	0.34	0.12	5	1.22	0.15	5	1.07	0.17
	F	6	4.70	0.28	6	0.27	0.06	6	0.11	0.07	6	0.34	0.09	6	1.42	0.24	6	1.28	0.23
	All	11	4.78	0.26	11	0.29	0.05	11	0.14	0.09	11	0.34	0.10	11	1.33	0.22	11	1.18	0.22

^a Organ weight as a percent of total body weight at 8 weeks of age.

SD=standard deviation

Effects of SBC-102 on Organ Weight Change in LAL-/- (Deficient) Rats

Table 2.6.2-6. Organ weight as percent of body weight in LAL-/- rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age every week for four weeks^a

Dose (mg·kg ⁻¹)	Sex	Liver			Spleen			Mesenteric LN			Duodenum			Jejunum			Ileum		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vehicle	M	4	10.88	0.25	3	0.80	0.25	2	0.66	0.14	2	0.71	0.22	2	3.49	0.32	2	2.37	0.10
	F	5	12.01	0.83	5	0.90	0.10	3	0.59	0.59	4	1.00	0.29	3	3.80	0.37	3	3.01	0.58
	All	9	11.50	1.62	8	0.86	0.16	5	0.62	0.08	6	0.90	0.29	5	3.67	0.35	5	2.75	0.54
0.35 qw	M	3	7.01	0.21	3	0.56	0.04	3	0.71	0.15	3	0.73	0.17	3	3.52	0.23	3	2.31	0.25
	F	2	6.87	1.27	2	0.61	0.09	2	0.73	0.00	2	0.56	0.07	2	3.71	0.06	2	2.29	0.24
	All	5	6.95	0.65	5	0.58	0.06	5	0.72	0.11	5	0.66	0.15	5	3.60	0.19	5	2.30	0.22
1 qw	M	2	6.23	0.92	2	0.50	0.09	2	0.63	0.17	2	0.17	0.17	2	2.65	0.43	2	1.94	0.27
	F	3	6.24	0.23	3	0.45	0.01	3	0.55	0.08	3	0.60	0.06	3	2.56	0.12	3	1.92	0.15
	All	5	6.24	0.49	5	0.47	0.05	5	0.58	0.11	5	0.58	0.10	5	2.59	0.22	5	1.93	0.17
5 qw	M	2	5.34	0.10	2	0.36	0.10	2	0.05	0.07	2	0.53	0.13	2	1.61	0.08	2	1.07	0.11
	F	2	5.65	0.52	2	0.33	0.02	2	0.36	0.03	2	0.49	0.01	2	2.17	0.10	2	1.62	0.19
	All	4	5.50	0.36	4	0.35	0.06	4	0.37	0.05	4	0.51	0.08	4	1.89	0.33	4	1.35	0.34
LAL +/+	M	5	4.87	0.24	5	0.31	0.04	5	0.18	0.10	5	0.34	0.12	5	1.22	0.15	5	1.07	0.17
	F	6	4.70	0.28	6	0.27	0.06	6	0.11	0.07	6	0.34	0.09	6	1.42	0.24	6	1.28	0.23
	All	11	4.78	0.26	11	0.29	0.05	11	0.14	0.09	11	0.34	0.10	11	1.33	0.22	11	1.18	0.22

^a Organ weight as a percent of total body weight at 8 weeks of age.

SD=standard deviation

Effects of SBC-102 on Tissue Cholesterol Concentration in LAL^{-/-} (Deficient) Rats**Table 2.6.2-7. Tissue cholesterol concentration ($\mu\text{g}/\text{mg}$ tissue)^a in LAL^{-/-} rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age**

Dose ($\text{mg}\cdot\text{kg}^{-1}$)	Sex	N	Liver				Spleen			
			Total Cholesterol		Cholesteryl Ester		Total Cholesterol		Cholesteryl Ester	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vehicle	M	2	5.51	0.71	2.94	1.03	8.09	1.27	4.15	0.76
	F	3	7.85	2.73	3.85	2.71	7.34	1.07	3.74	0.94
	All	5	6.91	2.34	3.48	2.04	7.71	0.14	3.95	0.13
0.2 qow	M	3	7.54	1.60	3.48	1.40	7.31	1.72	3.48	1.46
	F	4	8.43	4.59	4.44	2.61	6.46	0.25	3.06	0.13
	All	7	8.05	3.41	4.03	2.08	6.82	1.11	3.27	0.94
0.35 qw	M	3	5.93	1.04	1.99	0.27	3.68	1.08	0.43	0.25
	F	2	6.21	0.95	2.61	0.10	3.52	0.79	0.50	0.32
	All	5	6.04	0.89	2.28	0.36	3.62	1.57	0.46	0.24
1 qow	M	4	4.73	1.08	1.10	0.07	3.57	1.16	0.60	0.50
	F	2	5.16	0.83	1.39	0.24	4.42	0.64	0.75	0.39
	All	6	4.91	0.93	1.38	0.50	3.94	1.01	0.66	0.43
1 qw	M	2	3.38	0.29	0.82	0.04	3.43	0.47	0.21	0.25
	F	2	3.20	0.54	1.75	0.10	2.88	0.18	0.11	0.28
	All	4	3.29	0.37	0.94	0.15	3.51	0.43	0.16	0.23
3 qow	M	3	2.73	0.84	0.48	0.39	3.12	0.14	0.06	0.28
	F	4	2.60	0.48	0.43	0.19	3.43	1.11	0.36	0.90
	All	7	2.65	0.59	0.45	0.26	3.29	0.81	0.23	0.68
5 qow	M	1	2.56		0.20		3.64		0	-
	F	1	3.48		0.88		2.80		0	-
	All	2	3.02	0.65	0.54	0.48	3.22	0.59	0.54	0.17
5 qw	M	2	3.76	0.21	0.74		3.84	0.59	0.21	0.21
	F	2	3.64	0.79	1.17	0.30	2.81		0.15	
	All	4	3.70	0.48	1.03	0.60	3.49	0.72	0.19	0.15
LAL +/+	M	2	2.08	0.09	0.16	0.05	1.69	0.91	0	-
	F	3	2.61	0.46	0.26	0.24	2.70	0.28	0.05	0.26
	All	5	2.25	0.53	0.24	0.18	2.30	0.74	0	-

^a Micrograms of cholesterol per milligram of wet tissue at 8 weeks of age
SD = standard deviation

Effects of SBC-102 on Tissue Triglyceride Concentration in LAL^{-/-} (Deficient) Rats

Table 2.6.2-8. Tissue triglyceride concentration ($\mu\text{g}/\text{mg}$ tissue)^a in LAL^{-/-} rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age

Dose (mg·kg ⁻¹)	Sex	Liver			Spleen		
		N	Mean	SD	N	Mean	SD
Vehicle	M	2	21.03	2.50	2	5.38	3.04
	F	3	22.04	9.51	3	10.46	3.47
	All	5	21.63	4.01	5	8.43	4.01
0.2 qow	M	3	17.12	4.29	3	2.16	0.84
	F	4	14.81	3.27	4	2.77	0.61
	All	7	15.80	3.60	7	2.51	0.72
0.35 qw	M	3	18.38	0.17	3	1.06	0.17
	F	2	17.29	3.52	2	1.38	0.62
	All	5	17.83	2.37	5	1.22	0.32
1 qow	M	4	13.91	3.03	4	1.34	0.25
	F	3	14.11	3.46	3	2.63	2.76
	All	7	14.02	0.31	7	1.99	1.78
1 qw	M	2	14.85	1.93	2	1.29	0.34
	F	3	14.57	0.55	2	1.06	0.01
	All	5	14.71	1.17	4	1.18	0.24
3 qow	M	3	14.32	9.13	3	1.31	0.15
	F	4	16.94	3.57	4	2.43	2.09
	All	7	15.63	3.93	7	1.87	1.37
5 qow	M	1	15.93		1	5.76	
	F	1	14.63		1	1.23	
	All	2	15.28	0.92	2	3.50	3.21
5 qw	M	2	13.92	2.26	2	1.20	0.22
	F	2	16.19	1.06	1	0.95	
	All	4	15.05	1.95	3	1.11	0.21
LAL ^{+/+}	M	2	9.55	1.42	2	1.03	0.11
	F	3	11.52	1.52	3	1.89	1.88
	All	5	10.73	1.68	5	1.55	1.41

^a microgram per milligram of wet tissue at 8 weeks of age
SD = standard deviation

Serum anti-SBC-102 Antibody Activity in LAL^{-/-} (Deficient) Rats

Table 2.6.2-12. Serum anti-SBC-102 antibody assessment in LAL^{-/-} rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age

Dose (mg·kg ⁻¹)	N	Positive by initial screen ^a	Confirmed positive ^b
0.2 qow	5	0	NT
0.35 qw	5	0	NT
1 qow	6	2	0
1 qw	6	2	0
3 qow	8	1	0
5 qow	2	0	NT
5 qw	4	3	0

^a Positive by bridging ELISA

^b Positive by bridging ELISA after immunodepletion (competition) with SBC-102

NT: Not tested

Effects of SBC-102 on Serum AST and ALT in LAL^{-/-} (Deficient) Rats

Table 2.6.2-10. Serum aspartate aminotransferase levels (units/L)^a in LAL^{-/-} rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age

Dose (mg·kg ⁻¹)	Males			Females			All		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vehicle	3	155	44	4	183	30	7	176	40
0.2 qow	2	179	73	3	205	65	5	195	60
0.35 qw	3	154	18	2	185	100	5	166	55
1 qow	4	123	50	3	135	20	7	128	38
1 qw	3	170	132	3	111	32	6	140	92
3 qow	4	135	44	5	151	47	9	144	44
5 qow	1	63		1	89		2	76	19
5 qw	2	80	3	2	66	2	4	73	9
LAL +/+	4	74	12	6	60	6	10	65	11

SD=standard deviation

^aAST Enzymatic Assay Kit (ID Labs)

Table 2.6.2-11. Serum alanine aminotransferase levels (units/L)^a in LAL^{-/-} rats after 4 weeks administration SBC-102 at the indicated levels and schedules, determined at 8 weeks of age

Dose (mg·kg ⁻¹)	Males			Females			All		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Vehicle	3	65.7	13.2	4	52.3	20.0	7	58.0	17.6
0.2 qow	3	42.0	16.8	4	44.8	14.7	7	43.6	14.3
0.35 qw	3	23.7	3.2	2	41.0	33.9	5	30.6	19.6
1 qow	4	48.5	20.6	3	37.0	11.1	7	43.6	17.1
1 qw	2	26.0	1.4	3	26.0	2.0	5	26.0	1.6
3 qow	2	25.5	7.8	2	34.5	12.0	4	30.0	9.8
5 qow	1	33.0		1	18.0		2	25.5	10.6
5 qw	2	29.0	8.5	2	34.5	7.8	4	31.8	7.4
LAL +/+	3	48.7	10.8	6	42.0	4.3	9	44.2	7.2

SD=standard deviation

Thus, the administration of SBC-102 (5 mg/kg/week) for 4 weeks resulted in the restoration of LAL enzymatic activity and reversal of associated pathophysiology in LAL^{-/-} rats.

The improvements observed in disease markers (increased body weight gains, reduction in organ weights, decreased tissue (liver and spleen) lipid content, and reduction in serum ALT and AST levels) were dose dependent in LAL^{-/-} rats treated with SBC-102 (weekly for 4 weeks) at a dose level of 0.35 mg/kg or greater

Effects of a Single Dose of SBC-102 on Serum ALT and AST Concentration in Wild-Type (LAL+/+) and LAL-/- (Deficient) Rats

Table 2.6.1-13. Serum ALT and AST Concentrations in 8-week old LAL-/- Rats Administered A Single Dose of SBC-102 at 5 mg·kg⁻¹

Assay		LAL -/- SBC-102 (5 mg·kg ⁻¹)			LAL -/- Vehicle			LAL+/+		
		N	Mean	SD	N	Mean	SD	N	Mean	SD
ALT	U/L	8	33.75	15.41	7	58.00	17.57	9	44.22	7.19
AST	U/L	8	267.00	65.38	7	357.14	83.63	9	96.89	30.64

Effects of a Single Dose of SBC-102 on Liver Enzyme Activity in Wild-Type (LAL+/+) and LAL-/- (Deficient) Rats

Table 2.6.2-9. Hepatic LAL enzymatic activity (mU/mg protein) in 34-Day old LAL-/- rats at 1, 24 and 72 hours following the administration of a single dose of SBC-102 (5 mg·kg⁻¹)

	SBC-102 (mg·kg ⁻¹)	N	Time post dose (h)	LAL Enzymatic Activity (mU/mg liver protein)
LAL -/-	-	1	-	0.43
	5	1	1	51.49
	5	1	24	7.79
	5	1	72	2.70
LAL +/+	-	1	-	2.47

In conclusion, the administration of a single bolus injection of 5 mg/kg dose of SBC-102 to LAL-/- rats does not precipitate any toxicity as measured by comparable liver transaminase concentrations in the wildtype and enzyme deficient rats. In addition, the level of enzymatic activity measured at 1, 2, and 72 hrs to assess the duration of activity following a 5 mg/kg single dose of SBC-102 in LAL-/- rats was comparable to that in the LAL+/+ rat, suggesting that accumulation of the enzyme is unlikely to occur with chronic or repeated dosing.

Survival Analysis of Homozygous, LAL-Deficient Donryu Rats (SBC-102-P003)

Methods: The LAL (lysosomal acid lipase)-deficient Donryu (“Yoshida”) rat contains a spontaneous mutation in the LAL gene which abrogates LAL activity. This rat model demonstrates many phenotypic abnormalities seen in humans with LAL deficiency and shows pathological changes seen in LAL deficiency/CESD (cholesteryl ester storage disease) and Wolman phenotype patients. In this model, the underlying pathology resembles human disease with lipid accumulation in the gastrointestinal tract, liver, spleen and other tissues, and loss of body fat. In order to determine the survival profile of the LAL-deficient rats, mortality rates of LAL-/- and LAL+/+ rats were compared from 14 to 200 days of age. Animals were weaned at about three weeks of age and

maintained under standard conditions. Animals were surveyed daily and deaths were recorded. Survival analysis was performed using data from the first 20 LAL^{-/-} (10/sex) and first 20 LAL^{+/+} (10/sex) progeny.

Results: LAL^{-/-} rats showed a biphasic survival curve with an increase in mortality around the time of weaning and a second sharp increase beginning at 11 weeks of age. Fifty percent of LAL^{-/-} rats survived to 12 weeks of age and all 20 had died by 14 weeks. LAL^{+/+} rats all survived to at least 200 days, indicating that the increased mortality seen in the LAL^{-/-} rats is directly associated to the LAL deletion. This model of LAL-deficiency demonstrates increased mortality, which is very similar to the presentation of the disease in infants with LAL Deficiency/Wolman phenotype. The following figure (from page 3 of the report) and table (from page 3 of the report) shows the survival curve and mortality data, respectively.

Figure 1. Survival Curve (Kaplan-Meier plot) of LAL ^{-/-} and LAL ^{+/+} Rats

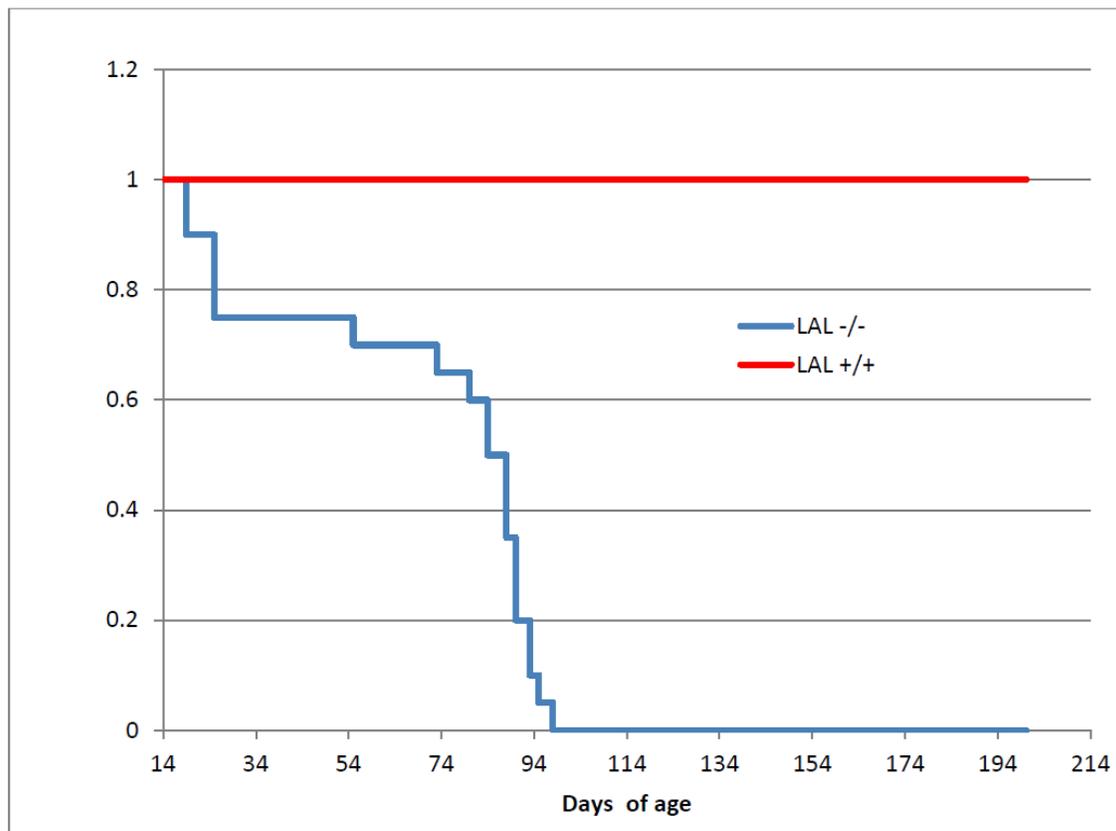


Table 1: Individual Animal Survival Data

(b) (4)

Pharmacodynamic Activity of Two Treatment Regimens of Sebelipase Alfa in the Rat Model of LAL Deficiency (SBC-102-P004)

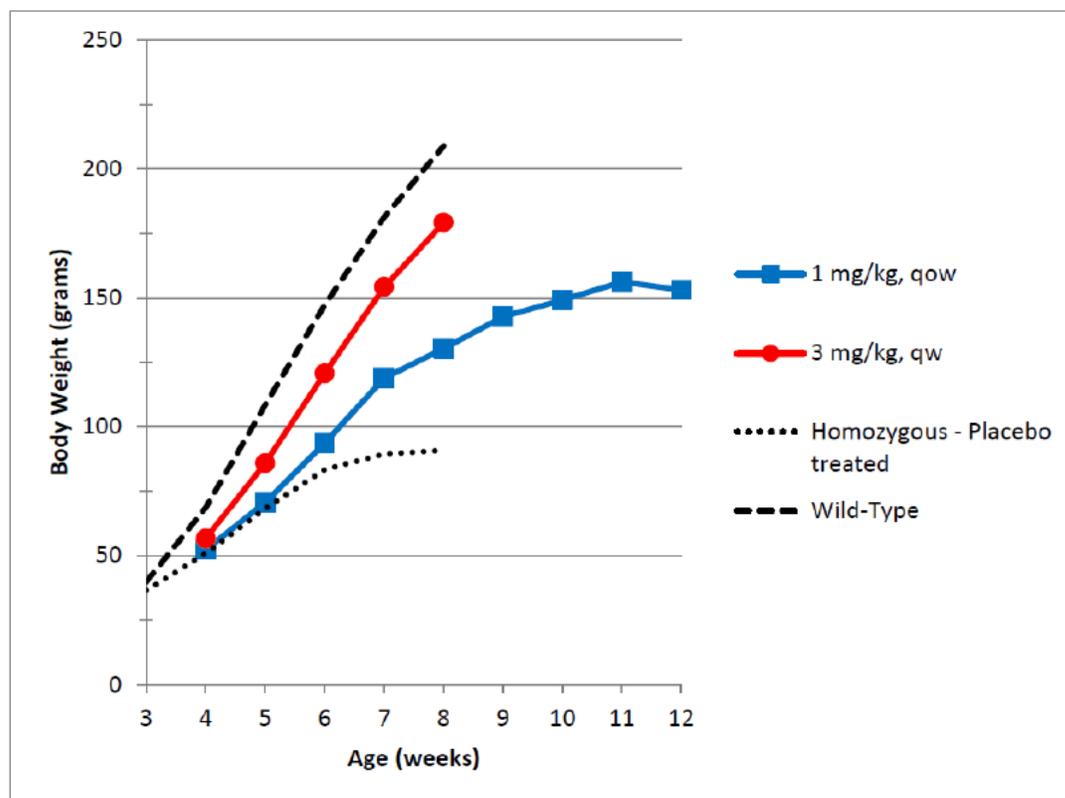
Methods: Previous studies in a rat model of LAL deficiency demonstrated that administration of sebelipase alfa (SBC-102) once weekly (QW) or every other week (QOW) at 4 weeks of age leads to dose related improvements in the phenotypic abnormalities of LAL deficiency.

In the present study, sebelipase alfa was administered by bolus IV injection to LAL-deficient rats ("Yoshida" rats) to examine the pharmacologic effects in this model. "Yoshida" rats are Donryu rats that are LAL deficient due to a spontaneous mutation in the LAL gene (Yoshida H and M Kuriyama, 1990, Genetic Lipid Storage Disease with Lysosomal Acid Lipase Deficiency in Rats, Lab Anim Sci., 40:486-9). Rats (4 weeks of age, n = 3/sex) were administered with sebelipase alfa at 1 mg/kg, QOW, and 3 mg/kg, QW, for 8 and 4 weeks, respectively. Normal saline was used as the vehicle. The 1 mg/kg, QOW, dosing regimen was used in this study to assess temporal effects of prolonged dosing. The 3 mg/kg, QW, dosing regimen was used to complete the dose-response evaluation of sebelipase alfa in the LAL-deficient rat, as this dosage was not previously tested in the LAL deficient rat model.

Results: When administered at 1 mg/kg (QOW for 8 weeks) and 3 mg/kg (QW for 4 weeks), sebelipase alfa demonstrated positive effects on growth (body weight and body weight gain), decreases in organomegaly, reductions in lipid accumulation in the liver, normalization of both the serum lipid profile and serum transaminases, and a restoration of normal hepatic architecture.

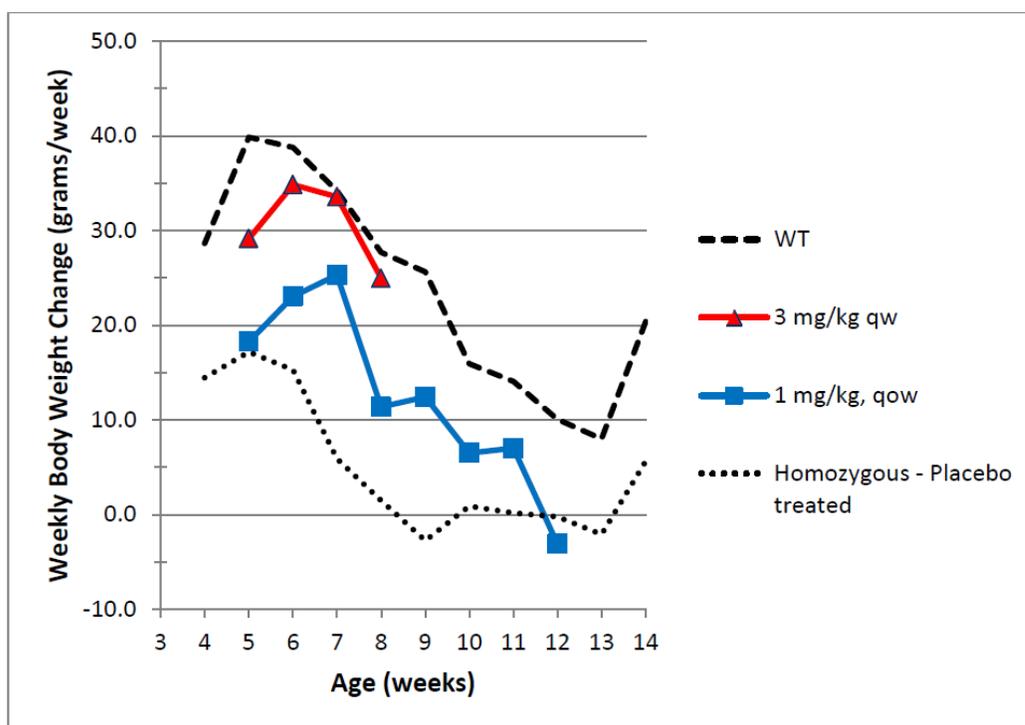
Body weight: Body weights increased in all rats in both dose groups throughout the dosing period until the terminal sacrifice. Growth velocity [body weight change (in grams) per week] at 3 mg/kg, QW dose approached that of wild-type (WT) rats (historical database) and greater than that of at 1 mg/kg, QOW. Growth velocity at 1 mg/kg, QOW was greater than that observed in the homozygous LAL-deficient rats (historical data from SBC102-P002). The following figures (from pages 12 and 13 of the report, respectively) show the body weight and body weight changes following treatment with sebelipase alfa.

Figure 1. Mean body weights of LAL-deficient rats following treatment with sebelipase alfa at 1 mg/kg, qow, and 3 mg/kg, qw.



Wild-type (historical data) and vehicle-treated homozygous LAL-deficient rat data (SBC102-P002) are included. Dosing occurred at weeks 4, 5, 6, and 7 for the 3 mg/kg, qw, group; and at weeks 4, 6, 8, and 10 for the 1 mg/kg, qow, group.

Figure 2. Mean weekly body weight change of LAL-deficient rats following treatment with sebelipase alfa at 1 mg/kg, qow and 3 mg/kg, qw.

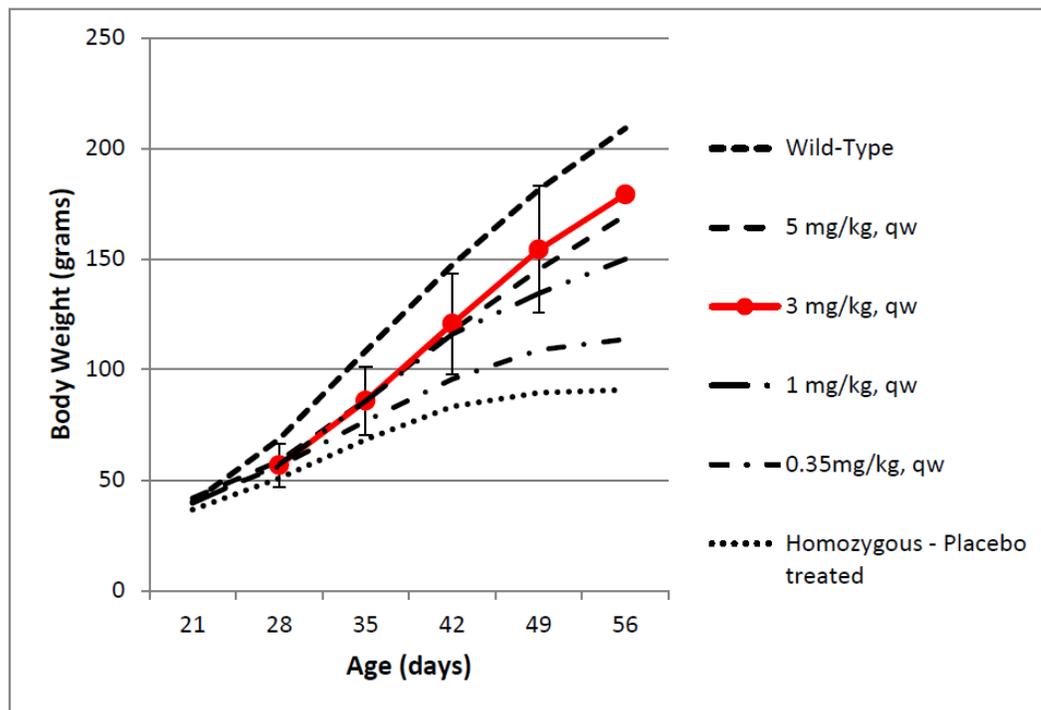


Wild-type (WT) (historical data) and vehicle-treated homozygous LAL-deficient rat data (SBC102-P002) are included.

Dosing occurred at weeks 4, 5, 6, and 7 for the 3 mg/kg, qw, group; and at weeks 4, 6, 8, and 10 for the 1 mg/kg, qow, group.

Mean body weights at 3 mg/kg, QW dose demonstrated a maximal effect of sebelipase alfa with once per week dosing. Mean body weights of this dose group did not differ from the previously reported data from LAL-deficient rats administered sebelipase alfa at 5 mg/kg QW, through 56 days of age as shown in the figure below (from page 14 of the report).

Figure 3. Mean body weights of LAL-deficient rats following treatment with sebelipase alfa at 3 mg/kg, qw, compared to the historical data (SBC102-P002).



Wild-type (historical data); 5, 1, and 0.35 mg/kg sebelipase alfa-treated, and vehicle-treated homozygous LAL-deficient rat data (SBC102-P002) are included.

Clinical Pathology: Serum chemistry parameters were measured at approximately 12 weeks of age (1 mg/kg, QOW) or 8 weeks of age (3 mg/kg, QW), approximately 2 weeks or 1 week after the last dose, respectively. At 1 and 3 mg/kg, lower serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were observed at the terminal sacrifice when compared to untreated LAL-deficient rats at 12-13 weeks of age. Serum ALT levels in both dosage groups were comparable to those of the WT (wild-type) rat at 12-13 weeks of age. However, AST levels at 1 mg/kg, QOW was lower than the untreated LAL-deficient rats (12-13 weeks of age), but remained elevated relative to the WT rats. The following table (from page 15 of the report) and figure (from page 16 of the report) show the effects of sebelipase alfa on liver enzymes.

