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Note: All figures and tables in this review were excerpted from the sponsor's submission

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1 Executive Summary

1.1 Discussion of Nonclinical Findings

Risdiplam (RO7034067, RIS) is an orally available small molecule SMN2 splicing modifier that is intended to increase SMN protein levels in spinal muscular atrophy (SMA) patients through modulation of SMN2 pre-mRNA splicing to include exon 7 into the mRNA transcript, thereby increasing expression of full-length protein from the SMN2 gene. The proposed indication is for the treatment of SMA in (b) (4) patients. The recommended human oral dose (RHD) of 5 mg/day (0.25 mg/kg for <20 kg) is associated with a plasma RIS exposure (AUC) of approximately 2000 ng.h/mL.

In primary pharmacology studies in cultured SMA patient-derived cells and in human whole blood samples from healthy volunteers, RIS was shown to dose-dependently shift the alternative splicing reaction towards the inclusion of SMN2 exon 7. RIS was shown to be specific for SMN2, as indicated by the lack of splicing modifying effect on SMN1. The effect on SMN2 pre-mRNA splicing resulted in the significant and dose-dependent increase of SMN protein levels in fibroblasts derived from Type 1 SMA patients, and in motor neurons derived from Type 1 SMA patient iPSCs. A maximum increase in SMN protein level of 60–80% was seen in both tested cell types.

A significant, dose-dependent increase in SMN protein levels was detected in vivo in 2 different SMA mouse models (SMNΔ7 and C/C), which both carry the human SMN2 transgene. SMN protein levels in muscle and CNS tissue were increased up to a maximum of 3–3.5-fold in SMNΔ7 mice and 1.5–2-fold in C/C mice, as compared to vehicle-treated controls. For C/C mice, the SMN protein levels induced by the highest dose reached those of the heterozygous positive control mice. In the more severe model, SMNΔ7 mice, heterozygous control SMN levels were never reached. RIS induced a prolongation of the median survival time of SMNΔ7 mice, increasing lifespan beyond 6 months of age compared to a lifespan of no longer than 3 weeks in vehicle-dosed SMNΔ7 mice. Dosing SMNΔ7 mice with RIS between PND3 and 14 rescued loss of vesicular glutamate transporter 1 [vGluT1] proprioceptive motor neuron inputs in lumbar sections L3–5, rescued loss of motor neurons in lumbar section L4, prevented neuromuscular junction denervation, and reduced muscle atrophy. RIS also improved motor function as measured by assessment of righting reflex (studied up to PND16), prevented necrosis of the tail, and reduced necrosis of tissues around the eye (studied up to PND220) in the SMNΔ7 model.

A transcriptional profiling analysis indicated that the splicing events of 2 additional genes, STRN3 and SLC25A17, were also affected to a similar extent at what was considered a therapeutically relevant concentration of 121 nM (EC_{90}). The specific function and consequences of alternative splicing for STRN3 and SLC25A17 is unknown. At the highest RIS concentration tested (5-fold the EC_{90}), splicing events of another 11 genes, including FOXM1 and MADD, were affected. FOXM1 and MADD are thought to be involved in cell cycle regulation and apoptosis, respectively.

When effects on FOXM1 marker mRNA expression and the cell cycle were examined in vitro in iPSCs from human and cynomolgus monkeys, treatment with RIS (0.64-10 µM) resulted in a concentration dependent down-regulation of FOXM1B/C transcript variants in human and monkey cells (IC50: 114 nM and 155 nM, respectively). RIS induced concentration-dependent cell cycle arrest in G2 phase in monkey iPSCs and in S phase in human iPSCs.

In both the 2-week rat and monkey dose range-finding studies, gene expression analysis showed splicing responses for MADD, STRN3, and APLP2 gene transcripts in spleen, duodenum, and testis. The transcript variants FOXM1B/C of the FOXM1 gene showed down-regulation in the testis in monkeys at the highest dose tested (6 mg/kg/day). Splicing analyses for the retina and choroid/RPE were integrated into a 7-day monkey study and showed splice variant changes for APLP2 and STRN3 at a dose of 3 mg/kg. Similar observations were made in a study conducted in albino and pigmented rats.

The major human metabolite M1 was assessed in vitro for pharmacological activity on SMN2 and FOXM1 splicing modification in SMA patient-derived fibroblasts and in whole blood samples from healthy volunteers. Both experiments indicated that the M1 metabolite had negligible effects in vitro and ex vivo at clinically-relevant concentrations.

When RIS and the metabolite M1 were tested for off-target activity in vitro at a single test concentration of 10 µM in radioligand binding and enzymatic assays, both inhibited cyclooxygenase 1 and 2 (IC50 ~2 µM) and acetylcholinesterase (IC50 ~0.6 µM for RIS, ~2 µM for M1) and RIS also inhibited binding at histamine H3 (IC50 ~4 µM) and muscarinic M1 (IC50 ~6 µM) receptors.

In safety pharmacology studies, RIS concentrations up to 5 mM (said to correspond to ~18000 ng/mL total plasma concentration in human) produced no significant reduction in the outward K^+ current in CHO cells expressing recombinant hERG K^+ channels. In cynomolgus monkeys, administration of single oral doses up to 7.5 mg/kg had no effects on hemodynamic or ECG parameters at up to 24 hours postdose. At the HD, a plasma concentration of 894 ng/mL was measured at 3h post-dose. In male Wistar rats, administration of single oral doses of up to 10 mg/kg had no effect on general behavior, locomotor activity, motor coordination, sensory/motor reflexes, or body temperature and single oral doses up to 9 mg/kg had no effect on respiratory parameters.

After oral administration, bioavailability was moderate (monkeys) to high (rats), half-life was short (~5 h), clearance low (5–10 mL/min/kg), and volume of distribution moderate (2–3 L/kg) in rats and monkeys. No significant sex difference or accumulation was observed with multiple oral dosing across nonclinical species and daily doses tested; exposure increased approximately dose-proportionally. Plasma protein binding was moderate (89%), with no significant species differences in adults.

RIS concentration in CSF in cynomolgus monkeys reflected free plasma concentration. Tissue distribution studies showed a wide distribution of RIS in both rat and monkey. Elimination kinetics from tissues paralleled the elimination from plasma. RIS levels

equivalent to or higher than total plasma levels were seen in brain, muscle, bone, mucosa of the GI tract, pancreas, liver, lung, heart, kidney, and spleen of rats and monkeys. Substantial distribution into melanin-containing parts of the eye (choroid + retinal pigment epithelium [RPE], iris, sclera) was demonstrated in rats and monkeys, and ocular RIS concentrations remained high for considerable periods after the end of dosing.

In rats, monkeys, and humans, parent drug was the primary drug-related component in plasma. The pharmacologically inactive (based on in vitro and ex vivo SMN2 or FOXM1 splicing) N-hydroxy metabolite M1 was identified as a major circulating metabolite in healthy volunteers and patients (median M1/parent ratio 0.334 in SMA patients). M1 was detected in mouse, rat, rabbit, and monkey plasma, but at lower levels relative to parent than in humans. All other metabolites were present at trace levels in plasma of nonclinical species. Human exposure to M1 appears to have been generally covered in the pivotal toxicology studies.

The toxicology package included the following oral (gavage) repeat-dose toxicity studies: dose range-finding and 4-week definitive toxicity studies in adult mice (previously reviewed under IND 128972), dose range-finding and 4- and 26-week definitive toxicity studies in adult Wistar rats, a 26-week non-GLP toxicity study in Brown Norway rats, dose range-finding and 2- and 39-week definitive toxicity studies in young cynomolgus monkeys, and dose range-finding and 4- and 13-week definitive studies in juvenile rats. TK analyses were included in the pivotal studies.

In the 4-week toxicity study in adult rats, findings included pronounced increases in incidences of micronucleated erythrocytes in the bone marrow and reversible microscopic findings in the GI tract (increased apoptosis/single cell necrosis), thymus (atrophy), and testis (increase in degenerated spermatocytes). At the end of the 4-week recovery period, testis seminiferous tubule atrophy was seen in a single (1/6) HD male. The NOAEL (1 mg/kg/day) was associated with Day 28 Cmax and AUC values of 217/200 ng/mL and 1540/1650 ng•h/mL in males/females, respectively.

In the 26-week study in albino rats, RIS administration resulted in clinical signs, decreased BW, and deaths that were attributed to microscopic findings in the GI tract (crypt single cell necrosis and/or degeneration/necrosis) and bone marrow (decreased cellularity) at the HD (7.5 mg/kg/day). Electroretinography (ERG) findings included a dose-related reduction in scotopic b-wave amplitudes in drug-treated females compared to control at Weeks 13 and 26. A similar trend of reduced amplitudes in drug-treated groups was observed for photopic responses in females. No clear ERG changes were observed in males, and no clear differences among groups of either sex were observed at the end of the recovery period. Drug-related microscopic findings in MD and HD survivors included single cell necrosis in the epithelia of the GI tract, hard palate, and pancreas; degeneration/atrophy of the seminiferous tubules in the testis and degeneration/necrosis of ductular epithelium in the epididymis; estrous cycle arrest; and cortical pigmentation of the adrenals. Following the 8-week recovery period, adrenocortical pigmentation (all doses) and testicular histopathology (HD) were still

present. The low-effect dose (1 mg/kg/day) was associated with Day 182 Cmax and AUC values of 203/295 ng/mL and 2200/2400 ng•h/mL in males/females, respectively.

Similar effects were observed in pigmented (Brown Norway) male rats administered RIS at a single dose level (7.5 mg/kg lowered to 5 mg/kg on Day 52/53) for 26 weeks (non-GLP study); these included: mortality due to decreased cellularity of the bone marrow in combination with intestinal tract findings, reduced body weight and food intake, red cell mass reductions, and microscopic findings in the GI tract (epithelial single cell necrosis), testis (degeneration/atrophy of the seminiferous tubule), and epididymis (degeneration and necrosis of the ductular epithelium). Ocular findings on ophthalmoscopic examination consisted of slight diffuse vitreous haziness in the retinal area in 9/18 animals examined starting at Week 19, and retinal vessel attenuation in 2 animals at Week 26. ERG evaluation showed a progressive reduction in photopic b-wave amplitudes in response to the 0.5 Hz and 29 Hz stimuli over the course of the dosing period at all doses. A similar trend was noted for scotopic a- and b-wave amplitude starting at Week 13. A reflective band was seen in the vitreous by SD-OCT which was thought to be related to the haziness in the retinal area observed during the ophthalmology examination. The plasma AUC associated with the single dose evaluated was 14500 ng.h/mL at the end of the dosing period. RIS concentrations (Cmax) in choroid and retina were orders of magnitude higher than in plasma.

Administration of RIS in the 2-week toxicity study in young (112-141 weeks) monkeys at doses up to 6 mg/kg/day resulted in clinical findings in the skin correlating with microscopic findings of parakeratosis, inflammation, erosion/ulcer, and epidermis hyperplasia at the HD. In addition, there were histopathological changes in the larynx (epithelial degeneration, erosion/ulcer, inflammation, squamous cell metaplasia), thymus (atrophy), and testis (increases in multinucleate cells, germ cell degeneration) at the MD and/or HD. A single minimal focus of retinal dysplasia was seen in one eye of 1 HD male. The anatomical findings in the skin, larynx, and thymus showed complete reversal, but reversal of the testicular effects could not be assessed due to sexual immaturity. The NOAEL (2 mg/kg/day) was associated with RIS exposures of 345 ng/mL (Cmax) and 2550 ng·hr/mL (AUC0-24 h) at Day 14 for both sexes combined.

In the 39-week study in young (24-26 months) monkeys, oral administration of RIS (initial HD of 7.5 reduced to 5 mg/kg/day on Day 26 due to shedding/peeling skin at various locations and reddening of the face) resulted in clinical signs in the skin of HD animals, which correlated with microscopic findings of epidermal hyperplasia. Hematology changes consisting of decreased RBC parameters and lymphocyte count and increased reticulocyte and platelet counts were reversible. C-reactive protein concentrations were increased at the HD and did not reverse by the end of the recovery period. Dose-related functional (as measured by ERG) and morphological (retinal degeneration as observed by OCT, histopathology, and EM) changes in the eye were observed at the MD and HD. In addition, thymic atrophy was seen in HD females. Testicular effects could not be assessed due to immaturity. After the recovery period, retinal findings in HD animals (retinal degeneration characterized by multifocal disorganization of the outer nuclear and photoreceptor layers of the retina, loss of photoreceptor layer, multifocal hypertrophy of

retinal pigment epithelial cells, thinning and disorganization of the inner nuclear layer) were similar to those seen at the end of treatment. The NOAEL (1.5 mg/kg/day) was associated with RIS exposures of 414 / 396 ng/mL (Cmax) and 1870 / 2060 ng·hr/mL (AUC0-24 h) during Week 39 of the dosing phase for males / females.

