CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211970Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 211970
Submission date: 12/19/2018
Drug: golodirsen
Applicant: Sarepta Therapeutics
Indication: treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the dystrophin gene that is amenable to exon 53 skipping

Reviewing Division: Division of Neurology Products

Discussion:
The pharm/tox reviewer and supervisor found the nonclinical information for golodirsen adequate to support approval for the above indication. As noted in the primary and secondary pharm/tox reviews, renal toxicity, particularly in the juvenile rat study, was the most prominent finding in the toxicity studies. Potential relevance to human could not be ruled out particularly because of the small margin between the human exposure and the exposure at which the toxicity occurred in animals.

No developmental and reproductive toxicity studies were conducted with golodirsen because the patient population is almost entirely male. Potential effects on male fertility were assessed by histopathological evaluation of male reproductive organs and sperm assessment in toxicity studies. No adverse effects were noted.

The pharm/tox reviewer and supervisor recommend that carcinogenicity studies be conducted as postmarketing requirements, which is reasonable.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that the information is adequate to support approval from a pharm/tox perspective.

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/s/

PAUL C BROWN
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MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: August 1, 2019

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 211-970 (VONDYS 53, golodirsen, SRP-4053)

NDA 211-970 was submitted by Sarepta Pharmaceuticals on December 19, 2018, to support marketing approval of golodirsen for the treatment of Duchenne muscular dystrophy in pediatric and adult patients who have a confirmed mutation of the *DMD* gene amenable to exon 53 skipping. Clinical development of golodirsen was conducted by Sarepta under IND 119982.

The nonclinical studies provided in the NDA are consistent with the division's recommendations and feedback to the sponsor during clinical development. The nonclinical studies conducted on golodirsen by the sponsor include:

- Primary, secondary, and safety pharmacology
- PK/ADME
- Toxicology: 12- and 26-week studies in mouse; 12- and 39-week studies in monkey; 4- and 10-week toxicity study in juvenile rat.
- Carcinogenicity: 4- and 13-week dose-ranging studies in mouse and rat, respectively.

Reproductive and developmental studies (except for the juvenile animal study) were not required because of the intended patient population, which is almost exclusively male. A focused assessment of male reproductive organs was incorporated into pivotal toxicity studies. If the NDA is approved, carcinogenicity studies in two species (26-week in transgenic mouse, 2-year in rat) are to be conducted post-approval, as previously agreed to by the division.

The nonclinical studies were reviewed in detail by Dr. Wilcox (Pharmacology/Toxicology NDA Review and Evaluation, NDA 211970, Barbara J. Wilcox, Ph.D., July 3, 2019). Based on that review, Dr. Wilcox has concluded the nonclinical data support approval of golodirsen for the proposed indication but does note the severe renal toxicity produced by golodirsen, particularly in juvenile animals.

This memo will briefly summarize selected nonclinical data but will focus on the renal toxicity and other toxicity, which appears unique to golodirsen. Discussion of eteplirsen (a 30-mer PMO ASO, approved under Accelerated Approval on September 19, 2016, for DMD patients amenable to exon 51 skipping) is based on information provided in the nonclinical NDA review of eteplirsen (Pharmacology/Toxicology NDA Review and Evaluation, NDA 206488, David B. Hawver, Ph.D., January 22, 2016).

Pharmacology

Golodirsen is a 25-mer phosphorodiamidate morpholino (PMO) antisense oligonucleotide (ASO) designed to bind to exon 53 of dystrophin pre-mRNA resulting in exclusion of exon 53 during mRNA processing in patients with mutations amenable to exon 53 skipping. The resulting dystrophin is internally truncated but potentially functional.

The sponsor conducted a single pharmacology study, which characterized the pharmacological activity of a series of PMO ASOs, including golodirsen, using a variety of cell types (rhabdomyosarcoma cells and immortalized and primary myoblast from DMD patients). All ASOs demonstrated activity, but golodirsen was selected for development based on "ease and cost of manufacture and IP status (freedom-to-operate) …" No in vivo nonclinical studies were conducted to assess the extent of exon 53 skipping by golodirsen. Minimal off-target activity was identified.

The published literature provided by the sponsor (and additional selected publications) regarding efficacy of PMO ASOs in animal models of DMD (mdx mouse) were reviewed under the sponsor's NDA for eteplirsen (see Memorandum, NDA 206-488, Lois M. Freed, Ph.D., May 25, 2016).

In the only in vivo safety pharmacology study, golodirsen (0, 5, 40, or 320 mg/kg IV) had no effects on CNS, cardiovascular, or respiratory parameters in four male cynomolgus monkeys. The IC_{50} in the hERG assay was >30 µM.

PK/ADME

The PK/ADME of golodirsen was assessed in a study in mdx mouse. Following single IV injection of ^{14}C-golodirsen (120 mg/kg), plasma $t_{1/2}$ and Vss were 1.46 and 324 g/kg. Peak total radioactivity in selected tissues (0.25 to 1 hr post dose) was highest in kidney. Peak levels in various skeletal muscles were 9-14% of that in kidney. The majority of drug (~75%) was excreted intact in urine. In vitro plasma protein binding was low (<40%) in all species tested (mouse, rat, monkey, and human). The PK/ADME profile of golodirsen is characteristic of PMO ASOs, i.e., low protein binding, limited tissue distribution, and high renal excretion.

Toxicology

The toxicity of golodirsen was assessed in adult male C57BL/6NCrl mouse and cynomolgus monkey and in male juvenile Sprague Dawley rat. (All pivotal studies were conducted in accordance with GLP.) Kidney was a target organ in all species tested. No adverse effects on male reproductive organs or sperm (motility and morphology) were observed in any species tested.

Mouse: Golodirsen was administered by weekly injection for 12 weeks (0, 12, 120, or 960 mg/kg IV) or 26 weeks (0, 120, 300, or 600 mg/kg SC); recovery periods were 4 and 8 weeks, respectively. A 23-week IV toxicity study in mouse (0, 60, 120, 300, or 600 mg/kg) was terminated prematurely because of severe injection site reactions. There were a number of premature deaths in the 12-week study, which were not considered drug-related because of a lack of dose-response or presumed cause of death (thoracic and abdominal cavity and/or subcutaneous masses) considered unrelated to drug. There were no premature deaths in the 26-week study.

Microscopic findings in kidney, which generally correlated with clinical pathology markers of renal function (e.g., increased serum urea N and creatinine), were observed in all studies, at all doses. Kidney findings consisted of renal basophilic or eosinophilic casts, tubular dilatation, basophilia, and vacuolation, and tubular degeneration/regeneration. Findings in ureter and urinary bladder, which also occurred at all doses, consisted of transitional epithelial hypertrophy and degeneration. Kidney and urinary bladder findings were still evident at the end of the recovery period in the 12- and 26-week studies. On Day 176 of the 26-week study, plasma exposures at the low, mid, and high doses were 64.4, 136, and 191 µg/mL for C_{max} and 138, 472, and 988 µg*hr/mL for $AUC_{(0-t)}$, respectively.

Monkey: Golodirsen was administered weekly for 12 weeks (0, 5, 40, or 320 mg/kg; IV bolus) or 39 weeks (0, 80, 200 or 400 mg/kg; 30-min IV infusion); recovery periods were 4 and 8 weeks, respectively. There were no premature deaths in either study.

No drug-related findings were observed in the 12-week study, except for transient (Day 8) increases in complement B6 fragment and C3a at the mid and high doses and a dose-related decrease in FSH (Day 80).

In the 39-week study, microscopic changes in kidney (proximal convoluted tubule basophilic granules, distal convoluted tubule/collecting duct basophilia, dilatation, and microvesicular vacuolation, and/or mononuclear cell infiltration) were observed at all doses. At the end of recovery, basophilic granules were still detected at all doses but were in distal convoluted tubules and the loop of Henle; distal convoluted tubule/collecting duct basophilia and vacuolation were detected at the high dose. Kidney degeneration or necrosis was not observed at the end of the dosing or recovery periods. Bone was also a target organ in monkey; synovial hyperplasia (0/6, 0/6, 1/6, and 5/6) and inflammation (0/6, 0/6, 3/6, and 5/6) of the femorotibial joint (not accompanied by cartilage degeneration) were observed at the mid and high doses. Mononuclear cell inflammation of the heart was detected at all doses (0/6, 1/6, 2/6, and 2/6); parasitic infection was considered the cause, although the sponsor could not rule out a drug-related effect. On Day 260, plasma exposures at the low, mid, and high doses were 571, 1250, and 2600 µg/mL for C_{max} and 833, 1620, and 4280 µg*hr/mL for $AUC_{(0-t)}$, respectively.

Juvenile Rat: Potential effects of golodirsen on postnatal development were assessed in male juvenile Sprague Dawley rat. Doses for the pivotal (GLP) study were based on findings in a non-GLP dose-ranging (DR) study in which golodirsen (0, 100, 300, 600, or 960 mg/kg) was administered by IV injection weekly from postnatal day (PND) 14 to PND 35.

In the DR study, one high dose animal was found dead on PND 29, with no cause of death identified. Microscopic changes in kidney (tubular vacuolation and basophilic granules) were observed at all doses. At the two highest doses (600 and 960 mg/kg), microscopic findings of tubular dilatation and degeneration/regeneration were associated with clinical pathology findings (increased urea N, creatinine, Ca, Pi, and K).

In the pivotal study, golodirsen (0, 100, 300, or 900 mg/kg) was administered by IV injection weekly from PND 14 to PND 77. Twenty-four of 96 high dose animals died (20) or were sacrificed moribund (4) during PNDs 29-59. Cause of death was attributed to primary renal impairment and/or renal failure. Microscopic findings in kidney (tubular basophilic casts, dilatation, vacuolation, and/or degeneration/regeneration) and urinary bladder (transitional epithelium hypertrophy) were observed primarily at the mid and high doses (renal tubule degeneration/regeneration was detected in 1 low-dose animal). Similar findings were observed in recovery animals, although one high-dose recovery animal was particularly affected. According to the pathology report, "mild capsular fibrosis…associated with marked bilateral tubular vacuolation and degeneration/regeneration, moderate tubular dilatation and mild eosinophilic casts and correlated macroscopically with mottled discoloration, irregular surface, abnormal consistent and thick" were observed in this recovery animal.

No adverse effects on developmental parameters were observed, except for decreases in bone area and mineral content and density (femur, lumbar spine) at the high dose.

On PND 77, plasma exposures at the low, mid, and high doses were 544, 1340, and 4620 µg/mL for C_{max} (at 5 min post dose, the first sampling time) and 239, 774, and 3650 µg*hr/mL for $AUC_{(0\text{-}t)}$, respectively.

Carcinogenicity

No carcinogenicity studies have been conducted. The sponsor has conducted dose-ranging (DR) studies (reviewed below) to support planned carcinogenicity studies.

4-week DR in CByB6F1-Tg(HRAS)2Jic WT mouse: Golodirsen (0, 100, 300, 600, or 1000 mg/kg) was administered weekly by IV bolus injection for 4 weeks. There were no deaths or clinical signs. Kidney was the only target organ identified, with tubular degeneration observed at 600 and 1000 mg/kg. Plasma golodirsen exposures on Day 22 were 165, 631, 1870, and 3050 µg/mL for C_{max} (at 5 min post dose, the first sampling time) and 142, 520, 1250, and 2660 µg*hr/mL for $AUC_{(0\text{-}24\ h)}$.

13-week DR in Sprague Dawley rat: Golodirsen (0, 60, 100, 300, or 600 mg/kg) was administered weekly by IV bolus injection for 13 weeks. There were no deaths or clinical signs. Kidney was the primary target organ. Microscopic findings in kidney consisted of tubular basophilic granules at all doses, tubular degeneration at all but the lowest dose, tubular regeneration and casts primarily at the high dose. On Day 85, plasma exposures were 288, 531, 1940, and 2630 µg/mL for C_0 and 126, 226, 933, and 1930 µg*hr/mL for $AUC_{(0\text{-}t)}$, respectively. Although the toxicity of golodirsen was substantially greater in juvenile rat, plasma exposures were <2-fold (~1.8 and 1.9 for C_0 and AUC, respectively) higher than those in adult rat at the

highest doses tested. Whether this relatively small difference in plasma exposure is responsible for the marked difference in toxicity between adult and juvenile rats is uncertain.

Genetic Toxicology

Golodirsen was negative in an adequately conducted standard battery of in vitro (Ames, chromosomal aberration in CHO cells) and in vivo (mouse bone marrow micronucleus) assays.

Conclusions and Recommendations

The primary target organ in all species and strains tested (C57BL/6NCrl mouse, CByB6F1-Tg(HRAS)2Jic WT mouse, Sprague Dawley rat, and cynomolgus monkey) was kidney. Kidney is a well-known target organ for ASOs (Engelhardt JA Nucl Acid Therap 26(4): 199-209, 2016), and golodirsen, a PMO ASO, is primarily distributed to the kidney and excreted intact in urine (~75% of dose) following parenteral administration. The sponsor states, in more than one location (e.g., Toxicology Written Summary), that the nonclinical safety profile of golodirsen is similar to (or consistent with) that of "the approved PMO eteplirsen." Any comparisons between golodirsen and eteplirsen are limited by several factors. They were not tested in the same studies, a 26-week toxicity study in mouse was not conducted for eteplirsen, and there were differences in the dosing regimen used for golodirsen and eteplirsen in the 39-week IV toxicity studies in cynomolgus monkey (30-min infusion vs bolus injection, respectively, and higher doses were tested for golodirsen [0, 8, 200, and 400 mg/kg vs 0, 5, 40, and 320 mg/kg QW for eteplirsen]). (In monkey, plasma eteplirsen exposures during Week 39 were 84, 746, and 4184 µg/mL for C_0 and 48, 434, and 2552 µg*hr/mL for $AUC_{(0\text{-}t)}$.) However, there are a few notable observations.

In monkey, eteplirsen (for 12 or 39 weeks) resulted in tubular basophilia, vacuolation, and basophilic granules at the highest dose tested; renal tubular basophilia, dilatation, degeneration (minimal-slight), and mononuclear inflammation were observed in the high-dose recovery group in the 39-week study. Therefore, the pattern of microscopic renal changes in monkey differed between golodirsen and eteplirsen (e.g. golodirsen resulted in a wider distribution of toxicity in kidney, and only eteplirsen resulted in degeneration) but were generally of similar severity. The femorotibial joint and heart findings observed with golodirsen were not reported for eteplirsen. The differences between golodirsen and eteplirsen may reflect differences in C_0/C_{max} (higher with eteplirsen) and AUC (higher with golodirsen).

The most notable difference between golodirsen and eteplirsen was observed in the juvenile rat. Following administration of golodirsen at a high dose of 900 mg/kg, there were multiple premature deaths due to renal toxicity/failure; tubular degeneration/regeneration was observed at the mid (300 mg/kg) and high doses. When eteplirsen was administered to male Sprague Dawley rats at the same doses (0, 100, 300, or 900 mg/kg) weekly by IV injection during the same postnatal period (PNDs 14-77), there were no deaths or drug-related clinical signs. Microscopic changes in kidney were observed, which were correlated with changes in clinical pathology markers of renal function (e.g., increased BUN, creatinine, and Pi). However, eteplirsen had no effect on urinary bladder/ureter, and no instance of the severe renal impairment or renal failure that were observed with golodirsen. As for golodirsen, no adverse effects on developmental parameters were observed with eteplirsen, except for effects on bone (reduced mineral density, mineral content, and area at all eteplirsen doses). On PND 77, plasma eteplirsen exposures were 449, 1293, and 4570 µg/mL for C_{max} (at 5 min post dose, the first sampling time) and 204, 768,

and 2937 µg*hr/mL for $AUC_{(0-t)}$, respectively, which are similar to the plasma golodirsen exposures in juvenile rat. As for golodirsen, no other adverse effects on developmental parameters were observed with eteplirsen.

Based on the available data in juvenile rat, it appears that golodirsen has a greater risk for renal toxicity than does eteplirsen; renal failure leading to death was observed only with golodirsen. While there are interspecies differences in the anatomical and functional development of the kidney (Frazier KS. Birth Defects Res 109(16):1243-1256, 2017), which need to be taken into consideration, rat is considered a relevant species for human (Trevisan A et al. Exp Opin Drug Metab Toxicol 6(12): 1451-1459, 2010). At the recommended human dose of 30 mg/kg, plasma exposure (AUC of ~90 µg*hr/mL) is only 2.6-fold lower than that at the no-adverse-effect-level in the most sensitive species (juvenile rat). The human relevance of the femorotibial joint synovial hyperplasia observed in monkey with golodirsen (but not eteplirsen) is uncertain. Based on the available information, it does not appear to be a toxicity typically observed with ASOs.

Recommendation: The nonclinical studies of golodirsen are adequate to support approval for the proposed indication, although the data suggest the potential for serious renal toxicity in humans. As previously agreed to by the division, carcinogenicity studies in two species may be conducted post-approval (as PMRs), based on the seriousness of the indication.

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LOIS M FREED
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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 211970
Supporting document/s:	SDN 4
Applicant's letter date:	12/19/2018
CDER stamp date:	12/19/2018
Product:	Golodirsen
Indication:	DMD (exon 53 mutations)
Applicant:	Sarepta Therapeutics
Review Division:	Division of Neurology Products
Reviewer:	Barbara J. Wilcox, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
Division Director:	Billy Dunn, M.D.
Project Manager:	Fannie Choy, R.Ph.

Disclaimer:

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 211970 are owned by Sarepta or are data for which Sarepta has obtained a written right of reference. Any data or information described or referenced from a previously approved application that Sarepta does not own (or from FDA reviews or summaries of a previously approved application) are for descriptive purposes only and are not relied upon for approval of NDA 211970.

(All tables and figures in this review are from the Sponsor, unless otherwise designated.)

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1 Executive Summary

1.1 Introduction

Duchenne muscular dystrophy (DMD) is a condition caused by disrupted dystrophin protein synthesis due to a genetic mutation in the dystrophin gene sequence resulting in an out-of-frame transcription product. The mutation leads to the lack of a functional dystrophin protein. 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. The successful skipping of the exon containing the mutation at the pre-mRNA level is expected to result in the production of a truncated but functional dystrophin protein.

Golodirsen (SRP-4053) is a 25-mer oligonucleotide with a sequence designed to produce skipping of exon 53 in DMD patients who have been genetically identified as having a mutation in the dystrophin gene in a location that is amenable to treatment by skipping of exon 53. Golodirsen is believed to be highly specific for the human dystrophin gene sequence and reportedly exhibits no hybridization affinity to any other sequence in the human genome that would present a safety concern for human use.

1.2 Brief Discussion of Nonclinical Findings

Pivotal toxicology studies were conducted in mouse, rat, and cynomolgus monkey. Kidney toxicity was the major adverse effect in all species. Studies were conducted in male mouse with up to 26 weeks in duration, and IV doses up to 960 mg/kg. Dose-related renal impairment was reflected in effects on clinical chemistry parameters including increase in creatinine, CK, urea nitrogen, potassium, and phosphorus. The clinical chemistry findings correlated with microscopic kidney findings of tubular dilatation, vacuolation of the tubular epithelium and basophilic casts. Hypertrophy of the transitional epithelium of the urinary bladder and ureter was also observed in mouse studies.

In monkey studies, the magnitude of the renal toxicity appeared to be less severe, but effects on clinical chemistry parameters and kidney histopathology were also apparent in a 39-week toxicity study in which male cynomolgus monkeys received weekly IV injections of golodirsen at doses up to 400 mg/kg. As in the mouse, dose-related elevation of plasma urea nitrogen and creatinine were observed. Urinalysis data showed increased urine creatinine, urea, and protein/creatinine ratio at the HD, in the 39-week study. In addition, the in the 39-week study, adverse effects on clinical chemistry in HD monkeys were correlated with microscopic kidney findings described as dilation of the distal convoluted tubules and collecting ducts and microvesicular vacuolation of the distal convoluted tubule and collecting ducts in the mid dose (MD) and high-dose (HD) groups. Synovial hyperplasia in bone was also observed at the MD and HD. The kidney findings persisted through the recovery period, but the bone findings did not.

In a juvenile animal toxicology study, male rat pups received weekly IV injections at doses up to 900 mg/kg for 10 weeks beginning on postnatal day 14. No adverse effects on neurobehavioral or immune system development were observed. However, severe renal toxicity was observed, which was lethal at the HD. There were 31 unscheduled deaths at the HD determined to be due to renal toxicity. At the end of the dosing period, dose-related microscopic findings in the kidney of (surviving) MD and HD pups were similar to those described in previous studies (minimal to marked tubular vacuolation, minimal to marked tubular degeneration/regeneration, minimal to moderate tubular dilatation, minimal to moderate eosinophilic casts). In addition to the adverse findings in the kidney, findings were observed that were considered secondary to impaired renal function. These included mineralization of multiple tissues, degeneration of coronary artery tunic media, atrial thrombosis, and reduced bone area and density. No effects of golodirsen were observed on male reproductive organs or sperm evaluations.

In ADME studies, no induction or inhibition of hepatic microsomal enzymes, no significant interactions with known human drug transporters, and no metabolism by in the presence of human hepatic microsomes were observed. Plasma protein binding was low (<50%) in mouse, rat, monkey, and human plasma. Distribution of radiolabeled golodirsen showed rapid and extensive distribution to all tissues examined except CNS. Urinary excretion of golodirsen, primarily as unmetabolized drug, was the major route of elimination.

Golodirsen was negative in a battery of genetic toxicology studies.

1.3 Recommendations

1.3.1 Approvability

The pivotal nonclinical studies indicate that severe renal impairment (including irreversible renal damage in the juvenile animals) can result from chronic exposure to golodirsen, with only a small safety margin based on exposure. However, because kidney function is monitorable, the nonclinical data are considered adequate to support approval of golodirsen for the treatment of DMD in patients with mutations amenable to exon 53 skipping therapies.