Table 2. Effects of sebelipase alfa on serum transaminases following administration at 1 mg/kg, qow, and 3 mg/kg, qw, in LAL-deficient rats.

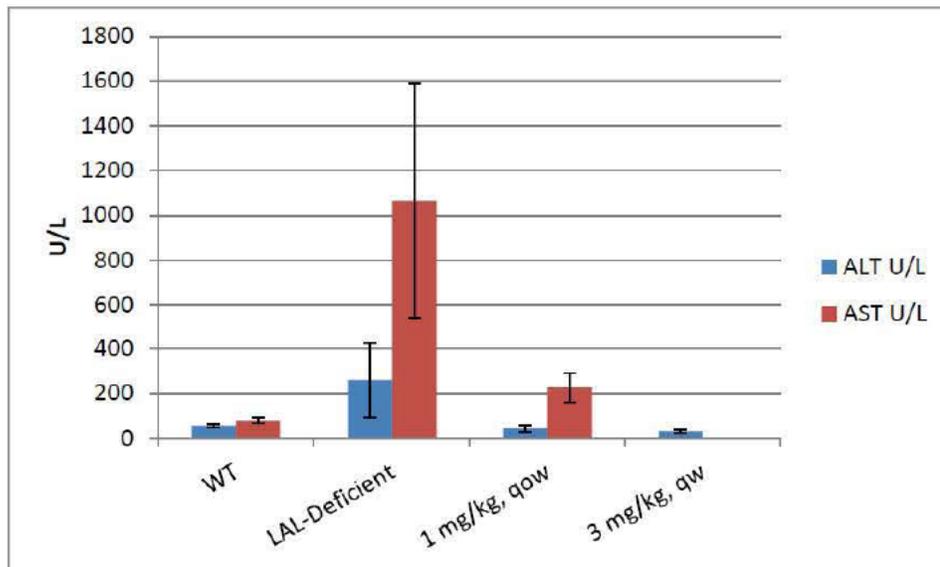
Treatment	Assay	units	Male		Female		M & F	
			Mean	SD	Mean	SD	Mean	SD
1 mg/kg, qow for 8 weeks*	ALT	U/L	42.3	13.1	47.0	19.1	44.7	14.8
	AST	U/L	182.7	34.8	270.0	57.3	226.3	63.9
	N		3		3		6	
3 mg/kg, qw for 4 weeks*	ALT	U/L	35.3	1.2	28.7	6.8	32.0	5.7
	AST	U/L	n/a	n/a	n/a	n/a	n/a	n/a
	N		3		3		6	
WT**	ALT	U/L	59.5	-	52	-	56	9
	AST	U/L	80	-	82.5	-	81	11
	N		2		2		4	
LAL-Deficient**	ALT	U/L	265.5	-	245	-	259	167
	AST	U/L	938	-	1321	-	1066	522
	N		2		1		3	

n/a: not available

*Dosing was initiated at approximately 4 weeks of age.

**Clinical serum transaminase data in untreated wild-type (WT) Donryu rats and untreated LAL-deficient rats were obtained at 12-13 weeks of age under study protocol [SBC102-P006](#) (WT: n=2 males, n=2 females; LAL-deficient: n=2 males; n=1 female).

Figure 4. Effects of sebelipase alfa on serum transaminases following administration at 1 mg/kg, qow, and 3 mg/kg, qw, in LAL-deficient rats.



Serum lipid parameters are shown in the following table (from page 17 of the report) and figure (from page 18 of the report). Treatment with sebelipase alfa resulted in lower serum cholesterol, triglyceride, and low density lipoprotein (LDL) levels, while the serum high density lipoprotein (HDL) levels were increased, compared to untreated LAL-deficient rats at 12-13 weeks of age.

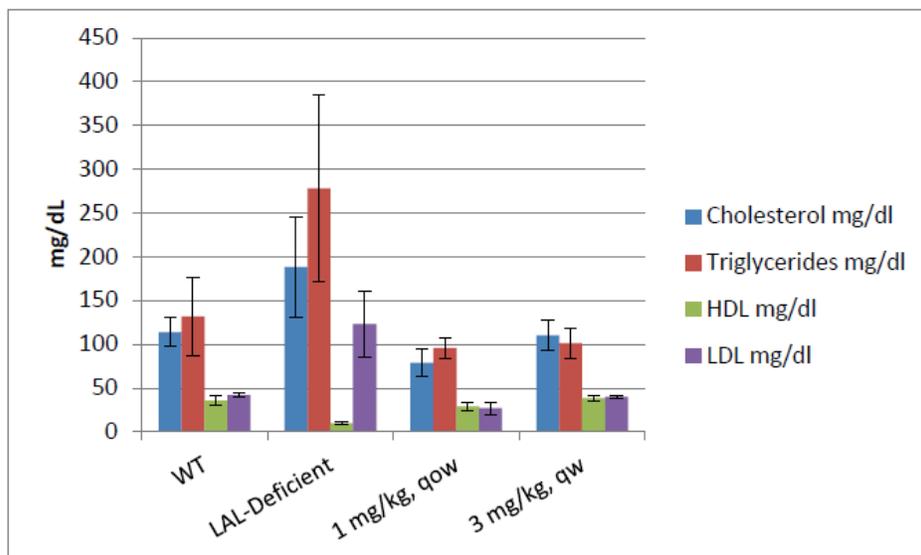
Table 3. Effects of sebelipase alfa on serum lipid parameters following administration at 1 mg/kg, qow, and 3 mg/kg, qw, in LAL-deficient rats.

Treatment	Assay	units	Males		Females		M & F	
			Mean	SD	Mean	SD	Mean	SD
1 mg/kg, qow for 8 weeks*	Cholesterol	mg/dL	79.7	17.7	78.0	18.2	78.8	16.1
	Triglycerides	mg/dL	96.0	11.0	95.3	15.0	95.7	11.8
	HDL	mg/dL	30.7	5.7	27.3	4.2	29.0	4.8
	LDL	mg/dL	23.7	1.2	30.7	9.0	27.2	6.9
	N		3		3		6	
3 mg/kg, qw for 4 weeks*	Cholesterol	mg/dL	99.0	17.7	121.3	9.1	110.2	17.5
	Triglycerides	mg/dL	110.7	20.8	92.0	5.3	101.3	17.0
	HDL	mg/dL	40.3	2.9	36.3	3.5	38.3	3.6
	LDL	mg/dL	40.0	1.7	40.0	2.6	40.0	2.0
	N		3		3		6	
WT**	Cholesterol	mg/dL	100.5	-	127.5	-	114	16
	Triglycerides	mg/dL	142.5	-	120.5	-	132	45
	HDL	mg/dL	31.5	-	39.5	-	36	5
	LDL	mg/dL	39.5	-	43.5	-	42	3
	N		2		2		4	
LAL-Deficient**	Cholesterol	mg/dL	166.5	-	232	-	188	58
	Triglycerides	mg/dL	250	-	333	-	278	106
	HDL	mg/dL	10.5	-	10	-	10	2
	LDL	mg/dL	108.5	-	153	-	123	38
	N		2		1		3	

*Dosing was initiated at approximately 4 weeks of age.

**Clinical serum lipid data in untreated wild-type (WT) Donryu rats and untreated LAL-deficient rats were obtained at 12-13 weeks of age under study protocol SBC102-P006 (WT: n=2 males, n=2 females; LAL-deficient: n=2 males; n=1 females)

Figure 5. Effects of sebelipase alfa on serum lipid parameters following administration at 1 mg/kg, qow, and 3 mg/kg, qw, in LAL-deficient rats.



Organ Weights: Relative (as percentage of body weight) liver, spleen, duodenum, mesenteric lymph node, jejunum, ileum, and brain weights were decreased in the sebelipase alfa treated rats compared to vehicle-treated rats. Maximum reduction was seen in the liver as shown in the following table (from page 19 of the report). These decreases in relative organ weights indicated a remediation of the disease state; however, other than the brain, relative organ weights were slightly higher than the average relative organ weights of the WT Donryu rat.

Table 4. Relative organ weights (% body weight) following administration of sebelipase alfa at 1 mg/kg, qow, and 3 mg/kg, qw, in LAL-deficient rats.

Treatment		Body weight (g)	Liver	Spleen	Mesenteric LN	Duodenum	Jejunum	Ileum	Brain
1 mg/kg, qow for 8 weeks*	Mean	169.3	6.7%	0.4%	0.7%	0.4%	3.6%	2.5%	0.8%
	SD	51.3	1.4%	0.1%	0.2%	0.2%	0.8%	0.8%	0.2%
	N	6	6	6	6	6	6	6	6
3 mg/kg, qw for 4 weeks*	Mean	179.2	5.7%	0.4%	0.5%	0.5%	2.8%	2.0%	-
	SD	28.7	0.3%	0.1%	0.1%	0.1%	0.8%	0.4%	-
	N	6	6	6	6	6	6	6	6
LAL-Deficient**	Mean	95.0	14.9%	0.9%	0.8%	1.0%	4.4%	3.2%	1.5%
	SD	19.4	4.4%	0.2%	0.2%	0.3%	1.2%	0.7%	0.3%
	N	34	34	18	13	14	14	14	10
WT**	Mean	226.5	4.6%	0.3%	0.2%	0.3%	1.2%	1.1%	0.7%
	SD	53.4	0.7%	0.1%	0.1%	0.1%	0.4%	0.3%	0.1%
	N	25	25	25	15	18	17	17	8

*Dosing was initiated at approximately 4 weeks of age.

**Vehicle-treated LAL-deficient rat data and wild type (WT) rat data extracted from historical data base, including animals up to 12.5 weeks of age.

Substrate Concentrations: Total cholesterol, free cholesterol, cholesteryl ester, triglyceride (liver tissue) were measured in the liver and small intestine (cholesteryl ester was determined as the difference between total cholesterol and free cholesterol). Treatment with sebelipase alfa decreased hepatic and jejunal cholesterol content (total, free and esterified) including liver triglyceride when compared to the vehicle at 1 and 3 mg/kg. The results are shown (from page 20-22 of the report) in the following tables and figures.

Table 5. Hepatic cholesterol content in sebelipase alfa-treated LAL-deficient rats.

Treatment	Gender	N		Free Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Total Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Cholesteryl Ester ($\mu\text{g}/\text{mg}$ tissue)
1 mg/kg, qow for 8 weeks*	M & F	6	Mean	3.03	7.44	4.42
			SD	0.74	1.70	1.28
3 mg/kg, qw for 4 weeks*	M & F	6	Mean	2.13	4.92	2.79
			SD	0.49	0.77	0.45
WT [†]	M & F	2	Mean	1.89	2.61	0.72
			SD	-	-	-
LAL-Deficient [†]	M & F	2	Mean	6.78	20.95	14.18
			SD	-	-	-

- = n < 3; SD not calculated.

*Dosing was initiated at approximately 4 weeks of age.

[†]Wild-type (WT) and LAL-deficient liver tissue samples measured at 8 weeks of age as assay controls in parallel with tissue samples from animals treated in this study.

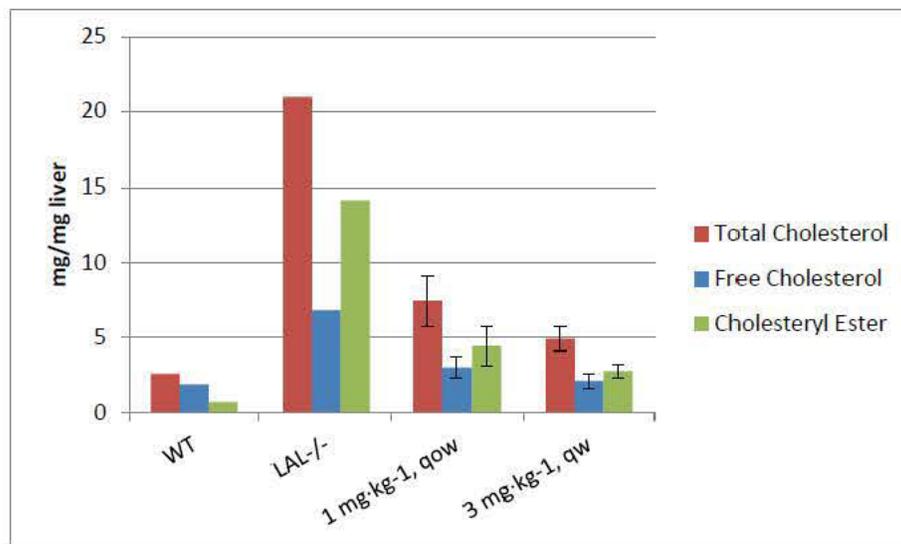
Figure 6. Hepatic cholesterol content in sebelipase alfa-treated LAL-deficient rats.

Table 6. Jejunal cholesterol content in sebelipase alfa-treated LAL-deficient rats.

Treatment	Gender	N	Free Cholesterol			Total Cholesterol		Cholesterol Ester	
				($\mu\text{g}/\text{mg}$ tissue)					
1 mg/kg, qow for 8 weeks*	M & F	6	Mean	2.64	4.56	1.92			
			SD	0.41	1.11	1.00			
3 mg/kg, qw for 4 weeks*	M & F	6	Mean	1.97	2.40	0.42			
			SD	0.48	0.54	0.42			
WT [†]	M & F	2	Mean	2.35	2.40	0.05			
			SD	-	-	-			
LAL-deficient [†]	M & F	2	Mean	2.07	7.17	5.10			
			SD	-	-	-			

*Dosing was initiated at approximately 4 weeks of age.

[†]Wild-type (WT) and LAL-deficient tissue samples measured at 8 weeks of age as assay controls in parallel with tissue samples from animals treated in this study.

Table 7. Liver triglyceride content of sebelipase alfa-treated LAL-deficient rats.

Treatment	Gender	N	Liver	
				(nmol/mg tissue)
1 mg/kg, qow for 8 weeks	M & F	6	Mean	23.34
			SD	7.14
3 mg/kg, qw for 4 weeks	M & F	6	Mean	14.57
			SD	2.65
WT*	M & F	5	Mean	10.73
			SD	1.68
LAL-deficient*	M & F	5	Mean	21.63
			SD	4.01
WT [†]		1		16.97
LAL-deficient [†]		1		107

n/a: Not available.

*Hepatic triglyceride levels in wild type (WT) Donryu rats and LAL-deficient rats were obtained under study protocol [SBC102-P002](#) (WT: n=2 males, n=3 females; LAL-deficient: n=2 males; n=3 females).

[†]Wild-type (WT) and LAL-deficient liver tissue samples measured at 8 weeks of age as assay controls in parallel with tissue samples from animals treated in this study.

Histopathology: Histopathological abnormalities in the liver were minimal (rare or occasional minute to small foci of lipid-laden macrophages) in the treated animals (3 mg/kg, QW) and there was no excess lipid accumulation in the cytoplasm of hepatocytes. The small intestine exhibited less severe histologic alterations (expansion of the lamina propria due to accumulation of lipid-laden macrophages) in the treated (3 mg/kg, QW) animals when compared to vehicle-treated homozygous control animals. These effects were observed at 4 and 8 weeks postdose.

Overall, in this rat model of LAL deficiency, sebelipase alfa demonstrated increased growth, decreased organomegaly, caused reductions in lipid accumulation in the liver, normalized both the serum lipid profile and serum transaminases, and minimal histopathological abnormalities in the liver and small intestine.

Efficacy of a Delayed Treatment Regimen with Sebelipase Alfa in a Rat Model of LAL Deficiency (SBC-102-P005)

Methods: Previous studies in LAL-deficient rats demonstrated that administration of sebelipase alfa once weekly (QW) or every other week (QOW) at 4 weeks of age caused dose related improvements in the phenotypic abnormalities of LAL deficiency.

In this study, the effectiveness of sebelipase alfa was examined in LAL-deficient rats at 8 weeks of age. The initiation of treatment was delayed until the rats were 8 weeks of age, at which time there was a much greater burden of disease compared to rats of 4 weeks of age. Prior to sebelipase alfa administration, rats were injected intraperitoneally with diphenhydramine at 5 mg/kg. Approximately 20 minutes after diphenhydramine pretreatment, rats (n = 3/sex/group) were administered sebelipase alfa or vehicle (0.9% NaCl) intravenously at 3 mg/kg, QW, for 19 weeks, beginning at 8 weeks of age, with final dose at 26 weeks of age.

Results: Sebelipase alfa treated rats showed long-term survival (scheduled sacrifice at 27 weeks of age) compared to vehicle-treated rats, which were sacrificed moribund at 9-12 weeks of age. Sebelipase alfa increased growth (body weight and body weight gain), decreased organomegaly, decreased serum AST and ALT (females), and caused reductions in lipid accumulation in the liver. Histologically, the appearance of the liver in the sebelipase alfa treated rats resembled that of the wild type (WT) rats. The following (from pages 15, 17, 18, 19, 20, 21 of the report) tables and figures show the results.

Figure 2. Mean weekly body weights of LAL-deficient rats following treatment with sebelipase alfa at 3 mg/kg, qw.

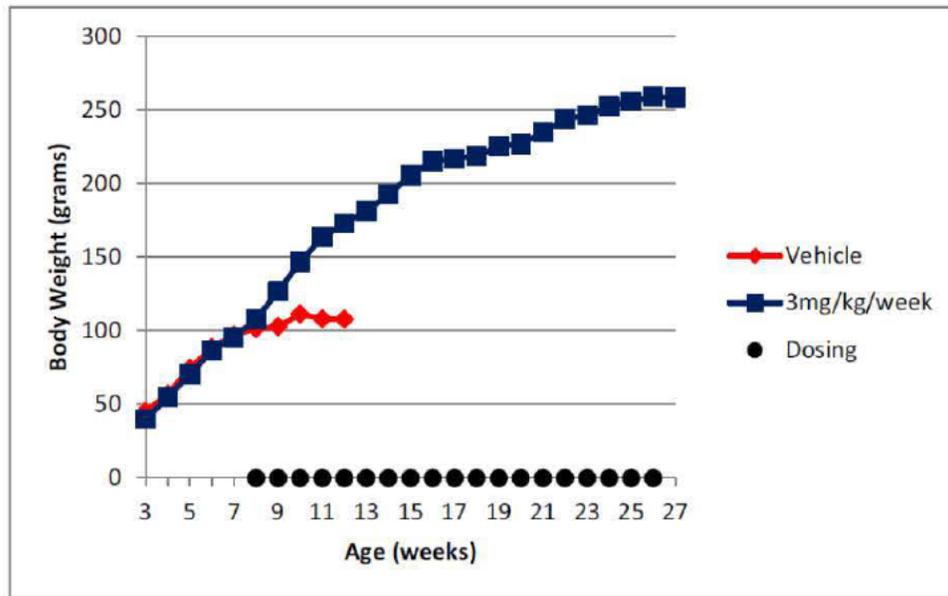


Table 3. Transaminases and alkaline phosphatase in 9 to 12-week-old vehicle-treated LAL-deficient rats.

Assay	units	Males		Females		M & F	
		Mean	SD	Mean	SD	Mean	SD
ALT	U/L	52	15	59	18	56	15
AST	U/L	287	49	359	39	323	56
ALP	U/L	153	42	184	26	169	36

Table 4. Transaminases and alkaline phosphatase in 27-week-old LAL-deficient rats following sebelipase alfa administered at 3 mg/kg, qw, for 19 weeks.

Assay	units	Males		Females		M & F	
		Mean	SD	Mean	SD	Mean	SD
ALT	U/L	53	5.0	29	8.4	41	15
AST	U/L	91	17	100	36	96	25
ALP	U/L	126	21	63	18	95	38

Table 6 Serum lipid parameters in 9 to 12-week-old vehicle-treated LAL-deficient rats.

		Males		Females		M & F	
N		3	3	6	N	3	
Assay	units	Mean	SD	Mean	SD	Mean	SD
Cholesterol	mg/dL	95.67	16.86	122.67	8.96	109.17	19.09
Triglycerides	mg/dL	80.33	12.42	126.33	25.58	103.33	30.96
HDL	mg/dL	14.33	1.53	15.33	1.15	14.83	1.33
LDL	mg/dL	20.33	4.51	27.00	5.29	23.67	5.72

Table 7. Serum lipid parameters in 27-week-old LAL-deficient rats following sebelipase alfa administered at 3 mg/kg, qw, for 19 weeks.

		Males		Females		M & F	
N		3	3	3	3	6	
Assay	units	Mean	SD	Mean	SD	Mean	SD
Cholesterol	mg/dL	74	1.7	67	54	71	34
Triglycerides	mg/dL	77	25	93	44	85	33
HDL	mg/dL	28	2.0	27	4.4	28	3
LDL	mg/dL	13	1.5	<7	-	-	-

Table 9. Relative organ weights (% body weight) of sebelipase alfa-treated LAL-deficient rats

Organ Weight as Percentage (%) Body Weight											
Dose	Gender	N	Body weight (g)	Mesenteric							
				Liver	Spleen	LN	Duodenum	Jejunum	Ileum	Brain	
Vehicle	M	3	Mean	126.27	14.60	1.12	0.81	1.09	3.94	3.76	1.15
			SD	8.94	2.01	0.14	0.04	0.11	0.32	0.92	0.04
	F	3	Mean	76.59	16.58	0.95	0.90	1.31	5.29	3.74	1.68
			SD	10.02	1.66	0.10	0.13	0.30	0.30	0.60	0.13
3 mg/kg qw	M	3	Mean	290.51	4.20	0.26	0.29	0.38	1.11	0.91	0.58
			SD	7.57	0.31	0.01	0.05	0.10	0.06	0.07	0.02
	F	3	Mean	226.25	4.97	0.38	0.41	0.57	1.87	1.35	0.71
			SD	11.36	0.68	0.04	0.10	0.17	0.46	0.43	0.04

Vehicle-treated LAL-deficient rats were sacrificed moribund at 9-12 weeks of age; sebelipase alfa-treated rats were sacrificed at 27 weeks of age.

Table 10. Hepatic cholesterol content in sebelipase alfa-treated LAL-deficient rats.

Treatment	Gender	N		Total Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Free Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Cholesterol Ester ($\mu\text{g}/\text{mg}$ tissue)
Vehicle	M	3	Mean	12.39	5.04	7.35
			SD	1.94	0.80	1.15
	F	3	Mean	11.86	4.87	6.99
			SD	2.26	0.83	1.45
	M & F	6	Mean	12.13	4.95	7.17
			SD	1.90	0.74	1.18
3 mg/kg, qw	M	3	Mean	3.20	1.62	1.59
			SD	0.14	0.32	0.29
	F	3	Mean	5.68	2.46	3.22
			SD	0.89	0.28	0.91
	M & F	6	Mean	4.44	2.04	2.41
			SD	1.47	0.53	1.08

Vehicle-treated LAL-deficient rats were sacrificed moribund at 9-12 weeks of age; sebelipase alfa-treated rats were sacrificed at 27 weeks of age.

Table 11. Jejunal cholesterol content in sebelipase alfa-treated LAL-deficient rats.

Treatment	Gender	N		Total Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Free Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Cholesterol Ester ($\mu\text{g}/\text{mg}$ tissue)
Vehicle	M	3	Mean	7.61	3.43	4.19
			SD	1.99	0.56	1.45
	F	3	Mean	8.83	4.02	4.81
			SD	1.98	0.59	1.40
	M & F	6	Mean	8.22	3.73	4.50
			SD	1.90	0.61	1.32
3 mg/kg, qw	M	3	Mean	4.55	2.90	1.66
			SD	2.55	1.19	1.37
	F	3	Mean	5.00	2.96	2.04
			SD	2.33	0.78	1.58
	M & F	6	Mean	4.78	2.93	1.85
			SD	2.20	0.90	1.34

Vehicle-treated LAL-deficient rats were sacrificed moribund at 9-12 weeks of age; sebelipase alfa-treated rats were sacrificed at 27 weeks of age.

Efficacy of a Once Monthly Treatment Regimen with Sebelipase Alfa in the LAL-Deficient Rat Model (SBC-102-P011)

Methods: In this study, sebelipase alfa was administered by bolus IV injection to LAL-deficient rats (n = 3/sex) at 3 mg/kg, once weekly (QW) for 4 doses, from 4 weeks of age until 7 weeks of age, followed by once monthly treatment beginning at 8 weeks of age. Sebelipase alfa treatment was continued through 27 weeks of age. Prior to sebelipase alfa administration, rats were treated intraperitoneally with diphenhydramine at 5 mg/kg.

Results: Sebelipase alfa showed positive effects in growth (body weight and body weight gain); however, positive growth velocity was not sustained throughout the course

of the treatment period, with marked fluctuation particularly after 13 weeks of age. Organomegaly and lipid accumulation in the liver were reduced, along with a normalization of both the serum lipid profile and serum transaminases. Histopathological changes in the liver (scattered, small foci of lipid-laden macrophages in the parenchyma, hepatocellular lipidosis) and jejunum (marked expansion of the lamina propria due to sheets of foamy macrophages) were observed at the completion of the treatment period. Thus, once monthly treatment did not appear to fully restore the normal hepatic architecture. The following tables (from pages 12, 15-18 of the report) show the results.

Figure 1. Mean body weights of LAL-deficient rats following treatment with sebelipase alfa at 3 mg/kg, qw, for 4 weeks and once monthly until 27 weeks of age.

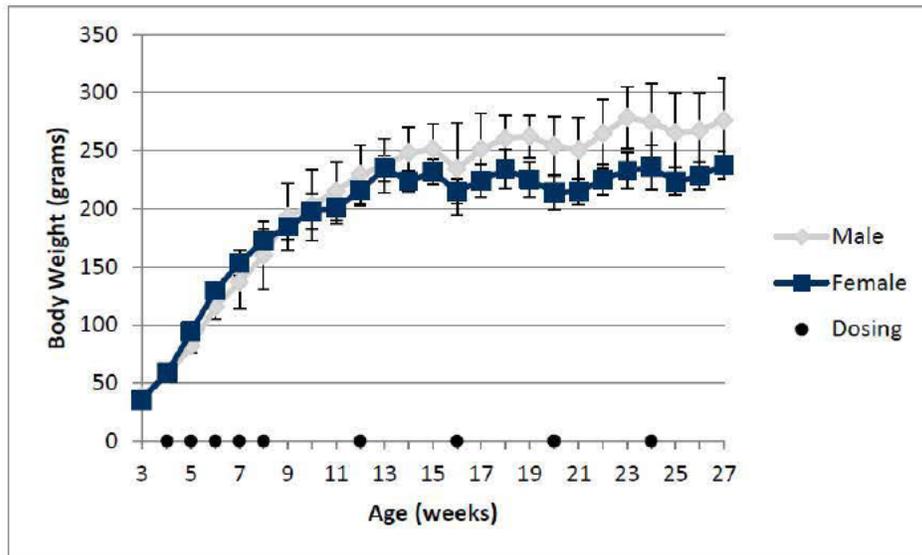


Figure 2. Mean body weights of LAL-deficient rats following treatment with sebelipase alfa at 3 mg/kg, qw, until 27 weeks of age (from study SBC102-P005).

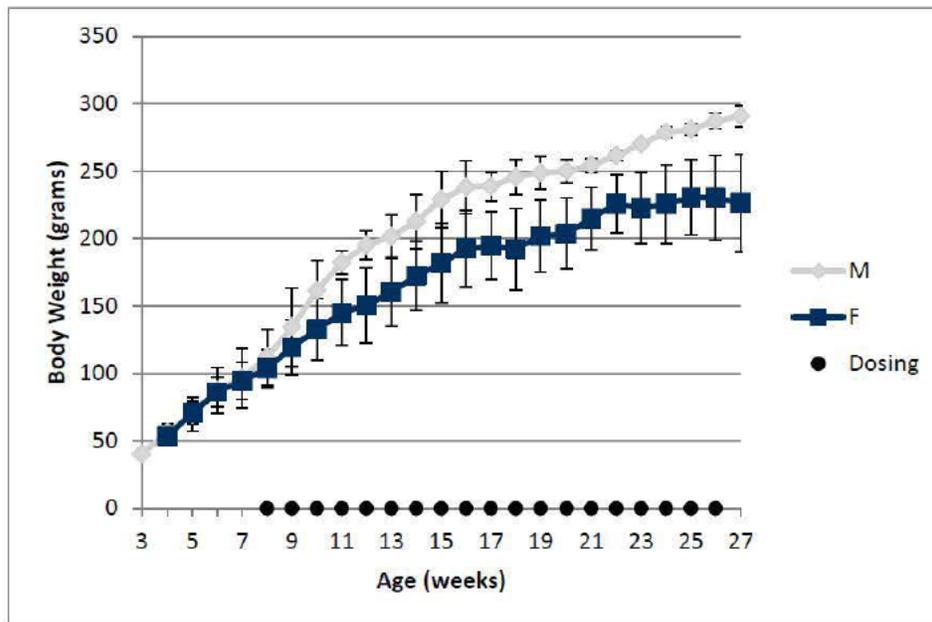


Table 2. Effects of sebelipase alfa on serum transaminases following administration at 3 mg/kg, qw, for 4 weeks and once monthly until 27 weeks of age.