RIS was negative for mutagenicity in an in vitro Ames test but positive for clastogenicity (micronucleus (MN) formation) in vitro in mouse lymphoma cells and in vivo in a combined bone marrow micronucleus and comet assay in rat. A pronounced effect on bone marrow MN formation was also seen the adult and juvenile rat toxicity studies. A 6-month carcinogenicity study in Tg.rasH2 mice showed no tumorigenic effects at oral doses up to 9 mg/kg/day, which was associated with exposures (AUC) of 15600 and 11800 ng.hr/mL in males and females, respectively. In rats administered RIS during the preweaning or postweaning periods of juvenile development, low incidences of a malignant renal neoplasm (nephroblastoma) and its precursor lesion (nephroblastomatosis) were observed at plasma drug exposures similar to or below that in humans at the RHD. A 2-year rat carcinogenicity study is planned to be conducted postmarketing.

A standard fertility and early embryonic development study was not conducted. The sponsor's justification was that assessment of fertility was integrated into the juvenile rat toxicity studies and reproductive organ histopathology was examined in the adult and juvenile toxicity studies. These studies demonstrated clear reproductive toxicity in both sexes. Male reproductive organ histopathology was seen in adult rats (degeneration/atrophy of the seminiferous tubules in the testis, degeneration and necrosis of the ductular epithelium of the epididymis), young monkeys (increased multinucleate cells and germ cell degeneration in the testis), and juvenile rats (delayed sexual maturation, degeneration/necrosis of the seminiferous tubule epithelium, oligo/aspermia in the epididymis, spermatic granulomas, decreased sperm concentration and motility, increased sperm morphology abnormalities). Female reproductive toxicity was seen in adult (estrous cycle arrest) and juvenile (decreased conception rate and fertility index) rats and delayed female sexual maturation and impaired reproductive performance (increased preimplantation loss) were seen in female offspring in the rat PPND development study.

In the rat embryofetal development study, fetal BWs were decreased and structural variations increased at a dose (HD of 7.5 mg/kg/day) that did not produce significant maternal toxicity. There was no evidence of embryolethality or increased fetal malformations. The no-effect dose for embryofetal toxicity (3 mg/kg/day) was associated with a maternal RIS exposure (AUC) of 4630 ng•h/mL on GD 15.

Embryofetal mortality (increased number of late resorptions) and increased incidences of fetal malformations (hydrocephaly) and variations were seen in the rabbit embryofetal development study at the HD (12 mg/kg), which was also maternally toxic. The no-effect dose for adverse effects on embryofetal development (4 mg/kg/day) was associated with a maternal AUC of 7990 ng•h/mL on GD 15.

In the pre- and postnatal development study in rats in which the highest dose tested (3 mg/kg) was limited by apparent effects on parturition (dystocia), gestation was lengthened at the HD (1 HD dam was euthanized on GD 22 after showing signs of prolonged labor) but there were no other signs of maternal toxicity. There were no apparent effects on offspring survival or growth. Sexual maturation (vaginal opening) was delayed in HD female offspring. When offspring were mated the numbers of corpora lutea, implantation sites, and live embryos were decreased at the HD. At necropsy of offspring, dose-related increases in total ovarian follicle number and ovarian follicles per section were noted. Locomotor activity tended to be increased in treated groups, but differences were not SS. No apparent group differences were observed in auditory startle response and habituation or in performance in the Cincinnati maze. The no-effect dose for adverse effects on pre- and postnatal development in rats (1.5 mg/kg/day) was associated with maternal exposures of 2360 and 1880 ng.h/mL on GD 6 and PND 7, respectively.

Separate juvenile rat studies were conducted to cover the pre- and postweaning periods of juvenile development. In a 4-week study in which oral doses up to 2.5 mg/day were administered from PND 4 through PND 31, there was no mortality; however, clinical signs of dehydration and thinness and decreased BW gain were seen at the MD and HD. BW remained significantly lower in HD males after the recovery period. Preputial separation was delayed at the MD and HD. Decreased tibia length was seen at the end of treatment and persisted up to the end of the recovery period at the MD and HD. Drug-related ophthalmic changes consisting of multiple small vacuoles in the anterior vitreous against the posterior capsule were seen at the HD. Neurobehavioral testing showed effects in the FOB at the end of the treatment period that partially recovered and possible effects on learning and memory in the Cincinnati water maze at the end of the recovery period. Impaired reproductive performance (decreases in day to mating, mating index, fertility index, and conception rate) was seen in HD females mated with proven breeder males. Decreases in the B lymphocyte relative percentages and absolute counts were observed in HD males and females at all doses. Decreases in testis and epididymis weights were seen at the MD and HD at the end of treatment and at all doses in the recovery groups. Histopathology findings noted in the testis at the end of dosing (seminiferous tubule degeneration at the MD and HD) were not evident after the recovery period. Nephroblastomatosis was observed at all doses at the end of treatment, and nephroblastomas were present in the kidney of 1 MD and 1 HD female after the recovery period. The low-effect dose (0.75 mg/kg/day), based on immunophenotyping and testicular weight effects, was associated with AUC values of 680 and 686 ng*hr/mL for RIS and 163 and 173 ng*hr/mL for RO7112063 on PND 31 in males and females, respectively. The results of this study were consistent with those of a previous preweaning rat study that was repeated due to an inadequate number of dose groups, lack of a mating assessment, and absence of a no-effect dose for testicular toxicity.

In a 13-week juvenile rat study with oral administration of doses up to 7.5 mg/kg from PND 22 through PND 112, there was no mortality and no effects on clinical signs, body weights, long bone growth, or sexual maturation. Neurobehavioral testing indicated possible effects on auditory startle and learning and memory in the HD group at the end of the treatment and recovery periods. Lymphocyte phenotyping showed dose-dependent

increases in total, helper, and cytotoxic T lymphocytes and decreases in the relative percentages of B cells at the end of the dosing period. Cytotoxic T cells remained elevated in HD animals at the end of the recovery period. Degeneration/necrosis of the seminiferous epithelium with accompanying oligo/aspermia in the epididymis was observed at the HD at the end of the dosing period, and these testicular effects had not fully recovered after 8 weeks. Renal tubular dilatation was increased in incidence and severity in HD animals at the end of the treatment period and was only seen in MD and HD animals after the recovery period. Malignant nephroblastoma was found in a single HD male at the end of the dosing period. Increases in the number of micronucleated immature erythrocytes in the bone marrow were observed in HD males and in females at all doses at this time. HD values exceeded those in vehicle controls by 10 to 12-fold and exceeded the cyclophosphamide positive control values. Decreased sperm concentrations and motility and increased morphological abnormalities were found in the HD reproductive subset males at the completion of the mating period, but there was no evidence of impairment in male or female reproductive performance. The low-effect dose (1 mg/kg/day), based on genotoxicity, was associated with AUC values of 600 and 511 ng*hr/mL on PND 22 in males and females, respectively.

In an effort to determine the mechanism of action for the retinal toxicity observed in cynomolgus monkeys, human retinal pigment epithelial cells (ARPE19) were treated with RIS and key RPE cell functions were analyzed. Results showed that RIS induced an increase in lysosomal mass and an accumulation of autophagosomes comparable to that caused by chloroquine, a positive control that is known to cause lysosomal dysfunction in RPE in vitro and in the human retina in vivo. These findings were similar to those seen with another SMN2 splicing modifier, RO6885247 (described in Ratni et al., 2018). The clinical development of RO6885247 was discontinued after retinal degeneration was seen in a 39-week study in cynomolgus monkeys. These results were thought to indicate that lysosomal malfunction and an impairment of autophagy may lead to a dysfunction of cellular degradation processes in RPE cells. This dysfunction was thought to be due to physicochemical properties, since pharmacologically inactive compounds with physicochemical properties comparable to RIS and RO6885247 induced similar changes in this RPE cell line in vitro.

1.2 Recommendations

The application is not approvable from a pharmacology/toxicology standpoint due to the serious, irreversible toxicity observed at clinically-relevant exposures. However, the serious indication of SMA and clinical safety assessment may shift the risk/benefit equation in favor of approval. If risdiplam is approved, the significant risks, based on animal studies, (b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number	1825352-65-5
Generic Name	Risdiplam (RO7034067)
Chemical Name	7-(4,7-Diazaspiro[2.5]octan-7-yl)-2-(2,8-dimethylimidazo[1,2-b]pyridazin-6-yl)-4H-pyrido-[1,2-a]pyrimidin-4-one
Molecular Formula/Molecular Weight	C22H23N7O/ 401.46 g/mol
Pharmacologic Class	SMN2 splicing modifier

2.2 Relevant IND

128972

2.3 Proposed Clinical Population and Dosing Regimen

Risdiplam is indicated for the treatment of spinal muscular atrophy (SMA) in (b) (4) patients. The recommended once daily dose of risdiplam for SMA patients is determined by age and body weight:

Age	Recommended Daily Dose
2 months to < 2 years of age	0.20 mg/kg
≥ 2 years of age (< 20 kg)	0.25 mg/kg
≥ 2 years of age (≥ 20 kg)	5 mg

3 Studies Reviewed

Pharmacology
- Primary, secondary, safety

Pharmacokinetics
- ADME

Repeat-Dose General Toxicity
- 4- and 26-week definitive toxicity studies in adult Wistar rats
- 26-week non-GLP toxicity study in Brown Norway rats
- 2- and 39-week definitive toxicity studies cynomolgus monkeys

Genotoxicity
- in vitro Ames and mouse lymphoma assays
- in vivo combined bone marrow micronucleus and comet assay in rat

Carcinogenicity
- 6-month carcinogenicity study in RasH2 mice

Reproductive and Developmental Toxicity
- Embryofetal development in rat and rabbit
- Pre- and postnatal development in rat
- 4-week preweaning juvenile development in rat
- 13-week postweaning juvenile development in rat

Special Toxicity
- Investigations of effects on cell cycle and MOA of retinal toxicity and micronucleus induction

4 Pharmacology

4.1 Primary Pharmacology

Risdiplam (RIS) is a small molecule SMN2 splicing modifier. In cultured SMA patient-derived cells and in human whole blood samples from healthy volunteers, RIS was shown to dose-dependently shift the alternative splicing reaction towards the inclusion of SMN2 exon 7 (1066984, 1078515). The shift in the alternative splicing reaction was studied from both directions, i.e. both the increase in the full-length (exon 7 including) SMN2 mRNA and the decrease in the truncated (exon 7 lacking) SMN2 mRNA levels were assessed. RIS was shown to be specific for SMN2, as indicated by the lack of splicing modifying effect on SMN1 (1066984). The effect on SMN2 pre-mRNA splicing resulted in the significant and dose-dependent increase of SMN protein levels in fibroblasts derived from Type 1 SMA patients, and in motor neurons derived from Type 1 SMA patient iPSCs. A maximum SMN protein level increase of 60–80% was comparable in both tested cell types (1066984). The M1 metabolite of RIS had negligible primary pharmacological effect in vitro and ex vivo at concentrations that can reasonably be expected at therapeutic doses of RIS (1078515).

The in vivo activity of RIS was investigated in 2 different SMA mouse models (SMNΔ7 and C/C), which both carry the human SMN2 transgene (1063288, 1065078). The SMNΔ7 mouse model is homozygous for the mouse Smn1 knock-out allele, combined with full-length and exon 7-lacking (Δ7) SMN2 transgenes. SMNΔ7 mice develop a SMA-like phenotype, including a shortened lifespan. The severity and prenatal onset of disease in these mice was thought to explain the deaths eventually observed in animals receiving RIS treatment postnatally. In C/C mice with a milder SMA-like phenotype, delayed dosing (i.e., at juvenile to adult age versus early postnatal in SMNΔ7 mice) and pharmacodynamic readouts in adults was possible. Heterozygous littermates which do not show any SMA-related phenotype served as positive controls for both strains (SMNΔ7: carry one copy of the mouse Smn1 allele; C/C: heterozygous for C-allele and mouse Smn1).