1.3.2 Additional Non-Clinical Recommendations

Carcinogenicity assessments should be conducted post marketing.

1.3.3 Labeling

8.4 Pediatric Use

Intravenous administration of golodirsen (0, 100, 300, and 900 mg/kg) to juvenile male rats once weekly for 10 weeks (b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity studies have not been conducted with golodirsen.

Mutagenesis

Impairment of Fertility

2 Drug Information

2.1 Drug

Generic Name: Golodirsen

Trade Name: Vyondys53

Code Name: SRP-4053

Molecular Formula/Molecular Weight:

MW: 8647.28 Da

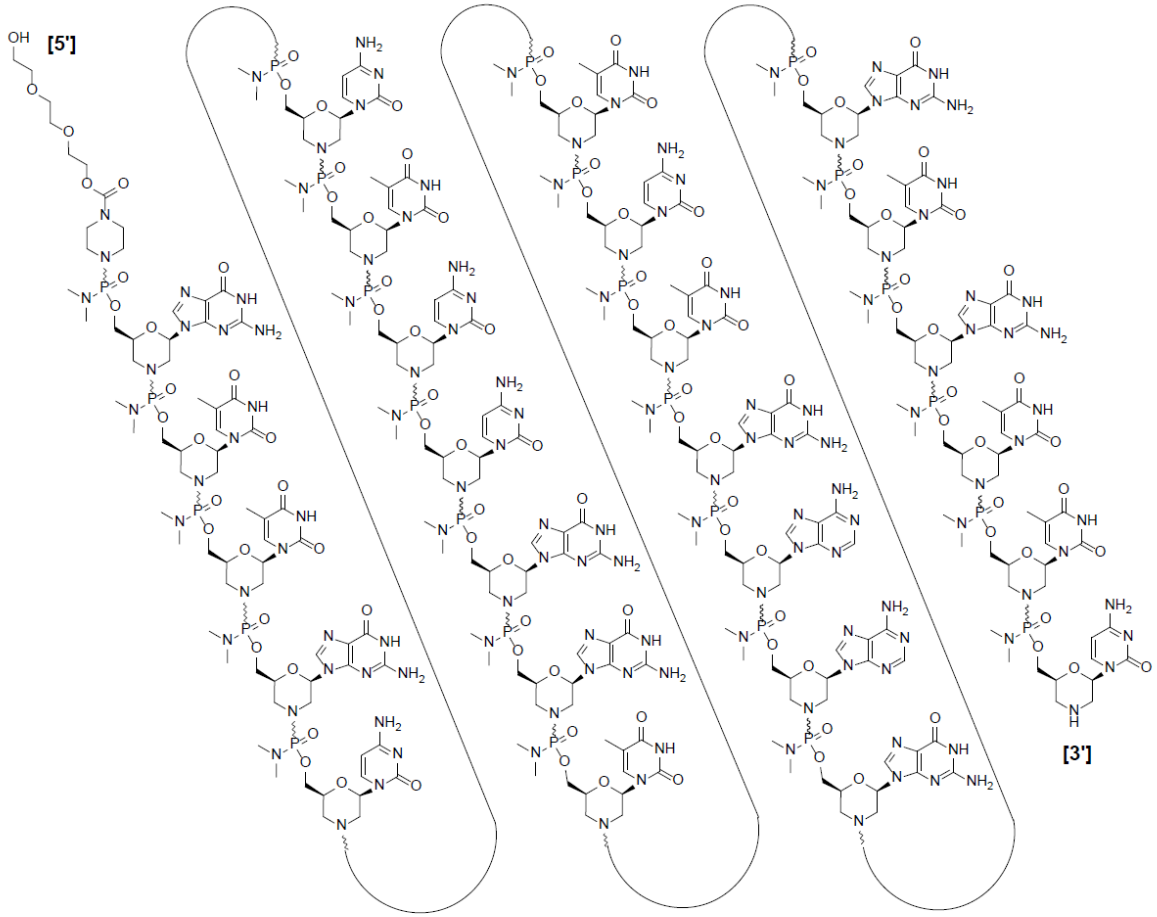
RNA, [*P*-deoxy-*P*-(dimethylamino)] (2′,3′-dideoxy-2′,3′-imino-2′,3′-seco) (2′a→ 5′) (G-m^{5}U-m^{5}U -G-C-C-m^{5}U-C-C-G-G-m^{5}U-m^{5}U-C-m^{5}U-G-A-A-G-G-m^{5}U-G-m^{5}U- m^{5}U-C), 5′-[*P*-[4-[[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]carbonyl]-1-piperazinyl]-*N*,*N*-dimethylphosphonamidate].

*Note that m^{5}U, which stands for 5-methyluracil, is the abbreviation for thymine base when thymine is named as part of RNA.

This document refers to the base as A, C, G, or T (m^{5}U), analogous to the standard usage in ribonucleic acids.

Structure or Biochemical Description: phosphorodiamidate morpholino oligomer (PMO)

Figure 1: Chemical Structure of Golodirsen



Pharmacologic Class:
Antisense oligonucleotide (ASO)

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND 77429, For treatment of DMD patients with mutations amenable to exon 51 skipping.
IND 118086, For treatment of DMD patients with mutations amenable to exon 45 skipping.

IND 119982, For treatment of DMD patients with mutations amenable to exon 53 skipping.
NDA 206488 (EXONDYS), for treatment of DMD patients with mutations amenable to skipping of exon 51.

2.3 Drug Formulation

Table 1: Drug Product Formulation, 50 mg/mL

Component	Reference to Standards	Quantity (mg/mL)	Function
Golodirsen	In-house specification	50	Active ingredient
Sodium chloride	USP; Ph. Eur.	8.0	(b) (4)
Potassium chloride	USP; Ph. Eur.	0.2	
Potassium phosphate monobasic	NF; Ph. Eur.	0.2	
Sodium phosphate dibasic, anhydrous	USP; Ph. Eur.	1.14	
Sodium hydroxide	NF; Ph. Eur.	q.s.[1]	pH adjustment
Hydrochloric acid	NF; Ph. Eur.	q.s.[1]	pH adjustment
Water for Injection	USP; Ph. Eur.	q.s.[2]	Solvent

[1] q.s. = quantity sufficient for pH adjustment
[2] q.s. = quantity sufficient to achieve final volume

2.5 Comments on Impurities/Degradants of Concern

All impurities are adequately qualified for the proposed Drug Substance specifications, based on data from the 39-week IV toxicity study in monkey. At the recommended dose of 30 mg/kg, the proposed specifications for Drug Product would result in slightly higher levels of human exposure for two impurities ((b) (4)) than the levels qualified in nonclinical studies. The impurities, (b) (4), are not considered to be of toxicological significance.

2.6 Proposed Clinical Population and Dosing Regimen

Patient population: DMD patients with confirmed mutations amenable to skipping of exon 53 of the dystrophin gene.
Dose: 30 mg/kg
Regimen: Weekly
Route of administration: IV infusion over a period of 35 to 60 minutes

2.7 Regulatory Background

SRP-4053 is one of three antisense oligonucleotide drugs with the same backbone chemistry being developed by Sarepta Therapeutics for treatment of DMD. A pre-IND meeting was conducted on February 12, 2014. IND 119982 was received October 7, 2014 and was allowed to proceed. On March 16, 2015, a safety report was received reporting multiple unscheduled deaths at the HD in an ongoing juvenile animal

toxicology study, with no cause of death identified. The clinical program was placed on full clinical hold. The Sponsor was asked to provide additional information about the unexpected deaths including a cause of death, if possible. The clinical hold was lifted after the Sponsor supplied sufficient data to determine that the cause of death for the HD animals was renal toxicity, and, therefore, monitorable in humans.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology:
- Selection of PMO sequence for SRP-4053 (Study #SR-14-172)
- Off-target sequence analysis for SRP-4053 (Study# SR-18-066)

Safety Pharmacology:
- CNS and Cardiovascular safety pharmacology (Study #4053-sph-001)
- Effects on hERG current by SRP-4053 (Study #SR-18-090)

ADME
- Plasma protein binding of SRP-4053 (Study #4053-pkd-001)
- Distribution of SRP-4053after single radiolabeled dose of SRP-4053 (Study #SR-17-007)
- Inhibitory potential of SRP-4053 on hepatic microsomal enzymes (Study #4053-pkd-002
- Potential induction of hepatic microsomal enzymes by SRP-4053 (Study #4053-pkd-003)
- Metabolism of SRP-4053 by hepatic microsomal enzymes (Study #4053-pkd-004)
- SRP-4053 as a substrate for human drug transporters (Study #SR-16-059)

General toxicology
- 12-mouse IV study (Study #4053-tox-002)
- Chronic SC mouse study (Study #SR-17-084)
- 23-week IV mouse study (Study #4053-tox-005)
- 12-week IV monkey study (Study # 4053-tox-001)
- 39-week IV monkey study (Study #SR-15-036)
- 13-week IV monkey study (Study #SR-16-019)
- 13-week IV study in rat (Study#SR-17-005)

Genetic toxicology
- In Vitro Ames Assay (Study #4053-gtx-001)
- In Vitro chromosomal aberration (Study #4053-gtx-002)
- In vivo mammalian erythrocyte micronucleus assay (Study #4053-gtx-003)

Reproductive and Developmental Toxicology
- IV dose-ranging study in juvenile rats (Study #4053-tox-003)
- 10-week IV study in juvenile rats (Study #4053-tox-004)

Studies not reviewed:
4-week IV toxicity study in CByB6F1 (Tg(HRAS) mice (Study # SR-17-004). This study was a preliminary study to test the tolerability of a transgenic mouse model for future carcinogenicity testing of SRP-4053.

3.3 Previous Reviews Referenced

NDA 206488 Pharmacology and Toxicology Review, David B. Hawver, PhD, (January 22, 2016)

4 Pharmacology

4.1 Primary Pharmacology

SRP-4053 is a phosphorodiamidate morpholino oligomer (PMO) antisense drug intended for use in the treatment of Duchennne muscular dystrophy (DMD). SRP-4053 is a 25-mer oligonucleotide designed to hybridize to the pre-mRNA transcript of the dystrophin gene, causing deletion of exon 53 from the mature mRNA.
Exon 53 skipping through this process induces production of a shortened but functional dystrophin protein in patients with genetic mutations that are amenable to exon 53 skipping

Study #SR-14-172
Title: Selection of PMO sequence for exon 53 SKIP-NMD Grant #305370

Testing Facility: Sarepta Bothell Research Biology
GLP Compliance: Non-GLP
Date of Study Initiation: 9/18/2013

This study was conducted to select the optimal PMO sequence for skipping of exon 53 of the dystrophin gene. Eight sequences were compared using a range of doses on multiple cell types (rhabdomyosarcoma, four different cultures of primary myoblasts, immortalized DMD patient myoblasts exhibiting deletion of exon 45-52, immortalized DMD cells carrying an exon 52 deletion, and primary DMD patient myoblasts with deletion of exon 52. After nucleofection of the PMOs into each cell type, RNA was harvested after 24 hours of incubation and RT-PCR was conducted to quantify the levels of exon 53 skipping. The optimal sequence was identified on the basis of efficiency of exon skipping at the range of doses investigated, as well as ease and cost of manufacture. The PMO designated NG-12-0163 (SRP-4053) was selected for further development.

Study # SR-18-066
Title: SRP-4053: Off-target sequence analysis

Testing Facility: (b) (4)

GLP Compliance: No

The potential for hybridization of SRP-4053 to off-target sites in the human genome was investigated. Using multiple bioinformatics tools, the binding affinity of SRP-4053 was calculated for potential off-target sites (within genes, at splice junctions, within non-gene sequences, and miRNAs). The investigations found no evidence of interactions that would cause concern for off-target binding of SRP-4053. Any potential off-target

binding should be sufficiently weak that it would be of minimal safety concern for human use of SRP-4053.

Proof of Concept:

Nonclinical studies demonstrating proof of concept for exon skipping were reviewed by Dr. David Hawver, under NDA 206488. The studies were re-submitted to NDA 211970 but were not re-reviewed in detail here. Studies AVI-4225-GLP-0903 and 4225-TOX-001 were conducted using an antisense oligonucleotide drug with a murine-specific sequence in the MDX mouse model of DMD, as well as in normal C57BL/6NCrl normal control mice. Both studies demonstrated skipping of the mutation-containing exon (exon 23) and showed dose-dependent decreases in muscle damage in the MDX mice.

Safety Pharmacology

Study #4053-sph-001

Title: Cardiovascular and neurological safety pharmacology evaluation of SRP-4053 in conscious, male, telemetered cynomolgus monkeys following acute intravenous injection

Testing Facility: (b) (4)

Date of Study Initiation: 12/2/2013
GLP Compliance: Yes
Drug/Lot#/ purity: SRP-4053/7001257/92%

Methods:

Animals: cynomolgus monkey, male, telemeterized (one group of 4 animals)
Doses and route of administration: 0, 5, 40, or 320 mg/kg IV
Age/weight: approximately 5 years old/ 5.29 to 7.19 kg
Study Design: Each monkey received each dose level only once (in a Latin square design). All animals were returned to the stock colony at the end of the study.

Experimental Design

Animal ID (supplier no.)	SRP-4053 Dose Level (mg/kg)			
	Day 1	Day 8	Day 15	Day 22
1M (0804999)	5	Vehicle	320[a]	40
2M (0809453)	40	320	Vehicle	5[b]
3M (0810197)	320	5	40	Vehicle
4M (0810177)	Vehicle	40	5	320[b]

[a] based on formulation analysis results from 19 Dec 13 and a dose volume of 3.9 mL/kg, an actual dose level of approximately 398 mg/kg SPR-4053 was administered to animal 1M.
[b] based on formulation analysis results from 19 Dec 13 and a dose volume of 3.2 mL/kg, actual dose levels of approximately 6 and 336 mg/kg were administered to animals 2M and 4M, respectively.

Observations/Results:
Formulation analysis: (Samples were collected from each dose formulation and tested for concentration and homogeneity.)

- Some results of formulation testing were out of acceptance range. However, retesting of back-up samples and dose/dilution adjustments resulted in no impact on study validity.

Mortality: (Observations were recorded twice daily.)

- No unscheduled deaths occurred.

Clinical observations: (Data were recorded not less than once per day.)

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

Body weight: (Data were recorded weekly prior to initiation of dosing and prior to dosing on each dosing day.)

- No test article-related effects on body weight were observed.

Neurobehavioral Testing: (All animals were tested prior to initiation of dosing and post dose at 4 and 7 hours on each day of dosing. Parameters were unrestrained activity in cage [mentation/behavior, posture, gait], detailed neurological examination of restrained animals [all reflexes].)

- No effects on neurobehavioral parameters were observed.

Telemetry: (Recording was started 1 hour prior to each dose administration and was continued for 24 hours. For each animal, at least 20 hours of baseline data were collected prior to study initiation.)
Parameters evaluated: Arterial blood pressure (SBP, DBP and MAP), Heart rate, Left ventricular pressure (LVSP, LVEDP, dP/dt+ and dP/dt-), Lead II ECG parameters (PR interval, QRS duration and QT interval and QTCR), QA Interval, Respiratory rate, Core body temperature.

- No effects on telemetry parameters related to the test article were noted.

Respiratory parameters:

- No effect on respiratory rate was noted.

Conclusion:
This study was inappropriately designed. Tissue washout prior to the next dose was not demonstrated. However, with the probable tissue retention over the duration of the study, no adverse effects related to the test article were observed.

Study #SR-18-090
Title: Effect of SRP-4053 on cloned hERG potassium channels expressed in human embryonic kidney cells

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation: 10/17/2018
Drug/Lot#/purity: SRP-4053/ 7700417/ 95%

Methods:
Cells: HEK/hERG 293
Vehicle control: hERG buffer (130mM NaCl, 1mM $CaCl_2$, 1mM $MgCl_2$, 10 mM HEPES, 12.5 mM dextrose, pH 7.4)
Positive control: Terfenadine, 60 nM
Positive control and reference buffer: 0.3% DMSO
Reference substance: E-4031

Results
Test article concentration-response group: 4 concentrations of SRP-4053 were tested (100uM, 300 µM, 1 mM, and 10 mM) (No useful data could be obtained from the 2 highest concentrations. No explanation was given.)

Mean percent inhibition at each SRP-4053 concentration (Mean), standard deviation (SD), standard error of the mean (SEM) and number of cells (N).

Concentration (µM)	Mean	SD	SEM	N
0	6.2%	0.8%	0.5%	3
100	9.4%	1.1%	0.6%	3
300	20.1%*	3.7%	1.9%	4

* Value is statistically different from vehicle alone ($P < 0.05$).

Vehicle control group:
Vehicle control runs (n=3) showed hERG inhibition of approximately 6.2%

Positive control: Terfenadine inhibited hERG potassium current by approximately 77%. Confirming the sensitivity of the test.

Conclusion:
The IC_{50} for the inhibitory effect of SRP-4053 was estimated to be greater than 300 µM.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Distribution:

Study #4053-pkd-001

Title: In vitro plasma protein binding of SRP-4053 in mouse, rat, monkey, and human

Testing Facility: (b) (4)
GLP Compliance: No
Date of Study Initiation: December 2013 (The exact date was not specified.)
Drug/Lot#/purity: SRP-4053/7001257/91%

Methods/Results:
Five concentrations of SRP-4053 (8, 24, 80, 240, and 800 ug/mL) were used.
Pooled plasma from at least 3/species (CD-1 mouse, Sprague-Dawley rat, cynomolgus monkey, and male humans).

Results demonstrated low non-specific binding of SRP-4053 in plasma from all species (0% in mouse, 1.0 to 1.7% in rat, 0.2 to 4.2% in monkey, and 0 to 0.5% in human).

Binding of golodirsen to plasma protein (expressed as percent bound) from mouse, rat, monkey, and human:

(Reviewer table)

Drug Concentration	Mouse	Rat	Monkey	Human
80 ug/mL	31.3	11.0	33.7	37.0
800 ug/mL	39.5	22.2	33.3	37.3

Overall, protein binding of SRP-4053 to plasma proteins across species was determined to be low and not concentration dependent. Binding was generally similar across species.

Study #SR-17-007
Title: Distribution, metabolism, and excretion of ^{14}C-SRP-4053 after a single intravenous injection to mice

Testing Facility: (b) (4)
GLP Compliance: No
Date of Study Initiation: 12/12/2013
Drug/Lot#/purity: SRP-4053/7003064/93%

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.
Notes: The radioactive dose was approximately 300 µCi/kg.

Results:

Radioactivity was rapidly and extensively distributed to most tissues. Over time, kidney retained the highest levels of radioactivity. CNS structures including spinal cord contained the lowest level of radioactivity of the tissues examined.

Pharmacokinetic parameters calculated are summarized in the table below:

Matrix	C_0 (µg-eq/g)	C_{max} (µg-eq/g)	T_{max} (h)	AUC_{0-t} (µg-eq·h/g)	AUC_{0-inf} (µg-eq·h/g)	$t_{1/2}$ (h)	CL (g/h/kg)	V_{SS} (g/kg)
Blood	677	339	0.0830	117	117	1.46	1020	325
Plasma	1130	544	0.0830	182	NC	NC	NC	NC

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.

The concentration of retained radioactivity in individual tissues over time post dose is summarized in the table below:

	Mean Concentration (ng-Equivalents ^{14}C-SRP-4053/g)								
	Sacrifice Time (Hours)								
Sample	0.25	1	4	8	24	48	96	120	144
Biceps brachii	40300	7740	1680	1630	521	884	841	444	909
Biceps femoris	41600	18100	2030	1990	1570	1510	1600	985	932
Quadriceps	38300	12300	1430	1140	864	1270	1010	833	589
Tibialis anterior	25100	13900	1190	816	715	722	788	506	846
Brain	1940	2290	121	66.8	21.0	18.6	18.1	16.4	16.8
Diaphragm	30300	7510	1650	1250	781	1020	667	743	784
Kidney (right)	291000	159000	137000	107000	63500	41400	23400	27800	20800
Heart	24500	4370	1230	1020	800	915	718	697	583

A summary of elimination data is found in the table below.

	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)	93.1 ± 0.503	74.9 ± 1.95	9.64 ± 2.05	0.780 ± 0.0158	4.93 ± 3.08	2.86 ± 0.638

a Cage wash and cage wipe.

- Overall recovery of the radioactivity was 93.1%.

Elimination is primarily via urinary excretion, accounting for 74.9% of the radioactive dose. The predominant component in urine was SRP-4053 and accounted for 60.1% of the dose over the 24 hours post dose.

Study#4053-PKD-002

Title: Inhibitory potential of SRP-4053 on human hepatic microsomal cytochrome P450 isoenzymes

Testing Facility: (b) (4)

GLP Compliance: No
Date of Study Initiation: 12/12/2013
Drug/Lot#/Purity: SRP-4053/7001257/91%

Summary

Pooled hepatic microsomes from 50 human donors (25 male, 25 female) were used to determined inhibitory potential of SRP-4053 on selected microsomal enzymes. Assays were conducted in the absence and presence of SRP-4053 (at concentrations from 0.00137 to 6.25 mg/mL). The enzymes selected are 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.

Positive control tests successfully demonstrated adequate assay sensitivity. Results demonstrated no significant inhibition of any of the selected enzymes greater than 50% by SRP-4053.