Treatment	Assay	units	Male		Female		M & F	
			Mean	SD	Mean	SD	Mean	SD
3 mg/kg once monthly*	ALT	U/L	128	79	66	33	97	64
	AST	U/L	223	113	182	119	203	106
	N		3		3		6	
WT**	ALT	U/L	59.5	-	52	-	56	9
	AST	U/L	80	-	82.6	-	81	11
	N		2		2		4	
LAL-Deficient**	ALT	U/L	265.5	-	245	-	259	167
	AST	U/L	938	-	1321	-	1066	522
	N		2		1		3	

n/a: not available

*Last monthly dose was at 24 weeks of age, and sacrifice was at 27 weeks of age.

Clinical serum transaminase data in wild type (WT) Donryu rats and untreated LAL-deficient rats were obtained under study protocol [SBC102-P006](#) (WT: n=2 males, n=2 females; LAL-deficient: n=2 males; n=1 female) at terminal sacrifice at 91 days (13 weeks).Table 3. Effects of sebelipase alfa on serum lipid parameters following administration at 3 mg/kg, qw, for 4 weeks then once monthly until 27 weeks of age.**

Treatment	Assay	units	Males		Females		M & F	
			Mean	SD	Mean	SD	Mean	SD
3 mg/kg once monthly*	Cholesterol	mg/dL	69	4	102	13	86	20
	Triglycerides	mg/dL	69	12	54	11	61	13
	HDL	mg/dL	23	4	31	7	27	7
	LDL	mg/dL	14	3	12	3	13	3
	N		3		3		6	
WT*	Cholesterol	mg/dL	100.5	-	127.5	-	114	16
	Triglycerides	mg/dL	142.5	-	120.5	-	132	45
	HDL	mg/dL	31.5	-	39.5	-	36	5
	LDL	mg/dL	39.5	-	43.5	-	42	3
LAL-Deficient*	Cholesterol	mg/dL	166.5	-	232	-	188	58
	Triglycerides	mg/dL	250	-	333	-	278	106
	HDL	mg/dL	10	-	10	-	10	2
	LDL	mg/dL	108.5	-	153	-	123	38

*Last monthly dose was at 24 weeks of age, and sacrifice was at 27 weeks of age.

**Clinical serum lipid data in wild type (WT) Donryu rats and untreated LAL-deficient rats were obtained under study protocol [SBC102-P006](#) (WT: n=2 males, n=2 females; LAL-deficient: n=2 males; n=1 female) at terminal sacrifice at 91 days (13 weeks).

Table 4. Relative organ weights (% body weight) following administration of sebelipase alfa at 3 mg/kg, qw, for 4 weeks and once monthly until 27 weeks of age.

Treatment		Body weight (g)	Liver	Spleen	Mesenteric LN	Duodenum	Jejunum	Ileum	Brain
3 mg/kg once monthly*	Mean	257.0	6.0%	0.4%	0.5%	0.5%	2.1%	1.5%	0.7%
	SD	31.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	N	6	6	6	6	6	6	6	6
LAL-Deficient**	Mean	95.0	14.9%	0.9%	0.8%	1.0%	4.4%	3.2%	1.5%
	SD	19.4	4.4%	0.2%	0.2%	0.3%	1.2%	0.7%	0.3%
	N	34	34	18	13	14	14	14	10
WT**	Mean	226.5	4.6%	0.3%	0.2%	0.3%	1.2%	1.1%	0.7%
	SD	53.4	0.7%	0.1%	0.1%	0.1%	0.4%	0.3%	0.1%
	N	25	25	25	15	18	17	17	8

*Last monthly dose was at 24 weeks of age, and sacrifice was at 27 weeks of age.

**Vehicle-treated LAL-deficient rat data and wild type (WT) rat data extracted from historical data base, including animals up to 12.5 weeks of age.

Table 5. Hepatic cholesterol content in sebelipase alfa-treated LAL-deficient rats following administration at 3 mg/kg, qw, for 4 weeks and once monthly until 27 weeks of age.

Treatment	Gender	N		Free Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Total Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Cholesteryl Ester ($\mu\text{g}/\text{mg}$ tissue)
3 mg/kg once monthly*	M & F	6	Mean	2.77	7.58	4.80
			SD	0.48	1.88	1.44
WT**	M & F	2	Mean	0.24	2.23	1.99
			SD	-	-	-
LAL-deficient**	M & F	2	Mean	4.57	17.27	12.71
			SD	-	-	-

- = n < 3; SD not calculated.

*Last monthly dose was at 24 weeks of age, and sacrifice was at 27 weeks of age.

** WT and LAL-deficient liver tissue samples measured as assay controls in parallel with tissue samples from animals treated in this study.

Table 6. Jejunal cholesterol content in sebelipase alfa-treated LAL-deficient rats following administration at 3 mg/kg, qw, for 4 weeks and once monthly until 27 weeks of age.

Treatment	Gender	N		Free Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Total Cholesterol ($\mu\text{g}/\text{mg}$ tissue)	Cholesterol Ester ($\mu\text{g}/\text{mg}$ tissue)
3 mg/kg, qw once monthly*	M & F	6	Mean	2.98	5.39	2.41
			SD	0.24	1.12	1.13
WT**	M & F	2	Mean	2.91	2.46	0
			SD	-	-	-
LAL-deficient**	M & F	2	Mean	3.32	7.32	4.00
			SD	-	-	-

*Last monthly dose was at 24 weeks of age, and sacrifice was at 27 weeks of age.

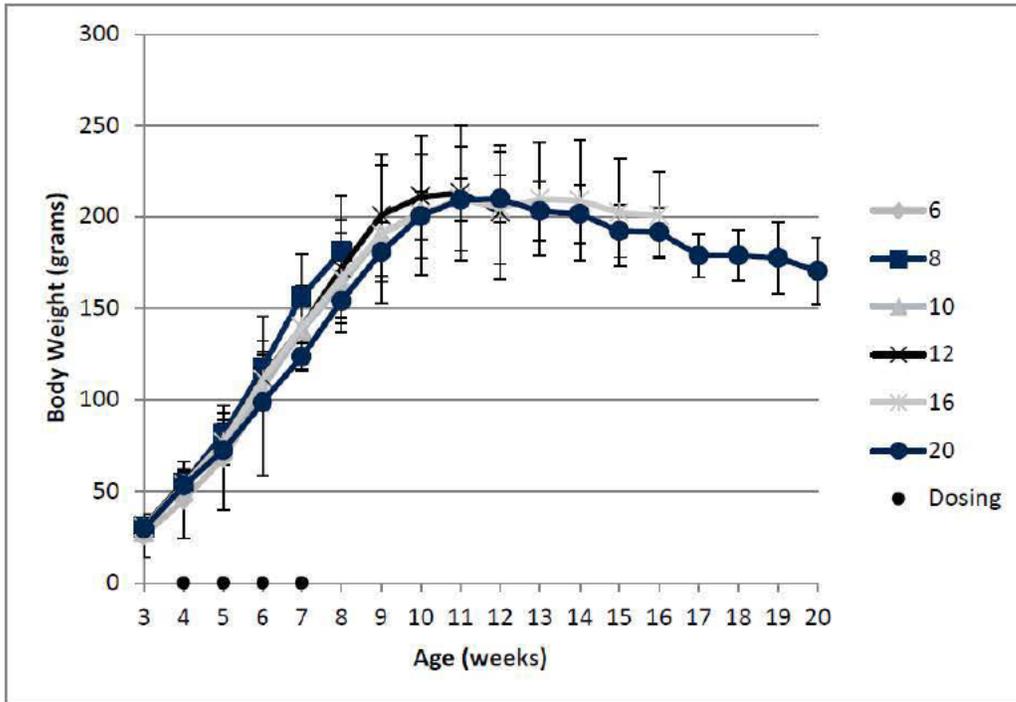
** WT and LAL-deficient liver tissue samples measured as assay controls in parallel with tissue samples from animals treated in this study.

Elimination and Re-Accumulation of Cholesterol and Triglyceride in the Liver of the LAL-Deficient Rat Following Sebelipase Alfa Treatment at 3 mg/kg, Once Weekly, for 4 Weeks (SBC-102-P012)

Methods: In this study, tissue content of cholesterol (total cholesterol, free cholesterol, and cholesterol ester) and triglyceride in the liver was determined during and following treatment with sebelipase alfa in LAL-deficient rats. The objective of this study was to demonstrate the efficacy of substrate reduction and the time course of re-accumulation of the abnormal tissue lipid. Sebelipase alfa was administered at 3 mg/kg once weekly (QW) for 4 weeks starting at 4 weeks of age, and postdose assessments were conducted through 13 weeks postdose.

Results: The results indicated that the benefits of sebelipase alfa may require maintenance of regular dosing. A general decline in the health of these animals was observed by a progressive decrease in growth velocity and subsequent body weight loss beginning 5 weeks following cessation of the treatment. Liver weights increased beginning 3 weeks after the last sebelipase alfa dose, with concomitant increases in hepatic cholesterol, free cholesterol, cholesterol ester, and triglyceride tissue concentrations. Serum triglyceride and cholesterol levels were also increased following cessation of treatment, beginning 5 and 8 weeks after the last dose, respectively. Serum high-density lipoprotein (HDL) progressively decreased beginning 3 weeks after cessation of treatment, while low-density lipoprotein (LDL) increased beginning 5 weeks after treatment cessation. In addition, histopathological lesions in the liver (accumulations of foamy macrophages replacing the parenchyma and microvesicular hepatic lipidosis) following cessation of treatment were similar to untreated LAL-deficient rats. These histopathological changes were accompanied by progressive elevations of serum ALT and AST levels. This reversal of the beneficial effects of sebelipase alfa therapy following cessation of the treatment indicated that continuous enzyme replacement may be necessary to maintain benefit. The following figures (from pages 14, 16, 17, 19, 22, and 23 of the report) show the results.

Figure 1. Mean body weights of LAL-deficient rats following cessation of sebelipase alfa administration, following treatment at 3 mg/kg, qw, for 4 weeks.



Legend indicates the age (in weeks) at which the rats were sacrificed.

Figure 2. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in LAL-deficient rats following cessation of sebelipase alfa administration, after treatment at 3 mg/kg, qw, for 4 weeks.

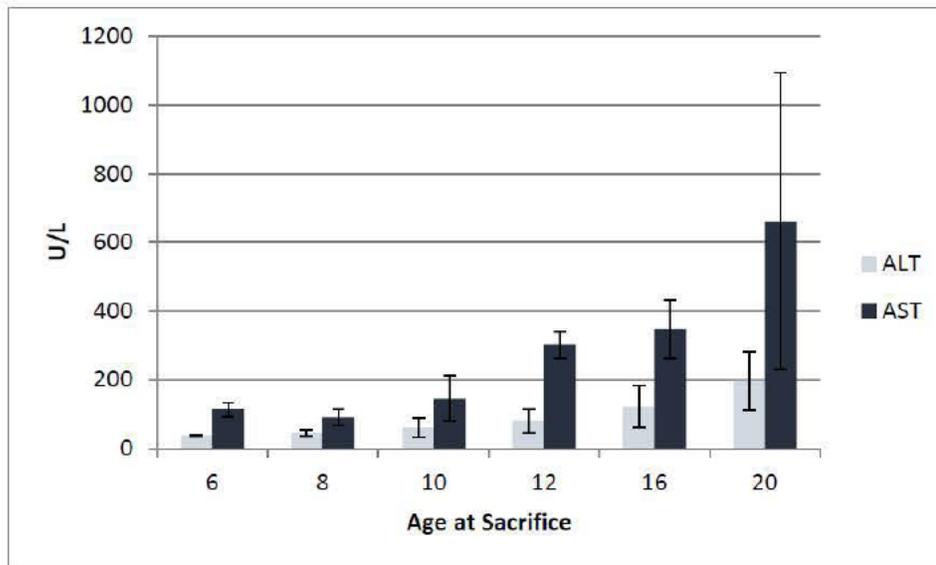


Figure 3. Serum high density lipoprotein (HDL) and low density lipoprotein (LDL) levels in LAL-deficient rats following cessation of sebelipase alfa administration, after treatment at 3 mg/kg, qw, for 4 weeks.

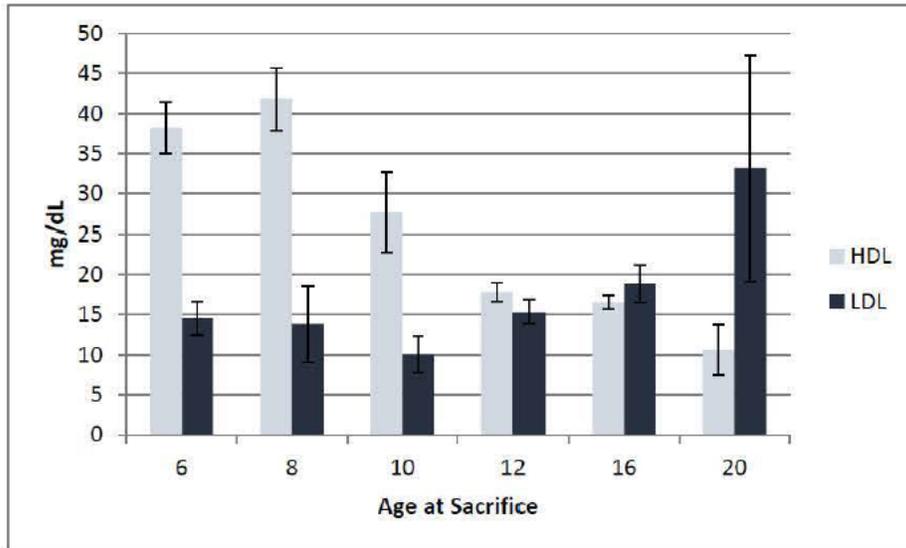


Figure 4. Serum cholesterol and triglyceride levels in LAL-deficient rats following cessation of sebelipase alfa administration, after treatment at 3 mg/kg, qw, for 4 weeks.

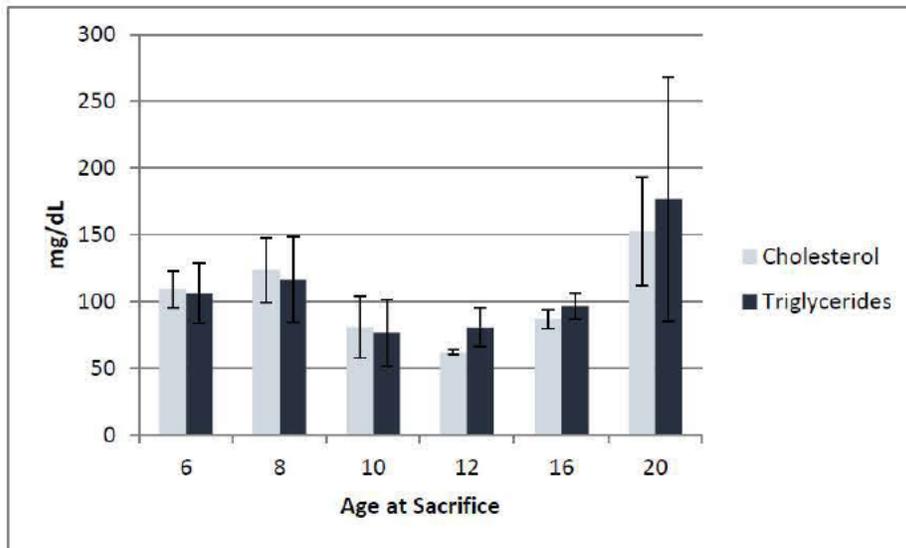


Figure 5. Relative liver weights (grams) of LAL-deficient rats following cessation of sebelipase alfa administration, after treatment at 3 mg/kg, qw, for 4 weeks.

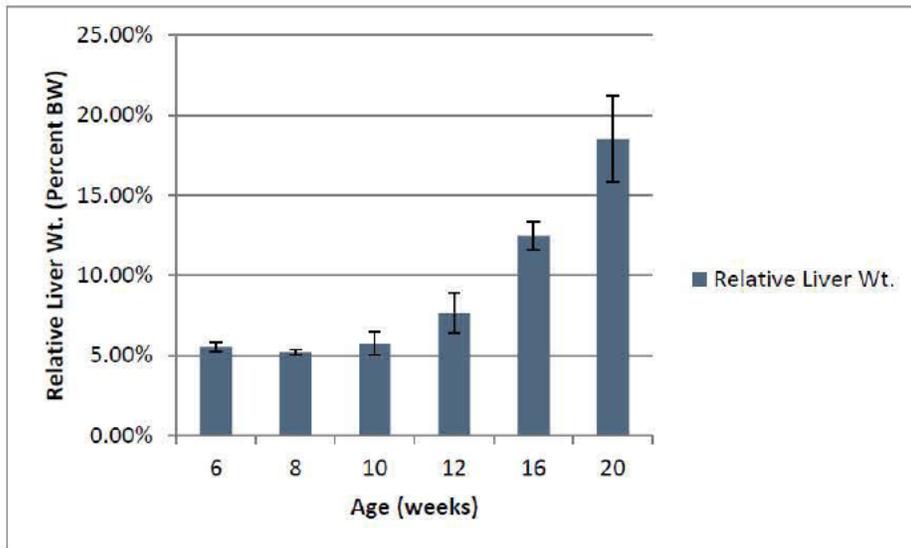


Figure 6. Hepatic cholesterol concentration of LAL-deficient rats following cessation of sebelipase alfa administration, after treatment at 3 mg/kg, qw, for 4 weeks.

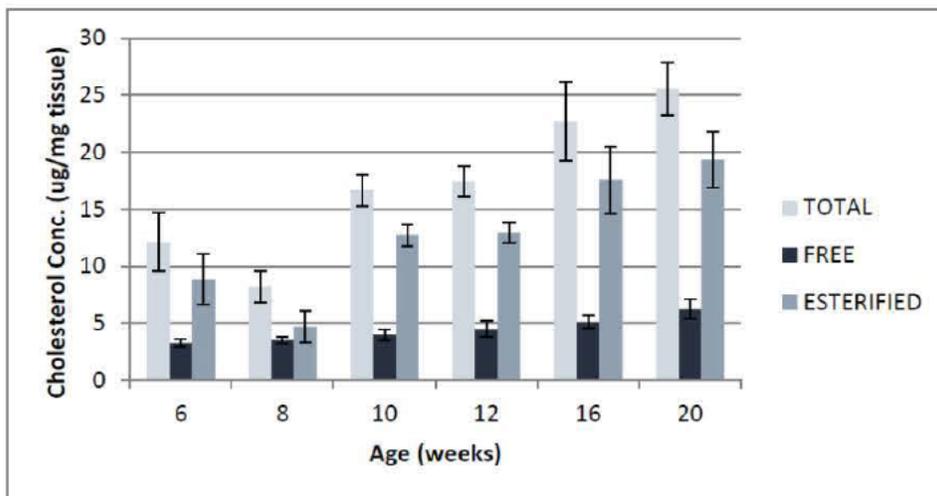
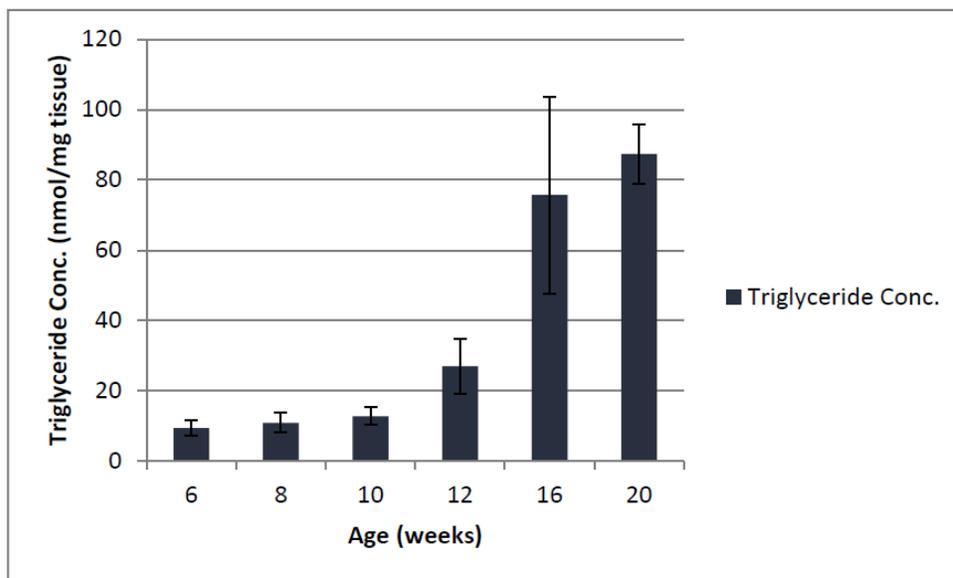


Figure 7. Hepatic triglyceride concentration of LAL-deficient rats following cessation of sebelipase alfa administration, after treatment at 3 mg/kg, qw, for 4 weeks.



Serum Clinical Chemistry Parameters Following Single and Repeated Doses of Sebelipase Alfa in the LAL-Deficient Rat (SBC-102-P013)

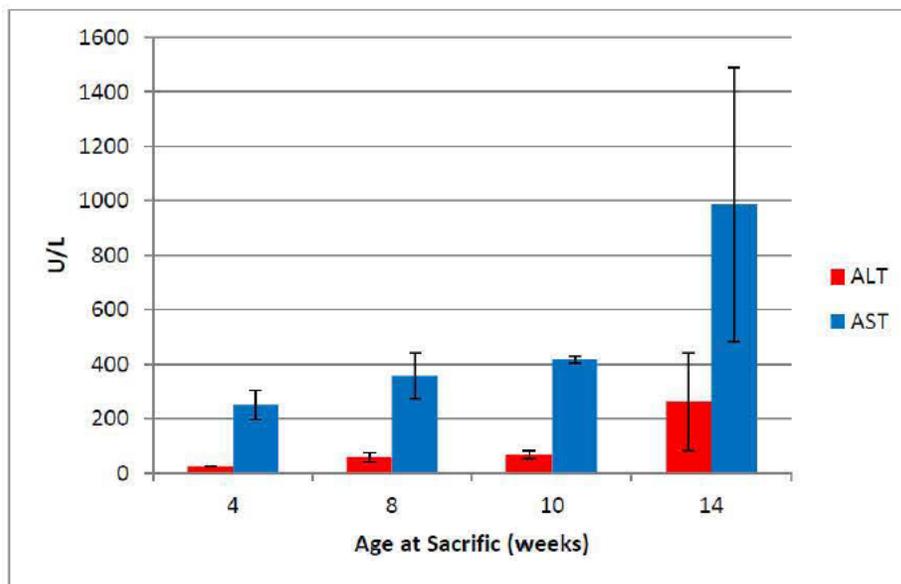
Methods: In this study, routine serum chemistry parameters were measured following (i) a single IV injection, or (ii) 4 repeated IV injections of sebelipase alfa in the LAL-deficient rats (n = 3/sex/group). Single IV injections were administered to male and female rats of 4, 8, and 12 weeks of age. In addition, male and female rats, at 4 weeks of age, received 4 weekly IV injections of sebelipase alfa. Sebelipase alfa was administered at a dose level of 3 mg/kg. Prior to sebelipase alfa administration, rats were injected intraperitoneally with diphenhydramine at 5 mg/kg. Whole blood was collected and serum was processed for clinical chemistry analyses at 24 hours following the single injections or 1 week following the final repeated injections.

Results: A single IV dose of sebelipase alfa at 3 mg/kg showed no apparent effect on serum ALT or AST levels at 24 hours postdose, as ALT and AST levels approximated the levels observed in age-matched, vehicle treated, and LAL-deficient rats. However, ALT and AST levels decreased following 4 weekly IV injections of sebelipase alfa at 3 mg/kg when compared to vehicle treated rats. The following table and figure (from page 15 of the report) show the serum chemistry changes.

Table 5: Serum clinical chemistry parameters in LAL-deficient rats (8 weeks of age) following single injection or 4 weekly intravenous injections of sebelipase alfa at 3 mg/kg.

		LAL-Deficient Rats - Age at Sacrifice (weeks)			
		8		8	
		Single Injection N=6		4 Weekly Injections N=6	
Assay	Units	Mean	SD	Mean	SD
Creatinine	mg/dL	0.23	0.06	0.28	0.08
Total Protein	g/dL	6.30	0.28	5.90	0.39
Albumin	g/dL	3.28	0.17	3.37	0.21
ALT	U/L	69.83	64.40	34.33	13.62
AST	U/L	395.67	252.53	137.83	82.89
Alk Phos	U/L	187.33	28.53	214.00	93.70
Calcium	mg/dL	13.15	1.05	12.65	0.87
Sodium	mmol/L	146.50	0.55	149.33	1.21
Potassium	mmol/L	8.50	0.84	6.68	0.54
Cholesterol	mg/dL	288.17	50.10	105.00	16.35
Triglycerides	mg/dL	74.67	19.91	80.17	20.60
Total Bilirubin	mg/dL	0.10	0.00	0.10	0.00
Urea Nitrogen	mg/dL	27.33	3.39	23.00	3.22
Glucose	mg/dL	257.00	48.43	236.83	64.82
HDL	mg/dL	39.17	6.49	41.17	6.08
LDL	mg/dL	51.50	12.47	17.00	5.62

Figure 1: Serum ALT and AST in vehicle-treated LAL-deficient rats.



4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

The following reviews are incorporated below from the pharmacology review of IND 108640 dated April 17, 2012 by Dr. Emmanuel Akinshola.

Central Nervous System (CNS) Safety Pharmacology Infusion Study with SBC-102 in Sprague-Dawley Rats (Study No. 8232-384)

Methods: SBC-102 at doses of 0 (vehicle control), 0 (diphenhydramine control), 5, 20, or 50 mg/kg were administered by IV infusion to 10 male and 10 female Sprague-Dawley (SD) rats randomly assigned to 5 dose groups. The 3 SBC-102 dose groups all received 5 mg/kg intramuscular (IM) injection of diphenhydramine, 30 minutes before the test article dosing. Animals were dosed weekly for four weeks, and SBC-102 was administered at an IV infusion rate of 10 ml/kg/hr for 5 hours.

The CNS safety study assessment was conducted as part of the 4-week repeat dose toxicity study in rats.

Observational measurements were assessed by home cage, hand-held and open-field observations conducted once before dosing, once post dosing on day 1. Body temperature measurement and complete toxicological evaluation of the animals were conducted.

Results: Clinical observations include dose-dependent, transient swelling of paws and/or nose and red skin of the paws. Observed limited use of the right hind limb was associated with the intramuscular injection of diphenhydramine.

Functional observational battery (home cage, hand-held, or open field) did not show any statistically significant or biologically important differences among the dosage groups in the measures of behavior, autonomic functions, or appearance. No test article-related adverse behaviors were observed in the study animals. The differences in other study parameters such as the greater "mean nociceptive reflex" in the high dose females, and greater mean body temperature in low dose females were not considered related to the administration of SBC-102.

In conclusion, SBC-102 did not produce any changes in the functional observational battery in this study following weekly administration of SBC-102 for 4 weeks at dose levels up to 50 mg/kg.

Respiratory Safety Evaluation using Head-Out Plethysmography of SBC-102 following Intravenous Administration to Male Sprague Dawley Rats (Study No. 8232-453)

Methods: Five groups each of 8 male (12.7 to 13.7 weeks old) Sprague-Dawley rats weighing 326 to 354 g were administered the vehicle or SBC-102 doses of 5, 20, or 50 mg/kg for 5 hours, at an IV infusion rate of 10 ml/kg/hr.

There were 2 control groups consisting of a vehicle control (group 1) and a diphenhydramine (5 mg/kg) control (group 2). The treated animals and the second control group animals all received 5 mg/kg of diphenhydramine, 30 mins before dosing of vehicle or test article.

Study animals were observed for clinical signs and body weight changes in addition to respiratory parameters of tidal volume, respiratory rate (breaths/minute) and minute volume, which were recorded while the animals were restrained in a plethysmography chamber. Necropsies were conducted at the end of the study.

Results: Clinical signs included swelling of the feet and/or head in all 8 animals of the high dose (50 mg/kg) group. Three of 8 animals in both the low and mid dose groups had limited use of the right hind limb which was associated with the intramuscular injection of the diphenhydramine.

There were no statistically significant or biologically relevant changes observed in tidal volume or respiration rate at any time point in any animal administered SBC-102 at 5, 20 or 50 mg/kg. Although a trend toward higher respiration rates was observed throughout the 5 hour infusion period in animals dosed at 20 or 50 mg/kg, it did not reach statistical significance, nor was it considered physiologically relevant. A trend toward higher minute volume in the 50 mg/kg dosed animals, and a significantly higher covariate-adjusted mean minute volume in animals dosed at 20 mg/kg were attributed to the observed trend toward higher respiration rates observed in the animals.

In conclusion, IV administration of SBC-102 to male S-D rats at dose levels of 5, 20, or 50 mg/kg for 5 hours did not produce significant changes in tidal volume, respiratory rate, and minute volume.

Cardiovascular Safety Evaluation of SBC-102 Administered by IV Infusion to Male Telemetry-Instrumented Conscious Cynomolgus Monkeys (Study No. 8232-454)

Methods: Eight 2 to 7 years old male cynomolgus monkeys weighing 3 to 6 kg were administered vehicle control solution (0 mg/kg/dose) and SBC-102 (50 mg/kg) in a two-dose crossover design, with a washout period of at least 6 days between doses. SBC-102 was administered to the monkeys at an infusion rate of 10 ml/kg/hr for 5 hours.

Animals were observed for changes in clinical signs, body weight, body temperature, heart rate, blood pressure (systolic, diastolic, mean arterial pressure; arterial pulse

pressure), qualitative and quantitative electrocardiogram (PR, QT, QTc intervals; QRS duration).

Results: There were no SBC-102-related adverse effects on body weights during the treatment period. There were no statistically significant or biologically relevant changes in hemodynamic or quantitative ECG parameters noted in animals administered SBC-102 at 50 mg/kg. No abnormal ECG waveforms or arrhythmias were attributable to SBC-102, and all of the segments of ECG data studied were qualitatively within normal limits.

