

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

APPLICATION NUMBER:

125151/0

PHARMACOLOGY REVIEW(S)

Comments BLA STN 125151 idursulfase eleprase

From A. Jacobs, Pharm/Tox AD

5/9/06

o-j 5/9/06

1. I concur with a pregnancy category C.
2. I concur that there are no outstanding nonclinical issues for approval
3. Not all the studies asked for in the pharm/tox supervisor's memo if eleprase is to be used in women with Hunter's syndrome, may be necessary. Because this disease is so serious and also extremely rare (in women), segment 1 and segment 3 studies would seem unnecessary. Although it would be nice to have studies in female monkeys, for this particular indication and product, the results in males should suffice. The only possibly desirable studies would seem to me to be a teratogenicity study, with a group that is followed until day 10 postnatal.

MEMORANDUM

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

DATE: May 7, 2006

FROM: Supervisory Pharmacologist
Division of Gastroenterology Products, HFD-180

SUBJECT: BLA STN 125151 (ELAPRASE) - Supervisory Addendum to Pharmacology Review

TO: BLA STN 125151

Under BLA STN 125151, the sponsor submitted reports of a preclinical program which included toxicology testing exclusively in male animals in support of the indication of Elaprase (idursulfase) for use in patients with Mucopolysaccharidosis II (MPS II, Hunter syndrome). The testing program in males is in accord with Agency's (CBER) previous recommendations and the X-limited recessive nature of Hunter syndrome. The submission included (1) pharmacology studies of Elaprase in IKO mouse model, (2) pharmacokinetic studies in Sprague-Dawley rats and cynomolgus monkeys, tissue distribution studies in ICR and IKO mice and toxicokinetics in rats and monkeys and (3) single dose i.v. toxicology studies in rats and monkeys, repeated dose 6-month i.v. toxicology study in monkeys and an i.v. Segment I. Fertility and reproductive performance study in male rats. These studies have been reviewed by Dr. Ronald Honchel of this Division and his review is attached.

Testing in single and repeated dose i.v. toxicology was done at limited doses (single dose at 5, 10 & 20 mg/kg and repeated dose at 0.5, 2.5 & 12.5 mg/kg/week). Doses employed in the i.v. reproductive toxicology study in male rats were also limited (0.5, 1.5 & 5.0 mg/twice a week). No target organ of toxicity was identified in the 6-month monkey repeated dose i.v. toxicology study and the highest tested dose was about 8 times the recommended human dose on surface area basis. While testing was done at very low doses (the high dose was 1.6 times the recommended human dose on surface area basis), idursulfase did not disrupt the fertility or reproductive performance of male rats.

RECOMMENDATIONS:

1. From a preclinical standpoint, approval of Elaprase for use in male patients with Hunter syndrome is recommended.
2. The following changes in the preclinical portion of labeling should be incorporated.

a) 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 ELAPRASE.

ELAPRASE at intravenous doses up to 5 mg/kg, administered twice weekly (about 1.6 times the recommended human weekly dose based on body surface area) had no effect on fertility and reproductive performance in male rats.

b) Pregnancy: Teratogenic Effects : Category C.

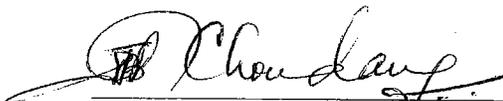
Animal reproduction studies have not been conducted with ELAPRASE. It is also not known whether ELAPRASE can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. ELAPRASE should be given to pregnant woman only if clearly needed.

c) OVERDOSAGE

There is no experience with overdosage of ELAPRASE in humans. Single intravenous doses of idursulfase up to 20 mg/kg were not lethal in male rats and cynomolgus monkeys (approximately 6.5 and 13 times respectively of the recommended human dose based on body surface area) and there were no clinical signs of toxicity.

3. The following postmarketing studies are recommended if Elaprase is intended to be tested or used in cases of Hunter syndrome in females.
 - a) 26-week chronic i.v. toxicology study in female cynomolgus monkeys.
 - b) Segment I. Fertility and reproductive performance study in female rats.
 - c) Segment II. Teratology studies in rats and rabbits.
 - d) Segment III. Prenatal and postnatal study in rats.

The dose selections for the above studies should be preceded by dose ranging studies. The highest doses selected should either produce mild toxicity or they should be the maximum feasible doses.


Jasti B. Choudary, B.V. Sc., Ph.D. Date May 7, 2006
Supervisory Pharmacologist, HFD-180

Cc:
BLA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary

HFD-180/Dr. Honchel
HFD-024/Dr. Jacobs
HFD-048/Dr. Viswanathan



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	BLA STN 125151
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	December 2, 2005
PRODUCT:	Elaprase (Iduronate-2-sulfatase, human, recombinant, human fibroblast cells)
INTENDED CLINICAL POPULATION:	Hunter syndrome
SPONSOR:	Transkaryotic Therapies
DOCUMENTS REVIEWED:	Electronic submission/Nonclinical sections
REVIEW DIVISION:	Division of Gastroenterology Products (HFD-180)
PHARM/TOX REVIEWER:	Ronald Honchel, Ph.D.
PHARM/TOX SUPERVISOR:	Jasti Choudary, B.V.Sc., Ph.D.
DIVISION DIRECTOR:	Brian Harvey, M.D., Ph.D.
PROJECT MANAGER:	Cristi Stark, M.S.

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: From a preclinical standpoint, this NDA may be approved.
- B. Recommendation for nonclinical studies: None.
- C. Recommendations on labeling: Recommended changes to proposed labeling are discussed on pages 69-70 in this Review.

II. Summary of nonclinical findings

- A. Brief overview of nonclinical findings: Since Hunter syndrome is an X-linked recessive disease, nonclinical studies were primarily performed in male animals. There were no significant safety pharmacology findings in monkeys administered single intravenous doses of up to 20 mg/kg idursulfase. There were no adverse effects observed in rats or monkeys in single-dose toxicity studies at intravenous doses of up to 20 mg/kg idursulfase. There were no adverse effects observed in a 6-month repeat-dose toxicity study in monkeys administered weekly intravenous doses of up to 12.5 mg/kg/dose idursulfase. Genotoxicity and carcinogenicity studies were not performed since idursulfase is a naturally occurring human protein that is not likely to have genotoxic or carcinogenic potential. Idursulfase had no effect on fertility or reproductive performance in male rats administered twice weekly doses of up to 5 mg/kg/dose idursulfase. Additional reproductive and developmental toxicity studies were not performed since females are not expected to be treated with idursulfase.
- B. Pharmacologic activity: Iduronate-2-sulfatase is a lysosomal enzyme that catabolizes glycosaminoglycans (GAGs) by cleaving oligosaccharide-linked sulfate moieties from dermatan sulfate and heparan sulfate. Hunter syndrome is an X-linked recessive disease produced by insufficient or defective levels of iduronate-2-sulfatase resulting in the lysosomal accumulation of GAGs in a variety of cells leading to cellular engorgement, dysfunction, and degeneration that eventually results in organ system dysfunction. Idursulfase (Elaprase™) is a purified recombinant analog to human iduronate-2-sulfatase. IKO mice have little or no tissue iduronate-2-sulfatase activity and exhibit many of the cellular and clinical effects observed in Hunter syndrome including increased tissue GAG levels and increased urinary excretion of GAGs compared to their wild type littermates. In a series of studies, male IKO mice were administered idursulfase at doses ranging from 0.1 to 5.0 mg/kg/dose. Idursulfase treatment reduced urinary and tissue (liver, spleen, kidney, and heart) GAG levels with weekly administration with a minimum weekly dose of 1 mg/kg/dose the most

effective dosing regimen (i.e., able to reduce urinary, liver, and spleen GAG levels in IKO mice to levels similar to those observed in wild type mice).

C. Nonclinical safety issues relevant to clinical use: None.

**APPEARS THIS WAY
ON ORIGINAL**

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125151

Review number: 01

Sequence number/date/type of submission: BLA

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Transkaryotic Therapies

Manufacturer for drug substance: ————

Reviewer name: Ronald Honchel, Ph.D.

Division name: Division of Gastroenterology Products

HFD #: 180

Review completion date: April 21, 2006

Drug:

Trade name: Elaprase

Generic name: Idursulfase

Code name: DX006A

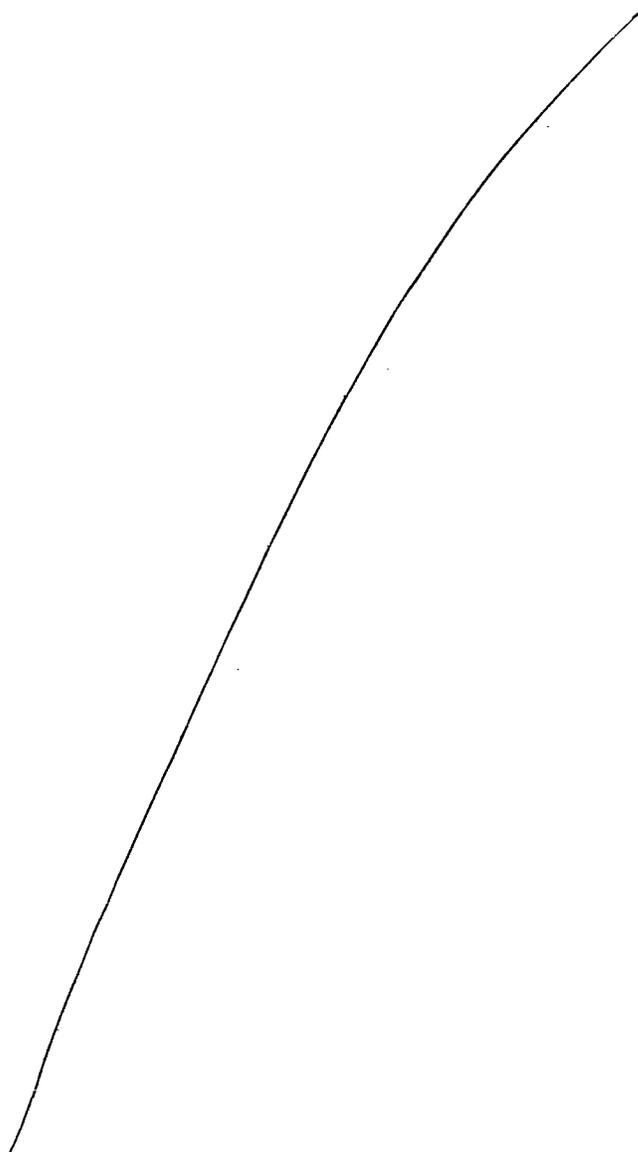
Chemical name: Iduronate-2-sulfatase

CAS registry number: N/A

Molecular formula/molecular weight: Idursulfase is a 525 amino acid glycoprotein with a molecular weight of approximately 76 kD

Structure: The amino acid sequence (provided by the sponsor) is shown in the Figure below.

Figure 3.2.S.3.1-2 Schematic Amino Acid Sequence of Idursulfase



Relevant INDs/NDAs/DMFs: IND — (Original IND submission for Idursulfase)

Drug class: Biological therapeutic/recombinant protein/replacement enzyme

Intended clinical population: Enzyme replacement therapy for patients with Hunter syndrome (Mucopolysaccharidosis II, MPSII).

Clinical formulation: Each single-use vial consists of a solution (3 mL, pH approximately 6) containing 6 mg (2 mg/mL) idursulfase, 24 mg NaCl, 6.75 mg sodium phosphate monobasic monohydrate, 2.97 mg sodium phosphate dibasic heptahydrate, and 0.6µL polysorbate 20.

Route of administration: Intravenous

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Type of Study	Study #	Test Site	Lot #	Review Page #
PHARMACOLOGY				9
PHARMACOKINETICS/ADME				30
ABSORPTION				
Pharmacokinetics of DRX006A following intravenous administration to male Sprague-Dawley rats	110-99-009	1	RD200-001	31
A pharmacokinetic study of DRX006A administered by a single intravenous injection to male cynomolgus monkeys	110-00-009	2	FD911-001	32
DISTRIBUTION				
Tissue distribution of radioactivity following single intravenous injection of ¹²⁵ I-DRX006A in the Sprague-Dawley rat	110-99-010	3	RD200-001	33
COMPARIBILITY				
Comparative biodistribution and pharmacodynamics of idursulfase development lot 3D-14-RC1: studies TKX26 and TKX27	720-110-03-443	4	FD911-001 3D-14-RC1	37
Biodistribution and pharmacodynamics of idursulfase development lot 0201G, Phase I lot FD911-001, and research lot 0202G: effect of content	720-110-03-441	4	0201G FD911-001 0202G	38
The effect of of I2S on pharmacokinetics in Sprague-Dawley rats	690-110-02-371	5	0202G	42
A crossover pharmacokinetic study of two lots of DRX006A administered by intravenous bolus injection to cynomolgus monkeys	110-02-002	2	0201G FD911-004	43
Biodistribution and pharmacodynamic comparison of a Phase II/III clinical drug product lot (FD911-004) with a Phase II/III clinical drug substance lot (D303-006): studies TKX41 and TKX42	720-110-03-444	4	FD911-004 D303-006	44
Pharmacodynamic comparison of a Phase II/III clinical drug product lot (FD924-001) with a commercial process drug product lot (FDB04-003): study TKX 52	720-110-04-634	4	FD924-001 FDB04-003	46
<i>In vivo</i> pharmacodynamic comparison of idursulfase manufactured by the Phase II/III process with the process intended for commercial use: duration of effect as measured by urinary glycosaminoglycan levels	720-110-05-635	4	FD924-001 FDB04-003	48
Biodistribution comparison of idursulfase Phase II/III clinical drug product lot (FD924-001) with a commercial process validation drug product lot (FDB04-003): study TKX51	720-110-04-633	4	FD924-001 FDB04-003	49

A 1-week crossover study of two lots of idursulfase administered by intravenous bolus injection to cynomolgus monkeys	110-05-002	6	FD924-002 FDB04-003	50
Pharmacodynamic comparison of Phase II/III clinical drug substance lot (D303-025) with a commercial process validation drug substance lot (DP04-003): study TKX48	720-110-04-607	4	D303-025 DP04-003	51
Reduction of tissue glycosaminoglycans in Hunter mice after 1, 3, and 5 injections of idursulfase: study TKX50	720-110-04-609	4	D303-025 DP04-003	51
Biodistribution comparison of a phase II/III clinical drug substance lot (D303-025) with a commercial process validation drug substance lot (DP04-003): study TKX47	720-110-04-606	4	D303-025 DP04-003	52
TOXICOLOGY				
Single-dose				
Rat (iv, male only)	6354-160	7	FDB04-003	53
Monkey (iv, male only)	6354-159	7	FDB04-003	56
Repeat-dose				
Monkey				
6-month (iv, male only)	110-99-011	2	RD200-001 RD200-002	58
REPRODUCTIVE TOXICOLOGY				
Segment I				
Rat (iv, male only)	110-04-010	8	FDB04-003	64

1. The animal portion of the study was performed by _____ The pharmacokinetic portion of the study was performed by the sponsor.
2. The animal portion of the study was performed by _____ The pharmacokinetic portion of the study was performed by the sponsor.
3. _____
4. Identified only as performed in the "In Vivo Resources" Department.
5. The animal portion of the study was performed at _____ The pharmacokinetic portion of the study was performed by the sponsor.
6. The animal portion of the study was performed at _____ The pharmacokinetic portion of the study was performed by the sponsor.
7. The animal portion of the study was performed at _____ The pharmacokinetic portion of the study was performed by the sponsor.
8. _____

Studies not reviewed within this submission:

1. Analytical Method Validation Report: Assay for Quantification of DRX006A Concentration (Study # 735-110-03-003).
2. Addendum to Analytical Method Validation Report: Assay for Quantification of DRX006A Concentration in Human Serum (Study # 735-110-03-003A).
3. Assay for DRX006A activity in Sera (Study # 635-110-02-002).
4. Assay for DRX006A activity in Sera – Addendum to Validation Report (Study # 635-110-02-002-A).

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Iduronate-2-sulfatase is a lysosomal enzyme that catabolizes glycosaminoglycans (GAGs). Hunter syndrome is an X-linked recessive disease produced by insufficient or defective levels of iduronate-2-sulfatase resulting in the lysosomal accumulation of GAGs in a variety of cells leading to cellular engorgement, dysfunction, and degeneration that eventually results in organ system dysfunction. Idursulfase is a purified recombinant analog to the naturally occurring enzyme designed for enzyme replacement therapy in Hunter syndrome. After intravenous administration, idursulfase is internalized by cells via membrane mannose-6-phosphate receptors binding to enzyme mannose-6-phosphate residues. Idursulfase is then taken up by lysosomes where it begins catabolizing accumulated GAGs.

IKO mice have little or no tissue iduronate-2-sulfatase activity and exhibit many of the cellular and clinical effects observed in Hunter's syndrome including increased tissue GAG levels and increased urinary excretion of GAGs compared to their wild type littermates. Therefore, primary pharmacodynamics studies were performed in the IKO mouse. Due to the X-linked recessive nature of Hunter syndrome, all pharmacology studies were performed using male animals. In a series of studies, IKO mice were administered varying intravenous dosing regimens at doses of idursulfase ranging from 0.1 to 5.0 mg/kg. Idursulfase treatment produced a reduction in urinary and tissue (liver, spleen, kidney, and heart) GAG levels with weekly administration and a minimum dose of 1 mg/kg/dose the most effective dosing regimen (i.e., urinary, liver, and spleen GAG levels in IKO mice were usually similar to wild type mice with a minimum weekly dose of 1 mg/kg/dose).

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

Iduronate-2-sulfatase is a lysosomal enzyme that catabolizes GAGs by cleaving oligosaccharide-linked sulfate moieties from dermatan sulfate and heparan sulfate. Hunter's syndrome is an X-linked recessive disease produced by insufficient or defective levels of iduronate-2-sulfatase resulting in the lysosomal accumulation of GAGs. Idursulfase is administered intravenously, internalized by cells via membrane mannose-6-phosphate receptors binding to enzyme mannose-6-phosphate residues, and then taken up by lysosomes where it begins catabolizing accumulated GAGs.

Drug activity related to proposed indication:

In Vivo

Preliminary Evaluation of the Effect of DRX006A in the Hunter Mouse Model of MPS II (Study # 720-110-00-180)

Methods:

The IKO mouse model is a genetic knockout model. Carrier females having one good and one knockout iduronate-2-sulfatase allele are bred to wild type males. Male pups then have a 50% chance of inheriting a good allele (wild type phenotype) or the knockout allele (IKO phenotype). IKO mice have little or no tissue iduronate-2-sulfatase activity and exhibit many of the cellular and clinical effects observed in Hunter's syndrome including increased tissue GAG levels and increased urinary excretion of GAGs compared to their wild type littermates.

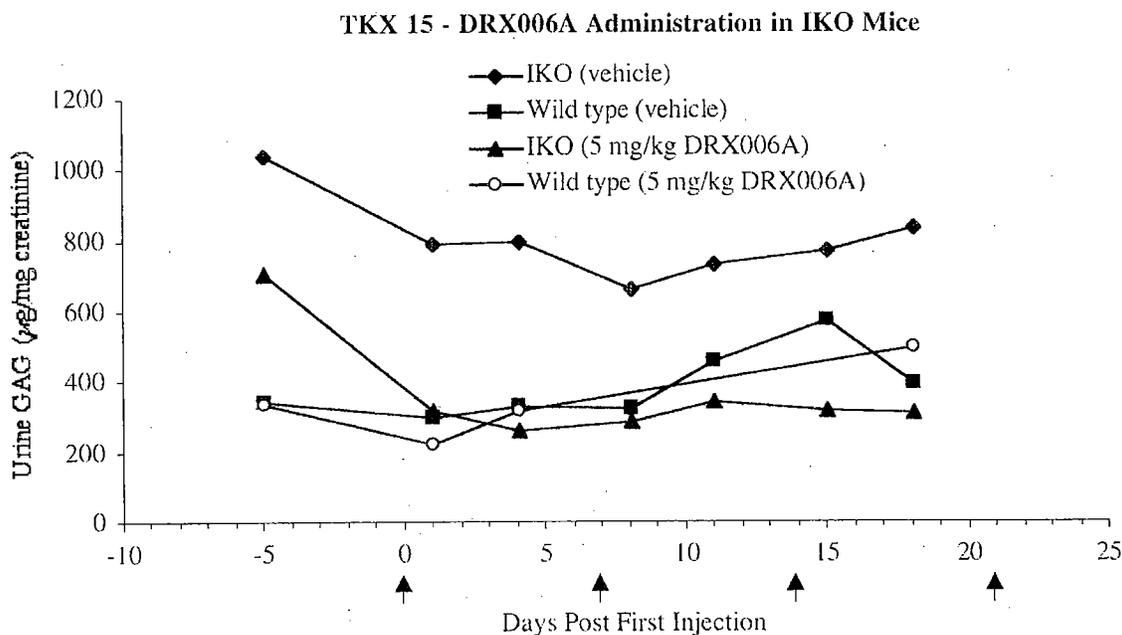
In experiment # TKX 15, 21-week old male IKO or wild type mice (n = 2/group) were administered weekly via intravenous injection 0 or 5 mg/kg/dose DRX006A for 4 consecutive weeks. Blood samples were collected 7 days prior to the first injection and 30 minutes after each injection for plasma iduronate-2-sulfatase activity measurements. Urine was collected and pooled for each group 5 days prior to initiation of dosing and 1 and 4 days after the first 3 injections and urinary GAG concentrations were determined. All animals were sacrificed within 2 days of the final injection. Liver, brain, heart, kidneys, spleen, skin, lungs, and skeletal muscle were collected and a portion from these tissues was fixed in methacarn, embedded in paraffin, and sections were stained with Alcian blue to detect tissue GAGs. Additionally, tissue GAG concentrations were determined on a portion of liver from one animal from the IKO 5 mg/kg group and wild type vehicle group and both animals from the IKO vehicle group. Two additional IKO and wild type mice were sacrificed prior to initiation of dosing to serve as pretreatment controls for histology.

In experiment # TKX 17, male IKO mice (n = 3/group) were administered weekly via intravenous injection 0, 0.1, 0.5, or 2.5 mg/kg/dose DRX006A for 5 consecutive weeks. In addition, wild type mice were administered 0 or 2.5 mg/kg/dose DRX006A. Blood samples for plasma iduronate-2-sulfatase activity determination and urine samples for GAG levels were collected before, during, and for 2 months after the dosing period.

