CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

213026Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

Date: February 22, 2021

From: Lois M. Freed, Ph.D.
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Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: NDA 213026 (Amondys 45; casimersen; SRP-4045)

NDA 213036 was submitted on January 20, 2020, for casimersen, a phosphorodiamidate morpholino oligomer (PMO) antisense oligonucleotide (ASO), for the treatment of Duchenne muscular dystrophy in patients with a confirmed mutation of the DMD gene that is amenable to exon 45 skipping. Casimersen is to be administered to patients at a dose of 30 mg/kg weekly by intravenous (IV) infusion, over 35-60 minutes.

The nonclinical studies conducted to support clinical development and the NDA consist of the following: pharmacology (primary, secondary), safety pharmacology, PK/ADME, general toxicology (12-, 22-, and 26-week IV in mouse; 12- and 39-week IV in monkey) studies, a juvenile animal toxicology (10-week IV) study, and a battery of genetic toxicology studies. Reproductive and developmental toxicology studies were not required because of the intended patient population (extremely rare in females), and carcinogenicity studies may be conducted post-approval, as previously agreed to by the division, because of the seriousness of the indication.

The nonclinical data were reviewed in detail by Barbara Wilcox (Pharmacology/Toxicology NDA Review and Evaluation, Barbara J. Wilcox, Ph.D., January 25, 2021). Based on her review, Dr. Wilcox has concluded that the nonclinical data are adequate to support approval of the NDA.

This memo briefly summarizes selected results of the studies of casimersen, with a focus on the studies providing data to support the nonclinical sections of labeling.

Summary

Casimersen is a 22-mer PMO ASO designed to hybridize with dystrophin pre-mRNA to cause skipping of exon 45, resulting in exclusion of exon 45 from mature mRNA, restoration of the open reading frame of dystrophin mRNA, and production of a truncated but potentially functional dystrophin protein. The pharmacological activity of casimersen was tested in vitro using human rhabdomyosarcoma cells and normal primary myoblasts, as there are no relevant animal models due to the species- and mutation-specific nature of PMO ASOs.

The PK/ADME of casimersen was fairly characteristic of PMOs. Protein binding was low (<37%) in animal and human plasma, and there was extensive tissue distribution, with highest and lowest levels of radioactivity in kidney and brain, respectively, following a single 150 mg/kg IV dose of ^{14}C-labeled casimersen.

The pivotal (GLP) general toxicity studies were conducted in adult male C57BL/6NCrl mouse and cynomolgus monkey.

In mouse, casimersen was administered at doses of 0, 12, 120, and 960 mg/kg IV QW for 12 weeks (+ 4-week recovery) and doses of 0, 300, 600, and 960 mg/kg SC QW for 26 weeks (+ 8-week recovery). A 22-week toxicity study (+ 8-week recovery) at doses of 0, 300, 960, and 2000 mg/kg IV QW was terminated prematurely (Week 22) because of dosing difficulties (dose-related local toxicity).

The primary findings were kidney histopathology, observed in all three studies, and local toxicity at the injection site, observed in the 22- and 26-week studies. In the 22-week IV study, toxicity at the injection site (dose-related thickening of the skin, swelling) resulted in partial or missed doses and early termination of the study. In the 26-week SC study, toxicity at the injection site (dermal fibroplasia, myofiber degeneration/regeneration) was also noted, but it did not interfere with dosing. Renal findings consisted of tubular basophilia and microvacuolation at the highest dose tested in the 12-week study, which progressed to tubular degeneration/regeneration at all doses in the 22- and 26-week studies. Microscopic findings in kidney are summarized in the following table.

STUDY	FINDING	DOSE (mg/kg)						
		0	12	120	300	600	960	2000
MAIN STUDY								
12-wk (IV)	cytoplasmic basophilia/microvacuolation							
	minimal	0/20	0/20	0/20			4/21	
	slight	0/20	0/20	0/20			11/21	
	moderate	0/20	0/20	0/20			6/21	
	total	**0/20**	**0/20**	**0/20**			**21/21**	
22-wk (IV)	cast							
	minimal	0/19			1/19		5/18	11/20
	mild	0/19			0/19		0/18	3/20
	total	**0/19**			**1/19**		**5/18**	**14/20**
	tubular degeneration/regeneration							
	minimal	0/19			1/19		11/18	9/20
	mild	0/19			0/19		0/18	7/20
	moderate	0/19			0/19		0/18	1/20
	total	**0/19**			**1/19**		**11/18**	**17/20**
26-wk (SC)	tubular degeneration/regeneration							
	minimal	**0/20**			**9/19**	**15/18**	**17/19**	
RECOVERY								
12-wk (IV)	cytoplasmic basophilia/microvacuolation							
	minimal	0/12	0/12	0/12			9/11	
	slight	0/12	0/12	0/12			2/11	
	moderate	0/12	0/12	0/12			0/11	
	total	**0/12**	**0/12**	**0/12**			**11/11**	
22-wk (IV)	cast							
	minimal	0/11			0/12		2/12	4/12
	mild	0/11			0/12		0/12	7/12
	total	**0/11**			**0/12**		**2/12**	**11/12**
	tubular degeneration/regeneration							
	minimal	0/11			2/12		6/12	2/12
	mild	0/11			0/12		0/12	10/12
	moderate	0/11			0/12		0/12	0/12
	total	**0/11**			**2/12**		**6/12**	**12/12**
26-wk (SC)	tubular degeneration/regeneration							
	minimal	**0/12**			**7/11**	**10/12**	**11/12**	

The 22- and 26-week studies included hormone analyses (testosterone, LH, and FSH) and an enhanced examination of the male reproductive system (sperm motility, morphology, and concentration; histological evaluation of the spermatogenic cycle); no adverse effects were observed.

Plasma exposure data in mouse (last sampling day: Day 78, Day 149, or Day 176) are summarized in the following table (C_{max} = first sampling time; units of µg/mL for C_{max} and µg*hr/mL for $AUC_{(0\text{-}t)}$).

STUDY	DOSE (mg/kg)											
	12		120		300		600		960		2000	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
12-week	9.63	8.05	85.6	69.8					1980	1160		
22-week					606	1060			3190	1490	7020	4120
26-week					185	460	345	991	332	1560		

In monkey, casimersen was administered at doses of 0, 5, 40, and 320 mg/kg weekly by IV bolus injection for 12 weeks (+ 4-week recovery) and doses of 0, 80, 320, and 640 mg/kg weekly by 30-min IV infusion for 39 weeks (+ 8-week recovery). Both studies included hormone analyses (testosterone, LH, and FSH) and an enhanced examination of the male reproductive system (testicular volume; sperm morphology, motility, count, and density; histological evaluation of the spermatogenic cycle).

The primary findings were complement activation (assessed only in the 12-week study) and kidney histopathology. In the 12-week study, complement analysis was conducted on blood samples collected during Days 1, 8, and 78. Dose-related increases in Bb and C3a were observed on Days 1 and 8, with the responses being greater on Day 8 but lower on Day 78; no effect on C5a were observed. Renal tubular basophilia and vacuolation were detected at doses >5 mg/kg, minimal-to-slight in severity in the 12-week study but with increased severity (mild to moderate) at the mid and high doses in the 39-week study; no degeneration or necrosis was detected. No drug-related effects on hormones or male reproductive assessments were observed in either study.

Plasma exposure data in monkey (last sampling day: Day 78 or Day 260) are summarized in the following table (for the 12-week study, Cmax represents the first sampling time; units of µg/mL for Cmax and µg*hr/mL for $AUC_{(0\text{-}t)}$).

STUDY	DOSE (mg/kg)									
	5		40		80		320		640	
	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
12-week	21.6	26.9	242	320			1490	1930		
39-week					582	782	1890	2870	4300	6290

In the juvenile animal toxicology study, casimersen (0, 100, 300, and 900 mg/kg IV) was administered weekly to male Sprague-Dawley rats from postnatal day (PND) 14 to PND 77. No drug-related deaths or clinical signs were observed, nor were there any adverse effects on developmental (e.g., neurobehavioral, immune function, male reproductive, bone densitometry) parameters. Kidney was the primary target organ, with tubular vacuolation detected at all doses and cytoplasmic basophilic granules and degeneration/necrosis at the mid and high doses. All findings (summarized in the following sponsor's table) were minimal, except for mild vacuolation in the majority of high-dose animals.

Text Table 3
Summary of Microscopic Findings – Scheduled Euthanasia (Day 78 pp) Subset A

	Males			
Group	1	2	3	4
Dose (mg/kg)	0	100	300	900
No. Animals Examined	32	32	32	31
Kidney (No. Examined)	32	32	32	31
Vacuolation; tubular	(0)[a]	(10)	(30)	(31)
Minimal	–	10	30	10
Mild	–	–	–	21
Cytoplasmic basophilic granules; tubular	(0)	(0)	(31)	(31)
Minimal	–	–	31	31
Degeneration/necrosis; tubular	(1)	(0)	(1)	(10)
Minimal	1	–	1	10

[a] Numbers in parentheses represent the number of animals with the finding

On PND 77, plasma exposures ($AUC_{(0\text{-}24h)}$) at the low, mid, and high doses were 200, 633, and 3430 µg*hr/mL, respectively.

Casimersen was tested in a standard battery of in vitro (bacterial reverse mutation, chromosomal aberration in CHO cells) and in vivo (mouse micronucleus) genetic toxicology assays. The studies were adequately conducted and negative.

As noted, carcinogenicity studies of casimersen are to be conducted post-approval. The sponsor did, however, conduct dose-ranging studies in adult male CBYB6F1 [CByB6F1-Tg(HRAS)2Jic (-/-homozygous c-Ha-ras)] mouse and Sprague Dawley rat.

In the 4-week IV toxicity study in mouse, casimersen was administered at doses of 0, 250, 500, 1000, and 2000 mg/kg QW. There were no deaths or drug-related effects on clinical signs, body weight, or food consumption. Microscopic findings consisted of renal tubular basophilia at doses >500 mg/kg and mineralization in the heart at the highest dose tested. Although male reproductive organs were examined, sperm parameters and spermatogenic cycle were not. Plasma exposures ($AUC_{(0\text{-}24h)}$) on Day 22 were 357, 824, 3520, and 7620 µ*hr/mL at 0, 250, 500, 1000, and 2000 mg/kg, respectively.

In the 13-week IV toxicity study in rat, casimersen was administered at doses of 0, 250, 500, 1000, and 2000 mg/kg QW. There were no deaths or drug-related effects on clinical signs or food consumption. Body weight gain was decreased (~8%) at the high dose. Drug-related microscopic findings were detected in lung (macrophage basophilic infiltrates), liver (hepatocyte basophilia, Kupffer cell vacuolation), lymph nodes (macrophage infiltrates), and heart (mononuclear cell infiltrate). However, kidney was the primary target organ, with tubular degeneration observed at all doses; microscopic findings (summarized in the table below) were associated with dose-related increases (12-22%) in kidney weight at >500 mg/kg and an increase in urea nitrogen at the high dose.

TISSUE	FINDING	DOSE (mg/kg)				
		0	250	500	1000	2000
Kidney	tubule degeneration					
	minimal	0/10	5/10	2/10	0/10	0/10
	slight	0/10	5/10	8/10	1/10	0/10
	moderate	0/10	0/10	0/10	9/10	4/10
	marked	0/10	0/10	0/10	0/10	6/10
	total	**0/10**	**10/10**	**10/10**	**10/10**	**10/10**
ureter	transitional cell vacuolation					
	minimal	**0/10**	**1/10**	**10/10**	**2/10**	**4/10**
Urinary bladder	Transitional cell vacuolation					
	minimal	**0/10**	**0/10**	**3/10**	**5/10**	**4/10**

A no-effect dose for renal tubular degeneration was not identified. Plasma exposure ($AUC_{(0\text{-}24h)}$) at the low dose was 726 µg*hr/mL on Day 85.

[Interspecies comparisons were calculated using a plasma $AUC_{(0\text{-}24h)}$ for humans of 182 µg*hr/mL (Study 4045-101, Week 60) at the recommended dose of 30 mg/kg/week.]

Conclusions and Recommendations

As observed with the sponsor's other PMO ASOs for Duchenne muscular dystrophy (eteplirsen and golodirsen), kidney was the primary target organ in all species and strains (C57BL/6NCrl mouse, CByB6F1-Tg(HRAS)2jJic WT mouse, Sprague Dawley rat, cynomolgus monkey) tested. Although a direct comparison of findings cannot be made, the renal toxicities observed with these three ASOs appear to differ, in terms of affected species and severity of effects.

The most direct comparison can be made in the juvenile animal. Eteplirsen, golodirsen, and casimersen were all tested in juvenile Sprague Dawley rat at IV doses of 0, 100, 300, and 900 mg/kg/week from PND 14 to PND 77. Plasma exposures (AUC) at each dose were similar among the studies. However, only with golodirsen were there drug-related deaths due to primary renal impairment and/or renal failure. The adverse effects on bone mineral content and density observed with eteplirsen (all doses) and golodirsen (high dose only) in juvenile rat (high dose only) were not detected with casimersen.

In addition, the synovial hyperplasia and inflammation of the femorotibial joint in adult cynomolgus monkey with golodirsen (doses of 200 and 400 mg/kg/week; plasma AUCs of 1620 and 4280 µg*hr/mL, respectively) were not detected with casimersen.

Recommendation: Overall, the battery of nonclinical studies conducted on casimersen is adequate; the primary toxicities identified in animals can be monitored for in humans. Therefore, the nonclinical data support approval of

casimersen for the intended indication, with appropriate labeling and postmarketing requirements for carcinogenicity studies in mouse and rat.

Labeling recommendations are provided in a separate document.

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/s/

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02/22/2021 08:09:03 AM

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 213026
Supporting document:	SDN1
Applicant's letter date:	1/10/2020
CDER stamp date:	1/20/2020
Product:	Casimersen (SRP-4045)
Indication:	Duchenne muscular dystrophy
Applicant:	Sarepta Therapeutics, Inc.
Review Division:	Division of Neurology 1
Reviewer:	Barbara J. Wilcox, PhD
Supervisor:	Lois M. Freed, PhD
DN1 Division Director:	Eric Bastings, MD
Project Manager:	Michael Matthews, MS, RAC-US

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All tables and figures are from the sponsor unless otherwise designated.

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1 Executive Summary

1.1 Introduction

SRP-4045 (casimersen) is a phosphorodiamidate morpholino oligomer (PMO) antisense drug intended for use in the treatment of Duchennne muscular dystrophy (DMD). SRP-4045 is designed to hybridize to the pre-mRNA transcript of the dystrophin gene, causing deletion of exon 45 from the mature mRNA. In DMD patients whose disease is caused by a mutation in exon 45, deletion of the exon leads to restoration of the mRNA reading frame and production of a truncated, but partially functional, dystrophin protein molecule. This mechanism of action is similar to that of eteplirsen and golodirsen, similar antisense oligonucleotide drugs with the same molecular backbone chemistry recently approved under Accelerated Approval for treatment of DMD.

1.2 Brief Discussion of Nonclinical Findings

A complete program of nonclinical safety studies was submitted to NDA 213026, including pharmacology, pharmacokinetics, safety pharmacology, genetic toxicology, and general toxicology studies in adult animals and a juvenile animal toxicology study.

Pivotal toxicology studies were conducted in mouse, rat, and cynomolgus monkey. Studies were conducted in male mouse with durations up to 26 weeks with doses up to 2000 mg/kg, and in male monkeys at durations of up to 39 weeks and dose levels of 640 mg/kg. Similar to the toxicities observed with golodirsen, the major target organ for SRP-4045 toxicity was kidney, although with significantly lower magnitude. In all species, dose-related histopathology was observed and described as cytoplasmic basophilia and vacuolization of renal tubular epithelium. At higher doses, the observations included regeneration/degeneration of the tubular epithelium. In most studies, no evidence of impairment of renal function was observed. However, in a 22-week study in male mice, initiated as a chronic intravenous (IV) study but terminated prematurely, the HD (2000 mg/kg) was associated with a small increase in plasma urea nitrogen, suggesting functional impairment at that dose. The magnitude of the microscopic findings in most of the toxicology studies were generally graded minimal to moderate at the end of dosing; however, in most cases, significant recovery was not observed.