In conclusion, there were no test article-related adverse cardiovascular effects observed in male cynomolgus monkeys following single dose IV administration of SBC-102 at a dose level of 50 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The following reviews are incorporated below from the pharmacology review of IND 108640 dated April 17, 2012 by Dr. Emmanuel Akinshola.

Absorption/Distribution

Single Dose Intravenous Pharmacokinetics of SBC-102 in Sprague-Dawley Rats (Study No SBC-102-POO2)

Methods: Rats were administered 1 or 5 mg/kg SBC-102 by single intravenous (IV) bolus injection, and serum samples collected at 0.5, 1, 2, 5, 15, and 30 minutes post injection for enzyme linked immunosorbent assay (ELISA) quantification of SBC-102. SBC-102 was administered IV for 2-3 minutes duration into the saphenous vein, and blood samples obtained by tail clip at the specified times.

Results: The mean maximum concentrations (C_{max}) of SBC-102 obtained at 0.5 min were 12.6 $\mu\text{g/ml}$ for the 1 mg/kg dose and 68.1 $\mu\text{g/ml}$ for the 5 mg/kg dose. SBC-102 was rapidly cleared from circulation, and its uptake appeared to be saturable. The elimination half-life of SBC-102 was estimated to be 6 minutes for the 1 mg/kg dose and 20 minutes for the 5 mg/kg dose. The overall exposures (AUC_{last}) achieved were 1.7 $\mu\text{g}\cdot\text{h/ml}$ for the 1 mg/kg dose and 17.3 $\mu\text{g}\cdot\text{h/ml}$ for the 5 mg/kg IV dose of SBC-102.

Pharmacokinetic Parameters for SBC-102 in Sprague-Dawley Rats after a Single Bolus IV Injection

Parameters (units)	1 mg·kg ⁻¹	5 mg·kg ⁻¹
C_{max} ($\mu\text{g}\cdot\text{mL}^{-1}$)	12.6	68.09
AUC_{last} ($\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$)	1.7	17.3

Four-Week Intravenous Repeated Dose Pharmacokinetics of SBC-102 in Sprague-Dawley Rats (Study No. SBC-102-T002)

Methods: Rats were administered 5, 20, or 50 mg/kg IV doses of SBC-102, once per week for 4 weeks, and blood samples collected on study Day 1 and Day 22 pre-dose, and at 0.5, 2, 4.75, 5.08, 5.5, 7, 9, 13, 29 hr from the start of infusion (SOI). SBC-102 was administered IV into the saphenous vein, and blood samples obtained by tail clip from the rats were processed for serum, and SBC-102 concentrations quantified by ELISA.

Results: The mean serum concentration-time profiles for SBC-102 on study days 1 and 22 show that the mean time to achieve maximum concentration (T_{max} to C_{max}) ranged between 0.5 and 4.75 hr post SOI and that the concentrations declined rapidly following the end of infusion (EOI). It was also observed that exposure (C_{max} and AUC_{last}) increased in a greater than dose-proportional manner.

On study day 1, SBC-102 doses of 5, 20, and 50 mg/kg provided maximum plasma concentration (C_{max}) of 0.973, 9.47 and 127 $\mu\text{g}/\text{ml}$ respectively, and total overall exposures (AUC_{last}) of 4.05, 42.2, and 413 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively.

On study day 22, 5, 20, and 50 mg/kg SBC-102 doses provided C_{max} levels of 1.0, 16.1 and 106 $\mu\text{g}\cdot\text{ml}$ respectively, and AUC_{last} levels of 3.29, 63.4 and 450 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively.

The Day 22 C_{max} and AUC_{last} values did not increase to more than 2 times (0.4- to 1.9-fold) their respective Day 1 values at all dose levels. Furthermore, the absence of measurable predose serum levels of SBC-102 on day 22 was indicative of its complete clearance from the serum.

The C_{max} and AUC_{last} values were greater for male rats (except for Day 1 C_{max} at 5 mg/kg dose) than for female rats by a factor of 1.42 to 3.10 for the C_{max} and by a factor of 1.56 to 3.2 for the AUC_{last} , suggesting a gender difference. Terminal half-lives were not calculated because of the lack of a distinct elimination phase due to the very rapid serum clearance.

Pharmacokinetic Parameters for SBC-102 in Sprague-Dawley Rats after 4-Weeks Repeated IV (Infusion) Administration

Parameters (units)	Day 1					
	Males			Females		
Dose (mg·kg ⁻¹ ·day ⁻¹)	5	20	50	5	20	50
C _{max} (µg·mL ⁻¹)	0.880	9.47	127	0.973	5.82	60.4
T _{max} (h)	0.5	2.0	4.75	4.75	2.0	4.75
AUC _{last} (µg·h·mL ⁻¹)	4.05	42.2	413	2.56	23.2	150

Parameters (units)	Day 22					
	Males			Females		
Dose (mg·kg ⁻¹ ·day ⁻¹)	5	20	50	5	20	50
C _{max} (µg·mL ⁻¹)	1.00	16.1	106	0.387	5.19	74.9
T _{max} (h)	2.0	2.0	2.0	4.75	0.5	4.75
AUC _{last} (µg·h·mL ⁻¹)	3.29	63.4	450	1.45	19.8	289

Four-Week IV Repeated Dose Pharmacokinetics of SBC-102 in Cynomolgus Monkeys (Study No. SBC-102-T001)

Methods: Cynomolgus monkeys were administered 5, 20, or 50 mg/kg IV doses of SBC-102, once per week for 4 weeks, and blood samples collected on study Day 1 and Day 22 pre-dose, and at 0.5, 2, 4.75, 5.08, 5.5, 7, 9, 13, 29 hr from the start of infusion (SOI). Serum obtained from blood samples were analyzed for SBC-102 concentrations by ELISA.

Results: The mean serum concentration-time profiles for SBC-102 on study days 1 and 22 show that the mean time to achieve maximum concentration (T_{max} to C_{max}) ranged between 1.35 and 5.27 hr post SOI and that the concentrations declined rapidly following the end of infusion (EOI). The exposure (C_{max} and AUC_{last}) increased in a greater than dose-proportional manner.

On study day 1, SBC-102 doses of 5, 20, and 50 mg/kg provided maximum plasma concentration (C_{max}) of 0.98, 20.9 and 179 µg/ml respectively, and total overall exposures (AUC_{last}) of 3.92, 93.0, and 493 µg·h/ml, respectively.

On study day 22, SBC-102 doses of 5, 20, and 50 mg/kg provided C_{max} levels of 1.16, 24.5 and 120 µg/ml respectively, and AUC_{last} levels of 4.87, 85.5 and 443 µg·h/ml, respectively.

The Day 22 C_{max} and AUC_{last} values did not increase to more than 1.5 times (0.8- to 1.5-fold) their respective Day 1 values at all dose levels. Furthermore, the absence of measurable predose serum levels of SBC-102 on day 22 was indicative of its complete clearance from the serum.

The C_{max} and AUC_{last} values were slightly greater for male monkeys at 20 and 50 mg/kg, marginally exceeding female monkey values by a factor of 1.1 – 1.5 and 1.1 – 1.4 for C_{max} and AUC_{last} respectively. However, it is unlikely that these variations represent a gender difference in cynomolgus monkeys.

Terminal half-lives at the 50 mg/kg (high dose) were estimated at 0.622 and 0.588 hr on day 1, and 0.419 and 0.342 hr on day 22 for male and female monkeys respectively. The terminal half-lives at 20 mg/kg (mid-dose) on Day 1, were shorter and represented by only 1 or 2 animals (males: $t_{1/2} = 0.182$, $n = 2$; females: $t_{1/2} = 0.187$, $n = 1$). On day 22, the mid dose terminal half-life was estimated at 0.319 hr ($n = 1$). Terminal half-lives at the 5 mg/kg (low dose) could not be calculated due to rapid clearance of SBC-102 from the blood.

Pharmacokinetic Parameters for SBC-102 in Cynomolgus Monkeys after 4 Weeks Repeated IV (Infusion) Administration

Parameters (units)	Day 1					
	Males			Females		
Dose (mg·kg ⁻¹ ·day ⁻¹)	5	20	50	5	20	50
C_{max} (µg·mL ⁻¹)	0.966	20.9	179	0.98	16.0	133
T_{max} (h)	2.50	3.72	4.88	1.70	1.70	5.03
$t_{1/2}$ (h)	NA	0.182 ^a	0.622 ^b	NA	0.187 ^c	0.588 ^b
AUC_{last} (µg·h·mL ⁻¹)	3.79	93.0	493	3.92	65.2	444
AUC (µg·h·mL ⁻¹)	3.76	91.5	493	3.90	65.1	443
Parameters (units)	Day 22					
	Males			Females		
Dose (mg·kg ⁻¹ ·day ⁻¹)	5	20	50	5	20	50
C_{max} (µg·mL ⁻¹)	1.16	24.5	120	1.16	16.7	108
T_{max} (h)	1.35	4.82	5.10	1.40	3.90	5.27
$t_{1/2}$ (h)	NA	0.319 ^c	0.419	NA	NA	0.342 ^b
AUC_{last} (µg·h·mL ⁻¹)	4.87	85.5	443	4.69	71.2	419
AUC (µg·h·mL ⁻¹)	4.86	84.7	443	4.67	70.9	419
^a N=2						
^b N=4						
^c N=1						

5.2 Toxicokinetics

(Included in toxicity studies)

6 General Toxicology

6.1 Single-Dose Toxicity

The following reviews are incorporated below from the pharmacology review of IND 108640 dated April 17, 2012 by Dr. Emmanuel Akinshola.

Single Dose IV Range-Finding Infusion Study of SBC-102 in Cynomolgus Monkeys (Study No 8234874)

Methods: Two male cynomolgus monkeys (1 animal/dose group) were injected intramuscularly with 5 mg/kg diphenhydramine 30 mins prior to being dosed with a single IV bolus of 5 or 40 mg/kg SBC 102 each. The test article was diluted to a volume of 40 ml/kg with sterile saline, and applied with infusion pump at 10 ml/kg/hr via the saphenous vein for 4 hours. Assessment of SBC-102 toxicity was based on mortality, clinical observations, body weight, and clinical pathology data.

Results: The male monkey dosed at 40 mg/kg showed redness at the dosing site, and other clinical signs, including scabs and transient nasal discharge. The clinical observations were not SBC-102 related.

Observed hematological changes of mild to minimal decrease in total leukocyte and neutrophil counts on the third day after the dosing were not considered test article-related effects.

Clinical chemistry changes observed at the 5 and 40 mg/kg doses included a mild, (non-dose dependent) increase in total bilirubin concentration, minimal increase in alanine aminotransferase (AST) activity, and minimal increase in alkaline phosphatase (ALP) activity. These changes may indicate SBC-102-related effects on the liver of the monkeys, but variations in Individual animal cannot be excluded.

Administration of SBC-102 at 5 or 40 mg/kg had no effect on coagulation or urinalysis test results.

In conclusion, the administration of single IV bolus doses of 5 or 40 mg/kg SBC-102 in male cynomolgus monkeys was well tolerated, but with increases in total bilirubin concentration, and with minimal increases in AST and ALP activity in the liver. Decreases in total leukocyte and neutrophil counts were observed, along with transient nasal discharge, redness and scabs at the injection site in the 40 mg/kg dosed monkey.

6.2 Repeat-Dose Toxicity

The following reviews are incorporated below from the pharmacology review of IND 108640 dated April 17, 2012 by Dr. Emmanuel Akinshola.

Study title: Four-Week Toxicity and Toxicokinetic Infusion Study in Rats with SBC-102 with a 2-Week Recovery Phase.

Study no.: 8232-384
Study report location: Volume 5, Pages 1 to 997
Conducting laboratory and location: (b) (4)
Date of study initiation: August 30, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SBC-102, lot #s 102-09-001, 102-09-002; Placebo SBC-102, lot #s DF-006 BUF-1729, DF-006 BUF-1751; 0.9 % Sodium Chloride, lot # 82-236-JT

Key Study Findings

Dose dependent clinical signs of swelling of the paws and nose and red skin of the paws, were noticed, predominantly on the day of dosing. Swelling of the paws occurred in males dosed with ≥ 5 mg/kg/week and females dosed with ≥ 20 mg/kg/week of SBC 102. Swelling of the nose was noted in males dosed with ≥ 20 mg/kg/week and females dosed with ≥ 50 mg/kg/week SBC 102, and redness of the paws was noted in animals dosed with 50 mg/kg/dose of the test article. The NOAEL dose was 50 mg/kg/week.

Methods

Doses: 0 (placebo), 0 (vehicle), 5, 20, 50 mg/kg/week.
Vehicle control and treatment groups received intramuscular injection of 5 mg/kg diphenhydramine 30 min before each dose.

Frequency of dosing: Once weekly
Route of administration: Intravenous
Dose volume: 50 ml/kg (10 ml/kg/hour) for 5 hours/day
Formulation/Vehicle: 0.9 % Sodium Chloride
Species/Strain: Rats/Sprague Dawley
Number/Sex/Group: 10/sex/group for placebo control and 15/sex/group for vehicle control and 5, 20, and 50 mg/kg/week groups.
Age: 9 to 10 weeks at dose initiation
Weight: At dose initiation: males: 260 to 334 g, females: 180 to 249 g.
Satellite groups: 5/sex/group for vehicle control, 5, 20 and 50 mg/kg/week groups for a 2-week recovery
Unique study design: None
Deviation from study protocol: Minor deviations from study protocol are provided as protocol amendments from difficulties arising from study procedures.

Observations and Results

Mortality

None

Clinical Signs

Animals were checked twice daily (a.m. and p.m.) for clinical signs including pain and distress. Cage side observations were done once daily during the dosing and recovery phases and approximately 1 and 4 hrs postdose during week 2 through week 4 of the dosing phase.

Although diphenhydramine was administered to the animals prior to administration of the test article, SBC 102-related clinical signs included swelling of the paws and nose and redness of the paws. The clinical signs were SBC-102-related, occurred predominantly on the day of dosing and were dose-dependent.

Swelling of the paws occurred in males dosed with ≥ 5 mg/kg/week and females dosed with ≥ 20 mg/kg/week of SBC 102. Swelling of the nose was noted in males dosed with ≥ 20 mg/kg/week and females dosed with ≥ 50 mg/kg/week, and redness of the paws was noted in animals dosed with 50 mg/kg/week of the test article. Clinical signs are not considered adverse because they are transient and are not associated with other adverse effects.

Limited use of the right hind leg observed in 3 to 12 animals per group in all the dose groups with the exception of the control group that was not injected with diphenhydramine, is attributed to the intramuscular injection of diphenhydramine.

Sores and scabs observed on the back of female rats is also attributed to subcutaneous diphenhydramine administration because this observation was not present in control females that were not injected with diphenhydramine.

Other clinical signs such as swelling of areas other than the paws and nose, discharge from the eyes or genitals, red haircoat appear infrequently, are transient and comparable with control animals, and are not considered SBC 102-related.

Body Weights

Body weights were recorded twice prior to dosing and weekly during the dosing phase.

There were no SBC 102-related alterations in mean body weight or mean body weight gain in animals dosed with up to 50 mg/kg/week. Although the mean body weight was significantly higher from days 8 to 15 and days 1 to 29 of the dosing phase in male rats dosed with 5, 20 or 50 mg/kg/dose when compared with control animals not injected with diphenhydramine, mean body weight was not significantly different from controls injected with diphenhydramine. The weight gain in male rats appears to be diphenhydramine-related. There were no significant differences in mean body weight or mean body weight change in male or female animals during the recovery phase.

Feed Consumption

Food consumption by animals was recorded weekly during the dosing and recovery phases.

There was no SBC 102-related alteration in food consumption by female rats. However, the mean food consumption in males dosed with SBC 102 \geq 20 mg/kg/week was significantly lower (9 to 13 %) at all dosing phase intervals, compared with control males given diphenhydramine. The mean food consumption for days 8 to 15 and 22 to 28 was significantly higher in control males injected with diphenhydramine compared to control males not injected with diphenhydramine. No significant difference in mean food consumption was observed during the recovery phase in male or female rats.

Ophthalmoscopy

Ophthalmic examination was performed once prior to dosing and during week four of the dosing phase.

There were no SBC-102-related ophthalmic effects. However, corneal dystrophy was observed in 5 of 15 male rats dosed with 5 mg/kg of SBC 102, and in all the control rats. The corneal dystrophy finding was not dose related.

ECG

No ECG recording was performed.

Hematology

Blood samples for hematology were collected from fasted animals on the last day of dosing, prior to sacrifice and on the last day of recovery.

No SBC-102-related on hematology were noted in treated animals. Statistically significant differences observed between control and treated animals were small in magnitude, not dose-related, and considered incidental.

Clinical Chemistry

Blood samples for clinical chemistry were collected from fasted animals on the last day of dosing, prior to sacrifice and on the last day of recovery.

No SBC-102-related effects on clinical chemistry were noted in treated animals. Statistically significant differences observed between control and treated animals were small in magnitude, not dose-related, and considered incidental.

Urinalysis

Urine samples were collected for urinalysis from fasted animals on the last day of dosing, prior to sacrifice and on the last day of recovery.

No SBC-102-related effects were noted in the urine of treated animals. Statistically significant differences observed between control and treated animals were small in magnitude, not dose-related, and considered incidental.

Gross Pathology

After 4 weeks of dosing, the main study animals were sacrificed and necropsied. Recovery animals were also sacrificed and necropsied at the end of the two weeks recovery period.

There were no SBC-102-related macroscopic findings at the dosing or recovery phase in treated animals. The incidence of crusted skin/subcutis sites seen at dorsal hind or dorsal front regions in SBC-102-treated animals was similar to that seen in control animals dosed with diphenhydramine.

The observation of a mass in the dorsal front skin/subcutis region of a female animal dosed with 20 mg/kg SBC-102 correlated microscopically with chronic-active inflammation in the subcutaneous region, and not an SBC-102-related effect. In recovery animals, the incidences of crusted skin at subcutaneous sites appear similar between control (diphenhydramine-treated) and SBC-102-treated animals.

Organ Weights

Organ weights of main study and recovery animals were recorded after necropsy.

The following organs were weighed:

adrenal (2)	prostate
brain	salivary gland [mandibular (2)]
epididymis (2)	seminal vesicle
heart	spleen
kidney (2)	testis (2)
liver	thymus
lung	thyroid (2 lobes) with parathyroid
ovary (2)	uterus
pituitary gland	

There were no SBC-102-related organ weight changes in animals at the dosing or recovery phase. The significant differences in organ weights in animals (shown in the figures below) did not have microscopic correlates, and therefore, were not considered SBC-102-related.

Mean Organ Weight and Organ/Terminal Body Weight Data

Group/ Sex		Terminal Body Weight (g)	Seminal Vesicles (g)	Seminal Vesicles Ratio(%)	Heart (g)	Heart Ratio (%)	Liver (g)	Liver Ratio (%)
1 Male (Control)	Mean SD	326 15.2	1.3738 0.21309	0.4189 0.06629	--	--	--	--
2. Male (Control)	Mean SD	348 16.2	1.2711 0.14667	0.3655 * 0.04648	--	--	--	--
3. Male 5 mg/kg	Mean SD	336 29.6	1.2289 0.20773	0.3667 0.05973	--	--	--	--
4. Male 20 mg/kg	Mean SD	338 21.3	1.4489 0.24407	0.4293 # 0.07262	--	--	--	--
5. Male 50 mg/kg	Mean SD	335 19.0	1.3928 0.10616	0.4158 # 0.02238	--	--	--	--
1. Female (control)	Mean SD	227 9.7	--	--	0.9772 0.06046	0.4300 0.02530	6.2428 0.46774	2.7740 0.14621
2. Female (control)	Mean SD	231 16.0	--	--	0.9540 0.08610	0.4131 0.02121	6.9471 0.61568	3.0093 * 0.17101
3. Female 5 mg/kg	Mean SD	217 9.8	--	--	0.9965 0.10100	0.4589# 0.04149	6.3582 0.41132	2.9285 * 0.15060
4. Female 20 mg/kg	Mean SD	223 16.0	--	--	0.9937 0.08978	0.4475# 0.04835	6.6361 0.59187	2.9967 * 0.21521
5. Female 50 mg/kg	Mean SD	231 18.6	--	--	0.9885 0.07995	0.4281 0.02951	7.1839 1.30901	3.0877 * 0.35538

* Statistically significant from group 1 at $p \leq 0.05$

Statistically significant from group 2 at $p \leq 0.05$

SD Standard deviation

Mean Organ Weight and Organ/Terminal Body Weight Data

Group/ Sex	Mean SD	Brain Weight (g)	Brain (g)	Brain (%)
2. Male (Control)	Mean SD	348 16.2	1.8578 0.09546	0.5207 0.02113
3. Male 5 mg/kg	Mean SD	336 29.6	1.8102 0.17988	0.4793# 0.03067
4. Male 20 mg/kg	Mean SD	338 21.3	1.9327 0.11248	0.5403 0.02100
5. Male 50 mg/kg	Mean SD	335 19.0	1.9257 0.05076	0.5484 0.02367

Statistically significant from group 2 at $p \leq 0.05$
SD Standard deviation

Mean Rat Organ Weight and Organ/Brain Weight Data

Group/ Sex		Brain Weight (g)	Thymus (g)	Thymus Ratio (%)	Heart (g)	Heart Ratio (%)	Liver (g)	Liver Ratio (%)
2. Male (Control)	Mean SD	1.8578 0.09546	0.2959 0.03669	15.9703 2.20049	1.3059 0.10715	70.4768 7.13643	--	--
3. Male 5 mg/kg	Mean SD	1.8102 0.17988	0.3734 0.03705	20.644# 0.83472	1.4735 0.09096	81.8512# 6.82203	--	--
4. Male 20 mg/kg	Mean SD	1.9327 0.11248	0.2919 0.02295	15.2003 2.13233	1.2912 0.10801	66.9737 6.41742	--	--
5. Male 50 mg/kg	Mean SD	1.9257 0.05076	0.2822 0.04238	14.7001 2.56727	1.3188 0.12243	68.4376 5.49348	--	--
1. Female (control)	Mean SD	1.8095 0.09588	--	--	--	--	6.2428 0.46774	345.6945 29.72100
2. Female (control)	Mean SD	1.7839 0.07656	--	--	--	--	6.9471 0.61568	391.6021* 32.65184
3. Female 5 mg/kg	Mean SD	1.8114 0.05635	--	--	--	--	6.3582 0.41132	351.3173# 25.22220
4. Female 20 mg/kg	Mean SD	1.7972 0.08848	--	--	--	--	6.6361 0.59187	373.2293 32.28824
5. Female 50 mg/kg	Mean SD	1.7521 0.10780	--	--	--	--	7.1839 1.30901	409.6498* 69.63840

* Statistically significant from group 1 at $p \leq 0.05$
Statistically significant from group 2 at $p \leq 0.05$
SD Standard deviation

Mean Rat Organ Weight and Organ/Brain Weight Data

Group/Sex	Mean SD	Brain Weight (g)	Salivary Gland (g)	Salivary Gland (%)
1. Female (control)	Mean SD	1.8095 0.09588	0.4684 0.06554	25.8581 2.99879
2. Female (control)	Mean SD	1.7839 0.07656	0.5027 0.06033	28.1546 2.86217
3. Female 5 mg/kg	Mean SD	1.8114 0.05635	0.4734 0.03550	26.1174 1.44506
4. Female 20 mg/kg	Mean SD	1.7972 0.08848	0.5197 0.04820	28.9673* 2.85234
5. Female 50 mg/kg	Mean SD	1.7521 0.10780	0.5002 0.05041	28.5758* 2.56034

* Statistically significant from group 1 at $p \leq 0.05$

Statistically significant from group 2 at $p \leq 0.05$

SD Standard deviation

Histopathology

Tissues were preserved in 10 % neutral-buffered formalin. Preserved tissues were paraffin embedded, sectioned and stained with hematoxylin and eosin for histopathology.

The following tissues were examined:

adrenal (2)
aorta
brain
cecum
cervix
colon

mammary gland (females)
muscle, biceps femoris
optic nerve (2)^a
ovary (2)
pancreas
pituitary gland

duodenum	prostate
epididymis (2)	rectum
esophagus	salivary gland [mandibular (2)]
eye (2) ^a	sciatic nerve
femur with bone marrow (articular surface of the distal end)	seminal vesicle
Harderian gland ^a	skin/subcutis
heart	spinal cord (cervical, thoracic, and lumbar)
ileum	spleen
infusion and catheterization sites	sternum with bone marrow
injection site(s)	stomach
jejunum	testis (2) ^a
kidney (2)	thymus
lesions	thyroid (2 lobes) with parathyroid
liver	tongue
lung with large bronchi	trachea
lymph node (mandibular)	urinary bladder
lymph node (mesenteric)	uterus
a Preserved in modified Davidson's fixative.	vagina

Adequate Battery

Yes

Peer Review

No

Histological Findings

No SBC-102-related microscopic findings were present in main study or recovery animals. Microscopic findings at the infusion or catheter sites included chronic-active inflammation of the blood vessel wall or surrounding tissue, intimal proliferation, medial fibrosis, and occasional thrombosis or abscess of the femoral vein (with partial or complete venal occlusion).

Chronic-active inflammation was observed to be similar between control (diphenhydramine-treated) and SBC-102-treated animals at the infusion sites.

At catheter sites, chronic-active inflammation was also similar between control (diphenhydramine-treated) males and SBC-102-treated males, and only in one control female dosed with diphenhydramine. Chronic-active inflammation was absent at infusion and/or catheter sites in control animals dosed with vehicle only.

Other histological findings of dermal inflammation, epidermal ulceration, and/or muscle degeneration from the skin/subcutis sections of the dorsal hind or dorsal front regions, consistent with macroscopic observations of crusted areas, are generally similar in severity in SBC-102-treated animals and in control (diphenhydramine-treated) animals. These findings appear to be secondary to diphenhydramine treatment, and not SBC-102-related, because it is absent in control animals dosed with vehicle only.

In recovery animals, histological findings of ulceration at the dorsal hind or dorsal front skin sections were present only as a single incidence in SBC-102-treated groups. Microscopic findings in sections of the skin/subcutis and infusion and/or catheter sites were also of single incidence and/or minimal to slight severity.

In conclusion, administration of four weekly doses of SBC-102 (with diphenhydramine pretreatment) was well tolerated in male and female Sprague Dawley rats at doses up to 50 mg/kg/dose. Dose-dependent swelling of the paws and nose and redness of the paws were noted in both male and female rats on dosing days, and mean food consumption was significantly lower during the dosing phase in males dosed with ≥ 20 mg/kg of SBC-102. These findings were transient and minimal in severity. Based on these findings, the NOAEL dose is 50 mg/kg/week.

Toxicokinetics

Blood samples for toxicokinetics (TK) were collected from all animals prior to each dose on dosing days 1, 8, 15, 22, 29, and 43. Three male and 3 female rats from vehicle control group and 9 male and female rats from 5, 20 and 50 mg/kg SBC-102 groups were designated as TK animals. All TK animals were pre-dosed with diphenhydramine.

Serum levels of SBC-102 in control animals were below the limit of quantitation. Exposure to SBC-102 increased with increasing dose of SBC-102 from 5 to 50 mg/kg/dose. The mean concentrations of SBC-102 in TK animals remained similar after multiple dosing.

Due to fluctuations in mean serum concentration, T_{max} values for SBC-102 ranged from 0.50 hrs to 4.75 hrs from the start of infusion on day 1 and day 22. After reaching the maximum concentration, C_{max} of SBC-102 readily declined without a distinct elimination phase. Estimation of half-life ($t_{1/2}$) was not done due to a limited number of dosing intervals, and lack of a distinct elimination phase. Consequently, no values were estimated for day 1 and day 22 exposure (AUC), total clearance (Cl), or volume of distribution (Vd) at steady state.

The C_{max} and AUC values for male rats were higher than values for female rats, with increases greater than dose proportional. C_{max} and AUC values were generally similar on day 1 and day 22, indicating that there was no accumulation of SBC-102 after multiple dosing.

Toxicokinetic Parameters for SBC-102 in Rat Serum

Group	Dose Level (mg/kg/dose)	Sex	C _{max} (µg/mL)	DN C _{max} [(µg/mL)/ (mg/kg/dose)]	T _{max} (hr)	AUC _{0-t} (µg·hr/mL)	AUC ₀₋₂₉ (µg·hr/mL)	DN AUC ₀₋₂₉ [(µg·hr/mL)/ (mg/kg/dose)]
Day 1								
7	5	M	0.880	0.176	0.500	4.04	4.05	0.810
		F	0.973	0.195	4.75	2.55	2.56	0.512
8	20	M	9.47	0.474	2.00	42.2	42.2	2.11
		F	5.82	0.291	2.00	23.0	23.2	1.16
9	50	M	127	2.54	4.75	413	413	8.25
		F	60.4	1.21	4.75	150	150	3.00
Day 22								
7	5	M	1.00	0.199	2.00	3.28	3.29	0.659
		F	0.387	0.0773	4.75	1.42	1.45	0.290
8	20	M	16.1	0.807	2.00	63.3	63.4	3.17
		F	5.19	0.260	0.500	19.7	19.8	0.988
9	50	M	106	2.11	2.00	450	450	9.01
		F	74.9	1.50	4.75	289	289	5.77

Anti-Drug Antibody

Blood samples were also collected from all animals on the last day of dosing, and from recovery animals on the last day of the recovery phase, for anti-drug antibody analysis.