Results:

In experiment # TKX 15, urinary GAG results are shown in the figure below (provided by the sponsor). DRX006A administration reduced urine GAG in IKO mice in to levels similar to the wild-type vehicle group whereas urine GAG levels were not affected in wild type mice after DRX006A administration. Liver GAG levels were 104 and 118 µg/mg protein in the IKO vehicle group, 20.9 µg/mg protein in the IKO 5 mg/kg group, and 13.5 µg/mg protein in the wild type vehicle group. Organ alcian blue staining was

similar between wild type treated and vehicle groups. However, alcian blue staining was decreased in all organs but the brain in treated IKO mice compared to vehicle IKO mice.

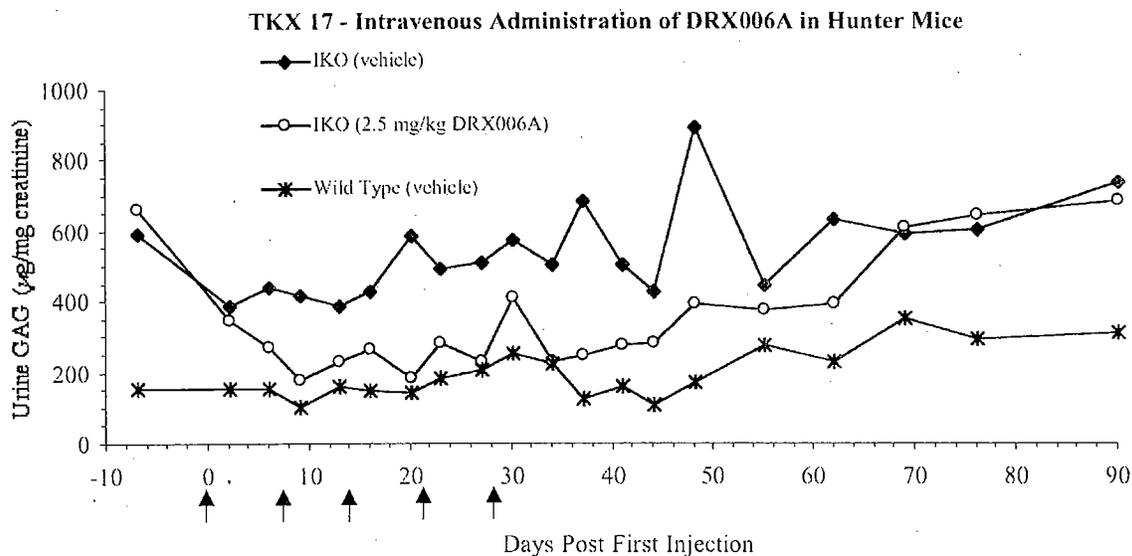


In experiment # TKX 17, plasma iduronate-2-sulfatase results are summarized in the table below (provided by the sponsor, results expressed as U/mL). Plasma iduronate-2-sulfatase activity was similar to vehicle control (approximately 1.5 U/mL) at the 0.1 mg/kg dose in IKO mice. Plasma iduronate-2-sulfatase then increased with increasing dose of DRX006A. Administration of 0.5 and 2.5 mg/kg/dose DRX006A (but not 0.1 mg/kg/dose) produced a decrease in urine GAG levels that remained decreased until 30-40 days after the last injection. Results from the IKO vehicle, IKO 2.5 mg/kg/dose, and wild type vehicle groups are summarized in the figure below (provided by the sponsor).

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IKO 0.1 mg/kg				IKO 0.5 mg/kg			
Injection	Day	Mean	SEM	Injection	Day	Mean	SEM
1	0	1.9	0.1	1	0	6.0	0.7
2	7	1.8	0.1	2	7	6.9	0.1
3	14	1.8	0.1	3	14	7.3	0.3
4	21	2.6	0.2	4	21	8.3	0.4
5	28	1.9	0.1	5	28	6.4	1.1

IKO 2.5 mg/kg				WT 2.5 mg/kg			
Injection	Day	Mean	SEM	Injection	Day	Mean	SEM
1	0	44.5	4.7	1	0	78.8	6.7
2	7	35.8	0.2	2	7	57.2	13.1
3	14	45.5	1.1	3	14	66.3	10.5
4	21	53.4	6.6	4	21	76.8	3.9
5	28	49.1	15.8	5	28	70.8	11.7



Reduction in GAG Levels following IV Dosing with Idursulfase (Study # 720-110-03-436)

Methods:

In experiment TKX 19, male IKO mice (n = 2-4/group) were administered intravenously 0 (vehicle), 0.1, 0.25, 0.5, and 1 mg/kg/dose idursulfase weekly for 5 consecutive weeks.

A separate group of wild type mice were administered vehicle. Urine was collected 6 days prior to the first injection as well as 2 and 6 days after the first 4 injections. Animals were euthanized 2 days after the final injection and liver, kidney, heart, spleen, lungs, skin, skeletal muscle, and brain were collected for tissue GAG determination.

In experiment TKX 21, male mice (n = 2-4/group) were administered intravenously one of the following regimens: 1) vehicle every other week for 8 weeks; 2) 1 mg/kg/dose idursulfase weekly for 8 consecutive weeks; 3) 1 mg/kg/dose idursulfase every other week over an 8 week period; 4) 1 mg/kg/dose idursulfase every 4 weeks over an 8 week period; and 5) a single 1/mg/kg/dose. A separate group of wild type mice were maintained for comparison. Urine was collected 4 days after the first injection, then weekly thereafter. Animals were euthanized on Day 56 and liver, kidney, heart, spleen, lungs, skin, skeletal muscle, and brain were collected for tissue GAG determination.

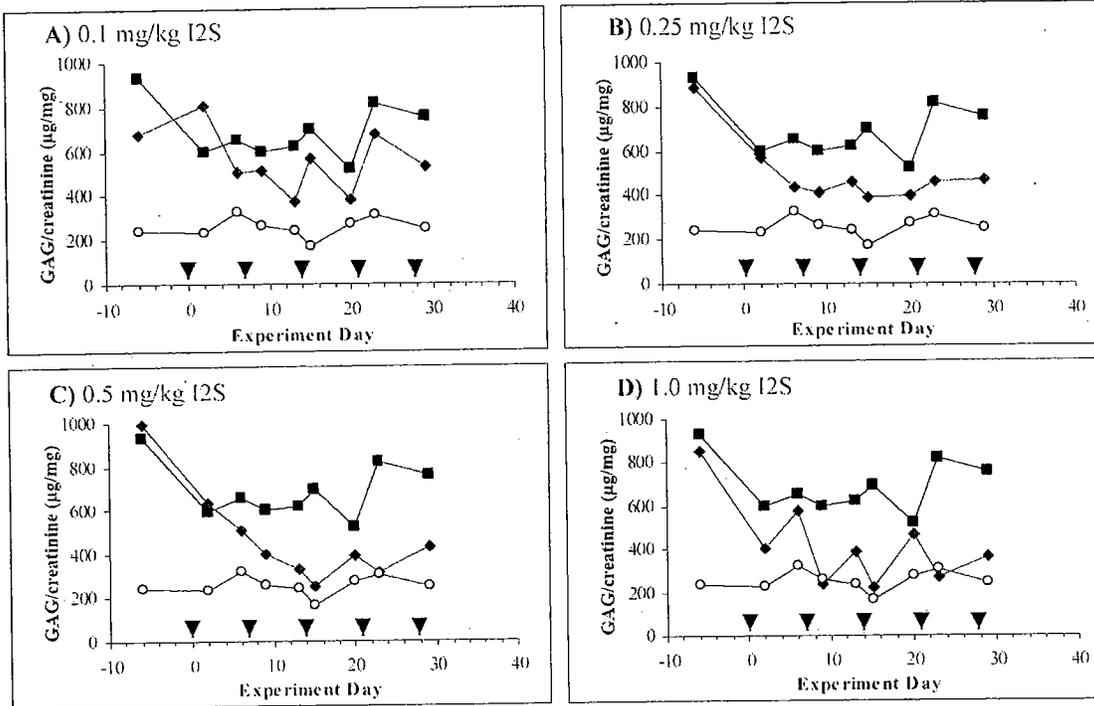
In experiment TKX 29, male mice (n = 6-8/group) were administered intravenously one of the following regimens over a 12-week or 24-week period: 1) vehicle every other week; 2) 1 mg/kg/dose idursulfase weekly; and 3) 1 mg/kg/dose idursulfase every other week. A separate group of wild type mice were maintained for comparison. Urine was collected pretreatment, 5 days after the first injection, and every 4 weeks thereafter. Animals receiving weekly injections were euthanized 7 days after their final injection and animals receiving biweekly injections were euthanized 14 days after their final injection. Liver, kidney, heart, spleen, lungs, skin, skeletal muscle, and brain were collected for tissue GAG determination. Liver and spleen weights were also recorded for animals with 24-week treatment regimens.

Results:

Results for experiment TKX 19 are summarized in Figures 1 and 2A below (provided by the sponsor). A dose-dependent decrease in urinary GAG levels was observed in idursulfase treated mice compared to vehicle controls. The sponsor stated that brain GAG levels were not reported due to observed wide type animal variability. Skeletal muscle GAG levels in IKO vehicle mice were similar to wild type. Tissue GAG levels were decreased at all dose levels in the liver, heart and spleen. Tissue GAG levels were decreased at the 1.0 mg/kg/dose levels in the kidney and skin (although the decrease was not significant in skin). Lung GAG levels were not decreased at any dose level.

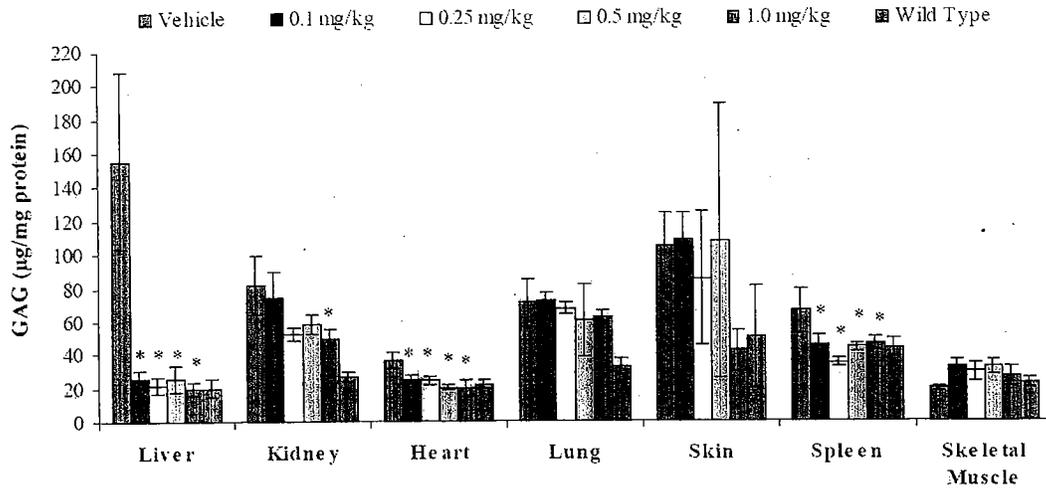
Figure 1: TKX 19 Dose Response Study: Urine GAG Levels Through 5 Weekly I2S Injections

■ IKO treated with vehicle; ◆ IKO treated with I2S; ○ WT control; ▼ indicates injection time point.



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Figure 2A: TKX 19 Dose Response Study: Tissue Extract GAG Levels Following 5 Weekly I2S Injections. Absolute GAG concentrations (error bars: ± 1 SD)



Data Table (Mean ± 1 SD)

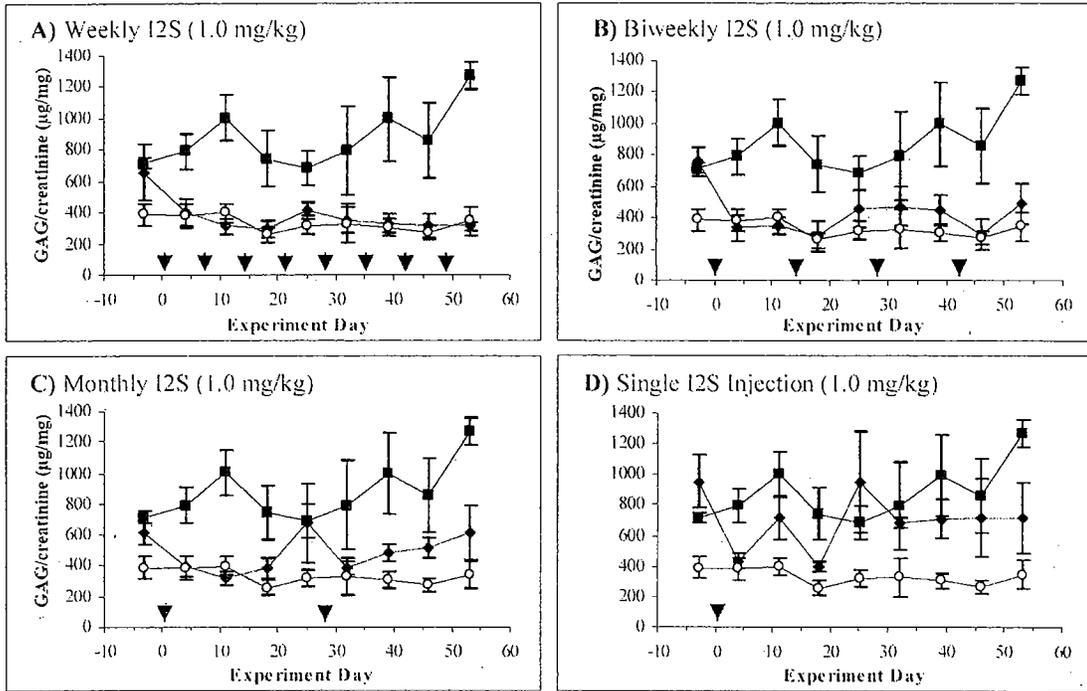
Results expressed as $\mu\text{g GAG} / \text{mg protein}$ in tissue extract

Tissue Extract	IKO Vehicle Controls	I2S Dose (mg/kg, 5 weekly injections)				Wild Type Controls
		0.1	0.25	0.5	1.0	
Liver	155.5 \pm 52.6	25.7 \pm 5.2 *	21.7 \pm 4.5 *	25.5 \pm 8.1 *	19.6 \pm 4.1 *	20.1 \pm 5.6
Kidney	81.7 \pm 17.4	73.6 \pm 16.0	52.3 \pm 4.0	58.2 \pm 5.9	49.0 \pm 6.3 *	26.3 \pm 2.7
Heart	36.3 \pm 5.0	24.8 \pm 2.5 *	24.1 \pm 2.6 *	20.1 \pm 1.0 *	19.8 \pm 4.6 *	21.9 \pm 2.4
Lungs	70.5 \pm 14.2	71.5 \pm 4.6	67.2 \pm 3.1	59.9 \pm 21.2	62.3 \pm 3.7	32.4 \pm 5.2
Skin	104.6 \pm 19.4	107.6 \pm 16.2	84.7 \pm 39.6	106.9 \pm 81.4	42.0 \pm 12.3	50.0 \pm 30.2
Spleen	65.4 \pm 12.9	45.3 \pm 5.7 *	34.6 \pm 2.3 *	43.8 \pm 2.4 *	45.9 \pm 3.8 *	43.4 \pm 5.3
Sk. Muscle	19.2 \pm 1.5	32.2 \pm 3.9	29.4 \pm 5.4	32.1 \pm 4.7	26.5 \pm 5.5	22.8 \pm 2.7

* statistically significant reduction compared to IKO vehicle controls ($p \leq 0.05$)

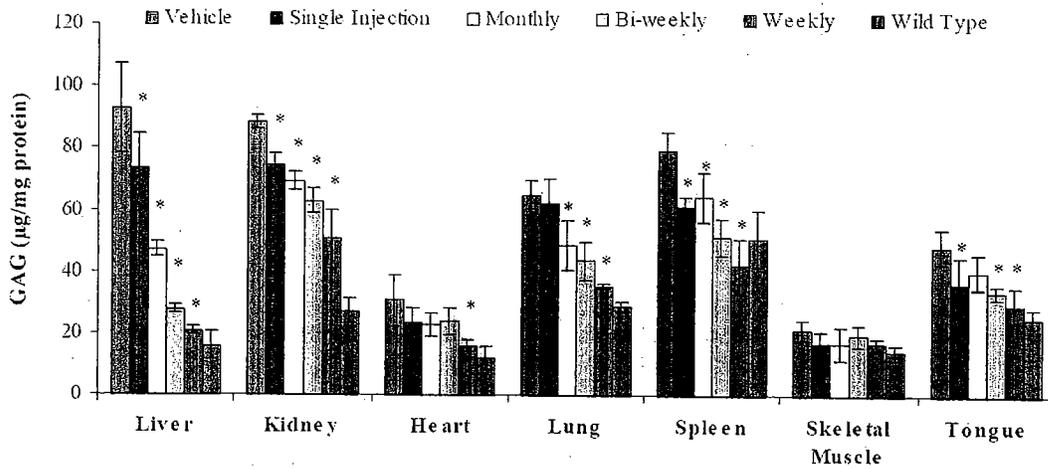
Results for experiment TKX 21 are summarized in Figures 3 and 4A below (provided by the sponsor). Weekly and biweekly 1 mg/kg/dose idursulfase administration to IKO mice reduced urine GAG to levels similar to wild type controls whereas reduced urine GAG levels were not maintained throughout the 8-week period after monthly or single idursulfase administration. The sponsor stated that brain GAG levels were similar to wild type (data not shown). Skeletal muscle GAG levels in IKO vehicle mice were only slightly higher than wild type and there was no significant effect on skeletal muscle GAG levels with idursulfase treatment. Tissue GAG levels for the other organs in the IKO mouse were decreased most effectively by weekly idursulfase administration.

Figure 3: TKX 21 Eight Week I2S Dose Frequency Study: Urine GAG Levels (error bars : \pm 1 SD)
 ■ IKO treated with vehicle; ◆ IKO treated with I2S; ○ WT control; ▼ indicates injection time point.



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Figure 4A: TKX 21 Eight Week I2S Dose Frequency Study: Tissue Extract GAG Levels. Absolute GAG Concentrations (error bars: ± 1 SD)



Data Table (Mean ± 1 SD)

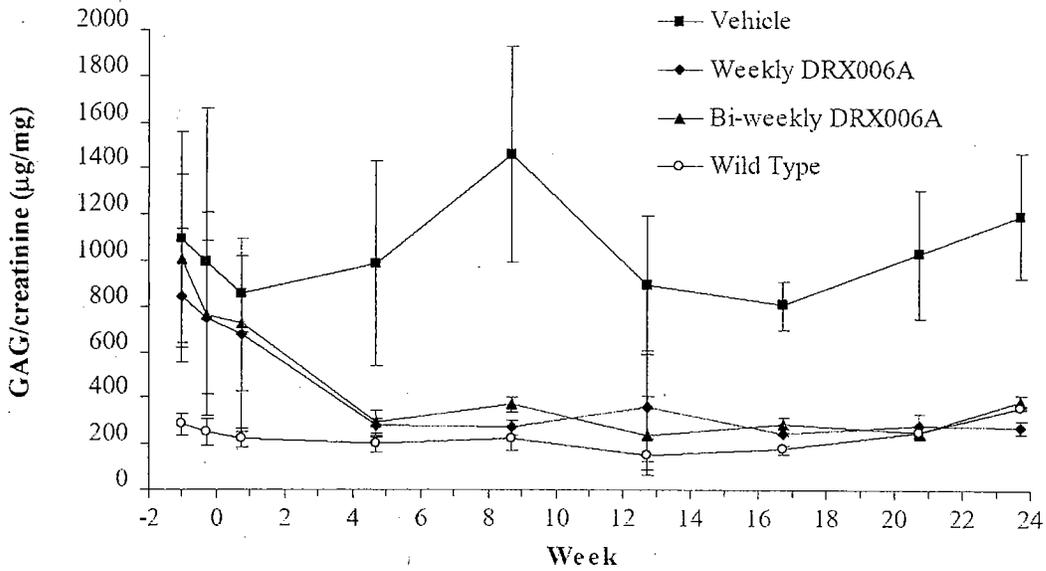
Results expressed as μg GAG / mg protein in tissue extract

Tissue Extract	IKO Vehicle Controls	Dose Frequency 1.0 mg/kg (IKO mice)				Wild Type Controls
		Single	Monthly	Bi-weekly	Weekly	
Liver	92.6 \pm 14.7	73.4 \pm 11.3 *	47.1 \pm 2.6 *	27.7 \pm 1.2 *	20.5 \pm 1.4 *	15.7 \pm 4.5
Kidney	88.1 \pm 2.1	74.5 \pm 3.7 *	69.0 \pm 2.9 *	62.6 \pm 4.0 *	50.4 \pm 9.6 *	27.2 \pm 4.2
Heart	30.4 \pm 8.1	22.9 \pm 5.3	22.5 \pm 3.9	23.6 \pm 4.3	15.7 \pm 2.0 *	11.9 \pm 3.6
Lungs	64.8 \pm 4.5	61.8 \pm 8.0	48.6 \pm 8.0 *	43.3 \pm 6.2 *	35.2 \pm 1.0 *	28.6 \pm 1.7
Spleen	79.3 \pm 5.9	60.7 \pm 3.3 *	64.1 \pm 8.1 *	51.1 \pm 5.9 *	41.9 \pm 8.8 *	50.6 \pm 9.2
Sk. Muscle	21.1 \pm 3.3	16.9 \pm 3.8	16.6 \pm 5.3	19.2 \pm 3.5	16.5 \pm 1.9	13.8 \pm 2.3
Tongue	48.1 \pm 5.5	35.9 \pm 9.0 *	39.9 \pm 5.7	33.3 \pm 2.2 *	29.1 \pm 5.7 *	24.8 \pm 2.9

* statistically significant reduction compared to IKO vehicle controls ($p \leq 0.05$)

Results for experiment TKX 29 are summarized in Figures 5, 6A, and 7A below (provided by the sponsor). Weekly and biweekly 1 mg/kg/dose idursulfase administration to IKO mice reduced urine GAG to levels similar to wild type controls. Tissue GAG levels in IKO mice were significantly decreased with weekly and/or biweekly regimens in the liver, kidney, heart, lungs, and spleen whereas skeletal muscle and tongue GAG levels were not significantly decreased with any regimen at any timepoint. Brain tissue GAG levels were not reported. There were no significant effects on spleen weights after 24 weeks of treatment. However, liver weight in IKO mice was similar to wild type controls after 24 weeks of weekly or biweekly idursulfase treatment (see Figure 8 below, provided by the sponsor).