To support use of SRP-4045 in pediatric patients, a juvenile animal toxicology study was conducted in which male rat pups received weekly IV injections of SRP-4045 of up to 900 mg/kg beginning on PND14 and continuing for 10 weeks. No effects on postnatal development were observed. Histopathology showed vacuolation of kidney tubular epithelium, graded minimal to mild, and cytoplasmic basophilia of tubular epithelium, graded mild. In addition, degeneration/necrosis of the renal tubular epithelium was observed at the high dose, although no correlating adverse clinical pathology findings were observed.

Carcinogenicity assessments were not conducted. PMRs covering the carcinogenicity assessments are recommended.

1.3 Recommendations

1.3.1 Approvability

The pivotal nonclinical studies indicate that renal impairment can result from chronic exposure to casimersen. However, because kidney function is monitorable, the nonclinical data are considered adequate to support approval of casimersen for the treatment of DMD in patients with mutations amenable to exon 45 skipping therapies.

1.3.2 Additional Nonclinical Recommendations

Carcinogenicity assessments should be conducted post marketing.

1.3.3 Labeling

8.4 Pediatric Use

Intravenous administration of casimersen (0, 100, 300, and 900 mg/kg) to juvenile male rats once weekly for 10 weeks (b) (4) resulted in (b) (4) (b) (4) renal tubular degeneration/necrosis at (b) (4) at the recommended human dose of 30 mg/kg/week.

12.1 Mechanism of Action

Casimersen is designed to bind to exon 45 of dystrophin pre-mRNA resulting in exclusion of this exon during mRNA processing in patients with genetic mutations that are amenable to exon 45 skipping. Exon 45 skipping is intended to allow for production of an internally truncated dystrophin protein in patients with genetic mutations that are amenable to exon 45 skipping.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Mutagenesis

(b) (4)

Impairment of Fertility

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

Generic Name: Casimersen

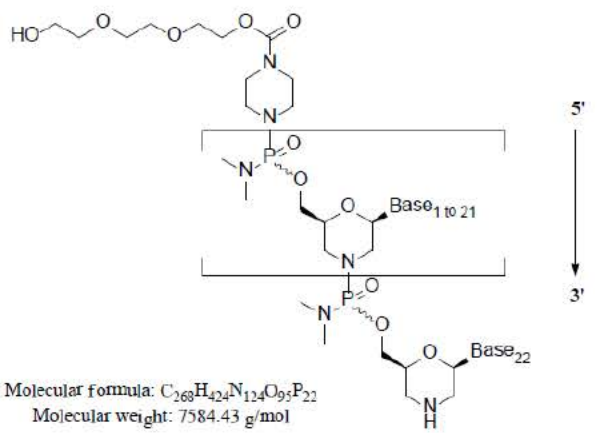
Code Name: SRP-4045

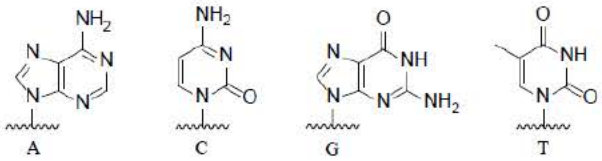
Molecular Formula/Molecular Weight:

$C_{268}H_{424}N_{124}O_{95}P_{22}$/ 7584.4 g/mol

Structure or Biochemical Description

Figure 1 **Structure of SRP-4045**





The sequence of bases from the 5' end to the 3' end is:
CAATGCCATCCTGGAGTTCCTG

Pharmacologic Class: Antisense oligonucleotide

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND 118086 (casimersen)
IND 119982 and NDA 211970 (golodirsen, Vyondys53) For treatment of DMD patients with mutations amenable to exon 53 skipping.

IND 77429 and NDA 206448 (eteplirsen, Exondys) For treatment of DMD patients amenable to skipping of exon 51.

2.3 Drug Formulation

Table 1: Formulation for SRP-4045 Drug Product, 50 mg/mL

Component	Reference to Standards	Quantity (mg/mL)	Function
SRP-4045	In-house specification	50 mg	Active ingredient
Sodium chloride	USP	8.0 mg	(b) (4)
Potassium chloride	USP	0.2 mg	
Potassium phosphate monobasic, anhydrous	NF	0.2 mg	
Sodium phosphate dibasic, anhydrous	USP	1.14 mg	
Sodium hydroxide	NF	q.s.	
Hydrochloric acid	NF	q.s.	
Water for Injection	USP	q.s. to volume	

2.4 Comments on Novel Excipients

The formulation contains no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

All impurities are adequately qualified or are not of toxicological concern.

2.6 Proposed Clinical Population and Dosing Regimen

Patient population: Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the *DMD* gene that is amenable to exon 45 skipping.
Dose: 30 mg/kg, weekly
Route: IV infusion

2.7 Regulatory Background

SRP-4045 is one of three PMO antisense oligonucleotide drugs developed by Sarepta Therapeutics for treatment of DMD. IND 118086 was submitted on June 23, 2014 and allowed to proceed (July 23, 2014). The pre-NDA meeting was conducted on June 5, 2019 (meeting minutes issued on June 27, 2019).

3 Studies Submitted

3.1 Studies Reviewed

Studies reviewed under IND 118086 included *in vitro* genetic toxicology, safety pharmacology (cardiovascular and CNS), plasma protein binding, and 12- week general toxicology studies in male mice and male cynomolgus monkeys. Proof-of-concept studies in the MDX mouse model of muscular dystrophy were submitted to IND 77429.

Pharmacology
Study #SR-14-119, Selection of PMO sequence for exon 45 development
Study #SR-18-112, Off-target sequence analysis

Safety Pharmacology
Study #SR-18-112, Effect of SRP-4045 on cloned hERG potassium channels expressed in human embryonic kidney cells

ADME
Study #SR-17-006, Distribution, metabolism, and excretion of ^{14}C-SRP-4045 after a single intravenous dose
Study #SR4045-PKD-002, Inhibitory potential of SRP-4045 on human hepatic cytochrome P450 isozymes
Study #4045-pkd-003, Evaluation of cytochrome P450 induction following exposure of primary cultures if human hepatocytes to SRP-4045
Study #4045-pkd-004, Metabolism of SRP-4045 in mouse, rat, monkey, and human microsomes
Study #SR-16-058, Evaluation of [^{14}C]SRP-4045 as a substrate and SRP-4045 as an inhibitor of a panel of human transporters.
Study #SR-17-050, Toxicokinetic profile and bioavailability following subcutaneous and intravenous administration in the male mouse

Repeat-Dose toxicology:
Study #SR-15-057, A 22-week intravenous injection toxicity and toxicokinetic study of SRP-4045 in the male mouse with an 8-week recovery period
Study #SR-17-085, 26-week subcutaneous injection toxicity and toxicokinetic study in male mice with an 8-week recovery period
Study #SR-15-037, A 39-week intravenous infusion toxicity study in male cynomolgus monkeys with an 8-week recovery period

Genetic Toxicology
Study #4045-gtx-003, In vivo mouse bone marrow micronucleus assay

Carcinogenicity
Study #SR-17-002, 4-week repeated dose intravenous toxicity and toxicokinetic study in male CByF1 mice
Study #SR-17-003, 13-week intravenous injection toxicity and toxicokinetic study in male rats with an 8-week recovery period

Developmental Toxicology:
Study #4045-tox-004, An intravenous dose range-finding study of SRP-4045 in juvenile male rats
Study #4045-tox-005, A 10-week intravenous juvenile toxicity study of SRP-4045 in male rats

3.3 Previous Reviews Referenced

IND 118086, Pharmacology and Toxicology Review, Barbara J Wilcox, PhD, 2/27/2017
IND 77429, Pharmacology and Toxicology Review, Barbara J Wilcox, PhD, 3/11/2013

4 Pharmacology

4.1 Primary Pharmacology

Casimersen (SRP-4045) is a 22-mer phosphorodiamidate morpholino oligomer (PMO) antisense drug developed for use in the treatment of Duchennne muscular dystrophy (DMD). SRP-4045 is designed to hybridize to the pre-mRNA transcript of the dystrophin gene and cause deletion of exon 45 from the mature RNA through normal RNA processing. In DMD patients whose disease is caused by a mutation in exon 45, deletion of the exon leads to restoration of the mRNA reading frame and production of a truncated but partially functional dystrophin molecule. This mechanism of action is similar to that of eteplirsen and golodirsen, oligonucleotide drugs with the same backbone chemistry but designed for deletion or "skipping" of differing dystrophin gene mutations.

Proof of concept studies for the exon skipping activity of the oligonucleotide drugs such as SRP-4045 was demonstrated in studies originally submitted and reviewed under IND 77429 in which the murine surrogate PMO, AVI-4225, was used in the *mdx* mouse, a model in which exon 23 of the dystrophin gene is mutated resulting in a DMD-like syndrome. AVI-4225 was administered to male and female *mdx* mice at doses up to 960 mg/kg weekly for up to 6 months. At all doses, a dose-related decrease in incidence and severity of myopathy-related myofiber degeneration in skeletal muscle was observed, relative to controls.

Study #SR-14-119
Title: Research Report: Selection of PMO sequence for exon 45 development

A collaborative study was conducted by Sarepta to determine the optimal sequence for targeting exon 45 of the dystrophin gene. Nine PMOs were screened for exon 45 skipping and compared in blinded studies in three independent laboratories using rhabdomyosarcoma cells and primary myoblasts. One PMO (SRP-4045) was identified as having the optimal sequence.

4.2 Secondary Pharmacology

The potential for off-target hybridization was evaluated in study #SR-18-108. The binding affinity of SRP-4045 was calculated for potential off-target sites in the entire human genome using a variety of *in silico* tools. The results showed that SRP-4045 has minimal predicted potential for off-target hybridization in the human genome.

4.3 Safety Pharmacology

Cardiovascular and neurological safety pharmacology studies were conducted with SRP-4045. Those studies were reviewed under IND 118086. Cynomolgus monkeys received IV injections of SRP-4045 weekly for 4 total doses of 0, 5, 40, or 320 mg/kg.

No adverse test article-related effects were observed on cardiovascular or neurological endpoints.

Study #SR-18-112
Title: Effect of SRP-4045 on cloned hERG potassium channels expressed in human embryonic kidney cells.

The effect of SRP-4045 on current across the hERG channel was evaluated using standard methods using HEK-293 cells expressing the hERG channel. Test article concentrations used were 100 µM, 300 µM, 1 mM, and 3 mM. Concentrations were verified in samples collected from perfusion outflow. The positive control was terfenadine. Vehicle control (hERG buffer) and positive control contained 0.3% DMSO.

Results:
The IC_{50} for the inhibitory effect of SRP-4045 on the hERG current was greater than 3 mM. Terfenadine inhibited hERG current by78.3% at 60 nM.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Distribution:
Plasma protein binding of SRP-4045 was evaluated *in vitro* in mouse, rat, monkey, and human (Study #4045-pkd-001, reviewed under IND 118086). The results demonstrated low protein binding, which was independent of drug concentration.

Study # SR-17-006
Title: Distribution, metabolism, and excretion of ^{14}C-SRP-4045 after a single intravenous dose

Testing Facility: (b) (4)

GLP Compliance: No
Date of Study Initiation: Not specified
Drug/Lot#/purity: SRP-4045/7003088/95%

Methods:
Species: mouse, C57BL/10ScSn-*Dmd*^{mdx}/J, males only
Dose: 120 mg/kg (300µCi/kg)
Regimen: Single IV dose.

Study design is summarized in the table below:

Group	Number of Male Animals	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Sample Collections
1	9	IV bolus	120	5	Urine, Feces, and Carcass
2	39	IV bolus	120	5	Blood and Tissues
3	22	IV bolus	120	5	Blood and Carcasses for QWBA

IV Intravenous.
QWBA Quantitative whole-body autoradiography.
Note: The radioactive dose was approximately 300 µCi/kg.

Results:

Maximum concentration of drug in blood and plasma was observed at 0.083 hours post dose. SRP-4045 was rapidly distributed to most tissues after a single dose of 120 mg/kg. C_{max} in tissues was observed at approximately 0.25 hours post dose. The primary route of elimination was urinary excretion and was considered complete by 336 hours post dose.

Pharmacokinetic parameters in blood and plasma estimated from total radioactivity are summarized below:

Matrix	C_0 (ng-eq/g)	C_{max} (ng-eq/g)	T_{max} (h)	AUC_{0-t} (ng-eq·h/g)	AUC_{0-inf} (ng-eq·h/g)	$t_{1/2}$ (h)	CL (g/h/kg)	V_{SS} (g/kg)
Blood	782000	363000	0.0830	123000	124000	0.291	971	237
Plasma	1230000	576000	0.0830	196000	196000	0.438	613	155

Abbreviations: AUC_{0-inf} = Area under the curve from time zero to infinity, AUC_{0-t} = Area under the curve from time zero to the time fo the last measureable concentration, C_0 = Back-extrapolated concentration at time zero, CL = Clearance, C_{max} = Maximum observed concentration, eq =Equivalents ^{14}C-SRP-4053, h = Hours, $t_{1/2}$ = Elimination half-life, T_{max} = Time of maximum observed concentration, Vss = Volume of distribution at steady-state.

Mean concentration in selected tissues is summarized below:

Sample	Mean Concentration (ng-Equivalents ^{14}C-SRP-4045/g) Sacrifice Time (Hours) 0.25	1	4	8	24	48	96	120	144
Brain	1980	462	81.2	43.1	39.5	14.0	26.5	14.3	11.7
Diaphragm	34700	19400	1060	1170	1470	837	900	609	675
Heart	24700	6040	533	566	1040	410	489	399	416
Biceps femoris	61200	27000	1900	1860	1520	1310	1300	1160	1660
Quadriceps	42300	11600	1220	1150	1320	745	859	861	612
Tibialis anterior	127000	11600	1760	1920	1310	1140	1060	898	577
Biceps brachii	34900	6390	1020	542	1330	680	970	417	529
Kidney	623000	245000	127000	99200	117000	56100	86700	34600	30100

Radioactivity was not detected in the following tissues at 1344 hours post dose: blood, eye lens, brown fat, pancreas, cerebellum, cerebrum, medulla, olfactory lobe, and spinal cord.

Recovery of radioactivity:

	Intravenous Administration					
	% Mean Total Recovery	% Dose in Urine	% Dose in Feces	% Dose in Carcass	% Dose in Cage Rinse	% Dose in Other[a]
Group 1 (0-336 h postdose)	90.3 ± 4.26	68.9 ± 2.26	12.5 ± 6.02	0.727 ± 0.0528	5.75 ± 2.59	2.46

a Cage wash and cage wipe.

Overall mean recovery of radioactivity over a two-week period after a single IV injection was 90.3 ± 4.26%

Metabolism

Study #4045-PKD-002

Title: Inhibitory potential of SRP-4045 on human hepatic cytochrome P450 Isozymes

Testing Facility: (b) (4)

GLP Compliance: No
Date of Study Initiation: 12/06/2013 (Date of protocol issue)
Drug/Lot#/Purity: SRP-4045/Dev-569, LY01/87.1%

Summary

Pooled hepatic microsomes from 50 human donors (25 male, 25 female) were used to determine the inhibitory potential of SRP-4045 (0.00129 to 5.89 mg/mL) on selected microsomal enzymes (listed in the table below with the positive control items used to assure assay sensitivity).