The majority of animals treated weekly with SBC-102 for 4 weeks were positive for anti-drug and neutralizing antibodies, whereas, all the placebo treated animals were negative for anti-drug antibodies.

Pre-dose samples from all animals dosed with 5, 20, or 50 mg/kg/dose of SBC-102 were negative for anti-drug antibodies except for a female rat dosed at 50 mg/kg, which was positive for both anti-drug and neutralizing antibodies.

All samples obtained on day 29 of the dosing phase and at the end of the recovery phase from animals dosed with 5 and 20 mg/kg/dose of SBC-102, were positive for both anti-drug and neutralizing antibodies.

On day 29 of the dosing phase, 7 of 15 male rats and 13 of 16 female rats were positive for anti-drug antibodies whereas, 6 of the male rats and 11 of the female rats were positive for neutralizing antibodies. Furthermore, all the three toxicokinetic males and three toxicokinetic females were positive for both anti-drug and neutralizing antibodies. However, at the end of the recovery period, all the male recovery animals were still positive for both antidrug and neutralizing antibodies whereas, 3 of 5 females were

positive for anti-drug antibodies, of which 2 were confirmed positive for neutralizing antibodies. The animal serum antibody data is summarized in the table below.

Anti-Drug Antibody Presence in Rat Serum Predose and on Dosing Day 29

Group Sex/ Dose	Anti-Drug Antibody Predose	Neutralizing Antibody Predose	Anti-Drug Antibody Dosing day 29	Neutralizing Antibody Dosing day 29	Anti-Drug Antibody on Recovery Day 14	Neutralizing Antibody on Recovery Day 14
1. Male (control)	Negative	Negative	Negative	Negative	Negative	Negative
2. Male (Control)	Negative	Negative	Negative	Negative	Negative	Negative
3. Male 5 mg/kg	Negative	Negative	Positive	Positive	Positive	Positive
4. Male 20 mg/kg	Negative	Negative	Positive	Positive	Positive	Positive
5. Male 50 mg/kg	Negative	Negative	7/15 were Positive	6/7 were positive	Positive	Positive
1. Female (control)	Negative	Negative	Negative	Negative	Negative	Negative
2. Female (control)	Negative	Negative	Negative	Negative	Negative	Negative
3. Female 5 mg/kg	Negative	Negative	Positive	Positive	Positive	Positive
4. Female 20 mg/kg	Negative	Negative	Positive	Positive	Positive	Positive
5. Female 50 mg/kg	9./10 were negative	9./10 were negative	13/16 were Positive	11/13 were Positive	3/5 were Positive	2/5 were Positive

Study title: Four-Week Toxicity and Toxicokinetic Infusion Study in Cynomolgus Monkeys with SBC-102 with a 2-Week Recovery Phase.

Study no.: 8232455
Study report location: Volume 4-5, Pages 1 to 1032
Conducting laboratory and location: (b) (4)
Date of study initiation: August 9, 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SBC-102, lot #s 102-09-001, 102-09-002; Placebo SBC-102, lot #s DF-006 BUF-1729, DF-006 BUF-1751; 0.9 % Sodium Chloride, lot # 82-236-JT

Key Study Findings

At terminal sacrifice, minimal to moderate vascular/perivascular inflammation was observed in one or more tissues in one female monkey dosed at 5 mg/kg, two male monkeys dosed at 20 mg/kg, and one female monkey dosed at 50 mg/kg/dose. At the recovery sacrifice, minimal vascular/perivascular inflammation was observed in one or more tissues in one male and one female control animal, two male animals dosed with 5 mg/kg, one female dosed with 20 mg/kg, and one male dosed with 50 mg/kg/week of SBC-102. Observed findings were not dose-related, and were also present in recovery control animals.

Fibrosis and/or chronic inflammation were observed in areas of the right/superior vena cava in heart sections from several control and SBC-102-treated monkeys. Chronic/chronic-active inflammation was also observed at the catheter site and/or infusion site in SBC-102-treated and control monkeys. At the recovery sacrifice, one male monkey dosed at 20 mg/kg had chronic-active inflammation in the skin/subcutis area overlying the infusion site.
The NOAEL dose was 50 mg/kg/week.

Methods

Doses: 0 (placebo control), 5, 20, or 50 mg/kg IV SBC-102 once a week with 5 mg/kg diphenhydramine intramuscular (IM) pretreatment

Frequency of dosing: Once weekly for 4 weeks (4 doses)

Route of administration: IV infusion via the jugular vein

Dose volume: 50 ml/kg (10 ml/kg/hr) for 5 hrs/day on days 1, 8, 15, 22 of the dosing phase

Formulation/Vehicle: SBC-102 formulation buffer (trisodium citrate dehydrate 13.7 mg/ml; citric acid monohydrate, 1.57 mg/ml; and human serum albumin, 10 mg/ml) or SBC-102 (1:1 dilution with 0.9 % saline).

Species/Strain: Cynomolgus monkeys: *Macaca fascicularis*

Number/Sex/Group: 5 animals/sex/group

Age: 2 to 3 years old

Weight: 2.3 to 3.1 kg (males); 2.4 to 3.0 kg (females)

Satellite groups: 2/sex/group from groups 1 to 4 was designated as recovery animals.

Unique study design: None

Deviation from study protocol: Deviations from study protocol are provided as protocol amendments from difficulties arising from study procedures.

Observations and Results

Mortality

None

Clinical Signs

Animals were checked twice daily (a.m. and p.m.) for clinical signs including pain and distress. Cage side observations were done once daily during the dosing and recovery phases and approximately 1 and 4 hrs postdose during week 2 through week 4 of the dosing phase.

No SBC-102-related clinical observations were noted during the dosing or recovery phase. Clinical observations were infrequent, transient, or were present to a comparable extent in treated and in control animals

Body Weights

Body weights of animals were recorded pre-dose, and weekly during the dosing and recovery phases. The initial and final mean body weights were 2.65 and 2.9 kg for the male animals and 2.6 and 2.78 kg for the female animals respectively. At the end of the recovery phase, the final mean body weights were 2.88 kg for male animals and 2.75 kg for the female animals.

No SBC-102-related alterations in mean body weight or mean body weight change were observed in animals during the dosing period.

Feed Consumption

Food consumption was not recorded.

Ophthalmoscopy

Ophthalmic examination was performed on animals once prior to dosing, and once on week 4 of the dosing phase. There were no ophthalmic lesions noted during the dosing phase. No ophthalmic examination was performed on the animals during the recovery period.

ECG

Not done

Hematology

Blood samples for hematology were collected from fasted animals twice during the pre-dose period, and on the days of scheduled sacrifices.

No SBC-102-related findings on hematology were observed.

Clinical Chemistry

Blood samples for clinical chemistry were collected from fasted animals twice during the pre-dose period, and on the days of scheduled sacrifices.

No SBC-102-related findings on clinical chemistry were observed.

Urinalysis

Samples for urinalysis were collected overnight from fasted animals once during the pre-dose period, and on the days of scheduled sacrifices.

No SBC-102-related findings on urine analysis were observed.

Gross Pathology

After 4 weeks of dosing, 3 animals/sex/group were sacrificed and necropsied, and following 2 weeks of recovery, the recovery animals (2/sex/group) were also necropsied.

No SBC-102-related macroscopic findings were noted at the terminal or recovery sacrifice.

Organ Weights

After necropsy of main study and recovery animals, the following organs were weighed:

adrenal (2)
 brain
 epididymis (2)
 heart
 kidney (2)
 liver
 lung
 ovary (2)
 pituitary gland

prostate
 salivary gland [mandibular (2)]
 seminal vesicle
 spleen
 testis (2)
 thymus
 thyroid (2 lobes) with parathyroid
 uterus

There were no SBC-102-related alterations in absolute or relative organ weights in any group after terminal or recovery sacrifice.

Histopathology

Representative samples of tissues were preserved in 10 % neutral-buffered formalin. The optic nerve and the testis were preserved in Davidson fixative, and modified Davidson's fixative respectively. Preserved tissues were paraffin embedded, sectioned and stained with hematoxylin and eosin for histopathology.

The following tissues were examined

adrenal (2)
 aorta
 brain
 cecum
 cervix
 colon
 duodenum
 epididymis (2)
 esophagus
 eye (2)^a
 femur with bone marrow (articular surface of the distal end)
 Harderian gland^a
 heart
 ileum
 infusion and catheterization sites
 injection site(s)
 jejunum
 kidney (2)
 lesions
 liver
 lung with large bronchi
 lymph node (mandibular)
 lymph node (mesenteric)
 a Preserved in modified Davidson's fixative.

mammary gland (females)
 muscle, biceps femoris
 optic nerve (2)^a
 ovary (2)
 pancreas
 pituitary gland
 prostate
 rectum
 salivary gland [mandibular (2)]
 sciatic nerve
 seminal vesicle
 skin/subcutis
 spinal cord (cervical, thoracic, and lumbar)
 spleen
 sternum with bone marrow
 stomach
 testis (2)^a
 thymus
 thyroid (2 lobes) with parathyroid
 tongue
 trachea
 urinary bladder
 uterus
 vagina

Adequate Battery

Yes

Peer Review

No

Histological Findings

Dispersed, segmental vascular/perivascular inflammation of minimal to moderate severity was observed microscopically in one or more tissues in one female monkey dosed at 5 mg/kg, two male monkeys dosed at 20 mg/kg, and one female monkey dosed at 50 mg/kg/. The inflammation was observed in small to medium sized vessels, and characterized by plump reactive endothelium and mixed inflammatory cells located primarily in the tunica adventitia and perivascular tissue. Because of the incidence and severity of vascular/perivascular inflammation in treated and in 2 control recovery animals, it was considered an adverse finding associated with a response to the vehicle control article, and not due to a direct toxic effect of the test article.

At the recovery sacrifice, minimal vascular/perivascular inflammation was observed microscopically in one or more tissues in one male and one female control animal, two male animals dosed with 5 mg/kg, one female dosed with 20 mg/kg, and one male dosed with 50 mg/kg/week of SBC-102. Observed findings were not dose-related, and were present in recovery control animals. The incidence and severity of tissue inflammation at terminal and recovery sacrifice are listed in the sponsor's table below.

Fibrosis and/or chronic inflammation were observed in areas of the right/superior vena cava in heart sections from several control and SBC-102-treated monkeys.

Chronic/chronic-active inflammation was also observed at the catheter site and/or infusion site in SBC-102-treated and control monkeys. The inflammation is considered a local reaction to the catheter or vascular access port, and not SBC-102-related. At the recovery sacrifice, one male monkey dosed at 20 mg/kg had chronic-active inflammation in the skin/subcutis area overlying the infusion site.

Incidence/Severity of Vascular/Perivascular Inflammation in Monkey Tissues – Terminal Sacrifice

	Sex	Males				Females				
		Dose Concentration SBC-102 (mg/kg/dose)	0	5	20	50	0	5	20	50
	No. Examined	3	3	3	3	3	3	3	3	
Heart										
Inflammation, vascular/perivascular										
	Not Present	3	3	1	3	3	3	3	2	
	Minimal	0	0	1	0	0	0	0	0	
	Slight	0	0	0	0	0	0	0	1	
	Moderate	0	0	1	0	0	0	0	0	
Gallbladder										
Inflammation, vascular/perivascular										
	Not Present	3	3	1	3	3	3	3	2	
	Minimal	0	0	1	0	0	0	0	1	
	Slight	0	0	1	0	0	0	0	0	
Colon										
Inflammation, vascular/perivascular										
	Not Present	3	3	1	3	3	2	3	3	
	Minimal	0	0	1	0	0	0	0	0	
	Slight	0	0	1	0	0	1	0	0	
Ileum										
Inflammation, vascular/perivascular										
	Not Present	3	3	1	3	3	3	3	3	
	Slight	0	0	2	0	0	0	0	0	
Duodenum										
Inflammation, vascular/perivascular										
	Not Present	3	3	1	3	3	3	3	3	
	Minimal	0	0	2	0	0	0	0	0	
Rectum										
Inflammation, vascular/perivascular										
	Not Present	3	3	2	3	3	3	3	3	
	Slight	0	0	1	0	0	0	0	0	
Stomach										
Inflammation, vascular/perivascular										
	Not Present	3	3	1	3	3	3	3	3	
	Slight	0	0	2	0	0	0	0	0	
Thyroid										
Inflammation, vascular/perivascular										
	Not Present	3	3	2	3	3	3	3	3	
	Moderate	0	0	1	0	0	0	0	0	
Kidney										
Inflammation, vascular/perivascular										
	Not Present	3	3	2	3	3	3	3	3	
	Slight	0	0	1	0	0	0	0	0	

Incidence/Severity of Vascular/Perivascular Inflammation in Monkey Tissues-Recovery

	Sex	Males				Females			
		0	5	20	50	0	5	20	50
	Dose Concentration SBC-102 (mg/kg/dose)	0	5	20	50	0	5	20	50
	No. Examined	2	2	2	2	2	2	2	2
Heart									
Inflammation, vascular/perivascular	Not Present	2	1	2	2	2	2	2	2
	Minimal	0	1	0	0	0	0	0	0
Kidney									
Inflammation, vascular/perivascular	Not Present	2	2	2	1	1	2	2	2
	Minimal	0	0	0	1	1	0	0	0
Colon									
Inflammation, vascular/perivascular	Not Present	1	1	2	1	2	2	1	2
	Minimal	1	1	0	1	0	0	1	0
Cecum									
Inflammation, vascular/perivascular	Not Present	2	2	2	2	1	2	2	2
	Minimal	0	0	0	0	1	0	0	0
Pancreas									
Inflammation, vascular/perivascular	Not Present	1	2	2	2	2	2	2	2
	Minimal	1	0	0	0	0	0	0	0
Epididymis									
Inflammation, vascular/perivascular	Not Present	2	1	2	2	NA	NA	NA	NA
	Minimal	0	1	0	0	NA	NA	NA	NA

NA – Not applicable.

In conclusion, IV administration of SBC-102 for four weeks at once weekly doses of 5, 20, and 50 mg/kg were well tolerated in cynomolgus monkeys. The observed microscopic vascular/perivascular inflammation in tissues were not dose-related, were also observed in recovery control animals and therefore not due to a direct effect of SBC-102 treatment. Similarly, the fibrosis and/or chronic inflammation observed in areas of the right/superior vena cava, and the skin/subcutis area of the catheter infusion site were present in treated and control animals, and not considered SBC-102-related. The NOAEL dose of SBC-102 in cynomolgus monkeys in this study is 50 mg/kg/dose.

Toxicokinetics

Blood was collected from all animals on dosing day 1 and week 4 pre-dose, and at 0.5 and 2 hrs after the start of infusion. Blood samples were also collected 15 min prior to the end of infusion, and 5 min, 0.5, 2, 4, 8, and 24 hrs post-dose.

Measurable concentrations of SBC-102 were found in the control group animals on day one and during the dosing week 4, with values ranging from 0.02 to 0.03 µg/ml. Measurable serum concentrations of SBC-102 ranging from 0.02 to 0.04 µg/ml were also detected pre-dose on day 1. The values were slightly higher than the lower limit of quantitation (<0.008 µg/ml). All the detectable values of SBC-102 in control animals were believed to be due endogenous lipase activity.

Exposure to SBC-102 increased with increasing dose from 5 to 50 mg/kg/dose. The mean concentrations of SBC-102 in TK animals remained similar after multiple dosing.

Following IV infusion, levels of SBC-102 declined generally in a multi-exponential manner, and serum levels readily declined to the background (endogenous) levels. The mean half-life ($t_{1/2}$) values ranged from 0.182 to 0.622 hrs on day 1 and from 0.319 to 0.419 hrs during week 4. The elimination half-life could not be estimated for most of the animal dose groups because of the lack of a distinct elimination phase.

The mean clearance (CL) values ranged from 107 to 239 ml/hr/kg and appear to be dose and time independent. The mean volume of distribution (V_{ss}) values ranged from 83.5 to 301 ml/kg and also appears to be dose independent. The mean V_{ss} did not exceed the total body water in a 5 kg monkey, indicating that SBC-102 is not highly distributed in monkey tissues after IV infusion.

The differences in mean C_{max} and AUC values on day 1 and week 4 between male and female monkeys were less than 2-fold. The mean values for C_{max} and AUC were generally similar on day 1 and during week 4, indicating that multiple dosing of SBC-102 in monkeys did not lead to accumulation. The increases in mean C_{max} and AUC values in male and female monkeys were greater than dose-proportional.

In conclusion, exposure of male and female monkeys to SBC-102 increased with increasing dose from 5 to 50 mg/kg/dose. The increases in C_{max} and AUC on day 1 and week 4 were greater than dose proportional, and the sex differences in mean C_{max} and AUC values were less than 2-fold. There was no accumulation of SBC-102 in monkeys following multiple dosing.

Summary of the Mean Toxicokinetic Parameters for SBC-102 in Monkey Serum: Day 1

Dose Level Group	(mg/kg/dose)	Sex		C_{max} ($\mu\text{g/mL}$)	DN C_{max} [($\mu\text{g/mL}$) ² / (mg/kg/dose)]		T_{max} (hr)	AUC_{0-1} ($\mu\text{g}\cdot\text{hr/mL}$)	AUC_{0-24} ($\mu\text{g}\cdot\text{hr/mL}$)	DN AUC_{0-24} [($\mu\text{g}\cdot\text{hr/mL}$) ² / (mg/kg/dose)]		$t_{1/2}$ (hr)	CL (mL/hr/kg)	V_{ss} (mL/kg)
					C_{max} ($\mu\text{g/mL}$)	[($\mu\text{g/mL}$) ² / (mg/kg/dose)]				AUC_{0-24} ($\mu\text{g}\cdot\text{hr/mL}$)	AUC_{0-24} ($\mu\text{g}\cdot\text{hr/mL}$)			
2	5	M	Mean	0.966	0.193	2.50	3.76	3.79	0.757	NA	NA	NA	NA	NA
			SD	0.216	0.043	2.14	0.50	0.50	0.100	NA	NA	NA	NA	NA
			N	5	5	5	5	5	5	0	0	0	0	0
		F	Mean	0.978	0.196	1.70	3.90	3.92	0.784	NA	NA	NA	NA	NA
			SD	0.217	0.043	0.67	0.90	0.90	0.181	NA	NA	NA	NA	NA
			N	5	5	5	5	5	5	0	0	0	0	0
3	20	M	Mean	20.9	1.05	3.72	91.5	93.0	4.65	99.8	0.182	239	301	
			SD	4.6	0.23	1.57	29.9	29.8	1.49	NA	NA	NA	NA	
			N	5	5	5	5	5	5	2	2	2	2	
		F	Mean	16.0	0.799	1.70	65.1	65.2	3.26	113	0.187	176	83.5	
			SD	8.6	0.432	0.67	36.8	36.9	1.84	NA	NA	NA	NA	
			N	5	5	5	5	5	5	1	1	1	1	
4	50	M	Mean	179	3.58	4.88	493	493	9.87	498	0.622	107	212	
			SD	65	1.31	0.18	138	138	2.75	158	0.095	29	46	
			N	5	5	5	5	5	5	4	4	4	4	
		F	Mean	133	2.67	5.03	443	444	8.87	429	0.588	117	213	
			SD	30	0.60	0.31	45	45	0.91	38	0.095	11	9	
			N	5	5	5	5	5	5	4	4	4	4	

NA Not applicable.

Summary of the Mean Toxicokinetic Parameters for SBC-102 in Monkey Serum: Week 4

Group	Dose Level (mg/kg/dose)	Sex		DN C _{max}			DN AUC ₀₋₂₀			t _{1/2} (hr)	CL ₉₅ (mL/hr/kg)	V ₉₅ (mL/kg)
				C _{max} (µg/mL)	[(µg/mL)/ (mg/kg/dose)]	T _{max} (hr)	AUC ₀₋₁ (µg·hr/mL)	AUC ₀₋₂₀ (µg·hr/mL)	[(µg·hr/mL)/ (mg/kg/dose)]			
2	5	M	Mean	1.16	0.232	1.35	4.86	4.87	0.974	NA	NA	NA
			SD	0.13	0.025	1.90	0.63	0.63	0.126	NA	NA	NA
			N	5	5	5	5	5	5	0	0	0
		F	Mean	1.16	0.232	1.40	4.67	4.69	0.938	NA	NA	NA
			SD	0.20	0.040	0.82	1.15	1.16	0.231	NA	NA	NA
			N	5	5	5	5	5	5	0	0	0
3	20	M	Mean	24.5	1.23	4.82	84.7	85.5	4.27	0.319	173	229
			SD	6.2	0.31	0.15	25.4	25.5	1.28	NA	NA	NA
			N	5	5	5	5	5	5	1	1	1
		F	Mean	16.7	0.837	3.90	70.9	71.2	3.56	NA	NA	NA
			SD	8.9	0.446	1.90	41.6	41.8	2.09	NA	NA	NA
			N	5	5	5	5	5	5	0	0	0
4	50	M	Mean	120	1.60	5.10	443	443	5.91	0.419	113	186
			SD	27	0.36	0.27	30	30	0.40	0.121	8	20
			N	5	5	5	5	5	5	5	5	5
		F	Mean	108	1.44	5.27	419	419	5.58	0.342	122	183
			SD	26	0.35	0.34	46	46	0.62	0.023	17	33
			N	5	5	5	5	5	5	4	4	4

NA Not applicable.

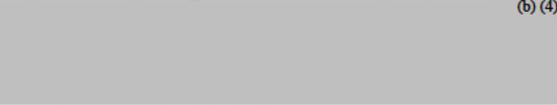
Anti-Drug Antibody

Blood samples were collected from all animals on day 1 and week 4 predose, and at 0.5 and 2 hours after the start of infusion; within 15 minutes prior to the end of infusion; and at approximately 5 min, 0.5, 2, 4, 8, and 24 hr postdose. Blood samples for anti-drug antibody were analyzed by ELISA.

Following 4 weeks of SBC-102 administration, 3 male monkeys from the 20 mg/kg dose group, and 1 male monkey from the 50 mg/kg dose group were positive for anti-drug antibodies. There were no animals from the 5 mg/kg dose group positive for anti-drug antibodies. No monkey was found to be positive for neutralizing antibodies. All pre-dose samples and samples from vehicle control animals were negative for anti-drug antibodies.

In conclusion, although 22 of 30 SBC-102-treated monkeys were initially positive for anti-drug antibodies in the screening assay (immunogenicity), only 4 males out of these animals were verified to be positive by the confirmatory assay (immunodepletion) for anti-drug antibodies after day 29 of treatment. No monkey was found to be positive for neutralizing antibodies, and all pre-dose samples and samples from vehicle control animals were negative for anti-drug antibodies.

Study title: Six-Month Toxicity and Toxicokinetic Study of SBC-102 by Intravenous Infusion in Cynomolgus Monkeys with a 2-Week Recovery Period

Study no.:	20006580
Study report location:	Volume 1, Pages 1 to 1279
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	September 21, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SBC-102, lot #s 102-09-003, 102-09-004; 102-09-005; 102-09-008; Placebo SBC-102, lot #s BUF-1803 BUF-1895, DF-006; 0.9 % Sodium Chloride, lot # C785667; C817635.

Key Study Findings

SBC-102-related decreased activity, reddened face, and frothing of the mouth was seen in one male monkey dosed with the 10 mg/kg dose on day 1 of drug administration. Diphenhydramine (DPH) treatment alleviated the symptoms, and subsequent DPH pretreatment abolished dose reaction in the animal.

A finding of scattered multifocal myocardial degeneration and fibrosis of minimal severity were observed in a female monkey dosed with 30 mg/kg/dose of SBC-102. A NOAEL dose of 30 mg/kg/dose was established in the study.

Methods

Doses: 0 (placebo control), 3, 10, or 30 mg/kg SBC-102 intravenous (IV) with
 Frequency of dosing: Once weekly for 6 months
 Route of administration: IV infusion via the jugular vein
 Dose volume: 30 ml/kg (10 ml/kg/hr) for 3 hrs/day
 Formulation/Vehicle: Placebo SBC-102 formulation buffer (trisodium citrate dehydrate ^{(b) (4)} mg/ml; citric acid monohydrate, ^{(b) (4)} mg/ml; and human serum albumin, ^{(b) (4)} mg/ml; ^{(b) (4)} mg/ml) or SBC-102 (1:1 dilution with 0.9 % saline).
 Species/Strain: Cynomolgus monkeys: *Macaca fascicularis*
 Number/Sex/Group: 3/sex/group
 Age: 2.3 to 2.9 years (males); 2.3 to 2.6 years (females)
 Weight: 2.4 to 2.8 kg (males); 2.4 to 3.0 kg (females)
 Satellite groups: 2/sex/group from groups 1 to 4 was designated as recovery animals.
 Unique study design: None
 Deviation from study protocol: Deviations from study protocol are provided as protocol amendments from difficulties arising from study procedures.

Observations and Results**Mortality**

None

Clinical Signs

Cageside observations were performed once daily in the morning. Detailed clinical observations of animals were done once prior to initiation of dosing, and weekly during the dosing and recovery periods.

One male monkey dosed with 10 mg/kg SBC-102 showed decreased activity, reddened face and frothing at the mouth on dosing day 1. Treatment with diphenhydramine (DPH) alleviated the symptoms, and pretreatment with DPH prior to subsequent dosing abolished the dosing reactions.

Periorbital swelling was observed in several animals dosed with 3 or 10 mg/kg SBC-102 on various occasions throughout the study duration. The observed periorbital swelling was sporadic, transient, and was not dose-responsive since it was not seen in animals dosed with 30 mg/kg SBC-102.

Body Weights

Body weights were recorded twice prior to the initiation of dosing, and weekly during the dosing and recovery periods. A final fasted body weight was recorded on the day of scheduled euthanasia.

No SBC-102-related body weight or body weight gain changes occurred in animals during the treatment or recovery periods. The initial group mean body weights of the male animals at the first week of dosing were 2.72 kg (control), 2.66 kg (3 mg/kg/week), 2.62 kg (10 mg/kg/week), and 2.68 kg (30 mg/kg/week), and during the last dosing week (week 28) were, 3.15 kg (control), 3.15 kg (3 mg/kg/week), 3.20 kg (10 mg/kg/week) and 3.35 kg (30 mg/kg/week). For the female monkeys, the initial group mean body weights at the first week of SBC-102 dosing were 2.58 kg (control), 2.48 kg (3 mg/kg/week), 2.48 kg (10 mg/kg/week), and 2.52 kg (30 mg/kg/week), and during the last dosing week were, 2.80 kg (control), 2.90 (3 mg/kg/week), 2.45 kg (10 mg/kg/week), and 2.60 kg (30 mg/kg/week).

Feed Consumption

Food consumption was evaluated once daily in the morning.

No SBC-102-related changes in food consumption were observed in the study animals.

Ophthalmoscopy

Ophthalmic examinations were performed prior to dose initiation and on week 26 of the dosing period.

No SBC-102-related ocular findings were observed in any of the study animals.

ECG

Electrocardiographic recordings were performed prior to the start of dosing, and on weeks 4 and 26 in all the study animals.

There were no abnormal electrocardiographic findings associated with the administration of SBC-102. All electrocardiograms were qualitatively and quantitatively within normal limits. There were no abnormalities in rhythm or waveform morphology at any dose level, in comparisons of pre-dose and post-dose electrocardiographic recordings.

However, analysis of RR interval and QTc segment recordings in animals are pending.

Hematology

Blood samples for hematology were collected from all animals prior to dosing initiation, or on weeks 4, 13 and 26 for main study animals, and on week 28 for recovery animals.

There were no SBC-102-related findings on hematology parameters during the study.

Clinical Chemistry

Blood samples for clinical chemistry were collected from fasted animals prior to dosing initiation, or on weeks 4, 13 and 26 for main study animals, and on week 28 for recovery animals.

There were no SBC-102-related findings on measured clinical chemistry parameters in study animals.

Urinalysis

Urine samples for urinalysis were collected from animals prior to dosing initiation, or on weeks 4, 13, 26 for main study animals, and on week 28 for recovery animals.

No SBC-102-related changes in urinalysis parameters occurred in the study animals.

Gross Pathology

All animals were sacrificed at the end of the dosing period, or at the end of the recovery period, and subjected to complete necropsy.

No SBC-102-related macroscopic findings were seen in the study animals.

Organ Weights

After necropsy of main study and recovery animals, the following organs were weighed:

Organs Weighed at Necropsy

Brain	Liver
Epididymis	Lung
Gland, adrenal	Ovary
Gland, pituitary	Spleen
Gland, prostate	Testis
Gland, thyroid	Thymus
Heart	Uterus
Kidney	

No SBC-102-related organ weight changes were seen. The mean spleen weight for female monkeys administered 10 mg/kg SBC-102 was significantly increased in comparison to the mean spleen weight of control animals. However, due to the absence of correlating changes or any specific trend or weight difference in any other groups, the spleen weight increase was considered incidental and of no toxicological relevance. The spleen weight differences were also attributed to differences in the attainment of sexual maturity in these animals.

No SBC-102-related organ weight changes were noted in recovery animals.