A significant, dose-dependent increase in SMN protein levels was detected in both mouse models in muscle and CNS tissue with a maximum of 3–3.5-fold SMN protein level increase for SMNΔ7 and a 1.5–2-fold SMN protein level increase for C/C mice, as compared to vehicle-treated controls. For C/C mice, the SMN protein levels induced by the highest dose reached those of the heterozygous positive control mice, unlike for the more severe model SMNΔ7 mice, in which heterozygous control SMN levels were not reached upon RIS exposure. However, RIS induced a prolongation of the median survival time, increasing the lifespan of SMNΔ7 mice beyond 6 months of age, while vehicle-dosed SMNΔ7 mice lived no longer than 3 weeks (1093133). Correlated with animal survival, RIS also increased body weight gain (studied up to postnatal day [PND] 220). Dosing with RIS between PND 3–14 restored the neuromuscular circuitry (rescued loss of vesicular glutamate transporter 1 [vGluT1] proprioceptive motor neuron inputs in lumbar section L3–5, rescued loss of motor neurons in lumbar section L4, prevented neuromuscular junction [NMJ] denervation) and reduced muscle atrophy. Similar beneficial effects on the neuromuscular circuitry were observed after long-term (PND 3–

220) RIS treatment. RIS increased the number of vGluT1 boutons in L3–5 and the number of L4 spinal nerve ventral root axons to near control levels and increased muscle innervation and muscle weights. RIS also improved motor function as measured by assessment of righting reflex (studied up to PND16), prevented tissue necrosis of the tail, and reduced necrosis of tissues around the eye (studied up to PND220).

Secondary (off-target) Pharmacology

A transcriptional profiling analysis indicated that the splicing events of 2 additional genes, STRN3 and SLC25A17, were also affected to a similar extent at what was considered a therapeutically relevant concentration of 121 nM (EC90) compared to control (1066987). The specific function and consequences of alternative splicing for STRN3 and SLC25A17 is unknown. At the highest RIS concentration tested (5-fold the EC90), splicing events of a total 11 genes, including FOXM1 and MADD, were affected. FOXM1 and MADD are thought to be involved in cell cycle regulation and apoptosis, respectively.

RNA sequencing analyses followed by detailed analysis of spliced genes were integrated into the 2-week dose-range finding repeat dose toxicity studies conducted in the rat and monkey (1061416, 1061651). Gene expression analysis was performed in spleen, duodenum, and testis in rats treated for 2 weeks with a focus on the genes, the splicing of which were most affected in vitro and with previously tested compounds, i.e., Madd, Strn3, Aplp2, Smn1 and Foxm1 genes (Ratni et al., 2018). Gene expression analysis of RIS-treated animals revealed a splicing response at the highest dose of 7.5 mg/kg/day for Madd and Strn3 gene transcripts and to lower extent for the Aplp2 gene transcript in spleen, duodenum, and testis. Aplp2 was not seen as a major secondary splice target in the in vitro pharmacology studies but surfaced as more affected in these in vivo study in rats and monkeys at the high doses at which these were conducted. Transcripts of the Smn1 and Foxm1 genes remained unchanged in alternative splicing.

Similar investigations were integrated into the 2-week dose-range finding toxicity study conducted in the cynomolgus monkey with RIS (1061651). Spleen, duodenum, and testis were analyzed from the animals treated with 0.75, 1.5, 3 and 6 mg/kg/day. The analysis with mRNA isoform specific qPCR assays revealed a pharmacological response to RIS treatment at the highest dose for the transcripts of the MADD and STRN3 genes, and to a lower extent for the APLP2 gene transcript in all three organs. The transcript variants FOXM1B/C of the FOXM1 gene showed a downregulation in testis in one animal at the highest dose. Transcripts of the SMN1 genes remained unchanged in alternative splicing in response to treatments. The spleen tissue was further analyzed for transcriptome-wide mRNA splicing or expression changes by RNA-sequencing. This analysis indicated that, except for the spliced transcripts already noted, the majority of mRNAs exhibited either no change in alternative splicing or no dose-dependent trend in such changes upon treatment with RIS.

Splicing analyses for the retina and choroid/RPE were also integrated into a monkey multiple dose PK study (1065261) with RIS and another SMN2 splice modifier, RO6885247, administered at 3 mg/kg/day over 7 days. Splice variants of the main

secondary splice targets FOXM1, STRN3, APLP2, and MADD were examined. In contrast to spleen and skin samples, splice variant changes were detected in the retina and choroid/RPE only for APLP2 and STRN3 and these changes were clearly weaker than in spleen and skin (1067982). Both compounds showed consistent results in this analysis. Splicing changes appeared to return to control levels when measured after 6 weeks of recovery.

Similar observations were made in a study conducted with albino and pigmented rats (1068196) in which spleen, skin, retina, and choroid/retinal pigment epithelium (RPE) were obtained from Wistar and Brown Norway rats treated for 4 days with RIS (3 mg/kg/day) and sacrificed after 4 days and during the recovery period. The analysis with mRNA isoform specific qPCR marker assays revealed changes in alternative splicing for the transcript variants of rat Foxm1, Aplp2, Madd, and Strn3 genes. There were no changes in either of the rat Foxm1 isoforms in the retina, but in choroid/RPE tissues the Foxm1b isoform appeared to be decreased in expression in both albino and pigmented rats treated with RIS. Changes in alternative splicing for Aplp2 were detected in retina and choroid/RPE of RIS-treated pigmented rats, but albino rats showed minimal changes in Aplp2 splicing after RIS administration. No splicing changes of the Madd transcripts were detected in retina and choroid/RPE tissues from either albino or pigmented rats. Treatment-related effects on Strn3 splicing were detected in the choroid/RPE tissue of both albino and pigmented rats. Splicing changes returned to control values when measured during the recovery period. In general, compound-related changes in alternative splicing were more pronounced in pigmented than in albino rats.

In addition to RIS, the major human metabolite M1 was assessed in vitro for SMN2 and FOXM1 splicing modification in SMA patient-derived fibroblasts and in whole blood samples from healthy volunteers. Both experiments indicated that the M1 metabolite had negligible pharmacological effects in vitro and ex vivo at concentrations that can be expected at therapeutic doses of RIS (1078515).

RIS and metabolite M1 were tested for off-target activity in vitro at a single test concentration of 10 µM in radioligand binding and enzymatic assays (1087510). In these studies, both RIS and M1 inhibited cyclooxygenase 1 (COX1) and 2 (COX2) by >84% (IC50 ~2 µM) and acetylcholinesterase by >77% (IC50 ~0.6 µM for RIS, and ~2 µM for metabolite M1) at 10 µM. RIS also inhibited radioligand binding at histamine H3 (78%, Ki=1.01 µM, IC50=4.2 µM) and muscarinic M1 (54%, Ki=5.6 µM, IC50=6.4 µM) receptors. RIS inhibited the δ-opioid receptor by 62% in the primary screening assay, but a follow-up assay identified only 35% inhibition at 10 µM. The functional properties, i.e. agonist or antagonist, were not explored due to solubility constraints.

Safety Pharmacology

RO7034067 concentrations up to 5 mM (said to correspond to ~18000 ng/mL total plasma concentration in human) produced no significant reduction in the outward K+ current in CHO cells expressing recombinant hERG K+ channels (1064272, 1075143).

In a CV safety pharmacology study in cynomolgus monkeys, single oral doses of 1, 3, or 7.5 mg/kg had no effects on hemodynamic or ECG parameters at up to 24 hours postdose. At the HD, a plasma concentration (n = 6; combined sex avg) of 894 ng/mL was measured at 3h post-dose (1062902).

Administration of single oral doses of 1, 3, or 10 mg/kg had no effect on general behavioral, locomotor activity, motor coordination, sensory/motor reflexes or body temperature in male Wistar rats (1064342). Single oral doses of 1, 3, or 9 mg/kg had no effect on respiratory parameters in male Wistar rats (1064343).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

PK parameters after single dose administration are shown in Table 1.

Table 1. Pharmacokinetics of Risdiplam After Single PO or IP Administration

Species (n) [Reference]	Actual Dose [mg/kg]	C_{max} [ng/mL]	t_{max} [h]	$t_{½}$ [h]	$AUC_{0-\infty}$ [ng·h/mL]	F [%]
Intraperitoneal						
PND10 mice (3)[a] [1063287]	3 (nominal)	208	2	nc	2160 (AUC_{0-24})	na
Oral gavage						
Adult rat (2)[b] [1064409]	5.5	1530, 1090	1, 2	5.2, 7.6	12200, 12300	116, 118
Adult rat (4)[a] [1064408]	5.1	775	3	6.0	10300	na
PND4 rat (17)[a] [1064408]	5.4	566	7	15.1	15600	na
PND12 rat (9)[a] [1064408]	6.1	461	7	20.4	16600	na
PND21 rat (6)[a] [1064408]	5.5	448	3	5.5	7230	na
Rabbit (3)[c] [1078251]	3.6	470 (13)	1.0 (1.0–2.0)	12.0 (26)	3890 (25)	na
Monkey (3)[c] [1064410]	0.5	79 (5)	2.0 (1.0–2.0)	5.4 (21)	699 (14)	43 (12)

n=number of animals; na=not applicable; nc=not calculated; PND=postnatal day

[a] Parameters derived from composite profile.

[b] Parameters show individual values for each animal.

[c] Parameters show mean value with coefficient of variation in % in parenthesis (median with range in parenthesis for t_{max}).

Absorption

RIS was well-absorbed with moderate to high oral bioavailability and an early Tmax after oral dosing in animals. A PBPK analysis predicted oral absorption in human to be above 90%. No significant sex difference or accumulation over time was observed after multiple oral administrations across all nonclinical species and daily doses tested; the exposure increased roughly dose proportionally. Half-life was short, clearance low, and volume of distribution moderate in monkeys and rats. Half-life in human was predicted to be between 10 to 70 h based on combined in vitro, in vivo preclinical data, and PBPK integrated analysis.

Distribution

Vd was moderate in monkeys and rats (2 to 3 L/kg). Plasma protein binding of RIS was moderate and it showed no significant species differences in adult. The difference in tolerability between the pre- and post-weaning rats was partly attributed to age dependent protein binding; a markedly higher free fraction and high inter-individual variability were seen juvenile rats (up to 90% in PND 4 rat versus 16% in adult). Combined with lower systemic clearance in juvenile rats, this led to significantly higher unbound plasma concentrations. The age-dependency was less pronounced in mice and monkeys (27% in PND 11 mice versus 12% adult and 25% in PND 10 monkeys versus 14% at 6 months of age in the same individuals).

RIS concentrations in monkey CSF reflected approximately those of the free, unbound compound in plasma. However, in rats the CSF-to-unbound plasma partition coefficient (Kp,uu) was 0.28. Drug levels decreased in parallel in plasma, brain, and CSF across species and were undetectable in long term studies after a washout period, confirming the lack of accumulation in tissues (1063584, 1065114).

In albino rats, tissue to plasma ratios in the different ocular substructures were ≤1. In pigmented rats and monkeys, distribution into melanin-containing parts of the eye (choroid and RPE, iris, sclera) was significantly higher than in non-pigmented ocular structures (retina, lens, vitreous body) or the rest of the body (muscle, brain, CSF; Table 2), which was in agreement with in vitro melanin binding results. Ocular concentrations remained high for considerable periods after the end of dosing, especially in melanin-containing structures (Table 3).

Table 2. Average Ocular Tissue / Plasma Ratios of Risdiplam in Albino and Pigmented Rats and Monkeys After 3 mg/kg/day Oral Daily Dosing

Ocular Tissue	Rat			Cynomolgus	
	Albino (Wistar)		Pigmented		
	28 days (C_{24h}) [a]	4 days (C_{1h})	4 days (C_{1h})	7 days (C_{2h})	39 weeks ($C_{3 \text{ or } 5h}$)[b]
Choroid + RPE[c]	nd	0.80	5220	172	2190
Iris	nd	0.92	2270	58	3180
Sclera	nd	0.63	1960	8.2	117
Retina	1.95	0.66	20	5.3	28
Lens	nd	0.05	0.93	0.17	1.3
Vitreous body	0.54	0.06	0.80	0.38	0.38[d]

nd=not determined (tissue not sampled); RPE=retinal pigment epithelium

Tissue/plasma ratios for rats and Cynomolgus monkeys are based on individual plasma and tissue concentrations (C) at given timepoints.

[a] Tissue / plasma ratios were equivalent in the low (1 mg/kg/day) and high (9 mg/kg/day) dose groups of the 4-week rat toxicology study.

[b] Tissue / plasma ratios were equivalent in the low (1.5 mg/kg/day) and high (5 mg/kg/day) dose groups of the monkey chronic toxicology study.

[c] Choroid and RPE were sampled and analyzed together.