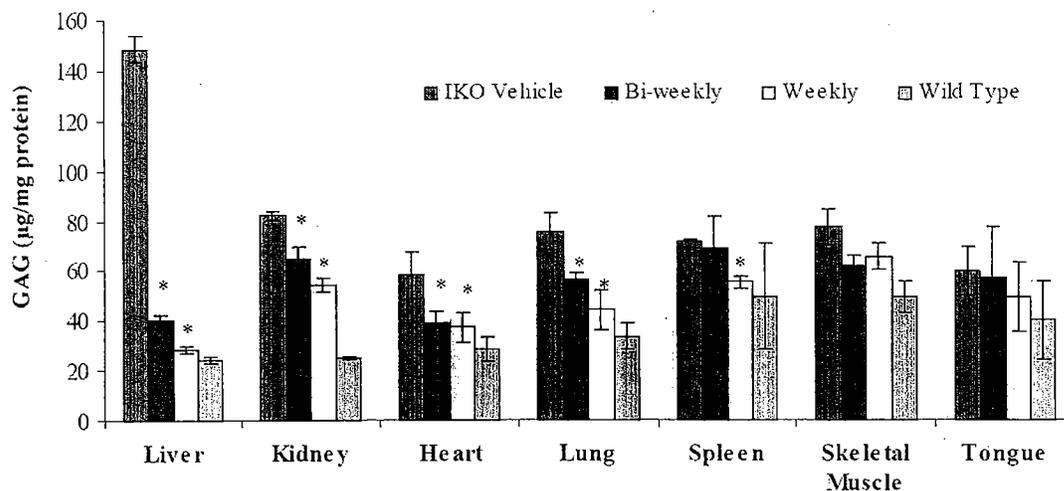
Figure 5: TKX 29 Long Term I2S Dose Frequency Study: Urine GAG Levels (error bars: \pm 1 SD)



All animals started receiving IV I2S (1.0 mg/kg) injections at day 0. Half of the animals in each group (3 in the vehicle group and 4 in the remaining groups) were sacrificed after 12 weeks and the remaining animals were sacrificed after 24 weeks for tissue extract GAG analysis.

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Figure 6A: TKX 29 Tissue Extract GAG reduction in IKO Mice Treated Weekly or Biweekly (1.0 mg/kg) for 12 Weeks. Absolute GAG Concentrations (error bars: ± 1 SD)



Data Table (Mean \pm 1 SD)

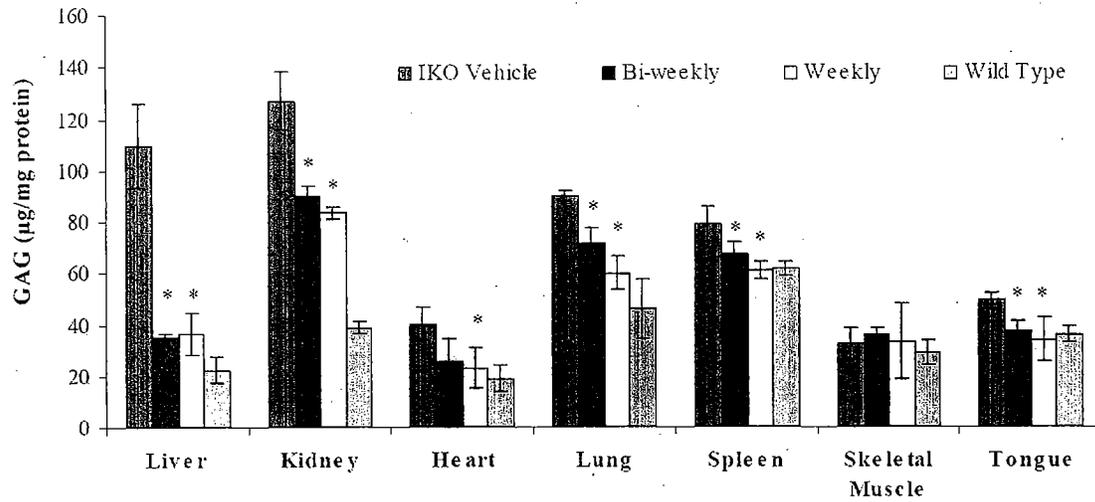
Results expressed as $\mu\text{g GAG} / \text{mg protein}$ in tissue extract.

Tissue Extract	IKO Vehicle Controls	Dose Frequency 1.0 mg/kg (IKO mice)		Wild Type Controls
		Bi-weekly	Weekly	
Liver	148.6 \pm 5.4	40.3 \pm 1.9 *	28.4 \pm 1.3 *	24.3 \pm 1.1
Kidney	82.1 \pm 1.6	64.4 \pm 5.0 *	54.3 \pm 2.8 *	25.0 \pm 0.9
Heart	58.0 \pm 9.4	38.7 \pm 4.7 *	37.1 \pm 6.1 *	28.4 \pm 5.1
Lungs	75.3 \pm 7.7	56.4 \pm 2.7 *	44.1 \pm 7.7 *	33.4 \pm 5.4
Spleen	71.6 \pm 0.4	68.7 \pm 13.0	55.2 \pm 2.4 *	49.5 \pm 21.3
Sk. Muscle	77.4 \pm 7.1	61.3 \pm 4.1	65.4 \pm 5.2	48.9 \pm 6.2
Tongue	59.5 \pm 9.8	57.0 \pm 20.4	49.3 \pm 14.0	40.0 \pm 15.7

* statistically significant reduction compared to IKO vehicle controls ($p \leq 0.05$)

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Figure 7A: TKX 29: Tissue Extract GAG reduction in IKO Mice Treated Weekly (1.0 mg/kg) or Biweekly for 24 Weeks. Absolute GAG Concentrations (error bars: ± 1 SD)



Data Table (Mean \pm 1 SD)

Results expressed as μg GAG / mg protein in tissue extract.

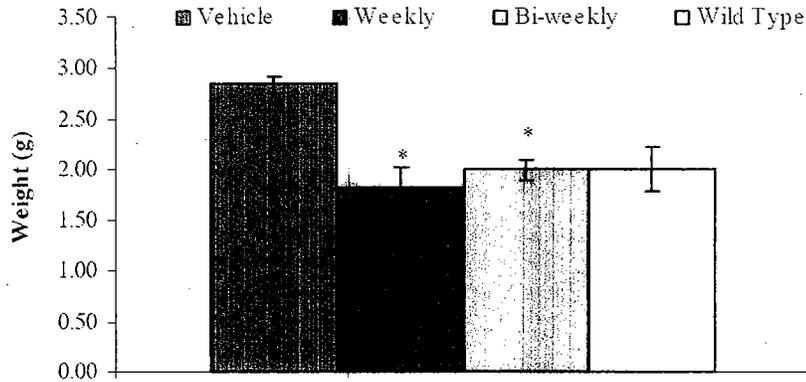
Tissue Extract	IKO Vehicle Controls	Dose Frequency 1.0 mg/kg (IKO mice)		Wild Type Controls
		Bi-weekly	Weekly	
Liver	109.5 \pm 16.3	35.2 \pm 1.2 *	36.3 \pm 8.0 *	22.0 \pm 5.0
Kidney	126.6 \pm 11.2	89.3 \pm 4.1 *	83.1 \pm 2.2 *	38.5 \pm 2.2
Heart	39.9 \pm 6.5	25.5 \pm 8.7	22.9 \pm 7.7 *	18.8 \pm 5.4
Lungs	89.7 \pm 2.2	71.1 \pm 6.3 *	59.7 \pm 6.5 *	45.9 \pm 11.6
Spleen	78.6 \pm 6.8	67.1 \pm 4.5 *	60.6 \pm 3.4 *	61.8 \pm 2.7
Sk. Muscle	32.2 \pm 6.1	35.3 \pm 3.2	33.1 \pm 14.8	28.7 \pm 4.9
Tongue	49.5 \pm 2.3	36.8 \pm 4.4 *	33.8 \pm 8.3 *	35.7 \pm 3.0

* statistically significant reduction compared to IKO vehicle controls ($p \leq 0.05$)

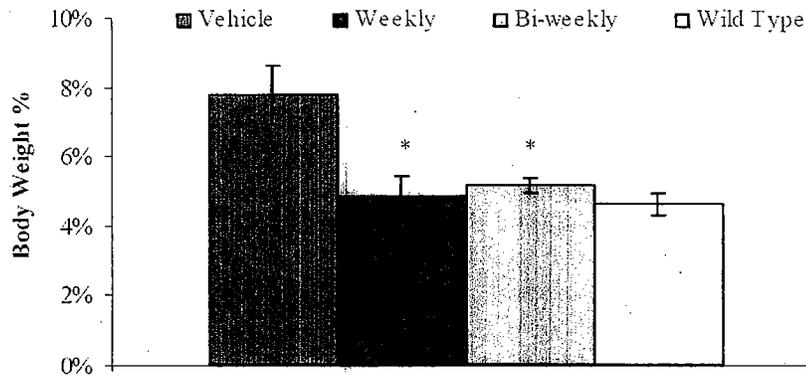
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Figure 8: TKX 29: Reduction of Liver Weights in IKO Mice Following 24 Weeks of I2S Treatment (error bars: ± 1 SD)

A. Absolute Liver Weights



B. Relative Liver Weights



Liver Weight Data Table (Mean \pm 1 SD)

Treatment	Weight (g)	Body Weight %
Vehicle	2.83 \pm 0.08	7.8 \pm 0.9
Weekly	1.81 \pm 0.21 *	4.9 \pm 0.5 *
Biweekly	1.99 \pm 0.10 *	5.2 \pm 0.2 *
Wild Type	2.00 \pm 0.22	4.6 \pm 0.3

* statistically significant reduction compared to IKO vehicle controls ($p \leq 0.05$)

The Effect of Dose Level and Frequency of Idursulfase Administration on GAG Reduction in IKO Mice (Study # 720-110-03-440)

Methods:

Male IKO mice were administered intravenously as shown in the figure (provided by the sponsor) below. Urinary GAG levels were measured prior to initiation of dosing and on Days 27, 55, 83, 108, 139, and 167. Animals were sacrificed 24 weeks after the first injection and tissue GAG levels were determined in liver, kidney, heart, lung, spleen, and brain. Spleen and liver weights were also recorded. The presence of antibodies to idursulfase was determined using an ELISA technique with plates coated with idursulfase.

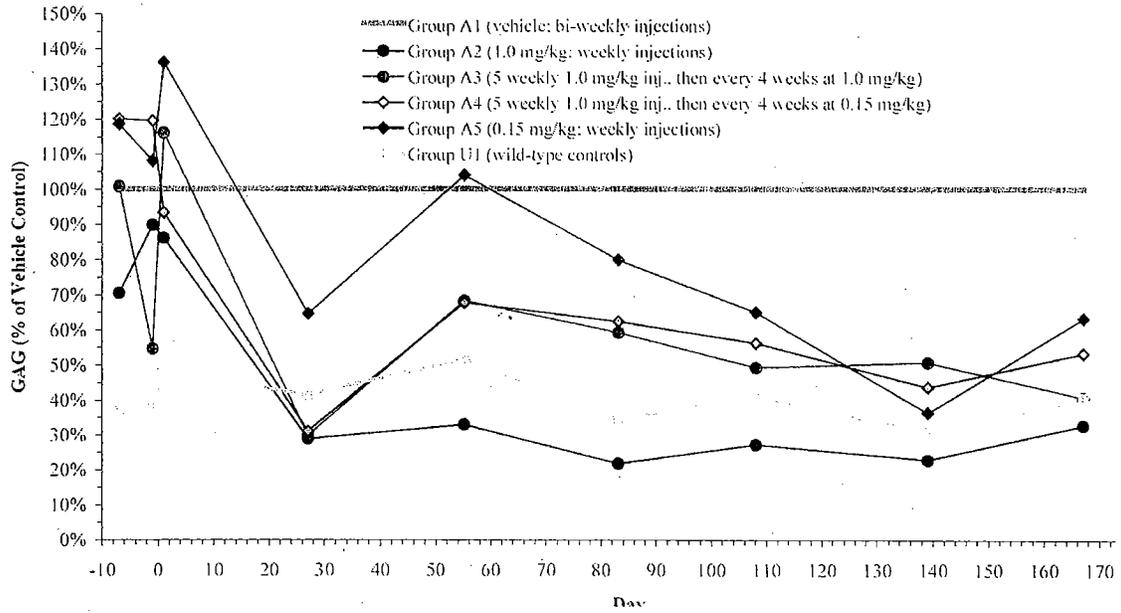
Group	No. of Mice	Dose (mg/kg)	
		Weeks 1-5	Weeks 6 - 23
A1	3	vehicle	vehicle
A2	3	1.0 weekly	1.0 weekly
A3	4	1.0 weekly	1.0 monthly
A4	4	1.0 weekly	0.15 monthly
A5	4	0.15 weekly	0.15 weekly

Wild type mice (n = 4) served as a control for normal urine and tissue GAG levels

Results:

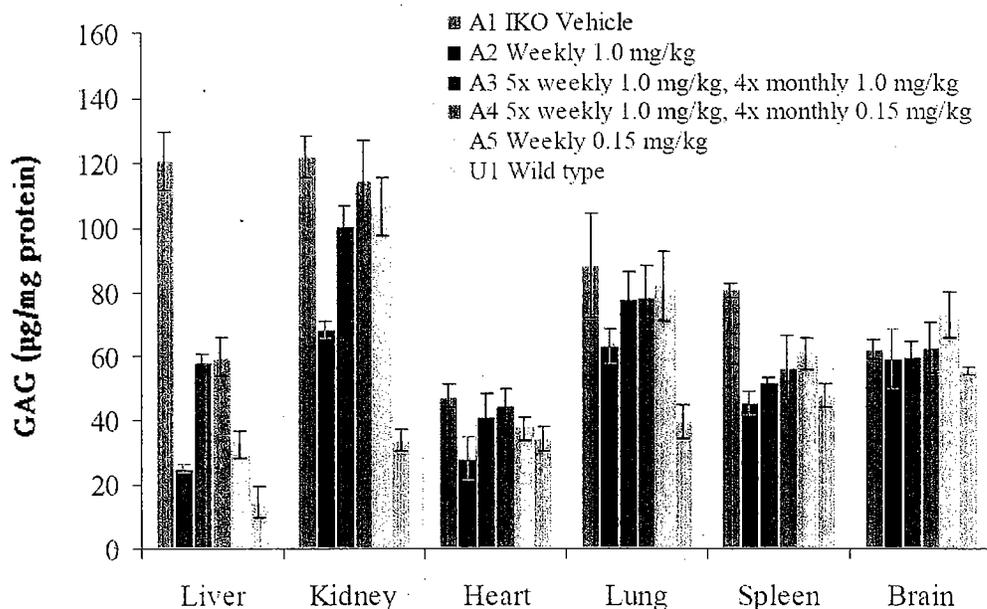
Urinary GAG levels were lower than wild type controls in IKO mice administered weekly 1 mg/kg/dose idursulfase (see Figure 2 below, provided by the sponsor). Urinary GAG levels were generally between wild type and IKO vehicle control levels for all other treatment regimens with IKO mice administered weekly 0.15 mg/kg/dose idursulfase taking the longest time to be consistently reduced. Brain GAG levels were similar between IKO and wild type mice (see Figure 3 below, provided by the sponsor). Tissue GAG levels were significantly reduced for all dosing regimens in liver and spleen. Tissue GAG levels were significantly reduced in kidney, heart, and lung in the weekly 1 mg/kg/dose regimen. Kidney GAG levels were also significantly decreased in the regimen 1 mg/kg/dose weekly for 5 weeks followed by 1 mg/kg/dose monthly for the remainder of the study. Mean spleen weights were not significantly decreased with any treatment regimen, whereas mean liver weights were significantly decreased compared to IKO vehicle controls in all treatment regimens (see Figure 5 below, provided by the sponsor). In the ELISA assay, a group A3 mouse was positive for anti-idursulfase at all dilutions evaluated (1:25, 1:50, and 1:100) and a group A4 mouse was positive at the 1:25 dilution only.

Figure 2: Urine GAG levels as a percent of the vehicle control group.



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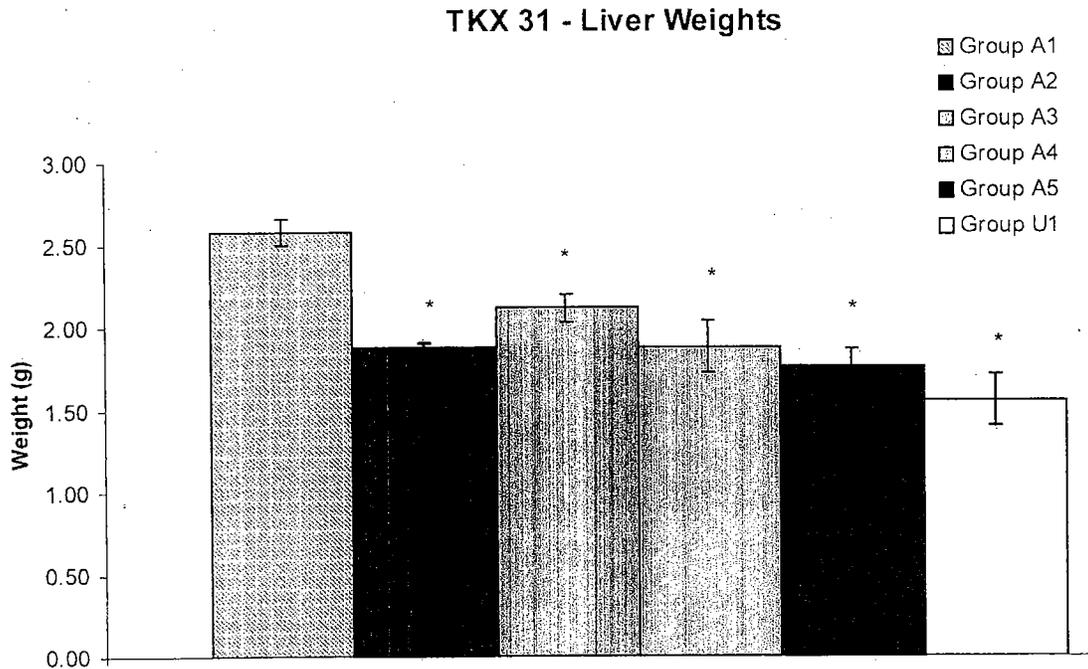
Figure 3: Tissue GAG levels.



Means significantly different from the vehicle control are bolded.

Tissue Extract	Dosage Group					
	A1 IKO Vehicle Control	A2 Weekly 1.0 mg/kg	A3 5 weekly 1.0 mg/kg 4x monthly 1.0 mg/kg	A4 5x weekly 1.0 mg/kg 4x monthly 0.15 mg/kg	A5 Weekly 0.15 mg/kg	U1 Wild Type
Liver	120.7 ± 9.2	25.1 ± 1.1	58.2 ± 2.3	59.9 ± 6.3	32.6 ± 4.2	14.6 ± 4.8
Kidney	121.9 ± 6.3	68.4 ± 2.7	100.1 ± 6.6	114.3 ± 12.5	106.4 ± 9.1	33.8 ± 3.6
Heart	47.7 ± 3.6	28 ± 6.5	41 ± 7.6	44.7 ± 5.1	37.5 ± 3.7	34.2 ± 4.1
Lung	88.1 ± 16.3	63 ± 5.3	77.9 ± 8.1	78.4 ± 9.5	81.7 ± 10.9	39.7 ± 5.5
Spleen	80.5 ± 2.3	45.3 ± 3.6	52.1 ± 1.1	56.3 ± 10	60.5 ± 4.9	47.8 ± 3.7
Brain	62.2 ± 3	59.3 ± 9.3	59.9 ± 4.6	62.6 ± 7.7	72.6 ± 7	55.4 ± 1.3

Figure 5: Liver weights



* Significantly different from Group A1 at $p < 0.05$

Intraperitoneal Injection of Idursulfase in Neonatal IKO Mice (Study # 720-110-03-449)

Methods:

The objective of this study was to determine if administration of idursulfase shortly after birth could reduce the incidence of skeletal abnormalities in aging IKO mice. Male IKO mice were administered intraperitoneally vehicle or 5 mg/kg/dose idursulfase on Days 4 and 11 of age (intravenous injection not possible due to small size). Animals were then administered intravenously vehicle or 1 mg/kg/dose idursulfase weekly beginning at approximately 4 weeks of age (when intravenous injections could be easily performed).

Results:

This study was halted when the mice were approximately 7 weeks old due to unexpected reactions occurring in 20 of 28 idursulfase treated mice. Three animals had died after intravenous idursulfase administration. Ataxia, dyspnea, and mild hypothermia was observed shortly after intravenous administration in 17 of the remaining 25 mice with animals typically recovering by 45 minutes after dosing. Animals administered vehicle appeared normal. The sponsor concluded that the unexpected reactions were likely due to intraperitoneal dosing of idursulfase in neonatal mice ultimately leading to an anaphylactic reaction too subsequent intravenous dosing. However, the sponsor did not

rule out that intravenous dosing to neonatal animals would not also produce the same unexpected reactions.

2.6.2.3 Secondary pharmacodynamics

There were no secondary pharmacodynamics studies submitted.

2.6.2.4 Safety pharmacology

Single-Dose Intravenous Injection Toxicity Study with Idursulfase in Male Cynomolgus Monkeys with Safety Pharmacology (Study # 6354-159)

This was a GLP study. Only the safety pharmacology portion of the study is reviewed in this section. Male cynomolgus monkeys (n = 4/group) were administered intravenously 0, 5, 10, and 20 mg/kg idursulfase via slow bolus injection (dose volume = 10 mL/kg). Clinical signs, body temperature, respiration rate, EKGs, blood pressure, and arterial oxygen saturation were recorded at least once prior to dosing and approximately 2 and 24 hours after dosing. There were no drug-related clinical signs of toxicity. There were no drug-related effects on respiration rate and body temperature (see Table 2 below, provided by the sponsor), EKG parameters including heart rate (see Table 3 below, provided by the sponsor), blood pressure (see Table 4 below, provided by the sponsor), and arterial oxygen saturation (see Table 5 below, provided by the sponsor) in this study.