Histopathology

Representative samples of tissues were preserved in 10 % neutral-buffered formalin. The optic nerve and the testis were preserved in Davidson fixative, and modified

Davidson’s fixative respectively. Preserved tissues were paraffin embedded, sectioned and stained with hematoxylin and eosin for histopathology. The following tissues were examined:

Tissue Collection and Preservation

Administration site	Large intestine, cecum
Animal identification	Large intestine, colon
Artery, aorta	Large intestine, rectum
Bone marrow smear	Liver
Bone marrow, femur	Lung
Bone marrow, sternum	Lymph node, inguinal
Bone, femur	Lymph node, mandibular
Bone, sternum	Lymph node, mesenteric
Brain	Muscle, skeletal psoas, and diaphragm
Cervix	Nerve, optic ^a
Epididymis	Nerve, sciatic
Esophagus	Ovary
Eye	Oviduct
Gallbladder	Pancreas
Gland, adrenal	Skin
Gland, lacrimal	Small intestine, duodenum
Gland, mammary	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary mandibular	Stomach
Gland, seminal vesicle	Testis ^b
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Ureter
Kidney	Urinary bladder
	Uterus
	Vagina

^a Preserved in Davidson’s fixative.
^b Preserved in Modified Davidson’s fixative.

Adequate Battery

Yes

Peer Review

No

Histological Findings

Minimal multifocal myocardial degeneration and fibrosis were observed in the heart of one female monkey dosed with 30 mg/kg SBC-102. The myocardial degeneration consisted of several small foci with enlarged cells dotted with clear cytoplasmic vacuoles. Cellular nuclei were also enlarged, with a few mononuclear inflammatory cells associated with the altered cells.

Other findings at the injection site and catheter entrance included fibrosis, chronic inflammation, thrombi, and granulomas.

One monkey dosed at 10 mg/kg had thrombus formation in the lung, which was consistent with frequently observed incidental findings in cynomolgus monkeys. The most frequently observed incidental findings were mononuclear cell infiltrates in various tissues and increased size or number of germinal centers in lymph nodes and spleen.

No SBC-102-related microscopic findings were seen in recovery animals.

In conclusion, administration of SBC-102 by once weekly infusion for 6 months at 3, 10 and 30 mg/kg doses resulted in reddened face and mouth frothing in a male animal dosed with the 10 mg/kg dose of SBC-102 on treatment day 1. This reaction was treatable and preventable with DPH. Transient and non dose-related periorbital swelling was observed in several animals dosed with 3 or 10 mg/kg SBC-102 on various occasions throughout the study duration. The mean spleen weight for female monkeys administered 10 mg/kg SBC-102 was significantly increased in comparison to the mean spleen weight of control animals. A finding of minimal multifocal myocardial degeneration and fibrosis were observed in the heart of one female monkey dosed with 30 mg/kg SBC-102. Other findings at the injection site and catheter entrance included fibrosis, chronic inflammation, thrombi, and granulomas. No SBC-102-related organ weight changes or microscopic findings were seen in recovery animals. The NOAEL dose was 30 mg/kg/week in this study.

Toxicokinetics

Blood samples for toxicokinetic (TK) analysis were taken from all animals on study days 1, 106, and 176 at pre-dose, and 0.5 and 2 hrs post-dose. Blood samples were also collected at 5 and 30 mins, 2, 4, and 8 hrs after the end of SBC-102 infusion.

Serum concentrations of SBC-102 were below the LLOQ for the control and pre-dose samples on Day 1, with the exception of 4 animals with quantifiable, marginal pre-dose concentrations ranging between 0.02-0.03 µg/ml.

SBC-102 serum concentration-time profiles for male and female monkeys were bi-exponential post infusion, with the exception of the 10 mg/kg dose level on Day 1 and the 30 mg/kg dose level on Day 176, which were mono-exponential. T_{max} occurred immediately at the end of the 3 hr infusion period, corresponding to the 3.033 hour post start of infusion (SOI) time point.

Elimination was generally not estimable for the 3 and 10 mg/kg/dose levels due to the lack of quantifiable post infusion concentrations, or because of the bi-exponential TK profiles. When measurable, $t_{1/2}$ estimates ranged from 0.19 to 3.73 hrs.

Volume of distribution (V_d) and clearance (CL) ranged from 27.1 ml/kg to 655 ml/kg, and 17.8 ml/h/kg to 158 ml/h/kg respectively, for male and female animals at all dose levels. The V_d estimates were comparable with the blood volume of monkeys (73.4 ml/kg), suggesting that SBC-102 may be confined within the vasculature.

The C_{max} and systemic exposure (AUC) increased in a greater than dose proportional manner from 3 to 30 mg/kg on Days 1 and 176, with no observed gender differences in systemic exposure. Mean SBC-102 systemic exposure increased 2-fold or greater with repeated dose administration, indicating the possibility of drug accumulation.

Toxicokinetic Parameters for SBC-102 in Cynomolgus Monkey Serum

Day 1							
Gender	Group No.	Dose Level (mg/kg/dose)	Fold Increase	Mean C_{max} ($\mu\text{g/mL}$)	Fold Increase	Mean AUC(0-t) ($\mu\text{g}\cdot\text{h/mL}$)	Fold Increase
Males	2	3	1.00	1.43	1.00	3.37	1.00
	3	10	3.33	17.6	12.3	41.2	12.2
	4	30	3.00	216	12.3	597	14.5
Females	2	3	1.00	1.16	1.00	2.94	1.00
	3	10	3.33	17.7	15.3	40.4	13.8
	4	30	3.00	207	11.7	598	14.8
Day 176							
Gender	Group No.	Dose Level (mg/kg/dose)	Fold Increase	Mean C_{max} ($\mu\text{g/mL}$)	Fold Increase	Mean AUC(0-t) ($\mu\text{g}\cdot\text{h/mL}$)	Fold Increase
Males	2	3	1.00	3.56	1.00	5.84	1.00
	3	10	3.33	43.8	12.3	103	17.6
	4	30	3.00	351	8.01	1086	10.5
Females	2	3	1.00	3.62	1.00	6.36	1.00
	3	10	3.33	47.8	13.2	107	16.8
	4	30	3.00	320	6.69	1040	9.74

Anti-Drug Antibody

Blood samples were collected on Days 1 and 176 from all animals (including recovery animals) for anti-drug antibody analysis by ELISA.

All pre-dose samples and samples from group 1 (placebo control) animals were negative for anti-drug antibodies.

All the SBC-102-treated (30) animals were positive for anti-drug antibodies in the screening (immunogenicity) assay. In the immunodepletion assay, 9 of 10 animals at 3

mg/kg, 9 of 10 animals at 10 mg/kg, and 7 of 10 animals at 30 mg/kg SBC-102 were confirmed positive for the anti-drug antibodies.

Five of the 10 mg/kg SBC-102 dosed monkeys, 2 of the 30 mg/kg SBC-102 dosed monkeys, and none of the 3 mg/kg SBC-102 dosed monkeys confirmed positive for anti-drug antibodies were also positive for neutralizing antibodies.

Note: The Applicant submitted the following amendments to the study reports:

Final Report Amendment No. 1 dated July 18, 2012

- New Report Section (Inserted Between Pages 33 and 34 of the Final Report), Section 12.16. Anti-ovalbumin Antibody Analysis to reflect the results of the anti-ovalbumin antibody analysis.
- Page 34, Section 13. Conclusion: The following (from page 8 of the amendment) new paragraph was inserted between paragraphs 3 and 4 to reflect the results of the anti-ovalbumin antibody analysis.

There was a dose dependent ovalbumin immune response to the administration of SBC-102 on Day 176, with 6, 8, and 10 animals confirmed positive for anti-ovalbumin antibodies from the 3 mg/kg, 10 mg/kg, and 30 mg/kg dose groups, respectively. Antibody titers ranged from 2 to 8 for the 3 mg/kg dose group, 4 to 128 for the 10 mg/kg dose group and 4 to 512 for the 30 mg/kg dose group. There was only one 30 mg/kg dosed animal that confirmed positive at the prestudy time point with a titer of 4-fold. No samples in the control group screened positive at either the prestudy or Day 176 time points. There was one 10 mg/kg dosed animal that had an apparent infusion reaction on Day 1 that may have been related to either the test article directly or residual host cell protein in the test article formulation. Given that this apparent infusion reaction was treatable and preventable in conjunction with the absence of immune-related pathology in this study, this immune response to ovalbumin does not appear to have any toxicologically relevant consequences.

Final Report Amendment No. 2 dated June 5, 2013

- List of changes included addition of anti-ovalbumin antibody analysis final report amendment no. 1 to Appendix 19 (placed in front of the Final Anti-ovalbumin Antibody Analysis Report). Anti-ovalbumin Antibody Analysis Final Report Amendment No. 1 was issued to include results of the long term stability analysis. The Final Report for the Main Study was updated to include this report amendment in Appendix 19.

Final Report Amendment No. 3 dated March 6, 2014

- Compliance statement to include exceptions to GLPs: The compliance section of the Final Report was updated to identify additional GLP exceptions from this

study. There was no impact from the GLP exceptions on the integrity of the data obtained or on the results and conclusions of this study.

- The GLP compliance statement of the Dose Formulation Report was updated in the Dose Formulation Report Amendment.
- The GLP compliance statement of the Bioanalytical Report was updated in the Bioanalytical Report Amendment.
- The Final Main Study Report was updated to include the Anti-drug Antibody Detection Report Amendment in Appendix 17. The following is incorporated below from page 52 of the amendment.

Conclusions

- ***SBC-102 Anti-Drug Antibodies***
 - All (30/30) of animals in Groups 2 - 4 were positive for anti-drug antibodies in the screening assay (immunogenicity); however, 23 of these animals were verified to be positive by the “confirmatory” assay (immunodepletion) after Day 176 of treatment. All pre-dose samples and samples from Groups 1 (Placebo) were negative for anti-drug antibodies (Appendix 1).
- ***SBC-102 Neutralizing Antibodies***
 - Six samples from the 23 confirmed positive animals contained neutralizing antibodies (Appendix 1).

7 Genetic Toxicology

Genotoxicity studies have not been performed with sebelipase alfa in accordance with the ICH S6 Guidance.

8 Carcinogenicity

Carcinogenicity studies have not been performed with sebelipase alfa in accordance with the ICH S6 Guidance.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Fertility and Early Embryonic Development to Implantation of SBC-102 by Intravenous Infusion in Male Rats

Report No.: SBC-102-T009

Study no.: 902526

Study report location: Section 4.2.3.5.1 of the submission

Conducting laboratory and location: (b) (4)

Date of study initiation: January 21, 2014

Date of study completion: August 19, 2014

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SBC-102, 13MM5535002. Purity date not provided. Concentration = 2.2 mg/mL

Key Study Findings:

- There was no treatment related mortality.
- Clinical signs indicative of hypersensitivity reaction were observed at all doses, including the control.
- In males, no significant treatment related changes were observed in any of the parameter evaluated including sperm motility, sperm morphology and sperm concentration.
- In females, the number of corpora lutea, implantation sites, live embryos and early resorptions and the pre and post implantation losses were unaffected by the treatment.
- There were no significant treatment related adverse effects on fertility and early embryonic development.

Methods: Males were treated with the test article or the vehicle formulations by 6-hour intravenous (IV) infusions, twice weekly beginning 28 days before cohabitation, during cohabitation and continuing through the day before euthanasia. At each dose level, naïve females were cohabited with treated males. Diphenhydramine (5 mg/kg) was administered by subcutaneous (SC) injection to all study animals to alleviate the clinical signs indicative of a hypersensitivity reaction, which were observed in the test article treated animals following initiation of the infusion period on Study Day 11. On the first day of administration (Study Day 11), diphenhydramine was injected during the 6-hour infusion period to the controls and treated males at 6 and 20 mg/kg and to all groups on subsequent days of dosing and animals received the injection at least 30 minutes before the 6-hour infusion was initiated.

Doses: 6, 20, 60 mg/kg
 Frequency of dosing: Twice weekly
 Dose volume: 10 mL/kg
 Route of administration: 6-hour IV infusion
 Formulation/Vehicle: Trisodium citrate dihydrate (13.7 mg/mL), citric acid monohydrate (1.57 mg/mL) and human serum albumin (10 mg/mL) diluted 1:1 in 0.9% Sodium Chloride Injection, USP
 Species/Strain: Sprague Dawley rats
 Number/Sex/Group: 22/sex/group
 Satellite groups: None
 Study design: Study design is shown below (from page 17 of the report)
 Deviation from study protocol: None of the protocol deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

Text Table 4
Experimental Design

Group No.	Dose Level (mg/kg)	Dose Volume ^a (mL/kg/hr)	Dose Concentration (mg/mL)	No. of Animals	
				M	F
1/ Vehicle Control	0	10	0	22	22 ^b
2/ SBC-102	6	10	0.10	22	22 ^b
3/ SBC-102	20	10	0.33	22	23 ^b
4/ SBC-102	60	10	1	22	22 ^b

^a Dose volume 60 mL/kg twice weekly (SBC-102 provided at a concentration of 2.2 mg/mL).

^b At each dose level, 22 (23 in Group 3) naïve females were cohoused with treated males.

Basis of dose selection: The IV route of exposure was selected because this is the intended route of human exposure. In addition, in the pharmacology study titled “A Study to Assess the Pharmacokinetics and Pharmacodynamic Effects of SBC-102 in a Rat Model of Lysosomal Acid Lipase Deficiency (Study No. SBC102-P002), the level of hepatic LAL enzymatic activity measured in the LAL-deficient rat at 1, 24 and 72 hours following a single 5 mg/kg dose of SBC-102 was 51.49, 7.79, and 2.70 mU/mg protein, respectively. At 3 days postdose, the enzymatic activity was comparable to that demonstrated in the wild type (WT) rat (2.47 mU/mg protein). The dose levels were selected based on the results of the following studies: 4-week toxicity and toxicokinetic study in rats with SBC-102 with a 2-week recovery phase (Study No. SBC102-T002), embryofetal dose range-finding study in rats (Study No. 902522) and anticipated clinical dose of 1 mg/kg.

Observations:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were observed twice weekly.

Body Weight: Body weights were recorded twice weekly.

Food Consumption: Food consumption was recorded twice weekly.

Toxicokinetics: Not conducted

Dosing Solution Analysis: Dose formulation samples were collected for analysis as shown in the following table (from page 14 of the report).

Dose Formulation Sample Collection Schedule

Interval	Concentration	pH, Osmolality and Density	Sampling from
First preparation	All groups	All groups	Dosing container
Week 5	All groups	N/A	Dosing container
Last preparation	All groups	N/A	Dosing container

N/A = Not applicable.

Results were considered acceptable if mean sample concentration results were within or equal to $\pm 10\%$ of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to $\pm 15\%$.

Necropsy: Males were euthanized (Week 9) after administration of 14 doses of the test article/or the vehicle and were subjected to a complete necropsy examination. Male reproductive system evaluation included sperm motility, concentration and morphology.

Fertility Parameters: Mated naive females were euthanized on Day 13 postcoitum. Ovaries and uterus were isolated from each female and examined for number and distribution of the following: corpora lutea, implantation sites, placentae (size, color or shape), and early resorptions, live and dead embryos.

Results:

Mortality: There was no treatment related mortality. Male No. 2014 at 6 mg/kg had a severe lesion on the scrotum and was euthanized for humane reasons on Day 43. Macroscopic examination revealed skin abrasion and/or scab at the urogenital and dorsal thoracic regions accompanied by enlargement of the lymph nodes draining these regions (iliac) as well as enlargement and firmness (abnormal consistency) of the mandibular salivary gland. These findings were considered likely incidental or related to experimental procedures.

Clinical Signs: Transient clinical signs included decreased activity, abnormal gait, hunched posture, animal lying on side or prostrate, decreased muscle tone, skin pallor, eyes partly closed (ptosis), labored/abnormal breathing and/or increased respiration. These signs were observed at ≥ 6 mg/kg following initiation of the infusion on Day 11 and were considered to be indicative of hypersensitivity reaction. Four males (Nos.

4007, 4008, 4019, and 4021) at 60 mg/kg did not complete the 6-hour infusion on Day 11 (received 5 to 9% of the intended dose), because of the severity of the clinical signs. Generally, the administration of diphenhydramine alleviated these symptoms on study Day 11 and on subsequent occasions. Ptosis and decreased activity were also observed in control males but appeared later on (Days 15 or 22). Additional transient signs were attributed to hypersensitivity reactions and were seen at all dose levels, including controls, which consisted of excessive scratching and swelling of the cranium and/or muzzle at ≥ 6 mg/kg.

Body Weight: Mean initial (Day-1) and final (Day 63) body weights of control males were 358 and 586 g, respectively. In males, final body weights were 98%, 98% and 98% of control at 6, 20 and 60 mg/kg, respectively. The mean initial (Day 0) and final (Day 13) body weights of control females were 288 and 374 g, respectively. In females, final body weights were 98%, 97% and 96% of control at 6, 20 and 60 mg/kg, respectively. There were no significant treatment related changes in either sex.

Food Consumption: Mean initial (Day-1) and final (Day 63) food consumption of control males was 32 and 33.5 g/animal/day, respectively. In males, final food consumptions were 103%, 103% and 91% of control at 6, 20 and 60 mg/kg, respectively. There were no significant treatment related changes.

Toxicokinetics: Not conducted

Dosing Solution Analysis: Results of all samples were found to be within or equal to the acceptance criteria of $\pm 10\%$ of their theoretical concentrations. The pH, density and osmolality of dosing formulation recorded on the first day of preparation are shown in the following table (from page 27 of the report).

Text Table 11
pH, density and osmolality measurements

Group No.	Dose Level (mg/kg)	Concentration (mg/mL)	pH	Density (g/cm ³)	Osmolality (mOsm/kg)
1/ Vehicle Control	0	0	5.79	1.0072	222
2/ SBC-102	6	0.10	5.77	1.0073	219
3/ SBC-102	20	0.33	5.76	1.0057	219
4/ SBC-102	60	1.0	5.75	1.0075	219

Necropsy: There were no significant treatment related findings.

Fertility Parameters: In males, no significant treatment related changes were observed in any of the parameter evaluated including sperm motility, sperm morphology and sperm concentration. In females, the number of corpora lutea, implantation sites, live embryos and early resorptions and the pre and post implantation losses were unaffected by the treatment. The following table (from page 72-73 of the report) shows the uterine findings.

Table 10 Summary of Ovarian and Uterine Findings

Group 1 - Vehicle Control		Group 3 - SBC-102 20 mg/kg		Group 4 - SBC-102 60 mg/kg	
Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Live Embryos	Dead Embryos
1	Mean	18.2	17.1	16.4	0.0
	SD	1.9	1.6	1.2	0.0
	N	21	21	21	21
2	Mean	18.5	16.7	15.8	0.0
	SD	2.0	2.9	2.7	0.0
	N	22	22	22	22
3	Mean	18.3	16.8	15.9	0.0
	SD	2.3	2.4	2.7	0.0
	N	22	22	22	22
4	Mean	18.4	16.6	15.7	0.0
	SD	2.7	3.0	3.5	0.0
	N	21	21	21	21

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 10 Summary of Ovarian and Uterine Findings

Group 1 - Vehicle Control		Group 3 - SBC-102 20 mg/kg		Group 4 - SBC-102 60 mg/kg	
Group	Summary Information	Early Resorptions	Preimplantation Loss %	Post Implantation Loss %	
1	Mean	0.8	5.72	4.20	
	SD	0.9	5.95	4.74	
	N	21	21	21	
2	Mean	1.0	9.50	5.44	
	SD	1.0	13.41	5.72	
	N	22	22	22	
3	Mean	0.9	8.41	5.37	
	SD	0.8	6.88	5.08	
	N	22	22	22	
4	Mean	0.9	10.30	6.34	
	SD	1.1	7.46	9.49	
	N	21	21	21	

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Study title: Fertility and Early Embryonic Development to Implantation of SBC-102 by Intravenous Infusion in Female Rats

Report No.: SBC-102-T010

Study no.: 902528

Study report location: Section 4.2.3.5.1 of the submission

Conducting laboratory and location: (b) (4)

Date of study initiation: April 1, 2014

Date of study completion: September 10, 2014

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: SBC-102, 13MM5535004. Purity date not provided. Concentration = 2.2 mg/mL

Key Study Findings:

- There was no SBC-102-related mortality.
- Clinical signs indicative of hypersensitivity reaction were observed at all doses, including the control.
- Number of corpora lutea, implantation sites, live embryos and early resorptions and the pre and post implantation losses were unaffected by the treatment.
- There were no significant treatment related adverse effects on fertility and early embryonic development.

Methods: Female rats were administered the test article/or the vehicle formulations by 6-hour IV infusions, twice weekly beginning 14 days before cohabitation, during cohabitation (14 days) and continuing through gestation day 7 (GD 7). At each dose level, naïve females were cohabited with treated males. In response to severe clinical signs observed at 6, 20, and 60 mg/kg following the 4th dose on Day 11, dosing was interrupted and animals were administered diphenhydramine (5 mg/kg) by subcutaneous (SC) injection and, dosing was initiated after a 30-minute waiting period. Group 3 (20 mg/kg) animals showed the same severe clinical signs after dosing re-initiation and Day 11 dosing was terminated for this group (animals received 9-24% of the nominal dose). Diphenhydramine was also administered to control animals on Study Day 11; however dosing was not interrupted as no severe clinical signs were noted. Subsequently, all females were administered diphenhydramine (5 mg/kg) by SC injection at least 30 minutes before each dosing.

Doses: 6, 20, 60 mg/kg
 Frequency of dosing: Twice weekly
 Dose volume: 10 mL/kg
 Route of administration: 6-hour IV infusion
 Formulation/Vehicle: Trisodium citrate dihydrate (13.7 mg/mL), citric acid monohydrate (1.57 mg/mL) and human serum albumin (10 mg/mL) diluted 1:1 in 0.9% sodium chloride injection, USP
 Species/Strain: Sprague Dawley rats
 Number/Sex/Group: 22/sex/group
 Satellite groups: None
 Study design: Study design is shown below
 Deviation from study protocol: None of the protocol deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

The following table (from page 19 of the report) shows the study design.

*Group No.	Test Material	Dose Level (mg/kg)	Dose Volume ^a (mL/kg/hr)	Dose Concentration (mg/mL)	No. of Animals
					Main Study Females
1	Control	0	10	0	1501-1514, 1715, 1516-1518, 1619, 1520-1522
2	SBC-102	6	10	0.1	2601, 2502-2517, 2618, 2519-2522
3	SBC-102	20	10	0.33	3501-3515, 3616, 3517-3522
4	SBC-102	60	10	1.0	4501-4510, 4611, 4512-4522

^a Dose Volume 60 mL/kg (SBC-102 provided at a concentration of 2.0 or 2.2 mg/mL).

Basis of dose selection: The IV route of exposure was selected because this is the intended route of human exposure. In addition, in the pharmacology study titled “A Study to Assess the Pharmacokinetics and Pharmacodynamic Effects of SBC-102 in a Rat Model of Lysosomal Acid Lipase Deficiency (Study No. SBC102-P002), the level of hepatic LAL enzymatic activity measured in the LAL-deficient rat at 1, 24 and 72 hours following a single 5 mg/kg dose of SBC-102 was 51.49, 7.79, and 2.70 mU/mg protein, respectively. At 3 days postdose, the enzymatic activity was comparable to that demonstrated in the wild-type rat (2.47 mU/mg protein). Dose levels were selected based on the results of the following studies: 4-week toxicity and toxicokinetic study in rats with SBC-102 with a 2-week recovery phase (Study No. SBC102-T002), embryofetal dose range-finding study in rats (Study No. 902522) and anticipated clinical dose of 1 mg/kg.

Observations:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were observed twice weekly.

Body Weight: Body weights were recorded twice weekly prior to mating, including the cohabitation period until mated, and on GD 0, 3, 7, 10, and 13.

Food Consumption: Food consumption was recorded twice weekly prior to mating, as well as on GD 0, 3, 7, 10, and 13. Food consumption was not measured during the mating period.

Toxicokinetics: Not conducted

Dosing Solution Analysis: Dose formulation samples were collected for analysis as shown in the following table (from page 16 of the report).

Text Table 3
Dose Formulation Sample Collection Schedule

Interval	Concentration	Stability	pH, Osmolality and Density	Sampling From
First Preparation	All groups	N/A	All groups	dosing container
Week 3	All groups	N/A	N/A	dosing container
Last Preparation	All groups	N/A	N/A	dosing container

N/A = Not applicable.

Results were considered acceptable if mean sample concentration results were within or equal to $\pm 10\%$ of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to $\pm 15\%$.

Necropsy: Females were subjected to a complete necropsy examination on Day 13 (scheduled euthanasia).

Fertility Parameters: Females were euthanized on Day 13 postcoitum. Each female was subjected to an ovarian/uterine examination. The reproductive tract was dissected from the abdominal cavity. The ovaries and uterus were examined for number and distribution of the following: corpora lutea, implantation sites, placentae (size, color or shape) including any abnormalities, early resorptions, live and dead embryos.

Results:

Mortality: On Study Day 5, Female No. 2618 at 6 mg/kg/day (Group 2) was euthanized for humane reasons due to a skin lesion at the femoral surgical site.

Clinical Signs: Transient clinical signs included decreased activity, partly closed eyes (ptosis), excessive scratching, soft swollen muzzle and head (cranium), red skin of the periorbital region and/or red pinnae. These were seen on treatment days at a similar incidence in all groups, including controls. These clinical signs were generally seen after the first dose and persisted during the gestation period. At 60 mg/kg, transient swelling of the forepaws and hindlimbs and red pinnae were also noted, indicating

hypersensitivity reaction, which may have been exacerbated by SBC-102 treatment. These clinical signs were transient, were not life-threatening, and did not interfere with the completion of daily dosing.

In addition to the clinical signs listed above, following the 4th dose (Day 11), uncoordinated and/or lying on side, limited usage of hindlimbs, labored breathing and/or increased respiration rate were observed in animals at 6 and 20 mg/kg following initiation of the infusion period. These signs were considered to be indicative of severe hypersensitivity reaction. Animals at 20 mg/kg did not complete the 6-hour infusion on Day 11, receiving 9 to 24% of the intended dose, because of the severity of the clinical signs. Administration of diphenhydramine generally alleviated these hypersensitivity reactions.

These clinical signs were anticipated due to the presence of human serum albumin (HSA) in the formulation, based on well-characterized response of the rat to polysaccharides and glycoproteins. Albino rats have been shown to react to intraperitoneal or IV injection of polysaccharides and glycoproteins with a resulting acute inflammatory response characterized as an anaphylactoid type reaction (e.g., hyperemia, itching, and edema of the extremities) (Parratt JR and West GB, 1958, Inhibition by Various Substances of Edema Formation in the Hind Paw of the Rat Induced by 5-Hydroxytryptamine, Histamine, Dextran, Egg white and Compound 48/80, Br J Chemother, 13: 65-70; Harris JM et al., 1967, The Influence of Molecular Weight and Structure on the Vascular Permeability Responses Induced by Glucose Polymers in Rat Skin, Br J Pharmacol Chemother, 29:16-24; Anker SI and West GB, 1968, Inhibition of the Anaphylactoid Reaction in Rats, Br J Pharmacol Chemother, 33:304-11; West GB, 1981, Histamine Release by Sugar Polymers in the Rat, Agents Actions, 11:75-6; Moodley I, 1982, Histamine Release Induced by Dextran: the Nature of the Dextran Receptor, Eur J Pharmacol, 83:69-81). Signs, such as reddening of the skin and scabs in the axillary, dorsal, and ventral thoracic regions seen across all groups and were attributed to the infusion procedures.

Body Weight: The mean initial (Day 0) and final (Day 13) body weights of control females were 293 and 365 g, respectively. In females, final body weights were 108%, 104% and 104% of control at 6, 20 and 60 mg/kg, respectively. There were no significant treatment related changes.

Food Consumption: The mean initial (Day 1) and final (Day 13) food consumption of control females were 24 and 26 g/animal/day, respectively. There were no significant treatment related changes.

Toxicokinetics: Not conducted

Dosing Solution Analysis: Results of all samples were found to be within or equal to the acceptance criteria of $\pm 10\%$ of their theoretical concentrations. The pH, density and osmolality of dosing formulation recorded on the first day of preparation are shown in the following table (from page 27 of the report).

Text Table 11
pH, density and osmolality measurements

Group No.	Dose Level (mg/kg)	Concentration (mg/mL)	pH	Density (g/cm ³)	Osmolality (mOsm/kg)
1/ Vehicle Control	0	0	5.79	1.0072	222
2/ SBC-102	6	0.10	5.77	1.0073	219
3/ SBC-102	20	0.33	5.76	1.0057	219
4/ SBC-102	60	1.0	5.75	1.0075	219

Necropsy: There were no significant treatment related findings.

Fertility Parameters: There were no significant treatment related effects on mean number of days to mating, mating index, fertility index, and conception rate. Number of corpora lutea, implantation sites, live embryos and early resorptions and the pre and post implantation losses were unaffected by the treatment. One female in each of the SBC-102 treated groups had total resorption. This incidence was within the historical control range (0-1 females/group/study). The mean number of early resorptions (2.0) and the post-implantation loss (12.2%) at 20 mg/kg (when including the animal with total resorption) was above the historical control (0.4 to 1.6 and 2.6 to 10.9%, respectively). However this was not considered treatment related due to the lack of a dose response. The following tables (from page 72-73 of the report) show the ovarian and uterine findings.