[d] Average of central (0.290) and central (0.476) vitreous humor.

Table 3. Approximation of Risdiplam Elimination Rate from Ocular Tissues in Pigmented Rats and in Monkeys After 3 mg/kg/day Oral Daily Dosing

Ocular Tissue	Rats (Pigmented)	Cynomolgus Monkeys	
	4 day treatment 42 day treatment-free	7 day treatment 42 day treatment-free[b]	39 week treatment 22 week treatment-free
Choroid + RPE [a]	$t_{½}$ ~65d	$t_{½}$ ~30d	~3-fold decrease
Iris	$t_{½}$ ~59d	nc	~2-fold decrease
Sclera	$t_{½}$ ~8d	$t_{½}$ ~20d	~4-fold decrease
Retina	$t_{½}$ ~13d	$t_{½}$ ~20d	~50-fold decrease
Lens	$t_{½}$ ~51d	nc	~25-fold decrease
Vitreous body	$t_{½}$ ~120d	$t_{½}$ ~9d	~11-fold decrease

nc=not calculated

An approximation of elimination rate (half-life or fold decrease from the last day of treatment) was done on the basis of tissue concentration at the end of the treatment-free period:

- 7, 14 and 42 days after the last dose (3 time points) for pigmented rats [1067462]
- 42 days after the last dose (1 time point) for monkeys treated for 7 days [1065261]
- 22 weeks after the last dose (1 time point) for monkeys treated for 39 weeks [1063584]

[a] Choroid and RPE were sampled and analyzed together

[b] Rough explorative estimates based on two data points; calculation not reported in 1065261

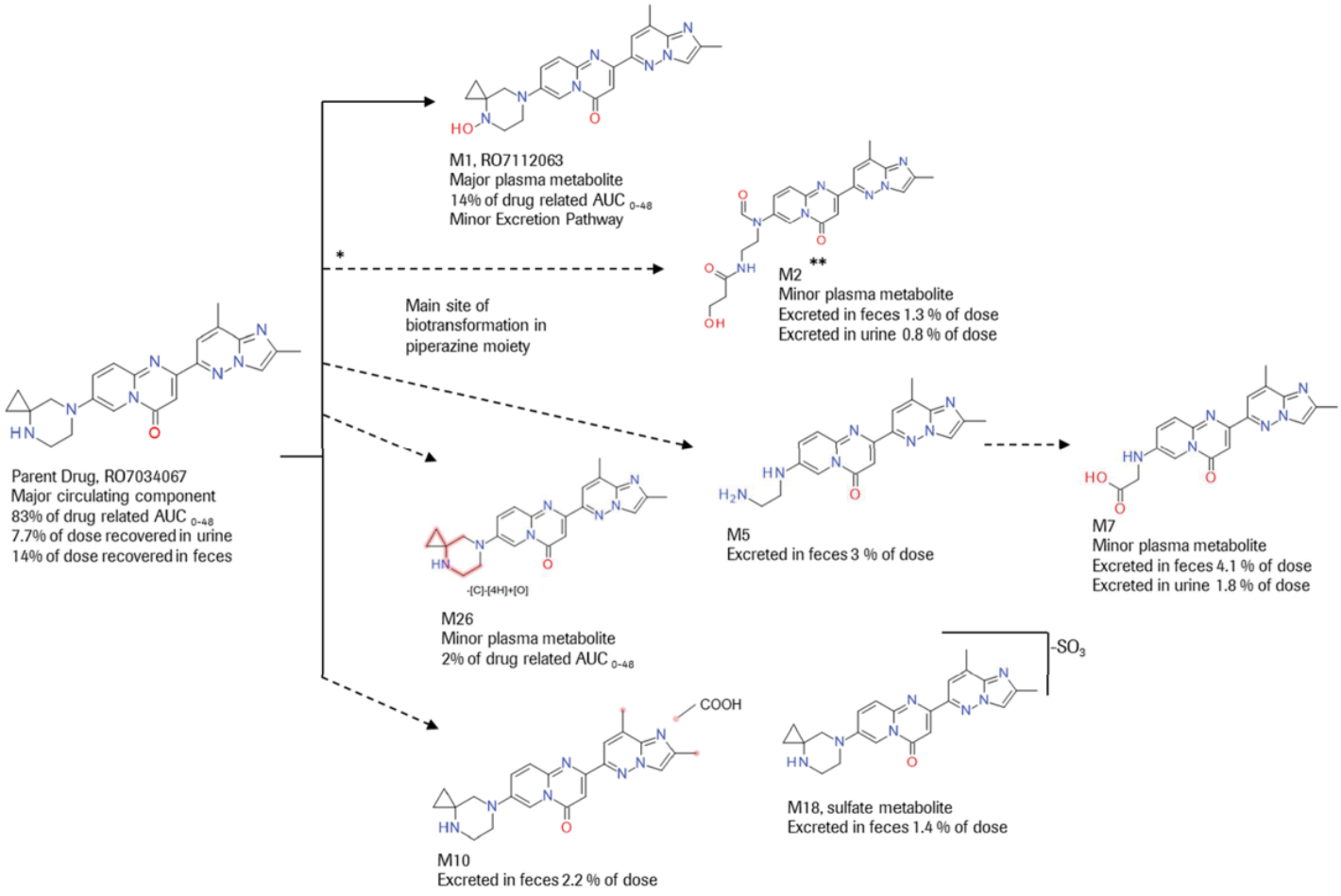
The presence of radioactivity in the fetus indicated that RIS-related material crossed the placenta in the rat.

Metabolism

The metabolic pathway of RIS in humans shown in Figure 1 was proposed based on the results of in vitro metabolism studies using human hepatocytes, human liver microsomes, and microsomes expressing human CYP isoforms, and the in vivo results of the radiolabeled human ADME study.

Figure 1.

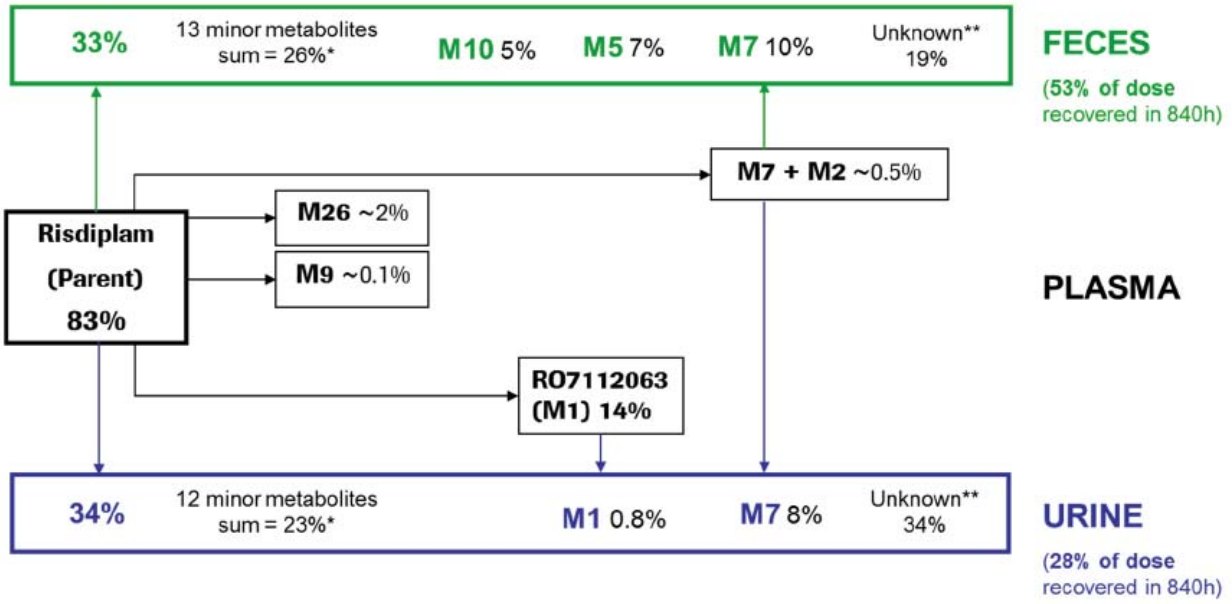
Proposed Main Metabolic Pathway for [^{14}C]-Risdiplam in Human



In human plasma samples after a single PO dose of 14C-risdiplam, parent drug was the major component, accounting for 83% of drug-related material in circulation (percent of AUC0-48, Figure 2). Parent drug was also the main drug-related component in excreta (urine and feces combined), accounting for 22% of the administered dose. M1 (N-hydroxy metabolite) accounted for 14% of total radioactivity (percent AUC0-48 h) in plasma; it was excreted in urine. Additional metabolites (M2, M7 and M26) resulting from biotransformation of the piperazine moiety were observed in plasma at lower levels. M5 and M7 (both resulting from biotransformation of the piperazine moiety), M10 (carboxylic

acid metabolite), and M18 (sulfate conjugate) were the most abundant metabolites in feces. M7 was the most abundant metabolite in urine. Plasma M1 levels were approximately 30% of parent in SMA patients.

Figure 2. Metabolism of risdiplam in human after single oral dose



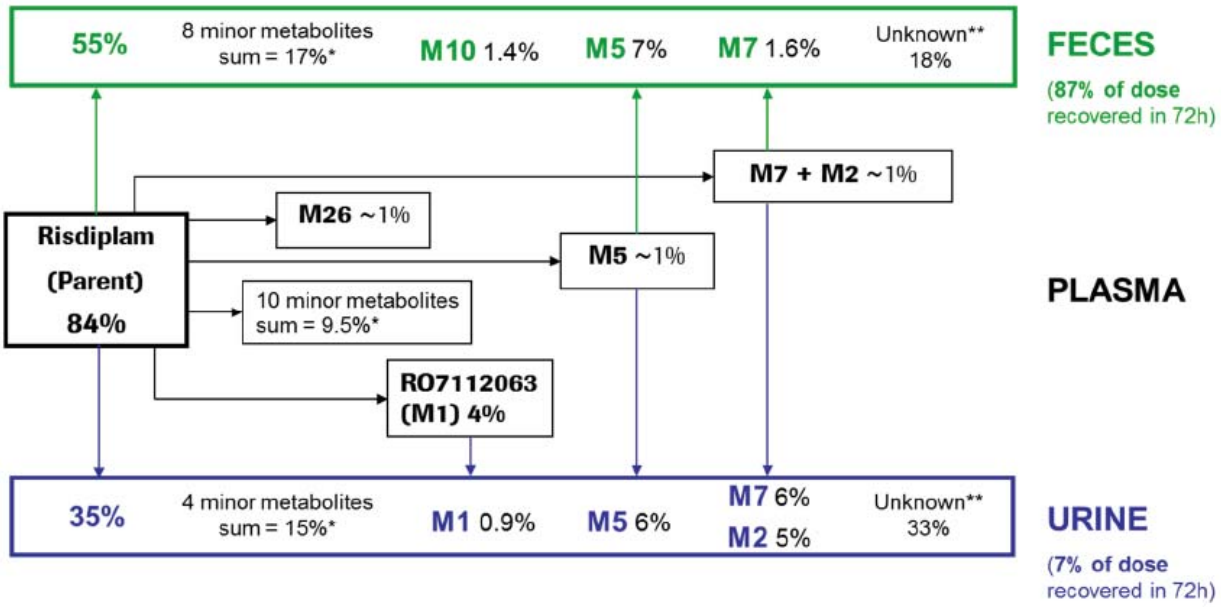
Unit (plasma, feces and urine): % of total drug-related material (radioactivity) in the given matrix (of AUC_{0-48} in plasma and over 0–168 h in excreta).

* individual contributions: 0.2–3.5% of drug-related material in feces (cumulated =9.6% of the dose), 0.4–4.5% of drug-related material in urine (cumulated = 5.3% of the dose)

** trace level components each accounting for <0.9% of the dose (feces) or ≤1.0% of the dose (urine)

Following 14C-risdiplam administration to naïve and bile-duct cannulated rats (1068338), the most abundant drug-related material in plasma, feces, and urine was unchanged parent drug (Fig. 3). The most abundant circulating metabolite was M1, representing 4% drug-related material in plasma 1 h after PO administration.

Figure 3. Metabolism of risdiplam in rat after single oral dose



Unit (plasma, feces and urine): % of total drug-related material (radioactivity) in the given matrix (of the 1 h sample in plasma and over 0–48 h in excreta).

* individual metabolite contributions: 0.3–1.8% of drug-related material in plasma, 1.4–3.4% of drug-related material in feces (cumulated = 14% of the dose), 2.6–5.1% of drug-related material in urine (cumulated = 1.0% of the dose).