**Table 2
Physical Examinations**

DAY:	PREDOSE PHASE		RESPIRATIONS BREATH/M	
	25		1	DOSING PHASE 2
M A L E S				
GROUP 1	- 0.00	MG/KG		
MEAN		62	58	53
SD		4.0	10.6	6.8
N		4	4	4
GROUP 2	- 5.00	MG/KG		
MEAN		52	49	47
SD		15.7	8.9	10.0
N		4	4	4
GROUP 3	- 10.00	MG/KG		
MEAN		65	61	51
SD		5.0	6.8	6.0
N		4	4	4
GROUP 4	- 20.00	MG/KG		
MEAN		59	68	51
SD		5.0	4.6	3.8
N		4	4	4

TABLE 2
 PHYSICAL EXAMINATIONS
 BODY TEMPERATURE (CELCIUS)
 DEGREES

DAY:	PREDOSE PHASE		DOSING PHASE	
	25	1	2	2
M A L E S				
GROUP 1 - 0.00 MG/KG				
MEAN	39.3	38.5		38.2
SD	0.31	1.17		0.27
N	4	4		4
GROUP 2 - 5.00 MG/KG				
MEAN	38.5	38.7		38.4
SD	0.43	0.16		0.32
N	4	4		4
GROUP 3 - 10.00 MG/KG				
MEAN	38.9	38.9		38.5
SD	0.29	0.20		0.15
N	4	4		4
GROUP 4 - 20.00 MG/KG				
MEAN	39.1	39.1		38.4
SD	0.55	0.33		0.50
N	4	4		4

Table 3
 Summary Electrocardiographic Examinations

INTERVAL: PRE (MALES)

ANIMAL NUMBER	HEART RATE (BEATS/MINUTE)	P-R INTERVAL (SECONDS)	QRS INTERVAL (SECONDS)	Q-T INTERVAL (SECONDS)	QTc (SECONDS)
Group 1 - 0.00 mg/kg					
N	4	4	4	4	4
MEAN	249	0.07	0.04	0.16	0.23
S.D.	19.9	0.010	0.000	0.000	0.002
Group 2 - 5.00 mg/kg					
N	4	4	4	4	4
MEAN	242	0.07	0.04	0.17	0.23
S.D.	12.4	0.012	0.000	0.010	0.009
Group 3 - 10.00 mg/kg					
N	4	4	4	4	4
MEAN	246	0.07	0.04	0.15	0.22
S.D.	11.0	0.010	0.000	0.012	0.012
Group 4 - 20.00 mg/kg					
N	4	4	4	4	4
MEAN	252	0.06	0.04	0.15	0.22
S.D.	28.1	0.000	0.000	0.012	0.009

TABLE 3
 SUMMARY ELECTROCARDIOGRAPHIC EXAMINATIONS

INTERVAL: DAY 1, 2-HR POST DOSE (MALES)

ANIMAL NUMBER	HEART RATE (BEATS/MINUTE)	P-R INTERVAL (SECONDS)	QRS INTERVAL (SECONDS)	Q-T INTERVAL (SECONDS)	QTc (SECONDS)
Group 1 - 0.00 mg/kg					
N	4	4	4	4	4
MEAN	236	0.07	0.04	0.17	0.23
S.D.	26.1	0.010	0.000	0.010	0.007
Group 2 - 5.00 mg/kg					
N	4	4	4	4	4
MEAN	203	0.08	0.04	0.18	0.24
S.D.	20.4	0.016	0.000	0.019	0.017
Group 3 - 10.00 mg/kg					
N	4	4	4	4	4
MEAN	230	0.07	0.04	0.17	0.23
S.D.	13.3	0.010	0.000	0.012	0.011
Group 4 - 20.00 mg/kg					
N	4	4	4	4	4
MEAN	246	0.07	0.04	0.15	0.22
S.D.	31.4	0.010	0.000	0.012	0.012

TABLE 3
SUMMARY ELECTROCARDIOGRAPHIC EXAMINATIONS
INTERVAL: DAY 1, 24-HR POST DOSE (MALES)

ANIMAL NUMBER	HEART RATE (BEATS/MINUTE)	P-R INTERVAL (SECONDS)	QRS INTERVAL (SECONDS)	Q-T INTERVAL (SECONDS)	QTc (SECONDS)
Group 1 - 0.00 mg/kg					
N	4	4	4	4	4
MEAN	233	0.07	0.04	0.18	0.24
S.D.	21.0	0.010	0.000	0.010	0.009
Group 2 - 5.00 mg/kg					
N	4	4	4	4	4
MEAN	230	0.07	0.04	0.17	0.23
S.D.	12.4	0.010	0.000	0.012	0.011
Group 3 - 10.00 mg/kg					
N	4	4	4	4	4
MEAN	230	0.07	0.04	0.17	0.23
S.D.	18.6	0.012	0.000	0.010	0.008
Group 4 - 20.00 mg/kg					
N	4	4	4	4	4
MEAN	227	0.06	0.04	0.17	0.23
S.D.	31.9	0.000	0.000	0.012	0.009

Table 4
Mean Blood Pressure Data

PREDOSE PHASE - DAY 25				
	SYS MM HG	DIAS MM HG	MAP	WDTH CM
M A L E S				
GROUP 1 - 0.00 MG/KG				
MEAN	150	86	107	4
SD	20.0	20.9	18.5	0.0
N	4	4	4	4
GROUP 2 - 5.00 MG/KG				
MEAN	142	99	113	4
SD	19.8	8.1	9.8	0.0
N	4	4	4	4
GROUP 3 - 10.00 MG/KG				
MEAN	131	61	84	4
SD	10.6	13.0	6.5	0.0
N	4	4	4	4
GROUP 4 - 20.00 MG/KG				
MEAN	152	95	114	4
SD	13.8	36.9	28.8	0.0
N	4	4	4	4

SYS - SYSTOLIC PRESSURE
DIAS - DIASTOLIC PRESSURE
MAP - MEAN ARTERIAL PRESSURE
WDTH - CUFF WIDTH

TABLE 4 BLOOD PRESSURE				
DOSING PHASE - DAY 1				
	SYS MM HG	DIAS MM HG	MAP	WDTH CM
M A L E S				
GROUP 1 - 0.00 MG/KG				
MEAN	143	100	114	3
SD	38.4	33.1	54.8	0.0
N	4	4	4	4
GROUP 2 - 5.00 MG/KG				
MEAN	140	60	87	3
SD	22.2	26.3	23.2	0.0
N	4	4	4	4
GROUP 3 - 10.00 MG/KG				
MEAN	139	63	88	3
SD	11.1	23.7	15.5	0.0
N	4	4	4	4
GROUP 4 - 20.00 MG/KG				
MEAN	124	67	86	3
SD	23.9	26.6	20.6	0.0
N	4	4	4	4

SYS - SYSTOLIC PRESSURE
DIAS - DIASTOLIC PRESSURE
MAP - MEAN ARTERIAL PRESSURE
WDTH - CUFF WIDTH

TABLE 4
BLOOD PRESSURE

		DOSING PHASE - DAY 2			
		SYS MM HG	DIAS MM HG	MAP	WDTH CM
M A L E S					
GROUP 1	- 0.00 MG/KG				
MEAN		128	84	99	3
SD		14.0	16.8	16.9	0.0
N		4	4	4	4
GROUP 2	- 5.00 MG/KG				
MEAN		128	72	91	3
SD		28.6	27.1	22.8	0.0
N		4	4	4	4
GROUP 3	- 10.00 MG/KG				
MEAN		98	73	81	3
SD		21.7	15.5	17.2	0.5
N		4	4	4	4
GROUP 4	- 20.00 MG/KG				
MEAN		112	63	79	3
SD		43.4	25.7	28.2	0.0
N		4	4	4	4

SYS - SYSTOLIC PRESSURE
DIAS - DIASTOLIC PRESSURE
MAP - MEAN ARTERIAL PRESSURE
WDTH - CUFF WIDTH

Table 5
Mean Pulse Oximetry Data

Day:	Pulse Oximetry Value (%)		
	Predose phase	Dosing Phase	
	25	1	2
Group 1 - 0.00 mg/kg			
N	4	4	4
Mean	88.5	83.5	77.8
S.D.	2.65	4.43	5.50
Group 2 - 5.00 mg/kg			
N	4	4	4
Mean	86.3	78.0	84.5
S.D.	2.99	5.60	9.40
Group 3 - 10.00 mg/kg			
N	4	4	4
Mean	85.8	86.5	88.0
S.D.	5.68	5.07	4.40
Group 4 - 20.00 mg/kg			
N	4	4	4
Mean	92.3	86.3	88.8
S.D.	3.50	9.64	11.62

2.6.2.5 Pharmacodynamic drug interactions

There were no pharmacodynamic drug interaction studies submitted.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

In humans, pharmacokinetic data was obtained from clinical studies where idursulfase was intravenously-administered to patients with Hunter syndrome. In one study (n = 3), mean C_{max} and systemic exposure (AUC) values were 5.8 µg/mL and 708 min·µg/mL, respectively, after the first single 1-hour intravenous infusion of 0.5 mg/kg idursulfase (the recommended weekly dose) with a t_{1/2} of approximately 2 hours and a clearance of 0.73 mL/minute/kg. In another study (n = 12), mean C_{max} and systemic exposure values were 1.5 µg/mL and 212 min·µg/mL, respectively, after the first single 3-hour intravenous infusion of 0.5 mg/kg idursulfase with a t_{1/2} of 46 minutes and a clearance of 2.8 mL/minute/kg. In this study, pharmacokinetic parameters were similar between Week 27 and Day 1.

In animals, single-dose intravenous pharmacokinetic studies were performed in rats and monkeys. In general, C_{max} and systemic exposure increased with increasing dose. Mean C_{max} values in rats were 15.1, 60.4, and 419 µg/mL and mean systemic exposure values were 17.9, 104, and 860 µg·hr/mL after single-dose intravenous administration of 0.5, 2.5, and 12.5 mg/kg idursulfase, respectively. Mean C_{max} values in monkeys were 2.6, 8.8, 12.7, and 41.4 µg/mL and mean systemic exposure values were 98, 257, 950, and 3095 µg·hr/mL after single-dose intravenous administration of 0.1, 0.3, 0.5, and 1.5 mg/kg idursulfase, respectively. The elimination half-life increased (0.32, 0.78, and 0.86 hr at 0.5, 2.5, and 12.5 mg/kg idursulfase, respectively, in rat; 4.2, 8.3, and 22.9 min at 0.1, 0.3, and 0.5 mg/kg idursulfase, respectively, in monkey) and clearance decreased (0.53, 0.41, and 0.24 mL/minute/kg at 0.5, 2.5, and 12.5 mg/kg idursulfase, respectively, in rat; 1.03, 1.18, 0.58, and 0.49 mL/minute/kg at 0.1, 0.3, 0.5, and 1.5 mg/kg idursulfase, respectively, in monkeys) with increasing dose. The sponsor suggested that this finding was possibly due to saturation of clearance mechanisms with increasing dose. A distribution study using ¹²⁵I-labeled idursulfase was performed in rats. Radioactivity was detectable in the blood, plasma, and urine through the 48 hour timepoint. The highest tissue radioactivity concentrations at the 4 hour timepoint were observed in the thyroid gland, liver, stomach, bone marrow, kidneys, spleen, bone (femur), and adrenal glands. Radioactivity concentration were decreased in all tissues except thyroid gland at the 24 hour timepoint compared to the 4 hour timepoint and in all tissues examined at the 48 hour timepoint compared to the 24 hour timepoint.

2.6.4.2 Methods of Analysis

Please see individual study reviews.

2.6.4.3 Absorption

Pharmacokinetics of DRX006A following Intravenous Administration to Male Sprague-Dawley Rats (Study # 110-99-009)

Methods: This was a GLP study. Male Sprague-Dawley rats (n = 5/group) were administered intravenously 0 (vehicle – 20 mM sodium phosphate, pH 6.5, 137 mM sodium chloride, 0.02% Tween 20), 0.5, 2.5, or 12.5 mg/kg ¹²⁵I-idursulfase (lot # D200-01). Blood samples were collected from the vehicle group 10 minutes and 24 hours after dosing. Blood samples were collected from treated groups prior to dosing, at 5, 10, 15 and 30 minutes after dosing, and at 1, 1.5, 2, 3, 4, 6, 8, and 24 hours after dosing. The methodology used to determine serum idursulfase levels was not provided.

Results: Pharmacokinetic results are summarized in Table 1 (provided by the sponsor) below. System exposure (AUC) increased with increasing dose. The elimination half-life ($t_{1/2}$ alpha) increased and clearance (CL) decreased with increasing dose.

Table 1
Summary of Mean Serum DRX006A Pharmacokinetic Parameters Following a Single Intravenous Dose of DRX006A to Male Sprague-Dawley Rats

Parameter (Units)	Parameter Value (SD)					
	Group 2 (0.5 mg/kg)		Group 3 (2.5 mg/kg)		Group 4 (12.5 mg/kg)	
	Mean	(SD)	Mean	(SD)	Mean ^a	(SD) ^a
C_{max} ($\mu\text{g/mL}$)	15.1	(3.6)	60.4	(2.0)	419	(27)
AUC ($\mu\text{g}\cdot\text{hr/mL}$)	17.9	(8.9)	104	(14)	860	(54)
$t_{1/2}$ alpha (hr)	0.32	(0.10)	0.78	(0.10)	0.86	(0.13)
$t_{1/2}$ beta (hr)	7.0 ^b	(9.2) ^b	6.2	(0.7)	4.4	(1.3)
CL (mL/min)	0.157	(0.049)	0.121	(0.012)	0.072	(0.005)
CL (mL/min/kg)	0.527	(0.164)	0.407	(0.045)	0.243	(0.017)
V_{ss} (mL)	46.1	(40.7)	30.8	(5.2)	15.5	(1.8)
V_{ss} (mL/kg)	156	(140)	104	(18)	52.9	(6.6)
C_{max}/Dose ($\mu\text{g/mL/mg/kg}$)	30	(7)	24	(1)	33	(2)
AUC/Dose ($\mu\text{g}\cdot\text{hr/mL/mg/kg}$)	36	(18)	42	(5)	69	(5)

- a: Excluding the values for Animal No. 20; serum DRX006A concentrations suggest that the animal may have received a lower dose than expected.
b: Excluding the value for Animal No. 6, which is markedly higher than for others in Group 2, the mean (SD) was 2.9 (0.7) hr.

A Pharmacokinetic Study of DRX006A Administered by a Single Intravenous Injection to Male Cynomolgus Monkeys (Study # 110-00-009)

Methods: This was a GLP study except there was no Quality Assurance auditing. Male cynomolgus monkeys (n = 2/male/group) were administered intravenously 0.1, 0.3, 0.5, and 1.5 mg/kg DRX006A (animal groups were called 1-4, respectively, drug lot # FD911-001) via slow bolus injection. The vehicle was phosphate buffer saline, pH 6.5, with 0.02% Tween-20. Blood samples were collected as indicated below. Serum DRX006A levels were determined using ELISA methodology. Animals were returned to the colony after the last blood sample was collected.

Group 1: Predose, 2, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600 min

Groups 2/3: Predose, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 1440 min

Group 4: Predose, 5, 15, 30, 60, 120, 180, 240, 360, 480, 600, 1440 min

Results: Pharmacokinetic results are summarized in the table below (provided by the sponsor). Systemic exposure (AUC) increased with increasing dose. A dose-related decrease in clearance (Cl) and increase in initial $t_{1/2}$ alpha was observed. The sponsor suggested that this finding was possibly due to saturation of clearance mechanisms with increasing dose.

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Group No.	Animal No.	Body Weight (Kg)	Cmax (µg/mL)	AUC (min* µg/mL)	T _{1/2} (α) (min)	T _{1/2} (β) (min)	MRT (min)	Cl (mL/min)	Normalized Cl (mL/min/kg)	V _{ss} (mL)	V _{ss} (% BW)
1	F13130M	4.4	2.9	104	4.2	92	117	4.23	0.96	493	11.2%
0.1 mg/kg	F13131M*	3.4	2.2	91	na	122	135	3.75	1.10	505	14.9%
Mean		3.9	2.6	98	4.2	107	126	3.99	1.03	499	13.0%
SD		0.71	0.5	9		21.2	12.7	0.34	0.10	8.5	2.6%
N		2	2	2	1	2	2	2	2	2	2
Group No.	Animal No.	Body Weight (Kg)	Cmax (µg/mL)	AUC (min* µg/mL)	T _{1/2} (α) (min)	T _{1/2} (β) (min)	MRT (min)	Cl (mL/min)	Normalized Cl (mL/min/kg)	V _{ss} (mL)	V _{ss} (% BW)
2	F13110M	3.7	7.9	277	11.2	99	93	4.00	1.08	373	10.1%
0.3 mg/kg	F13143M	4.1	9.7	236	5.9	45	52	5.21	1.27	273	6.7%
Mean		3.9	8.8	257	8.3	72	73	4.61	1.18	323	8.4%
SD		0.28	1.3	29	4.2	36.5	29.0	0.85	0.13	70.7	2.4%
N		2	2	2	2	2	2	2	2	2	2
Group No.	Animal No.	Body Weight (Kg)	Cmax (µg/mL)	AUC (min* µg/mL)	T _{1/2} (α) (min)	T _{1/2} (β) (min)	MRT (min)	Cl (mL/min)	Normalized Cl (mL/min/kg)	V _{ss} (mL)	V _{ss} (% BW)
3	F13114M	3.4	16.9	1244	21.4	320	305	1.37	0.40	417	12.3%
0.5 mg/kg	F13165M	4.4	9.1	655	24.3	153	148	3.36	0.76	499	11.3%
Mean		3.9	12.7	950	22.9	237	227	2.36	0.58	458	11.8%
SD		0.71	5.1	416	2.1	118.1	111.0	1.41	0.26	58.0	0.7%
N		2	2	2	2	2	2	2	2	2	2
Group No.	Animal No.	Body Weight (Kg)	Cmax (µg/mL)	AUC (min* µg/mL)	T _{1/2} (α) (min)	T _{1/2} (λ ₂) (min)	MRT (min)	Cl (mL/min)	Normalized Cl (mL/min/kg)	V _{ss} (mL)	V _{ss} (% BW)
4	F13112M*	4.0	32.0	2659	na	151	140	2.10	0.52	293	7.3%
1.5 mg/kg	F13155M*	3.7	50.8	3930	na	257	151	1.67	0.45	251	6.8%
Mean		3.9	41.4	3095		204	146	1.88	0.49	272	7.1%
SD		0.21	13.3	333		75.0	7.8	0.31	0.05	29.7	0.4%
N		2	2	2		2	2	2	2	2	2
* = Noncompartmental Analysis Performed and used elimination half life on T _{1/2} (λ ₂)											

2.6.4.4 Distribution

Tissue Distribution of Radioactivity following Single Intravenous Injection of ¹²⁵I-DRX006A in the Sprague-Dawley Rat (Study # 110-99-010)

Methods: This was a GLP study. Male Sprague-Dawley rats (n = 12/group) were administered intravenously (vehicle – 20 mM sodium phosphate, pH 6.5, 137 mM sodium chloride, 0.02% Tween 20) 0.5 and 12.5 ¹²⁵I-DRX006A (lot # RD200-001; specific activity = 14 x 10⁶ CPM/mL of dosing solution). The test article was formulated for a targeted radioactivity level of 5-10 µCi/rat in a dose volume of 5 mL/kg. The specific activity of the test article was 173,240,633 dpm/mg for the 0.5 mg/kg group and 7,317,839 dpm/mg. Four animals from each group were euthanized at 4, 24, and 48 hours after dosing. In addition to blood and urine, the following were collected at the 4 and 24 hour timepoints:

adipose tissue (epididymal)	kidneys
adrenal glands	liver
bone (femur)	lungs
bone marrow (femur)	lymph nodes (mesenteric)
brain	muscle (leg adductor)
epididymides	pancreas
eyes	pituitary gland
gastrointestinal tract (GIT):	prostate gland
stomach	salivary glands
small intestine	skin
large intestine	spleen
GIT contents (collected separately)	testes
harderian glands	thymus
heart	thyroid gland (and parathyroid glands)

In addition to blood and urine, the following were collected at the 48 hour timepoint:

- bone (femur)
- heart
- kidneys
- liver
- lungs
- spleen
- testes
- thyroid gland (and parathyroid glands)

Blood, urine, and tissues were also collected from a single vehicle injected control animals in order to determine background radioactivity levels. Tissue samples were first solubilized and then mixed with liquid scintillation fluid. Plasma and urine radioactivity were directly added to liquid scintillation fluid. Radioactivity levels were determined using liquid scintillation counting.

Results: Mean radioactivity concentrations results are summarized in the tables below (provided by the sponsor). Radioactivity was detectable in the blood, plasma, and urine through the 48 hour timepoint. The highest tissue radioactivity concentrations at the 4 hour timepoint were observed in the thyroid gland, liver, stomach, bone marrow, kidneys, spleen, bone (femur), and adrenal glands. Radioactivity concentrations were decreased in all tissues except thyroid gland at the 24 hour timepoint compared to the 4 hour timepoint and in all tissues examined at the 48 hour timepoint compared to the 24 hour timepoint. Approximately 72-73% of administered radioactivity had been excreted via urine by 48 hours after dosing. The radioactivity observed in intestinal contents suggested that a portion of the radioactivity was excreted by the biliary route.