Activity Assay (Cytochrome P450)	Substrate (μM)	Protein (mg/mL)	Time (Minutes)	Analyte	Positive Control (μM)
Phenacetin *O*-deethylase (CYP1A2)	30	0.1	15	Acetaminophen	Fluvoxamine (1)
Bupropion hydroxylase (CYP2B6)	65	0.1	15	Hydroxybupropion	ThioTEPA (100)
Amodiaquine *N*-deethylase (CYP2C8)	1.0	0.025	10	Desethylamodiaquine	Montelukast (0.1)
Diclofenac 4′-hydroxylase (CYP2C9)	3.5	0.025	10	4′-Hydroxydiclofenac	Sulfaphenazole (3)
S-mephenytoin 4′-hydroxylase (CYP2C19)	25	0.1	15	4′-Hydroxymephenytoin	Nootkatone (30)
Bufuralol 1′-hydroxylase (CYP2D6)	11	0.1	15	1′-Hydroxybufuralol	Quinidine (0.3)
Testosterone 6β-hydroxylase (CYP3A4/5)	45	0.25	5	6β-Hydroxytestosterone	Ketoconazole (0.2)
Midazolam 1′-hydroxylase (CYP3A4/5)	2.0	0.1	5	1′-Hydroxymidazolam	Ketoconazole (0.2)

Notes: The stopping solution for the CYP1A2, CYP2C8, CYP2C9, and CYP3A4/5 (midazolam 1′-hydroxylase) assays was 10% acetic acid:acetonitrile (1:1, v/v). The stopping solution for the CYP2B6 and CYP2D6 assays was 5% acetic acid. The stopping solution for the CYP2C19 and CYP3A4/5 (testosterone 6β-hydroxylase) assays was 7% formic acid. The protein concentration is the final concentration of microsomal protein in the assay.

Results:

Incubation Conditions for Ki Determinations

Activity Assay (Cytochrome P450)	Substrate Concentrations (μM)	SRP-4045 Concentrations (mg/mL)
Diclofenac 4′-hydroxylase (CYP2C9)	2.5, 5, 10, 20, 40	0, 1, 2, 4, 8, and 10
S-mephenytoin 4′-hydroxylase (CYP2C19)	10, 20, 40, 80, 160	0, 1, 2, 4, 8, and 10
Testosterone 6β-hydroxylase (CYP3A4/5)	15, 30, 60, 120, 240	0, 1, 2, 4, 8, and 10

Positive control tests successfully demonstrated adequate assay sensitivity.

Results for SRP-4045 are summarized in the table below:

Table 1
Summary of inhibition on human hepatic CYP isoenzymes by SRP-4045

	Direct Inhibition			
CYP Isoenzyme	Conclusion	IC_{50} (mg/mL)	K_i (mg/mL)	Type of Inhibition
CYP1A2	No	NA	NA	NA
CYP2B6	No	NA	NA	NA
CYP2C8	No	NA	NA	NA
CYP2C9	Yes	5.45	1.37 ± 0.15	Competitive
CYP2C19	Yes	2.26	2.02 ± 0.13	Competitive
CYP2D6	No	NA	NA	NA
CYP3A4/5[a]	Yes	5.32	10.6 ± 1.6	Competitive
CYP3A4/5[b]	No	NA	NA	NA

IC_{50} The concentration of the test article that inhibits 50% of the CYP isoenzyme activity.
K_i Inhibition constant.
NA Not applicable.
Note: No metabolism-dependent inhibition by SRP-4045 was observed; K_I and K_{intact} not determined.
a Testosterone 6β-hydroxylase.
b Midazolam 1′-hydroxylase.

Study #4045-pkd-003

Title: Evaluation of cytochrome P450 induction following exposure of primary cultures of human hepatocytes to SRP-4045

Testing Facility: (b) (4)

GLP Compliance: No
Date of Study Initiation: 1/8/2014 (Date of protocol issue)
Drug/Lot#/purity: SRP-4045/DEV-427, LY01/87.1%

Summary and results:
Test system: Primary cultures of human hepatocytes (3 donors)
Negative control: solvent control
SRP-4045 concentrations: 0.00137 to 6.25 mg/mL
Positive controls: known inducers of each selected enzyme

CYP Enzyme Induced	Prototypical Inducer/ Non-Inducer	Vehicle	Concentration (μM)
CYP1A2	Omeprazole	1% (v/v) ACN in sHMM	25[a]
CYP2B6	Phenobarbital	1% (v/v) ACN in sHMM	1000
CYP3A4	Rifampicin	1% (v/v) ACN in sHMM	50[a]
Non-Inducer	Flumazenil	1% (v/v) ACN in sHMM	20

Note: The vehicle, 1% (v/v) acetonitrile (ACN) in supplemented hepatocyte maintenance medium (sHMM), was also used as a solvent control.
a Protocol Deviation.

Hepatocytes were incubated for 72 hours and assessed for cytotoxicity, gene expression, and enzyme activity in the presence of a known inducer, a non-inducer, and

varying concentrations of SRP-4045. Cytotoxicity was determined by measurement of LDH levels.

Results:
Expected results were observed with positive control cultures.
No cytotoxicity was observed at any concentration of SRP-4045.
No increase in mRNA for enzymes CYP1A2, CYP2B6, or CYP3A4 was observed. SRP-4045 is not considered to be an inducer of these enzymes.

Study #4045-pkd-004
Title: Metabolism of SRP-4045 in mouse, rat, monkey, and human hepatic microsomes

Testing Facility:	(b) (4)
GLP Compliance:	No
Date of Study Initiation:	12/12/2013
Drug/Lot#/Purity:	SRP-4053/7001257/91%

Summary and Results:

Metabolically active mouse, rat, monkey, and human hepatic microsomes were incubated with target concentrations of SRP-4045 of 8 and 80 µg/mL in the presence and absence of NADPH. Incubations were conducted for 0, 30, 45, 60, or 120 minutes. SRP-4045 levels were measured at the end of the incubation period. Control cultures were incubated for 0 or 120 minutes without NADPH and for 120 minutes in the presence of heat-inactivated microsomes with NADPH for 120 minutes.

Mean concentrations of SRP-4045 at each target concentration showed no significant change at each time point up to 120 minutes for any of the microsomal cultures from each species, in the absence and presence of NADPH. Therefore, the results indicate that no significant time- or NADPH-dependent metabolism of SRP-4045 occurred in hepatic microsomes from mouse, rat, monkey, or human.

Study #SR-16-058
Title: Evaluation of [^{14}C]SRP-4045 as a substrate and SRP-4045 as an inhibitor of a panel of human drug transporters

Testing Facility:	(b) (4)
GLP Compliance:	No
Date of Study Initiation:	5/12/2017
Drug/Lot#/Purity:	Radiolabeled SRP-4045/CFQ43053/90.6% SRP-4053/7003064/93%

Summary and Results:

Potential drug transporter interactions were evaluated using radiolabeled SRP-4045 as substrate and unlabeled SRP-4045 as an inhibitor for known human drug transporters. The transporters evaluated were organic anion transporter (OAT)1 and

OAT3, organic cation transporter (OCT)2, organic anion transporting polypeptide (OATP)1B1 and OATP1B3, multidrug and toxin extrusion (MATE)1 and MATE2-K, key ATP-binding cassette (ABC) transporters P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance protein (MRP)2.

Assays were conducted using HEK293 transfected with each transporter gene. Negative control cells were transfected with vector only.

Uptake of SRP-4045 by each transporter was evaluated by incubation of the cell cultures in the presence of vehicle or selective inhibitor, and in cultures with the vector only control. Uptake of a probe substrate was also measured as a control.

The table below summarizes the various transporters evaluated with the corresponding probe substrate and inhibitor.

Uptake Transporter Control Substrates and Inhibitors

Transporter	Probe Substrate (µM)	Selective Inhibitor (µM)
OAT1	^{14}C-para-Aminohippurate (1)	Probenecid (200)
OAT3	^{3}H-Estrone-3-sulfate (1)	Probenecid (200)
OCT2	^{14}C-Metformin (1)	Quinidine (256)
OATP1B1	^{3}H-Estradiol-17β-D-glucuronide (0.5)	Cyclosporine A (10)
OATP1B3	^{3}H-Cholecystokinin octapeptide (1)	Cyclosporine A (10)
MATE1	^{14}C-Tetraethylammonium (5)	Cimetidine (100)
MATE2-K	^{14}C-Tetraethylammonium (5)	Cimetidine (100)

Results indicated that SRP-4045 is not a substrate for transporters OAT1, OAT3, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, P-gp, BCRP, or MRP2 at the test concentrations of 10 or 100 µg/mL.

Weak inhibition of MATE1 was observed. The estimated IC_{50} value was greater than 1000 µg/mL. No inhibition of uptake was observed for OAT1, OAT3, OCT2, OATP1B1, MATE2-K, P-gp, BCRP, or MRP2 at concentrations of SRP-4045 of 100 and 1000 µg/mL.

Table 1
Summary of SRP-4045 as a substrate or inhibitor of human drug transporters

Transporter	Substrate	Inhibitor	IC_{50} (μM)
OAT1	No	No	NA
OAT3	No	No	NA
OCT2	No	No	NA
OATP1B1	No	No	NA
OATP1B3	No	No	NA
MATE1	No	Yes (Weak)	ND
MATE2-K	No	No	NA
P-gp	No	No	NA
BCRP	No	No	NA
MRP2	No	No	NA

NA Not applicable.
ND Not determined.

Study# SR-17-050

Title: SRP-4045: Toxicokinetic profile and bioavailability following subcutaneous and intravenous administration in the male mouse.

Summary

This study was conducted to determine the toxicokinetic profile of SRP-4045 following subcutaneous (SC) and IV administration in male mice. Groups of male mice (C57BL/6J) received weekly injections of SRP-4045 at doses of 0, 300, or 2000 mg/kg IV or SC for 4 weeks. Results demonstrated that SC bioavailability was 100% at 300 mg/kg but, at 2000 mg/kg was 20-30%. No accumulation was noted, and $AUC_{0-\infty}$ increased with dose for both routes of administration.

Toxicokinetic Parameters in Male Mouse Plasma

Day	Dose Group	Route of Administration	Test Item	Dose (mg/kg/dose)	C_{max} (ug/mL)	$AUC_{0-\infty}$ (ug·hr/mL)	Cmax/Dose	AUC/Dose
1	2	IV	SRP-4045	300	2000	653	6.69	2.18
1	3	IV	SRP-4045	2000	14900	13100	7.47	6.54
1	4	SQ	SRP-4045	300	1030	1090	3.42	3.65
1	5	SQ	SRP-4045	2000	705	2640	0.353	1.32
22	6	SQ	SRP-4045	2000	893	3650	0.446	1.82

6 General Toxicology

6.1 Single-Dose Toxicity

No single dose toxicology studies were conducted.

6.2 Repeat-Dose Toxicity

General toxicology studies of 12 weeks' duration in mouse and cynomolgus monkey were reviewed under IND 118086 and are summarized here.

In study # 4045-tox-003, male mice (C57BL/6NCrl) received IV injections of SRP-4045 weekly for 12 weeks at doses of 0, 12, 120, or 960 mg/kg. Two HD animals died early (SD43 and SD50). The animal found dead on SD50 was a TK animal; no cause of death was identified on necropsy. No cause of death was determined for the main-study animal found dead on SD43; but the sponsor indicated that the death was most likely due to the injection procedure because of the proximity in time. However, because both deaths occurred in the HD group, a relationship to SRP-4045 could not be ruled out.

In surviving animals, no adverse clinical signs or test article-related in-life effects were observed. Histopathology examinations showed kidney was the target organ, with increases in kidney tubular cytoplasmic basophilia, graded minimal to moderate in HD animals. No adverse effects were observed on male reproductive parameters including hormone levels (testosterone, LH, and FSH) and sperm parameters. The NOAEL was 120 mg/kg (AUC_{0-t} = 69,800 ng•hr/mL)

In study #4045-tox-001, male cynomolgus monkeys received weekly IV injections of 0, 5, 40, or 320 mg/kg for 12 weeks. No unscheduled deaths occurred and no adverse clinical signs or adverse effects on clinical pathology parameters were observed. Activation of complement, dose-related in magnitude, was observed in all SRP-4045 dose groups, as evidenced by increases in Bb and C3a.

Histopathology showed kidney to be the target organ for toxicity with tubular epithelium cytoplasmic basophilia, graded minimal to slight, observed in the MD and HD groups. Trends toward recovery were observed, with findings only in the HD group at the end of the recovery period.

No adverse effects of SRP-4045 were observed on male reproductive organs, hormone values (testosterone, LH, or FSH), or sperm parameters.

Conclusion:

The NOAEL was the HD of 320 mg/kg ($AUC_{0-\infty}$ = 1930,000 ng•hr/mL)

Study #: SR-15-057

Title: A 22-week intravenous injection toxicity and toxicokinetic study of SRP-4045 in the male mouse with a [*sic*] 8-week recovery period

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation: 7/64/2016

Drug/ lot#/purity: Casimersen/7002071/91.4%

Methods:

Species: Mouse, male, C57BL/6NCrl
Number: 20/group for the main study, 12/group for recovery
Age/weight: 10 weeks old/ 21.5 to 28.6 grams at initiation of dosing
Doses: 0, 300, 960, or 2000 mg/kg
Route of administration: IV, slow bolus
Vehicle: DPBS without magnesium or calcium
Frequency/duration: Weekly for 22 weeks
Study design:

This study was originally designed as a 26-week study. However, due to technical difficulties (poor condition of the injection site) resulting in increasing incidence of partial or missed doses at the LD, MD, and HD, the study was terminated at 22 weeks. The increases in dosing difficulties were dose-related in time of onset and in incidence.

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Males		
					Main Study	Recovery Study	Toxicokinetic[a] /Hormone[b] Study
1	Vehicle control	0	10	0	20	12	6+20[c]
2	SRP-4045	300	10	30	20	12	63+20[c]
3	SRP-4045	960	10	96	20	12	63+20[c]
4	SRP-4045	2000	10	200	20	12	63+20[c]

[a] Toxicokinetic animals were used for toxicokinetic evaluation only (6 animals in Group 1 and 63 animals/group in Groups 2-4), numbers reflect the original assignments.
[b] Animals were used for hormone evaluation only (20 animals/group), numbers reflect the original assignments.
[c] Due to the dosing difficulties encountered, animals originally assigned to the Hormone cohort were re-assigned to the Toxicokinetic cohort and some animals originally assigned to the Toxicokinetic Cohort were reassigned to the Hormones cohort. Any animals that could not be used for Toxicokinetic assessments or Hormone assessments were sent to euthanasia at the end of the study.

Text Table 6
Incidence of Doses Received for Main and Recovery Study Animals

Item	Main Study (N= 20)				Recovery Study (N= 12)			
	0 mg/kg/dose	300 mg/kg/dose	960 mg/kg/dose	2000 mg/kg/dose	0 mg/kg/dose	300 mg/kg/dose	960 mg/kg/dose	2000 mg/kg/dose
No. of Males Receiving ≥ 20 doses	19	17	6	7	11	11	3	1
No. of Males Receiving 17-19 doses	0	3	7	12	0	1	6	11
No. of Males Receiving < 17 doses	1	0	7	1	1	0	3	0
No. of Males Receiving < 80% of overall intended dose	0	6	13	13	12	4	8	9
No. of Males Dosed[a] at Dose 22	18/19	15/20	2/18	0/20	10/11	9/12	2/12	3/12
No. of Males Receiving a Partial Dose[b] at Dose 22	5/19	2/20	0/18	0/20	0/11	1/12	0/12	1/12

[a] Including partial doses and taking into account early decedents
[b] Taking into account early decedents

Observations/Results

Formulation Analysis: Formulation samples were collected according to the schedule summarized below.

Text Table 3
Dose Formulation Sample Collection Schedule

Interval	Concentration	Homogeneity	Stability	pH, Osmolality and Density	Sampling From
Day 1	All groups	N/A	N/A	All groups	Dosing container
Day 15[a]	Group 4	N/A	N/A	N/A	Dosing container
Day 85	All groups	N/A	N/A	N/A	Dosing container
Day 148	All groups	N/A	N/A	N/A	Dosing container

[a] Sample collected to confirm that sample size was the reason for out of specification results on Day 1

All samples were determined to be within the pre-established acceptance criteria, with the exception of the sample for group 4 in SD1. That group mean was +19.5%. Because the result was greater than nominal and represented a single day of dosing, the study validity was not affected.

Mortality: (Observations were recorded twice daily.)

Eleven unscheduled deaths occurred during the study: 3 controls, 2 LD, 4 MD, and 2 HD. The deaths are summarized in the table below. No clear dose relationship was observed in the incidence of the deaths. No cause of death was identified for 7 of the deaths. However, due to the lack of dose relationship, a relationship to the test article is doubtful.