** Multiple trace drug-related components for which no structure information was generated.

Following oral administration of 6 mg/kg to cynomolgus monkeys for 2 weeks (collected in toxicology study 8306338), unchanged drug accounted for ≥ 84% of the identified drug-related material in plasma samples collected at 1h and 7h. The N-hydroxy metabolite M1 was identified as a major circulating metabolite (14%) in monkey plasma collected at 7h and as a minor circulating component (6%) in monkey plasma collected at 1h. Metabolites resulting from cyclopropyl cleavage and/or oxidation (M3 and M4) and metabolites involving cleavage of the piperazine ring (M2, M5, M6 and M7) were identified at trace levels (≤1.6%) in monkey plasma samples collected at 1h and 7h.

Human exposure to M1 appears to have been generally covered in the pivotal toxicology studies. Plasma samples taken prior to the incorporation of M1-preservation measures into the bioanalytical methods could not be used to assess M1 levels due to M1 instability. But after refinement of the plasma collection and assay conditions to address the ex vivo instability of M1, PK bridging studies were performed with a single administration in rats and 3 days repeat-dosing in monkeys (Table 4). M1 was also measured in the remaining toxicity testing in rats (embryofetal and pre- and postnatal development, juvenile toxicity), mice (carcinogenicity), and rabbit (embryofetal development).

Table 4. M1 plasma exposure in mice, rats, rabbits, and monkeys

Species [Reference]	Study Type	Daily Dose [mg/kg/day]	Treatment Duration	AUC_{0-24} [ng·h/mL] (M/F)		M1/Parent AUC Ratio [%]
				Parent	M1	
PK Bridging Studies						
Male Adult Rats [1078302]	SDPK	3.2	Single dose	3980	1440	36
Male Monkeys [1078303]	MDPK	3	3 days	11300	1720	15
Toxicity Studies (all GLP)						
Female Rats GD15 (dams) [1084123]	EFD	1 3 7.5	10 days (GD6–17)	1540 4630 10700	235 576 1210	15 12 11
Female Rats PP7 (dams) [1088289]	PPND	0.75 1.5 3	23–25 days (GD6 to PP20)	954 1880 4230	112 266 490	12 14 12
Juvenile Rats [1088815]	Juvenile Toxicity	0.75 1.5 2.5	4 weeks (PND4–31)	680/668 1570/1880 1950/2190	163/173 439/406 457/485	24/26 28/22 24/22
RasH2 Mice [1081793]	Carcino-genicity	1 3 9	26 weeks	1730/797 4850/3630 15600/11800	122/102 423/472 1690/1580	7.1/13 8.7/13 11/13
Rabbits GD15 [1084122]	EFD	1 4 12	10 days (GD6–19)	1880 7990 37900	63 303 760	3.4 3.8 2.0

EFD=embryofetal development; F=female; GD=gestational day; M=male; MDPK=multiple dose PK; PND=postnatal day; PP=days post-partum; PPND=pre- and post-natal development; SDPK=single dose PK.

The nonclinical coverage of M1 in some of the pivotal nonclinical studies at the NOAEL is shown in Table 5. M1 exposure slightly greater than the estimated human exposure at the dose used in pivotal clinical trials in rats and mice, while the exposures at the NOAEL in monkeys and rabbits were ~50% of the human exposure. Based on the available data, it appears that M1 exposures equivalent to (rabbit) or exceeding that in humans were achieved at higher doses in most of the pivotal studies but not in the rat PPND or 4-week juvenile rat studies.

Table 5. M1 exposure margins in toxicity studies at the respective NOAELs

Species [Reference]	Dose at NOAEL [mg/kg/day]		M1 Exposure at NOAEL [AUC_{0-24} in ng·h/mL]		Animal vs Human M1 Exposure Ratio[a]	
	M	F	M	F	M	F
Monkeys [1063584]	1.5	1.5	285[b]	314[b]	0.43	0.47
Adult Rats [1067365]	1	3	796[b]	3195[b]	1.19	4.78
Rats (PND31) [1088815]	1.5	1.5	439	406	0.66	0.61
RasH2 Mice [1081793]	9	9	1690	1580	2.53	2.37
Rabbits [1084122]	na	4	na	303	na	0.45

F=females; M=males; na=not applicable; NOAEL=no observed adverse effects level; PND=postnatal day

[a] The M1 $AUC_{0-24,ss}$ at the mean exposure guidance in SMA patients (2000 ng·h/mL; parent $AUC_{0-24,ss}$, see 2.4 Nonclinical Overview, Section 5.10) was extrapolated by multiplying the parent $AUC_{0-24,ss}$ with the median M1/parent ratio (0.334) in SMA patients at pivotal doses (i.e. M1 $AUC_{0-24h,ss}$ = 2000 ng·h/mL×0.334 = 668 ng·h/mL). The animal versus human M1 exposure ratio is the ratio of the animal M1 $AUC_{0-24,ss}$ on the last day of dosing at the NOAEL dose divided by the extrapolated M1 $AUC_{0-24,ss}$ at the mean exposure guidance in SMA patients (668 ng·h/mL).

[b] Plasma samples taken before incorporation of M1-preservation measures into bioanalytical methods. The M1 exposure was estimated from a corresponding PK bridging study as follows: M1 AUC_{0-24} = (parent AUC_{0-24} on the last day of dosing at the NOAEL dose)× (M1/parent ratio from PK bridging study).

Excretion

In rats and monkeys, the main route of excretion after oral administration of RIS was in the feces, while excretion in urine accounted for a lower amount. After administration of unlabeled RIS to rats (IV or PO) or cynomolgus monkeys (PO), a maximum of 2.4% (rats) or less than 1% of the dose (monkey) was excreted unchanged in urine (1065261, 1068338). RIS was excreted into milk of lactating rats.

6 General Toxicology

6.1 Repeat-Dose Toxicity

Study title: RO7034067: 4 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 4 Week Treatment-Free Period

Study no.:	1064057
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09 March 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 024780-026/ 97.66%

Key Study Findings

Oral (gavage) administration of RIS (0 (vehicle), 1, 3, or 9 mg/kg/day; 10 mL/kg) to Wistar rats for 4 weeks resulted in decreased BW at the HD, pronounced increases in incidences of micronucleated erythrocytes in the bone marrow at the MD and HD (% MN at HD greater than CPA positive control), and microscopic findings in the GI tract (increased apoptosis/single cell necrosis), thymus (atrophy), and testis (increase in degenerated spermatocytes) at the HD. At the end of the 4-week recovery period, testis seminiferous tubule atrophy was seen in a single (1/6) HD male. The NOAEL (1 mg/kg/day) was associated with Day 28 Cmax and AUC values of 217/200 ng/mL and 1540/1650 ng•h/mL in males/females, respectively.

Methods

Doses:	0 (vehicle), 1, 3, or 9 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Rat / Crl: WI (Han)
Number/Sex/Group:	10/sex/group
Age:	7 Weeks
Weight:	M: 156-206 g, F: 126-180.5 g
Satellite groups:	6/sex/grp TK and recovery, 10 MD males for tissue levels, 3/sex CPA positive control (see table below)
Unique study design:	Positive control group administered cyclophosphamide (20 mg/kg, 5 mL/kg) orally by gavage 24 hours prior to necropsy.
Deviation from study protocol:	None that impacted study quality or integrity

Group number	Group description	Dose level (mg/kg/day)	Number of animals in group: Main group Male	Main group Female	Toxicokinetics + Recovery group (treatment-free) Male	Toxicokinetics + Recovery group (treatment-free) Female
1	Control	0	10	10	6	6
2	Low	1	10	10	6	6
3	Intermediate	3	20#	10	6	6
4	High	9	10	10	6	6
5@	Micronucleus Positive Control (CPA)*	20	3	3	-	-

Additional 10 males included for evaluation of plasma, eye, brain and skin levels.

@ For micronucleus investigations only; no other experimental observation data from these animals has been reported.

* Cyclophosphamide

Dose selection was based on the results of a range-finding study in the rat (Roche Reference Number 8300P14) in which doses of 0, 0.75, 2.5, or 7.5 mg/kg/day were administered for 2 weeks. Decreased BW gain (19% in males) and increased apoptosis in the small intestine and caecum (1 female) were seen at the HD, which may be associated with alternative splicing effects on the apoptosis-related gene MADD. A toxicogenomics analysis with mRNA isoform specific qPCR marker assays revealed a splicing response to RIS at the HD for the transcripts of the MADD and STRN3 genes, and to lower extents for the APLP2 gene transcript in spleen, duodenum, and testis. Transcripts of the SMN1 and FOXM1 genes remained unchanged in alternative splicing in response to RIS.The NOAEL (7.5 mg/kg/day) was associated with AUC(0-24h) and Cmax values of 19600/17100 (ng·h)/mL and 1820/1640 ng/mL for males/females on day 14, respectively.

In an acute dose range-finding study for the bone marrow micronucleus test, singe oral doses up to 100 mg/kg were tolerated, but ≥ 50 mg/kg produced pronounced hematoxicity in the bone marrow. The HD selected for the 4-week study (9 mg/kg/day) was expected to produce and exposure (AUC) multiple of at least 10, compared to the exposure in human which would give the near maximum therapeutic increase in SMN protein of 150% (based on total, i.e. bound + unbound, plasma concentrations of RO7034067).

Observations and Results

Mortality

There were no unscheduled deaths during the course of the study.

Clinical Signs

There were no drug-related clinical signs.

Body Weights

BW gain was decreased at the HD in both sexes during the dosing period (M: -17%, F: -27%; Table 1). During the recovery period, BW gain was increased in HD males (15%) but decreased by a similar amount in females at all doses (27, 30, 31% at LD, MD, and HD, respectively).

Table 1. BW gain during dosing period

Test Item		Control RO7034067				CPA Positive Control	
Group		1	2	3	4	5	
Dose level (mg/kg/day)		0	1	3	9	20	
		Data Presented in "g" Interval X through X					
Group/ Sex	Phase DSNG Day	1 - 8	8 - 15	15 - 22	22 - 28	1 - 28	1 - 29
1/M	Mean	34.8	32.8	27.2	14.3	107.2	118.0
	SD	5.28	5.16	5.10	4.24	10.94	17.34
	N	16	16	16	10	10	6
2/M	Mean	31.3	36.5	26.2	12.1	104.3	116.6
	SD	7.31	5.22	5.38	3.44	9.76	19.29
	N	16	16	16	10	10	6
3/M	Mean	36.5	32.1	26.0	16.3	108.4	117.9
	SD	5.63	5.53	5.20	3.46	15.96	15.25
	N	26	26	26	10	10	16
4/M	Mean	33.3	26.9**	21.8*	6.4***	89.1**	91.3*
	SD	4.86	5.69	6.47	3.37	9.24	18.29
	N	16	16	16	10	10	6
	Statistics	A	A	A	A	A	A
1/F	Mean	16.4	16.1	15.4	7.5	54.0	55.0
	SD	6.52	3.24	5.79	4.65	7.63	10.22
	N	16	16	16	10	10	6
2/F	Mean	18.4	16.8	12.3	10.0	56.1	64.4
	SD	5.13	3.67	4.00	3.92	7.23	4.10
	N	16	15	15	10	10	5
3/F	Mean	19.9	17.8	14.3	7.4	58.4	61.4
	SD	5.46	3.43	3.16	4.14	7.17	9.36
	N	16	16	16	10	10	6
4/F	Mean	18.8	12.7*	9.7**	0.4***	39.6**	54.7
	SD	4.92	3.54	4.96	2.95	10.68	6.19
	N	16	16	16	10	10	6
	Statistics	A	A	A	A	A	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's

Food Consumption

There was no clear effect on food consumption.

Ophthalmic Examination

No drug-related ophthalmic findings were noted. One HD female (149F) had an opaque right eye at Week 4, but because there were no pathological correlates, this finding was considered to be incidental by the sponsor.

Hematology

There were no drug-related changes in hematology parameters.

Clinical Chemistry

There were no apparent drug-related changes in clinical chemistry parameters. Some samples from all groups were considered unsuitable for analysis due to hemolysis; however, there were enough remaining in the groups (minimum of 8/grp) to enable data interpretation and identification of potential effects.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Micronucleus Assessment

Bone marrow was sampled approximately 24 hours following the final dose. Vehicle and positive control values (%PCE and micronucleated PCE [MN PCE]) were acceptable. There was a decrease in group mean %PCE in HD males, but this was not marked enough to indicate excessive bone marrow toxicity. SS increases in MN PCE frequency were seen at the MD and HD compared to the concurrent vehicle control, and there was a statistically significant linear trend indicating a dose response (Tables 2 and 3). Frequencies at the MD were generally within the laboratory's historical vehicle control distribution, but individual MN PCE frequencies at the HD all clearly exceeded the laboratory's historical control distribution. It was concluded that RIS induced micronuclei in bone marrow PCEs of male and female rats at doses of 3 and 9 mg/kg/day.