Group 2: At a Mean Dose of 0.54 mg/kg

Concentration of Radioactivity, $\mu\text{g eq/g}$ ^a			
Sample	4 h	24 h	48 h
Plasma ^b	0.690 \pm 0.138	0.103 \pm 0.011	0.053 \pm 0.004
Blood ^b	0.508 \pm 0.106	0.071 \pm 0.007	0.037 \pm 0.003
Adrenal Glands	0.606 \pm 0.207	0.143 \pm 0.052	-
Bone (Femur)	0.335 \pm 0.116	0.066 \pm 0.022	0.035 \pm 0.010
Bone Marrow (Femur)	0.552 \pm 0.238	0.105 \pm 0.044	-
Brain	0.016 \pm 0.003	0.004 \pm 0.001	-
Epididymal Fat (Adipose tissue)	0.056 \pm 0.026	0.007 \pm 0.008	-
Epididymides	0.184 \pm 0.044	0.035 \pm 0.008	-
Eyes	0.087 \pm 0.020	0.016 \pm 0.001	-
Heart	0.156 \pm 0.045	0.032 \pm 0.009	0.014 \pm 0.001
Harderian Glands	0.150 \pm 0.026	0.024 \pm 0.005	-
Kidneys	0.390 \pm 0.045	0.103 \pm 0.008	0.067 \pm 0.006
Large Intestine	0.108 \pm 0.014	0.022 \pm 0.003	-
Liver	1.445 \pm 0.521	0.427 \pm 0.098	0.291 \pm 0.034
Lymph Nodes (Mesenteric)	0.269 \pm 0.077	0.062 \pm 0.008	-
Lungs	0.233 \pm 0.057	0.038 \pm 0.006	0.025 \pm 0.002
Muscle (Leg Adductor)	0.048 \pm 0.010	0.011 \pm 0.001	-
Pancreas	0.133 \pm 0.016	0.023 \pm 0.004	-
Pituitary Gland	0.139 \pm 0.059	0.013 \pm 0.015	-
Prostate Gland	0.254 \pm 0.143	0.032 \pm 0.008	-
Salivary Glands	0.154 \pm 0.038	0.027 \pm 0.004	-
Small Intestine	0.200 \pm 0.053	0.039 \pm 0.005	-
Skin (Whole body)	0.229 \pm 0.018	0.046 \pm 0.008	-
Spleen	0.543 \pm 0.218	0.151 \pm 0.073	0.085 \pm 0.028
Stomach	0.627 \pm 0.134	0.075 \pm 0.004	-
Testes	0.216 \pm 0.060	0.035 \pm 0.007	0.017 \pm 0.001
Thymus	0.100 \pm 0.021	0.014 \pm 0.002	-
Thyroid Gland (and parathyroid glands)	430 \pm 166	740 \pm 244	479 \pm 220

^a Mean \pm SD, N=4. The values for individual animals are presented in Appendices 2, 3 and 4.

^b Plasma and blood concentration units are $\mu\text{g eq/mL}$.

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Group 3: At a Mean Dose of 12.5 mg/kg

Concentration of Radioactivity, $\mu\text{g eq/g}$ ^a				
Sample	4 h	24 h	48 h	
Plasma ^b	23.584 \pm 7.926	2.262 \pm 0.078	1.164 \pm 0.240	
Blood ^b	19.289 \pm 1.632	1.640 \pm 0.113	0.793 \pm 0.149	
Adrenal Glands	7.101 \pm 2.783	1.523 \pm 0.096	-	
Bone (Femur)	7.346 \pm 2.683	1.909 \pm 0.184	0.857 \pm 0.118	
Bone Marrow (Femur)	11.378 \pm 3.672	1.867 \pm 0.471	-	
Brain	0.457 \pm 0.126	0.080 \pm 0.011	-	
Epididymal Fat (Adipose tissue)	2.221 \pm 1.152	0.234 \pm 0.163	-	
Epididymides	5.099 \pm 1.787	1.022 \pm 0.133	-	
Eyes	2.195 \pm 0.633	0.429 \pm 0.062	-	
Heart	4.617 \pm 1.799	0.655 \pm 0.173	0.300 \pm 0.032	
Harderian Glands	3.610 \pm 1.187	0.594 \pm 0.116	-	
Kidneys	9.884 \pm 2.070	2.301 \pm 0.130	1.668 \pm 0.172	
Large Intestine	3.045 \pm 0.742	0.654 \pm 0.029	-	
Liver	25.319 \pm 6.131	5.648 \pm 0.695	2.372 \pm 0.057	
Lymph Nodes (Mesenteric)	5.854 \pm 6.966	1.776 \pm 0.273	-	
Lungs	6.015 \pm 1.903	1.032 \pm 0.132	0.517 \pm 0.024	
Muscle (Leg Adductor)	1.414 \pm 0.214	0.259 \pm 0.090	-	
Pancreas	3.367 \pm 0.786	0.503 \pm 0.050	-	
Pituitary Gland	4.985 \pm 1.994	0.000 \pm 0.000	-	
Prostate Gland	4.951 \pm 0.823	0.885 \pm 0.432	-	
Salivary Glands	3.577 \pm 1.007	0.650 \pm 0.085	-	
Small Intestine	5.327 \pm 0.794	0.870 \pm 0.102	-	
Skin (Whole body)	4.843 \pm 0.690	0.937 \pm 0.587	-	
Spleen	9.477 \pm 2.961	3.490 \pm 0.093	1.459 \pm 0.298	
Stomach	18.198 \pm 11.935	1.648 \pm 0.372	-	
Testes	5.907 \pm 2.341	0.911 \pm 0.055	0.337 \pm 0.020	
Thymus	2.156 \pm 0.467	0.328 \pm 0.034	-	
Thyroid Gland (and parathyroid glands)	7243 \pm 2607	14418 \pm 7169	14231 \pm 2888	

^a Mean \pm SD, N=4. The values for individual animals are presented in Appendices 2, 3 and 4.

^b Plasma and blood concentration units are $\mu\text{g eq/mL}$.

Mean Content of Radioactivity in Urine Excreted by Male Sprague-Dawley Rats
Following Single Intravenous Injection of ¹²⁵I-DRX006A

Percent of Dose					
Group 2			Group 3		
4 h	24 h	48 h	4 h	24 h	48 h
12.66 \pm 4.44	49.72 \pm 5.77	9.95 \pm 3.08	9.02 \pm 5.21	56.32 \pm 9.98	8.03 \pm 1.77

2.6.4.5 Metabolism

There were no metabolism studies submitted. After intravenous administration, idursulfase is removed from the circulation via cellular uptake. Once internalized into the cell, idursulfase is thought to be transported to its site of action within cellular lysosomes where idursulfase is thought to eventually be degraded via protein hydrolysis.

2.6.4.6 Excretion

There were no specific studies submitted to determine the extent of idursulfase excretion or the routes of elimination. After idursulfase is degraded via protein hydrolysis the peptide/amino acid products are thought to be incorporated into the body's amino acid pool.

2.6.4.7 Pharmacokinetic drug interactions

There were no pharmacokinetic drug interaction studies submitted.

2.6.4.8 Other Pharmacokinetic Studies

Comparative Biodistribution and Pharmacodynamics of Idursulfase Development Lot 3D-14-RC1: Studies TKX 26 and TKX 27 (Study # 720-110-03-443)

Methods: The objective of these experiments was to compare the biodistribution and efficacy of Phase I/II clinical lot # 3D-14-RC1 to Phase II/III development lot # FD911-001 (this lot was used in clinical studies # TKT008 and TKT018). The GLP status of these experiments was not stated.

In experiment TKX 26, female ICR mice were administered intravenously 1 mg/kg Idursulfase lot # 3D-14-RC1 (n = 6), 1 mg/kg idursulfase lot # FD199-001 (n = 6), or vehicle (n = 3). Three animals from each group were sacrificed 2 hours after dosing and the remaining animals were sacrificed at 24 hours after dosing. A blood sample was collected on each animal at sacrifice and the liver, kidneys, heart, lungs, spleen, and brain were removed, weighed, and snap frozen. Idursulfase levels in tissue extracts and serum were then determined using ELISA.

In experiment TKX 27, male IKO mice (n = 4/group) were administered intravenously vehicle and 0.25 or 1.0 mg/kg of each of idursulfase lot weekly on Days 0, 7, 14, and 21. A group of wild type littermates were also administered vehicle. Urine was collected at various timepoints throughout the study for urine GAG level determination. All animals were sacrificed on Day 28 and the liver, kidneys, heart, lungs, spleen, and brain were removed, weighed, and snap frozen. Urine and tissue extract GAG levels were then determined using a dimethylmethylene blue assay.

Results: Results from experiment TKX 26 are summarized in Table 2 (provided by the sponsor) below. Serum idursulfase (I2S) levels were significantly decreased at the 2 hour

timepoint, and liver and kidney idursulfase levels were significantly increased at the 24 hour timepoint, for the 3D-14-RC1 lot compared to the FD911-001 lot.

Table 2. I2S Comparative Tissue Biodistribution in ICR Mice (Study TKX 26)

Tissue	Hours Post-Injection	Mean I2S (ng) Recovered (\pm 1SD)		Mean % of Administered Dose (\pm 1SD) †	
		Lot FD911-001	Lot 3D-14-RC1	Lot FD911-001	Lot 3D-14-RC1
Liver	2	10408.4 \pm 334.7	11592.6 \pm 1896.0	33.6% \pm 0.79%	37.3% \pm 5.0%
	24	2969.8 \pm 504.3	4388.2 \pm 759.1	9.9% \pm 1.0%	14.1% \pm 1.7% *
Kidneys	2	202.1 \pm 29.8	168.8 \pm 10.6	0.65% \pm 0.06%	0.55% \pm 0.04%
	24	19.0 \pm 0.6	31.8 \pm 5.4	0.06% \pm 0.01	0.10% \pm 0.02% *
Heart	2	30.0 \pm 2.3	24.4 \pm 1.5	0.097% \pm 0.012%	0.079% \pm 0.002%
	24	2.8 \pm 0.8	2.7 \pm 1.0	0.009% \pm 0.002%	0.009% \pm 0.003%
Lungs	2	66.6 \pm 25.6	35.8 \pm 3.8	0.22% \pm 0.09%	0.12% \pm 0.01%
	24	5.8 \pm 0.6	7.4 \pm 5.7	0.02% \pm 0.003%	0.02% \pm 0.02%
Spleen	2	431.7 \pm 148.8	395.8 \pm 141.1	1.38% \pm 0.40%	1.29% \pm 0.50%
	24	172.4 \pm 7.4	169.4 \pm 25.2	0.58% \pm 0.08%	0.55% \pm 0.06%
Brain	2	3.9 \pm 0.3	2.6 \pm 2.2	0.013% \pm 0.001%	0.008% \pm 0.007%
	24	ND	ND	ND	ND
Serum	2	2154.0 \pm 89.0	501.4 \pm 57.8	7.0% \pm 0.58%	1.6% \pm 0.21% *
	24	24.9 \pm 6.4	ND	0.08% \pm 0.01%	ND

ND = not detectable

n=3 mice per time point per treatment

* significantly different from FD911-001 at $p \leq 0.05$ (statistical analysis was only performed on % of administered dose)

† Total amount recovered was 41-43% of the administered dose at 2 hours and 11-15% at 24 hours. No I2S was detected in vehicle treated controls (n=3).

In experiment TKX-27, there were no significant differences in tissue GAG levels between lots. In addition, a dose-dependent decrease in urine GAG levels was observed in IKO treated groups compared to IKO vehicle controls and the decrease in urine GAG levels was similar between lots.

Biodistribution and Pharmacodynamics of Idursulfase Development Lot 0201G, Phase I Lot FD911-001, and Research Lot 0202G: Effect of α content (Study # 720-110-03-441)

Methods: The GLP status of these experiments was not stated. In experiment TKX 34, idursulfase lot 0202G was chromatographically fractionated by α

content (moles/mole idursulfase) for test articles used in this study is shown in Text Table 2 (provided by the sponsor) below. Groups of ICR mice (sex and number not stated) were administered intravenously vehicle or the test article identified in Text Table 2, and then sacrificed 2 hours after injection. Liver, spleen, kidneys, heart and lung were removed, weighed and tissue extract idursulfase concentrations were determined using ELISA.

Text Table 2: Test Article Concentrations

Animal Group No.	Test Article Designation	I2S Concentration (mg/mL)	Content (moles/mole I2S)
A	Phase I/II lot FD911-001	5.0	—
B	0202G 0.25M NaCl	3.4	—
C	0202G 1M NaCl	2.5	—
D	0202G Pool 3	3.9	not determined
E	Vehicle	0	

Pool 3: 280 mL of — fraction + 277 mL of — fraction + 276 mL of — fraction + 268 mL of — fraction

In experiment TKX 40, the treatment groups are shown in Text Table 3 (provided by the sponsor) below. Male IKO mice were treated intravenously weekly on Days 0, 7, 14, 21, 28, and 35. Urine was collected at various timepoints throughout the study. All mice were sacrificed on Day 35 two hours after the final dose was administered and liver, spleen, kidney and heart were collected. Tissue extract idursulfase concentrations were determined by ELISA. GAG levels were then determined using a dimethylmethylene blue assay.

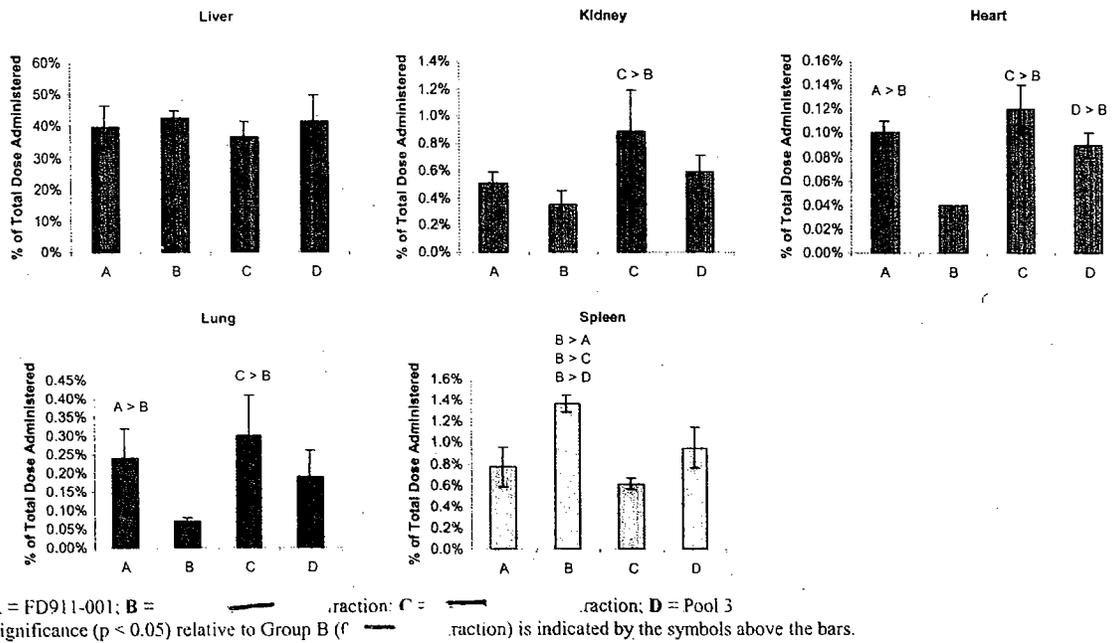
Text Table 3: Experimental Design using IKO Mice

Group No.	Test Article	No. of Mice	Dose (mg/kg)
A1	0202G — fraction	4	—
A2	0202G — fraction	4	—
A3	0201G	4	—
A4	0201G	4	—
A5	FD911-001	4	—
A6	FD911-001	4	—
A7	Vehicle control	4	0
U1	Wild type mice	4	

Results:

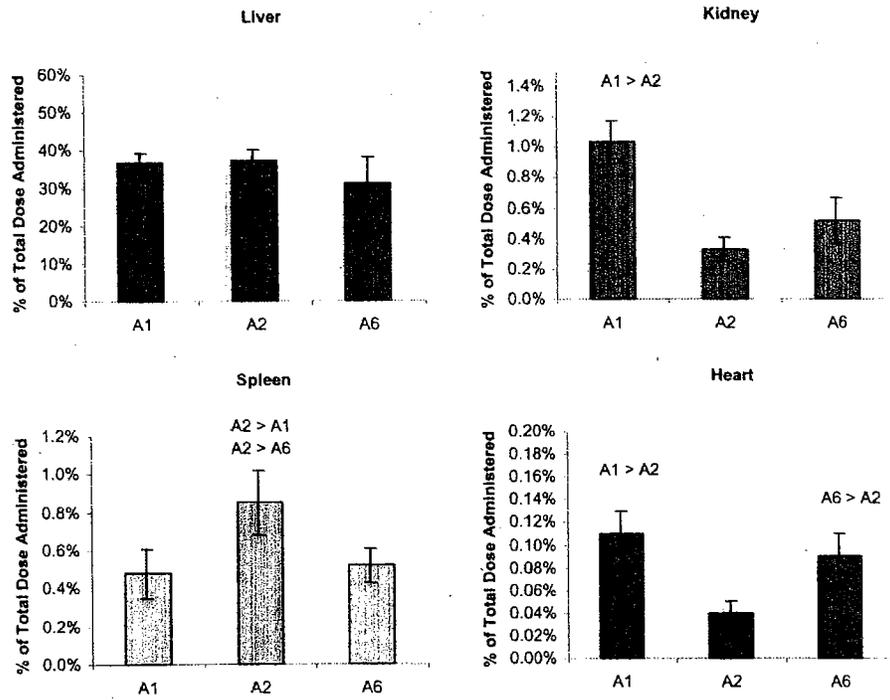
Results for experiment TKX 34 are summarized in Figure 1 (provided by the sponsor) below. Lower — content had no effect on liver idursulfase tissue concentrations, whereas lower — resulted in significantly decreased idursulfase uptake in kidney, heart and lung and increased uptake in spleen.

Figure 1: Role of [redacted] on I2S Tissue Biodistribution in ICR Mice (TKX 34)



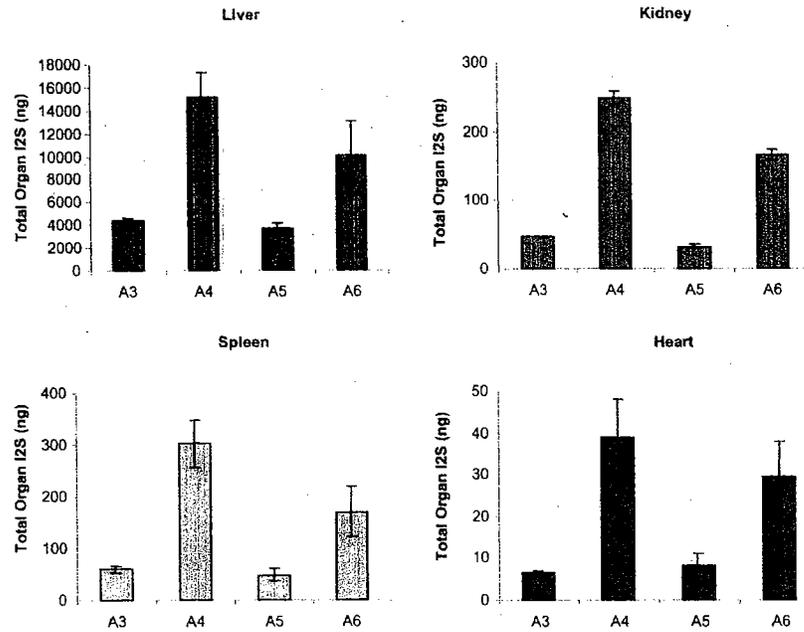
For experiment TKX 40, lower [redacted] content also had no effect on liver idursulfase tissue concentrations; whereas lower [redacted] content again resulted in significantly decreased idursulfase uptake in kidney and heart and increased uptake in spleen (see Figure 2 provided by the sponsor below). Tissue biodistribution of idursulfase was similar between lots 0201G and FD911-001 at the [redacted] mg/kg dose, whereas tissue idursulfase concentrations were decreased (although not significantly) at the [redacted] mg/kg dose for lot # FD911-001 compared to lot # 0201G (see Figure 3 provided by the sponsor below). Urine GAG levels were reduced (compared to IKO vehicle controls) by a similar amount in IKO mice administered [redacted] idursulfase from lot # FD911-001, lot # 0202G low [redacted] content and lot # 202G high [redacted] content. However, urinary GAG levels for all IKO treatment groups were similar to wild type vehicle control by Day 6. As shown in Figure 7 (provided by the sponsor) below, tissue GAG levels were decreased in all organs except the heart. Idursulfase [redacted] concentration had no affect liver or spleen GAG levels. However, kidney GAG levels were significantly increased in animals administered idursulfase with lower [redacted] content compared to animals administered idursulfase with higher [redacted] content. Tissue GAG concentrations was similar between lots 0201G and FD911-001 at both the [redacted] mg/kg dose.

Figure 2: Role of β on I2S Tissue Biodistribution in IKO Mice (TKX 40).



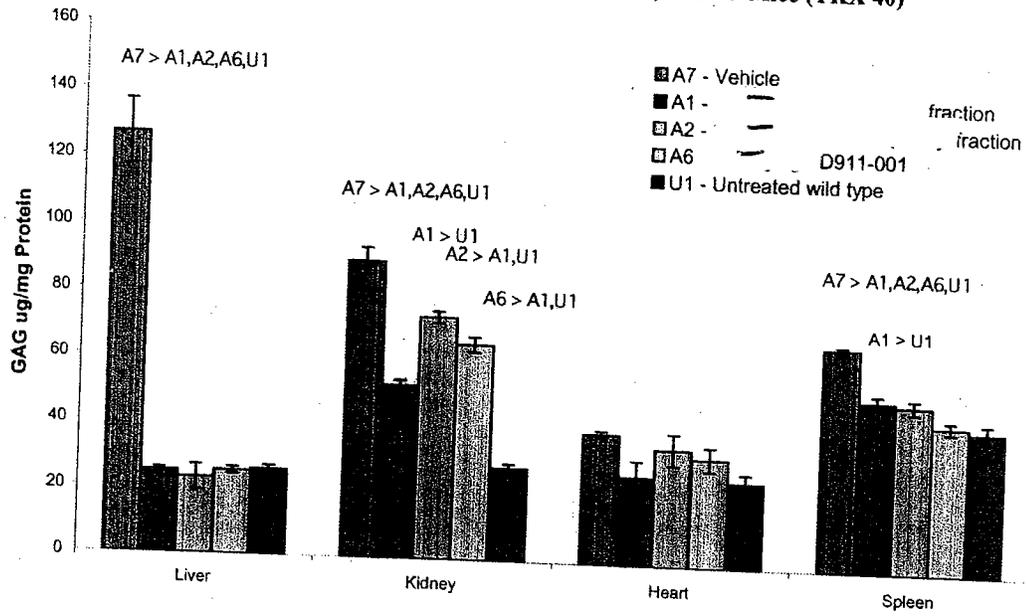
A1 = β fraction; A2 = β fraction; A6 = FD911-001. All treatments dosed at β mg. Significance ($p < 0.05$) relative to Group A2 (β fraction) is indicated by the symbols above the bars.

Figure 3: Comparative Absolute Tissue Biodistribution in IKO Mice Between FD911-001 and 0201G (TKX 40).