Study # 4053-PKD-003

Title: Evaluation of cytochrome P450 induction following exposure of primary culture of human hepatocytes to SRP-4053

Testing Facility: (b) (4)

GLP Compliance: No
Date of Study Initiation: 12/12/2013
Drug/Lot#/purity: SRP-4053/7001257/91%

Summary and results:

Test system: Primary cultures of human hepatocytes
Positive controls: known inducers of each selected enzyme
Negative control: solvent control

- Cytotoxicity was tested in preliminary testing by incubating cultures of hepatocytes for 72 hours in the presence of the test article and appropriate controls. Cytotoxicity was determined by measurement of LDH levels.
- Hepatocyte cultures were dosed with aliquots of SRP-4053 to give final test article concentrations of 0.00137, 0.0975, 0.188, 0.375, 0.75, 1.5, 3.15, and 6.25 mg/mL.

Hepatocyte cultures were dosed with solutions of known non-inducers and prototypical inducers of each CYP enzyme. Summarized in the table below:

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

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

- Cultures were exposed for 72 hours with dosing solutions of SRP-4053 and the designated inducer or non-inducer.

Test and control cultures processed at completion of the incubations for RNA in and collected using standard methods. Relative amounts of mRNA for the selected CTP enzymes were determined using real time PCR.

- Activity of each of the selected CYP enzymes was determined in separate cultures by measuring the production of enzyme-specific metabolites.

The table below lists the chemicals used for detection of CYP enzyme activity:

Cytochrome P450	Cytochrome P450 Activity	Analyte
CYP1A2	Phenacetin *O*-deethylase	Acetaminophen
CYP2B6	Bupropion hydroxylase	Hydroxybupropion
CYP3A4/5	Testosterone 6β-hydroxylase	6β-Hydroxytestosterone

Results:
Cytotoxicity: No concentration-dependent cytotoxicity was observed in response to the increasing concentrations of SRP-4053. Cell viability was greater than 91% of control at the highest concentration of the test article (6.25 mg/mL).

No substantive induction of CYP1A2, CYP2B6, CYP3A4 mRNA was observed.
A two-fold increase in CYP1A2 activity was detected. However, although elevated, the induction was well below that of the positive control drug omeprazole, and therefore, is most likely not of clinical significance for potential drug-drug interaction.

Study #4053-PKD-004
Title: Metabolism of SRP-4053 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 hepatic mouse, rat, monkey, and human microsomes were incubated with target concentrations of SRP-4053 of 8 and 80 µg/mL in the presence and absence of NADPH. Incubations were conducted for 0 or 120 minutes. At the end of the incubation period, SRP-4053 levels were measured. Mean concentrations of SRP-4053 at each target concentration showed no significant changes at the end of 120 minutes, relative to 0 minutes of incubation, for any of the species-specific microsomal cultures. Mean concentrations of SRP-4053 after 120 minutes of incubation in the absence of NADPH were similar to concentrations after 120-minute incubation in the presence of NADPH. Therefore, the results indicate that no significant time- or NADPH-dependent metabolism of SRP-4053 occurred in hepatic microsomes from mouse, rat, monkey or human.

Study #SR-16-059

Title: Evaluation of [^{14}C] SRP-4053 as a substrate and 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-4053/CFQ43053/90.6% SRP-4053/7003064/93%

Summary and Results:

Potential drug transporter interactions were evaluated using radiolabeled SRP-4053 as substrate and unlabeled SRP-4053 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 and were obtained from a commercial supplier. Negative control cells were transfected with vector only.

Uptake of the test article 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-4053 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.

At concentrations of 100 and 1000 µg/mL, SRP-4053 showed weak inhibition of uptake by OATP1B3 and MATE2-K. The estimated IC_{50} values were greater than 1000 µg/mL. No inhibition of uptake was observed for OAT1, OAT3, OCT2, OATP1B1, MATE1, P-gp, BCRP, or MRP2 at the higher concentrations of SRP-4053.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study # 4053-tox-002

Title: A 12-week intravenous injection toxicity and toxicokinetic study of SRP-4053 in the male mouse with a 4-week recovery period

Testing Facility: (b) (4)
Canada
GLP Compliance: Yes
QA Report: Yes
Date of Study Initiation: 10/1/2013
Drug/Lot #: SRP-4053/7001122

Methods:
Species: Mouse (C57BL/6NCrl), males only
Number per Group: 20 for main study, 12 for recovery, satellite groups of 63 mice for groups 2 - 4 and 6 for group 1 were designated for TK
Age: 9-10 weeks old, 21.7 to 29.3 g
Doses: 0, 12, 120, or 960 mg/kg
Vehicle: PBS
Route of administration: Intravenous bolus
Regimen/Duration: Weekly for 12 weeks, followed by 4-week recovery period
Study Design:

Text Table 3
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	10.4	0	20	12	6+20
2	SRP-4053	12	10.4	1.15	20	12	63+20
3	SRP-4053	120	10.4	11.5	20	12	63+20
4	SRP-4053	960	10.4	92.3	20	12	63+20

[a] Toxicokinetic animals were used for toxicokinetic evaluation only (6 animals in group 1 + 63 animals/group in Groups 2-4).
[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	Homogeneity	Stability	pH, Osmolality and Density	Sampling From
Day 1	All groups	Groups 2 and 4[a]	N/A	All groups	Dosing container
Each preparation	All groups	N/A	N/A	N/A	Dosing container

N/A: Not applicable.
[a] The homogeneity results obtained from the top, middle and bottom for the Group 2 and 4 preparations were averaged and utilized as the concentration results.

The results of formulation analysis were within pre-determined acceptance criteria with one exception: the stock solution for week one was slightly lower in concentration (-16.1%) than the predetermined lower limit of -15%. To accommodate the lower concentration, the dose volume was raised accordingly to deliver the correct dose levels. Therefore, the validity of the study was not affected.

Mortality: (Data were recorded twice daily.)

- Nine unscheduled deaths (4 from the main study and recovery groups, 5 from the TK groups) (1 LD, 7 MD and 1 HD) were reported between study days (SD) 22 and 78. Animals #3001, 3006, 3013 and 3025 (main study and recovery) were found dead on SD 22, 78, 35, and 78, respectively. No clinical observations were reported prior to finding the animals dead. No cause of death was determined for 6 of the 9 early deaths.
- TK animals #2075, 3077, and 3097 were found dead on SD 36, 42 and 42, respectively. Clinical signs prior to death included thinness and soft/swollen skin lesion in the urogenital region for #3077. Subcutaneous mass was considered the cause of death for #3097.
- Two TK/hormone animals (#3082 and 4112) were euthanized early on SD 30 and 70, respectively. Clinical signs prior to death included thinness, pale skin, cold to touch, suspected dehydration, weak, decreased activity, and/or

abdominal distention. Thoracic mass was found for #3082 and abdominal mass for animal 4112. (Further discussion of the abdominal masses is found below under *Clinical observations*).

- Of the 5 deaths in TK animals, cause of death for 3 was considered to be the thoracic and abdominal masses. No cause of death was determined for 2 of the 5 TK animals (# 3077 or 2075).

Summary of unscheduled deaths/group: (Reviewer table)

	Group 1 Control	Group 2 12 mg/kg	Group 3 120 mg/kg	Group 4 960 mg/kg	Comment
Found Dead	0	1(TK)	4 (MS) 2(TK) 1 (TK)	1 (TK)	No COD COD=mass No COD
Total		1	7	1	

9 found dead

6 No COD

MS= main study

TK= toxicokinetics

COD= Cause of death

Clinical observations: (Cageside observations were recorded once daily. Detailed observations were recorded once prior to the initiation of dosing, then weekly thereafter through the dosing and recovery periods.)

- Masses observed in the urogenital and abdominal regions were noted in all groups including control. These masses were considered by the sponsor to be abscesses caused by preputial adenitis. They report that this strain of mice is highly susceptible to this type of infection. However, the Sponsor did not provide sufficient information to support this claim. Masses were detected in all groups with incidence slightly greater in the LD and MD groups, relative to control. The data below summarize the incidence of masses observed for groups 1, 2, 3, and 4 (left to right).

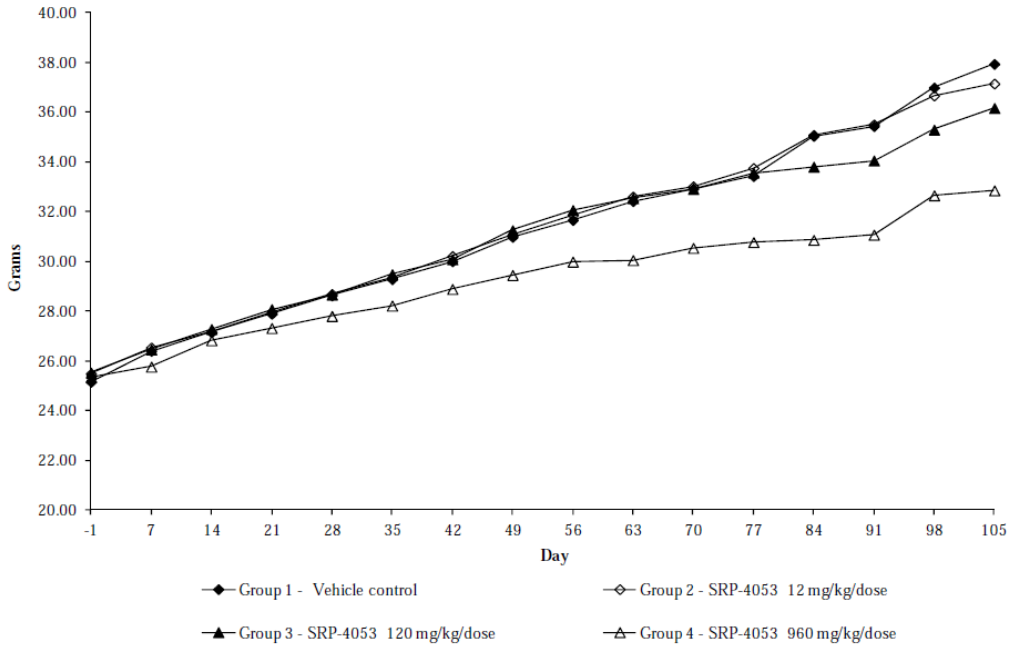
Mass Present				
Number of Observations	121	133	181	111
Number of Animals	7	9	11	7
Days from - to	1 106	1 106	8 106	1 106

Body weight: (Data were recorded once or twice prior to initiation of dosing, on SD 1, then weekly thereafter.)

- No test article-related effect on body weight or body weight gain was noted for the LD group.
- Although not statistically significant, the MD group showed a trend toward reduced body weight gain toward the end of the dosing period.
- At the HD, decreases in body weight gain were observed beginning on SD 35 and persisted into the recovery period.
- At the end of the dosing period, mean body weight for the HD group was approximately 12% lower than the control mean.

- Weight gain was similar to control during the recovery period, although the absolute body weight in the HD group remained lower than control.

Figure 1 **Summary of Body Weights - Males**



Food consumption: (Food was measured weekly starting on SD 8 and continued through the dosing and recovery periods.)

- The reduced body weight gain in the HD group was correlated with slightly reduced food intake during the dosing period (although variable, the range was approximately -4% to -16%, relative to control). Food consumption improved during the recovery period but remained slightly reduced in the HD group.

Ophthalmology: (Testing was conducted once prior to initiation of dosing and during week 11 of treatment.)

- 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]	Urinalysis
1 to 4[b]	Day 79	X	X	-
Main animals	At termination	-	-	X
1 to 4	Day 106	X	X	-
Recovery animals	At termination	-	-	X

X = Sample collected
[a] At scheduled occasions, half the animals were prioritized for hematology and half the animals were prioritized for biochemistry.
[b] Animals scheduled for euthanasia only.

Hematology: (A standard battery of parameters was monitored.)

- In the HD group, small decreases (0.92X) in red cell mass were observed (RBC, HGB, HCT).
- Reduced reticulocytes (0.86X) was observed at the end of the dosing period.
- Similar findings in HD animals were observed in red cell mass parameters (except for reticulocytes) at the end of the recovery period.

Clinical Chemistry: (Full battery of parameters was monitored)

End of dosing period:

- Increased UREAN was observed for all dose groups relative to control, but was ~2-fold greater at the HD. Most HD animals were affected by the increase in UREAN.
- Other changes noted for all dose groups included increased AST (1.27X, HD), increased CK (1.17X, HD), increased CREAT (1.55X, HD), reduced TRIG (0.66 to 0.87X), increased PHOS (1.24X, HD), increased K (1.05X), and reduced Cl (0.98X, HD), relative to control.

End of recovery:

- Elevated UREA N (1.3X) was noted in the HD group at the end of the recovery period. No other significant findings were observed at the end of recovery.

Urinalysis: (Appearance, pH, volume, specific gravity)

- No effects were observed in LD or MD groups.
- The HD group showed slightly decreased specific gravity (0.66X) at the end of the dosing period.
- No difference was noted among groups at the end of recovery.

Hormones: (Blood samples were collected on SDs 79 and 106 from 10 animals/group for hormone analysis including testosterone, FSH and LH.)

- No test article-related effects on hormone levels were observed.

Toxicokinetics

TK results:

Table 2.1

Summary Mean (± SE) SRP-4053 Toxicokinetic Parameters in Male C57BL/6NCrl Mouse Plasma Following 12 mg/kg IV Bolus Administration of SRP-4053 on Days 1 and 78

Day	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)	$T_{1/2}$ (hr)	C_{max}/D (ng•kg/mL/mg)	$AUC_{(0-t)}/D$ (ng•hr/mL/mg/kg)
1	0.08	39,500 ± 5770	13,500 ± 926	*	3290	1130
78	0.08	30,900 ± 1900	13,200 ± 427	1.68	2580	1100

*Result not reported because extrapolation exceeds 20%, or R-squared is less than 0.800

Text Table 17
Mean (±SE) Toxicokinetic Parameters of SRP-4053 in Plasma

Parameter	Study Day	12 mg/kg/dose	120 mg/kg/dose	960 mg/kg/dose
$AUC_{(0-t)}$ (ng•hr/mL)	1	13,500 ± 926	159,000 ± 14,000	2,390,000 ± 138,000
	78	13,200 ± 427	118,000 ± 29,100	4,270,000 ± 1,450,000
Cmax (ng/mL)	1	39,500 ± 5770	369,000 ± 34,200	3,970,000 ± 309,000
	78	30,900 ± 1900	258,000 ± 209,000	2,970,000 ± 1,470,000

- AUC increased dose proportionally between 12 and 120 mg/kg but more than dose proportionally at the HD.
- V_d, which ranged from 677 to 2120 mL/kg, indicates penetration beyond the vascular space.
- $T_{1/2}$ (plasma) ranged from 0.624 hours to 4.67 hours.

Necropsy:
Terminal procedures are summarized in the table below.

Text Table 11
Terminal Procedures

Group No.	No. of Males	Scheduled Euthanasia Day	Necropsy Procedures			Histology[a]	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	20	79	X	X	X	Full Tissue	Full Tissue[a]
2	20					Full Tissue	Gross Lesions, Target Tissues, Brain, testis and kidneys
3	20					Full Tissue	Gross Lesions, Target Tissues, Brain, testis and kidneys
4	20					Full Tissue	Full Tissue[a]
1	12	106	X	X	X	Full Tissue	Full Tissue[a]
2	12					Full Tissue	Gross Lesions, Target Tissues, Brain, testis and kidneys
3	12					Full Tissue	Gross Lesions, Target Tissues, Brain, testis and kidneys
4	12					Full Tissue	Full Tissue[a]
Unscheduled Deaths			X	X	-	Full Tissue	Full Tissue[a]

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

- Of the surviving mice, no macroscopic lesions were reported at the end of the dosing period or recovery.

Organ weights:

- Increased (absolute and relative) kidney and spleen weights were observed in the HD group (see tables below). The increased kidney weight correlated with microscopic findings of tubular dilatation and basophilic casts. No microscopic correlates for increased spleen weight were reported.
- At the end of recovery, increased kidney weight was observed. Spleen weights were not different from control values. However, increased adrenal weights in the HD group were observed (relative and absolute). The microscopic correlate for the increased weight of adrenal gland was increased incidence of pigmented cells (graded minimal, multifocal, cytoplasmic).

Text Table 3
Summary of Organ Weight Data – Scheduled Euthanasia (Day 79)

	Males		
Group	2	3	4
Dose (mg/kg/dose)	12	120	960
No. Animals per Group	20	17	20
Kidney (No. Weighed)[a]	20	17	20
Absolute value	5	9	**30**
% of body weight	3	3	**39**
% of brain weight	5	9	**31**
Spleen (No. Weighed)	20	17	20
Absolute value	5	12	**20**
% of body weight	2	6	**28**
% of brain weight	5	12	**21**

[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.

Text Table 4
Summary of Organ Weight Data – Scheduled Euthanasia (Day 106)

	Males		
Group	2	3	4
Dose (mg/kg/dose)	12	120	960
No. Animals per Group	12	11	12
Kidney (No. Weighed)[a]	12	11	12
Absolute value	5	3	**14**
% of body weight	7	9	**31**
% of brain weight	5	4	**15**
Adrenal gland (No. Weighed)	12	11	12
Absolute value	14	9	**26**
% of body weight	16	15	**45**
% of brain weight	14	9	**27**

[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.

Histopathology: (Signed and dated Pathology Report is provided.)

Detailed microscopic evaluation was conducted on control and HD groups. Gross lesions and target organs from LD and MD animals were evaluated microscopically. Full microscopic evaluation was also conducted on tissues from main study animals that died prematurely.

Results at the end of dosing:

- Kidney: Basophilic casts graded minimal (MD) to marked (HD), eosinophilic casts (all dosed groups, dose-related in incidence and severity), tubular dilatation (MD and HD), vacuolation of the tubular epithelium (all groups including control, increased severity and incidence in the HD group)
- Ureter: hypertrophy of transitional epithelium (MD and HD)
- Urinary bladder: hypertrophy of transitional epithelium (observed in all dosed groups, 100% incidence at MD and HD).
- Ureter: hypertrophy of the transitional epithelium was observed in MD and HD groups.

Microscopic findings observed at the end of dosing are summarized in the table below.

Text Table 5
Summary of Microscopic Findings – Scheduled Euthanasia (Day 79)

	Males			
Group	1	2	3	4
Dose (mg/kg/dose)	0	12	120	960
No. Animals Examined	20	20	20	20
Kidney (No. Examined)	20	20	20	20
Cast; basophilic (focal/multifocal)	(0)[a]	(0)	(2)	(20)
Minimal	0	0	2	4
Mild	0	0	0	11
Moderate	0	0	0	4
Marked	0	0	0	1
Cast; eosinophilic (focal/multifocal)	(0)	(1)	(3)	(5)
Minimal	0	1	3	5
Dilatation; tubular (focal/multifocal)	(0)	(0)	(2)	(20)
Minimal	0	0	2	4
Mild	0	0	0	11
Moderate	0	0	0	4
Marked	0	0	0	1
Vacuolation; tubular, epithelium (multifocal)	(20)	(17)	(18)	(20)
Minimal	14	16	15	5
Mild	6	1	3	9
Moderate	0	0	0	2
Marked	0	0	0	4
Ureter (No. Examined)	20	20	20	20
Hypertrophy; transitional epithelium	(0)	(0)	(10)	(7)
Minimal	0	0	10	7
Urinary Bladder (No. Examined)	20	20	20	20
Hypertrophy; transitional epithelium, multifocal	(0)	(4)	(20)	(20)
Minimal	0	4	20	20
Site, Injection (No. Examined)	20	20	20	20
Thrombosis; chronic (focal)	(2)	(3)	(1)	(6)
Minimal	2	2	0	5
Mild	0	1	1	1
Hemorrhage; perivascular	(1)	(0)	(0)	(4)
Minimal	1	0	0	2
Mild	0	0	0	2
Infiltration, neutrophilic (perivascular)	(4)	(2)	(2)	(4)
Minimal	4	2	2	4

[a] Numbers in parentheses represent the number of animals with the finding and includes unscheduled deaths during main phase.

Text Table 21
Summary of Microscopic Findings – Scheduled Euthanasia (Day 106)

	Males			
Group	1	2	3	4
Dose (mg/kg/dose)	0	12	120	960
No. Animals Examined	12	12	12	12
Kidney (No. Examined)	12	12	12	12
Cast; basophilic (focal/multifocal)	(0)[a]	(0)	(1)	(12)
Minimal	0	0	1	5
Mild	0	0	0	7
Dilatation; tubular (focal/multifocal)	(0)	(0)	(1)	(12)
Minimal	0	0	1	5
Mild	0	0	0	7
Basophilia; tubular (multifocal)	(0)	(3)	(0)	(10)
Minimal	0	3	0	8
Mild	0	0	0	2

	Males			
Group	1	2	3	4
Dose (mg/kg/dose)	0	12	120	960
No. Animals Examined	12	12	12	12
Ureter (No. Examined)	12	12	11	12
Hypertrophy; transitional epithelium	(0)	(0)	(1)	(2)
Minimal	0	0	1	2
Urinary Bladder (No. Examined)	12	12	12	12
Hypertrophy; transitional epithelium, multifocal	(0)	(0)	(6)	(11)
Minimal	0	0	6	11
Site, Injection (No. Examined)	12	12	12	12
Thrombosis; chronic (focal)	(1)	(4)	(2)	(0)
Minimal	0	2	0	0
Mild	1	2	2	0

[a] Numbers in parentheses represent the number of animals with the finding and includes animal No. 3025 (unscheduled death).

Male reproductive assessments: (sperm motility, concentration, and morphology)

No effects were observed.

Conclusion:
No NOAEL could be determined due to adverse microscopic changes in kidney, ureter, and bladder in the MD and HD groups, and clinical chemistry changes reflecting adverse renal effects at all dose levels.

Study #SR-17-084
Title: SRP-4053: 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: 11/23/2017
Drug/Lot#: SRP-4053/7003295 and 7003066

Methods:
Animals: Mouse, C57BL/6NCrl, males only
Number: see table below
Doses: 0, 120, 300, or 600 mg/kg
Route: SC
Regimen: Weekly
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-4053	120	10	12	20	12	63+20
3	SRP-4053	300	10	30	20	12	63+20
4	SRP-4053	600	10	60	20	12	63+20

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

Observations/Results:
Formulation Analysis: (Samples of dosing formulations were collected on SD1, 85, 169, and 176.)