Group	Group 1 - Control Group 3 - SBC-102 20 mg/kg/day		Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
	Number Placed for Mating Males	Females						
1	22	22	22	2.3 1.5 (N = 21)	20	100.0	90.9	90.9
2	21	21	21	3.7 3.7 (N = 21)	21	100.0	100.0	100.0
3	22	22	22	2.6 1.4 (N = 21)	22	100.0	100.0	100.0
4	22	22	21	3.0 3.4 (N = 20)	20	95.5	90.9	95.2

Significantly different from control group (Group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn - day to mating only)
* - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Group 1 - Control				Group 2 - SBC-102 6 mg/kg/day	
Group 3 - SBC-102 20 mg/kg/day				Group 4 - SBC-102 60 mg/kg/day	
Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Live Embryos	Dead Embryos
1	Mean	16.6	15.0	13.6	0.0
	SD	2.9	3.4	3.4	0.0
	N	19	19	19	19
2	Mean	17.0	14.9	14.2	0.0
	SD	4.4	5.4	5.4	0.0
	N	21	21	21	21
2	Mean	17.7	15.6	14.9	0.0
	SD	3.3	4.5	4.4	0.0
	N	20	20	20	20

A - Including animal with total resorption / ammonium sulfide
 B - Excluding animal with total resorption / ammonium sulfide
 Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Group 1 - Control				Group 2 - SBC-102 6 mg/kg/day	
Group 3 - SBC-102 20 mg/kg/day				Group 4 - SBC-102 60 mg/kg/day	
Group	Summary Information	Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss (%)	Post Implantation Loss (%)
1	Mean	1.4	1.4	10.12	9.21
	SD	1.5	1.5	11.66	9.71
	N	19	19	19	19
2	Mean	0.7	0.7	15.79	8.80
	SD	0.8	0.8	24.72	21.46
	N	21	21	21	21
2	Mean	0.7	0.7	12.83	4.25
	SD	0.8	0.8	21.20	5.00
	N	20	20	20	20

A - Including animal with total resorption / ammonium sulfide
 B - Excluding animal with total resorption / ammonium sulfide
 Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Group 1 - Control Group 3 - SBC-102 20 mg/kg/day			Group 2 - SBC-102 6 mg/kg/day Group 4 - SBC-102 60 mg/kg/day			
Group	Summary Information		Early Resorptions	Sum of Early Resorptions and Dead Embryos	Preimplantation Loss (%)	Post Implantation Loss (%)
3	Mean	A	2.0	2.0	9.30	12.18
	SD		3.7	3.7	9.71	22.59
	N		21	21	21	21
3	Mean	B	1.3	1.3	9.21	7.79
	SD		1.8	1.8	9.95	10.53
	N		20	20	20	20
4	Mean	A	1.5	1.5	11.57	9.33
	SD		1.2	1.2	9.27	7.07
	N		18	18	19	18
4	Mean	B	1.5	1.5	10.82	9.33
	SD		1.2	1.2	8.93	7.07
	N		18	18	18	18

A - Including animal with total resorption / ammonium sulfide

B - Excluding animal with total resorption / ammonium sulfide

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

9.2 Embryonic Fetal Development

An Intravenous Infusion Range-Finding Embryofetal Development Study in Rats (Study No. 902522, Report No. SBC-102-T007)

Methods: In this dose ranging study, pregnant female rats (n = 6/dose) were treated with SBC-102 daily by 6-hour IV infusion at 5, 15, 30, and 60 mg/kg on GD 6, 9, 12, 15, and 17. The following parameters were evaluated: maternal mortality and clinical signs, body weights, body weight changes, food consumption, gross necropsy findings, ovarian and uterine findings, fetal body weights and fetal external abnormalities.

Results: There were no mortalities. Treatment related clinical signs included swelling (paws, forelimbs/hindlimbs, muzzle, ventral and cervical region, head, etc.), which was observed on treatment days. There were no significant treatment related effects on body weight. A transient treatment related reduction in food consumption (< 10% of control) was seen ≥ 30 mg/kg/day between GD 6 and 9; however, food consumption was subsequently returned to control values. There was no significant treatment related effects on the numbers of corpora lutea, implantations, live and dead fetuses, resorptions or the sex ratio and pre- and post-implantation losses. SBC-102 administration had no effect on fetal weights or external fetal abnormalities. There were no gross pathologic findings considered to be related to SBC-102. Based on these results, dose levels for the following embryofetal development study in rats was set at 6, 20, and 60 mg/kg/day.

Study title: Embryo-Fetal Development Study in Rats by Intravenous Infusion

Study no.: 902524
 Report no.: SBC-102-T011
 Study report location: EDR Section 4.2.3.5.2
 Conducting laboratory and location: (b) (4)
 Date of study initiation: March 2, 2014
 Date of study completion: August 28, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: SBC-102, Batch No. 13MM5535004, purity data not provided

Key Study Findings:

- In an IV embryofetal development study in rats, animals were treated at 6, 20, or 60 mg/kg by 6-hour IV infusion on GD 6, 9, 12, 15, and 17.
- There were no treatment related mortalities.
- Treatment related clinical signs included swelling of the forepaws, hindpaws and/or muzzle on days of dosing, as well as clinical signs indicative of a hypersensitivity reaction starting on GD 15 in all treated groups.
- There were no significant treatment related fetal external, visceral or skeletal changes.
- There were no significant treatment related adverse effects on embryofetal development.

Methods: The objective of this study was to examine potential adverse effects of SBC-102 (sebelipase alfa) on embryofetal development of pregnant Sprague Dawley rats following IV infusion from implantation to closure of the hard palate.

Doses: 6, 20, 60 mg/kg
 Frequency of dosing: Reference and test article were administered by IV infusion for 6 hours on GD 6, 9, 12, 15, and 17
 Dose volume: 10 mL/kg/hr
 Route of administration: IV infusion
 Formulation/Vehicle: Trisodium citrate dihydrate (13.7 mg/mL), citric acid monohydrate (1.57 mg/mL) and human serum albumin (HSA, 10 mg/mL) diluted 1:1 in 0.9% sodium chloride injection, USP
 Species/Strain: Sprague Dawley (SD) rats
 Number/Sex/Group: 22/group
 Satellite groups: Toxiokinetic (2-8/group)
 Study design: Study design is shown below
 Deviation from study protocol: None of the deviations were considered to have

impacted the overall integrity of the study or the interpretation of the study results.

The following tables (from page 11 and 18 of the report) show the study design.

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg/hr)	Dose Concentration (mg/mL)	No. of Animals	
					Main Study	Toxicokinetic Study
1	Vehicle Control ^b	0	10	0	22	2
2	SBC-102	6	10	0.1	22	8
3	SBC-102	20	10	0.33	22	8
4	SBC-102	60	10	1	22	8

^a Dose Volume 60 mL/kg on Days 6, 9, 12, 15, and 17 pc (SBC-102 provided at a concentration of 2.0 mg/mL).

^b Placebo formulation buffer (trisodium citrate dihydrate [13.7 mg/mL], citric acid monohydrate [1.57 mg/mL] and human serum albumin [10 mg/mL]) diluted 1:1 in 0.9% Sodium Chloride Injection, USP.

Experimental Design

Group No.	Test Materials	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg/h)	Dose Concentration (mg/mL)	Main Study Animal Nos.	Toxicokinetic Animal Nos.
1	Vehicle Control	0	10	0	1501-1522	1523-1524
2	SBC-102	6	10	0.1	2601, 2502-2522	2523-2530
3	SBC-102	20	10	0.33	3501-3522	3523-3530
4	SBC-102	60	10	1	4501-4512, 4613, 4514-4522	4523-4524, 4625, 4526-4530

^a Dose Volume 60 mL/kg on Days 6, 9, 12, 15, and 17 pc (SBC-102 provided at a concentration of 2.0 mg/mL).

Note: In response to severe clinical signs observed in animals in Groups 2, 3, and 4 following treatment on GD Day 12 and 15, dosing was interrupted, animals were administered diphenhydramine (5 mg/kg) by SC injection and, following at least a 30 minute waiting period, dosing was re-initiated. Diphenhydramine was also administered to control animals; however dosing was not interrupted as no severe clinical signs were observed. Subsequently, all females were administered diphenhydramine (5 mg/kg) by SC injection at least 30 minutes before each dosing.

Basis of route, frequency of dosing and dose selection: The IV route of exposure was selected because this is the intended route of human exposure. In addition, in the pharmacology study titled "*A Study to Assess the Pharmacokinetics and Pharmacodynamic Effects of SBC-102 in a Rat Model of Lysosomal Acid Lipase Deficiency* (Study No. SBC102-P002), the level of hepatic LAL enzymatic activity measured in the LAL-deficient rat at 1, 24 and 72 hours following a single 5 mg/kg dose of SBC-102 was 51.49, 7.79, and 2.70 mU/mg protein, respectively. At 3 days postdose, enzymatic activity was comparable to that demonstrated in the wild-type rat (2.47 mU/mg protein). Dose levels were selected based on the results of the above dose ranging embryofetal development study (No. 902522; Report No. SBC-102-T007) in rats and anticipated clinical dose of 1 mg/kg.

Observations:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were observed on GD 6, 9, 12, 15, 18, and 21.

Body Weight: Body weights were recorded on GD 0, 3, 6, 9, 12, 15, 18, and 21.

Food Consumption: Food consumption was recorded between GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21.

Toxicokinetics (TK): Blood samples were collected from TK animals per the following (from page 22 of the report) schedule.

Text Table 6
TK Sample Collection Schedule

Group No.	Subgroup	No. of Females	Sample Collection Time Points (Time Postdose) on Day 17 pc					
			0 hr ^a	0.5 hrs SOI	2 hrs SOI	EOI	2 hrs EOI	4 hrs EOI
1	A	2	X	-	-	X	-	-
2 to 4	A	4	X	-	X	-	X	-
	B	4	-	X	-	X	-	X

x = Sample collected; - = Not applicable.

^a Sample collected before dosing.

EOI = End of Infusion.

SOI = Start of Infusion.

Antidrug Antibody Analysis (ADA): Blood samples were collected for ADA analysis from TK animals prior to treatment initiation and at termination on GD 18.

Dosing Solution Analysis: Dosing samples were collected from all groups for concentration, pH, osmolality and density. Concentration results were considered acceptable if mean sample concentration results were within or equal to $\pm 10\%$ of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to $\pm 10\%$ of theoretical concentration. On the first formulation occasion, the pH, density and osmolality was measured from one sample of each dose formulation concentration.

Necropsy: Main study animals were sacrificed on GD 21 and subjected to a complete necropsy examination.

Cesarean Section Data: Animals were examined for the number and distribution of corpora lutea, implantation sites, placental abnormalities, live and dead fetuses, early and late resorptions.

Offspring (Malformations, Variations, etc.): Fetuses were examined for external, visceral and skeletal abnormalities.

Results:

Mortality: There were no SBC-102-related mortalities.

Clinical Signs: Treatment related clinical signs included transient soft swelling of the forepaws, hindpaws, and muzzle, which were observed starting on GD 9 at 6 mg/kg, on GD 6 at 20 mg/kg, and on GD 6 at 60 mg/kg in all animals. In addition, following clinical signs were observed during dosing on GD 15 at 6, 20, and 60 mg/kg: decreased activity (4, 7, and 8 animals respectively), partly closed eyes (4, 4, and 2 animals respectively), animals lying on their side (1, 3, and 6 animals respectively) or prostrate (2, 0, and 2 animals, respectively). Labored breathing, increased respiration or abnormal breathing sounds were also observed in one animal in each treated group. These clinical signs were indicative of hypersensitivity reaction. As mentioned above, diphenhydramine was administered to alleviate these symptoms on GD 15 and 17. Signs such as reddening of the skin and scabs in the axillary, dorsal and ventral thoracic regions were seen across all groups, which were attributed to infusion procedures.

Body Weight: Mean initial (Day 0) and final (Day 21) body weights of control females were 233 and 428 g, respectively. Final body weights were 99%, 97% and 100% of control at 6, 20 and 60 mg/kg, respectively. There were no significant treatment related changes.

Food Consumption: Mean initial (Day 3-6) and final (Day 18-21) food consumption of control females was 26 and 102 g/animal/day, respectively. There were no significant treatment related changes.

Toxicokinetics: Peak SBC-102 serum concentrations were reached at 2 hours postdose. Systemic exposure was greater than dose proportional (104-fold increase for the 10-fold dose increase from 6 mg/kg to 60 mg/kg). The following table (from page 506 of the report) shows the TK data.

Table 2.1

Summary Mean (\pm SE) SBC-102 Toxicokinetic Parameters in Pregnant Female Sprague-Dawley Rat Serum Following 6 mg/kg, 20 mg/kg, and 60 mg/kg IV Infusion Administration of SBC-102 on Day 17 pc

Dose (mg/kg)	T _{max} (hr)	C _{max} (ng/mL)	AUC _(0-t) (ng•hr/mL)	C _{max} /D (ng•kg/mL/mg)	AUC _(0-t) /D (ng•hr/mL/mg/kg)
6	2.00	560 \pm 146	2160 \pm 420	93.4	360
20	2.00	2850 \pm 190	12,100 \pm 1370	143	603
60	2.00	57,900 \pm 7690	227,000 \pm 32,000	964	3780

Antidrug Antibody Analysis (ADA): Five of 22 samples collected on GD 18 were confirmed positive to ADA. However, there was no apparent correlation between ADA status and SBC-102 systemic exposure. All ADA positive animals were from the 6

mg/kg and 20 mg/kg dose groups with a higher incidence of confirmed samples (4 of 4) in the 20 mg/kg group.

Dosing Solution Analysis: Results of all samples were found to be within or equal to the acceptance criteria of 10% of their theoretical concentrations, (individual values within or equal to 15% of their theoretical concentrations), except for Group 3 at 20 mg/kg/day (0.33 mg/mL) from the first occasion (mean recovery: 89.1%). The investigation demonstrated that Group 3 from the first occasion was within specification and the initial failed results were likely due to issues during the processing of the samples in the analytical chemistry laboratory. The pH, density and osmolality of dosing formulation recorded on the first day of preparation are shown in the table below (from page 29 of the report).

pH, Density and Osmolality Measurements

Group No.	Dose Level (mg/kg)	Concentration (mg/mL)	pH	Density (g/cm ³)	Osmolality (mOsm/kg)
1/ Vehicle Control	0	0	5.80	1.0077	216
2/ SBC-102	6	0.10	5.79	1.0059	219
3/ SBC-102	20	0.33	5.80	1.0076	217
4/ SBC-102	60	1.0	5.77	1.0077	216

Necropsy: There were no significant treatment related findings.

Cesarean Section Data: There were no significant treatment related effects on the numbers of corpora lutea, implantation sites, live fetuses, dead fetuses, and early and late resorptions, on the sex ratio (% males), and pre- and post-implantation losses. The following table (from pages 53-55 of the report) shows the uterine data.

Table 7 Summary of Ovarian and Uterine Findings

Group	Summary Information	Group 1 - Vehicle Control Group 2 - SBC-102 6 mg/kg		Group 3 - SBC-102 20 mg/kg Group 4 - SBC-102 60 mg/kg		Sex Ratio (% Males)
		Total Number of Corpora Lutea	Total Number of Implantation Sites	Male Fetuses	Female Fetuses	
1	Mean	15.0	13.2	6.8	6.0	53.85
	SD	2.9	3.0	2.0	2.5	12.58
	N	22	22	22	22	22
2	Mean	15.1	13.9	6.4	6.7	48.27
	SD	2.5	2.0	2.0	1.4	11.31
	N	22	22	22	22	22
3	Mean	14.9	12.9	6.0	5.8	50.15
	SD	1.8	1.9	2.4	2.1	19.09
	N	22	22	22	22	22
4	Mean	15.5	14.0	7.0	6.4	51.68
	SD	2.7	2.3	2.6	2.2	14.77
	N	22	22	22	22	22

Significantly different from control group (group 1) value: D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Table 7 Summary of Ovarian and Uterine Findings

Group 1 - Vehicle Control Group 2 - SBC-102 6 mg/kg		Group 3 - SBC-102 20 mg/kg Group 4 - SBC-102 60 mg/kg				
Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Late Resorptions	Sum of Resorptions
1	Mean	12.8	0.0	0.4	0.0	0.4
	SD	3.2	0.0	0.7	0.0	0.7
	N	22	22	22	22	22
2	Mean	13.1	0.0	0.8	0.0	0.8
	SD	1.9	0.0	0.9	0.0	0.9
	N	22	22	22	22	22
3	Mean	11.8	0.0	1.0	0.1	1.1
	SD	2.5	0.0	1.4	0.3	1.5
	N	22	22	22	22	22
4	Mean	13.3	0.0	0.7	0.0	0.7
	SD	2.7	0.0	1.0	0.0	1.0
	N	22	22	22	22	22

Significantly different from control group (group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 7 Summary of Ovarian and Uterine Findings

Group 1 - Vehicle Control Group 2 - SBC-102 6 mg/kg		Group 3 - SBC-102 20 mg/kg Group 4 - SBC-102 60 mg/kg		
Group	Summary Information	Preimplantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	12.53	4.27	104.9
	SD	13.46	11.01	24.7
	N	22	22	22
2	Mean	7.52	5.37	104.8
	SD	8.08	5.73	11.9
	N	22	22	22
3	Mean	12.09	8.97	95.0
	SD	13.89	11.78	18.4
	N	22	22	22
4	Mean	9.06	5.30	107.1
	SD	9.59	9.20	18.3
	N	22	22	22

Significantly different from control group (group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Offspring (Malformations, Variations, etc.): The following malformations were observed:

- Severe dilatation of the lateral ventricles (hydrocephaly) for control fetus No. 1520-4 and an enlarged left eye for control fetus no. 1504-12.
- At 6 mg/kg, abnormal flexure of the hindlimbs was noted for fetuse no. 2516-15 and 2518-13.
- At 20 mg/kg, situs inversus, absent aortic arch, absent descending arch, ventricular septum defect, absent accessory lung lobe and fused lung lobes were

noted for fetus no. 3508-16, anal atresia and thread-like tail for fetus no. 3520-8, abnormal flexure of the hindlimbs for fetus no. 3510-7, and absent digits of the forepaw, shortened digits of the hindpaw and fused digits of the hindpaws for fetus No. 3509-2.

- At 60 mg/kg, misshapen lens was noted for fetus no. 4509-5.

Malformations were observed in fetuses of each group, including the control group. There was neither a dose-response in the incidence nor a pattern in the types of malformations indicative of a treatment related effect.

A statistically higher number of fetuses with incomplete supraoccipital bone ossification were seen at 6 and 60 mg/kg. This was not considered treatment related as the incidence remained within the historical control range (0-6.8%). A statistically significantly higher number of fetuses at 60 mg/kg with incomplete ossification of the hyoid bone (47%) fell outside the historical control range (0-30% fetuses affected); however the number of affected fetuses in concurrent controls was also out of the historical control range (32%). Therefore the increased incidence was attributed to biological variation and not to the treatment.

Overall, there were no significant treatment related fetal external, visceral or skeletal malformations.

An Intravenous Infusion Embryo-fetal Development Dose Range-finding Study in the Rabbit (Study No. 902523, Report No. SBC-102-T008)

Methods: In this dose ranging study, pregnant New Zealand white rabbits (n = 5/dose) were treated with SBC-102 daily by 5-hour IV infusion at 3, 10, 25, and 50 mg/kg on GD 7, 10, 13, 16, and 19. The following parameters were evaluated: maternal mortality and clinical signs, body weights, body weight changes, food consumption, gross necropsy findings, ovarian and uterine findings, fetal body weights, fetal external abnormalities, toxicokinetics (TK) and anti-drug antibody (ADA) formation.

Results: There were no treatment-related mortalities or clinical signs. There were no significant treatment related effects on body weight or food consumption. There were no gross pathologic findings considered to be related to SBC-102. At 50 mg/kg, number of late resorptions was increased; consequently, the number of live fetuses was decreased and the post-implantation loss was increased. This was mainly due to a litter (No. 5501) with a high number of late resorptions and a second litter (No. 5504) with total resorption (8 late resorptions). As a result, there was lower number of live fetuses and an increased post-implantation loss at 50 mg/kg compared to controls and to the historical control data from the test facility (late resorptions: 0 to 0.7 per litter; no. of live fetuses per litter: 5.8 to 8.9; post implantation loss: 2.5 to 29.1% per litter). This was seen for both "A" values (all litters, i.e., including No. 5504) and "B" values (litters with live fetuses, i.e., excluding No. 5504). The number of corpora lutea and implantation sites and the preimplantation loss were unaffected at each dose level. There were no

dead fetuses at any dose level. The following tables (from pages 52-54 of the report) show the ovarian and uterine data.

Table 7 Summary of Ovarian and Uterine Findings

Group	Summary Information	Total Number of Corpora Lutea	Total Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)
1	Mean	12.0	9.8	5.2	4.2	55.76
	SD	4.4	2.7	1.3	1.5	10.88
	N	5	5	5	5	5
2	Mean	11.0	9.5	4.0	4.5	48.50
	SD	2.6	1.7	1.4	2.4	17.19
	N	4	4	4	4	4
3	Mean	10.5	8.8	5.0	3.8	57.05
	SD	1.3	1.3	2.6	2.5	27.31
	N	4	4	4	4	4
4	Mean	12.3	10.0	6.3	3.5	63.65
	SD	1.7	2.7	2.2	1.3	6.43
	N	4	4	4	4	4
5	Mean	10.4	7.4	2.2	2.4	-
	SD	2.1	2.5	1.6	2.1	-
	N	5	5	5	5	4
	Mean	11.0	7.3	2.8	3.0	49.38
	SD	1.8	2.9	1.3	1.8	9.21
	N	4	4	4	4	4

A - Including animal(s) with total resorption
 B - Excluding animal(s) with total resorption

Table 7 Summary of Ovarian and Uterine Findings

Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Late Resorptions	Sum of Resorptions
1	Mean	9.4	0.0	0.0	0.4	0.4
	SD	2.2	0.0	0.0	0.5	0.5
	N	5	5	5	5	5
2	Mean	8.5	0.0	0.8	0.3	1.0
	SD	1.9	0.0	1.0	0.5	0.8
	N	4	4	4	4	4
3	Mean	8.8	0.0	0.0	0.0	0.0
	SD	1.3	0.0	0.0	0.0	0.0
	N	4	4	4	4	4
4	Mean	9.8	0.0	0.3	0.0	0.3
	SD	3.2	0.0	0.5	0.0	0.5
	N	4	4	4	4	4
5	Mean	A	4.6	0.0	0.2	2.8
	SD		3.6	0.0	0.4	3.3
	N		5	5	5	5
	Mean	B	5.8	0.0	0.3	1.3
	SD		2.9	0.0	0.5	1.9
	N		4	4	4	4

A - Including animal(s) with total resorption
 B - Excluding animal(s) with total resorption

Table 7 Summary of Ovarian and Uterine Findings

Group	Summary Information	Number of Empty Implantation Sites	Preimplantation Loss (%)	Post implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	0.0	16.02	3.24	508.4
	SD	0.0	11.34	4.49	72.2
	N	5	5	5	5
2	Mean	0.0	12.43	10.80	464.3
	SD	0.0	10.15	7.69	104.4
	N	4	4	4	4
3	Mean	0.0	16.58	0.00	516.8
	SD	0.0	8.20	0.00	14.4
	N	4	4	4	4
4	Mean	0.0	19.20	4.18	570.5
	SD	0.0	16.54	8.35	142.3
	N	4	4	4	4
5	Mean	A	0.0	25.16	37.76
	SD		0.0	30.86	38.97
	N		5	5	5
	Mean	B	0.0	31.45	22.20
	SD		0.0	31.71	20.27
	N		4	4	4

A - Including animal(s) with total resorption
 B - Excluding animal(s) with total resorption

Other uterine and ovarian parameters were unaffected by the treatment. There were no SBC-102-related effects on fetal weights and there were no significant treatment related fetal external malformations.

Fourteen of the 20 SBC-102 treated samples were positive for anti-drug antibodies (ADA); however, 12 of these animals were verified to be positive by the “confirmatory” assay. One sample from the 12 confirmed positive animals contained neutralizing antibodies. However, there was no notable effect of positive ADA on SBC-102 exposure. The following table (from page 29 of the report) shows the ADA measurements on GD 20.

Text Table 10
Anti-drug Antibody Measurements - Day 20 pc

Group No.	Dose Level (mg/kg/day)	No. of Animals Testing Positive		
		ADA Screening Assay	ADA Confirmation Assay	Neutralizing Antibody Assay
1/ Vehicle Control	0	0 OF 5	0 OF 5	0 OF 5
2/ SBC-102	3	5 OF 5	5 OF 5	0 OF 5
3/ SBC-102	10	3 OF 5	3 OF 5	1 OF 5 ^A
4/ SBC-102	25	5 OF 5	3 OF 5	0 OF 5
5/ SBC-102	50	1 OF 5	1 OF 5	0 OF 5

^a Animal No. 3504.

Peak SBC-102 concentrations occurred between 0.5 and 5 hours postdose. $T_{1/2}$ ranged from 0.310 to 0.676 hours. Systemic exposure was not dose proportional except between 25 and 50 mg/kg. Exposure was markedly greater than dose proportional between 3 and 25 mg/kg. Exposure appeared to increase following repeated dosing for the 3 and 10 mg/kg groups but showed a decrease for the 25 and 50 mg/kg groups from GD 7 to 19. Accumulation ratios ranged from 0.52 to 5.19. The following table (from page 28 of the report) shows the TK parameters.

Text Table 9
Toxicokinetic Parameters

Day 7 pc				
Group No.	Dose Level (mg/kg)	Tmax (hr)	Cmax (ng/mL)	AUC(0-t) (ng·hr/mL)
2/ SBC-102	3	0.500	207	780
3/ SBC-102	10	0.500	2700	11100
4/ SBC-102	25	5.00	147000	451000
5/ SBC-102	50	5.00	246000	978000
Day 19 pc				
Group No.	Dose Level (mg/kg/day)	Tmax (hr)	Cmax (ng/mL)	AUC(0-t) (ng·hr/mL)
2/ SBC-102	3	2.00	1070	4050
3/ SBC-102	10	0.500	5970	22900
4/ SBC-102	25	3.50	58900	237000
5/ SBC-102	50	5.00	146000	653000

Based on these results, the dose levels for the following embryofetal development study in rabbits was set at 10, 25, and 50 mg/kg/day.

Study title: Embryo-Fetal Development Study in Rabbits by Intravenous Infusion

Study no.: 902525
 Report no.: SBC-102-T012
 Study report location: EDR Section 4.2.3.5.2
 Conducting laboratory and location: [REDACTED] (b) (4)
 Date of study initiation: April 21, 2014
 Date of study completion: September 11, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: SBC-102, Batch No. 13MM5535006A, purity data not provided (concentration = 2.2 mg/mL)

Key Study Findings:

- In an intravenous embryofetal development study in rabbits, animals were treated at 10, 25, or 50 mg/kg by 6-hour IV infusion on GD 7, 10, 13, 16, and 19.
- There were no treatment related mortalities or clinical signs.
- Ovarian and uterine parameters were unaffected by the treatment.
- There were no significant treatment related fetal external, visceral or skeletal changes.
- There were no significant treatment related adverse effects on embryofetal development.

Methods: The objective of this study was to examine potential adverse effects of SBC-102 (sebelipase alfa) on embryofetal development of pregnant New Zealand White rabbits following IV infusion from implantation to closure of the hard palate.

Doses: 10, 25, 50 mg/kg
 Frequency of dosing: SBC-102 was administered by IV infusion for 6 hours on GD 7, 10, 13, 16, and 19
 Dose volume: 10 mL/kg/hr
 Route of administration: IV infusion
 Formulation/Vehicle: Trisodium citrate dihydrate (13.7 mg/mL), citric acid monohydrate (1.57 mg/mL) and human serum albumin (HSA, 10 mg/mL) diluted 1:1 in 0.9% sodium chloride injection, USP
 Species/Strain: New Zealand White rabbits

Number/Sex/Group: 22/group
 Satellite groups: Toxiokinetic (2-4/group)
 Study design: Study design is shown below
 Deviation from study protocol: None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results.

The following tables (from page 21 of the report) show the study design.

Text Table 4
 Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume ^a (mL/kg/hr)	Dose Concentration (mg/mL)	No. of Animals	
					Main Study	Toxicokinetic Study
1	Vehicle Control ^b	0	10	0	22	2
2	SBC-102	10	10	0.17	22	4
3	SBC-102	25	10	0.42	22	4
4	SBC-102	50	10	0.83	22	4

^a Dose Volume 60 mL/kg on Days 7, 10, 13, 16 and 19 pc (SBC-102 provided at a concentration of 2.2 mg/mL)

^b Placebo formulation buffer (trisodium citrate dihydrate [13.7 mg/mL], citric acid monohydrate [1.57 mg/mL] and human serum albumin [10 mg/mL]) diluted 1:1 in 0.9% Sodium Chloride Injection, USP

Basis of route, frequency of dosing and dose selection: The IV route of exposure was selected because this is the intended route of human exposure. The dosing regimen of approximately every third day (i.e., twice weekly) was based on the anticipated clinical regimen of every other week dosing as discussed before. Dose levels were selected based on the results of the above dose ranging embryofetal development study (Study No. 902523, Report No. SBC-102-T008) and anticipated clinical dose of 1 mg/kg.

Observations:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were observed on GD 0, 4, 7, 10, 13, 16, 20, 23, 26 and 29.

Body Weight: Body weights were recorded on GD 0, 4, 7, 10, 13, 16, 20, 23, 26 and 29.

Food Consumption: Food consumption was quantitatively measured daily starting on GD 0.

Toxicokinetics (TK): Blood samples were collected from TK animals per the following (from page 23 of the report) schedule on GD 7 and 19.

Group No.	No. of Females	Sample Collection Time Points (Time Post Dose) on Days 7 and 19 pc					
		0 ^a hr	0.5 hrs SOI	2 hrs SOI	EOI	2 hrs EOI	4 hrs EOI
1	2	X	-	-	-	-	-
2, 3 and 4	4	X	X	X	X	X	X

X = Sample collected; - = Not applicable; SOI = Start of infusion; EOI = End of infusion

^a Sample collected before dosing.