A3 = β ng/kg 0201G; A4 = β 0201G; A5 = β ; FD911-001; A6 = β , FD911-001

Figure 7: Role of I2S on Tissue GAG Levels (µg/mg protein) in IKO Mice (TKX 40)



The Effect of I2S on Pharmacokinetics of Sprague-Dawley Rats (Study # 690-110-02-371)

Methods: The GLP status of this study was not stated. Male Sprague-Dawley rats (n = 3/group) were administered 2.5 mg/kg idursulfase lot 0202G chromatographically fractionated by

as shown in Text Table 2 (provided by the sponsor) below. Blood samples were collected predose, and 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 1440 minutes after dosing. Serum idursulfase concentrations were determined using an ELISA method.

Text Table 2: Test Article Concentrations

Group No.	Test Article Designation	I2S Concentration (mg/mL)	Content (moles/mole I2S)
1		2.5	
2		2.7	
3		2.9	
4		3.4	
5	Pool 1	3.4	
6	Pool 2	3.6	
7	Pool 3	3.9	
8	Water pool	2.5	
Pool 1:	fraction +	fraction.	
Pool 2:	fraction +	fraction +	
Pool 3:	fraction +	fraction +	fraction.
	fraction	fraction +	fraction +

Results: Mean pharmacokinetic results for the — ractions are summarized in Table 1 below (provided by the sponsor). Systemic exposure (AUC) and half-life ($T_{1/2}$) decreased, and clearance (Cl) increased, with decreased — content suggesting idursulfase cellular uptake decreases with increasing — content. Pharmacokinetic parameters for the 3 — fraction pools were similar to the — fraction.

Table 1: Pharmacokinetic Parameters of Differentially — Idursulfase in Rats

Test Article	moles/mole I2S	C_{max} ($\mu\text{g/mL}$)	AUC ($\text{min} \cdot \mu\text{g/mL}$)	$T_{1/2}$ (λ_z) (min)	Cl (mL/min/kg)	V_{ss} (% body weight)
		52.7	4558	132	0.57	6.9%
		66.3	3598	110	0.71	6.1%
		72.0	1635	85	1.57	6.8%
		74.6	1153	79	2.23	7.1%

All fractions were produced from I2S development lot 0202G.

A Crossover Pharmacokinetic Study of Two Lots of DRX006A Administered by Intravenous Bolus Injection to Cynomolgus Monkeys (Study # 110-02-002)

Methods: This was a GLP study. Cynomolgus monkeys (2/sex/group) were administered intravenously 1 mg/kg idursulfase lot # 0201G or idursulfase lot # FD911-004 (used in clinical study TKT018) on Day 1. Animals were then administered idursulfase in cross-over fashion on Day 8. Blood samples were collected predose and 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 1440 minutes after dosing. Serum idursulfase concentrations were determined using an ELISA method.

Results: Pharmacokinetic results are summarized in Table 3 (provided by the sponsor) below. C_{max} and V_{ss} were similar between lots. Systemic exposure (AUC) was slightly decreased and clearance (Cl) was slightly increased for the 0201G lot compared to the FD911-004 lot.

Table 3: Summary of Pharmacokinetic Parameters in Cynomolgus Monkeys Dosed with 2 Lots of DRX006A

Lot		Body Wt (Kg)	C_{max} ($\mu\text{g/mL}$)	AUC ($\text{min} \cdot \mu\text{g/mL}$)	$T_{1/2}(\alpha)$ (min)	$T_{1/2}(\beta)$ (min)	MRT (min)	Cl (mL/min)	Cl (mL/min/kg)	V_{ss} (mL)	V_{ss} % BW
0201G	Mean	3.3	29.1	1492	25.2	221	131	2.30	0.70	303	9.4%
	SD	0.5	6.5	377	4.4	104	54.1	0.49	0.20	166	6.0%
	CV (%)	15%	22%	25%	17%	47%	41%	22%	28%	55%	64%
FD911-004	Mean	3.3	28.3	1966	26.5	211	174	1.83	0.56	318	9.8%
	SD	0.6	3.3	409	4.3	10.9	13.8	0.25	0.12	40.2	2.4%
	CV (%)	18%	12%	21%	16%	5%	8%	13%	22%	13%	24%
P value			N/A	N/A	0.55	0.78	0.05	0.03	0.13	0.82	0.88

N/A, not applicable
P values calculated using Student's t-test

Biodistribution and Pharmacodynamic Comparison of a Phase I/II Clinical Drug Product Lot (FD911-004) with a Phase II/III Clinical Drug Substance Lot (D303-006): Studies TKX 41 and TKX 42 (Study # 720-110-03-444)

Methods: The GLP status of these experiments was not stated. In experiment TKX 41, female ICR mice (n = 6/group) were administered intravenously 1 mg/kg idursulfase lot # FD911-004 or lot # D303-006. Animals were sacrificed at 2 hours after dosing. Blood samples were collected and liver, kidneys, heart, and spleen were removed and weighed. Serum and tissue extract idursulfase levels were then determined using an ELISA method. In experiment TKX 42, male IKO mice (n = 6/group) were administered intravenously vehicle and 0.25 or 1.0 mg/kg of each idursulfase lot weekly on Days 0, 7, 14, 21, and 28. A group of wild type littermates were also administered vehicle. Urine was collected at various timepoints throughout the study for urine GAG level determination. All animals were sacrificed on Day 30 and the liver, kidneys, heart, lungs, spleen, and brain were removed, weighed, and snap frozen. Urine and tissue extract GAG levels were then determined using a dimethylmethylene blue assay.

Results:

Results for experiment 41 are summarized in Table 2 (provided by the sponsor) below. The biodistribution patterns were similar between lots.

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Table 2. I2S Comparative Tissue Biodistribution in ICR mice (Study TKX 41).

A. Absolute Percent of the Administered Dose.

Tissue	Mean % of Administered Dose (± 1SD)	
	FD911-004	D303-006
Liver	32.8% ± 2.5%	30.0% ± 4.5%
Spleen	0.90% ± 0.18%	0.61% ± 0.08%
Kidneys	0.59% ± 0.06%	0.48% ± 0.08%
Heart	0.122% ± 0.02%	0.088% ± 0.01%
% Recovered	34.4%	31.2%

n=6 mice per treatment

All animals were sacrificed 2 hours post-injection.

B. Relative Amount of Recovered Dose.

Tissue	Mean % of Recovered Dose (± 1SD)	
	FD911-004	D303-006
Liver	95.3% ± 0.60%	96.2% ± 0.46%
Spleen	2.6% ± 0.57%	1.9% ± 0.13%
Kidneys	1.7% ± 0.16%	1.5% ± 0.31%
Heart	0.36% ± 0.05%	0.29% ± 0.08%

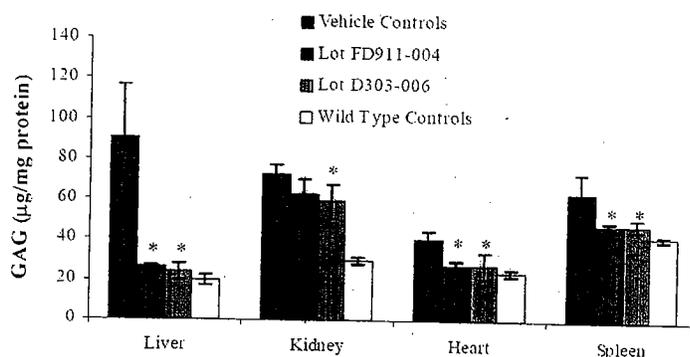
n=6 mice per treatment

All animals were sacrificed 2 hours post-injection.

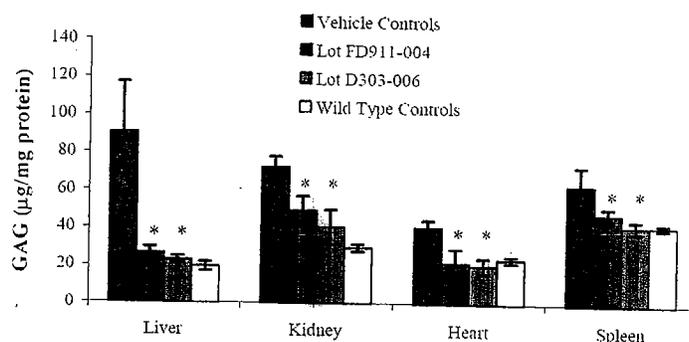
Results for experiment 42 are summarized in Figure 3 (provided by the sponsor) below. Although kidney GAG levels were not significantly decreased in 0.25 mg/kg idursulfase lot FD911-004 animals compared to IKO vehicle controls, tissue GAG levels were similar between the two lots at corresponding doses. A dose-dependent decrease in urine GAG levels was observed. Urine GAG levels in 1 mg/kg/dose groups were similar to wild type vehicle controls from Day 9 on and urine GAG levels in 0.25 mg/kg groups were in between IKO and wild type vehicle control values. Urine GAG levels were similar between lots for corresponding doses.

Figure 3. TKX 42 I2S Lot Comparison: Reduction of Tissue GAG (μg GAG/mg protein) in IKO Mice (results expressed as mean \pm 1 SD)

A. Dose: 0.25 mg/kg (5 weekly injections)



B. Dose: 1.0 mg/kg (5 weekly injections)



Data Table for Figure 3 (mean \pm 1 SD)

Tissue Extract	Vehicle Controls	0.25 mg/kg Dose		1.0 mg/kg Dose		Wild Type Controls
		FD911-004	D303-006	FD911-004	D303-006	
Liver	90.7 \pm 26.5	26.0 \pm 1.5*	24.1 \pm 4.0*	26.4 \pm 3.3*	22.9 \pm 1.9*	19.4 \pm 2.5
Kidney	72.3 \pm 5.5	62.4 \pm 7.3	59.2 \pm 8.7*	49.4 \pm 7.5*	40.5 \pm 9.2*	29.5 \pm 1.9
Heart	40.6 \pm 3.6	26.8 \pm 2.9*	27.2 \pm 6.5*	21.7 \pm 7.4*	20.2 \pm 4.1*	23.7 \pm 1.6
Spleen	63.3 \pm 9.9	47.4 \pm 1.9*	48.1 \pm 2.6*	48.0 \pm 3.5*	41.7 \pm 3.1*	41.7 \pm 1.4

* Significantly reduced compared to vehicle control ($p \leq 0.05$)

Pharmacodynamic Comparison of a Phase II/III Clinical Drug Product Lot (FD924-001) with a Commercial Process Drug Product Lot (FDB04-003): Study TKX 52 (Study 720-110-04-634)

Methods: The GLP status of this study was not stated. Male IKO mice ($n = 6/\text{group}$) were administered intravenously vehicle and 0.25 or 1.0 mg/kg/dose of idursulfase lot FD924-001 (used in clinical studies) and FDB04-003 (used in preclinical toxicology and clinical studies) weekly on Days 0, 7, 14, 21, and 28. A group of wild type littermates were also administered vehicle. Urine was collected at various timepoints throughout the study for urine GAG level determination. All animals were sacrificed on Day 30 and the

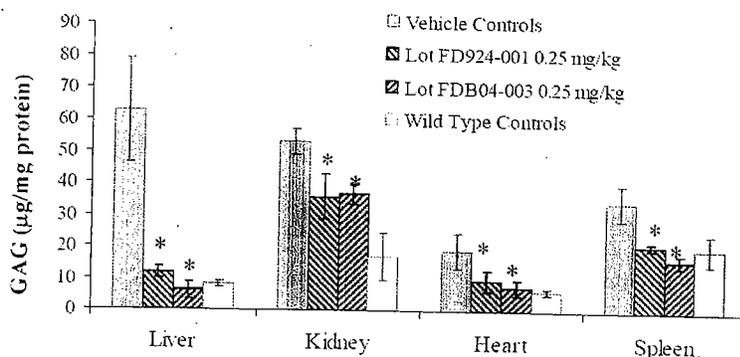
liver, kidneys, heart, lungs, spleen, and brain were removed, weighed, and snap frozen. Urine and tissue extract GAG levels were then determined using a dimethylmethylene blue assay.

Results: Tissue GAG results are summarize in Figure 2 (provided by the sponsor) below. Tissue GAG levels were similar between the two lots at corresponding doses. Urine GAG levels in 1 mg/kg/dose treated groups were similar to wild type vehicle controls from Day 2 on and urine GAG levels in 0.25 mg/kg groups were in between IKO and wild type vehicle control values except on Day 29 when urine GAG levels for both lots were similar to IKO vehicle control. Urine GAG levels were similar between lots for corresponding doses.

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Figure 2. Idursulfase Lot Comparison: Reduction of Tissue GAG ($\mu\text{g GAG}/\text{mg protein}$) in IKO Mice (results expressed as mean \pm 1 SD)

A. Dose: 0.25 mg/kg (5 weekly injections)



B. Dose: 1.0 mg/kg (5 weekly injections)

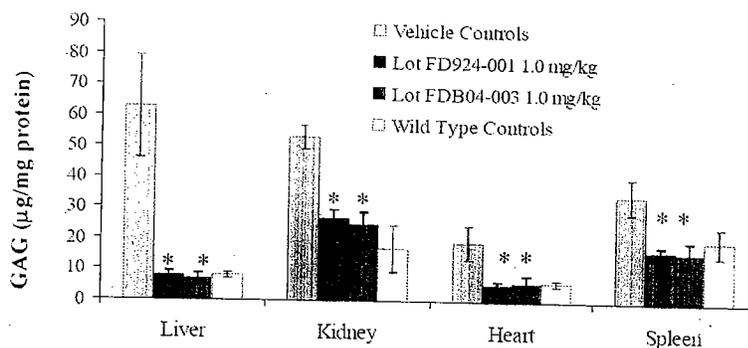


Table 4. Mean (\pm 1 SD) Tissue Extract GAG Levels in $\mu\text{g GAG}/\text{mg Protein}$

Tissue Extract	Vehicle Controls	0.25 mg/kg Dose		1.0 mg/kg Dose		Wild Type Controls
		FD924-001	FDB04-003	FD924-001	FDB04-003	
Liver	62.8 \pm 16.5	11.9 \pm 1.9*	6.2 \pm 2.9*	7.7 \pm 1.7*	6.8 \pm 1.9*	8.2 \pm 0.9
Kidney	53.1 \pm 4.0	35.9 \pm 7.1*	36.7 \pm 3.2*	26.4 \pm 2.9*	24.6 \pm 4.3*	17.1 \pm 7.6
Heart	19.0 \pm 5.5	9.5 \pm 3.4*	7.4 \pm 2.5*	5.4 \pm 1.0*	6.0 \pm 2.6*	6.2 \pm 1.0
Spleen	34.5 \pm 5.6	21.0 \pm 0.9*	16.3 \pm 2.0*	16.6 \pm 1.6*	16.0 \pm 4.0*	19.9 \pm 4.8

Results are from an n of 5 instead of 6 (animal 365 was found dead on Day 29 of the study)
 * Significantly reduced compared to vehicle controls ($p \leq 0.05$)

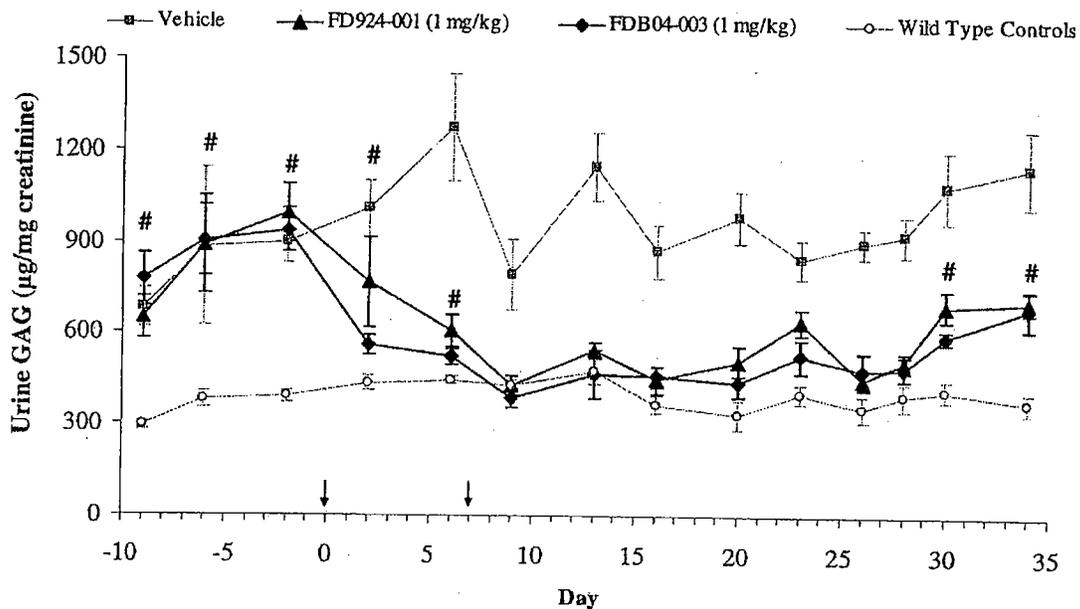
In Vivo Pharmacodynamic Comparison of Idursulfase Manufactured by the Phase II/III Process with the Process Intended for Commercial Use: Duration of Effect as Measured by Urinary Glycosaminoglycan Levels (Study # 720-110-05-635)

Methods: The GLP status of this study was not stated. Male IKO mice ($n = 8/\text{group}$) were administered 2 weekly intravenous injections of vehicle, 1 mg/kg/dose idursulfase lot # FD924-001 (used in clinical studies) and 1 mg/kg/dose idursulfase lot # FDB04-003

(used in both clinical and preclinical studies). Urine samples were collected throughout the study for urinary GAG and creatinine determination. The experiment concluded 4 weeks after the final injection. Urine was also collected from wild type littermates for comparison.

Results: Results are summarized in Figure 1 (provided by the sponsor) below. Urinary GAG levels were reduced to wild type levels in IKO mice after the second idursulfase dose with no significant difference observed between lots.

Figure 1: Urinary GAG levels (\pm standard error of the mean) in mice after injection of 1 mg/kg of idursulfase lots FD924-001 or FDB04-003.



Arrows indicate dosing of IKO mice with idursulfase (1mg/kg)

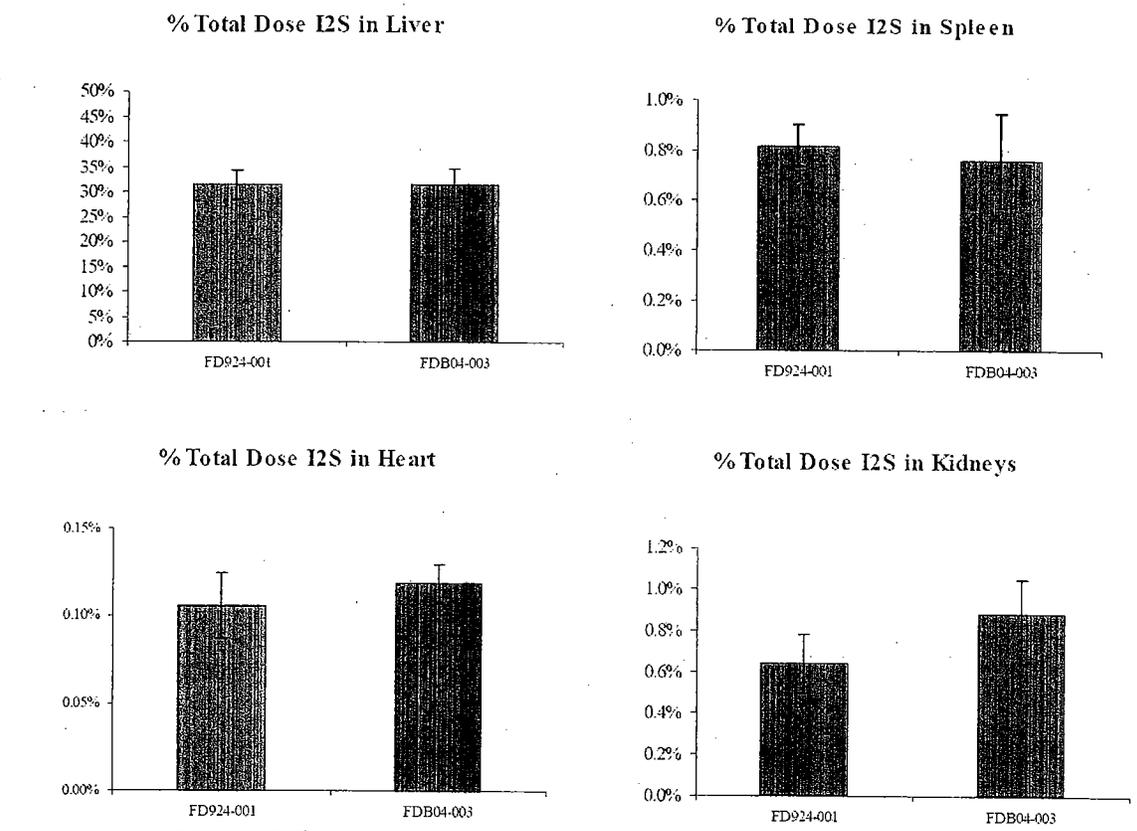
= Elevated GAGs for both idursulfase drug product lots as compared to wild-type urinary GAG levels ($p < 0.05$)

Biodistribution Comparison of a Phase II/III Clinical Drug Product Lot (FD924-001) with a Commercial Process Validation Drug Product Lot (FDB04-003): Study TKX 51 (Study # 720-110-04-633)

Methods: The GLP status of this experiment was not stated. Female ICR mice (n = 6/group) were administered a single intravenous dose of 1.0 mg/kg idursulfase lot # FD924-001 or lot # FDB04-003 I2S. A separate group of ICR mice (n = 3/group) were administered vehicle. *The two lots evaluated were not used in submitted preclinical toxicology or clinical studies.* Animals were sacrificed 2 hours after dosing. The liver, heart, kidney, and spleen were collected, weighed and tissue extract idursulfase levels were determined using an ELISA method.

Results: Idursulfase was not detectable in tissue extracts from vehicle control mice. The percentages of administered idursulfase dose recovered in liver, heart, kidney, and spleen were comparable between lots as shown in Figure 1 (provided by the sponsor) below.

Figure 1: Idursulfase Comparative Tissue Biodistribution in ICR mice.