Table 2 Summary of Micronucleus Data – Males

Group / Treatment (mg/kg/day)	PCE Scored	%PCE	No. MN PCE	%MN PCE	SD	Heterogeneity		Wilcoxon Rank	
						X^2	S	P-value	S
1M / Vehicle (0)	20000	52.12	19	0.10	0.02	0.74	NS		
2M / RO7034067 (1)	20000	50.12	19	0.10	0.05	4.43	NS	0.6190	NS
3M / RO7034067 (3)	20000	54.72	34	0.17	0.03	1.00	NS	0.0079	p≤0.01
4M / RO7034067 (9)	20000	43.40	924	4.62	1.87	127.41	p≤0.001	0.0040	p≤0.01
5M / CPA (20)	12000	25.53	150	1.25	0.17			0.0179	p≤0.05

Terpstra-Jonckeere trend test (Groups 1,2,3,4; upper tail) P-value: <0.0001, p≤0.001

Table 3 Summary of Micronucleus Data – Females

Group / Treatment (mg/kg/day)	PCE Scored	%PCE	No. MN PCE	%MN PCE	SD	Heterogeneity		Wilcoxon Rank	
						X^2	S	P-value	S
1F / Vehicle (0)	20000	56.92	29	0.15	0.05	3.25	NS		
2F / RO7034067 (1)	20000	54.72	35	0.18	0.11	11.73	p≤0.05	0.5397	NS
3F / RO7034067 (3)	20000	52.26	72	0.36	0.11	5.80	NS	0.0040	p≤0.01
4F / RO7034067 (9)	20000	21.68	1324	6.62	0.33	2.90	NS	0.0040	p≤0.01
5F / CPA (20)	12000	35.53	157	1.31	0.55			0.0179	p≤0.05

Terpstra-Jonckeere trend test (Groups 1,2,3,4; upper tail) P-value: <0.0001, p≤0.001

Organ Weights

Thymus to BW ratios and thymus to brain weight ratios were decreased at the HD. This correlated with findings seen macroscopically and microscopically in the thymus. At the end of the recovery period thymus weights were still lower in HD males (NS).

Absolute and/or relative liver weights and adrenal and kidney to BW ratios were also increased at the HD, but there were no associated microscopic findings.

Gross Pathology

Small thymus was found in in 2/10 HD males and 3/10 HD females, which correlated with findings seen microscopically. Large adrenal was seen in 2/10 HD females given 9 mg/kg/day, but there was no microscopic correlate.

Histopathology

Thymic atrophy was seen at the HD, which correlated with the lower organ weight ratios and the findings of small thymus seen at necropsy. Atrophy was characterized by a generalized reduction in cellularity in both cortex and medulla.

Increased apoptosis/single cell necrosis, characterized by occasional necrotic cells and/or cellular debris in the crypt epithelium, was seen in the duodenum, jejunum, ileum, caecum, and rectum of HD animals (rectum findings only in females).

Increased degenerated spermatocytes in the testis was noted in 4/10 HD males. The finding was characterized by increased numbers of degenerated spermatocytes, mainly in stage XIV tubules, above background levels.

Table 4. Summary of Microscopic Findings – Terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	1	3	9	0	1	3	9
Thymus	No. examined:	10	10	10	10	10	10	10	10
Atrophy	Grade -	10	10	10	2	10	10	10	0
	1	0	0	0	5	0	0	0	2
	2	0	0	0	2	0	0	0	5
	3	0	0	0	1	0	0	0	3

Duodenum	No. examined:	10	10	10	10	10	10	10	10
Increased apoptosis/single cell necrosis	Grade -	10	10	10	6	10	10	10	1
	1	0	0	0	4	0	0	0	9
Jejunum	No. examined:	10	10	10	10	10	10	10	10
Increased apoptosis/single cell necrosis	Grade -	10	10	10	2	10	10	10	1
	1	0	0	0	8	0	0	0	9
Ileum	No. examined:	10	10	10	10	10	10	10	10
Increased apoptosis/single cell necrosis	Grade -	10	10	10	1	10	10	10	0
	1	0	0	0	9	0	0	0	9
	2	0	0	0	0	0	0	0	1
Caecum	No. examined:	10	10	10	10	10	10	10	10
Increased apoptosis/single cell necrosis	Grade -	10	10	10	4	10	10	10	1
	1	0	0	0	6	0	0	0	9
Rectum	No. examined:	10	0	0	10	10	10	10	10
Increased apoptosis/single cell necrosis	Grade -	10	0	0	10	10	10	10	6
	1	0	0	0	0	0	0	0	4
Testis	No. examined:	10	10	10	10	0	0	0	0
Increased degenerated spermatocytes	Grade -	10	10	10	6	0	0	0	0
	1	0	0	0	4	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate.

At the end of the recovery period, microscopic findings in the thymus, duodenum, jejunum, ileum, caecum, and rectum of previously treated animals were similar to controls. However, testis seminiferous tubule atrophy was seen in 1/6 HD males and seminal vesicle contraction was increased at the HD (Table 5).

Table 5. Summary of Microscopic Findings – Recovery necropsy

Test Item	Control	RO7034067		CPA Positive Control				
Group	1	2	3	4	5			
Dose level (mg/kg/day)	0	1	3	9	20			
Tissue/ Observation / Group/Sex: Number of Animals:	1/M 6	2/M 6	3/M 6	4/M 6	1/F 6	2/F 5	3/F 6	4/F 6
Testis — Number Examined:	6	6	6	6	0	0	0	0
Unremarkable:	6	6	6	5	0	0	0	0
Atrophy, tubule — finding not present -	6	6	6	5	0	0	0	0
minimal 1	0	0	0	1	0	0	0	0
Total Incidence:	0	0	0	1	0	0	0	0
Ovary — Number Examined:	0	0	0	0	0	1	0	0
Unremarkable:	0	0	0	0	0	0	0	0
Cystic bursa — finding not present -	0	0	0	0	0	0	0	0
Present	0	0	0	0	0	1	0	0
Seminal Vesicle — Number Examined:	0	1	0	4	0	0	0	0
Unremarkable:	0	0	0	0	0	0	0	0
Contraction — finding not present -	0	0	0	0	0	0	0	0
Present	0	1	0	4	0	0	0	0

Toxicokinetic

TK data for RIS are shown in Table 6. The highest levels in tissues, excluding plasma, were observed in the skin, followed by the retina, brain and vitreous humor. Tissue to plasma ratios ranged from 0.404 to 0.938 for brain, from 1.32 to 28.5 for skin, from 0.430 to 2.61 for retina, and from 0.100 to 1.01 for vitreous humor.

Table 6. Plasma TK parameters for risdiplam

Occasion	Dose (mg/kg/day)	C_{max} (ng/mL) M/F	$AUC_{(0\text{-}24h)}$ ((ng·h)/mL) M/F
Day 1	1	127/139	821/1150
	3	499/628	2970/3650
	9	1630/2010	11100/15500
Day 28	1	217/200	1540/1650
	3	584/684	5450/5890
	9	1370/1730	15300/17700

Study title: RO7034067: A 26-Week Oral Gavage Toxicity Study in the Albino Rat Followed By an 8-Week Recovery

Study no.:	8001273
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02 December 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 1508SA01/ 99%

Key Study Findings

Daily oral (gavage) administration of RIS (0 (vehicle), 1, 3, or 7.5 mg/kg/day; 10 mL/kg) to Wistar rats (20/sex/grp + 6/sex/grp TK) for 26 weeks resulted in clinical signs, decreased BW, and deaths at the HD that were attributed to microscopic findings in the intestinal tract (crypt single cell necrosis and/or degeneration/necrosis) and bone marrow (decreased cellularity). Upon retinal examination, there was a dose-related reduction in scotopic b-wave amplitudes in drug-treated females compared to control at Weeks 13 and 26. A similar trend of reduced amplitudes in drug-treated groups was observed for photopic responses in females. No clear ERG changes were observed in males, and no clear differences among groups of either sex were observed at the end of the recovery period. Drug-related microscopic findings included single cell necrosis in the epithelia of the GI tract (MD and HD), hard palate (HD), and pancreas (all doses); degeneration/atrophy of the seminiferous tubules in the testis and degeneration/necrosis of ductular epithelium in the epididymis (MD and HD); estrous cycle arrest (MD and HD); and cortical pigmentation (consistent with lipofuscin/ceroid and correlating with the gross change of dark discoloration) of the adrenals (all doses). Following the 8-week recovery period, adrenocortical lipofuscin/ceroid pigmentation (all doses) and testis changes (HD) were still present. The low-effect dose (1 mg/kg/day) for ERG changes was associated with Day 182 Cmax and AUC values of 203/295 ng/mL and 2200/2400 ng•h/mL in males/females, respectively.

Methods

Doses:	0 (vehicle), 1, 3, or 7.5 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Rat / Crl: WI (Han)
Number/Sex/Group:	20/sex/group
Age:	7 Weeks
Weight:	M: 164 to 207 g, F: 111 to 156 g
Satellite groups:	6/sex/grp TK and recovery
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Group No.	Dose Level (mg/kg/day) [b]	No. of Animals			
		Main Study		Recovery (Toxicokinetic)[a]	
		Males	Females	Males	Females
1	0	20	20	6	6
2	1	20	20	6	6
3	3	20	20	6	6
4	7.5	20	20	6	6

[a] Recovery animals were sacrificed 8 weeks following the last dose and served also as Toxicokinetic animals.

Dose selection was based on the results of the 4-week oral gavage rat study (Roche Study # 8356P15) with doses of 1, 3 and, 9 mg/kg/day in which decreased BW gain (17/27% in male/females), induction of micronuclei in polychromatic erythrocytes of the bone marrow, and histopathology changes (thymic atrophy, increased apoptosis/single cell necrosis in the duodenum, jejunum, ileum, cecum and rectum, and an increase of degenerated spermatocytes in the testis of males) were seen primarily at the HD.

Observations and Results

Mortality

There were 7 drug-related early deaths: 4 HD males and 3 HD females were either found dead or euthanized due to poor and/or deteriorating condition between Days 27 and 178. The cause of death was attributed to the drug-related marked decreased cellularity of the bone marrow in combination with intestinal tract findings. Findings in the intestinal tract consisted of single cell necrosis and/or minimal to marked degeneration/necrosis of the crypts and rarely the surface epithelium of the intestinal tract (duodenum, jejunum, ileum,

cecum, colon, and rectum). This intestinal tract change was occasionally associated with regenerative crypt hyperplasia that inconsistently correlated with the gross finding of thick wall. Drug-related single cell necrosis was also noted in the hard palate epithelium, exocrine pancreas, lacrimal gland, and mandibular salivary gland. Other drug-related histopathologic changes were noted in the adrenal gland (minimal cortical pigmentation correlating with the gross dark discoloration), mammary gland atrophy in 1 male, testis in 3 males (tubular degeneration/atrophy), epididymis in 1 male (sperm depletion), incisor tooth of all males and 2 females (degeneration/necrosis of odontoblast), and spleen (decreased cellularity of the red pulp correlating with the macroscopic observation of reduced splenic size/small considered secondary to decreased cellularity in the bone marrow). Finally, decreased lymphoid cellularity was observed in the thymus (marked and correlating with the gross finding of small), spleen, GALT, and mandibular and mesenteric lymph node. Stress and the moribund condition might have contributed to these latter findings. Clinical pathology changes noted in the 3 euthanized HD rats included markedly decreased white blood cell counts, absolute neutrophil counts and absolute lymphocyte counts, moderate to marked decreases in the red cell mass parameters with no evidence of regeneration and mild to marked decreases in platelet counts, correlating with the decreased cellularity in the bone marrow (sternum and femur), indicating a markedly decreased hematopoiesis. There were clinical chemistry changes consisting of markedly increased urea and creatinine concentrations noted in 1 female that were observed concurrent with tubular degeneration/necrosis in the kidney.