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

Mortality: (Observations were recorded at least twice daily.)

Three early deaths occurred during the study: One control animal was found dead on SD85, animal #2102 (LD) was euthanized early on SD84, and animal #2105 (LD) was euthanized early on SD162.

Animal #2102 had urogenital skin lesions (graded moderate to severe). Macroscopic examination showed an ulcerated firm mass in the skin with core material described as pale and thick. Also noted was bilateral enlargement of the inguinal lymph nodes. Microscopically, the lesion was described as ulcerated abscess with mild plasmacytosis in the lymph nodes. Pre-terminal clinical signs for animal # 2105 were decreased activity, tremors, cold to touch, labored breathing, and thinness. Macroscopic findings reported were enlarged lymph nodes, pericardial adhesions, and dark focus in the cerebellum as well as small thymus. The macroscopic observations correlated with microscopic findings related to leukemia. The dark focus in the brain was correlated with chronic hemorrhage. Clinical pathology findings at the time of early euthanasia were increases in alkaline phosphatase, sodium, chloride, and urea nitrogen.

Decreases were observed in cholesterol, triglycerides, phosphorus, and glucose. These findings were considered suggestive of dehydration, decreased food intake and cholestasis.

The deaths were not dose-related and are not considered related to the test article.

Clinical observations: (Observations were recorded on day of dosing at 1, 2, 3, and 4 hours post dose. Detailed observations were recorded weekly.)

- No test article-related observations were reported.

Body weight: (Data were recorded weekly beginning 2 weeks prior to initiation of dosing.)

- No test article-related effects on body weight were observed.

Food consumption: (Consumption was measured qualitatively on a weekly basis beginning 7days prior to initiation of dosing.)

- No effects on food consumption were observed.

Ophthalmology: (Testing was conducted on all animals once prior to initiation of dosing and again during the final week of dosing.)

- No effects on ophthalmoscopy parameters were observed.

Clinical Pathology:

(Samples were collected according to the table below.)

Text Table 4
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 27	X	X	X	-
Main animals	Week 26	-	-	-	X
1 to 4	Week 34	X	X	X	-
Recovery animals	Week 33	-	-	-	X
Unscheduled Euthanasia [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 was: Clinical Chemistry, Hematology, based on available volume.

[d] Collected from selected Hormone study animals.

Hematology:
The table below lists the hematology parameters monitored.

Text Table 5
Hematology Parameters

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

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

Clinical Chemistry:
The table below lists the parameters monitored.

Text Table 6
Clinical Chemistry Parameters

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

[a] These parameters were prioritized for analysis.

- No statistically significant effects on clinical chemistry parameters were observed. However, a small trend toward increased UREAN and CK was noted in HD males (+9.9% and 6%, respectively). These trends were no longer noted at the end of the recovery period.

Urinalysis:

Text Table 7
Urinalysis Parameters

Appearance/Clarity	Volume
Specific gravity	pH

- No effects on urinalysis parameters were observed.

Hormones: (FSH, LH, and testosterone)

- A small reduction in testosterone in HD males (not statistically significant) was observed. No other changes in monitored hormones were apparent.

Toxicokinetics:

Results:

Table 2.1: Summary (± SE) SRP-4053 Toxicokinetic Parameters in Male Mouse Plasma Following SC Injection of SRP-4053 on Day 1

Day	Gender	Dose (mg/kg/dose)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	$AUC_{(0-t)}$ (hr*ng/mL)	SE $AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}/D$ (hr*ng/mL/ (mg/kg))	$AUC_{(0-24)}$ (hr*ng/mL)	$AUC_{(0-24)}/D$ (hr*ng/mL/ (mg/kg))
1	Male	120	0.5	97700	17600	132000	7620	1100	132000	1100
		300	0.5	155000	10700	293000	21200	976	293000	975
		600	1	297000	20500	851000	50400	1420	850000	1420

Table 2.2: Summary (± SE) SRP-4053 Toxicokinetic Parameters in Male Mouse Plasma Following SC Injection of SRP-4053 on Day 176

Day	Gender	Dose (mg/kg/dose)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	$AUC_{(0-t)}$ (hr*ng/mL)	SE $AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}/D$ (hr*ng/mL/ (mg/kg))	$AUC_{(0-24)}$ (hr*ng/mL)	$AUC_{(0-24)}/D$ (hr*ng/mL/ (mg/kg))	R_{AUC}
176	Male	120	0.25	64400	3820	138000	8580	1150	136000	1140	1.05
		300	0.5	136000	13900	472000	27300	1570	470000	1570	1.61
		600	1	191000	29300	988000	82300	1650	982000	1640	1.16

R_{AUC} = Day 176 $AUC_{(0-t)}$/ Day 1 $AUC_{(0-t)}$.

Terminal procedures:

Text Table 10
Terminal Procedures for Main Study and Recovery Animals

Group No.	No. of Males	Scheduled Euthanasia Week	Necropsy Procedures			Histology	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	20	Week 27	X	X	X	Full Tissue	Full Tissue [a]
2	20					Full Tissue	Gross lesions Target Tissues
3	20					Full Tissue	Gross lesions Target Tissues
4	20					Full Tissue	Full Tissue
1	12	Week 34	X	X	X	Full Tissue	Full Tissue
2	12					Full Tissue	Gross lesions Target Tissues
3	12					Full Tissue	Gross lesions Target Tissues
4	12					Full Tissue	Full Tissue
Unscheduled Deaths			X	X	-	Full Tissue	Full Tissue
Replaced animals (prestudy)			X	Standard Diagnostic List	-	-	-

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

Text Table 11
Terminal Procedures for Hormone Study Animals

Group No.	No. of Males	Scheduled Euthanasia Week	Necropsy Procedures			Histology[a]	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	10[a]	Week 27	Male reproductive organs only[c]	Testes only	Testes and epididymides only[b]	Testes only	Testes only
2	10[a]					Testes only	Testes only
3	10[a]					Testes only	Testes only
4	10[a]					Testes only	Testes only
1	10	Week 34	Male reproductive organs only[c]	Testes only	Testes and epididymides only[b]	Testes only	Testes only
2	10					Testes only	Testes only
3	10					Testes only	Testes only
4	10					Testes only	Testes only

X = Procedure conducted; - = Not applicable.
[a] First surviving 10 animals.
[b] Weighed separately.
[c] Testes, epididymides, prostate gland and seminal vesicle glands.

Macroscopic observations:

- No macroscopic observations were reported.

Organ weights:

Text Table 12
Organs Weighed at Necropsy

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

[a] Weighed post-fixation
[b] Epididymides and testis were weighted separately for the Hormone Study Animals only

- No organ weight changes related to the test article were observed.

Male reproductive assessment:

- No test article-related macroscopic observations or changes in organ weight were observed in male reproductive organs at the end of the dosing or recovery periods.
- No findings were observed upon microscopic examination of male reproductive organs at the end of the dosing or recovery periods.
- No test article-related effects were observed on sperm motility, concentration, morphology, or spermatogenic cycle.

Histopathology:

Text Table 13
Tissue Collection and Preservation

Animal identification	Kidney
Artery, aorta	Large intestine, cecum
Body cavity, nasal	Large intestine, colon
Bone marrow smear	Large intestine, rectum
Bone marrow	Larynx
Bone, femur	Liver
Bone, sternum	Lung
Brain	Lymph node, mandibular
Epididymis	Lymph node, mesenteric
Esophagus	Muscle, skeletal
Eye[a]	Nerve, optic[a]
Gallbladder	Nerve, sciatic
Gland, adrenal	Pancreas
Gland, harderian	Site, Injection
Gland, lacrimal (ocular accessory gland)	Skin/Subcutis
Gland, mammary	Small intestine, duodenum
Gland, parathyroid	Small intestine, ileum
Gland, pituitary	Small intestine, jejunum
Gland, prostate	Spinal cord
Gland, salivary (mandibular)	Spleen
Gland, salivary (parotid)	Stomach
Gland, salivary (sublingual)	Testis[b]
Gland, seminal vesicle	Thymus
Gland, thyroid	Tongue
Gross lesions/masses	Trachea
Gut-associated lymphoid tissue	Urinary bladder
Heart	

[a] Preserved in Davidson's fixative. (for exception, see Appendix 1).
[b] Preserved in Modified Davidson's fixative.

At the end of the dosing period, microscopic findings were observed in kidney and urinary bladder.

Kidney: Dose-related (incidence and severity) effects on kidney tubules (regeneration/degeneration) were observed in all dose groups at the end of the dosing period.

Urinary bladder: Degeneration of the transitional epithelium, dose-related in incidence and severity, was observed in all dose groups. At the HD, all animals showed these changes.

Histopathology findings in kidney and urinary bladder are summarized in the tables below:

Text Table 17
Summary of Microscopic Findings – Terminal Euthanasia (Week 27)

	Males			
Group	1	2	3	4
Dose (mg/kg/dose)	0	120	300	600
No. Animals Examined	19	20	20	20
Kidney (No. Examined)	19	20	20	20
Degeneration/regeneration, tubular	(0)[a]	(3)	(3)	(15)
Minimal	0	2	2	4
Mild	0	1	1	9
Moderate	0	0	0	2
Urinary bladder (No. Examined)	17	20	20	20
Degeneration, transitional epithelium	(0)	(19)	(16)	(20)
Minimal	0	17	16	19
Mild	0	2	0	1

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

At the end of recovery, the microscopic findings listed above persisted but with reduced severity.

Text Table 18
Summary of Microscopic Findings – Recovery Euthanasia (Week 34)

	Males			
Group	1	2	3	4
Dose (mg/kg/dose)	0	120	300	600
No. Animals Examined	12	10	12	12
Kidney (No. Examined)	12	10	12	12
Degeneration/regeneration, tubular	(0)[a]	(1)	(4)	(3)
Minimal	0	1	3	3
Urinary bladder (No. Examined)	12	10	12	12
Degeneration, transitional epithelium	(0)	(0)	(1)	(11)
Minimal	0	0	1	11

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

Conclusions:
The NOAEL for this study was determined to be the LD of 120 mg/kg due to kidney histopathology that correlated with clinical chemistry trends demonstrating effects on renal function.

Study #4053-tox-005
Title: A 23-week intravenous injection toxicity and toxicokinetic study of SRP-4053 in the male mouse with an 8-week recovery period

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation: June 14, 2016
Drug/Lot#/Purity: SRP-4053/7002670/93%

Methods:

Animals: Mouse, C57BL/6NCrl, males only
Age/weight: 9 weeks old, 20.3 to 28.8 g at initiation of dosing
Number: 20/group for main study 12/group for recovery (satellite groups were designated for TK and hormone evaluations)
Doses:0, 60, 120, 300, or 600 mg/kg
Route: IV
Regimen: Once per week
Study design:
This study was originally planned as a 26-week chronic study. However, due to the increasing number of partial or missed doses as the study proceeded, the decision was made to stop the study after 23 doses. Difficulty in dosing was encountered in all dose groups, but the onset of dosing difficulty appeared to be dose-related. Partial and missed doses began with dose 14 for the HD, dose 15 for the LMD and HMD, and dose 17 for the LD.

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-4053	60	10	6	20	12	63+20 [c]
3	SRP-4053	120	10	12	20	12	63+20 [c]
4	SRP-4053	300	10	30	20	12	63+20 [c]
5	SRP-4053	600	10	60	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 2-5), 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.

Observations/Results:
Formulation Analysis:
(Formulation samples were collected prior to dosing on SD1, 85, and 155.)

On SD85, the dosing samples for the LD and HD groups were slightly out of specification (-10.2 and -11.7% of nominal). The small deviation for a single dose did not have had a significant effect on the total dose received by the LD and HD groups over the course of the study and did result in an adverse effect on the validity of the study.

Mortality: (Data were collected twice daily.)
Multiple unscheduled deaths occurred during the study in all groups including control. No observations (macroscopic or microscopic were recorded that could explain the continuing difficulty with dosing.

The table below summarizes the early deaths and cause of death when it was determined.

Text Table 22
Individual Causes of Death

Animal No.	Dose level (mg/kg/dose)	Sex	Study Day	Cohort	Found Dead/ Unscheduled Euthanasia	Comment
1012	0	Male	29	Main Study	Found Dead	Cause of death: undetermined
1013	0	Male	57	Main Study	Found Dead	Cause of death: undetermined
1039	0	Male	148	Hormone Study	Found Dead	Cause of death: undetermined
2002	60	Male	75	Main Study	Found Dead	Cause of death: moderate seminal vesicle gland inflammation
2097	60	Male	146	Hormone Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
2104	60	Male	5	Hormone Study	Found Dead	Cause of death: undetermined
3005	120	Male	146	Main Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
3021	120	Male	146	Recovery Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
3036	120	Male	146	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
3038	120	Male	146	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
3051	120	Male	145	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
3061	120	Male	106	Toxicokinetics	Found Dead	Cause of death: undetermined
3065	120	Male	144	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
3109	120	Male	145	Hormone Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
4010	300	Male	113	Main Study	Found Dead	Cause of death: undetermined
4024	300	Male	52	Recovery Study	Unscheduled Euthanasia	Cause of death: marked skin ulceration in the urogenital area
4044	300	Male	130	Toxicokinetics	Unscheduled Euthanasia	Cause of death: undetermined
4052	300	Male	145	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
4056	300	Male	8	Toxicokinetics	Found Dead	Cause of death: undetermined
4111	300	Male	124	Hormone Study	Found Dead	Cause of death: undetermined
4115	300	Male	144	Hormone Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5003	600	Male	146	Main Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5004	600	Male	115	Main Study	Found Dead	Cause of death: undetermined
5006	600	Male	64	Main Study	Found Dead	Cause of death: undetermined
5021	600	Male	146	Recovery Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5027	600	Male	77	Recovery Study	Unscheduled Euthanasia	Cause of death: undetermined
5034	600	Male	146	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses

Animal No.	Dose level (mg/kg/dose)	Sex	Study Day	Cohort	Found Dead/ Unscheduled Euthanasia	Comment
5040	600	Male	146	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5047	600	Male	145	Toxicokinetics	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5056	600	Male	8	Toxicokinetics	Found Dead	Cause of death: undetermined
5097	600	Male	146	Hormone Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5100	600	Male	146	Hormone Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses
5108	600	Male	145	Hormone Study	Unscheduled Euthanasia	Cause of death: >4 missed consecutive doses

Clinical signs: (Observations were recorded daily with detailed observations recorded weekly beginning one week prior to initiation of dosing.)

- A dose-related increase in incidence and severity of skin findings was reported, described as thickening, skin flaking/dry, red/blue skin, and/or swelling at the injection site in animals from all dose groups, except control. No microscopic correlates for the skin findings were reported, and the findings had generally subsided by the end of the recovery period.

Body weight: (Data were recorded weekly prior to dosing.)

- No effects on body weight or body weight gain were reported.

Food consumption: (Qualitatively estimated weekly.)

- No significant effects on food consumption related to SRP-4053 were observed.

Ophthalmology: (Testing was conducted on main-study and recovery animals once prior to initiation of dosing and during week 23.)

- No effects related to SRP-4053 were observed.

Clinical Pathology: (Samples were collected according to the table below.)

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 5[b]	Week 24/25	X	X	X	-
Main animals	Week 23/24	-	-	-	X
1 to 5	Day 225[e]	X	X	X	-
Recovery animals	Week 32	-	-	-	X
Unscheduled Euthanasia (When Possible)[c]	Before Euthanasia	X	X	-	-

X = Sample to be 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.
[e] See Appendix 1 for exceptions

Hematology: (A standard battery of hematological parameters were monitored.)

- Reduced neutrophil counts (graded minimal) were observed in HD animals (mean = -33%) at the end of the dosing period but was similar to control at the end of the recovery period.

Clinical Chemistry: (A standard battery of clinical chemistry parameters were monitored.

- No SRP-4053-related effects on clinical chemistry were observed.

Urinalysis: (Urine was tested for appearance, volume, specific gravity, and pH.)

- No effects related to SRP-4053 on urinalysis parameters were observed.

Hormones: (Blood samples from 10 animals /group were collected at scheduled euthanasia.) The following hormones were measured:

Text Table 11
Hormone Parameters

Testosterone Follicle-stimulating hormone	Luteinizing hormone

- No SRP-4053-related effects were observed on levels of LH or FSH.
- A trend described as increased pulsatility (individual high values) of testosterone was observed at all dose levels but was not observed during the recovery period. No microscopic correlate for the effects on testosterone was observed.

Toxicokinetics:

Text Table 23
Summary of Selected Toxicokinetic Parameters

Parameter	Occasion	Group 2 60 mg/kg Males	Group 3 120 mg/kg Males	Group 4 300 mg/kg Males	Group 5 600 mg/kg Males
C_{max} (ng/mL)	Day 1	161000	661000	1590000	2150000
	Day 155 (Dose 23)	267000	627000	113000	2900000
$AUC_{(0-t)}$ (ng*hr/mL)	Day 1	73000	223000	618000	1120000
	Day 155 (Dose 23)	95600	261000	477000	833000
$AUC_{(0-t)}$ /Dose (ng*hr/mL/(mg/kg))	Day 1	1220	1860	2060	1860
	Day 155 (Dose 23)	1590	2170	1590	1390
T ½ (hr)	Day 1	NRR	0.634	7.87	NRR
	Day 155 (Dose 23)	NRR	NRR	0.522	0.152
CL (mL/hr/kg)	Day 1	NRR	537	485	NRR
	Day 155 (Dose 23)	NRR	NRR	601	16.7
Vd (mL/kg)	Day 1	NRR	491	5510	NRR
	Day 155 (Dose 23)	NRR	NRR	452	3.66

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

Terminal Procedures: (Signed, dated Pathology Report is provided)
Main study animals (20/group) were euthanized during weeks 24 and 25. Recovery animals (12/group) were euthanized on SD 225 (week 32). Full necropsy was conducted on all animals. A standard battery of tissues was collected, but histopathology was limited to gross lesions, brain, injection sites, urinary bladder, testis, and kidneys (as summarized in the table below).

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 24/25	X	X	X	Full Tissue	Full Tissue[a]
2	20					Full Tissue	Gross Lesions, Brain, injection sites, urinary bladder, testis and kidneys
3	20					Full Tissue	Gross Lesions, Brain, injection sites, urinary bladder, testis and kidneys
4	20					Full Tissue	Gross Lesions, Brain, injection sites, urinary bladder, testis and kidneys
5	20					Full Tissue	Full Tissue[a]
1	12	Day 225 (for exceptions, see Appendix 1)	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	Gross Lesions, Brain, testis and kidneys
5	12					Full Tissue	Full Tissue[a]
Unscheduled Deaths			X	X	-	Full Tissue	Full Tissue[a]
Replaced animals (prestudy)			X	Standard Diagnostic List	-	-	-
Replaced animals (after dosing start) (for exceptions, see Appendix 1)			X	X	-	-	-

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

Animals designated for hormone investigations were euthanized and processed according to the schedule below.

Text Table 16
Terminal Procedures for Hormone Study Animals

Group No.	No. of Males	**Scheduled Euthanasia Week/Day**	Necropsy Procedures			Histology[a]	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	10[a]	Week 24/25	Male reproductive organs only[c]	Testes only	Testes and epididymides only[b]	Testes only	Testes only
2	10[a]					Testes only	Testes only
3	10[a]					Testes only	Testes only
4	10[a]					Testes only	Testes only
5	10[a]					Testes only	Testes only
1	10	Day 225	Male reproductive organs only[c]	Testes only	Testes and epididymides only[b]	Testes only	Testes only
2	10					Testes only	Testes only
3	10					Testes only	Testes only
4	10					Testes only	Testes only
5	10[a]					Testes only	Testes only

X = Procedure to be conducted; - = Not applicable.
[a] First surviving 10 animals.
[b] Weighed separately.
[c] Testes, epididymides, prostate gland and seminal vesicle glands (for exceptions, see Appendix 1).

Macroscopic observations

- No macroscopic findings related to SRP-4053 were observed.

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.

Male reproductive assessments:

- No SRP-4053-related effects on sperm evaluations (motility, concentration, and morphology) were observed.

Spermatogenesis staging: Analysis was conducted on section of testes from control and HD terminal sacrifice hormone study groups.

- No microscopic findings were observed in examination of the testis, and there was no evidence of abnormalities in the spermatogenic cycle.

Histopathology:
At the end of the dosing period, test article-related effects were observed in kidney and urinary bladder at all dose levels of SRP-4053.

Text Table 24
Summary of Microscopic Findings – Scheduled Euthanasia (Week 24/25)

	Males				
Group	1	2	3	4	5
Dose (mg/kg/dose)	0	60	120	300	600
No. Animals Examined	18	19	19	19	17
Kidney (No. Examined)	(18)	(19)	(19)	(19)	(17)
Cast	(0)[a]	(1)	(1)	(14)	(16)
Minimal	0	1	1	14	9
Mild	0	0	0	0	7
Degeneration/regeneration; tubular	(0)	(0)	(0)	(12)	(14)
Minimal	0	0	0	12	8
Mild	0	0	0	0	6

	Males				
Group	1	2	3	4	5
Dose (mg/kg/dose)	0	60	120	300	600
No. Animals Examined	18	19	19	19	17
Urinary bladder (No. Examined)	(18)	(19)	(19)	(19)	(17)
Degeneration; transitional epithelium	(0)	(10)	(12)	(16)	(17)
Minimal	0	10	12	12	11
Mild	0	0	0	4	6

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

- The adverse effects summarized above increased with dose, in incidence and severity, and were typically observed bilaterally in the kidney. The affected tubules were randomly distributed in the renal cortex.
- Similar findings were observed in kidney and urinary bladder at the end of the recovery period, with a slight decrease in incidence.