Text Table 22
Individual Causes of Death

Animal No.	Dose level (mg/kg/dose)	Sex	Study Day	Cohort	Found Dead/ Unscheduled Euthanasia	Comment
1014	0	Male	40	Main Study	Unscheduled Euthanasia	Cause of death: undetermined
1021	0	Male	58	Recovery	Found dead	Cause of death: moderate degeneration/necrosis of the aorta at the base of the heart
1038	0	Male	41	Toxicokinetics	Unscheduled Euthanasia	Cause of death: undetermined
2006	300	Male	141	Main Study	Found dead	Cause of death: undetermined
2106	300	Male	15	Hormones	Found dead	Cause of death: undetermined
3012	960	Male	84	Main Study	Unscheduled Euthanasia	Euthanized due to skin lesion in the urogenital region that correlated with severe skin ulceration with inflammation
3015	960	Male	121	Main Study	Found dead	Cause of death: thrombus in the atrium of the heart with associated lung findings including interstitial inflammation and macrophage aggregates
3064	960	Male	99	Toxicokinetics	Unscheduled Euthanasia	Cause of death: dark fluid accumulation in the abdominal cavity with enlarged and firm seminal vesicles, dilatation of the urinary bladder and spleen enlargement, which were compatible with chronic inflammation in the seminal vesicles with urinary track obstruction
3103	960	Male	71	Hormones	Found dead	Cause of death: undetermined
4063	2000	Male	29	Toxicokinetics	Found dead	Cause of death: undetermined
4084	2000	Male	35	Toxicokinetics	Found dead	Cause of death: undetermined

Clinical observations: (Cageside observations were recorded on dosing days at 1, 2, 3, and 4 hours post dose. Detailed observations were recorded once weekly and on days of scheduled euthanasia.)

- Skin findings of dry/flaking skin, thickening of skin with discoloration (red/blue), and swelling at the injection sites in animals in the MD and HD groups. These findings were dose-related in incidence beginning at approximately SD85 (dose 13) and involved a majority of animals by dose 22. The skin findings were not observed at the end of the recovery period.

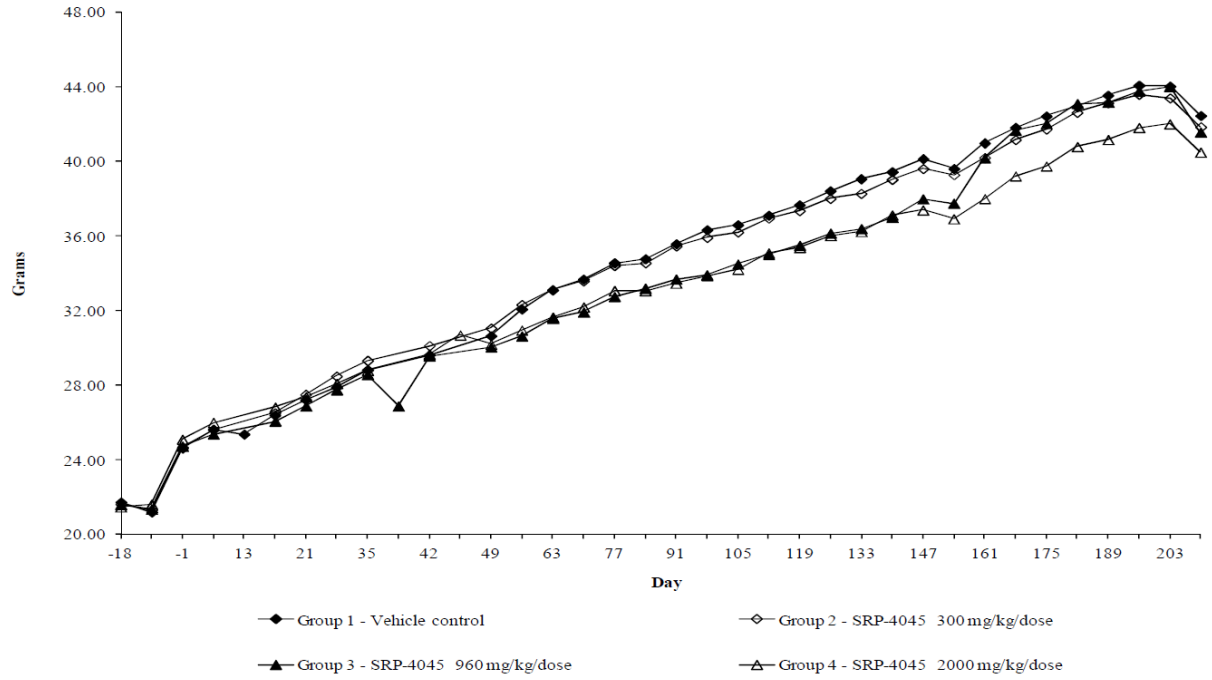
Body weight: (Data were recorded weekly.)

- Slightly reduced body weight gain was observed in MD and HD animals. The reduced gain resulted in slightly lower mean body weights for the MD and HD groups at the end of the dosing period (-4.7 and -6.8%, respectively). The

sponsor suggests that the reduced weight gain for the MD and HD animals began at approximately the same time as the difficulty with dosing and may have been associated with the stress of the procedure. By the end of the recovery period, the difference in mean group body weight was reduced, suggesting recovery.

Figure 1

Summary of Body Weights - Males



Food consumption: (Food consumption was quantitatively recorded weekly beginning one week prior to initiation of dosing.)

- No effect on food consumption related to the test article was observed.

Ophthalmology: (Testing was conducted once prior to initiation of dosing and once during week 22 of dosing.)

- No test article-related effects were observed.

<u>Clinical Pathology:</u>

Text Table 7
Samples for Clinical Pathology Evaluation Main and Recovery Animals

Group Nos.	Time Point	Hematology[a]	Clinical Chemistry[a]	Hormones[d]	Urinalysis
1 to 4[b]	Week 23	X	X	X	-
Main animals	Week 22	-	-	-	X
1 to 4	Day 211	X	X	X	-
Recovery animals	Week 30	-	-	-	X
Unscheduled Euthanasia (When Possible)[c]	Before Euthanasia	X	X	-	-

X = Sample collected
[a] At scheduled occasions, approximately 1/2 of the animals were assigned for hematology and 1/2 of the animals were assigned for biochemistry.
[b] Animals scheduled for euthanasia only.
[c] Priority order of collection for unscheduled euthanasia were: Clinical Chemistry, Hematology, based on available volume.
[d] Collected from selected Hormone study animals.

Hematology:

- A decrease in neutrophil counts (0.53X) was observed in HD animals at the end of the dosing phase. The finding was no longer observed at the end of the recovery period. No histological correlates were observed.

Clinical chemistry:

- Dose-related changes in serum urea nitrogen and creatinine levels (graded minimal) were observed in the MD and HD groups. The values are summarized in the table below. The changes correlated with increased kidney weight and degeneration/regeneration (graded minimal to moderate) of kidney tubular epithelium and minimal to mild casts. The increased urea nitrogen was again observed at the end of the recovery period for the HD group, but serum creatinine was similar to control.

Text Table 23
SRP-4045-Related Clinical Chemistry Changes

Group Dose (mg/kg/dose) Sex	1 0 M	2 300 M	3 960 M	4 2000 M
UREA NITROGEN				
Week 23	–	–	–	**1.23**
Day 211	–	–	–	**1.29**
CREATININE				
Week 23	–	–	1.46	1.31

M = Males
A dash (—) indicates absence of change. Numerical values indicate fold changes of treated group value relative to reference item group mean value. Bolded values are statistically significant at $P \leq 0.05$.

Urinalysis:

- At the end of the dosing period, increases in urine volume and specific gravity, associated with increased serum urea nitrogen, were observed at the HD.

- Increased urine volume and decreased specific gravity (graded minimal) were observed in HD animals at the end of the recovery period.

Hormones:

Text Table 11
Hormone Parameters

Testosterone Follicle-stimulating hormone	Luteinizing hormone

- No test article -related changes in hormone levels were observed.

Toxicokinetics:

Text Table 2
Summary of Selected Toxicokinetic Parameters

Parameter	Occasion	Group 2 300 mg/kg Males	Group 3 960 mg/kg Males	Group 4 2000 mg/kg Males
C_{max} (ng/mL)	Day 1	1140000	4670000	9620000
	Day 149 (Dose 22)	606000	3190000	7020000
$AUC_{(0-t)}$ (ng*hr/mL)	Day 1	435000	2630000	7080000
	Day 149(Dose 22)	1060000	1490000	4120000
$AUC_{(0-t)}$ /Dose (ng*hr/mL/(mg/kg))	Day 1	1450	2740	3540
	Day 149 (Dose 22)	3550	1550	2060

Terminal Procedures: Signed, dated Pathology Report was provided.

Text Table 15
Terminal Procedures for Main Study and Recovery Animals

Group No.	No. of Males	Scheduled Euthanasia Week/Day	Necropsy Procedures			Histology[a]	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	20	Week 23	X	X	X	Full Tissue	Full Tissue[a]
2	20					Full Tissue	Gross Lesions, Brain, testis and kidneys
3	20					Full Tissue	Gross Lesions, Brain, testis and kidneys
4	20					Full Tissue	Full Tissue[a]
1	12	Day 211	X	X	X	Full Tissue	Full Tissue[a]
2	12					Full Tissue	Gross Lesions, Brain, testis and kidneys
3	12					Full Tissue	Gross Lesions, Brain, testis and kidneys
4	12					Full Tissue	Full Tissue[a]
Unscheduled Deaths			X	X	-	Full Tissue	Full Tissue[a]
Replaced animals (prestudy)			X	Standard Diagnostic List	-	-	-

X = Procedure conducted; - = Not applicable.
[a] See Tissue Collection and Preservation table for listing of tissues.

Macroscopic observations:

- No macroscopic findings were reported.

Organ weights:

Text Table 17
Organs Weighed at Necropsy for Main and Recovery Animals

Brain	Heart
Epididymis[a]	Kidney[a]
Gland, adrenal[a]	Liver
Gland, pituitary	Lung
Gland, prostate	Spleen
Gland, salivary (mandibular)	Testis[a]
Gland, thyroid	Thymus[a]

[a] Paired organ weight.

End of dosing period:

- At the end of the dosing period, kidney weights were elevated in the MD and HD groups. The increased weight correlated with microscopic findings of tubular degeneration/regeneration and clinical chemistry findings.

Text Table 25
Summary of Organ Weight Data – Scheduled Euthanasia (Day 157/158)

	Males		
Group	**2**	**3**	**4**
Dose (mg/kg/dose)	**300**	**960**	**2000**
No. Animals per Group	**19**	**18**	**20**
Kidney (No. Weighed)[a]	(19)	(18)	(20)
Absolute value	1.3	6.8	**13.3**
% of body weight	2.3	**13.1**	**18.4**
% of brain weight	1.5	**7.2**	**11.8**

[a] All values expressed as percent difference of control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Male reproductive assessments: No test article-related effects were observed on male reproductive assessments (sperm concentration, morphology, or spermatogenesis [assessed in a stage-aware manner]).

Histopathology: (Full battery of tissues was collected for microscopic evaluation.)

- At the end of the dosing period, SRP-4045-related microscopic findings were observed in the kidney. The findings were observed in all dose groups and were described as degeneration/regeneration of the tubular epithelium (graded minimal to moderate) with minimal to mild casts. The severity and incidence were dose-related and occurred bilaterally. The effects on the tubular epithelium were observed in the convoluted tubules and collecting ducts and were randomly distributed in the renal cortex. Occasional cysts filled with casts or cellular debris were detected.

Text Table 26
Summary of Microscopic Findings – Scheduled Euthanasia (Day 157/158)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg/dose)	**0**	**300**	**960**	**2000**
No. Animals Examined	**19**	**19**	**18**	**20**
Kidney (No. Examined)	(19)	(19)	(18)	(20)
Cast	(0)[a]	(1)	(5)	(14)
Minimal	0	1	5	11
Mild	0	0	0	3
Degeneration/regeneration; tubular	(0)	(1)	(11)	(17)
Minimal	0	1	11	9
Mild	0	0	0	7
Moderate	0	0	0	1

[a] Numbers in parentheses represent the number of animals with the finding.

At the end of the recovery period, renal tubular epithelial regeneration/degeneration (graded minimal to mild) was observed at all dose levels of SRP-4045. Casts were observed in animals from the MD and HD groups. These findings were of similar incidence and severity as were observed at the end of the dosing period. Therefore, no significant recovery was observed.

Text Table 27
Summary of Microscopic Findings – Scheduled Euthanasia (Day 211)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg/dose)	**0**	**300**	**960**	**2000**
No. Animals Examined	**11**	**12**	**12**	**12**
Kidney (No. Examined)	(11)	(12)	(12)	(12)
Cast	(0)[a]	(0)	(2)	(11)
Minimal	0	0	2	4
Mild	0	0	0	7
Degeneration/regeneration; tubular	(0)	(2)	(6)	(12)
Minimal	0	2	6	2
Mild	0	0	0	10

[a] Numbers in parentheses represent the number of animals with the finding.

Conclusion: The NOAEL was the LD of 300 mg/kg.

Study #SR-17-085

Title: SRP-4045: 26-week subcutaneous injection toxicity and toxicokinetic study in male mice with an 8-week recovery period

Testing Facility: (b) (4)
GLP Compliance: Yes
Date of Study Initiation: 10/31/2017
Drug/Lot#/Purity: SRP-4045/7003492/95%

Methods:
Animals: Mouse, males only, C57BL/6NCrl
Age/weight: 8 weeks old at start of dosing, 20.3 to 28.6 grams.
Number: 20/group for main study, 12/group for recovery
Doses: 0, 300, 600, or 960 mg/kg
Frequency: Weekly
Route: SC
Study design:

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals		
					Main Study	Recovery Study	Toxicokinetic[a]+ Hormone[b] Study
1	Vehicle Control	0	10	0	20	12	6+20
2	SRP-4045	300	10	30	20	12	63+20
3	SRP-4045	600	10	60	20	12	63+20
4	SRP-4045	960	10	96	20	12	63+20

[a] Toxicokinetic animals were used for toxicokinetic evaluation only (6 animals in Group 1 + 63 animals/group in Groups 2 to 4).
[b] Animals were used for hormone evaluation only (20 animals/group).

Observations/Results:
Formulation analysis:

Text Table 2
Dose Formulation Sample Collection Schedule

Interval	Concentration	pH, Osmolality and Density	Sampling From
Day 1	All groups	All groups	Dosing container
Day 85	All groups	N/A	Dosing container
Day 176	All groups	N/A	Dosing container

All samples were determined to be within the pre-established acceptance criteria for concentration.

Mortality: (Data were recorded twice daily.)

- Unscheduled euthanasia was conducted on 3 main study and recovery animals: #2104 (LD, recovery), #3090 (MD, MS, SD109), and #4090 (HD, MS, SD130). The animals were euthanized on SD 109 or 130 due to poor and deteriorating condition characterized by skin lesions and/or tremors. The skin lesions (ventral cervical and thoracic regions) correlated with microscopic findings of marked ulceration. The sponsor considered these ulcerations secondary and associated with injection sites.
- Clinical pathology results for these animals were similar and generally reflected the inflammation related to the poor condition and skin lesions. Findings consisted of increased neutrophils, increased platelets, increased globulin and urea, decreased total protein and albumin, reduced lymphocytes, decreased red cell mass, increased reticulocytes, and increased myeloid cellularity in bone marrow and extramedullary hematopoiesis.
- Histopathology showed plasmacytosis in multiple lymph nodes and extramedullary hematopoiesis in liver, kidney, and adrenal gland. These findings were considered secondary to the inflammatory status due to the skin ulcerations.

Clinical observations: (Cageside observations were recorded on dosing days at 1, 2, 3, and 4 hours post dose. Detailed observations were recorded weekly throughout the study.)

- All dose groups showed skin lesions or scabs with thin fur cover in multiple regions. The sponsor considered these findings secondary to the SC dosing procedure and to be non-adverse. However, one animal in each group was euthanized early due to these skin ulcerations.

Body weight: (Data were recorded weekly.)

- No effects related to the test article were observed.

Food consumption: (Food was measured weekly.)

- No test article-related effects were observed.

Ophthalmology: (Testing was conducted once prior to initiation of dosing and once during week 26.)

- No test article-related effects were observed.