Clinical Signs

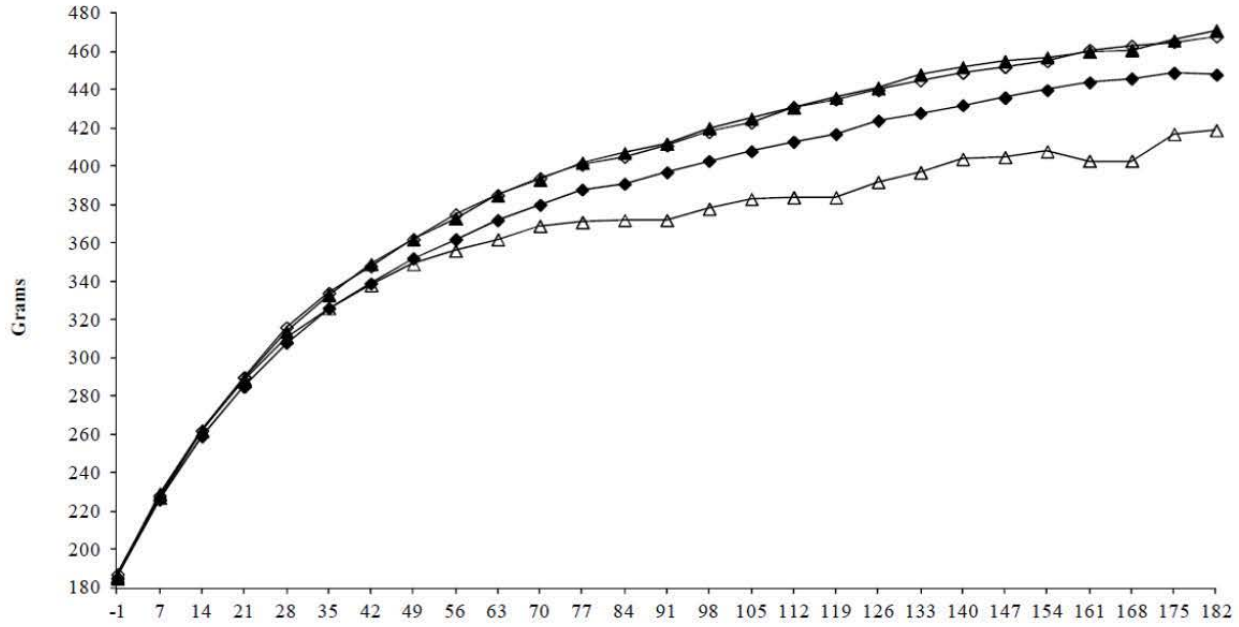
Drug-related clinical signs at the HD included prominent backbone (16/52 animals), hunched posture (48/52 animals), dehydration (10/52 animals), weakness (5/52 animals), and decreased activity (24/52 animals), which led to the death or pre-terminal euthanasia of 4 males and 3 females at that dose.

Body Weights

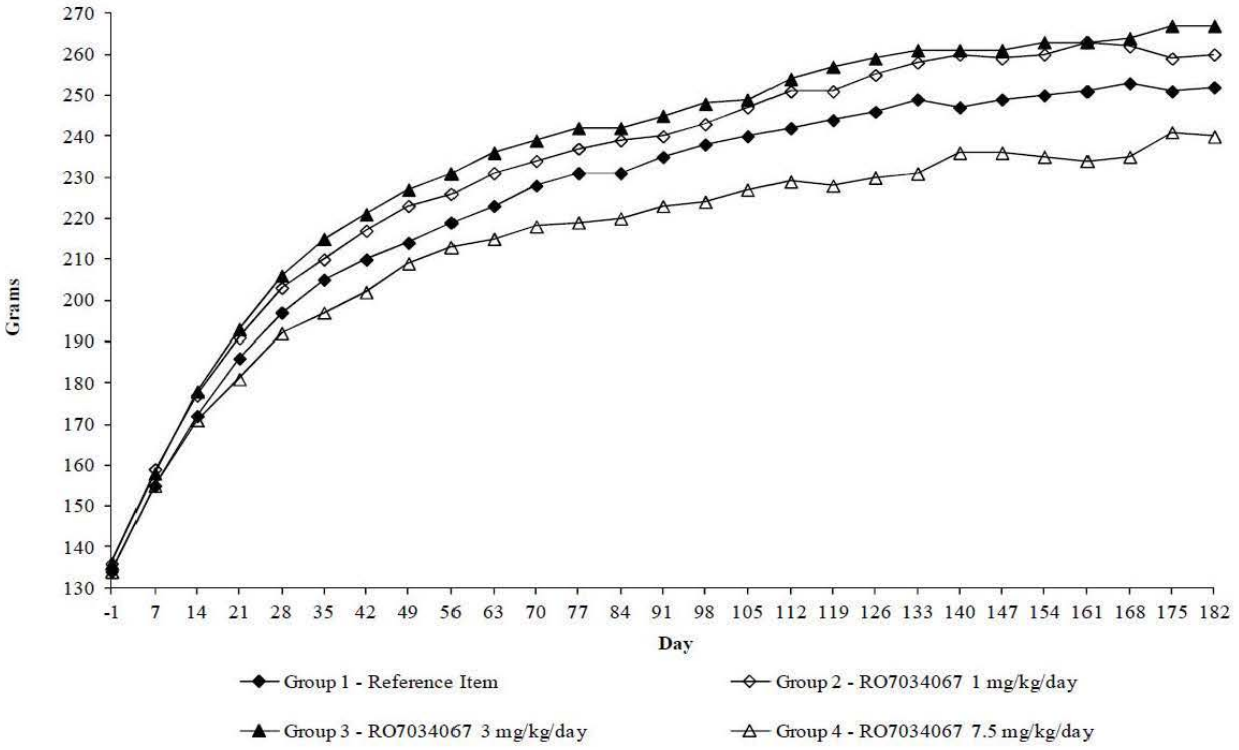
BW gain and BWs were decreased at the HD (Figures 1). HD males and females gained 12/10% less than controls over the dosing period. By the end of the recovery period, there were no differences in BW or BW gain across groups.

Figure 1

Summary of Body Weights - Males



Summary of Body Weights - Females



Food Consumption

There was no clear effect on food consumption.

Ophthalmic Examination

No drug-related ophthalmic findings were noted.

Hematology

There were no drug-related changes in hematology or coagulation parameters.

Clinical Chemistry

Drug-related decreases in potassium and chloride concentrations were seen at all doses. These findings were thought possibly related to concurrent GI tract single cell necrosis. These changes were no longer seen at the end of the recovery period.

Urinalysis

Decreases in urine specific gravity, associated with increases in urine volume, were observed in MD and HD females at 12 and 26 weeks despite suspected dehydration at the same doses throughout the dosing period. However, these findings had no histological correlates, so they were not considered adverse. The changes were no longer seen at the end of the recovery period.

Electroretinography Evaluation

There was a dose-related reduction in scotopic b-wave amplitudes in drug-treated females compared to control at Weeks 13 and 26 (Table 1). At Week 26, group mean b-waves were reduced by 34-40% at the HD, and by 32-39% at the MD, compared to C for each stimulus. A similar trend of reduced amplitudes in drug-treated groups was observed for photopic responses in the females. These findings were dismissed in the study report based on the observed variability and the absence of any clinical signs related to loss of visual function, correlating ophthalmology or histopathological findings, or similar effect in males. However, given the consistent, dose-related pattern across stimulus conditions, it seems likely that the effect was drug-related. No clear ERG changes were observed in males, and no clear differences among groups of either sex were observed at the end of the recovery phase.

Table 1.

Summary of Electroretinography

Scotopic Single Flash -20dB - B-Wave Amplitude (µV)/Implicit Time (ms)
Week 26
Females

Group 1 - Reference Item
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Amplitude Combined Eyes	Implicit Time Combined Eyes
1	Mean	464.56	70.04
	SD	135.78	3.71
	N	12	12
2	Mean	369.26	70.05
	SD	75.81	2.76
	N	10	10
3	Mean	295.75	71.46
	SD	114.67	4.40
	N	12	12
4	Mean	279.44	70.58
	SD	68.69	5.87
	N	12	12

Scotopic Single Flash 10dB - B-Wave Amplitude (µV)/Implicit Time (ms)
Week 26
Females

Group 1 - Reference Item
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Amplitude Combined Eyes	Implicit Time Combined Eyes
1	Mean	743.45	82.29
	SD	161.93	5.88
	N	12	12
2	Mean	601.62	87.10
	SD	107.18	5.08
	N	10	10
3	Mean	506.31	82.67
	SD	201.10	10.04
	N	12	12
4	Mean	493.06	81.67
	SD	101.06	5.72
	N	12	12

Organ Weights

A decrease in absolute testis weight was seen in HD males, which correlated with a gross finding of reduced size (small) and microscopically with tubular degeneration/necrosis. HD males also showed a decreased absolute and relative (to brain) epididymis weights that correlated with a gross finding of small epididymis and histopathologically with decreased sperm. A decrease in uterine absolute and relative weights was noted in MD and HD females, associated with a shift toward estrous cycle arrest. Increased adrenal

weights were seen at the HD but with no microscopic correlate. Decreased thymic weights in HD males correlated with reduced thymus size and decreased lymphoid cellularity observed microscopically. Decreased absolute and relative uterine weights persisted in HD females after the 8-week recovery period, but there were no other organ weight changes at that time.

Gross Pathology

Drug-related gross findings were noted in the testis, epididymis, adrenal gland, and thymus in males and/or females (see above).

Histopathology

In the GI tract, single cell necrosis of epithelial tissue was noted in the crypt of the duodenum, jejunum, ileum, cecum, colon, and rectum mainly in HD males and females but also in 1 MD male (Table 2). Single cell necrosis was observed in the hard palate epithelium at the HD and in pancreatic acini at the MD and HD as well as in 1 LD male.

Microscopic changes in the testes of MD and HD males consisted of minimal to marked degeneration/atrophy of the seminiferous tubules characterized by segmental to circumferential germ cell depletion, sertoli cell vacuolation, occasional single cell necrosis of germ cell, primarily meiotic stage XIV spermatocytes, and rare spermatid retention. Minimal to marked decreased sperm content was observed in the epididymis of 4 HD males and was considered secondary to the degeneration/atrophy of the seminiferous tubules in the testis. In addition, minimal degeneration/necrosis of ductular epithelium, with interstitial mononuclear cell infiltration, was present in the epididymis of one of the affected HD males.

Mammary gland microscopic changes characterized by minimal to moderate glandular atrophy, associated with increased intracellular brown granular pigment consistent with hemosiderin, were seen in HD males.

Adrenocortical pigmentation was noted in males and females at all doses. This was characterized by brown granular pigment within the cells of the zona reticularis and fascicularis, consistent with lipofuscin/ceroid and correlated with the dark discoloration seen macroscopically. Adrenocortical pigmentation was not associated with any degenerative lesion. In addition, cortical hemorrhage was noted in MD and HD females. No accumulation of hemosiderin was associated with the hemorrhage.

Drug-related findings in the thymus consisted of decreased lymphoid cellularity in HD males and females.

At the MD and HD, females were more often in diestrus compared to their respective controls. This was considered a shift toward an estrous cycle arrest.