A 1-Week Crossover Study of Two Lots of Idursulfase Administered by Intravenous Bolus Injection to Cynomolgus Monkeys (Study # 110-05-002)

Methods: This was a GLP study. In a cross-over design, male cynomolgus monkeys (n = 8) were administered 1 mg/kg single-dose idursulfase lot # FDB04-003 on Day 1 and lot # FD924-002 on Day 8. A second group of monkeys were administered 1 mg/kg idursulfase lot # FD924-002 on Day 1 and lot # FDB04-003 on Day 8. Blood samples were collected at predose and 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 1440 minutes after dosing. Serum idursulfase concentrations were then determined using ELISA.

Results: Results are summarized in Table 3 (provided by the sponsor) below. Mean pharmacokinetic parameters were nearly identical for both lots.

Table 3: Summary of Pharmacokinetic Parameters in Cynomolgus Monkeys Dosed with 2 Lots of Idursulfase

Lot		Body Wt (Kg)	C ₀ (µg/mL)	AUC _{last} (min*µg/mL)	AUC _∞ (min*µg/mL)	T _{1/2} (λz) (min)	MRT (min)	Cl (mL/min)	Cl (mL/min/kg)	V _{ss} (mL)	V _{ss} % BW
FDB04-003	Mean	2.6	27.5	1398	1436	177	92	1.90	0.75	172	6.7%
	SD	0.2	5.4	251	255	40.5	13.7	0.39	0.17	27.8	1.3%
	CV (%)	7%	19%	18%	18%	23%	15%	21%	22%	16%	19%
FD924-002	Mean	2.6	26.4	1376	1418	187	98	1.91	0.75	186	7.3%
	SD	0.2	3.4	226	226	41.9	12.6	0.35	0.14	31.6	1.4%
	CV (%)	6%	13%	16%	16%	22%	13%	18%	19%	17%	19%
P value	N/A	N/A	N/A	N/A	N/A	0.52	0.17	0.17	N/A	0.19	0.30

N/A, not applicable
P values calculated using Student's t-test

Pharmacodynamic Comparison of a Phase II/III Clinical Drug Substance Lot (D303-025) with a Commercial Process Validation Drug Substance Lot (DP04-003): Study TKX 48 (Study # 720-110-04-607)

Methods: The GLP status of this experiment was not stated. *The two lots evaluated were not used in submitted preclinical toxicology or clinical studies.* Male IKO mice (n = 6/group) were administered intravenously vehicle and 0.25 or 1.0 mg/kg/dose idursulfase lot # D303-05 or lot # DP04-003 weekly on Days 0, 7, 14, 21, and 28. A group of wild type littermates were also administered vehicle. Urine was collected at various timepoints throughout the study for urine GAG level determination. All animals were sacrificed on Day 30 and the liver, kidneys, heart, lungs, spleen, and brain were removed, weighed, and snap frozen. Urine and tissue extract GAG levels were then determined using a dimethylmethylene blue assay.

Results: Urinary and tissue GAG reductions in treated compared to vehicle control IKO mice were similar between lots at corresponding doses.

Reduction of Tissue Glycosaminoglycans in Hunter Mice after 1, 3, and 5 Injections of Idursulfase: Study TKX 50 (Study # 720-110-04-609)

Methods: The GLP status of this experiment was not stated. Male IKO mice (n = 3/group) were administered intravenously 1, 3, and 5 weekly injections of 0.1 or 1.0 mg/kg/dose idursulfase lot # D303-025 or lot # DP04-003. A separate group of IKO mice were administered vehicle. *The two lots evaluated were not used in submitted preclinical toxicology or clinical studies.* Animals were sacrificed 2 days after final dose. The liver and spleen were collected and tissue GAG levels were determined. Liver and spleen were also collected from wild type littermates for comparison.

Results: Both lots produced comparable reductions in liver and spleen GAG levels.

Biodistribution Comparison of a Phase II/III Clinical Drug Substance Lot (D303-025) with a Commercial Process Validation Drug Substance Lot (DP04-003): Study TKX 47 (Study # 720-110-04-606)

Methods: The GLP status of this experiment was not stated. Female ICR mice (n = 6/group) were administered a single intravenous dose of 1.0 mg/kg idursulfase lot # D303-025 or lot # DP04-003. A separate group of ICR mice (n = 3/group) were administered vehicle. *The two lots evaluated were not used in submitted preclinical toxicology or clinical studies.* Animals were sacrificed 2 hours after dosing. The liver, heart, kidney, and spleen were collected, weighed and tissue extract idursulfase levels were determined using an ELISA method.

Results: Idursulfase was not detectable in tissue extracts from vehicle control mice. The percentages of administered idursulfase dose recovered in liver, heart, kidney, and spleen were comparable between lots.

2.6.4.9 Discussion and Conclusions

When administered intravenously, idursulfase was widely distributed and rapidly removed from the serum (mean elimination half-life was less than 1 hour in rats and less than 30 minutes in monkeys), presumably due cell internalization via membrane mannose-6-phosphate receptors binding to enzyme mannose-6-phosphate residues. A large number of comparability studies were performed in mice, rats, and monkeys. Different lots were generally found comparable, although occasional differences were noted.

2.6.4.10 Tables and figures to include comparative TK summary

A comparative TK summary was not provided by the sponsor.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

There were no adverse effects observed after single intravenous doses of up to 20 mg/kg idursulfase in male rats and monkeys. In a 6-month monkey toxicity study, there were no adverse effects observed after weekly intravenous doses of up to 12.5 mg/kg/dose. Genetic toxicology and carcinogenicity studies were not submitted since idursulfase is a recombinant endogenous human protein that is not expected to interact with cellular DNA. In the lone reproductive toxicity study submitted, there was no effect on fertility, reproductive performance, or early embryonic development after twice weekly doses of up to 5 mg/kg/dose to male rats.

2.6.6.2 Single-dose toxicity

Study title: Single-Dose Intravenous Injection Toxicity Study with Idursulfase in Male Rats

Key study findings: In an acute toxicity study, male rats (n = 10/group) were administered intravenously 0, 5, 10, and 20 mg/kg idursulfase via slow bolus injection. There were no adverse effects observed in this study and a target organ of toxicity was not identified.

Study no.: 6354-160

Volume #, and page #: N/A

Conducting laboratory and location: 

Date of study initiation: March 1, 2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Idursulfase, lot # FDB04-003, — purity as a monomer

Methods

Doses: 0 (vehicle), 5, 10, and 20 mg/kg

Species/strain: Rat/ CD@ (SD) IGS BR

Number/sex/group (main study): 10/male/group

Route, formulation, volume, and infusion rate: Intravenous, solution (vehicle – phosphate-buffered saline), 10 mL/kg, and slow bolus injection

Satellite groups used for toxicokinetics: 8/male/group

Age: Approximately 7 weeks at initiation of dosing.

Weight: 214-272 g at initiation of dosing.

Sampling times: Blood samples for toxicokinetic analyses (4 rats/timepoint) were collected predose and approximately 5, 15, and 30 minutes and 1, 2, 4, 8, 24, 36, and 48 hours after dosing. Plasma idursulfase levels were determined using ELISA methodology. Toxicokinetic animals were euthanized without necropsy after the final blood collection.

Observations and times:

Mortality: All animals were observed twice daily.

Clinical signs: All animals were observed twice daily.

Body weights: Body weights were recorded on all animals prior to dosing (Day 1) and on Days 8, 14, and 15 for main study animals.

Food consumption: Food consumption was measured for the intervals Day 1 to Day 8 and Day 8 to Day 13.

Hematology: Blood samples for hematology analyses (including PT and APTT) were collected on Day 15.

Clinical chemistry: Blood samples for clinical chemistry analyses were collected on Day 15.

Gross pathology: Main study animals were euthanized on Day 15 and full necropsies were performed.

Organ weights:

At scheduled sacrifice, the following organs (when present) will be weighed; paired organs will be weighed together.

adrenal (2)	lung
brain	pituitary
epididymis (2)	spleen
heart	testis (2)
kidney (2)	thymus
liver	

Histopathology: Histopathological evaluation was performed only on control and high dose animals as well as any abnormal tissue/organ from low or mid dose animals.

The following tissues (when present) from each animal will be preserved in 10% neutral-buffered formalin (unless otherwise noted).

adrenal (2)	lung with mainstem bronchi
brain	lymph node (mesenteric)
cecum	optic nerve (2) ^a
colon	pancreas
duodenum	pituitary gland
epididymis (2) ^a	prostate
esophagus	rectum
eye (2) ^a	salivary gland [mandibular (2)]
femur with bone marrow (articular surface of the distal end)	seminal vesicle (2)
Harderian gland ^a	skin
heart	spinal cord (cervical, thoracic and lumbar)
ileum	spleen
implantable microchip identification device ^b	sternum with bone marrow
injection site(s)	stomach
jejunum	testis (2) ^a
kidney (2)	thymus
lesions	thyroid (2 lobes) with parathyroid
liver	tongue
	trachea
	urinary bladder

^a These tissues will be fixed in modified Davidson's.

^b The implantable microchip identification device will be collected and retained with the preserved tissues. The device will not be processed.

Adequate Battery: yes (X), no ()—explain

Peer review: yes (), no (), Not stated (X)

Results

Mortality: There were no unscheduled deaths in this study.

Clinical signs: There were no drug-related clinical signs of toxicity.

Body weights: The mean initial and final body weight of control males was 244 and 340 g, respectively. There were no drug-related effects on body weights.

Food consumption: Mean food consumption in control males for study days 1-7 was 223 g. Mean food consumption for study days 8-13 was 200 g. There were no drug-related effects on food consumption.

Hematology: There were no drug-related changes in hematology parameters. Mean monocytes were significantly decreased in the 5 mg/kg group ($0.13 \times 10^3/\mu\text{L}$ compared to $0.23 \times 10^3/\mu\text{L}$ for controls, $p < 0.05$). However, monocytes were not significantly decreased in the 10 mg/kg ($0.17 \times 10^3/\mu\text{L}$) and 20 mg/kg ($0.18 \times 10^3/\mu\text{L}$) groups. Therefore, the decrease in monocytes observed in the 5 mg/kg group was not considered drug-related.

Clinical chemistry: Mean serum potassium levels were significantly decreased in the 20 mg/kg group (4.6 mmol/L compared to 5.2 mmol/L for controls, $p < 0.05$). This decrease was not considered toxicologically significant due to small magnitude and that all potassium levels were within the normal range for Sprague-Dawley rats. There were no other significant changes in clinical chemistry parameters.

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

Organ weights: There were no drug-related effects on organ weights.

Histopathology: There were no drug-related microscopic findings.

Adequate Battery: yes (X), no ()—explain

Peer review: yes (), no (), Not stated (X)

Toxicokinetics: Toxicokinetic results are summarized in Table 1 below (provided by the sponsor). Systemic exposure (AUC) increased with increasing dose. Elimination half-life and volume of distribution increased, and clearance decreased, with increasing dose. The sponsor suggested that clearance mechanisms may be saturable.

Table I. Toxicokinetic Parameters after Single-Dose Intravenous Idursulfase Administration in Male Sprague Dawley Rats.

Toxicokinetic Parameters	5 mg/kg	10 mg/kg	20 mg/kg
$t_{1/2}$ (hr)	3.3 ± 2.90	3.7 ± 0.59	5.0 ± 1.36
C_{max} (g/ml)	73.2 ± 25.09	138.1 ± 34.07	245.2 ± 43.87
Cl (ml/hr/kg)	36.34 ± 5.99	32.8 ± 9.11	26.8 ± 6.08
Vd (ml/kg)	168.5 ± 147.49	177.5 ± 62.84	201.9 ± 106.64
Vss (ml/kg)	65.3 ± 28.0	70.0 ± 21.8	70.6 ± 11.1
AUC _{0-∞} (hr* g/ml)	141.0 ± 24.68	321.7 ± 70.43	777.9 ± 159.97
AUC/Dose (hr*kg* g/ml/mg)	28.2 ± 4.94	32.2 ± 7.04	38.9 ± 8.00

$t_{1/2}$, elimination half-life; C_{max} , maximal idursulfase concentration; Cl, clearance; Vd, volume of distribution; Vss, volume of distribution at steady state; AUC_{0-∞}, area under

Study title: Single-Dose Intravenous Injection Toxicity Study with Idursulfase in Male Cynomolgus Monkeys with Safety Pharmacology

Key study findings: In an acute toxicity study, male monkeys (n = 4/group) were administered intravenously 0, 5, 10, and 20 mg/kg idursulfase via slow bolus injection. The safety pharmacology portion of this study was reviewed earlier (see Section 2.6.2.4). There were no adverse effects observed in this study and a target organ of toxicity was not identified. However, animals were removed from the study and returned to the stock colony on Day 15. Therefore, macroscopic and microscopic evaluations were not performed.

Study no.: 6354-159

Volume #, and page #: N/A

Conducting laboratory and location: _____

Date of study initiation: February 15, 2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Idursulfase, lot # FDB04-003, _____ purity as a monomer

Methods

Doses: 0 (vehicle), 5, 10, and 20 mg/kg

Species/strain: Monkey/cynomolgus

Number/sex/group (main study): 4/male/group

Route, formulation, volume, and infusion rate: Intravenous, solution (vehicle – phosphate-buffered saline), 10 mL/kg, and slow bolus injection

Satellite groups used for toxicokinetics or recovery: none

Age: 3-5 years old

Weight: 2.4-3.3 kg

Sampling times: Blood samples for toxicokinetic analyses were collected predose and approximately 5, 15, and 30 minutes and 1, 2, 4, 8, 24, 36, and 48 hours after dosing. Plasma idursulfase levels were determined using ELISA methodology.

Observations and times:

Mortality: Animals were observed twice daily.

Clinical signs: Animals were observed twice daily.

Body weights: Body weights were recorded on Days 1, 4, 8, 11, and 15.

Hematology: Blood samples for hematology analyses (including PT and APTT) were collected predose, Day 2, and Day 15.

Clinical chemistry: Blood samples for clinical chemistry analyses were collected predose, Day 2, and Day 15.

Urinalysis: Urine samples for urinalysis were collected predose, Day 2, and Day 15.

Gross pathology: Animals were not euthanized, therefore, necropsies were not performed. Animals were removed from the study and returned to the stock colony on Day 15.

Organ weights: Not performed.

Histopathology: Not performed.

Results

Mortality: There were no unscheduled deaths.

Clinical signs: There were no drug-related clinical signs noted.

Body weights: The mean initial and final body weight for control males was 2.725 and 2.800 kg. There were no drug-related effects on body weights.

Hematology: There were no drug-related effects on hematology parameters.

Clinical chemistry: There were no drug-related effects on clinical chemistry parameters.

Urinalysis: Urine protein concentration on Day 2 was either trace or 1+ (on a scale from negative to 4+) for two 5 mg/kg, two 10 mg/kg, and three 20 mg/kg monkeys compared to negative for all control monkeys. There were no other drug-related changes in urinalysis parameters.

Toxicokinetics: Toxicokinetic results are summarized in Table 1 below (provided by the sponsor). Systemic exposure (AUC) increased with increasing dose. Elimination half-life and volume of distribution increased, and clearance decreased, with increasing dose. The sponsor suggested that clearance mechanisms may be saturable.

Table I. Toxicokinetic Parameters after Single-Dose Intravenous Idursulfase Administration in Male Cynomolgus Monkeys.

Toxicokinetic Parameters	5 mg/kg	10 mg/kg	20 mg/kg
$t_{1/2}$ (hr)	10.2 ± 1.54	13.7 ± 4.05	15.2 ± 0.96
C_{max} (g/ml)	112 ± 12.0	241 ± 70.7	566 ± 68.8
Cl (ml/hr/kg)	16.1 ± 11.0	14.7 ± 1.30	9.4 ± 1.28
Vd (ml/kg)	252 ± 170	288 ± 79.7	206 ± 27.2
Vss (ml/kg)	44.1 ± 30.3	50.4 ± 4.87	37.5 ± 3.18
AUC _{0-∞} (hr* g/ml)	231 ± 24.6	686 ± 57.7	2159 ± 292
AUC/Dose (hr*kg* g/ml/mg)	46.9 ± 5.66	68.6 ± 5.77	108 ± 14.6

$t_{1/2}$, elimination half-life; C_{max} , maximal idursulfase concentration; Cl, clearance; Vd, volume of distribution; Vss, volume of distribution at steady state; AUC_{0-∞}, area under concentration curve extrapolated to infinity; AUC/Dose, AUC divided by dose. Each study group contained 4 animals.

2.6.6.3 Repeat-dose toxicity

Study title: A Six-Month Toxicity Study of DRX006A Administered Weekly by Intravenous Injection to Male Cynomolgus Monkeys, with a 4-Week Recovery Period

Key study findings: In a 6-month toxicity study, male cynomolgus monkeys (n = 4/group) were administered intravenously 0, 0.5, 2.5, and 12.5 mg/kg/dose idursulfase weekly with half the animals sacrificed on Day 91. Two additional control and high dose animals were included and sacrificed after a 4-week recovery period. There were no adverse effects observed in this study and a target organ of toxicity was not identified.

Study no.: 110-99-011

Volume #, and page #: N/A

Conducting laboratory and location: _____

Date of study initiation: November 12, 1999

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Idursulfase, lot #s RD200-001 and RD200-002,

Methods

Doses: See table below for the dosing regimens.

Group No.	Number of Animals (all male)	Dose Level (mg/kg/week)	Dose Vol. (mL/kg)	Dose Solution Conc. (mg/mL)	Number Sacrificed on:		
					Day 91	Day 182	Day 210
1	6	0 (control)	2.5	0	2	2	2
2	4	0.5	2.5	0.2	2	2	
3	4	2.5	2.5	1	2	2	
4	6	12.5	2.5	5	2	2	2

Species/strain: Monkey/cynomolgus

Number/sex/group (main study): 4/male/group

Route, formulation, volume, and infusion rate: Intravenous, solution (vehicle – phosphate-buffer saline, pH 6.5, with 0.02% Tween 20), 2.5 mL/kg, and slow bolus injection.

Satellite groups used for recovery: 2/male/group for control and high dose only.

Age: 2-4 years old

Weight: 1.8-2.7 kg

Sampling times: Blood samples for toxicokinetic analyses were collected on Days 1, 8, 85, and 176 at predose and 5, 10, 15, 30, 60, 120, 180, 340, 360, 480, 600, and 1440 minutes after dosing. Single blood samples were also collected 30 minutes after dosing on Days 29, 57, 113, and 141. Plasma idursulfase levels were determined using ELISA methodology.

Unique study design or methodology: Blood samples were collected on non-dosing days in Weeks 2, 4, 13, 26, and 30 antibody titers against idursulfase were determined.

Observations and times:

Mortality: Animals were observed at least twice daily throughout the study.

Clinical signs: Animals were observed at least twice daily throughout the study.

Body weights: Body weights were recorded weekly.

Food consumption: Food consumption was qualitatively assessed during cageside observations and a notation was made when less than half of the rations were consumed.

Ophthalmoscopy: Ophthalmic examinations were performed on all animals prior to initiation of dosing and Week 13 (on non-dosing days). Ophthalmic examinations were also performed on all remaining animals on Week 26 and Week 30.

EKG: EKGs were recorded prior to initiation of dosing and in Weeks 13 and 26 on non-dosing days.

Hematology: Blood samples for hematology analyses (including PT, APTT, and fibrinogen) were collected prior to initiation of dosing, on Day 2, and on non-dosing days in Weeks 4, 13, 20, 26, and 30 (recovery animals).

Clinical chemistry: Blood samples for clinical chemistry analyses were collected prior to initiation of dosing, on Day 2, and on non-dosing days in Weeks 4, 13, 20, 26, and 30 (recovery animals).

Urinalysis: Urine samples for urinalysis were collected prior to initiation of dosing, on Day 2, and on non-dosing days in Weeks 4, 13, 20, 26, and 30 (recovery animals).

Gross pathology: Full necropsies were performed after scheduled sacrifice.

Organ weights: The following organs were weighed:

Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Spleen
Pituitary (post fixation)	Thymus
Testes	Thyroid with parathyroids
Salivary Glands (mandibular)	Prostate

Histopathology: The following tissues/organs were collected, preserved, and prepared for histopathological evaluation. A bone marrow smear was collected from the 7th rib of all animals but was not evaluated.

Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Ureter***
Salivary Gland (mandibular, parotid***, sublingual***)	Testes
Tongue	Epididymis
Esophagus	Prostate
Stomach	Seminal Vesicles
Small Intestine	Endocrine
Duodenum	Adrenals
Jejunum	Pituitary
Ileum [Peyer's patch (2)***]	Thyroid/Parathyroids*
Large Intestine	Skin/Musculoskeletal
Cecum	Skin/Mammary Gland
Colon	Bone (femoral head)
Rectum	Bone (7th rib)
Pancreas	Joint, femoral-tibial***
Liver	Skeletal Muscle (thigh)
Gallbladder	Nervous/Special Sense
Respiratory	Eyes with optic nerve
Trachea	Sciatic Nerve
Larynx***	Brain
Lung	Spinal Cord (thoracic)
Lymphoid/Hematopoietic	Other
Bone Marrow (sternum)	Animal Number Tattoo
Thymus	Gross Lesions
Spleen	Injection Site**
Lymph Nodes	* The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section
Mandibular	** Cephalic and saphenous veins
Mesenteric	*** Only collected on Days 182 and 210

Adequate Battery: yes (X) no ()—explain

Peer review: yes () no (X) not stated (X)

Results

Mortality: There were no unscheduled deaths.

Clinical signs: There were no drug-related clinical signs noted. Slight tremors were observed in one 10 mg/kg male and one 20 mg/kg male but were transient and considered likely due to stress associated with study procedures.

Body weights: The mean initial and final (last day of dosing) body weight of control males was 2.3 and 2.7 kg, respectively. There were no drug-related effects on body weights.

Food consumption: There were apparent drug-related effects on food consumption based on cageside observation.

Ophthalmoscopy: There were no drug-related ophthalmic findings.

EKG: There were no drug-related effects on EKG parameters noted.

Hematology: There were no drug-related changes in hematology parameters.

Clinical chemistry: There were no toxicologically significant drug-related changes in clinical chemistry parameters. Mean total bilirubin was significantly increased in 10 mg/kg/dose animals on Week 4 (0.6 mg/dL compared to 0.4 mg/dL for controls) and creatinine was significantly increased in 20 mg/kg/dose animals on Week 20 (0.7 mg/dL compared to 0.6 mg/dL for controls) but the increases were of small magnitude, within normal ranges and were not considered toxicologically significant.

Urinalysis: There were no drug-related effects on urinalysis parameters.

Gross pathology: There were no apparent drug-related macroscopic findings.