Text Table 25
Summary of Microscopic Findings – Scheduled Euthanasia (Day 225)

	Males				
Group	1	2	3	4	5
Dose (mg/kg/dose)	0	60	120	300	600
No. Animals Examined	12	12	11	11	10
Kidney (No. Examined)	(12)	(12)	(11)	(11)	(10)
Cast	(0)[a]	(1)	(0)	(1)	(6)
Minimal	0	1	0	1	5
Mild	0	0	0	0	1
Degeneration/regeneration; tubular	(0)	(0)	(2)	(2)	(6)
Minimal	0	0	2	2	5
Mild	0	0	0	0	1
Urinary bladder (No. Examined)	(12)	(0)	(0)	(1)	(10)
Degeneration; transitional epithelium	(0)	-	-	(0)	(3)
Minimal	0	-	-	0	3

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

Conclusion:
Due to the kidney findings (and lack of recovery), no NOAEL was determined. With the dosing difficulties, it is not possible to clearly assess how much drug each animal received. Therefore, toxicities may be underestimated.

Study #4053-tox-001
Title: 12-week intravenous injection toxicity and toxicokinetic study with SRP-4053 in sexually mature male cynomolgus monkeys with a 4 week recovery

Testing Facility: (b) (4)

GLP Compliance: Yes.
Date of Study Initiation: 10/2013
Drug/Lot #: SRP-4053/7001122 and 7001257

Methods:
Animals: cynomolgus monkey, male, 6/group plus 3/group for recovery
Age: 5 to 6 years old
Weight: 5.2 to 8.9 kg
Vehicle: Dulbecco's PBS
Route of administration: IV
Doses: 0, 5, 40, or 320 mg/kg
Regimen/Duration: weekly for 12 weeks (13 total doses)

Text Table 3
Experimental Design

Group No.	Test Item	Dosage Level (mg/kg)	Dosage Volume (mL/kg)	Dosage Concentration (mg/mL)	Number of Animals	
					Main Study	Recovery Study
1	Control	0	3.2	0	6	3
2	SRP-4053	5	3.2	1.56	6	3
3	SRP-4053	40	3.2	12.5	6	3
4	SRP-4053	320	3.2	100	6	3

Observations/Results:

Formulation analysis: All formulation samples were determined to be within the pre-determined acceptance criteria (± 10%) for concentration.

Mortality: (Observations made twice daily.)
- All animals survived to scheduled euthanasia.

Clinical observations: (Observations were recorded at least once daily, detailed observations were recorded weekly; on dosing days, animals were observed regularly throughout the day after completion of dose administration.)

- No test article-related clinical observations were reported.

Body weight: (Data were collected weekly starting 3 weeks prior to initiation of dosing.)

- No dose-related effects on body weight were observed.

Food Consumption: (Daily visual check was conducted.) Not reported.

Ophthalmology: (Testing was conducted once prior to initiation of dosing and once during week 11.)

- No test article-related effects were observed.

ECG: (Testing was conducted once prior to initiation of dosing, twice during week 11, and once during the last week of recovery.)

- 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

Predose Phase		
Timepoint	Week -1	Week -2
Clinical Pathology		
Haematology	X	X
Coagulation	X	X
Clinical Chemistry	X	X
Urinalysis and Urine Chemistry		X

	Dosing Phase						Recovery Phase
Days	2	9	23	30[a]	79	80	Day 107[b]
Clinical Pathology							
Haematology				X		X	X
Coagulation				X		X	X
Clinical Chemistry				X		X	X
Plasma Creatinine (a separate sample)	X	X	X		X		
Urinalysis and Urine Chemistry	X	X	X	X	X		X

x = sample collected
[a] blood samples taken ca +24h post dose given on Day 29.
[b] 29 days after last dose

Hematology: (Standard battery of parameters was monitored.)

- No test article-related effects were observed.

Coagulation:

Text Table 6
Coagulation Parameters

Activated partial thromboplastin time	Prothrombin time	Fibrinogen

- No test article-related effects were observed.

Clinical chemistry: (Standard battery was conducted.)
- No test article-related effects were observed.

Urinalysis:

Text Table 8
Urinalysis Parameters

Microscopic evaluation of spun deposit Colour Turbidity Specific gravity Volume pH Protein	Glucose Bilirubin Ketones Leukocytes Blood Pigments Urobilinogen

- No effects on urinalysis parameters were observed.

Complement activation: (Samples were collected once prior to initiation of dosing and on SDs 1, 8, and 78 at 15 minutes, 1 hour, and 4 hours post dosing. Final samples were collected on SD 113.)

Text Table 10
Complement Factor Parameters

Parameter	Number of aliquots	Volume of plasma	Volume of Futhan (500 µg/mL)
C3a des Arg	3	45 µL	5 µL
C5a des Arg	3	405 µL	45 µL
sC5b-9	3	50 µL	-
Bb	3	100 µL	-

- No changes in Bb, C3a or C5a were noted in the LD group. (One LD animal showed increased C3a at 15 minutes post-dose).
- On SD 8, the MD and HD groups showed increases in Bb, relative to baseline, peaking at 15 minutes to 1 hour post dose and returned to baseline after 4 hours. The magnitude of the increases did not appear to be dose-related, but the incidence was greater in the HD group.
- No effects on Bb levels were noted on SD 78 (near the end of dosing) or during recovery.

Text Table 6
Incidence and Magnitude of Bb fragment Changes Compared to the Overall Baseline (Males).

Male Bb	SRP-4053					
	5 mg/kg/dose		40 mg/kg/dose		320 mg/kg/dose	
	Inc	Fold	Inc	Fold	Inc	Fold
Pre-Study	0/9	-	0/9	-	0/9	-
D1 predose	0/9	-	0/9	-	0/9	-
D1 15 min. postdose	0/9	-	0/9	-	0/9	-
D1 1 hour postdose	0/9	-	0/9	-	0/9	-
D1 4 hour postdose	0/9	-	1/9	1.9	0/9	-
D8 predose	0/9	-	0/9	-	0/9	-
D8 15 min. postdose	0/9	-	3/9	2.2-3.7	5/9	1.9-3.2
D8 1 hour postdose	0/9	-	2/9	4.0-4.1	4/9	1.9-2.1
D8 4 hour postdose	0/9	-	2/9	2.8-3.0	2/9	1.9-2.1
D78 predose	0/9	-	0/8	-	0/9	-
D78 15 min. postdose	0/9	-	0/9	-	0/7	-
D78 1 hour postdose	0/9	-	0/9	-	0/9	-
D78 4 hour postdose	0/9	-	0/9	-	0/9	-
Recovery	0/3	-	0/3	-	0/3	-

Inc = Incidence.
Fold = Fold increase above overall prestudy baseline mean + 2 SD (2225.75 ng/mL).

- Increases (relative to baseline and control) in C3a were observed on SD 8 in the MD and HD groups, which peaked at 15 minutes post dose and returned to baseline within 4 hours. The magnitude and incidence were dose-related. On SD 78, a single HD animal showed a sharp increase. No effects on C3a were observed during the recovery period.

Text Table 7
Incidence and Magnitude of C3a Changes Compared to the Overall Baseline (Males).

Male C3a	SRP-4053					
	5 mg/kg/dose		40 mg/kg/dose		320 mg/kg/dose	
	Inc	Fold	Inc	Fold	Inc	Fold
Pre-study	0/9	-	0/9	-	0/9	-
D1 predose	0/9	-	0/9	-	0/9	-
D1 15 min. postdose	0/9	-	0/9	-	1/9	5.3
D1 1 hour postdose	0/9	-	0/9	-	0/9	-
D1 4 hour postdose	0/9	-	0/9	-	0/9	-
D8 predose	1/9	38.9	0/9	-	0/9	-
D8 15 min. postdose	0/9	-	3/9	5.8-8	6/9	5.8-12.0
D8 1 hour postdose	0/9	-	0/9	-	1/9	5.2
D8 4 hour postdose	0/9	-	0/9	-	0/9	-
D78 predose	0/9	-	0/9	-	0/9	-
D78 15 min. postdose	0/9	-	0/9	-	0/9	-
D78 1 hour postdose	0/9	-	0/9	-	1/9	96.8
D78 4 hour postdose	0/9	-	0/9	-	0/9	-
Recovery	0/3	-	0/3	-	0/3	-

Inc = Incidence.
Fold = fold increase above overall prestudy baseline mean + 2 SD (21238.87 pg/mL).

- Increased C5a was observed in one MD animal 15 minutes post-dose on SD 78 and in 3 HD animals at 1 hour post-dose on SD 78.

Text Table 8
Incidence and Magnitude of C5a Changes Compared to the Overall Baseline (Males)

Male C5a	SRP-4053					
	5 mg/kg/dose		40 mg/kg/dose		320 mg/kg/dose	
	Inc	Fold	Inc	Fold	Inc	Fold
Pre-study	0/9	-	0/9	-	0/9	-
D1 predose	0/9	-	0/9	-	0/9	-
D1 15 min. postdose	0/9	-	0/9	-	0/9	-
D1 1 hour postdose	0/9	-	0/9	-	0/9	-
D1 4 hour postdose	0/9	-	0/9	-	0/9	-
D8 predose	0/9	-	0/9	-	0/9	-
D8 15 min. postdose	0/9	-	0/9	-	0/9	-
D8 1 hour postdose	0/9	-	0/9	-	0/9	-
D8 4 hour postdose	0/9	-	0/9	-	0/9	-
D78 predose	0/9	-	0/9	-	1/9	2.2
D78 15 min. postdose	0/9	-	1/9	2.4	0/9	-
D78 1 hour postdose	0/9	-	0/9	-	2/9	2.7-2.8
D78 4 hour postdose	0/9	-	0/9	-	1/9	2.5
Recovery	0/3	-	0/3	-	0/3	-

Inc = Incidence.
Fold = Fold increase above overall prestudy baseline mean + 2 SD (8.00 ng/mL).

Toxicokinetics:

TK Results:

Text Table 18
Summary Mean (± SD) SRP-4053 Toxicokinetic Parameters in Male Monkey Plasma Following 5 mg/kg, 40 mg/kg, and 320 mg/kg IV Bolus Administration of SRP-4053 on Day 1

Dose (mg/kg)	C0 (ng/mL)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)	$AUC_{(0-48h)}$ (ng•hr/mL)	$AUC_{(0.25-48h)}$ (ng•hr/mL)
5	57000 ± 15500	26600 ± 7660	31700 ± 10100	31800 ± 10100	21400 ± 7800
40	398000 ± 74000	188000 ± 17700	233000 ± 27800	233000 ± 27800	160000 ± 24100
320	4050000 ± 2170000	1580000 ± 475000	1900000 ± 676000	1900000 ± 676000	1190000 ± 524000

Text Table 19
Summary Mean (± SD) SRP-4053 Toxicokinetic Parameters in Male Monkey Plasma Following 5 mg/kg, 40 mg/kg, and 320 mg/kg IV Bolus Administration of SRP-4053 on Day 78

Dose (mg/kg)	C0 (ng/mL)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)	$AUC_{(0-48h)}$ (ng•hr/mL)	$AUC_{(0.25-48h)}$ (ng•hr/mL)
5	52100 ± 10100	25600 ± 5190	33500 ± 8740	33600 ± 8770	23900 ± 7610
40	485000 ± 106000	200000 ± 36700	230000 ± 41400	230000 ± 41300	144000 ± 32500
320	3250000 ± 693000	1790000 ± 495000	2550000 ± 764000	2550000 ± 764000	1910000 ± 683000

Testicular size: (Measurement of each testis was taken in all animals twice prior to initiation of dosing, once during week 11, and once during the last week of recovery.)

- Testicular volume was increased in HD animals at the end of the dosing period relative to control (+26%). No microscopic correlate was identified.

Sperm analysis: (Sperm samples were collected 3 times prior to initiation of dosing, during week 6 and week 11, and during the last week of recovery.)

- No dose-related effects on sperm count, motility, or abnormal sperm, and no differences in pH, color, or volume of semen were observed among groups.

Hormone analysis: (Blood was collected from all animals for hormone analysis: testosterone, FSH and LH. Samples were collected once prior to initiation of dosing, during weeks 6 and 11, and during the last week of recovery.)

- On SD 80, decreases in LH concentrations were observed in all dose groups but were statistically significant in the MD and HD groups (-33% relative to baseline and -27% relative to control).
- Decreases in FSH (-46% relative to baseline and 52% relative to control) in the MD and HD groups were observed on SD 80.
- No test article-related effects on testosterone levels were observed.
- No effects were noted on hormones at the end of the recovery period.

Summary of Hormones : Day 80

Group 1 - Control
Group 2 - SRP-4053 5 mg/kg
Group 3 - SRP-4053 40 mg/kg
Group 4 - SRP-4053 320 mg/kg

Group / Sex		Testos ng/dL	LH ng/mL	FSH ng/mL
1M	Mean	697	1.1	19.7
	SD	282	0.3	2.9
	N	9	9	9
2M	Mean	702	0.8	15.4
	SD	377	0.1	6.3
	N	9	9	9
3M	Mean	750	0.8b	10.2b
	SD	452	0.1	2.5
	N	9	9	9
4M	Mean	741	0.8a	9.5b
	SD	421	0.0	1.7
	N	9	9	9

Significantly different from control group 1 value :a=p≤0.05,b=p≤0.01,c=p≤0.001 (Dunn)

Necropsy: (All animals received complete necropsy.)

Text Table 13
Terminal Procedures

Group Number	Number of Animals	Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology[a]
			Necropsy	Tissue Collection	Organ Weights		
1	6	80	X	X	X	Full Tissue	Full Tissue
2	6					Full Tissue	Full Tissue
3	6					Full Tissue	Full Tissue
4	6					Full Tissue	Full Tissue
1	3	117	X	X	X	Full Tissue	Full Tissue
2	3					Full Tissue	Full Tissue
3	3					Full Tissue	Full Tissue
4	3					Full Tissue	Full Tissue

X = procedure conducted
[a] See Tissue Collection and Preservation table for listing of tissues.

Macroscopic Findings:

- No macroscopic dose-related findings were observed.

Organ weights:

Text Table 14
Organs Weighed at Necropsy

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

[a] Paired organ weight.

Testicular weights are summarized below:

Text Table 20
Summary Group Mean Organ Weight Data – Scheduled Euthanasia (Day 80)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**5**	**40**	**320**
No. animals per group	**6**	**6**	**6**	**6**
Testis (No. of pairs weighed)	6	6	6	6
Absolute value (g)	32.92	24.71	31.79	50.98
% of body weight	0.4478	0.3984	0.4834	0.6726
% of brain weight	44.0706	35.2073	45.1477	71.8375

- Increase in testicular weight was observed in HD males (50%). This increase in testicular weight was not observed at the end of the recovery period.

Histopathology:
(A full battery was examined for all animals. Peer review was conducted. Signed, dated Pathology Report was provided.)

- Diffuse follicular cell hypertrophy in the thyroid gland in one HD animal (graded minimal). This finding was considered related to the test article and was not observed at the end of the recovery period.
- One of 6 HD animals showed mild capsular fibrosis of the liver. This finding was not observed at the end of recovery.
- Kidney: One of 6 HD animals showed tubular cysts (graded minimal). This finding was observed in one control animal at the end of recovery.
- No microscopic correlates were observed that would account for the increased testicular weight.

Conclusion:
The NOAEL for this study is the MD of 40 mg/kg due to changes in hormone levels and microscopic findings in thyroid and liver at the HD.

Study #SR-15-036
Title: 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:	September 6, 2017
Drug/Lot#/Purity:	SRP-4053/7003064, 7003066, and 7700417/93%

Methods:
Animals: Cynomolgus monkeys, males only
Age/weight: 5-7 years old, 5.2 to 9.5 kg
Number: 6/group for main study, 3/groups for recovery
Doses: 0, 80, 200, or 400 mg/kg
Route of administration: IV, 30-minute infusion
Regimen: Weekly
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-4053	80	3.2	25	6.4	6	3
3	SRP-4053	200	3.2	62.5	6.4	6	3
4	SRP-4053	400	3.2	125	6.4	6	3

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

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 260	All groups	N/A	dosing container

N/A = not applicable.

- All formulation samples were found to be within the pre-established acceptance criteria for all parameters.

Mortality: (Observations were recorded twice daily.)
- No unscheduled deaths occurred.

Clinical observations: (Observations were recorded once daily on dosing days, 10 to 20 minutes after end of infusion. Detailed observations were recorded at least weekly during the dosing period.)
- No test article-related observations were reported.

Body weight: (Data were recorded at least every 2 weeks prior to initiation of dosing and weekly thereafter.)
- No significant test article-related effect on body weight was observed.

Food consumption: (Data were recorded daily.)
- No test article-related effects were noted.

Ophthalmoscopy: (Testing was conducted once prior to initiation of dosing and once during week 38 of dosing.)
- No test article-related effects were observed.

ECG: (Data were recorded once prior to initiation of dosing and during weeks 1 and 38 of the dosing period at 1 to 4 hours post dose.)

- No test article-related effects were observed.

Reproductive assessments: (Semen samples were collected prior to initiation of dosing, during weeks 13, 26, and 38 of the dosing period, and once near the end of the recovery period during week 46.)

- No effects related to SRP-053 were observed on sperm motility, morphology, or count. Spermatogenic cycle assessment did not demonstrate any effects related to SRP-053.

Clinical Pathology

Sample collection schedule is summarized in the table 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:

Text Table 5
Hematology Parameters

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

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

Coagulation:

Text Table 6
Coagulation Parameters

Activated partial thromboplastin time Fibrinogen	Prothrombin time Sample Quality

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

Clinical chemistry:

Text Table 7
Clinical Chemistry Parameters

Alanine aminotransferase	Total protein
Aspartate aminotransferase	Albumin
Alkaline phosphatase	Globulin
Gamma-glutamyltransferase	Albumin/globulin ratio
Creatine Kinase	Glucose
Total bilirubin	Cholesterol
Urea nitrogen	Triglycerides
Creatinine	Sodium
Calcium	Potassium
Phosphorus	Chloride
	Sample Quality

- Increased UREA N and CREAT were observed in HD males during weeks 13 and 38 (graded mild to moderate). Results are summarized in the tables below. These findings were no longer observed at the end of the recovery period.

Text Table 20
SRP-4053-related Clinical Chemistry Changes

Group	1	2	3	4
Dose (mg/kg)	0	80	200	400
Sex	M	M	M	M
UREAN				
Week 13	–	–	–	1.40x (3/9)
Week 26	–	–	–	–
Week 38	–	–	–	1.39x
Week 46	–	–	–	–

Group	1	2	3	4
Dose (mg/kg)	0	80	200	400
Sex	M	M	M	M
CREAT				
Week 13	–	–	–	1.67x (4/9)
Week 26	–	–	–	–
Week 38	–	–	–	1.54x
Week 46	–	–	–	–

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:

Text Table 8
Urinalysis Parameters

Color Appearance/Clarity Specific gravity Volume pH	Glucose[a] Bilirubin Ketones Blood

[a] Semi-quantitative measurement

Text Table 9
Urine Chemistry Parameters

Protein[a] Creatinine Protein/Creatinine ratio

[a] Quantitative measurement

Results:

- During week 38, increased glucose and blood (+3) were observed in the urine of 1 HD male.
- Also noted in HD males was increased CREAT, UREA, and protein/creatinine ratio (4.02X).
- These findings correlated with microscopic kidney findings observed at the end of the dosing period.
- Findings were no longer observed at the end of the recovery period.

Hormones:

Text Table 10
Hormone Parameters

Testosterone (Testos) Follicle-stimulating hormone (FSH) Luteinizing hormone (LH)

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

Toxicokinetics:

Results:

Text Table 21
Mean Toxicokinetic Parameters of SRP-4053

Parameter	Period	Dose of SRP-4053 (mg/kg)		
		80	200	400
$AUC_{(0-t)}$ (hr*µg/mL)	Day 1	410	1050	2760
	Day 85	653	1440	4100
	Day 260	836	1620	4280
C_{max} (µg/mL)	Day 1	327	722	1470
	Day 85	512	1170	2160
	Day 260	571	1250	2600
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).

Terminal Procedures: Signed and Dated Pathology Report is provided.

Text Table 14
Terminal Procedures

Group No.	No. of Animals Males	Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	6	268	X	X	X	Full Tissue[a]	Full Tissue[a]
2	6					Full Tissue[a]	Full Tissue[a]
3	6					Full Tissue[a]	Full Tissue[a]
4	6					Full Tissue[a]	Full Tissue[a]
1	3	323	X	X	X	Full Tissue[a]	Full Tissue[a]
2	3					Full Tissue[a]	Full Tissue[a]
3	3					Full Tissue[a]	Full Tissue[a]
4	3					Full Tissue[a]	Full Tissue[a]

X = Procedure conducted
[a] See Tissue Collection and Preservation table for listing of tissues.

Macroscopic observations:

- No macroscopic findings related to the test article were observed at the end of dosing or recovery.

Organ weights:

Text Table 15
Organs Weighed at Necropsy

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

[a] Paired organ weight.

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

Histopathology:

Tissues collected are listed in the table below.