Clinical pathology: (Samples were collected according to the schedule below.)

Text Table 4
Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology[a]	Clinical Chemistry[a]	Hormones[d]	Urinalysis
1 to 4[b]	Week 27	X	X	X	-
1 to 4, Main Study animals	Week 26	-	-	-	X
1 to 4	Week 34	X	X	X	-
1 to 4, Recovery Study animals	Week 33	-	-	-	X
Unscheduled Euthanasia[c]	Before Euthanasia	X	X	-	-

X = Sample collected.

[a] At scheduled occasions, approximately half of the animals were assigned to hematology and half of the animals were assigned to biochemistry.

[b] Animals scheduled for euthanasia only.

[c] Priority order of collection for unscheduled euthanasia was: clinical chemistry, hematology, based on available volume.

[d] Collected from selected Hormone Study animals.

Hematology: (A standard battery of hematology parameters was assessed.)

- No test article-related effects were observed.

Clinical chemistry: (A standard battery of clinical chemistry parameters was assessed.)

- No test article-related effects were observed.

Coagulation: Not evaluated.

Urinalysis: (Volume and specific gravity)

- No test article-related effects were observed.

Hormone analysis:

- No test article-related effects were observed on hormone levels (testosterone, luteinizing hormone, and follicle stimulating hormone).

Toxicokinetics:

Table 2.1: Summary (± SE) SRP-4045 Toxicokinetic Parameters in Male Mouse Plasma Following SC Injection of SRP-4045 on Day 1

Day	Gender	Dose (mg/kg/dose)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	T_{last} (hr)	$AUC_{(0-t)}$ (hr*ng/mL)	SE $AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}/D$ (hr*ng/mL/ (mg/kg/dose))	$AUC_{(0-24)}$ (hr*ng/mL)	$AUC_{(0-24)}/D$ (hr*ng/mL/ (mg/kg/dose))	$T_{1/2}$ (hr)
1	Male	300	0.5	157000	2960	48.0	257000	12500	858	257000	857	24.7
		600	0.5	267000	13800	48.0	590000	49100	983	589000	982	NRR
		960	1	407000	68800	48.0	953000	68100	993	952000	992	NRR

NRR = Not reported because Rsq was less than 0.800 and/or the extrapolation of the AUC to infinity represented more than 20% of the total area.

Table 2.2: Summary (± SE) SRP-4045 Toxicokinetic Parameters in Male Mouse Plasma Following SC Injection of SRP-4045 on Day 176

Day	Gender	Dose (mg/kg/dose)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	T_{last} (hr)	$AUC_{(0-t)}$ (hr*ng/mL)	SE $AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}/D$ (hr*ng/mL/ (mg/kg/dose))	$AUC_{(0-24)}$ (hr*ng/mL)	$AUC_{(0-24)}/D$ (hr*ng/mL/ (mg/kg/dose))	$T_{1/2}$ (hr)	R_{AUC}
176	Male	300	0.5	185000	28000	48.0	460000	25300	1530	459000	1530	8.77	1.79
		600	1	345000	49900	48.0	991000	63600	1650	988000	1650	NRR	1.68
		960	0.5	332000	30300	48.0	1560000	53500	1630	1560000	1620	4.01	1.64

NRR = Not reported because Rsq was less than 0.800 and/or the extrapolation of the AUC to infinity represented more than 20% of the total area.
R_{AUC} = Day 176 $AUC_{(0-t)}$/ Day 1 $AUC_{(0-t)}$.

Terminal Procedures

Signed, dated Pathology Report: Yes.
Adequate battery of tissues was examined.

Gross Pathology: (Main-study animals were euthanized and necropsied on SD183; recovery animals were euthanized and necropsied on SD232.)

- No test article-related findings were observed.

Organ weights:

- No test article-related effects on organ weights were observed.

Histopathology:

- At the end of the dosing period, test article-related findings were observed in the kidney at all dose levels. The findings were characterized as tubular degeneration/regeneration (graded minimal). The affected tubules were randomly distributed through the cortex and were observed in the convoluted tubules and collecting ducts, sometimes associated with dilatation with intratubular accumulation of substance described as “eosinophilic proteinaceous material”. Findings were dose-related in incidence.
- Kidney findings were not considered reversible.
- Injection site reactions were also observed at all dose levels. At the end of the recovery period, dermal fibroplasia was observed at the injection site in HD animals.

Text Table 21
Summary of Microscopic Findings – Scheduled Euthanasia (Day 183)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg/dose)	**0**	**300**	**600**	**960**
No. Animals per Group	**20**	**20**	**19**	**19**
Kidney (No. Examined)	20	19	18	19
Degeneration/regeneration; tubular	(0)[a]	(9)	(15)	(17)
Minimal	-	9	15	17
Site, injection (No. Examined)	20	19	18	19
Degeneration/regeneration; myofiber	(0)	(5)	(9)	(11)
Minimal	-	5	9	11
Infiltration, mononuclear cell	(0)	(5)	(10)	(12)
Minimal	-	5	10	12
Fibroplasia; dermal	(0)	(1)	(3)	(2)
Minimal	-	1	1	1
Mild	-	0	2	1

[a] Numbers in parentheses represent the number of animals with the finding.

Text Table 22
Summary of Microscopic Findings – Scheduled Euthanasia (Day 232)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg/dose)	**0**	**300**	**600**	**960**
No. Animals per Group	**12**	**11**	**12**	**12**
Kidney (No. Examined)	12	11	12	12
Degeneration/regeneration; tubular	(0)[a]	(7)	(10)	(11)
Minimal	-	7	10	11
Site, injection (No. Examined)	12	11	12	12
Fibroplasia; dermal	(0)	(0)	(0)	(1)
Mild	-	-	-	1

[a] Numbers in parentheses represent the number of animals with the finding.

Conclusion:
The NOAEL is 960 mg/kg.

Study #SR-15-037
Title: SRP-4045: A 39-week intravenous infusion toxicity study in male cynomolgus monkeys with an 8-week recovery period

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation:8/10/2017
Drug/Lot#/Purity: SRP-4045/7003088, 7003123, 7003125, 7700412, and 7003354/95%, 98%, 97%, 99%, 101%

Methods:

Species: Cynomolgus monkey, males only
Number: 9/ group (6/group main study, 3/group for recovery)
Age/weight: 5 to 7 years old, 5.1 to 9.4 kg
Doses: 0, 80, 320, or 640 mg/kg
Route: IV
Frequency: Once weekly
Duration: 39 weeks plus 8 weeks recovery
Vehicle: DPBS
Study design:

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Dose Rate (mL/kg/hr)	No. of Animals	
						Main Study	Recovery Study
						Males	
1	Vehicle Control[a]	0	3.2	0	6.4	6	3
2	SRP-4045	80	3.2	25	6.4	6	3
3	SRP-4045	320	3.2	100	6.4	6	3
4	SRP-4045	640	3.2	200	6.4	6	3

[a] Animals received the Reference Item: Dulbecco's Phosphate Buffered Saline (1x) without magnesium or calcium

Observations/Results:
Formulation analysis: Samples were collected after each preparation.

Text Table 2
Dose Formulation Sample Collection Schedule

Interval	Concentration	pH, Osmolality and Density	Sampling From
Day 1	All groups	All groups	dosing container
Day 85	All groups	N/A	dosing container
Day 260	All groups	N/A	dosing container

N/A = not applicable.

Mortality: (Data were recorded twice daily.)

- No unscheduled deaths occurred during this study.

Clinical signs: (Cageside observations were conducted once daily; on dosing days between 10 and 20 minutes after infusion. Detailed observations were recorded weekly.)

- No test article-related clinical signs were observed.

Body weight: (Data were recorded weekly.)

- No test article-related effects were observed.

Food consumption: (Food consumption was monitored daily.)

- No test article-related effects were related.

ECG: (Testing was conducted once prior to initiation of dosing, once during week 1, and once during week 38 of the dosing period. Testing was conducted at the T_{max}, 1 to 4 hours post infusion.)

- No test article-related effects were observed.

Ophthalmology: (Examinations were conducted once prior to initiation of dosing and once during week 38.)

- No test article-related effects were observed.

Clinical Pathology: Collection schedule is summarized below.

Text Table 4
Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry	Hormones	Urinalysis and Urine Chemistry
All animals	Week -2	X	X	X	X	-
All animals	Week -1	X	X	X	X	X
1 to 4	Week 13	X	X	X	X	X
1 to 4	Week 26	X	X	X	X	X
1 to 4	Week 38	X	X	X	X	X
1 to 4	Week 46	X	X	X	X	X

X = Sample collected; - = Not applicable.

Hematology:

- No significant test article-related effects were observed.

Clinical Chemistry:

- No significant test article-related effects were observed.

Coagulation:

- No test article-related effects were observed.

Urinalysis:

- No significant test article-related effects were observed.

Hormone analysis: (Testosterone, follicle-stimulating hormone, and luteinizing hormone.)

- No test article-related effects were observed.

Toxicokinetics:

Text Table 20
Mean Toxicokinetic Parameters of SRP-4045

Parameter	Timepoint	Dose of SRP-4045 (mg/kg)		
		80	320	640
$AUC_{(0-t)}$ (hr*µg/mL)	Day 1	457	1810	4170
	Day 85	592	2140	4880
	Day 260	782	2870	6290
C_{max} (µg/mL)	Day 1	363	1450	3350
	Day 85	456	1860	3630
	Day 260	582	1890	4300
T_{max}[a] (hr)	Day 1	0.583	0.583	0.583
	Day 85	0.583	0.583	0.583
	Day 260	0.583	0.583	0.583

a = T_{max} reported as median (min-max).

$T_{1/2}$ ranged from 5.05 ± 3.51 to 19.5 ± 20.5 hours

Male reproductive assessments: (Semen samples were collected twice prior to initiation of dosing, during weeks 13, 26, 38 of the dosing period, and once at the end of recovery during week 46. Sperm motility, morphology, and counts were assessed.)

- No test article-related effects were observed.

Spermatogenic cycle was assessed by microscopic examination after necropsy.
No test article-related effects on the spermatogenic cycle were observed.

Anatomic Pathology: Signed, dated Pathology report was provided.
Adequate panel of tissues was collected and examined microscopically.

Macroscopic observations:

- No test article-related macroscopic findings were observed.

Organ weights:

- No test article-related effects on organ weights were observed.

Histopathology:

- Kidney: At the end of the dosing period, dose-related increases in incidence and severity of tubular basophilia (graded minimal to mild), tubular vacuolation (graded minimal to moderate) in the all dose groups, and vacuolated macrophage aggregation in mesenteric lymph nodes at the MD and HD (graded minimal to mild) were observed. At the end of the recovery period, findings of tubular basophilia (minimal) and tubular vacuolation were observed in HD animals only.
- Degeneration/atrophy of seminiferous tubules was observed in all dose groups including controls. Due to lack of a dose-relationship, this finding was not considered test-article related. Similar findings were not observed at the end of the recovery period.

Text Table 21
Summary of Microscopic Findings – Scheduled Euthanasia (Day 268)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**80**	**320**	**640**
No. Animals per Group	**6**	**6**	**6**	**6**
Kidneys (No. Examined)	6	6	6	6
Basophilia; tubular	(0)[a]	(1)	(5)	(6)
Minimal	0	1	4	1
Mild	0	0	1	5
Vacuolation; tubular	(0)	(1)	(4)	(6)
Minimal	0	1	4	3
Mild	0	0	0	2
Moderate	0	0	0	1
Lymph node, mesenteric (No. Examined)	6	6	6	5
Macrophage aggregation	(0)	(0)	(1)	(2)
Minimal	0	0	1	1
Mild	0	0	0	1

[a] Numbers in parentheses represent the number of animals with the finding.

Recovery

Text Table 22
Summary of Microscopic Findings – Scheduled Euthanasia (Day 323)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**80**	**320**	**640**
No. Animals per Group	**3**	**3**	**3**	**3**
Kidneys (No. Examined)	3	3	3	3
Basophilia; tubular	(0)[a]	(0)	(0)	(2)
Minimal	0	0	0	2
Vacuolation; tubular	(0)	(0)	(0)	(2)
Minimal	0	0	0	1
Mild	0	0	0	1
Lymph node, mesenteric (No. Examined)	3	3	3	3
Macrophage aggregation	(0)	(0)	(0)	(2)
Mild	0	0	0	2

[a] Numbers in parentheses represent the number of animals with the finding.

Conclusion:
The NOAEL is 640 mg/kg.

7 Genetic Toxicology

In vitro genetic toxicology studies were reviewed under IND 118086:
Study # 4045-gtx-001 (In vitro reverse mutation assay in bacterial cells (Ames)
Study # 4045-gtx-002 (Chromosomal aberrations in Chinese hamster ovarian (CHO) cells

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study #: 4045-gtx-003

Title: SRP-4045: *In vivo* mouse bone marrow micronucleus assay

Testing Facility and location:	(b) (4)
Date of study initiation:	7/08/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #/purity:	SRP-4045/DEV-569, LY01/87.1%

Methods:

Species: CD-1 mouse, male

Doses used in definitive study: 0, 500, 1000, or 2000 mg/kg IV; single dose

Basis of dose selection: Dose-range finding preliminary study

Negative controls: Vehicle (Dulbecco's PBS)

Positive controls: cyclophosphamide (80 mg/kg)

Incubation and sampling times: bone marrow was harvested at 24 and 48 hours post dose.

Study validity:
All criteria for validity were met.

Results:
No evidence of micronucleus induction was observed. Negative and positive concurrent controls were within the expected historical range. One animal in the 1000 mg/kg group showed increased % micronucleated PCEs (0.25%). Because this finding was observed in a single animal and was not dose-related and mean % micronucleated PCEs in the 1000 mg/kg group was within the historical vehicle control range, this finding was considered to have no biological relevance.

Test Article: SRP-4045 Species/Strain: Mouse/CD-1
Initiation of Dosing: 29 July 2013

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean ± SD Male	Ratio PCE:NCE Mean ± SD Male
Vehicle	20 mL/kg	24	0.04 ± 0.02	0.60 ± 0.22
		48	0.07 ± 0.04	0.70 ± 0.12
Positive	80 mg/kg	24	3.86 ± 0.63*	0.68 ± 0.11
Test Article	500 mg/kg	24	0.03 ± 0.03	0.68 ± 0.20
	1000 mg/kg	24	0.13 ± 0.08**	0.67 ± 0.12
	2000 mg/kg	24	0.07 ± 0.04	0.52 ± 0.18
		48	0.06 ± 0.04	0.90 ± 0.09*

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly greater than the corresponding vehicle control, $p \leq 0.05$.
NCE = Normochromatic erythrocyte
PCE = Polychromatic erythrocyte
Positive = Cyclophosphamide
Vehicle = Dulbecco's Phosphate Buffered Saline (DPBS, 1x) without magnesium or calcium

Conclusion:

SRP-4045 was negative for micronucleus formation.

All genetic toxicology studies met the OECD criteria for study validity and results showed no evidence of mutagenicity or clastogenicity for SRP-4045.

8 Carcinogenicity

Carcinogenicity assessments are deferred to post-marketing for the DMD indication. The following studies were conducted as dose-range finding for future carcinogenicity assays in TgRas mice and Sprague-Dawley rat.

Study # SR-17-002
Title: SRP-4045: 4-week repeated dose intravenous toxicity and toxicokinetic study in male CByB6F1 mice

Testing Facility: (b) (4)

Date of Study Initiation: 5/4/2017
GLP Compliance: Yes
Drug/Lot#/Purity: SRP-4045/7003088/95%

Methods:

Animals: Mouse CByB6F1-Tg(HRAS)2Jic (-/- homozygous c-Ha-ras) males only
Number: 10/group in main study, 8/group for TK
Age/weight: 7 weeks old at initiation of dosing/ 23.2 to 26.6 grams (main study animals)
Doses: 0, 250, 500, 1000, or 2000 mg/kg (HD considered to be MFD)
Route: IV
Vehicle: PBS
Frequency: once weekly for 4 weeks
Study design:

Study Design

Group	Dose Levels (mg/kg)	Number of Males	
		Main Cohort	TK Cohort*
1 (Vehicle control)	0	10	8
2	250	10	38
3	500	10	38
4	1000	10	38
5	2000** [1950]	10	38
Total		**50**	**160**

*Extra animals (2/group) were used to ensure adequate animals were available for TK bleeding.
** = Original dose level; 1950 mg/kg was the actual level achieved, based on dose analysis results (see Section 3.6).