Organ weights: The organ weight changes that were possibly drug-related are shown in the table (created by the reviewer based on the sponsors data, mean absolute weight (g) on top and mean relative weight (%) on bottom) below. However, because the study was designed to have only 2 animals/group/sacrifice timepoint, meaningful interpretation of organ weight data is limited. Mean absolute and relative spleen weights were increased in treated groups compared to controls for animals sacrificed on Day 91. This increase in spleen weight was not associated with any microscopic findings and there were no apparent increases in spleen weights for animals sacrificed on Day 182 or Day 210. Absolute and relative testicular weights were decreased in 20 mg/kg/dose animals on Day 91, all treatment groups on Day 182 and in recovery treated animals on Day 210. Absolute and relative epididymides weights were decreased in all treatment groups on Day 182 and in recovery treated animals on Day 210. Decreases in testes and epididymides weights were not associated with and microscopic findings.

Organ/sacrifice day	0 mg/kg	0.5 mg/kg	2.5 mg/kg	12.5 mg/kg
Spleen/Day 91	2.54 (g) 0.99 (%)	4.84 1.95	4.29 1.52	3.98 1.55
Spleen/Day 182	4.78 1.83	3.59 1.40	3.37 1.48	3.45 1.41
Spleen/Day 210	4.08 1.48			4.02 1.62
Testes/Day 91	1.27 0.50	1.12 0.46	1.31 0.48	0.61 0.25
Testes/Day 181	3.27 1.20	0.95 0.37	0.79 0.34	0.50 0.20
Testes/Day 210	1.22 0.44			0.69 0.28
Epididymides/Day 91	0.60 0.24	0.85 0.34	0.76 0.27	1.00 0.37
Epididymides/Day 182	1.43 0.54	0.66 0.26	0.45 0.19	0.39 0.16
Epididymides/Day 210	0.88 0.32			0.39 0.16

Histopathology: Granuloma of the liver was observed in one high dose male and sinus histiocytosis of the mesenteric lymph nodes was observed in one mid dose male on Day 182. Otherwise, all noted histopathological changes were considered incidental and there were no apparent drug-related microscopic findings.

Adequate Battery: yes (X), no ()—explain

Peer review: yes (), no (X) not stated (X)

Toxicokinetics: Pharmacokinetic/toxicokinetic parameters for Days 1, 8, 85, and 176 are summarized in Table 5 below (provided by the sponsor). Systemic exposure (AUC) increased in a slightly greater than dose-proportional manner. Systemic exposure levels at each dose were similar throughout the study. Serum DRX006A concentrations were within expected ranges at 30 minutes after dosing on Days 29, 57, 113, and 141.

Table 5: Comparison of Average Pharmacokinetic Parameters for Days 1, 8, 85, and 176

Parameter	Day 1	Day 8	Day 85	Day 176
Low Dose (0.5 mg/kg)				
C_{max} ($\mu\text{g/mL}$)	16.6	15.1	21.4	22.1
AUC ($\text{min} \cdot \mu\text{g/mL}$)	999	997	1087	1205
$T_{1/2} (\alpha)$ (min)	20.8	22.7	17.4	19.8
$T_{1/2} (\beta)$ (min)	207	225	199	215
Cl (mL/min/kg)	0.513	0.532	0.462	0.417
V_{ss} (% body weight)	9.3%	9.8%	7.3%	7.3%
Mid Dose (2.5 mg/kg)				
C_{max} ($\mu\text{g/mL}$)	61.8	97.1	84.2	51.4
AUC ($\text{min} \cdot \mu\text{g/mL}$)	8,720	9,164	9,595	7,606
$T_{1/2} (\alpha)$ (min)	71.3	70.0	67.4	85.6
$T_{1/2} (\beta)$ (min)	552	465	304	336
Cl (mL/min/kg)	0.289	0.273	0.281	0.344
V_{ss} (% body weight)	9.2%	7.3%	4.4%	6.6%
High Dose (12.5 mg/kg)				
C_{max} ($\mu\text{g/mL}$)	377	382	437	397
AUC ($\text{min} \cdot \mu\text{g/mL}$)	80,466	78,396	74,520	82,775
$T_{1/2} (\alpha)$ (min)	88.0	128.5	87.7	na
$T_{1/2} (\beta)$ (min)	295	358	216	193
Cl (mL/min/kg)	0.161	0.167	0.175	0.152
V_{ss} (% body weight)	4.7%	4.9%	3.8%	3.9%

na, not applicable

Other: During the study, 6 animals (2/4 mid dose and 4/6 high dose) developed IgG antibodies to DRX006A. As shown in Table 9 below (provided by the sponsor), as expected, onset of IgG antibodies to DRX006A was associated with increased clearance of DRX006A. However, adequate systemic exposure was still maintained throughout the study.

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Table 9: Comparison of Antibody Response to Changes in Clearance

Group No.	Animal No.	Absorbance (Antibody Assay)						Clearance (mL/min/kg)			
		1 st Analysis				2 nd Analysis		Day 1	Day 8	Day 85	Day 176
		Prestudy	Day 9	Day 23	Day 87	Prestudy	Day 177				
3	F11735M	0.084	0.076	0.094	0.227*	-	-	0.272	0.260	0.433 (+59%)	-
	F13101M	0.030	0.037	0.041	0.057	0.086	0.166*	0.264	0.277	0.221	0.415 (+57%)
4	F11703M	0.091	0.116	0.178	0.424*	-	-	0.179	0.164	0.130	-
	F11722M	0.094	0.080	0.160	0.445*	-	-	0.158	0.172	0.248 (+57%)	-
	F13144M	0.210	0.118	0.106	0.572*	0.129	0.236	0.119	0.132	0.180 (+51%)	0.146
	F13171M	0.166	0.109	0.115	0.414*	0.110	0.241*	0.136	0.152	0.165	0.146

* positive antibody response

significant increase in clearance

2.6.6.4 Genetic toxicology

Genotoxicity studies were not submitted. Genotoxicity studies are not usually required for biological therapeutics. Idursulfase is not likely to be genotoxic since it is a naturally occurring human protein.

2.6.6.5 Carcinogenicity

Carcinogenicity studies were not submitted. Carcinogenicity studies are often not required for biological therapeutics. Idursulfase is a replacement enzyme that is taken into the cell by endocytosis, remains within the lysosomal system, and is not likely to have carcinogenic potential.

2.6.6.6 Reproductive and developmental toxicology

Since females are not expected to be treated with idursulfase (Hunter syndrome is an X-linked recessive disease), the only reproductive toxicity study performed was a Segment I study in males rats.

Fertility and early embryonic development

Study title: I2S: Intravenous Male Fertility Study in Sprague Dawley Rats

Key study findings: In a fertility and early embryonic development study, male rats (n = 22/group) were administered intravenously 0, 0.5, 1.5, and 5 mg/kg/dose idursulfase (I2S) twice weekly (3-4 days between doses) beginning 4 weeks prior to cohabitation, during cohabitation, and until the day of necropsy (Day 64). There were no adverse effects, effects on male fertility, or effects on early embryonic development observed in this study.

Study no.: 110-04-010

Volume #, and page #: N/A

Conducting laboratory and location:

Date of study initiation: November 9, 2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: I2S, FDB04-003, ← purity as a monomer

Methods

Doses: 0 (vehicle), 0.5, 1.5, and 5 mg/kg/dose administered twice weekly. The basis of dose selection was not stated. The sponsor did state that idursulfase was administered twice weekly in order to maintain steady-state levels in tissues and to provide an additional safety factor compared to weekly drug administration.

Species/strain: Rat/Sprague Dawley

Number/sex/group: 22/male/group

Route, formulation, volume, and infusion rate: Intravenous, solution (vehicle – 20 mM Sodium Phosphate, pH 6.5, 137 mM NaCl, 0.002% polysorbate-20), 2.5 mL/kg, bolus injection

Satellite groups used for toxicokinetics: None

Study design: Male rats were administered intravenously 0, 0.5, 1.5, and 5 mg/kg/dose I2S twice weekly (3-4 days between doses) beginning 4 weeks prior to cohabitation (males approximately 11 weeks old at initiation of dosing), during cohabitation, and until the day of necropsy (Day 64). After 4 weeks of dosing, males were cohabitated (1:1) with untreated virgin females until conformation of mating or for 14 days, whichever came first. The day of conformation was Gestation Day (GD) 1 for the females.

Parameters and endpoints evaluated: Males were observed twice daily for clinical signs. Body weights and food consumption were recorded twice weekly. All males were euthanized on Day 64 and all females were euthanized on GD 13. Full necropsies were performed on all animals. Testes, epididymides, prostate and seminal vesicles were collected on male rats. For females, the uterus and ovaries were excised and examined for number of corpora lutea, number and position of live fetuses, number and position of dead fetuses, number and position of early resorptions, and abnormalities of the placenta or embryonic sac. Uteri without implantation were stained for detection of early embryonic death. Histopathology was performed on the left testis and epididymis of all males. The right vas deferens was used for sperm motility analysis. The right cauda epididymis was used for sperm density analysis.

Results

Mortality: There were no unscheduled deaths.

Clinical signs: There were no drug-related clinical signs noted.

Body weight: The mean initial and final body weight in control males was 356 and 521 g, respectively. There were no significant drug-related effects on mean body weights. Significant differences in mean body weight gain were occasionally observed during the

study, but were not considered drug-related because the changes were transient and not dose-related.

Food consumption: The initial and final mean daily food consumption in control males was 28.3 and 25.6 g/day. Food consumption was sporadically increased in treated animals compared to controls throughout the study. These increases were transient, small in magnitude, and not associated with effects on body weight. Therefore these sporadic increases in food consumption were not considered drug related.

Toxicokinetics: Not performed.

Necropsy: There were no drug-related macroscopic findings.

Fertility parameters:

Absolute and relative organ weights are shown in Tables 8 & 9 below (provided by the sponsor). Mean absolute and relative weights for right cauda epididymis and right epididymis were significantly increased in the high dose group. Absolute, but not relative, right cauda epididymis, right epididymis, and seminal vesicle weights were increased in the low dose group. Changes in organ weights were not associated with any gross pathology or histopathological findings.

Table 8
Summary of Organ Weights (g)
T28: Intravenous Male Fertility Study in Sprague Dawley Rats

Day: 65 Relative to Start Date

Group Sex	Animal Number	Prostate	Right Cauda Epididymis	Right Epididymis	Right Testis	Seminal Vesicles
1m	Mean	1.1410	0.2799	0.5800	1.8619	2.4534
	S.D.	0.2017	0.0384	0.0644	0.1805	0.4057
	N	22	22	22	22	22
2m	Mean	1.2870	0.3120*	0.7470*	1.8602	2.7818*
	S.D.	0.3023	0.0348	0.0634	0.1538	0.4371
	N	22	22	22	22	22
3m	Mean	1.1700	0.2628	0.6475	1.8410	2.3885
	S.D.	0.2100	0.0443	0.1048	0.3193	0.3850
	N	22	22	22	22	22
4m	Mean	1.2455	0.3170*	0.7334*	1.8698	2.6962
	S.D.	0.2976	0.0306	0.1210	0.1533	0.3510
	N	22	22	22	22	22

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Table 9
Summary of Organ-to-Body Weight Ratios (x100)
I28: Intravenous Male Fertility Study in Sprague Dawley Rats

Group Sex	Animal Number	BW (g)	Day: 65 Relative to Start Date				
			Prostate/BW	Right Cauda Epididymis/BW	Right Epididymis/BW	Right Testis/BW	Seminal Vesicles/BW
1m	Mean	520.71	0.216634	0.053498	0.131115	0.359282	0.471948
	S.D.	41.62	0.033845	0.007270	0.014271	0.042057	0.074523
	N	22	22	22	22	22	22
2m	Mean	542.96	0.237306	0.057857	0.136316	0.344960	0.511234
	S.D.	48.89	0.052026	0.007816	0.044222	0.039066	0.093157
	N	22	22	22	22	22	22
3m	Mean	543.66	0.216537	0.048837	0.120220	0.342200	0.439275
	S.D.	40.79	0.042443	0.009716	0.022508	0.069183	0.064533
	N	22	22	22	22	22	22
4m	Mean	534.73	0.237081	0.059724*	0.133194*	0.350944	0.506091
	S.D.	41.61	0.051072	0.006807	0.024828	0.051725	0.067559
	N	22	22	22	22	22	22

* p<0.05

Group 1, 2, 3, and 4 correspond to 0, 0.5, 1.5, and 5 mg/kg/dose, respectively.

Reproductive performance and uterine observations are presented in Tables 11 and 12, respectively. There were no drug-related effects on reproductive performance, fertility or early embryonic development.

Table 11
Summary of Reproductive Performance
I28: Intravenous Male Fertility Study in Sprague Dawley Rats

Group	Dose Level			
	0 mg/kg/dose	0.5 mg/kg/dose	1.5 mg/kg/dose	5 mg/kg/dose
<u>Number of Days to Mating</u>				
Mean	2.0	2.1	2.5	2.0
S.D.	0.73	1.32	2.56	1.21
N	22	20	19	22
<u>Mating Index (%)</u>				
	27/22 (100.0)	17/22 (77.3)	18/22 (81.8)	22/22 (100.0)
<u>Pregnancy Index (%)</u>				
	15/22 (68.2)	14/22 (63.6)	16/22 (72.7)	5/22 (22.7)
<u>Fertility Index (%)</u>				
	14/22 (63.6)	14/22 (63.6)	14/19 (73.7)	2/22 (9.1)

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Group	Number of Animals	Number of Sperm	Mean Sperm Density	SD	Mean Sperm Morphology	SD	Mean Sperm Motility	SD	Mean Epididymis Histology	SD
Control	10	10	17.1 (3.0)	3.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0
Group 1	10	10	17.1 (3.0)	3.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0
Group 2	10	10	17.1 (3.0)	3.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0
Group 3	10	10	17.1 (3.0)	3.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0
Group 4	10	10	17.1 (3.0)	3.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0	1.0 (0.0)	0.0

Total: 40
 Mean: 17.1 (3.0)
 SD: 3.0
 N: 40

Total: 40
 Mean: 1.0 (0.0)
 SD: 0.0
 N: 40

Total: 40
 Mean: 1.0 (0.0)
 SD: 0.0
 N: 40

Total: 40
 Mean: 1.0 (0.0)
 SD: 0.0
 N: 40

Total: 40
 Mean: 1.0 (0.0)
 SD: 0.0
 N: 40

There were no drug-related effects on sperm density, morphology, or motility. There were no drug-related histopathological findings in the left testis and epididymis.

2.6.6.7 Local tolerance

There were no specific studies submitted to evaluate local tolerance to intravenous administration of idursulfase. There were no histological drug-related effects observed at injection sites in single dose toxicity studies in rats and monkeys, or in the 6-month monkey repeat-dose toxicity study.

2.6.6.8 Special toxicology studies

There were no special toxicology studies submitted. In particular, there were no specific studies submitted to evaluate the immunogenicity of intravenously administered idursulfase.

2.6.6.9 Discussion and Conclusions

Based on submitted toxicology studies, the intravenous use of idursulfase proposed in the draft labeling is safe.

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LABELING

The non-clinical portions of the labeling are evaluated below.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's version:

Evaluation:

Information on dose levels should also be included in the labeling.

Recommended version:

not been per

Pregnancy

Sponsor's version:

ian

Evaluation: Since the only reproductive toxicology study performed was a Segment I study in male rats, the correct Pregnancy category is C. The labeling text should be as close as accordance as possible with 21CFR 201.57 (f)(6)(i)(c).

Recommended version:

Pregnancy: Category C

have not been conducted with ELAPRASE. It is also not known whether ELAPRASE can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. ELAPRASE should be given to a pregnant woman only if clearly needed.

OVERDOSAGE

Sponsor's version:

There is no experience with overdose of ELAPRASE. Single-dose studies of idursulfase

Evaluation: The end of the first sentence should be clarified with "in humans". Although implied in the sponsor's version, we recommend that the text clearly indicate that idursulfase was not lethal in single-dose toxicity studies.

Recommended version:

There is no experience with overdose of ELAPRASE in humans. Single intravenous doses of 20 mg/kg idursulfase were not lethal in male rats and cynomolgus monkeys, and there were no signs of toxicity at this dose level.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Hunter syndrome (mucopolysaccharidosis II or MPS II) is an X-linked recessive disease produced by insufficient or defective levels of iduronate-2-sulfatase resulting in the lysosomal accumulation of GAGs in a variety of cells leading to cellular engorgement, dysfunction, and degeneration that eventually results in organ system dysfunction. Currently, no medical therapy has been shown effective for this disease. Idursulfase (Elaprase™) is a purified recombinant analog to the naturally occurring enzyme designed for enzyme replacement therapy in Hunter syndrome. The sponsor is seeking marketing approval for idursulfase as enzyme replacement therapy in patients with Hunter syndrome.

Preclinical studies were performed primarily using the intravenous route of administration to male animals only. The sponsor submitted the following preclinical studies in support of this NDA: Pharmacology; Absorption (rat and monkey), Distribution (rat), and Comparability (mouse, monkey, and rat); Toxicology – single-dose (rat and monkey) and repeat-dose (6-month monkey); and Reproductive Toxicology – segment I (rat).

Primary pharmacodynamics studies were performed in the male IKO mouse. IKO mice have little or no tissue iduronate-2-sulfatase activity and exhibit many of the cellular and clinical effects observed in Hunter's syndrome. In a series of studies, IKO mice were administered idursulfase intravenously at doses ranging from 0.1 to 5.0 mg/kg/dose. Idursulfase treatment produced a reduction in urinary and tissue (liver, spleen, kidney, and heart) GAG levels with weekly administration and a minimum dose of 1 mg/kg/dose the most effective dosing regimen.

Single-dose pharmacokinetic studies were performed in rats and monkeys. In general, C_{max} and systemic exposure (AUC) increased with increasing dose. After single-dose intravenous administration of 0.5, 2.5, and 12.5 mg/kg idursulfase in rats, mean C_{max} values were 15.1, 60.4, and 419 µg/mL, respectively, and mean AUC_{0-24h} values were 17.9, 104, and 860 µg·hr/mL, respectively. After single-dose intravenous administration of 0.1, 0.3, 0.5, and 1.5 mg/kg idursulfase in monkeys, mean C_{max} values were 2.6, 8.8, 12.7, and 41.4 µg/mL, respectively, and mean AUC_{0-24h} values were 98, 257, 950, and 3095 min·µg/mL, respectively. The elimination half-life (t_{1/2} alpha) increased and clearance (CL) decreased with increasing dose. The sponsor stated that this finding suggests saturation of clearance mechanisms with increasing dose. However, systemic exposure was only slightly increased with increasing dose. A distribution study using ¹²⁵I-labeled idursulfase was performed in rats. Radioactivity was detectable in the blood, plasma, and urine through the 48 hour timepoint. The highest tissue radioactivity concentrations at the 4 hour timepoint were observed in the thyroid gland, liver, stomach, bone marrow, kidneys, spleen, bone (femur), and adrenal glands. Radioactivity concentration were decreased in all tissues except thyroid gland at the 24 hour timepoint compared to the 4 hour timepoint and in all tissues examined at the 48 hour timepoint compared to the 24 hour timepoint. A large number of comparability studies were performed in mice, rats, and monkeys. Different lots were generally found to be comparable, although slight differences were sometimes noted. In humans, pharmacokinetic data was obtained from clinical studies where idursulfase was intravenously-administered to patients with Hunter syndrome. In one study (n = 3), mean C_{max} and systemic exposure (AUC) values were 5.8 µg/mL and 708 min·µg/mL, respectively, after the first single 1-hour intravenous infusion of 0.5 mg/kg idursulfase (the recommended weekly dose) with a t_{1/2} of approximately 2 hours and a clearance of 0.73 mL/minute/kg. In another study (n = 12), mean C_{max} and systemic exposure values were 1.5 µg/mL and 212 min·µg/mL, respectively, after the first single 3-hour intravenous infusion of 0.5 mg/kg idursulfase with a t_{1/2} of 46 minutes and a clearance of 2.8 mL/minute/kg. In this study, pharmacokinetic parameters were similar between Week 27 and Day 1.

In an acute toxicity study, male rats were administered intravenously 0, 5, 10, and 20 mg/kg idursulfase via slow bolus injection. There were no adverse effects observed in this study and a target organ of toxicity was not identified.

In an acute toxicity study, male monkeys were administered intravenously 0, 5, 10, and 20 mg/kg idursulfase via slow bolus injection. There were no adverse effects observed in this study and a target organ of toxicity was not identified. However, animals were removed from the study and returned to the stock colony on Day 15. Therefore, macroscopic and microscopic evaluations were not performed.

In a 6-month toxicity study, male cynomolgus monkeys (n = 4/group) were administered intravenously 0, 0.5, 2.5, and 12.5 mg/kg/dose idursulfase weekly with half the animals sacrificed on Day 91. Two additional control and high dose animals were included and sacrificed after a 4-week recovery period. There were no adverse effects observed in this study and a target organ of toxicity was not identified.

In a fertility and early embryonic development study, male rats were administered intravenously 0, 0.5, 1.5, and 5 mg/kg/dose idursulfase (I2S) twice weekly (3-4 days between doses) beginning 4 weeks prior to cohabitation, during cohabitation, and until the day of necropsy (Day 64). There were no adverse effects on male fertility or reproductive performance observed in this study. Additionally, there were no effects on early embryonic development in the pups of treated male rats.

A number of changes were recommending to the nonclinical portion of the label in order to conform to the format specified under 21CFR, Subpart B. Otherwise, this submission contains adequate nonclinical studies for marketing approval of idursulfase. From a preclinical standpoint, idursulfase is safe for the proposed use as indicated in the draft labeling and this NDA may be approved.

Recommendations:

1. From a preclinical standpoint, this NDA may be approved.
2. Labeling changes should be made as recommended in the text of this review.

Reviewer Signature Ronald Honchel 4/21/06
Ronald Honchel, Ph.D. Date
Pharmacologist, HFD-180

See the accompanying Supervisory Addendum

Supervisor Signature J. B. Choudary May 7, 2006
Jasti B. Choudary, B.V.Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

Concurrence Yes ___ No ___

cc:

Original NDA

HFD-180

HFD-181/CSO

HFD-180/Dr. Choudary

HFD-180/Dr. Honchel

HFD-48/Dr. Viswanathan

R/D Init. J. Choudary 4/18/06 J. Choudary

APPENDIX/ATTACHMENTS

NONE

**APPEARS THIS WAY
ON ORIGINAL**