Text Table 16
Tissue Collection and Preservation

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Bone marrow smear	Large intestine, rectum
Bone marrow	Liver
Bone, femur	Lung
Bone, sternum	Lymph node, mandibular
Brain	Lymph node, mesenteric
Epididymis	Small intestine, duodenum
Esophagus	Small intestine, ileum
Eye[a]	Small intestine, jejunum
Gallbladder	Muscle, skeletal
Gland, adrenal	Nerve, optic[a]
Gland, mammary	Nerve, sciatic
Gland, parathyroid	Pancreas
Gland, pituitary	Site, Infusion
Gland, prostate	Skin
Gland, salivary	Spinal cord
Gland, seminal vesicle	Spleen
Gland, thyroid	Stomach
Gross lesions/masses	Testis[b]
Gut-associated lymphoid tissue	Thymus
Heart	Tongue
Kidney	Trachea
	Urinary bladder

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

Histopathology Results:

- SRP-4053-related adverse findings were observed in kidney and bone. The findings, summarized in the tables below, are described as basophilia of the tubular epithelium in all dose groups, dose-related in incidence and severity, and dilatation of the distal convoluted tubules and collecting ducts, microvesicular vacuolation of the distal convoluted tubule and collecting ducts, and inflammation and synovial hyperplasia in the MD and HD groups.
- Findings in the kidney correlated with changes in clinical chemistry at the HD and are considered adverse.

Text Table 22
Summary of Microscopic Findings – Scheduled Euthanasia (Day 268)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**80**	**200**	**400**
No. Animals per Group	**6**	**6**	**6**	**6**
Kidney (No. Examined)	6	6	6	6
Basophilic granules; proximal convoluted tubule	(0)[a]	(4)	(6)	(6)
Minimal	0	4	3	3
Mild	0	0	3	3
Basophilia; distal convoluted tubule; collecting duct	(0)	(1)	(5)	(6)
Minimal	0	1	4	3
Mild	0	0	1	3
Dilatation; distal convoluted tubule; collecting duct	(0)	(0)	(2)	(4)
Minimal	0	0	2	3
Mild	0	0	0	1
Vacuolation; microvesicular; distal convoluted tubule; collecting duct	(0)	(0)	(4)	(5)
Minimal	0	0	4	2
Mild	0	0	0	2
Moderate	0	0	0	1
Inflammation, vascular/perivascular	(0)	(0)	(1)	(0)
Moderate	0	0	1	0
Infiltration, mononuclear cell	(1)	(0)	(0)	(5)
Minimal	1	0	0	3
Mild	0	0	0	2
Liver (No. Examined)	6	6	6	6
Basophilic granules; Kupffer cell	(0)	(0)	(1)	(5)
Minimal	0	0	1	4
Mild	0	0	0	1
Inflammation, vascular/perivascular	(0)	(0)	(1)	(0)
Mild	0	0	1	0

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**80**	**200**	**400**
No. Animals per Group	**6**	**6**	**6**	**6**
Bone, femur (No. Examined)	6	6	6	6
Hyperplasia; synovium	(0)	(0)	(1)	(5)
Minimal	0	0	1	2
Mild	0	0	0	3
Inflammation; synovium	(0)	(0)	(3)	(5)
Minimal	0	0	1	4
Mild	0	0	2	1
Heart (No. Examined)	6	6	6	6
Inflammation, mononuclear cell	(0)	(1)	(1)	(2)
Minimal	0	0	0	1
Mild	0	1	0	0
Moderate	0	0	1	1
Parasitism	(0)	(0)	(1)	(0)
Present	0	0	1	0
Inflammation, vascular/perivascular	(0)	(0)	(1)	(0)
Minimal	0	0	1	0
Esophagus (No. Examined)	6	6	6	6
Degeneration/regeneration; myofiber	(0)	(0)	(0)	(1)
Minimal	0	0	0	1
Infiltration, mononuclear cell; myofiber	(0)	(0)	(0)	(1)
Mild	0	0	0	1
Gallbladder (No. Examined)	6	6	6	6
Inflammation, vascular/perivascular	(0)	(0)	(1)	(0)
Minimal	0	0	1	0
Muscle, skeletal (No. Examined)	6	6	6	6
Degeneration/regeneration	(0)	(0)	(0)	(1)
Minimal	0	0	0	1
Infiltration, mononuclear cell	(0)	(0)	(0)	(1)
Mild	0	0	0	1
Site, infusion (No. Examined)	6	6	6	6
Degeneration/regeneration; myofiber	(0)	(0)	(0)	(1)
Moderate	0	0	0	1
Tongue (No. Examined)	6	6	6	6
Degeneration/regeneration; myofiber	(0)	(0)	(0)	(1)
Minimal	0	0	0	1
Infiltration, mononuclear cell; myofiber	(0)	(0)	(0)	(1)
Mild	0	0	0	1
Large intestine, colon (No. Examined)	6	6	6	6
Inflammation, vascular/perivascular	(0)	(0)	(1)	(0)
Minimal	0	0	1	0
Stomach (No. Examined)	6	6	6	6
Infiltration, mononuclear cell; smooth muscle	(0)	(0)	(0)	(1)
Mild	0	0	0	1
Inflammation, vascular/perivascular	(0)	(0)	(1)	(0)
Mild	0	0	1	0

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

Histopathology at the end of recovery:

Text Table 23
Summary of Microscopic Findings – Scheduled Euthanasia (Day 323)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**80**	**200**	**400**
No. Animals per Group	**3**	**3**	**3**	**3**
Kidney (No. Examined)	3	3	3	3
Basophilic granules; distal convoluted tubule, loop of Henle	(0)	(2)	(2)	(2)
Minimal	0	2	2	1
Mild	0	0	0	1
Basophilia; distal convoluted tubule; collecting duct	(0)	(0)	(0)	(2)
Minimal	0	0	0	2
Dilatation; distal convoluted tubule; collecting duct	(0)	(1)	(0)	(1)
Minimal	0	1	0	1
Vacuolation; microvesicular; distal convoluted tubule; collecting duct	(0)	(0)	(0)	(3)
Minimal	0	0	0	3

- Findings in the kidney persisted through recovery, but a trend toward recovery was observed in incidence.
- Bone findings were not observed at the end of recovery.

Conclusions:

The NOAEL was determined to be the LD of 80 mg/kg.

Study #SR-16-019

Title: A 13-week intravenous injection toxicity study of SRP-4053 in the male mouse with a 4-week recovery period (Impurities qualification, (b) (4))

Testing Facility: (b) (4)

GLP Compliance: Yes.
Date of Study Initiation: 1/17/2017
Drug/ Lot #/purity: SRP-4053/RD-027-080, LY01/94%

Methods:
Species: Mouse, C57BL/6NCrl, male
Age/weight: 8.5 to 9.5 weeks old, 21.7 to 28.7g
Number: 20/group, 12/group recovery
Doses: 0, 60, 120, 300, or 600 mg/kg (Control, LD, LMD, HMD, and HD, respectively)
Route: IV slow bolus
Regimen: Daily for 13 doses
Study Design:

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 Study[a]
1	Vehicle control	0	10	0	20	12	3
2	SRP-4053	60	10	6	20	12	30
3	SRP-4053	120	10	12	20	12	30
4	SRP-4053	300	10	30	20	12	30
5	SRP-4053	600	10	60	20	12	30

[a] Toxicokinetic animals will be used for toxicokinetic evaluation only.

Observations/Results:

Formulation analysis: All samples were determined to be within the pre-established acceptability limits for all parameters.

Text Table 3
Dose Formulation Sample Collection Schedule

Interval	Concentration	Stability	pH, Osmolality and Density	Sampling From
Day 1	All groups	Dosing container	All groups	Dosing container
Day 36	All groups	Dosing container	N/A	Dosing container
Day 85	All groups	Dosing container	N/A	Dosing container

Mortality: (Observations were recorded twice daily.)
Two unscheduled deaths occurred during the study. Animal #2015 (LD) was euthanized due to poor and deteriorating condition on SD 35. The cause of death was "multicentric lymphoma" in brain, liver, spleen, and bone marrow. Animal #4018 (HMD) was found dead on SD 64. No cause of death was identified. Histopathology showed no findings, and there were no clinical signs of deteriorating health prior to death. Because the death was in the MD group, the sponsor did not consider this death related to the test article.

Clinical observations: (Observations were recorded once daily. On dosing days, observations were also recorded 1-2 hours post dose. Detailed clinical signs were recorded weekly.)

- Increased tail skin dryness was reported in the HD group. No other test article-related observations were recorded.

Food consumption: (Data were recorded weekly.)

- No effects on food consumption were reported.

Body weight: (Data were recorded weekly.)

- No effects on body weight related to the test article were observed.

Ophthalmoscopy: (Conducted once prior to initiation of dosing and again during week 13 of dosing.)

- No effects related to the test article were observed.

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

Text Table 5
Samples for Clinical Pathology Evaluation Main and Recovery Animals

Group Nos.	Time Point	Hematology[a]	Clinical Chemistry[a]	Urinalysis
1 to 5[b]	Day 92	X	X	-
Main animals	Week 13	-	-	X
1 to 5	Day 113	X	X	-
Recovery animals	Week 16	-	-	X
Unscheduled Euthanasia		-	X	-

X = Sample to be 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.

Hematology:

Text Table 6
Hematology Parameters

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

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

Clinical chemistry:

Text Table 7
Clinical Chemistry Parameters

Urea nitrogen	Globulin
Total protein[b]	Albumin/globulin ratio
Alkaline phosphatase[b]	Calcium
Chloride[b]	Cholesterol
Creatinine[b]	Glucose
Alanine aminotransferase[b]	Inorganic Phosphorus
Creatine kinase[b]	Potassium
Triglycerides[b]	Sodium
Albumin[b]	Total bilirubin
Gamma-glutamyltransferase	Sample Quality

[b] These parameters were prioritized for analysis (for exceptions see Appendix 1).

- In HD males, a small increase in UREAN was observed, which was graded mild (1.19X) at the end of the dosing period. No effect was observed at the end of recovery.
- Small increases in globulin levels (graded mild) were observed in the HMD and HD groups at the end of the dosing period. No microscopic correlate was identified.

Urinalysis:

Text Table 8
Urinalysis Parameters

Appearance (Clarity and color) Volume	Specific gravity pH

- At the HMD and HD, increases in urine volume and decreases in specific gravity (graded mild) were observed. These changes correlated with microscopic findings of renal tubular lesions. No differences in urinalysis parameters were observed at the end of the recovery period.
- These findings indicate a reduced ability to concentrate urine and were correlated with renal histopathology finding of tubular lesions.

Toxicokinetics:

TK results are summarized in the following table:

Text Table 18
Summary of Selected Toxicokinetic Parameters

Parameter	Occasion	Group 2 60 mg/kg Males	Group 3 120 mg/kg Males	Group 4 300 mg/kg Males	Group 5 600 mg/kg Males
C_{max} (ng/mL)	Day 1	196000	426000	1180000	2680000
$AUC_{(0-t)}$ (ng*hr/mL)	Day 1	718000	164000	520000	1190000
$AUC_{(0-t)}$ /Dose (ng*hr/mL/(mg/kg))	Day 1	1200	1370	1730	1980
T ½ (hr)	Day 1	0.393	0.558	7.73	13.7
CL (mL/hr/kg)	Day 1	835	732	577	506
Vd (mL/kg)	Day 1	474	589	6430	10000

Terminal procedures: Signed and dated Pathology Report is provided

Macroscopic observations:
No gross findings related to the test article were observed.

Text Table 12
Terminal Procedures for Main Study and Recovery Animals

Group No.	No. of Animals M	Scheduled Euthanasia Day	Necropsy Procedures			Histology	Histopathology
			Necropsy	Tissue Collection	Organ Weights		
1	20	92	X	X	X	Full Tissue	Full Tissue[a]
2	20					Full Tissue	Gross Lesions Brain, urinary bladder, testis and kidneys
3	20					Full Tissue	Gross Lesions Brain, urinary bladder, testis and kidneys
4	20					Full Tissue	Gross Lesions Brain, urinary bladder, testis and kidneys
5	20					Full Tissue	Full Tissue[a]
1	12	113	X	X	X	Full Tissue	Full Tissue[a]
2	12					Full Tissue	Gross Lesions Brain, urinary bladder, testis and kidneys
3	12					Full Tissue	Gross Lesions Brain, urinary bladder, testis and kidneys
4	12					Full Tissue	Gross Lesions Brain, urinary bladder, testis and kidneys
5	12					Full Tissue	Full Tissue[a]
Unscheduled Deaths			X	X	-	Full Tissue	Full Tissue[a]

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

Organ weights:

Text Table 13
Organs Weighed at Necropsy

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

[a] Paired organ weight.

- Increased kidney weight was observed in the HMD and HD groups at the end of the dosing period. The increased weight correlated with microscopic findings of tubular microvesicular vacuolation, degeneration/necrosis, and casts.

Text Table 19
Summary of Organ Weight Data – Scheduled Euthanasia (Day 92)

	Males			
Group	**2**	**3**	**4**	**5**
Dose (mg/kg/dose)	**60**	**120**	**300**	**600**
No. Animals per Group	**20**	**20**	**20**	**20**
Kidney (No. Weighed)[a]	19	20	19	20
Absolute value	-1	2	**10**	**12**
% of body weight	1	2	6	7
% of brain weight	-1	3	**9**	**12**

[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.

Histopathology:

Text Table 14
Tissue Collection and Preservation

Animal identification	Large intestine, cecum
Artery, aorta	Large intestine, colon
Body cavity, nasal	Large intestine, rectum
Bone marrow smear	Larynx
Bone marrow	Liver
Bone, femur	Lung
Bone, sternum	Lymph node, mandibular
Brain	Lymph node, mesenteric
Epididymis	Muscle, skeletal
Esophagus	Nerve, optic[a]
Eye[a]	Nerve, sciatic
Gallbladder	Pancreas
Gland, adrenal	Site, Injection
Gland, harderian	Skin/Subcutis
Gland, lacrimal (ocular accessory gland)	Small intestine, duodenum
Gland, mammary gland	Small intestine, ileum
Gland, parathyroid	Small intestine, jejunum
Gland, pituitary	Spinal cord
Gland, prostate	Spleen
Gland, salivary (mandibular, parotid and sublingual)	Stomach
Gland, seminal vesicle	Testis[b]
Gland, thyroid	Thymus
Gross lesions/masses	Tongue
Gut-associated lymphoid tissue	Trachea
Heart	Ureter
Kidney	Urinary bladder

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

Results:

- Dose-related increase (incidence and severity) of tubular microvesicular vacuolation was observed in the LMD, HMD, and HD groups (graded minimal to mild). Also observed in the same groups were tubular casts (graded minimal to mild) and degeneration/necrosis of the tubular epithelium.
- Degeneration of the transitional epithelium of the urinary bladder (graded minimal to mild) was observed in all groups, except control.

Text Table 20
Summary of Microscopic Findings – Scheduled Euthanasia (Day 92)

	Males				
Group	1	2	3	4	5
Dose (mg/kg/dose)	0	60	120	300	600
No. Animals Examined	20	19	20	19	20
Kidney (No. Examined)	20	19	20	19	20
Vacuolation, microvesicular	(0)[a]	(0)	(5)	(12)	(19)
Minimal	0	0	5	12	13
Mild	0	0	0	0	6
Cast	(0)	(0)	(3)	(9)	(19)
Minimal	0	0	3	9	16
Mild	0	0	0	0	3
Degeneration/necrosis, tubular	(0)	(0)	(2)	(3)	(14)
Minimal	0	0	2	3	13
Mild	0	0	0	0	1
Urinary bladder (No. Examined)	20	19	20	19	20
Degeneration, transitional epithelium	(0)	(12)	(14)	(17)	(19)
Minimal	0	12	14	14	14
Mild	0	0	0	3	5

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

Conclusion:

Due to the adverse urinary tract (bladder and kidney) microscopic findings, no NOAEL was determined.

Study title: SRP-4053: A 13-week intravenous toxicity and toxicokinetic study in male Sprague-Dawley rats

Study no.:	SR-17-005
Testing Facility:	(b) (4)
Date of study initiation:	4/18/2017
GLP compliance:	Yes.
QA statement:	Provided
Drug, lot #, and % purity:	SRP-4053/7003064/93%

Methods

Doses:	0, 60, 100, 300, or 600 mg/kg (Control, LD, LMD, HMD, and HD, respectively)
Frequency of dosing:	Weekly, for 13 weeks
Route of administration:	IV
Dose volume:	10 mL/kg
Formulation/Vehicle:	PBS
Species/Strain:	Rat, Sprague-Dawley, males only
Number/Sex/Group:	10/group
Age:	6 weeks old at arrival
Weight:	219 to 250 grams at randomization
Unique study design:	

Table A. Study Design

Group Number	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Male Animals
Main Study				
1	0	10	0	10
2	60	10	6	10
3	100	10	10	10
4	300	10	30	10
5	600	10	60	10
Toxicokinetic				
6	0	10	0	3
7	60	10	6	6
8	100	10	10	6
9	300	10	30	6
10	600	10	60	6

Observations and Results

Mortality: (Observations were recorded twice daily)

No unscheduled deaths occurred.

Clinical Observations: (Data were recorded once daily. On dosing days, observations were recorded 1 hour post dose.)

- No test article-related effects were observed.

Body Weights: (Data were recorded at randomization and weekly thereafter.)

- A small reduction in mean body weight (-2%) and mean body weight gain (-3%) was observed at the HD (graded minimal) on SD91.

Food Consumption: (Data were recorded weekly.)

- A dose-related decrease in food intake was observed for all dose groups, relative to control. Data are summarized in the table below.

Table F. Summary of Mean Total Food Consumption

Dose Level (mg/kg)	0	60	100	300	600
Food Consumption (g/animal)[a]	393.2	387.8	382.2	378.8	374.5
% of control	NA	-1%	-3%	-4%	-5%

NA-Not applicable
[a]Total of mean weekly food consumption values.

Ophthalmoscopy: Testing was conducted once prior to initiation of dosing and once at the end of dosing.)

- No test article-related effects were observed.

Clinical Pathology: (Testing was conducted on all main study animals prior to necropsy.)

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

- A small reduction in RET (graded mild) was observed in HD animals (-19%)
- Increase in "other cells" (not defined) in all dose groups relative to control.

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

- Increased urea/nitrogen in HD group (graded mild) correlated with kidney histopathology (+37%).

Gross Pathology: A signed and dated Pathology Report was provided.

Necropsy was conducted on all main study animals at the end of the dosing period.

Organ Weights

- A dose-related increase in kidney weight (absolute and relative) was observed in the HD group (+47%).
- HD animals showed a decrease in thymic weight (absolute and relative), which the sponsor considered due to stress and not related to the test article. Because the effect is clearly dose-related, a relationship to the test article cannot be ruled out.

Histopathology: A full battery of tissues was examined microscopically.

Histological Findings

Test article-related findings were observed in multiple organs (kidney, lymph nodes, lung, testes, epididymides, cecum, stomach, and duodenum.

Kidney findings are listed in the table below.

Table G. Test Article-related Kidney Microscopic Observations - Terminal Male

Dose level: mg/kg	0	60	100	300	600
Number Examined	10	10	10	10	10
Kidneys					
basophilic granules, tubular cell	0	4	8	10	10
-minimal	0	4	8	0	0
-mild	0	0	0	10	6
-moderate	0	0	0	0	4
cast, tubular	0	0	0	0	10
-minimal	0	0	0	0	6
-mild	0	0	0	0	4
degeneration, tubular	0	0	5	10	10
-minimal	0	0	5	9	1
-mild	0	0	0	1	5
-moderate	0	0	0	0	4
dilation, tubular	0	0	0	4	9
-minimal	0	0	0	4	1
-mild	0	0	0	0	5
-moderate	0	0	0	0	2
-marked	0	0	0	0	1
hyaline, droplets, increased	0	1	2	2	2
-minimal	0	1	1	2	2
-mild	0	0	1	0	0
regeneration, tubular	0	1	1	1	10
-minimal	0	1	1	1	7
-mild	0	0	0	0	3

Macrophages containing basophilic granules were observed in multiple tissues in the HD group (epididymides, lung, lymph nodes, duodenum, stomach, testes. Lymphoid cell depletion was noted in the thymus of the HD and HMD groups.

Toxicokinetics: (Samples were collected from 3 TK animals/group at 0.083, 0.5, 1, 4, 8, and 24 hours post dose on SDs 1 and 85. Samples were collected from control TK animals at 0.083 hours post dose on SDs 1 and 85.)

MPI Research Study Number 1152-087
SRP-4053: A 13-Week Intravenous Toxicity and Toxicokinetic Study in Male Sprague-Dawley Rats

Table 1: SRP-4053 Toxicokinetic Parameters on Days 1 and 85 Following Weekly IV Injection of 60, 100, 300, and 600 mg/kg SRP-4053 to Male Rats

Dose (mg/kg)	Day	C_0 (ng/mL)	C_0/Dose (kg*ng/mL/mg)	C_{max} (ng/mL)	T_{last} (hr)	AUC_{Tlast} (hr*ng/mL)	AUC_{0-24hr} (hr*ng/mL)	AUC_{0-24hr}/Dose (hr*kg*ng/mL/mg)	AUC_{INF} (hr*ng/mL)	AUC_{INF}/Dose (hr*kg*ng/mL/mg)	R[a]	$T_{1/2}$ (hr)
60	1	190000	3170	145000	8	84900	85100	1420	84900	1420	NA	0.642
60	85	288000	4800	214000	8	126000	126000	2110	NA	NA	1.48	0.679
100	1	315000	3150	299000	24	255000	255000	2550	NA[b]	NA	NA	NA[b]
100	85	531000	5310	388000	24	226000	226000	2260	NA	NA	0.886	NA[b]
300	1	1300000	4330	971000	24	739000	739000	2460	NA[b]	NA	NA	NA[b]
300	85	1940000	6450	1410000	24	933000	933000	3110	NA	NA	1.26	NA[b]
600	1	2720000	4530	2010000	24	3030000	3030000	5060	NA[b]	NA	NA	NA[b]
600	85	2630000	4380	1930000	24	1930000	1930000	3220	NA	NA	0.638	5.24

NA - Not applicable
a: $R = AUC_{0-24hr\ Day\ 85}/AUC_{0-24hr\ Day\ 1}$
b: Secondary parameters ($T_{1/2}$ and AUC_{INF}) not reported due to insufficient plasma-concentration time data and/or multiphasic elimination

Dosing Solution Analysis

Table B. Dosing Formulation Analysis Sample Collection

Sample Type	Dose Concentration Sampled (mg/mL)	Stratum	Number of Samples per Concentration: Collected	Analyzed	Backup	Sample Volume (mL)	Intervals (Days)
Stock Formulation Concentration[a, b]	110	Middle	4	2	2	0.5	-7, 29, 57
Dose Formulation Concentration[a]	0, 6, 10, 30, 60	Middle	4	2	2	0.5	-3, 82

[a]The samples, including backup samples, were stored refrigerated at 2 to 8°C pending analyses or final disposition.
[b]The average of the two results from the stock formulation analysis was used in the dose formulation preparation calculations.