Observations/Results:
Formulation analysis:

Sample Type	Groups	First formulation	Last formulation	Storage temperature
Retention	All groups	One 1 mL sample from the middle	One 1 mL sample from the middle	2-8°C
Stock Formulation Concentration	N/A	Four (4) 0.25 mL samples from the middle[a]	N/A	2-8°C
Dose Formulation Concentration	All groups	Four (4) 0.25 mL samples from the middle[b]	Four (4) 0.25 mL samples from the middle[b]	2-8°C

[a] Samples were collected from the stock prepared on 09 May 2017. All samples were analyzed for test article content. The results were provided to the Study Director and approved by the Study Monitor, before being used in the dose formulation preparation calculations.
[b] Two samples from each of the dose formulation preparations were analyzed to determine actual concentration; the second set of samples from each dose formulation preparation served as backup.

All formulation samples were within the pre-established acceptance criteria for concentration.

Mortality: (Data were recorded twice daily.)

- All main study animals survived to scheduled necropsy.
- One TK animal was found dead on SD15 (HMD group) after dosing. No COD or data regarding this death were provided.

Body weight: (Data were recorded on dosing days, pre-dose and on the day of necropsy.)

- No significant effects on body weight or body weight change was observed.

Food consumption: (Data were collected weekly for main study animals.)

- No significant test article-related effects on food consumption were observed.

Toxicokinetics:

Toxicokinetic Blood Collection

Group	Number of Animals/ Time Point	Time Points	Optimal Volume/ Collection Tube	Days of Blood Collection
1	3	5 minutes post dose	600 µL per animal /K_2EDTA	Day 1 and Day 22
2-5	3	5 minutes and 0.5, 1, 4, 8, and 24 hours post dose	600 µL* per animal /K_2EDTA	Day 1 or 2 (24 hours) and Day 22 or 23 (24 hours)

* = When the blood volume collected was less than 600 µL, the approximate volume collected was recorded.

TK results are summarized in the table below:

Text Table 3: TK Parameters

Parameter	Dose Level (mg/kg)			
	250	500	1000	2000
Study Day 1				
C_0 (µg/mL)	680	1,380	2,970	8,330
C_{max} (µg/mL)	506	1,120	2,750	7,150
T_{max} (h)	0.0833	0.0833	0.0833	0.0833
AUC_{0-24h} (µg·h/mL)	257	745	2,780	5,610
AUC_{0-inf} (µg·h/mL)	NC	745	2,780	5,610
$T_{1/2}$ (h)	NC	4.81	3.66	2.88
CL (h/kg)	NC	0.671	0.359	0.356
V_{ss} (kg)	NC	0.332	0.252	0.255
Study Day 22				
C_0 (µg/mL)	414	1,230	1,970	5,650
C_{max} (µg/mL)	354	954	1,970	5,260
T_{max} (h)	0.0833	0.0833	0.0833	0.0833
AUC_{0-24h} (µg·h/mL)	357	824	3,520	7,620
$T_{1/2}$ (h)	NC	4.66	3.25	2.92
C_0 Ratio (Day 22/Day 1)	0.608	0.891	0.663	0.678
AUC_{0-24} Ratio (Day 22/Day 1)	1.39	1.11	1.27	1.36

C_0 = projected concentration at time zero
C_{max} = observed maximum plasma concentration after dosing
T_{max} = the time to reach the C_{max}
$T_{1/2}$ = terminal half-life
$AUC_{(0-24h)}$ = area under the plasma concentration-time curve from time zero to 24 hour post-dose
AUC_{0-inf} = area under the plasma concentration-time curve from time zero to infinity
CL = systemic clearance (only calculated for the single dose on Day 1)
V_{ss} = volume of distribution at the steady state (only calculated for the single dose on Day 1)
NC = Not Calculated

Clinical Pathology
Samples were collected on the day of necropsy

Hematology:

Hematology Parameters	
Erythrocyte count	Mean Corpuscular Volume
Hematocrit	Mean Platelet Volume
Hemoglobin	Platelet count
Leukocyte count, total and differential	Reticulocyte count
Mean Corpuscular Hemoglobin	Red cell distribution width
Mean Corpuscular Hemoglobin Concentration	

- No significant test article-related effects on hematology parameters were observed.

Clinical chemistry:

Clinical Chemistry Parameters	
Albumin/Globulin ratio	Creatinine
Alanine aminotransferase	Creatine kinase
Albumin	Globulin
Alkaline phosphatase	Glucose
Aspartate aminotransferase	Potassium
Blood Urea nitrogen	Sodium
Calcium	Total bilirubin
Chloride	Total cholesterol
Phosphorus	Total protein

- No significant effects on clinical chemistry parameters were observed.

Terminal procedures:
Main study animals were euthanized on SD29 and full necropsy was conducted.

Organ weights: (Adrenal glands, brain, heart, kidneys, liver, spleen, and testes)

- Absolute heart weights were slightly higher for the LD group (+7.7%, relative to control). No histological correlate was observed. This finding was considered to be toxicologically insignificant due to the lack of dose relationship and lack of histological correlate.

Histopathology:

Tissues/Organs	
Adrenal glands	Pancreas
Aorta	Parathyroid glands
Bone (femur and sternum)	Pituitary gland
Bone marrow (femur and sternum)	Prostate gland
Brain	Salivary gland
Epididymides	Sciatic nerve
Esophagus	Seminal vesicles
Eyes	Skeletal muscle (thigh)
Gall bladder	Small intestine (duodenum, jejunum, and ileum)
Gross lesions	Spinal cord (cervical, thoracic, and lumbar)
Skin from mammary area	
Harderian gland	Spleen
Heart	Stomach
Kidneys	Testes
Large intestine (cecum, colon, rectum)	Thymus
Liver	Thyroid glands
Lungs and bronchi	Trachea
Lymph nodes (mesenteric and mandibular)	Urinary bladder
Nasal cavity	Injection site (tail)

Kidneys and heart were considered to be target organs for SRP-4045 toxicity. Dose related findings were observed in both organs. Findings are summarized in the tables below.

Text Table 1: Kidney, tubular basophilia, bilateral

	Group 1	Group 2	Group 3	Group 4	Group 5
Dose Level (mg/kg)	**0**	**250**	**500**	**1000**	**2000**
Number Examined	**10**	**10**	**10**	**10**	**10**
Minimal	0	0	0	3	2
Mild	0	0	0	2	3
Moderate	0	0	0	1	5
Combined Incidence	0	0	0	6	10

Text Table 3: Heart, mineralization

	Group 1	Group 2	Group 3	Group 4	Group 5
Dose Level (mg/kg)	**0**	**250**	**500**	**1000**	**2000**
Number Examined	**10**	**10**	**10**	**10**	**10**
Minimal	0	0	0	0	2
Combined Incidence	0	0	0	0	2

Conclusion:
Due to the dose-related increase in mineralization in the heart, the NOAEL is 1000 mg/kg.

Study # Sr-17-003
Title: SRP-4045: 13-week intravenous injection toxicity and toxicokinetic study in male rats.

Testing Facility: (b) (4)

GLP Compliance: Yes.
Date of Study Initiation: 3/27/2017
Drug/Lot#/purity: SRP-4045/7003088/95%

Methods:
Animals: Rat, male, Sprague-Dawley
Age/weight: 7 weeks old at initiation of dosing/159 to 192 grams
Number: 10/group
Doses: 0, 250, 500, 1000, or 2000 mg/kg
Route: IV injection
Frequency: Once weekly for 13 weeks.
Study design:

Group[a,b]	Subgroup	No. of Animals[c]	Dose Level (mg/kg)	Dose Concentration[d] (mg/mL)
1 (Control)	1 (Toxicity)	10	0	0
	2 (Toxicokinetic)	4		
2 (Low)	1 (Toxicity)	10	250	25
	2 (Toxicokinetic)	10		
3 (Low-Mid)	1 (Toxicity)	10	500	50
	2 (Toxicokinetic)	10		
4 (Mid-High)	1 (Toxicity)	10	1000	100
	2 (Toxicokinetic)	10		
5 (High)	1 (Toxicity)	10	2000	200
	2 (Toxicokinetic)	10		

a Group 1 was administered vehicle control article only.
b All animals were dosed at a volume of 10 mL/kg.
c One toxicokinetic animal/group was designated as a possible replacement animal.
d Concentrations were corrected for lot-specific purity and water. A correction factor of 1.096 was used.

Observations/Results:

Formulation analysis: (Samples were collected from all dosing formulations in SD1 and 85 for verification of concentration.)

- All formulation samples were determined to be within the pre-established acceptance criteria for concentration

Mortality: (Data were recorded twice daily.)

- No unscheduled deaths occurred.

Clinical observations: (Clinical observations were recorded once daily. Detailed examinations were conducted weekly through the study. On each dosing day, observations were recorded at approximately 1 hour post dose.)

- No test article-related observations were recorded.

Body weight: (Data were recorded twice prior to initiation of dosing, prior to dosing on SD1, and weekly thereafter.)

- Body weight gain was slightly reduced in the HD group (8% less than control).
- Animals in the LD, LMD, and HMD groups gained slightly more, relative to control (+4, 5, and 11%, respectively). Because the increases in body weight gain were "inconsistent" with the reduction in body weight gain observed in HD animals, these findings were not considered by the sponsor to be related to the test article. However, the changes in the lower dose groups seem dose-related in magnitude and the reduced gain in the HD group may reflect a threshold effect or toxicity at the HD. The differences among groups are small, animal numbers per group was small, and changes are not statistically significant. However, the reduction in body weight gain for the very high dose of 2000 mg/kg could result in significant body weight reduction in a longer duration study.

Food consumption: (Amount of food was recorded weekly, by cage.)

- No test article-related effects on food consumption were observed.

Ophthalmology: (Testing was conducted once prior to initiation of dosing and once on SD89.)

- No adverse test article-related effects were observed.

Clinical Pathology

Samples were collected during week 4 of the dosing period and at necropsy.

Hematology: A standard battery of parameters was monitored.

- No significant test article-related effects on hematology parameters were observed.

Clinical chemistry: A standard battery of parameters was assessed.

- Urea nitrogen was elevated (graded mild, +29%) in HD animals on SD92, and correlated with microscopic finding of renal tubular degeneration.
- Findings suggesting dehydration were observed on SD92 and SD24 in LMD, HM, and HD groups: higher red cell mass, higher albumin, higher total protein, higher calcium, and higher phosphorus. All differences were small and graded mild in magnitude.

Toxicokinetics:

Group	Subgroup	Set	Dosing Phase Day	Time Points[a]
1	2	1st three animals	1, 85	5 minutes post-dose
2 through 5	2	1st three animals/group	1, 85	Pre-dose (Day 85 only) and 0.5 and 24 hours post-dose
2 through 5	2	2nd three animals/group	1, 85	5 minutes and 1 hour post-dose
2 through 5	2	3rd three animals/group	1, 85	4 and 8 hours post-dose

a Blood collection times were approximate and were based on the completion of dosing for each animal.

TK results:

Text Table 4.1: Summary of the SRP-4045 Toxicokinetic (TK) Parameters in Male Rat Plasma

Day	Dose Group	Dose Level (mg/kg)	C_0 (µg/mL)	C_{max} (µg/mL)	T_{max} (h)	AUC_{0-24} (µg·h/mL)
1	2	250	1250	951	0.0833	584
	3	500	2410	1850	0.0833	1160
	4	1000	4380	3550	0.0833	2900
	5	2000	8890	7240	0.0833	8340
85	2	250	1490	1140	0.0833	726
	3	500	2550	1960	0.0833	1430
	4	1000	5500	4300	0.0833	3170
	5	2000	10600	8260	0.0833	7780

Terminal Procedures:

Necropsies were conducted on all toxicity animals and organs and tissue were collected according to the table below.

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	lymph node (mandibular)		P,E
animal identification			lymph node (mesenteric)		P,E
aorta		P,E	muscle, biceps femoris		P,E
bone, femur with bone marrow (articular surface of the distal end to include stifle joint)		P,E	optic nerve (2)[a]		P,E
bone, sternum with bone marrow		P,E	pancreas		P,E
brain	W	P,E	pituitary gland	W	P,E
cecum		P,E	prostate	W	P,E
coagulating gland (intact with seminal vesicles [2])		P,E	rectum		P,E
colon		P,E	salivary gland (mandibular [2])		P,E
duodenum		P,E	sciatic nerve		P,E
epididymis (2)	W	P,E	seminal vesicle		P,E
esophagus		P,E	skin/subcutis		P,E
eye(2)[a]		P,E	spinal cord (cervical, thoracic, and lumbar)		P,E
gut-associated lymphoid tissue (GALT) - (Peyer's Patch)		P,E	spleen	W	P,E
Harderian gland[a]		P,E	stomach		P,E
heart	W	P,E	testis (2)[a]	W	P,E
ileum		P,E	thymus	W	P,E
injection site (tail)		P,E	thyroid (2 lobes) with parathyroid	W	P,E
jejunum		P,E	tongue		P,E
kidney (2)	W	P,E	trachea		P,E
lesions		P,E	ureter		P,E
liver	W	P,E	urinary bladder		P,E
lung with large bronchi		P,E			

E = Examined microscopically; P = Processed; W = Weighed.

a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

Macroscopic observations:

- No macroscopic findings were observed.

Organ weights:

- Absolute kidney weight was elevated in the HMD group and the HD group.

Data are summarized in the table below.

Text Table 4.2: SRP-4045-Related Changes in Organ Weight Parameters

		SRP-4045				
	Sex	Males				
	Dose Level (mg/kg)	0	250	500	1000	2000
Kidney						
	Absolute Weight (g)	1.9956	101	106	112*	122*
	Body Weight Ratio (%)	0.6007	100	102	105	128*
	Brain Weight Ratio (%)	108.7028	100	102	110	121*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for dosed groups expressed as percentage control mean value.

Histopathology: Dose-related effects were observed in multiple organs after SRP-4045 dosing for 13 weeks:

- Microscopic findings were observed in kidney and ureter in all SRP-4045 dose groups. The findings in kidney were described as kidney tubule degeneration (minimal to marked) “characterized by dilated tubules with vacuolated and intensely basophilic epithelium.” The kidney effects were dose-related in severity and incidence.
- Transitional cell vacuolation (graded minimal) was observed in the ureter in all SRP-4045 dose groups and in the urinary bladder in LMD, HMD, and HD groups.
- Basophilic macrophages in the lung were observed in the HMD and HD groups.
- In the liver of HD animals, cytoplasmic basophilia (minimal or slight) was observed in hepatocytes with Kupffer cell vacuolation.
- Mononuclear cell infiltrates were observed in the heart of animals in the LD, HMD, and HD groups.
- Vacuolated macrophages were observed in the mandibular (HD) and mesenteric (HMD, HD) lymph nodes

Conclusion:
The NOAEL was 1000 mg/kg due to “marked” severity of the kidney tubular degeneration. (The sponsor’s NOAEL was 2000 mg/kg.)

Text Table 4.3: Incidence and Severity of SRP-4045-Related Microscopic Findings

	SRP-4045				
Sex	Males				
Dose Level (mg/kg)	0	250	500	1000	2000
Number Examined[a]	10	10	10	10	10
Kidney					
Degeneration, tubule					
Minimal	0	5	2	0	0
Slight	0	5	8	1	0
Moderate	0	0	0	9	4
Marked	0	0	0	0	6
Ureter					
Number Examined	10	10	9	10	10
Vacuolation, transitional cell					
Minimal	0	1	1	2	4
Urinary Bladder					
	10	9	9	9	10
Vacuolation, transitional cell					
Minimal	0	0	3	5	4
Lung					
Infiltrate, macrophages, vacuolated, basophilic					
Minimal	0	0	0	3	5
Slight	0	0	0	0	1
Liver					
Basophilia, hepatocyte					
Minimal	0	0	0	0	1
Slight	0	0	0	0	1
Vacuolation, Kupffer cell					
Minimal	0	0	0	0	4
Slight	0	0	0	0	1
Lymph Node, Mandibular					
Infiltrate, macrophages, vacuolated					
Minimal	0	0	0	0	5
Lymph Node, Mesenteric					
Infiltrate, macrophages, vacuolated					
Minimal	0	0	0	4	3
Heart					
Infiltrate, mononuclear cell					
Minimal	0	1	0	1	4

a Number examined for each tissue unless otherwise specified.