Antidrug Antibody Analysis (ADA): Blood samples were collected for ADA analysis from TK animals prior to initiation of the treatment and on GD 20.

Dosing Solution Analysis: Dosing samples were collected from all groups for concentration, pH, osmolality and density. Concentration results were considered acceptable if mean sample concentration results were within or equal to $\pm 10\%$ of theoretical concentration. Each individual sample concentration result was considered acceptable if it was within or equal to $\pm 15\%$ of theoretical concentration. On the first formulation occasion, the pH, density and osmolality was measured from one sample of each dose formulation concentration.

Necropsy: Main study animals were sacrificed on GD 29 and subjected to a complete necropsy examination.

Cesarean Section Data: Animals were examined for the number and distribution of corpora lutea, implantation sites, placental abnormalities, live and dead fetuses, early and late resorptions.

Offspring (Malformations, Variations, etc.): Fetuses were examined for external, visceral and skeletal abnormalities.

Results:

Mortality: There were no treatment related deaths.

Clinical Signs: There were no significant treatment related clinical signs.

Body Weight: The mean initial (GD 0) and final (GD 23) body weights of control females were 3.37 and 3.56 kg, respectively. Final body weights were 101%, 101% and 99% of control at 10, 25 and 50 mg/kg, respectively. There were no significant treatment related changes.

Food Consumption: The mean initial (GD 0-1) and final (Day 28-29) food consumption of control females were 132 and 112 g/animal/day, respectively. Final food consumptions were 101%, 100%, and 89% of control at 10, 25 and 50 mg/kg, respectively. At the high dose, food consumption was reduced by 11%.

Toxicokinetics: Quantifiable levels of SBC-102 were observed in 2 of 4 samples (Animal Nos. 1523 and 1524) collected from the vehicle control group on GD 19 at predose. The

highest individual level of exposure (187.69 ng/mL) was < 98% of the mean SBC-102 concentration observed in the serum at the low dose at the same sampling occasion. These concentrations observed in the control animals were slightly above the LOQ (125 ng/mL) and were not considered to have an impact on the TK interpretation.

T_{max} ranged from 0.5 to 6 hours. Terminal half-life ($T_{1/2}$) ranged from 0.399 to 2.17 hr. Volume of distribution (Vd) and clearance (CL) ranged between 31.4 and 5210 mL/kg and between 46.2 and 1660 mL/hr/kg, respectively. SBC-102 systemic exposure (C_{max} and AUC_{0-t}) increased with increasing dose levels in a greater than dose proportional manner on GD 7 but was dose proportional between 25 and 50 mg/kg on GD 19. For the 5-fold increase in dose from 10 mg/kg to 50 mg/kg, exposure increased 60-fold on GD 19. The exposure to SBC-102 on GD 19 did not change substantially when compared to GD 7 and accumulation ratios ranged from 0.642 to 2.06. The following table (from page 34 of the report) shows the TK parameters.

Text Table 13
Toxicokinetic Parameters

Day 7 pc				
Group No.	Dose Level (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	$AUC(0-t)$ (ng·hr/mL)
2/ SBC-102	10	2.33	3680	12200
3/ SBC-102	25	6.00	48700	250000
4/ SBC-102	50	4.00	147000	863000
Day 19 pc				
Group No.	Dose Level (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	$AUC(0-t)$ (ng·hr/mL)
2/ SBC-102	10	2.83	2620	12200
3/ SBC-102	25	6.00	69500	379000
4/ SBC-102	50	6.00	132000	730000

Antidrug Antibody Analysis (ADA): All control and pretreatment samples were negative for ADA. Samples collected from SBC-102-treated doe Nos. 2523 (10 mg/kg) and 3525 (25 mg/kg) on GD 20 were confirmed positive for ADA. There was no notable correlation between ADA status and SBC-102 systemic exposure in the tested animals. The following table (from page 34 of the report) shows the ADA analysis results.

Text Table 14
Anti-drug Antibody Measurements – Day 19 pc

Group No.	Dose Level (mg/kg)	No. of Animals Testing Positive	
		ADA Screening Assay	ADA Confirmation Assay
1/ Vehicle Control	0	0 OF 2	0 OF 2
2/ SBC-102	10	1 OF 4	1 OF 4
3/ SBC-102	25	1 OF 3	1 OF 3
4/ SBC-102	50	0 OF 4	0 OF 4

Dosing Solution Analysis: All dose formulations were within the specification. The pH, density and osmolality of dosing formulation recorded on the first day of preparation are shown in the table below (from page 31 of the report).

Text Table 12
pH, Density and Osmolality Measurements

Group No.	Dose Level (mg/kg)	Concentration (mg/mL)	pH	Density (g/cm ³)	Osmolality (mOsm/kg)
1/ Vehicle control	0	0	5.70	1.0076	221
2/ SBC-102	10	0.17	5.68	1.0076	221
3/ SBC-102	25	0.42	5.68	1.0077	220
4/ SBC-102	50	0.83	5.67	1.0078	220

Necropsy: There were no significant treatment related findings.

Cesarean Section Data: One control rabbit (No. 1521) and one rabbit (No. 4513) at 50 mg/kg aborted. This was not considered SBC-102-related since the incidence of abortion at 50 mg/kg was within the historical control range (0 to 4 rabbits per control group). There were no significant treatment related effects on the numbers of corpora lutea, implantation sites, live fetuses, dead fetuses, and early and late resorptions, on the sex ratio (% males), and pre- and post-implantation losses. The following table (from pages 50-52 of the report) shows the uterine data.

Table 6

Summary of Ovarian and Uterine Findings

Group 1 - Vehicle Control		Group 2 - SBC-102 10 mg/kg/day		Group 3 - SBC-102 25 mg/kg/day		Group 4 - SBC-102 50 mg/kg/day	
Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)	
1	Mean	10.1	9.4	4.4	4.2	52.48	
	SD	2.1	2.2	1.8	2.2	18.86	
	N	21	21	21	21	21	
2	Mean	10.6	9.5	4.6	4.5	51.16	
	SD	2.6	2.4	2.2	1.9	18.23	
	N	22	22	22	22	22	
3	Mean	9.8	8.8	4.8	3.7	55.18	
	SD	2.0	1.8	1.8	1.4	15.13	
	N	22	22	22	22	22	
4	Mean	10.6	8.4	3.7	4.4	47.66	
	SD	2.2	2.7	1.9	2.0	23.87	
	N	21	21	21	21	21	

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 6**Summary of Ovarian and Uterine Findings**

Group 1 - Vehicle Control		Group 2 - SBC-102 10 mg/kg/day		Group 3 - SBC-102 25 mg/kg/day		Group 4 - SBC-102 50 mg/kg/day	
Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Late Resorptions	Sum of Resorptions	
1	Mean	8.6	0.0	0.8	0.0	0.8	
	SD	2.5	0.0	2.0	0.2	2.0	
	N	21	21	21	21	21	
2	Mean	9.1	0.0	0.3	0.2	0.5	
	SD	2.7	0.0	0.6	0.5	0.8	
	N	22	22	22	22	22	
3	Mean	8.5	0.0	0.2	0.0	0.2	
	SD	2.0	0.2	0.4	0.2	0.4	
	N	22	22	22	22	22	
4	Mean	8.1	0.0	0.2	0.1	0.3	
	SD	2.5	0.0	0.5	0.3	0.7	
	N	21	21	21	21	21	

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Table 6**Summary of Ovarian and Uterine Findings**

Group 1 - Vehicle Control		Group 2 - SBC-102 10 mg/kg/day		Group 3 - SBC-102 25 mg/kg/day		Group 4 - SBC-102 50 mg/kg/day	
Group	Summary Information	Number of Empty Implantation Sites	Preimplantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)		
1	Mean	0.0	7.05	7.55	503.0		
	SD	0.0	8.83	17.70	101.5		
	N	21	21	21	21		
2	Mean	0.0	9.70	5.30	556.0		
	SD	0.0	9.28	9.76	136.9		
	N	22	22	22	21		
3	Mean	0.0	9.48	3.70	514.9		
	SD	0.0	10.35	7.81	96.5		
	N	22	22	22	22		
4	Mean	0.0	19.18	3.34	492.8		
	SD	0.0	20.56	6.58	128.1		
	N	21	21	21	21		

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Offspring (Malformations, Variations, etc.): Overall incidence of malformations in each group was within the historical control range (litters affected 0 to 27.8%; fetuses affected 0 to 6.1%). One fetus at 25 mg/kg (4.5% of litters; 0.53% of fetuses) and 3 fetuses from 3 different litters at 50 mg/kg (14.3% of litters; 1.76% of fetuses) had malformations. These included a blunt tail and hydrocephaly for the fetus no. 3520-3 at 25 mg/kg; and hydrocephaly for the fetus no. 4509-4; small upper jaw (maxillary micrognathia), absent naris, teeth and philtrum, and misplaced, small eye bulges (microphthalmia) for the

fetus no. 4517-4; and hyper flexion of the hindpaws for the fetus no. 4514-4 at 50 mg/kg. There was no dose response for these findings. There was increased incidence of litters and fetuses per litter at 50 mg/kg with incomplete hyoid bone ossification (14.3% and 2.9% affected, respectively, versus 0% in controls); however, this was not considered SBC-102-related as it was within the historical control ranges (0 to 43.5% of the fetuses affected per litter; 0 to 85.7% litters affected). Overall, there were no significant treatment related fetal external, visceral or skeletal aberrations.

9.3 Prenatal and Postnatal Development

Study title: An Intravenous Infusion Pre and Postnatal Study of SBC-102 in the Rat

Study no.: 902527
Report no.: SBC-102-T013
Study report location: EDR Section 4.2.3.5.3
Conducting laboratory and location: (b) (4)
Date of study initiation: January 13, 2014
Date of study completion: September 11, 2014
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SBC-102, 13MM5535004, purity data not provided, concentration = 1.6-2.4 mg/mL

Key Study Findings:

- In a pre and postnatal development study in rats, SBC-102 was administered by 6-hour IV infusion at 6, 20, and 60 mg/kg on GD 6, 9, 12, 15, 18, and 20 and Days 4, 7, 10, 14, and 17 post partum (pp).
- There were no significant treatment related effects on maternal body weight, food consumption, and reproductive performance.
- For the F1 generation, there were no significant treatment related effects on survival, physical and sensory/behavioral development and reproductive parameters.
- There were no significant treatment related adverse effects on pre and postnatal development.

Methods: The objective of this study was to examine the effects of SBC-102 (sebelipase alfa) in female Sprague Dawley rats during gestation, parturition and lactation and the development of the pups and their survival, physical development, behavior and reproductive performance.

Doses: 6, 20, and 60 mg/kg
 Frequency of dosing: The time-mated female rats were administered vehicle control (placebo formulation buffer) or SBC-102 by 6-hour IV infusion on GD 6, 9, 12, 15, 18, and 20, and GD 4, 7, 10, 14, and 17
 Dose volume: 10 mL/kg
 Route of administration: Intravenous infusion
 Formulation/Vehicle: Trisodium citrate dihydrate (13.7 mg/mL), citric acid monohydrate (1.57 mg/mL) and human serum albumin (10 mg/mL)
 Species/Strain: Sprague Dawley (SD) rats
 Number/Sex/Group: 23-24/group
 Satellite groups: None
 Study design: Shown in the table below
 Deviation from study protocol: None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

The following table (from page 21 of the report) shows the study design.

Text Table 4
 Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume ^a (mL/kg/h)	Dose Concentration (mg/mL)	No. of Animals
1	Vehicle Control ^b	0	10	0.00	1501-1523,1624
2	SBC-102	6	10	0.10	2501-2515,2517-2519, 2521-2524, 2616, 2620
3	SBC-102	20	10	0.33	3501-3504, 3506-3514, 3516-3524, 3605, 3615
4	SBC-102	60	10	1.00	4501-4524

^a Dose Volume 60 mL/kg (SBC-102 provided at a concentration of 2.0 mg/mL).

^b Placebo formulation buffer (trisodium citrate dihydrate [13.7 mg/mL], citric acid monohydrate [1.57 mg/mL] and human serum albumin [10 mg/mL]) diluted 1:1 in 0.9% Sodium Chloride Injection, USP.

Basis of dose selection: Dose levels were selected based on the results of an embryo fetal dose range finding study (Study No. 902522/SBC102-T007), and an anticipated clinical dose of 1 mg/kg. In the above dose ranging study, pregnant female rats were administered SBC-102 by IV infusion at 5, 15, 30, and 60 mg/kg on GD Days 6, 9, 12, 15, and 17. There was significant treatment related effect on embryofetal development. Based on these, doses of 6, 20, and 60 mg/kg were selected for the current pre and postnatal development study.

Observations:**F₀ Dams:**

- Survival: Twice daily
- Clinical signs: Once daily
- Body weight: Animals were weighed on GD 0, 3, 6, 9, 12, 15, 18, and 20 and lactation days (LDs) 0, 4, 7, 10, 14, 17, and 21
- Feed consumption: Food consumption was measured on GD 3 to 6, 6 to 9, 9 to 12, 12 to 15, and 15 to 18
- Uterine content: Dams were euthanized on LD 21 or 23 and the number of implantation sites were recorded
- Necropsy observation: Necropsy was conducted on LD 21 or 23
- Toxicokinetics: Not conducted
- Dosing Solution Analysis: Dosing solutions were analyzed for concentration, pH, osmolality and density at first preparation, Week 3 and 6

F₁ Generation:

- Survival: Twice daily
- Clinical signs: Once weekly
- Body weight: Days 0, 4, 7, 14, 17, and 21 post partum (pp).
- Feed consumption: Not recorded
- Physical development: Pinna unfolding was examined daily from Day 2 pp until all pups in the litter had a positive response. Eye opening was examined daily from Day 12 pp until the pup had a positive response. Starting from Day 26 pp (females) and from Day 35 pp (for males), vaginal opening and preputial separation were examined until development.
- Neurological assessment: Righting reflex was examined daily from Day 2 pp until all pups in the litter had a positive response. Auricular startle response was examined daily from Day 12 pp until the pup had a positive response. Locomotor activity was assessed on Days 35 and 60 pp. The startle habituation was measured on Day 55 pp. Water maze tests were performed on Days 60 and 70 pp.
- Reproduction: Beginning at 85 days of age, 1 female was placed with 1 male for a maximum of 14 days. Females were examined for mating and estrus cycle by examination of the vaginal lavage for spermatozoa/cell types. The day of positive identification of spermatozoa or presence of a vaginal plug was termed GD 0. Reproductive tract of each F₁ generation female euthanized on GD 13 was dissected from the abdominal cavity. Uterus was opened and the contents examined for number and distribution of corpora lutea, implantation sites, placentae (abnormalities in size, color or shape were recorded), early resorptions, and live and dead embryos.

Results:**F₀ Dams:**

Survival: There were 8 unscheduled deaths, however, none of these deaths were considered SBC-102-related. The following table shows the mortalities.

Animal No.	Sex	Dose	Day of Death/Euthanasia	Cause of Death
1513	Female	Control	Not mentioned	Euthanized due to cannibalization of their own litter at parturition
3517	Female	20 mg/kg	Not mentioned	
3524	Female	20 mg/kg	Not mentioned	
4506	Female	60 mg/kg	Not mentioned	
4520	Female	60 mg/kg	GD 24	Euthanized due to dystocia noted at parturition
2510	Female	6 mg/kg	GD 7	Death/euthanasia was interpreted to be secondary to technical procedures at the infusion site, and unrelated to the administration of SBC-102
2524	Female	6 mg/kg	GD 8	
4516	Female	60 mg/kg	GD 13	

Females Nos. 1513 (control), 3517, 3524 (20 mg/kg), and 4506 (60 mg/kg) were euthanized due to cannibalization of their own litter at parturition. All gross and histopathological findings in those females were interpreted to be physiological changes secondary to the parturition (e.g. edema of the cervix with macroscopic correlate of thick) or due to technical procedures at the infusion site and unrelated to the administration of SBC-102.

Female No. 4520 (60 mg/kg) was euthanized on GD 24 due to dystocia noted at parturition. Marked vascular/perivascular inflammation at the infusion site (mass with abscess) was interpreted to be the most probable cause of the dystocia. This change was considered to be secondary to technical procedures and unrelated to the administration of SBC-102.

Female Nos. 2510, 2524 (6 mg/kg) and 4516 (60 mg/kg) were found dead or euthanized on GD 7, 8, and 13, respectively. For Female No. 2510, clinical signs included skin pallor, decreased activity, weak, irregular/increased heart rate, and increased respiratory rate, blue abdominal skin and blue gums. For Female No. 2524, clinical signs included blue, swollen soft inguinal region and limited usage of the right hindlimb. For Female No. 4516, clinical signs included decreased muscle tone, skin pallor and decreased activity. These clinical signs were considered to be unrelated to SBC-102. All three females showed gross and histopathological findings at the infusion site (vascular/perivascular inflammation, thrombosis, hemorrhage and/or intimal proliferation with a mass). The death of Female No. 2524 was attributable to marked hemorrhage at the infusion site (mass), while marked liver necrosis (with macroscopic correlates of pale foci and/or irregular surface) was considered the main cause of euthanasia in Female Nos. 2510 and 4516. Liver necrosis was considered to be likely induced by inflammation at the infusion site (probable emboli) seen in those females. For all three females, death/euthanasia was interpreted to be secondary to technical procedures at the infusion site, and unrelated to the administration of SBC-102.

Clinical signs: During the gestation period, transient clinical signs were seen at all dose levels on days of treatment following initiation of the 6-hour infusion periods, including controls. These signs consisted of excessive scratching and swelling of the cranium, forelimbs, forepaws, hindlimbs, hindpaws and/or muzzle. These signs were first being

observed starting on GD 6 at ≥ 6 mg/kg and on GD 9 for control animals. These transient clinical signs persisted during the lactation period, but at a lower incidence and severity. These observations were suggestive of a hypersensitivity reaction exacerbated by the administration of SBC-102. These clinical observations were anticipated due to the presence of human serum albumin (HSA) in the formulation, based on the well-characterized response of the rat to polysaccharides and glycoproteins. As discussed before, albino rats have been shown to respond to the IP or IV injection of polysaccharides and glycoproteins with a resulting acute inflammatory response characterized as an anaphylactoid reaction (e.g., hyperemia, itching, and edema of the extremities).

Body weight: The mean initial (GD 0) and final (GD 20) gestational body weight of control animals were 242 and 399 g, respectively. There were no significant treatment related changes.

Food consumption: The mean initial (GD 3-6) and final (GD 15-18) gestational food consumption of control animals were 24 and 30 g/animal/day, respectively. There were no significant treatment related changes.

Uterine content: There were no significant treatment related effects on the length of gestation, pregnancy rate, gestation index, sex ratio or number of live, dead or malformed pups. Higher number of dead pups (3, 3, 23, and 3 at 0, 6, 20, and 60 mg/kg, respectively) were seen at 20 mg/kg when compared to the control group. This was largely due to the cannibalism of 2 litters (Nos. 3517 and 3524) that resulted in 22 of these 23 pup deaths. Maternal cannibalism of pups was also noted in one control dam (No. 1513) and one dam at 60 mg/kg (No. 4506). This was not dose related. Based on this, this increase in the number of dead pups was not considered related to SBC-102 administration. The number of implant scars was slightly lower (-8%) at 20 mg/kg than controls; this was not considered SBC-102 related as dose administration began following implantation. These factors both resulted, at least in part, in the lower number of live pups at birth and decreased live birth index at 20 mg/kg (-18% and -8% lower than controls, respectively). The following tables (from pages 71-72 of the report) show the uterine data.

Table 7 Summary of Maternal Performance

		Incidence Data F0 Generation						
Group 1 - Vehicle Control		Group 3 - SBC-102 20 mg/kg/day						
Group 2 - SBC-102 6 mg/kg/day		Group 4 - SBC-102 60 mg/kg/day						
Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups		Malformed Pups	
					Litters Affected	Pups Affected	Litters Affected	Pups Affected
1	24	23	95.8	95.65	3	3	0	0
2	24	22	91.7	100.0	3	3	0	0
3	24	22	91.7	90.91	3	23 **	0	0
4	24	21	87.5	90.48	3	3	0	0

Significantly different from control group (Group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ (Fisher's)

Table 7 Summary of Maternal Performance

		Group Mean Data F0 Generation						
Group 1 - Vehicle Control		Group 3 - SBC-102 20 mg/kg/day						
Group 2 - SBC-102 6 mg/kg/day		Group 4 - SBC-102 60 mg/kg/day						
Group	Summary information	Length of Gestation (days)	Sex Ratio (% males)	Number of Pups at Birth/Litters			Number of Implant Scars	Live Birth Index (%)
				Live	Dead	Malformed		
1	Mean	21.4	53.15	12.4	0.1	0.0	13.0	92.43
	SD	0.7	12.32	3.4	0.3	0.0	3.5	20.53
	N	23	22	23	23	23	23	23
2	Mean	21.5	54.11	12.7	0.1	0.0	13.9	91.35
	SD	0.5	13.63	3.0	0.4	0.0	2.4	11.09
	N	22	22	22	22	22	22	22
3	Mean	21.9	43.50	10.2	1.1	0.0	12.0	84.50
	SD	1.1	22.84	4.3	3.4	0.0	3.0	28.09
	N	22	19	21	21	21	21	21
4	Mean	21.7	52.22	12.8	0.2	0.0	13.2	91.01
	SD	0.6	11.82	3.5	0.4	0.0	3.8	16.07
	N	21	19	19	19	19	20	19

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ (Dunn)

Necropsy: There were no significant treatment related findings.

Dosing Solution Analysis: The results of all samples were found to be within or equal to the acceptance criteria of $\pm 10\%$ of their theoretical concentrations, (individual values within or equal to $\pm 15\%$ of their theoretical concentrations).

F1 Generation:

Survival: Control Male No. 117 was found dead on Day 63 pp. The exact cause of the death remained undetermined upon gross and histopathological evaluation.

Clinical signs: There were no significant treatment related clinical signs.

Food consumption: Data not provided

Physical development: There were no significant differences in the day of preputial separation or of vaginal opening.

Neurological assessment: There were no significant treatment related effects on motor activity, auditory startle habituation, and water maze performance.

Reproduction: There were no SBC-102 related effects on mean day to mating, mating index, fertility index or conception rate. There were no SBC-102 related effects on the numbers of corpora lutea, implantation sites, live embryos, dead embryos, early resorptions, or pre- and post-implantation loss for the F1 generation females. The following tables (from pages 154 and 155) show the reproductive performance and uterine data.

Table 29 Summary of Parental Performance

Group	Number Placed for Mating		Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
	Males	Females						
1	21	21	21	2.3 1.2 (N = 19)	21	100.0	100.0	100.0
2	20	20	20	2.6 1.4 (N = 19)	19	100.0	95.0	95.0
3	18	18	18	2.7 1.2 (N = 17)	18	100.0	100.0	100.0
4	18	18	18	2.5 1.3 (N = 17)	18	100.0	100.0	100.0

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ (Dunn - day to mating only)
* - $P \leq 0.05$ ** - $P \leq 0.01$ (Fisher's)

Table 30 Summary of Ovarian and Uterine Findings

F1 Generation Adults					
Group 1 - Vehicle Control			Group 3 - SBC-102 20 mg/kg/day		
Group 2 - SBC-102 6 mg/kg/day			Group 4 - SBC-102 60 mg/kg/day		
Group	Summary Information	Total Number of Corpora Lutea	Total Number of Implantation Sites	Live Embryos	Dead Embryos
1	Mean	18.7	16.1	15.1	0.0
	SD	2.9	2.8	2.6	0.0
	N	19	19	19	19
2	Mean	17.8	15.7	14.3	0.0
	SD	2.5	2.4	2.6	0.0
	N	18	18	18	18
3	Mean	17.9	15.8	15.3	0.0
	SD	2.7	1.9	2.1	0.0
	N	17	17	17	17
4	Mean	17.7	16.4	15.6	0.0
	SD	2.4	1.9	2.2	0.0
	N	17	17	17	17

Significantly different from control group (Group 1) value: D - $P \leq 0.05$ E - $P \leq 0.01$ (Dunn)

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Sebelipase alfa (Kanuma™) is a recombinant human lysosomal acid lipase (rhLAL) enzyme. Under BLA 125561, the Applicant is seeking approval of sebelipase alfa for the treatment of Lysosomal Acid Lipase (LAL) deficiency. In pediatric and adult patients with LAL deficiency, the recommended dose is 1 mg/kg administered as an intravenous (IV) infusion once every other week. In patients with rapidly progressive LAL deficiency within the first 6 months of life, the recommended dosage is 1 mg/kg as an IV infusion once weekly as an initial dose followed by escalation to 3 mg/kg once weekly.

Sebelipase alfa has been evaluated in a comprehensive program of nonclinical studies which included pharmacology, safety pharmacology, pharmacokinetics, acute toxicology (Cynomolgus monkey), 4-week intravenous toxicology studies in rats and Cynomolgus monkeys and chronic toxicology (6-month) study in Cynomolgus monkeys, and reproductive toxicology studies (fertility and early embryonic development in male and female rats, embryofetal development in rats and rabbits and pre and postnatal development in rats).

Primary pharmacology of sebelipase alfa was evaluated in in vitro and in vivo studies in a rat (LAL-deficient rat) model of LAL deficiency (Yoshida rats). In vitro studies using fluorescent sebelipase alfa demonstrated MMR (macrophage mannose receptor)-dependent endocytosis and lysosomal localization in a rat macrophage cell line that is

known to express the MMR receptor. In vivo animal efficacy studies were conducted using a rat model of LAL deficiency. The “Yoshida” rat is a Donryu rat that contains a spontaneous 3' deletion mutation in the LIPA (Lipase A) gene, which produces LAL deficiency (Yoshida H and Kuriyama M, 1990, Genetic Lipid Storage Disease with Lysosomal Acid Lipase Deficiency in Rat, Lab Anim Sci, 40:486-9). This homozygous LAL-deficient rat exhibits multiple abnormalities analogous to the human disease, including marked organomegaly such as hepatomegaly, elevated serum transaminases, growth failure, and a shortened life-span. Sebelipase alfa was evaluated in this rat model of LAL deficiency at IV doses ranging from 0.2 to 5 mg/kg. Sebelipase alfa administered once weekly or every other week caused improvements in several disease related parameters in this rat disease model, e.g., body weight gain, reduction in organomegaly, reduction in cholesteryl esters and triglycerides in the liver and spleen, and in serum transaminase levels. Results from these studies also indicated that the benefits of sebelipase alfa require maintenance of regular dosing, as the animals showed general decline in the health associated with a progressive decrease in growth velocity and subsequent body weight loss following cessation of sebelipase alfa treatment.

In safety pharmacology studies, sebelipase alfa did not show any significant CNS (rat), respiratory (rat) or cardiovascular (monkeys) effects up to 50 mg/kg, IV.

The pharmacokinetics of sebelipase alfa was studied after a single intravenous bolus dose in rats at 1 and 5 mg/kg. The elimination half-life was 6 and 20 minutes at 1 and 5 mg/kg, respectively. Sebelipase alfa was rapidly cleared from the circulation.

In acute intravenous toxicity study in Cynomolgus monkeys, maximum nonlethal dose was 40 mg/kg. Repeated dose intravenous toxicity studies have been conducted with sebelipase alfa in rats (4-week) and in Cynomolgus monkeys (1- and 6-month). The No Observed Adverse Effect Levels (NOAELs) in 4-week intravenous toxicity studies in rats and monkeys were 50 mg/kg/day in both species (approximately 267 and 310 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week, respectively). The NOAEL of 30 mg/kg/day (highest tested dose) in the 6-month intravenous toxicity study in Cynomolgus monkeys was approximately 766 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week. No significant organ toxicities were identified in these studies.

Sebelipase alfa at intravenous doses up to 60 mg/kg administered twice weekly (approximately 164 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week) was found to have no adverse effect on fertility and reproductive performance of male and female rats. In embryofetal development studies, sebelipase alfa administered during the period of organogenesis to rats (on gestation days 6, 9, 12, 15 and 17) and rabbits (on gestation days 7, 10, 13, 16 and 19) at intravenous doses up to 60 and 50 mg/kg, respectively, (approximately 164 and 526 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week, respectively) did not cause any adverse effects on embryofetal development. A pre and postnatal development study in rats showed no evidence of any adverse effect

on pre and postnatal development at intravenous doses (administered on gestation days 6, 9, 12, 15, 18, and 20 and days 4, 7, 10, 14, and 17 postpartum) of sebelipase alfa up to 60 mg/kg/day (approximately 164 times the human AUC of 1387 ng.h/mL at 1 mg/kg dose administered once every other week).

In conclusion, this application contains adequate nonclinical studies and satisfies the criteria for marketing authorization of sebelipase alfa. From a nonclinical perspective, this BLA is recommended for approval for its proposed use as indicated in the label.

12 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TAMAL K CHAKRABORTI
06/08/2015

SUSHANTA K CHAKDER
06/08/2015

Comments on BLA 125561 sebelipase alfa

From A. Jacobs AD

Date 5/8/15

1. I concur that there are no pharm-tox approval issues and that the non clinical labeling is appropriate
2. Other comments have been conveyed to the reviewer and they will be addressed as appropriate.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ABIGAIL C JACOBS
05/14/2015

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125561

Applicant: Synegeva BioPharma Corp **Stamp Date: October 21, 2014**

Drug Name: Sebilipase Alfa **BLA Type:**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	x		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Sruthi Tallapragada King	12-17-14
Reviewing Pharmacologist	Date
Sushanta Chakder	12-17-14
Team Leader/Supervisor	Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

SRUTHI T KING
01/20/2015

SUSHANTA K CHAKDER
01/20/2015