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

Conclusion:

The NOAEL is the LD of 60 mg/kg due to the adverse effects on kidney.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study # 4053-gtx-001

Title: SRP-4053: Mutagenic activity study with *salmonella typhimurium* TA 1535, TA 100, TA 1537, TA 98 and *Escherichia coli* WP2*urv*A (ICH with in-house dose solution analysis)

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation: 11/12/2013
Drug/Lot#: SRP-4053/7001257

Methods:

Test strains: *S. typhimurium* TA 1535, TA 100, TA 1537, TA 98, and *Escherichia coli* WP2*uvr*A

Concentrations used in definitive study:
5, 17, 50, 167, 500, 1667, and 5000 µg per plate of SRP-4053 in the presence and absence of S9 mix. S9 enzymes from the liver of Aroclor 1254-treated adult male Fischer rats were characterized by testing with *S. typhimurium* TA 1538.

Basis of concentration selection: Absence of cytotoxicity at test article concentrations up to 5000 µg per plate.

Negative controls: Vehicle (Dulbecco's PBS without calcium or magnesium)

Positive controls:

With S9 mix: 2-aminoanthracene (2AAN): 2 µg per plate with *S. typhimurium* TA 1535 and TA 1537, 0.5 µg per plate with *S. typhimurium* TA 98 and TA 100, and 20 µg per plate with *E. coli* WP2*uvr*A

Without S9: Sodium azide (NaN_3): 1µg per plate with *S. typhimurium* TA 1535, TA 100
9-Aminoacridine (9-AA): 80 per plate with *S. typhimurium* TA 1537
2-Nitrofluorene (2-NF): 1µg per plate with *S. typhimurium* TA 98
N-Ethyl-N-nitro-N-nitrosoguanidine (ENNG): 2 µg per plate with *E. coli* WP2*uvr*A

Incubation and sampling times: 3 days at 37 degrees C.

Study validity:
The study design was in compliance with relevant guidelines. However, analysis of dose formulations demonstrated that the 0.05 and16.7 target concentrations were out of specification (-10.2 and -20.4%, respectively).

Because the highest concentration (50 mg/mL/5000 µg per plate) was within acceptance criteria and showed no mutagenic effect, the samples shown to be outside the acceptance criteria had no impact on the validity of the study.

Results:
No mutagenic effect was observed at any concentration with or without metabolic activation. Vehicle control values were within historical ranges for the testing facility. The positive controls values were within the historical control values for the testing facility. No toxicity to the bacterial strains was observed. No test item precipitation was observed.

Conclusion: Criteria for a valid study were met. SRP-4053 showed no mutagenic activity with or without metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Title: SRP-4053: *in vitro* Mammalian chromosome aberration test in Chinese hamster ovary cell cultures

Testing Facility and location:	(b) (4)
Date of study initiation:	12/9/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	SRP-4053/ 7001257

Methods

Cell line: Chinese hamster ovary cells (CHO 10 B_4)

Concentrations used in definitive study: 31.3, 62.5, 125, 250, and 500 µg/mL

Basis of concentration selection: results of preliminary test (Test 1) in which 9 concentrations were tested.

Negative controls: Vehicle (Dulbecco's PBS)

Positive controls:
With S9 mix: cyclophosphamide (concentration range of 20-50 µg/mL)
Without S9 mix: Methyl methanesulphonate (concentration range of 10-40 µg/mL)

Incubation and sampling times:

Treatment schedule is summarized in the table below, from the study report.

S9 Mix	Cultures Established	Test	Treatment Period	Recovery Period	Colcemid	Harvest
Presence of S9 mix	20-24 h before exposure	Tests 1 and 2	0-6 h	6-22 h	22-24 h	24 h
Absence of S9 mix		Test 1 only				
		Test 2 only	0-22 h	None	22-24 h	24 h
				22-46 h	46-48 h	48 h

Study validity:
All criteria for study validity were met.

Results:
The concentrations of all formulation samples were within the acceptance criteria except one. The concentrations of the 25 mg/mL samples from Test 1, with S9 were slightly lower than target (-12.5%). This small deviation had no impact on the study.

No cytotoxicity was observed in any culture up to 500 µg/mL of test item.
The sensitivity of the test was confirmed by induction of aberrations in the positive control cultures at frequencies consistent with historical data.

Vehicle control cultures were negative and within the 95% limits of historical negative control data.

Levels of structural aberrations were consistent with negative control data. No clastogenicity was observed.

Conclusions:
SRP-4053 was not clastogenic when incubated with CHO cells in vitro.

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

Study #: 4053-gtx-003
Title: SRP-4053: Mammalian erythrocyte micronucleus test in mouse bone marrow: 0 and 24 h intravenous dosing and 48 h sampling

Testing Facility and location: (b) (4)

Date of study initiation: 11/21/2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #/purity: SRP-4053/7001122/87.8%

Methods:

Species: CD-1 mouse, male

Doses used in definitive study: 0, 500, 1000, or 2000 mg/kg

Basis of dose selection: Dose-range finding preliminary study

Negative controls: Vehicle (Dulbecco's PBS)

Positive controls: cyclophosphamide (50 mg/kg)

Incubation and sampling times: Mice were dosed at 0 and 24 hours, by IV injection. Bone marrow samples were taken 48 hours after the initial 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.

Conclusion
SRP-4053 did not induce micronucleus formation.

8 Carcinogenicity

Carcinogenicity assessment was not conducted.

9 Reproductive and Developmental Toxicology

Study #4053-tox-003
Title: An intravenous dose range-finding toxicity study of SRP-4053

Testing Facility: (b) (4)

GLP Compliance: No
Date of Study Initiation: 11/20/2014
Drug/Lot#: SRP-4053/7001836

Methods:
Animals: Rat (Sprague-Dawley)
Age/weight: PND 14 at start of dosing; 30.9 to 43.7 g
Number/group: 9 males/group
Doses: 0, 100, 300, 600, or 960 mg/kg
Vehicle: PBS
Route: IV bolus
Regimen: Once weekly
Study design:

Text Table 3
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-4053	100	10	10	6 + 3 (1)
3	SRP-4053	300	10	30	6 + 3 (1)
4	SRP-4053	600	10	60	6 + 3 (1)
5	SRP-4053	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, it 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 dosing formulation samples were determined to be within the pre-determined acceptance criteria.

Mortality: (Dams were observed twice daily through the pre-weaning period. Pups were monitored twice daily throughout the study.)

- One HD pup was found dead on PND 29. No adverse signs were observed prior to death. Kidney showed findings similar to those of the other animals in this group. (See below).

Clinical observations: (During the pre-weaning period, dams were observed cageside for clinical signs daily and detailed observations were recorded on days of body weight measurement.)

- No SRP-4053- related clinical observations were reported.

Body weight: (Pups were weighed on PND4, 7, 10, 14, 17, 21, 24, 28, 32, and 35.)

Food Consumption: (Food consumption was not monitored.)

Clinical Pathology: Samples were collected according to the schedule 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[a]	-	X
All groups / 6	Day 36 pp	X	X

X = sample collected; - = not applicable.
[a] By error, control animal 151-5 was bled at Day 16 pp.

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

- No SRP-4053-related effects on hematology parameters were observed.

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

- SRP-4053-related effects were observed in the HMD and HD group that reflect renal toxicity: increase in urea nitrogen in HMD at PND 15 (graded slight), decreased sodium and chloride, increased urea nitrogen, increased creatinine, increased phosphorus, and increased potassium. Increased calcium was observed only at the HD. These findings correlated with microscopic findings in kidney.

Terminal Procedures

Three animals per dose group were euthanized on PND 15 and 6 per group were euthanized on PND 36. Necropsy was conducted on animals euthanized on PND 36. Tissue collection is summarized in the table below. Histopathology was limited to kidney evaluation only.

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
Unscheduled Deaths			X	X	-	Gross Lesions and Kidneys	Gross Lesions and Kidneys
Dosed spares			-	-	-	-	-

X = Procedure conducted; - = Not applicable

Macroscopic observations:
Macroscopic observations related to SRP-4053 were reported for kidney in the HMD and HD groups. Observations were described as enlargement and discoloration of the kidneys, with abnormally firm consistency.

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**	**5**
Kidney (No. Examined)	6	6	6	6	5
Discoloration, pale	0	0	0	3	3
Abnormal consistency, firm	0	0	0	1	4
Enlargement	0	0	0	3	3
Focus, pale	0	0	0	0	2

Histopathology:

- SRP-4053-related microscopic effects were observed in the kidney and are summarized in the table below.

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	5
Kidney (No. Examined)	6	6	6	6	5
Vacuolation, tubular	(0)[a]	(4)	(6)	(6)	(5)
Minimal	0	4	6	3	0
Mild	0	0	0	3	4
Moderate	0	0	0	0	1
Basophilic granules, tubular	(0)	(1)	(6)	(6)	(5)
Minimal	0	1	6	3	0
Mild	0	0	0	3	4
Moderate	0	0	0	0	1
Dilatation, tubular	(0)	(0)	(1)	(5)	(4)
Minimal	0	0	1	1	0
Mild	0	0	0	3	0
Moderate	0	0	0	1	4
Degeneration/regeneration, tubular	(0)	(0)	(3)	(6)	(5)
Minimal	0	0	3	2	2
Mild	0	0	0	4	3

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

Conclusions:

The NOAEL is the LMD of 300 mg/kg. The HD of 960 mg/kg appears exceed an MTD in juvenile animals.

Study #4053-tox-004

Title: A 10-week intravenous juvenile toxicity study of SRP-4053 in male rats with an 8-week recovery period

Testing Facility: (b) (4)
Canada
GLP Compliance: Yes
Date of Study Initiation: January 22, 2015
Drug/Lot #: SRP-4053/7001836 and 7001837

Methods:
Animals: Rat, Sprague-Dawley, males only
Age: PND14 at initiation of dosing
Weight: 21.6 to 47.1 g
Doses: 0, 100, 300, or 900 mg/kg
Route: IV
Regimen: Once weekly
Study design:
Dams were received with 3-day old cross-fostered litters of 8 male pups each. Dosing

was initiated on PND14. Pups were weaned on PND21.Animals were assigned to the following groups as described in the tables below:

Text Table 2
Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Males
1	Vehicle Control	0	10	0	112
2	SRP-4053	100	10	10	140
3	SRP-4053	300	10	30	140
4	SRP-4053	900	10	90	140

Phase I - Toxicology Subsets

Group No.	Test Material	No. of Males	
		Subset A	Subset B
		Main Study	Recovery/Behavior/Reproduction
1	Vehicle Control	32	32
2	SRP-4053	32	32
3	SRP-4053	32	32
4	SRP-4053	32	32

Phase II - Laboratory Investigation Subsets

Group No.	Test Material	No. of Males				
		Subset C	Subset D	Subset E	Subset F	Subset G
		TK		Clinical Pathology	Immunology	
		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-4053	36	8	12	10	10
3	SRP-4053	36	8	12	10	10
4	SRP-4053	36	8	12	10	10

Observations/Results:

Formulation analysis:

Formulation samples were collected as described in the table below.

Text Table 1
Dose Formulation Sample Collection Schedule

Interval	Concentration	pH, Osmolality and Density	Sampling From
First preparation	All groups	All groups	Preparation vessel
Subsequent preparations	All groups	N/A	Dosing container

N/A: Not applicable.

- Several out of specification results for concentration observed. Repeat of the testing with back-up samples demonstrated these formulations to be within specification. Therefore, there were no formulation concentration results that affected the study validity.

Mortality: (Observations were recorded twice daily.)
A total of 31 early deaths occurred across all HD subgroups. In Subgroups A and B, 22 animals were found dead (2 during the recovery period) and 4 were euthanized early due to moribund condition. (One LD animal was found dead during study week 16 and 2 MD animals were found dead during study week 4. No post mortem investigations were conducted for these 3 animals.)

For 13 of the 26 HD animals, no clinical signs were recorded prior to death. For the remaining 13, clinical signs consistent of decreased activity, dehydration, cold to touch, lying on side, reduced muscle tone, labored or shallow breathing, skin pallor, and prostrate. The cause of death was renal toxicity, with histopathology findings of moderate to severe tubular vacuolation, moderate to severe tubular dilatation, mild to moderate basophilic casts, and mild to moderate tubular degeneration/regeneration. These findings correlated with macroscopic observations of enlarged and pale colored kidneys. Other findings that correlated with renal toxicity and uremia were mineralization of the adrenal glands, glandular mucosa of the stomach, tunica media of the aorta, and degeneration of the tunica media of the coronary arteries and arterioles and arterioles of the tongue.

Clinical observations: (Cageside observations were recorded at least once daily. Detailed clinical observations were recorded daily pre-weaning and weekly after weaning {Subsets A and B} and at scheduled euthanasia.)

- Beginning on PND 29 (one day after the third dose), HD animals showed clinical signs of abnormal gait, decreased activity, cold to the touch, suspected dehydration, prostrate position, decreased muscle tone, weakness, piloerection, pallor, and labored breathing. These signs continued up to PND 58 in animals that were found dead or euthanized in moribund condition. Similar clinical signs occurring later in the study were observed in 10 additional HD animals that survived to scheduled necropsy (main study and recovery).

Body weight: (Pups were weighed on PNDs 4, 7, 10, 14, 17, and 21. After weaning, pups were weighed twice weekly from PND 21 to 28 and once weekly thereafter).

- No effects on body weight were observed during the preweaning period. After weaning, reduced body weight and body weight gain were observed in HD animals. Reduced body weight gain was observed for the 3 days following each dose but return to control values for the 4 remaining days until the next dose. During the recovery period, body weight gains were similar to control.

Food consumption: (After weaning, food consumption was measured on a cage-by-cage basis twice weekly from PND 21 to 28, and weekly thereafter.)

The reduced body weight gain in HD animals was correlated with reduced food intake (approximately -10%) during the dosing period. During recovery, HD food intake remained lower than control (-12%).

Physical development: (Pups were observed daily from PND 35 until preputial separation was observed.)

- The day of preputial separation was not affected by SRP-4053.

Neurobehavioral assessment (Subsets A and B):

Functional observation battery: Testing was conducted once at the end of dosing (PND 73 ± 4, Subset B) and at the end of recovery (PND129 ± 4, Subset B).

Testing was conducted on Subset B at the end of dosing and at the end of recovery.

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

- No SRP-4053-related effects were observed on qualitative FOB parameters.

Text Table 5
Quantitative FOB Parameters

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

Grip strength forelimb and hindlimb (Subset B):

- Hindlimb and forelimb grip strength was reduced (graded slight) in HD animals (-11% and 13%, respectively).

Motor activity (Subset B): Testing was conducted once at the end of dosing (PND 73 ± 4), and at the end of recovery (PND129 ± 4).

- A reduction in motor activity (graded slight) was observed in HD animals during the dosing period. At the end of recovery, results for all groups were similar.

Auditory startle: (Testing was conducted on Subset B between PND 49 and PND 62, and during recovery, between PND 113 and PND 127.)

- No effects attributable to the test article were observed.

Cincinnati water maze: (Testing was conducted on Subset A between PND 56 and PND 69 during the dosing period and on Subset B between PND 120 and PND 133 during the recovery period.)

- No effects attributable to the test article were observed.

Clinical Pathology

Samples were collected as described in the table below.

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]	Day 78 pp	X	X	X	X
Groups 1 to 4/Subset B[b]	Day 134 pp	X	-	X	X
Unscheduled euthanasia (when possible)[c]	Before euthanasia	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] Subsets A and B males only; A blood sample for clinical chemistry was collected first followed by a sample for hematology.

Hematology:

Text Table 8
Hematology Parameters

Red blood cell count	Platelet count
Hemoglobin concentration	Mean platelet volume
Hematocrit	White blood cell count
Mean corpuscular volume	Neutrophil count (absolute)
Red blood cell distribution width	Lymphocyte count (absolute)
Mean corpuscular hemoglobin concentration	Monocyte count (absolute)
Mean corpuscular hemoglobin	Eosinophil count (absolute)
Reticulocyte count (absolute and percent)	Basophil count (absolute)

- In surviving HD animals, hematology findings were decreases (graded mild) in RBC, HGB, and HCT (-6 to 9% across dose groups, relative to control).
- Reductions in reticulocyte counts (-24%) were observed in HD animals.
- Increased neutrophils were observed in HD animals, graded mild (+37%) at the end of the dosing period. No effects on hematology parameters were observed in the LD or MD groups.
- At the end of recovery, all hematology values were similar to control values.

Coagulation: (PT and APTT were evaluated.)

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

Clinical Chemistry:

Text Table 10
Clinical Chemistry Parameters

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

[a] When total bilirubin was > 0.5 mg/dL, indirect and direct bilirubin were also measured.
[b] These parameters were prioritized for analysis.

Unscheduled deaths: The 3 HD animals euthanized early (allowing blood collection) showed marked increases in urea nitrogen, creatinine, phosphorus, and potassium. Also noted were decreased chloride and increased RBC, HGB, and HCT. The findings support renal toxicity, with dehydration, as the COD.

- At the end of the dosing period, the HD group showed SRP-4053-related effects indicative of kidney toxicity, i.e., increased urea nitrogen (+291%), creatinine (+175%), phosphorous (+17%), potassium (+9%), and chloride (+2%) and decreased total protein (-4%).
- At the end of the recovery period, similar findings were reported in the HD group but at lower magnitude.

Urinalysis:

Text Table 11
Urinalysis Parameters

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

[a] Semi-quantitative measurement.

4.12.1.6. Urine Chemistry

Text Table 12
Urine Chemistry Parameters

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

[a] Quantitative measurements.

Text Table 13
Urinary Biomarkers

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

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

SRP-4053-related alterations in urinalysis parameters were observed at the HD at the end of the dosing period. These findings were correlated with the dose-related effects on clinical chemistry and with the microscopic kidney findings. Increases in urine volume and trends toward decreased specific gravity were observed, suggesting decreased ability to concentrate urine.

At the MD and HD, urinalysis showed increased protein/creatinine ratio (+33% and 165% at the MD and HD, respectively, relative to control). Decreased creatinine clearance was also observed at the HD (-63%). Reduced Na+ and Cl- in urine (graded mild to moderate (-42% and -49% for the MD and HD groups, respectively) was also observed.

At the end of the recovery period, SRP-4053-related effects persisted at the MD and HD, with little recovery. No effects on urinalysis parameters were observed at the LD.

No SRP-4053-related effects on urinary biomarkers were observed at the end of the dosing period. However, at the end of the recovery period, increased cystatin C/creatinine ratio (+150%) was observed at the HD, suggesting continuing kidney toxicity through the 8-week recovery period.

TDAR:

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 effects related to the test article on TDAR results were observed.

Immunophenotyping: (Samples were collected at scheduled necropsy from Subsets A and B)

- No effects on immunophenotyping were observed.

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

Toxicokinetics:

TK results are summarized in the tables below:

Text Table 24
Summary Mean (± SE) SRP-4053 Toxicokinetic Parameters
Following IV Bolus Administration of SRP-4053 on Day 14 pp

Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24} (ng•hr/mL)	AUC_{0-t} (ng•hr/mL)	$T_{1/2}$ (hr)
100	0.0833	595,000 ± 26,800	338000 ± 20,600	338,000 ± 20,600	11.6
300	0.0833	2,260,000 ± 163,000	1,080,000 ± 42,100	1,080,000 ± 42,100	3.38
900	0.0833	4,830,000 ± 407,000	3,080,000 ± 227,000	3,090,000 ± 227,000	2.70

Text Table 25
Summary Mean (± SE) SRP-4053 Toxicokinetic Parameters
Following IV Bolus Administration of SRP-4053 on Day 77 pp

Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24} (ng•hr/mL)	AUC_{0-t} (ng•hr/mL)	$T_{1/2}$ (hr)	R_{AUC}
100	0.0833	544,000 ± 27,800	198,000 ± 9360	239,000 ± 9390	23.1	0.587
300	0.0833	1,340,000 ± 107,000	684,000 ± 47,700	774,000 ± 47,700	25.6	0.635
900	0.0833	4,620,000 ± 291,000	3,120,000 ± 220,000	3,650,000 ± 245,000	7.07	1.01

[a] R_{AUC} = Day 77 pp AUC_{0-24}/ Day 14 pp AUC_{0-24}.

Terminal Procedures:

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

Group No.	Targeted 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 to be conducted; - = Not applicable.
[a] See Tissue Collection and Preservation table for listing of tissues.
[b] Target tissues (other than kidneys): lungs and injection sites; and potential target tissues: aorta, adrenal gland, heart, stomach, tongue, thymus, urinary bladder.
[c] See Section 4.16.9 Histopathology.

Text Table 20
Terminal Procedures for TK and Clinical Pathology Animals (Subsets C, D and E)

Group No.	Targeted No. of Animals / Males	Scheduled Euthanasia Day	Necropsy Procedures / Necropsy	Tissue Collection	Organ Weights	Histology	Histopathology
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.
[a] Single dose TK males.
[b] Repeat dose TK males.
[c] Single dose clinical pathology males.