9 Reproductive and Developmental Toxicology

Standard reproductive and developmental toxicology studies were not conducted.

Study# 4045-tox-004

Title: An intravenous dose range-finding toxicity study of SRP-4045 in juvenile male rats

Testing Facility: [redacted] (b) (4)

Date of Study Initiation: 11/27/2014
GLP Compliance: No
Drug/Lot#/Purity: SRP-4045/7001064/89%

Methods
Animals: Rat, male, Sprague-Dawley
Age/weight: 14 days old at initiation of dosing/33.7 to 43.9 grams
Doses: 0, 100, 300, 600, or 960 mg/kg
Route: IV
Vehicle: DPBS
Frequency: Once per week (4 doses)

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Males[a]
1	Vehicle Control	0	10	0	6 + 3 (1)
2	SRP-4045	100	10	10	6 + 3 (1)
3	SRP-4045	300	10	30	6 + 3 (1)
4	SRP-4045	600	10	60	6 + 3 (1)
5	SRP-4045	960	10	96	6 + 3 (1)

[a] In each group, 3 and 6 main study males were euthanized at Days 15 and 36 pp, respectively. In addition, 1 spare male was dosed and, since unassigned to the main study, was euthanized at weaning on Day 21 pp.

Observations/Results
Formulation analysis:

Text Table 2
Dose Formulation Sample Collection Schedule

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

N/A: Not applicable

All formulation samples were determined to be within the pre-established acceptance criteria for concentration.

Mortality: (Data were collected on dams twice daily through the pre-weaning period. Pups were checked twice daily.)

- No unscheduled deaths occurred.

Clinical observations: (Cageside observations were recorded twice daily. Detailed observations of dams were conducted on days that body weight was measured. F1 pups were monitored once daily on non-dosing days beginning PND15. On dosing days, pups were examined pre-dosing and within one hour post dose.)

- No adverse clinical signs related to the test article were observed.

Body weight: (Dams were weighed weekly pre-weaning. F1 pups were weighed on PND 4, 7, 10, 14, 17, 21, 24, 28, 32, and 35.)

- No test article-related effects on body weight or body weight gain were observed or F1 pups.

Pups were weaned on PND 21.

Clinical Pathology

Samples were collected according to the schedule summarized below.

Text Table 4
Samples for Clinical Pathology Evaluation

Groups / No. of Males per Group	Time Point	Hematology	Clinical Chemistry
All groups / 3	Day 15 pp	-	X
All groups / 6	Day 36 pp	X	X

X = sample collected; - = not applicable

Hematology:

Text Table 5
Hematology Parameters

Red blood cell count	Platelet count
Hemoglobin concentration	White blood cell count
Hematocrit	Neutrophil count (absolute)
Mean corpuscular volume	Lymphocyte count (absolute)
Red Blood Cell Distribution Width	Monocyte count (absolute)
Mean corpuscular hemoglobin concentration	Eosinophil count (absolute)
Mean corpuscular hemoglobin	Basophil count (absolute)
Reticulocyte count (absolute)	Large unstained cells

- No test article-related effects on hematology parameters were observed.

Clinical chemistry:

Text Table 6
Clinical Chemistry Parameters

Urea nitrogen	Albumin/globulin ratio
Total protein	Calcium
Alkaline phosphatase	Cholesterol
Chloride	Glucose
Creatinine	Inorganic phosphorus
Alanine aminotransferase	Potassium
Creatine kinase	Sodium
Triglycerides	Total bilirubin[a]
Albumin	Sample quality
Globulin	

[a] Direct and indirect bilirubin were measured but only reported in samples having total bilirubin values > 0.5 mg/dL.

- No test article-related effects on clinical chemistry parameters were observed.

Terminal Procedures

Text Table 7
Terminal Procedures

Group No.	No. of Males	Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	3	15 pp	-	-	-	-	-
2	3					-	-
3	3					-	-
4	3					-	-
5	3					-	-
1	6	36 pp	X	X	-	Gross Lesions and Kidneys	Gross Lesions and Kidneys
2	6					Gross Lesions and Kidneys	Gross Lesions and Kidneys
3	6					Gross Lesions and Kidneys	Gross Lesions and Kidneys
4	6					Gross Lesions and Kidneys	Gross Lesions and Kidneys
5	6					Gross Lesions and Kidneys	Gross Lesions and Kidneys
Dosed spares			-	-	-	-	-

X = Procedure conducted; - = Not applicable

Main study animals were euthanized on PND 36 and full necropsy was conducted.

Macroscopic observations:

- Pale discoloration of the kidneys was observed in all SRP-4045 dose groups.

Text Table 10
Summary of Gross Pathology Findings – Scheduled Euthanasia (Day 36 pp)

	Males				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg)	**0**	**100**	**300**	**600**	**960**
No. Animals Examined	**6**	**6**	**6**	**6**	**6**
Kidney (No. Examined)	6	6	6	6	6
Discoloration; pale	0	3	1	2	5

Text Table 8
Tissue Collection and Preservation

Animal identification Injection sites	Gross lesions/masses Kidney

Histopathology:

- Kidney tubular vacuolation (graded minimal to mild) was observed in LMD, HMD, and HD groups, dose related in incidence and severity.

Text Table 11
Summary of Microscopic Findings – Scheduled Euthanasia (Day 36 pp)

	Males				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg)	**0**	**100**	**300**	**600**	**960**
No. Animals Examined	**6**	**6**	**6**	**6**	**6**
Kidney (No. Examined)	6	6	6	6	6
Vacuolation; tubular (unilateral/bilateral)	(0)[a]	(0)	(5)	(6)	(6)
Minimal	0	0	5	6	5
Mild	0	0	0	0	1

[a] Numbers in parentheses represent the number of animals with the finding.

Conclusion:
The NOAEL was 960 mg/kg. The dose-related changes in kidney were considered non-adverse under the conditions of this study.

Study #4045-tox-005
Title: A 10-week intravenous juvenile toxicity study of SRP-4045 in male rats with an 8-week recovery period

Testing Facility: (b) (4)

Date of Study Initiation: 2/5/2015
GLP Compliance: Yes
Drug/Lot#/Purity: SRP-4045/7001978 and 7001979/94.8% and 94.4%, respectively

Methods
Animals: Rat, male, Sprague-Dawley CD
Age/weight: 14 days old at initiation of dosing/24.3 to 45.6 grams
Doses: 0, 100, 300, or 900 mg/kg
Route: IV
Frequency: Once weekly
Vehicle: DPBS
Study design: Pups were randomly assigned to dose groups on PND4. Subgroups are summarized in the table below.
Pups were weaned on PND 21. Pups in Subsets A, B, and D received 10 weekly doses.

Text Table 2
Experimental Design

Group No.	Test Material	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Males
1	Vehicle Control	0	10	0	112
2	SRP-4045	100	10	10	140
3	SRP-4045	300	10	30	140
4	SRP-4045	900	10	90	140

Phase I – Toxicology Subsets

Group No.	Test Material	Number of Males	
		Subset A	Subset B
		Main Study	Recovery/ Behavior/ Reproduction
1	Vehicle Control	32	32
2	SRP-4045	32	32
3	SRP-4045	32	32
4	SRP-4045	32	32

Phase II – Laboratory Investigation Subsets

Group No.	Test Material	Number of Males				
		Toxicokinetics		Clinical Pathology	Immunology	
		Subset C	Subset D	Subset E	Subset F	Subset G
		Day 14/15 pp	Day 77/78 pp	Day 15 pp	Dosing Period	Recovery Period
1	Vehicle Control	8	8	12	10	10
2	SRP-4045	36	8	12	10	10
3	SRP-4045	36	8	12	10	10
4	SRP-4045	36	8	12	10	10

Observations/Results:
Formulation Analysis:

Text Table 1
Dose Formulation Sample Collection Schedule

Interval	Concentration	pH, Osmolality, and Density	Sampling From
First preparation	All groups	All groups	Dosing container
Each subsequent preparation	All groups	N/A	Dosing container

N/A = Not applicable.

Text Table 1
Dose Formulation Sample Collection Schedule

Interval	Concentration	pH, Osmolality, and Density	Sampling From
First preparation	All groups	All groups	Dosing container
Each subsequent preparation	All groups	N/A	Dosing container

N/A = Not applicable.

All formulation samples were demonstrated to be within the pre-established acceptance criteria for concentration.

Mortality: (Dams were observed twice daily during the pre-weaning period. After weaning, F1 pups were observed twice daily.)

- No unscheduled deaths considered related to SRP-4045 occurred. One HD animal was found dead shortly after dosing on PND28, considered due to the dosing procedure.
- 4 MD animals were found dead, 3 shortly after dosing, and one due to obstructive uropathy.

Clinical observations: (Cageside observations were recorded once daily during the pre-weaning period and after weaning, F1 pups were observed once daily. Detailed examinations of F1 pups were conducted daily during the pre-weaning period and weekly after weaning.)

- No test article-related clinical signs were observed.

Body weight: (F1 pups were weighed on PND4, 7, 10, 14, 17, and 21. After weaning, body weights were measured twice weekly for the remainder of the dosing period and weekly during the recovery period.)

- No SRP-4045-relted effects on body weight or body weight change were observed.

Food consumption: (For Subgroups A and B, food consumption was measured per cage twice weekly from PND21 to 28, and weekly thereafter.)

- No test article-related effects on food consumption were observed.

Physical development:

Preputial separation: Assessments were made from PND35 until the goal was reached.

- No test article-related effect on timing of preputial separation was observed.

Behavioral performance: (Subsets A and B)

1. *Functional observational battery: (*Subset B, Conducted on PND73±4 and PND 129±4)

Text Table 4
Qualitative FOB Parameters

Posture	Palpebral closure
Tremors, tonic spasms and convulsions	Eye prominence
Bizarre behavior	Pupil size
Ease of removal	Pupillary response
Rearing	Lacrimation
Alertness	Salivation
Gait	Body tone
Piloerection	Extensor thrust
Respiratory rate/pattern	Pinna reflex
Grooming	Tactile reflex
Defecation	Overall animal reactivity
Urination	Auricular startle
Tail Pinch	Air righting reflex

Text Table 5
Quantitative FOB Parameters

Grip strength - fore and hind limbs Hind limb splay	Body temperature (rectal)

- No test article-related effects on qualitative or quantitative FOB parameters were observed.

2. *Motor activity:* (Subset B, conducted on PND73±4 and PND 129±4)
- No test article-related effects on motor activity were observed.

3. *Auditory startle:* (Subset B, During dosing between PND49 to 62, and during recovery between PND 113 to 127)
- No test article-related effects on auditory startle habituation were observed.

4. *Learning and Memory:* (Cincinnati water maze, Subset A, testing was conducted during dosing between PND 56 to 69; Subset B, testing was conducted during recovery between PND 120 to 133.)
- No adverse effects on performance in the water maze test were observed.

5. *Bone densitometry: (*Conducted at the end of the dosing period on 12 /group from Subset B. Testing used was DXA to measure bone mineral density, bone mineral content and area.)

Text Table 6
Bone Evaluation Using Dual Energy X-Ray Absorptiometry

Scan Site	**Reporting** **Area (cm^2), BMC (g), BMD (g/cm^2)**
Right Femur (Single scans)	Global, proximal, mid, distal
Lumbar spine (Single scans)	Global, L1-L4

- No test article-related effects were observed on femur length, femur and lumber spine area, bone mineral content, or bone mineral density.

<u>Clinical Pathology</u>
Samples were collected as summarized below at scheduled termination:

Text Table 7
Samples for Clinical Pathology Evaluation

Group Nos. / Subsets	Time Point	Hematology	Coagulation	Clinical Chemistry	Urinalysis/ Urine Chemistry (Biomarker)
Groups 1 to 4 / subset E[b]	Day 15 pp	X[a]	-	X[a]	-
Groups 1 to 4 / subset A[b,c]	Day 78 pp	X	X	X	X
Groups 1 to 4 / subset B[b,c]	Day 134 pp	X	-	X	X

X = Sample collected; - = Not applicable
[a] The blood collected from one set of 6 males per group was analyzed for clinical chemistry and the blood collected from a second set of 6 males in each group was analyzed for hematology.
[b] Samples were only collected from those males scheduled for euthanasia at the corresponding time point.
[c] Subset A: A blood sample for clinical chemistry was first collected followed by a sample for coagulation.

Hematology:

Text Table 8
Hematology Parameters

Red blood cell count	Mean corpuscular hemoglobin
Hemoglobin concentration	Reticulocyte count (absolute and percent)
Hematocrit	Platelet count
Mean corpuscular volume	Mean platelet volume
Red blood cell distribution width	White blood cell count (total, absolute and percent differential)
Mean corpuscular hemoglobin concentration	

- No test article-related effects on hematology parameters were observed.

Clinical chemistry:

Text Table 10
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin
Urea nitrogen	Albumin/globulin ratio
Creatinine	Sample quality

Clinical Chemistry Parameters Measured when Sufficient Serum Was Available

Calcium	Triglycerides
Total bilirubin[a]	Sodium
Phosphorus	Potassium
Glucose	Chloride
Cholesterol	

[a] Direct and indirect bilirubin were measured but only reported in samples having total bilirubin values > 0.5 mg/dL

- No test article-related effects on clinical chemistry parameters were observed.

Coagulation:

Text Table 9
Coagulation Parameters

Activated partial thromboplastin time Sample quality	Prothrombin time

- No test article-related effects on coagulation parameters were observed.

Urinalysis:

Text Table 11
Urinalysis Parameters

Color Appearance Specific gravity Total Volume pH	Glucose[a] Bilirubin Ketones Blood Urobilinogen

[a] Semi-quantitative measurement

Urine chemistry:

Text Table 12
Urine Chemistry Parameters

Creatinine Creatinine clearance Calcium Chloride	Protein[a] Total protein/creatinine ratio Potassium Sodium

[a] Quantitative measurements

- No test article-related effects on urinalysis or urine chemistry parameters were observed.

Urinary biomarker:

Text Table 13
Urinary Biomarkers

Cystatin C[a]	Cystatin C/creatinine ratio[b]

[a] Measured by the Test Site
[b] Calculated by the Test Facility using cystatin C values reported by the Test Site.

- No test article-related effects on cystatin C or cystatin c/creatinine ratio were observed.

TDAR:
KLH was administered, IV, at two time points to males in the Immunology Subsets F and G. The primary and secondary IgM and IgG responses to the KLH immunization were assessed according to the schedule below:

Text Table 14
Sample Collection Occasions for TDAR

Group Nos. / Subsets	Day 43 pp	Day 50 pp	Day 57 pp	Day 120 pp	Day 127 pp	Day 134 pp
Groups 1, 2, 3 and 4 / Subset F	Ig KLH	Ig KLH	Ig	-	-	-
Groups 1, 2, 3 and 4 / Subset G	-	-	-	Ig KLH	Ig KLH	Ig

- = Not applicable; KLH = KLH administration
Ig = Blood sample collection before KLH injection, where applicable

- No test article-related effects on IgM and IgG antibody responses to KLH immunization were observed.

Immunophenotyping: (Samples were collected from surviving males in Subsets A and B at scheduled necropsy.)

Text Table 15
Cellular Antigens and Cell Populations

Antigen Markers	Cell Population Identified
CD3+	Total T lymphocytes
CD3+/CD4+	Helper T lymphocytes
CD3+/CD8a+	Cytotoxic T lymphocytes
CD3-/CD45RA+	B lymphocytes
CD3-/CD161a+	Natural-killer lymphocytes

- No significant effects on lymphocyte subpopulations were observed.