Text Table 21
Terminal Procedures for Immunology Animals (Subsets F and G)

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

- = Not applicable.

F0 generation: Dams were euthanized following weaning on PND 21.

For early deaths in the F1 generation (deaths or moribund euthanasia after initiation of dosing), necropsy and collection of tissues was conducted on pups from Subgroups A and B. Early deaths in other subgroups were discarded without further evaluation.

Scheduled euthanasia:
Pups were euthanized and, where applicable, blood was collected.

The table below summarizes the terminal procedures.

Text Table 22
Tissue Collection and Preservation - Toxicology Subsets A and B

Tissue	Weight	Collection	Microscopic Evaluation	Comment
Animal identification	-	X	-	-
Artery, aorta	-	X	X	From thoracic segment.
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X	-	Bone marrow smears (3) collected from the femur at scheduled necropsies only.
Bone marrow, femur	-	X	X	Collected with bone, femur, decalcified before sectioning.
Bone marrow, sternum	-	X	X	Collected with bone, sternum, decalcified before sectioning.
Bone, femur	-	X	X	Right collected: distal end included femoral tibial joint, decalcified before sectioning (for histopathological evaluation) Left: complete femur collected intact for measurements; Wrapped in saline-soaked gauze and plastic wrap and frozen as soon as possible (within approximately 4 hours following euthanasia) in a freezer set to maintain -20°C, for possible future evaluation (euthanized animals only).
Bone, sternum	-	X	X	Decalcified before sectioning.
Bone, lumbar vertebra L5[a], L6[a]	-	X	-	Wrapped in saline-soaked gauze and plastic wrap and frozen as soon as possible (within approximately 4 hours following euthanasia) in a freezer set to maintain -20°C (euthanized animals only).
Brain	X	X	X	7 levels
Epididymis	X[c]	X	X	Paired examination; Fixed in modified Davidson's fluid (euthanized animals only).[b]
Esophagus	-	X	X	-
Eye	-	X	X	Paired examination; Fixed in Davidson's fixative (euthanized animals only).
Gland, adrenal	X	X	X	Paired weight and examination
Gland, harderian	-	X	X	Paired examination
Gland, lacrimal	-	X	X	Only 1 required for examination.
Gland, mammary	-	X	X	Collected with inguinal skin; Examined only when present in the routine section of skin.
Gland, parathyroid	-	X	X	Collected with thyroid.
Gland, pituitary	X	X	X	-
Gland, preputial	-	X	-	-
Gland, prostate	X	X	X	-
Gland, salivary	-	X	X	Submandibular; Only 1 required for examination.
Gland, seminal vesicle	X[c]	X	X	Paired examination[b]. Individual weight with fluid[c].
Gland, thyroid	X	X	X	Paired weight and examination; weight included parathyroid.
Gross lesions/masses	-	X	X	-
Gut-associated lymphoid tissue	-	X	X	Collected with small intestine
Heart	X	X	X	-

Tissue	Weight	Collection	Microscopic Evaluation	Comment
Injection site	-	X	X	Decalcified before sectioning.
Kidney	X	X	X	Paired weight and examination.
Large intestine, cecum	-	X	X	-
Large intestine, colon	-	X	X	-
Large intestine, rectum	-	X	-	-
Liver	X	X	X	Sample of 2 lobes.
Lung	X	X	X	Infused with 10% neutral buffered formalin after weighing (when applicable). All lobes retained. Sample of 2 lobes examined.
Lymph node, mandibular	-	X	X	Only 1 required for examination.
Lymph node, mesenteric	-	X	X	-
Muscle, skeletal	-	X	X	Left biceps femoris.
Nerves, optic	-	X	X	Fixed in Davidson's fixative (euthanized animals only); Examine only if present in the routine section of the eye.
Nerve, sciatic	-	X	X	Only 1 required for examination.
Pancreas	-	X	X	-
Skin	-	X	X	Inguinal; Collected with mammary gland.
Small intestine, duodenum	-	X	X	-
Small intestine, jejunum	-	X	X	-
Small intestine, ileum	-	X	X	-
Spinal cord	-	X	X	Cervical, thoracic, lumbar.
Spleen	X	X	X	-
Stomach	-	X	X	Glandular and nonglandular regions.
Testis	X	X	X	Paired weight and examination; Fixed in Modified Davidson's fixative (euthanized animals only)[b] Individual weight and examination; Paired collection; Fixed in Modified Davidson's fixative (euthanized animals only)[c]
Thymus	X	X	X	-
Tongue	-	X	X	-
Trachea	-	X	X	-
Urinary bladder	-	X	X	-

X = Procedure conducted; - = Not applicable.
[a] Bone cleaned of excess tissue (not scraped).
[b] Subset A animals only.
[c] Subset B animals only.

Male reproductive assessment: sperm evaluations were conducted on Subset B (sperm count, morphology and motility) but not in a stage-aware manner.

- No SRP-4053-related effects on sperm evaluation parameters were observed.

Macroscopic observations:
SRP-4053-related macroscopic observations are summarized in the table below.

Text Table 3
Summary of Gross Pathology Findings - Scheduled Euthanasia (Day 78)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**100**	**300**	**900**
No. Animals Examined	**32**	**32**	**32**	**16**
Artery, Aorta (No. Examined)	32	32	32	16
Thick	0	0	0	2
Gland, Adrenal (No. Examined)	32	32	32	16
Enlargement	0	2	1	8
Kidney (No. Examined)	32	32	32	16
Enlargement	0	0	0	16
Discoloration, mottled	0	0	0	13
Discoloration, pale	0	0	0	2
Focus, raised	0	0	0	6

At the end of the recovery period, macroscopic findings similar to those described at the end of the dosing period were observed.

In the HD, additional macroscopic findings were observed that were not observed at the end of the dosing period. Specifically, findings in the heart described as enlargement, discoloration, and abnormal consistency were observed and are summarized in the table below.

Text Table 27
Summary of Gross Pathology Findings - Scheduled Euthanasia (Day 134 pp)

Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**100**	**300**	**900**
No. Animals Examined	**32**	**31**	**32**	**23**
Artery, Aorta (No. Examined)	32	31	32	23
Abnormal consistency; firm	0	0	0	5
Focus; pale	0	0	0	6
Heart (No. Examined)	32	31	32	23
Focus; pale	0	0	0	1
Mass	0	0	0	1
Dilatation; ventricle	0	0	0	1
Kidney (No. Examined)	32	31	32	23
Enlargement	0	0	0	23
Discoloration, mottled	0	0	0	18
Discoloration, pale	0	0	0	2
Irregular surface	0	0	0	19
Abnormal consistency; firm	0	0	0	3
Adhesion	0	0	0	1
Thick	0	0	0	1

Organ weights:

- SRP-4053-related effects on organ weights are summarized in the table below.

Text Table 28
Summary of Organ Weight Data - Scheduled Euthanasia (Day 78 pp)

Group	2	3	4
Dose (mg/kg)	100	300	900
No. Animals per Group	32	32	16
Adrenal gland (No. Weighed)[a]	32	32	16
Absolute value	1	-7	**26**
% of body weight	-1	-7	**40**
% of brain weight	-1	-7	**26**
Kidney (No. Weighed)	32	32	16
Absolute value	1	5	**147**
% of body weight	-1	4	**175**
% of brain weight	0	5	**146**
Thymus (No. Weighed)	32	32	16
Absolute value	2	9	**-15**
% of body weight	0	10	-5
% of brain weight	1	9	**-15**

[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.

Organ weights at the end of the recovery period.

Text Table 29
Summary of Organ Weight Data - Scheduled Euthanasia (Day 134 pp)

Group	2	3	4
Dose (mg/kg)	100	300	900
No. Animals per Group	31	32	23
Kidney (No. Weighed)	31 32		16
Ab solute value	-2	1	**68**
% of body weight	-2	2	**87**
% of brain weight	-4	-1	**70**
Thymus (No. Weighed)	31 32		16
Ab solute value	-9	**-15**	**-44**
% of body weight	-10	**-14**	**-38**
% of brain weight	-10	**-16**	**-43**

[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.

Bone evaluation:
Femur length and width were measured in all Subset A and B animals. Dual energy X-ray absorptiometry (DXA) was conducted in vivo on Subset B (12 per group).
Bone mineral content (BMC) and bone mineral density (BMD) were assessed (right femur and lumbar spine). Animals from the Control and HD groups were re-scanned at the end of the recovery period.

Bone densitometry: (12 pups/group from Subset B were scanned at the end of the dosing period. The same pups from the control and HD groups were scanned at the end of the recovery period)

DXA:

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

At the end of the dosing period, decreases in area, bone mineral content and bone mineral density were observed in femur and lumbar spine in HD animals.
BMC and BMD in femur was reduced 11 and 9%, respectively, relative to control.
These effects persisted through the recovery period and were considered related to the renal malfunction.

- No effects on bone growth were observed.

Histopathology:
At the end of the dosing period, SRP-4053-related effects were observed in kidney, adrenal, gland, aorta, heart, stomach, tongue, and thymus.

- Kidney findings were largely limited to the HD group.

Histopathology findings are summarized in the tables below:

End of Dosing:

Text Table 30
Summary of Microscopic Findings in Kidney and Urinary Bladder - Scheduled Euthanasia (Day 78 pp)

Group	1	2	3	4
Dose (mg/kg/day)	0	100	300	900
No. Animals Examined	32	32	32	16
Kidney (No. Examined)	32	32	32	16
Cast; basophilic; tubular	(0)[a]	(0)	(1)	(14)
Minimal	0	0	1	0
Mild	0	0	0	13
Moderate	0	0	0	1
Dilatation; tubular (unilateral/bilateral)	(0)	(0)	(7)	(16)
Minimal	0	0	6	0
Mild	0	0	1	0
Moderate	0	0	0	2
Marked	0	0	0	14
Vacuolation; tubular (unilateral/bilateral)	(0)	(18)	(32)	(16)
Minimal	0	18	20	0
Mild	0	0	12	0
Moderate	0	0	0	2
Marked	0	0	0	14
Degeneration/regeneration; tubular (bilateral)	(0)	(1)	(5)	(16)
Minimal	0	0	3	0
Mild	0	1	1	2
Moderate	0	0	1	13
Marked	0	0	0	1
Urinary Bladder (No. Examined)	32	32	32	16
Hypertrophy; transitional epithelium	(0)	(0)	(2)	(16)
Minimal	0	0	2	8
Mild	0	0	0	8

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

End of recovery:

Text Table 32
Summary of Microscopic Findings in Kidneys – Scheduled Euthanasia (Day 134 pp)

Group	1	2	3	4
Dose (mg/kg/day)	0	100	300	900
No. Animals Examined	32	31	32	23
Kidney (No. Examined)	32	31	32	23
Cast; eosinophilic; tubular	(0)[a]	(0)	0	(23)
Minimal	0	0	0	14
Mild	0	0	0	8
Moderate	0	0	0	1
Dilatation; tubular (unilateral/bilateral)	(0)	(0)	(1)	(23)
Minimal	0	0	1	0
Mild	0	0	0	11
Moderate	0	0	0	12
Vacuolation; tubular (unilateral/bilateral)	(0)	(7)	(25)	(23)
Minimal	0	7	25	0
Mild	0	0	0	7
Moderate	0	0	0	15
Marked	0	0	0	1
Degeneration/regeneration; tubular (bilateral)	(0)	(1)	(1)	(23)
Minimal	0	1	1	0
Mild	0	0	0	5
Moderate	0	0	0	11
Marked	0	0	0	7
Fibrosis; capsular	(0)	(0)	(0)	(1)
Mild	0	0	0	1

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

Text Table 33
Summary of Microscopic Findings Non-Urinary tract tissues - Scheduled Euthanasia (Day 134 pp)

Group	1	2	3	4
Dose (mg/kg/day)	0	100	300	900
No. Animals Examined	32	31	32	23
Artery, aorta (No. Examined)	32	31	32	23
Mineralization; tunica media (regionally extensive)	(0)[a]	(0)	(0)	(12)
Minimal	0	0	0	1
Mild	0	0	0	2
Moderate	0	0	0	9
Adrenal Gland (No. Examined)	32	31	32	23
Mineralization	(0)	(0)	(0)	(6)
Minimal	0	0	0	2
Mild	0	0	0	3
Moderate	0	0	0	1
Necrosis	(0)	(0)	(0)	(1)
Mild	0	0	0	1
Heart (No. Examined)	32	31	32	23
Degeneration; coronary artery; tunica media	(0)	(0)	(0)	(10)
Minimal	0	0	0	1
Mild	0	0	0	6
Moderate	0	0	0	3
Degeneration; myofiber	(0)	(0)	(0)	(2)
Mild	0	0	0	1
Moderate	0	0	0	1
Mineralization; aortic arch	(0)	(0)	(0)	(17)
Mild	0	0	0	7
Moderate	0	0	0	8
Marked	0	0	0	2
Thrombosis; atrial	(0)	(0)	(0)	(1)
Moderate	0	0	0	1
Stomach (No. Examined)	32	31	32	23
Mineralization; mucosal; glandular	(0)	(0)	(0)	(20)
Minimal	0	0	0	6
Mild	0	0	0	4
Moderate	0	0	0	10
Tongue (No. Examined)	32	31	32	23
Degeneration; arteriole; tunica media	(0)	(0)	(0)	(11)
Minimal	0	0	0	3
Mild	0	0	0	8
Thymus (No. Examined)	32	31	32	23
Depletion; lymphoid	(0)	(0)	(0)	(12)
Minimal	0	0	0	6
Mild	0	0	0	5
Moderate	0	0	0	1

No significant recovery was observed in kidney findings in HD animals. Findings observed in tissues other than urinary tract are considered by the Sponsor to be secondary to renal impairment. Lymphoid depletion was considered stress-related and persisted in HD animals through the recovery period. The renal impairment was

considered irreversible in the HD group. The few findings reported in the MD group at the end of the dosing period were not observed at the end of the recovery period.

Conclusions:
The NOAEL was the LD of 100 mg/kg due to renal toxicity at higher doses.

11 Integrated Summary and Safety Evaluation

SRP-4053 (golodirsen) is a 25-mer phosphorodiamidate morpholino oligomer (PMO) antisense drug developed for use in the treatment of Duchennne muscular dystrophy (DMD). SRP-4053 is designed to hybridize to the pre-mRNA transcript of the dystrophin gene, causing deletion of exon 53 from the mature mRNA. This process is expected to give rise to a shortened, but functional, dystrophin protein.

Proof of concept for the exon-skipping mechanism was demonstrated in studies originally submitted and reviewed under IND 77429 and NDA 206488 (EXONDYS). These *in vivo* studies were conducted using a murine surrogate PMO, AVI-4225, in the *mdx* mouse, an animal model of DMD. In this model, 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. A dose-related decrease in incidence and severity of myopathy-related myofiber degeneration in skeletal muscle were observed, relative to controls, at all doses.

Specificity of SRP-4053 was evaluated in Study #SR-18-066 in which multiple available bioinformatics tools were used to assess binding affinity of SRP-4053 for potential off-target sites in the human genome. No evidence of potential off-target binding sites that would be of safety concern for humans was identified.

Cardiovascular and CNS safety pharmacology were assessed in a single study using telemeterized male monkeys, as well as in a standard hERG assay. No adverse test article-related effects were observed. Results of the hERG assay gave an IC_{50} of greater than 300 µM. Therefore, SRP-4053 is not predicted to result in hERG-mediated cardiotoxicity.

Metabolism and plasma protein binding of SRP-4053 were evaluated in a series of standard *in vitro* assays. Metabolism of oligonucleotide drugs such as SRP-4053 is typically mediated by tissue-specific nucleases. Therefore, as expected, no significant induction or inhibition of selected CYP450 microsomal enzymes was observed in the *in vitro* metabolism studies conducted. In addition, when incubated with hepatic microsomes from multiple species in the presence or absence of NADPH, no significant time-or NADPH-dependent metabolism of SRP-4053 was observed. Plasma protein binding was investigated using plasma from rat, monkey, mouse, and human. In all species tested (mouse, rat, monkey, and human), plasma protein binding was low (11-39%) and not concentration dependent.

Distribution of SRP-4053 was evaluated *in vivo* after a single IV dose of radiolabeled SRP-4053 at 120 mg/kg in mice. The drug was rapidly and extensively distributed to most tissues except CNS (brain and spinal cord). Highest levels of radioactivity were observed in kidney. The $t_{1/2}$ in blood was 1.46 hours, (tissue $t_{1/2}$ was not determined). Excretion of SRP-4053 was primarily urinary, accounting for approximately 75% of the radioactive dose. The predominant component in urine was unmetabolized SRP-4053.

Toxicology

General toxicology studies were conducted in C57BL/6NCrl mouse, cynomolgus monkey, and Sprague-Dawley rat. In all species, administration of SRP-4053 resulted in significant renal impairment, observed both functionally via clinical chemistry and urinalysis results as well as anatomically through histopathology observations.

Pivotal studies using adult male mice were conducted for durations of 12 and 26 weeks. SRP-4053 was administered at doses up to 960 mg/kg IV in the 12-week study and 600 mg/kg SC in the 26-week study. (A 23-week study was initiated with IV doses up to 600 mg/kg. This study was originally intended as a 26-week study but was terminated early after technical problems with the IV injection procedure resulted in partial and missing doses beginning in week 14.) In the 12-week mouse study, dose-related renal toxicity was evidenced by clinical chemistry results (increases in urea nitrogen, creatinine, CK, phosphorus, potassium, and reduced chloride) that correlated with dose-related increases in kidney weight and microscopic findings of tubular dilatation, vacuolation of the tubular epithelium, with basophilic casts. In the ureter and urinary bladder, hypertrophy of the transitional epithelium was observed, which was dose-related in severity and incidence. Trends toward recovery were observed. No NOAEL was identified for the 12-week study because of adverse kidney effects at all dose levels.

Similar renal toxicity, but with lower severity, was observed in the 26-week mouse study. Trends toward increased urea nitrogen and CK correlated with histopathology findings of dose-related degeneration/regeneration of the tubular epithelium. Trends toward recovery were observed in the chronic study. The lower severity of renal toxicity in the 26-week SC study (compared to the 12-week IV study) may be explained by the difference in route of administration. The NOAEL for the 26-week study was the LD of 120 mg/kg but because the route of administration was not consistent with the clinical route of IV infusion, safety margin provided by this dose is not considered relevant.

Multiple unscheduled deaths occurred in the mouse studies, which were not dose-related and, thus, are not considered related to SRP-4053.

Administration of SRP-4053 to monkeys via weekly IV injections for durations of 12 and 39 weeks resulted in dose-related renal toxicity only in the longer duration study. In the 12-week study, dose-related alterations in hormone levels were observed (decreased LH and FSH, no effects on testosterone) at the end of dosing but not at the end of recovery. Histopathology showed thyroid (follicular hypertrophy) and liver (capsular fibrosis) findings at low incidence at the HD at the end of dosing, but these findings were not observed at the end of recovery. The relationship of the hormone and histopathology findings to SRP-4053 is not clear since similar changes were not observed in the longer duration study testing higher doses. In the 12-week study, complement activation was observed, which peaked at 15 minutes post dose and resolved to baseline by 4 hours post dose. (Complement activation was not evaluated in the chronic monkey study.)

In the 39-week monkey study (doses up to 400 mg/kg IV), dose-related functional renal impairment was observed in clinical chemistry results (increases in urea nitrogen and creatinine) and urinalysis evaluation (increased urine blood, glucose, creatinine, urea, and protein/creatinine ratio). The clinical chemistry findings correlated with dose-

related microscopic findings in kidney (described as basophilia of the tubular epithelium in all dose groups, dilatation and microvesicular vacuolation of the distal convoluted tubules and collecting ducts. of the distal convoluted tubule and collecting ducts. An additional dose-related microscopic finding study was synovial hyperplasia noted at the end of dosing. The adverse kidney effects persisted through the recovery period, but the bone effects did not. The NOAEL was 80 mg/kg.

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-4053 at doses up to 900 mg/kg beginning on PND14 and continuing for 10 weeks. Results of this study demonstrated no SRP-4053-related adverse effects on neurobehavioral development or immune function parameters. However, severe renal toxicity was observed. A total of 31 unscheduled deaths occurred at the HD that were determined to be due to renal toxicity. Renal toxicity was demonstrated in clinical chemistry findings of increased urea nitrogen, creatinine, phosphorus, potassium, and chloride. Dose-related impairment of renal function was also noted in urinalysis evaluations (increased protein/creatinine ratio and reduced urine NA+, urinary Cl-, and creatinine clearance). The urinalysis effects persisted through the recovery period. The clinical chemistry and urinalysis findings correlated with dose-related microscopic findings in kidneys. These findings were similar to those described in previous studies in adult animals (minimal to marked tubular vacuolation, minimal to marked tubular degeneration/regeneration, minimal to moderate tubular dilatation, and minimal to moderate eosinophilic casts) but at greater severity (lethal at the HD). The kidney findings at the HD were considered irreversible. In addition to the adverse findings in the kidney, multiple additional findings were observed that were considered secondary to renal impairment (mineralization of multiple tissues, degeneration of coronary artery tunica media, and atrial thrombosis).

SRP-4053 effects on bone development were also observed. Bone length was not affected, but dose-related decreases in bone area, bone mineral content, and bone mineral density were observed. These findings persisted through the recovery period.

The NOAEL for this study was the LD of 100 mg/kg. The $AUC_{0\text{-}t}$ at this dose was 239,000 ng•hr/mL. This dose provides a safety margin of approximately 2.6X over the recommended human dose of 30 mg/kg, based on plasma exposure.

A standard battery of genetic toxicology studies was conducted (Ames, *in vitro* chromosome aberration, and *in vivo* micronucleus tests). The studies met all validity criteria. The results were negative for mutagenicity and clastogenicity.

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.

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