Toxicokinetics:

Text Table 16
TK Sample Collection Schedule: Subset C

Group No.	No. of Males per Timepoint	Sample Collection Time Points (Time Postdose) on Day 14 post partum								
		5 min	15 min	30 min	1 hr	3 hrs	8 hrs	24 hrs	36 hrs	48 hrs
1	8	X	-	-	-	-	-	-	-	-
2	4	X	X	X	X	X	X	X	X	X
3	4	X	X	X	X	X	X	X	X	X
4	4	X	X	X	X	X	X	X	X	X

X = Sample collected; - = Not applicable

Text Table 17
TK Sample Collection Schedule: Subset D

Group No.	No. of Males per Timepoint	Sample Collection Time Points (Time Postdose) on Day 77 post partum									
		0[a] hr	5 min	15 min	30 min	1 hr	3 hrs	8 hrs	24 hrs	36 hrs	48 hrs
1	7	-	X[b]	-	-	-	-	-	-	-	-
2	4	X	-	X	-	X	-	X	-	X	-
	4	-	X	-	X	-	X	-	X	-	X
3	3	X	-	X	-	X	-	X	-	X	-
	4	-	X[c]	-	X	-	X	-	X	-	X
4	4	X	-	X	-	X	-	X	-	X	-
	4	-	X	-	X	-	X	-	X	-	X

X = Sample collected; - = Not applicable
[a] Sample collected before dosing.
[b] One out of 7 samples was collected 4 minutes later than the theoretical time.
[c] Three out of 4 samples was collected 2 minutes later than the theoretical time.

TK results:

Text Table 23
Toxicokinetic Summary

Parameter	Day	SRP-4045 Dose 100 mg/kg	300 mg/kg	900 mg/kg
C_{max}	Day 14 pp	571	1540	4660
(µg/mL)	Day 77 pp	437	967	4160
$AUC_{(0-24h)}$	Day 14 pp	333	1020	2930
(µg•h/mL)	Day 77 pp	200	633	3430

Plasma $t_{1/2}$ ranged from 8.7 to 12 hours, independent of dose. No evidence of accumulation was reported.

Terminal Procedures:

Text Table 19
Terminal Procedures for Main Study and Recovery/Behavioural/Reproductive Animals (Subsets A and B)

Group No.	No. of Animals Males	Scheduled Euthanasia Day	Necropsy Procedures Necropsy	Tissue Collection	Organ Weights	Histology	Histopathology
1	32	78 pp	X	X	X	Full Tissue[a]	Full Tissue[a]
2	32					Gross Lesions and Kidneys, Target Organs[b]	Gross Lesions and Kidneys, Target Organs[b]
3	32					Gross Lesions and Kidneys, Target Organs[b]	Gross Lesions and Kidneys, Target Organs[b]
4	32					Full Tissue[a]	Full Tissue[a]
1	32	134 pp	X	X	X	Full Tissue[a]	Full Tissue[a]
2	32					Gross Lesions and Kidneys, Target Organs[b]	Gross Lesions and Kidneys, Target Organs[b, c]
3	32					Gross Lesions and Kidneys, Target Organs[b]	Gross Lesions and Kidneys, Target Organs[b, c]
4	32					Full Tissue[a]	Full Tissue[a]
Unscheduled Deaths			X	X	-	Full Tissue[a]	Full Tissue[a]

X = Procedure conducted; - = Not applicable
[a] See Tissue Collection and Preservation table for listing of tissues.
[b] Target tissues (other than kidneys): lungs and injection sites for Subset A; Subset B (134 pp).
[c] See Section 4.18.2 Histopathology.

Subsets C, D, and E, were euthanized according to the schedule below:

Terminal Procedures for Toxicokinetic and Clinical Pathology Animals (Subsets C, D, and E)

Group No.	No. of Animals Males	Scheduled Euthanasia Day	Necropsy Procedures		
			Necropsy	Tissue Collection	Organ Weights
1[a]	8	14/15/16 pp	-	-	-
2[a]	32				
3[a]	32				
4[a]	32				
1[b]	8	77/78/79 pp	-	-	-
2[b]	8				
3[b]	8				
4[b]	8				
1[c]	12	15 pp	-	-	-
2[c]	12				
3[c]	12				
4[c]	12				
Unscheduled Deaths[a, b, c]			-	-	-

- = Not applicable pp = post partum
[a] Single dose toxicokinetic males
[b] Repeat dose toxicokinetic males
[c] Single dose clinical pathology males

Subsets F and G were euthanized according to the table below:

Terminal Procedures for Immunology Animals (Subsets F and G)

Group No.	No. of Animals Males	Scheduled Euthanasia Day	Necropsy Procedures		
			Necropsy	Tissue Collection	Organ Weights
1	10	57 pp	-	-	-
2	10				
3	10				
4	10				
1	10	134 pp	-	-	-
2	10				
3	10				
4	10				

- = Not applicable; pp = post partum

Organ weights:

Text Table 20
Organs Weighed at Necropsy

Brain	Heart
Epididymis[a, b]	Kidney[a]
Gland, adrenal[a]	Liver
Gland, pituitary	Lung
Gland, prostate	Spleen
Gland, seminal vesicle[b]	Testis[a]
Gland, thyroid[a]	Thymus

[a] Paired organ weight.
[b] Subset B animals only

- No SRP-4045 effects on organ weights were observed.

Bone: (Length and width of the left femur were measured at necropsy for all Subset A and B animals.)

- No test article-related effects on bone growth were observed.

Histopathology: Microscopic examination was conducted on a full tissue panel from control and HD animals.

Text Table 24
Summary of Microscopic Findings in Kidney - Terminal Euthanasia (Day 78 pp)

Group	1	2	3	4
Dose (mg/kg)	0	100	300	900
No. Animals Examined	32	32	32	31
Kidney (No. Examined)	32	32	32	31
Vacuolation; tubular	(0)[a]	(10)	(30)	(31)
Minimal	—	10	30	10
Mild	—	—	—	21
Cytoplasmic basophilic granules; tubular	(0)	(0)	(31)	(31)
Minimal	—	—	31	31
Degeneration/necrosis; tubular	(1)	(0)	(1)	(10)
Minimal	1	—	1	10

[a] Numbers in parentheses represent the number of animals with the finding.

Text Table 25
Summary of Microscopic Findings in Kidney - Recovery Euthanasia (Day 134 pp)

Group	1	2	3	4
Dose (mg/kg)	0	100	300	900
No. Animals Examined	30	32	29	32
Kidney (No. Examined)	30	32	29	32
Vacuolation; tubular	(0)[a]	(8)	(20)	(32)
Minimal	—	8	20	31
Mild	—	—	—	1
Cytoplasmic basophilic granules; tubular	(0)	(0)	(6)	(17)
Minimal	—	—	6	17
Degeneration/necrosis; tubular	(0)	(0)	(0)	(1)
Minimal	—	—	—	1

[a] Numbers in parentheses represent the number of animals with the finding.

Male reproductive assessments: (Subset B males, testicular pathology examinations were conducted on control and HD males.)

- No test article-related effects were observed on testicular histology, sperm motility, concentration, or morphology:

Spermatogenesis staging: (Subsets A and B)

- This analysis was not conducted because histology of male reproductive organs did not show test item-related effects.

Conclusion:

The NOAEL for this study was the MD of 300 mg/kg.

11 Integrated Summary and Safety Evaluation

Casimersen (SRP-4045) is a 22-mer phosphorodiamidate morpholino oligomer (PMO) antisense drug designed for use in patients with Duchennne muscular dystrophy (DMD) whose condition is amenable to skipping of exon 45. The goal of antisense oligonucleotide-based therapy is to alter the post-transcriptional splicing process of the pre-mRNA to restore the reading frame of the resulting mRNA by "skipping" of the mutated exon during processing of the mature mRNA. Deletion of the mutation-containing exon leads to restoration of the mRNA reading frame and production of a truncated, but partially functional dystrophin molecule. The mechanism of action of casimersen is similar to that of eteplirsen and golodirsen, antisense oligonucleotide drugs with the same molecular backbone chemistry recently approved under Accelerated Approval for treatment of DMD.

Pharmacology:

The optimal sequence for SRP-4045 was identified in preliminary studies in which several candidates were evaluated for pharmacodynamic activity using rhabdomyosarcoma and primary myoblast cells.

Proof of concept for the exon-skipping mechanism was demonstrated in studies conducted under IND 77429 using the *MDX* mouse model of muscular dystrophy. In that model, the mutation is located in exon 23 of the dystrophin gene; therefore, a surrogate murine antisense oligonucleotide was developed (AVI-4225) to demonstrate the pharmacology of the exon-skipping activity. Results of those studies showed dose-related reduction (incidence and severity) of myofiber degeneration in skeletal muscle in animals that received AVI-4225.

The potential for off-target hybridization of SRP-4045 was evaluated using *in silico* analysis of the entire human genome. The analyses suggested that SRP-4045 has minimal predicted potential for off-target hybridization.

Safety pharmacology studies (CNS, cardiovascular, and hERG assay) were conducted with SRP-4045 and showed no adverse test article effects.

Pharmacokinetics:

A series of ADME studies was conducted with SRP-4045 demonstrating that SRP-4045 is rapidly distributed to most tissues, with tissue C_{max} reached at approximately 0.25 hours after a single IV dose of 120 mg/kg. The primary route of elimination was urinary excretion. Clearance of radioactive SRP-4045 was considered complete at 336 hours post dose. Low protein binding was observed in mouse, rat, monkey, and human plasma and was independent of drug concentration. In metabolism studies, SRP-4045 did not show significant induction or inhibition of major CYP450 enzymes and was not a substrate or an inhibitor of major selected human drug transporters. The ADME characteristics demonstrated for SRP-4045 in these studies were similar to those demonstrated for the other related drugs, golodirsen and eteplirsen.

Toxicology:

General toxicology studies were conducted in male mouse (C57BL/6NCrl) for durations of 12-, 22-, and 26-weeks; and male cynomolgus monkey for durations of 12- and 39-weeks. In all studies, the target organ for toxicity was kidney.

The 22-week IV mouse study was initiated as a 26-week study in which weekly doses of 0, 300, 960, or 2000 mg/kg were administered. The study was terminated early at week 22 after injection site reactions resulted in partial or missed doses. The injection site reactions were dose-related in severity. Clinical observations showed findings of dry/flaking skin, thickening of skin with discoloration in the MD and HD groups and were dose-related in incidence. Also observed in the MD and HD groups was a small reduction in body weight gain, which was dose-related in magnitude at the end of the dosing period but was not observed at the end of the recovery period. Small increases in plasma urea nitrogen and creatinine (+23% and 31%, respectively) were observed in HD animals at the end of the dosing period. MD animals showed a 46% increase in creatinine only at the end of the dosing period. At the end of the recovery period, urea/nitrogen and creatinine remained increased in HD animals. These clinical pathology effects were accompanied by increased kidney weight and regeneration/degeneration of kidney tubular epithelium, graded minimal to moderate in the HD group and minimal in the MD group at the end of dosing. At the end of recovery, renal tubular epithelial regeneration/degeneration with casts (graded minimal to mild) was again observed in MD and HD animals. Significant recovery was not observed at the MD and HD. The NOAEL was 300 mg/kg (AUC0-t = 1060000 ng• hr/mL).

Because the chronic IV study was terminated early, a 26-week mouse study was initiated using the SC route of administration and lower doses. Although the clinical route of administration is IV, the adverse effects at the injection site in the 22-week mouse IV study, indicated that the IV route for the 26-week duration is not feasible.

In the 26-week SC study in mouse, animals received weekly injections of SRP-4045 at doses of 0, 300, 600, or 960 mg/kg. Skin lesions (associated with the injection site) were observed in all groups that received SRP-4045. The sponsor considered these lesions to be due to the SC dosing route and not adverse. However, one animal in each SRP-4045 dose group was euthanized early due to the skin ulceration. Therefore, they are considered related to the test article and adverse.

No in-life adverse effects were observed. Microscopic findings at the end of dosing showed injection site fibroplasia (minimal to mild) with myofiber degeneration/regeneration (minimal) in all SRP-4045 dose groups, dose related in incidence. In the kidney, tubular degeneration/regeneration, graded minimal, was observed in all SRP-4045 dose groups, dose related in incidence. Similar kidney findings were observed at the end of recovery. Because the kidney microscopic findings apparently were not sufficiently severe to result in impaired renal function, the NOAEL was considered to be the HD of 960 mg/kg (AUC 0-t = 1560000 ng• hr/mL. Plasma exposure (AUC), achieved after SC dosing, is higher than that observed at the NOAEL of 300 mg/kg IV in the 22-week study but resulted in kidney toxicity of lower magnitude.)

In a 39-week toxicity study in male cynomolgus monkeys, SRP-4045 was administered weekly by IV injection at doses of 0, 80, 320, or 640 mg/kg for 39 weeks followed by an 8-week recovery period. No in-life SRP-4045-related adverse effects were observed throughout the study. Histopathology showed effects in kidney described as cytoplasmic basophilia of the tubular epithelium (graded minimal to mild), tubular vacuolation (graded minimal to moderate) in all SRP-4045 dose groups and were dose-related in severity and incidence. Vacuolated macrophage aggregation was observed in mesenteric lymph nodes in the MD and HD groups (graded minimal to mild). At the end of the recovery period, renal tubular basophilia and vacuolation were observed only in HD animals. Cytoplasmic basophilia and vacuolation of renal tubular epithelium are common microscopic findings observed after treatment with antisense oligonucleotide drugs and are not necessarily considered adverse unless associated with degeneration/ necrosis or impairment of renal function.

Carcinogenicity:

Carcinogenicity assessments are deferred to post marketing. However, in anticipation of carcinogenicity protocol submission, dose-ranging studies were conducted in Sprague-Dawley rat and male CByB6F1-Tg(HRAS)2Jic (-/- homozygous c-Ha-ras) mice.

Male CByB6F1-Tg mice received weekly IV injections for 4 weeks at doses of 0, 250, 500, 1000, or 2000 mg/kg. The HD was reported to be the MFD. No in-life adverse effects were observed. Histopathology showed expected effects on the kidney described as cytoplasmic basophilia of the kidney tubular epithelium, graded minimal to moderate. In 2 of 10 HD animals, mineralization of the heart, graded minimal, was observed. The mechanism for this finding is not clear but due to the dose-related incidence, is considered related to SRP-4045.

A 13-week study in male Sprague-Dawley rats was conducted in which RP-4045 was administered by IV injection weekly at doses of 0, 250, 500, 1000, or 2000 mg/kg. A small reduction in body weight gain was observed in the HD group, but a small increase in body weight gain was observed in the LD, LMD, and NMD groups. The effects were small and not statistically significant. Although the reduction in body weight gain was small in the HD group, this effect could result in a more significant body weight loss at this dose in a longer duration study.

Microscopic findings were observed in kidney and ureter in all SRP-4045 dose groups. Findings were described as kidney tubular degeneration (graded minimal to marked) associated with tubule dilation and vacuolated epithelium. These findings were dose-related in severity and incidence. Transitional cell vacuolation was also observed in ureter in all SRP-4045 dose groups and in the urinary bladder at the HD. Cytoplasmic basophilia was observed in macrophages in various tissues (lung, liver, lymph nodes.) as well as liver hepatocytes.

Reproductive and development toxicology:

Reproductive toxicology studies are typically not conducted for the DMD indication. Therefore, detailed examination of male reproductive organs and sperm evaluations were conducted as part of the chronic toxicity studies. No test article-related effects were observed on sperm motility, concentration, morphology, or spermatogenic cycle.

Juvenile Animal Toxicology:
To support use of SRP-4053 in pediatric patients, a juvenile animal toxicology study was conducted in which male rat pups received weekly IV injections of SRP-4045 of 0, 100, 300, or 900 mg/kg beginning on PND14 and continuing for 10 weeks. No adverse effects on postnatal development were observed (clinical pathology, physical development, neurobehavioral function, bone development, or immune function). Histopathology showed vacuolation of kidney tubular epithelium, graded minimal to mild, and cytoplasmic basophilia of tubular epithelium, graded mild in all SRP-4045 dose groups. At the HD, degeneration/necrosis of tubular epithelium was observed in 10 of 32 HD animals. Due to the observation of degeneration and necrosis in renal tubular epithelium in a significant number of HD animals, the NOAEL for this study was the MD of 300 mg/kg ($AUC_{(0\text{-}24h)}$ = 633 µg•h/mL on SD77)

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