Table 2. Summary of Microscopic Findings – Terminal necropsy

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**1**	**3**	**7.5**	**0**	**1**	**3**	**7.5**
No. Animals Examined	**20**	**20**	**19**	**17**	**20**	**20**	**20**	**17**
Large intestine, cecum (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial	(0)[a]	(0)	(0)	(4)	(0)	(0)	(0)	(3)
Minimal	0	0	0	4	0	0	0	3
Large intestine, colon (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial	(0)	(0)	(1)	(4)	(0)	(0)	(0)	(4)
Minimal	0	0	1	4	0	0	0	4
Large intestine, rectum (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial	(0)	(0)	(0)	(4)	(0)	(0)	(0)	(3)
Minimal	0	0	0	4	0	0	0	3
Small intestine, duodenum (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial	(0)	(0)	(0)	(7)	(0)	(0)	(0)	(6)
Minimal	0	0	0	7	0	0	0	6
Small intestine, ileum (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial	(0)	(0)	(0)	(13)	(0)	(0)	(0)	(12)
Minimal	0	0	0	13	0	0	0	12
Small intestine, jejunum (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial	0	0	0	(13)	0	0	0	(9)
Minimal	0	0	0	13	0	0	0	9
Body cavity, nasal (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; epithelial; hard palate	(0)	(0)	(0)	(12)	(0)	(0)	(0)	(10)
Minimal	0	0	0	12	0	0	0	10
Pancreas (No. Examined)	20	20	19	17	20	20	20	17
Single cell necrosis; acinar	(0)	(1)	(1)	(7)	(0)	(0)	(4)	(5)
Minimal	0	1	0	5	0	0	2	4
Mild	0	0	1	2	0	0	2	1

Testis (No. Examined)	20	20	19	17	N/A	N/A	N/A	N/A
Degeneration/atrophy; tubular	(0)	(0)	(2)	(10)	(-)	(-)	(-)	(-)
Minimal	0	0	2	5	-	-	-	-
Mild	0	0	0	3	-	-	-	-
Marked	0	0	0	2	-	-	-	-
Epididymis (No. Examined)	20	19	19	17	N/A	N/A	N/A	N/A
Decreased; sperm	(0)	(0)	(0)	(4)	(-)	(-)	(-)	(-)
Minimal	0	0	0	1	-	-	-	-
Mild	0	0	0	1	-	-	-	-
Moderate	0	0	0	1	-	-	-	-
Marked	0	0	0	1	-	-	-	-
Degeneration/necrosis; ductular	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
Minimal	0	0	0	1	-	-	-	-
Gland, adrenal (No. Examined)	20	20	19	17	20	20	20	17
Pigmentation; cortical	(0)	(2)	(19)	(17)	(0)	(20)	(19)	(17)
Minimal	0	2	18	13	0	20	9	1
Mild	0	0	1	4	0	0	10	8
Moderate	0	0	0	0	0	0	0	8
Hemorrhage; cortical	(0)	(0)	(0)	(0)	(0)	(0)	(8)	(9)
Minimal	0	0	0	0	0	0	6	8
Mild	0	0	0	0	0	0	2	1
Thymus (No. Examined)	20	20	19	17	20	20	20	17
Decreased cellularity; lymphoid	(0)	(0)	(0)	(13)	(0)	(0)	(0)	(12)
Minimal	0	0	0	6	0	0	0	7
Mild	0	0	0	6	0	0	0	4
Moderate	0	0	0	1	0	0	0	1
Gland, mammary (No. Examined)	18	20	17	16	20	N/A	N/A	17
Atrophy	(0)	(0)	(0)	(12)	(0)	-	-	(0)
Minimal	0	0	0	3	0	-	-	0
Mild	0	0	0	6	0	-	-	0
Moderate	0	0	0	3	0	-	-	0
Vagina (No. Examined)	N/A	N/A	N/A	N/A	20	20	20	17
Diestrus	(-)	(-)	(-)	(-)	5	5	8	9
Proestrus	-	-	-	-	4	6	4	2
Estrus	-	-	-	-	4	6	4	2
Metestrus	-	-	-	-	7	3	4	4

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

Microscopic findings in the testis and cortical pigmentation in the adrenal gland remained after the recovery period (Table 3). Testicular changes remained with similar incidence but lower severity. Degeneration/atrophy of the seminiferous tubules in the testis in individual HD recovery animals was occasionally associated with secondary minimal luminal debris in the epididymis.

Adrenocortical pigmentation (consistent with lipofuscin/ceroid) was still present after the recovery period in all dose groups with similar incidence and severity.

Table 3. Summary of Microscopic Findings – Recovery necropsy

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**1**	**3**	**7.5**	**0**	**1**	**3**	**7.5**
No. Animals Examined	**6**	**6**	**6**	**5**	**6**	**5**	**6**	**6**
Testis (No. Examined)	6	6	6	5	N/A	N/A	N/A	N/A
Degeneration/atrophy; tubular	(1)[a]	(0)	(0)	(4)	(-)	(-)	(-)	(-)
Minimal	0	0	0	3	-	-	-	-
Marked	1	0	0	1	-	-	-	-
Epididymis (No. Examined)	6	6	6	5	N/A	N/A	N/A	N/A
Cellular debris; lumen	(0)	(0)	(0)	(2)	(-)	(-)	(-)	(-)
Minimal	0	0	0	2	-	-	-	-
Gland, adrenal (No. Examined)	6	6	6	5	6	5	6	6
Pigmentation; cortical	(0)	(6)	(6)	(5)	(0)	(5)	(6)	(6)
Minimal	0	6	6	4	0	5	1	0
Mild	0	0	0	1	0	0	5	3
Moderate	0	0	0	0	0	0	0	3

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

Toxicokinetic

TK data for RIS are shown in Table 4.

Table 4. Plasma TK parameters for risdiplam

Day	Dose (mg/kg)	Gender	Cmax ± SE (ng/mL)		AUC(0-24) ± SE (hr*ng/mL)		R_{AUC}
182	1	Female	295	27.0	2400	378	2.39
	3	Female	768	13.0	8830	228	2.71
	7.5	Female	1170	135	15700	799	1.61
	1	Male	203	19.0	2200	140	2.71
	3	Male	651	143	7010	244	2.67
	7.5	Male	1140	125	16100	164	1.85

Study title: A 26- Week Oral Gavage Retinopathy Study of RO7034067 and RO6885247 in Pigmented Rats

Study no.:	5700653
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	30 Mar 2016
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	RIS/ (b) (4) 024780-026/ 97.66%

Key Study Findings

Daily oral (gavage) administration of RIS (0 (vehicle) or 7.5/5 mg/kg/day; 10 mL/kg) to male Brown Norway rats (24/sex/grp + 6/sex/grp TK) for 26 weeks resulted in mortality due to decreased cellularity of the bone marrow in combination with intestinal tract findings, reduced body weight and food intake, and red cell mass reductions. Macroscopic findings included small and soft testis, small epididymis, and small thymus and spleen. Microscopic findings consisted of single cell necrosis of the crypts and the surface epithelium of the GI tract, single cell necrosis of the pharyngeal epithelium and pancreas, adrenocortical pigmentation, degeneration/atrophy of the seminiferous tubules in the testis, and degeneration and necrosis of the ductular epithelium with decreased sperm with luminal cellular debris in the epididymis. Decreased lymphocytes were observed in the thymus, spleen, GALT, and mandibular and mesenteric lymph node. Ocular findings on ophthalmoscopic examination consisted of slight diffuse vitreous haziness in the retinal area in 9/18 animals examined starting at Week 19, and retinal vessel attenuation in 2 animals at Week 26. Electroretinography evaluation showed a progressive reduction in photopic b-wave amplitudes in response to the 0.5 Hz and 29 Hz stimuli over the course of the dosing period. A similar trend was noted for scotopic a- and b-wave amplitude starting at Week 13. A reflective band was seen in the vitreous by SD-OCT which was thought to be related to the haziness in the retinal area observed during the ophthalmology examination. Plasma levels associated with the single dose level evaluated were 14500 ng.h/mL at the end of the dosing period. RIS concentrations in choroid were at least 4 orders of magnitude higher than comparable plasma Cmax, and retina concentrations were about 1 order of magnitude higher than comparable plasma Cmax.

Methods

Doses:	0 (vehicle) or 7.5/5 mg/kg/day RIS
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Rat / Brown Norway
Number/Sex/Group:	24 males/group
Age:	8.5 Weeks
Weight:	M: 139 to 182 g
Satellite groups:	6 males/grp TK
Unique study design:	One dose level of 2 different drugs tested
Deviation from study protocol:	None that impacted study quality or integrity

Experimental Design

Group No.	Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Concentration (mg/mL)	No. of Animals (Males)	
					Main Study	Toxicokinetic
1	Reference Item	0	10	0	24	6
2	RO7034067	7.5 / 5[a]	10	0.75 / 0.5[a]	24	6
3	RO6885247	18	10	1.8	24	6

[a] Dosing holiday: Effective from 17 May 2016 (Day 47/46), Group 2 animals were not dosed due to excessive effects. Animals were given a treatment-free period and dosing resumed 23 May 2016 (Day 53/52) at a lower dose level (5 mg/kg).

Dose selection for RIS was based on the results of the 4-week oral gavage rat study (Roche # 8356P15) with doses of 1, 3 and, 9 mg/kg/day in which decreased BW gain (17/27% in male/females), induction of micronuclei in polychromatic erythrocytes of the bone marrow, and histopathology changes (thymic atrophy, increased apoptosis/single cell necrosis in the duodenum, jejunum, ileum, cecum and rectum, and an increase of degenerated spermatocytes in the testis of males) were seen primarily at the HD.

Observations and Results

Mortality

There were 7 drug-related early deaths in the main study group between Days 42 and 47 and in 2 TK animals on Days 48 or 49. After the dose was reduced to 5 mg/kg/day beginning on Day 52/53, 3 additional main study animals were found dead between Days 176 and 182, which prompted the scheduled termination of the remaining animals in this group a few days earlier than in controls. Deaths were attributed to moderate to marked decreased cellularity of the bone marrow of femur and sternum in combination with intestinal tract findings.

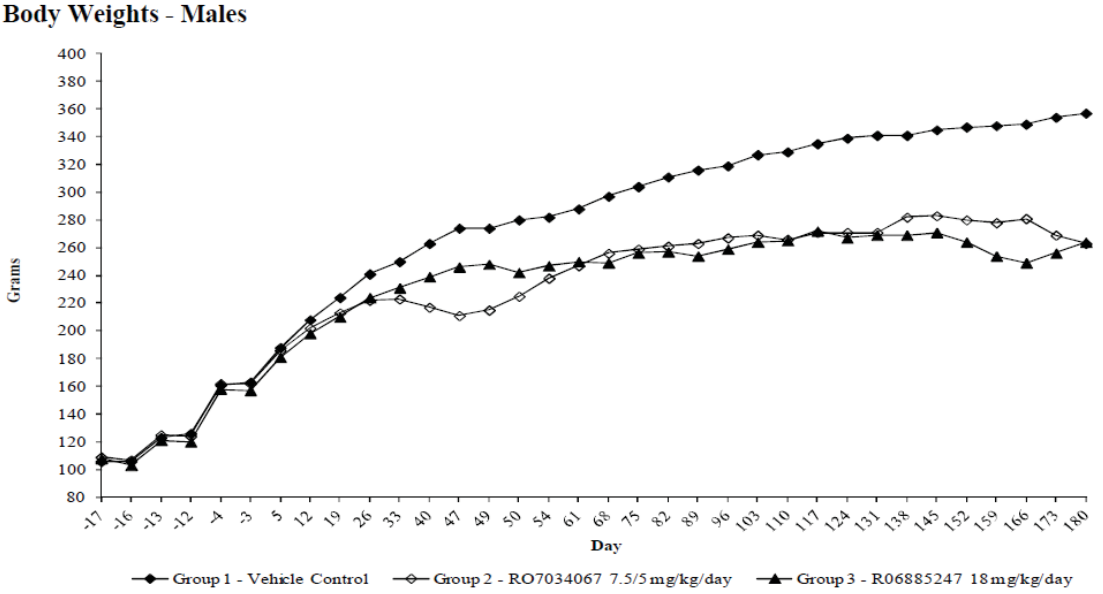
Clinical Signs

Drug-related clinical signs consisted of dehydrated with prominent thin/backbone starting at Day 40. Within a week of the onset of these findings, the condition had deteriorated, and several animals were found dead, and signs of weakness, decreased activity and/or muscle tone, lying on side, erect fur, and labored breathing resulted in euthanasia of 2 additional animals. After dose reduction to 5 mg/kg/day, the condition of the remaining animals improved somewhat, but animals generally remained dehydrated and thin until termination and appeared to deteriorate during the last week of dosing.

Body Weights

BW gain and BWs were decreased in the drug-treated group (Figures 1). By the end of the dosing period, BW in the RIS-treated group was 26% below C (SS).

Figure 1.



Food Consumption

Marked reductions in food consumption were present from Day 56 and correlated with decreases BWs.

Ophthalmic Examination

Ocular findings were observed starting at Week 19 in 9/18 animals given RIS (RO7034067) and at Week 8 or 9 in all animals given the related splicing modifier RO6885247 (for which clinical development was discontinued as a result of retinal toxicity observed in a chronic 39-week monkey toxicity study [described in Ratni et al. 2018]). This change was described as a diffuse retinal haziness, which became more prominent over time but was graded ‘slight’ in most eyes, while other ocular structures appeared normal. Retinal vessel attenuation was observed in 3 RIS-treated animals at Week 26.

Hematology

Red cell mass (count, hemoglobin, hematocrit, reticulocytes) was decreased by 30-35% at Week 22 and by 50-60% at Week 26/27 in animals given RIS compared to C. White blood cell count was also reduced by ~25%, mainly due to fewer circulating lymphocytes.

Clinical Chemistry

There were no clearly drug-related changes.

Electroretinography Evaluation

Photopic b-wave amplitudes in response to the 0.5 Hz and 29 Hz stimuli showed a progressive reduction in amplitude over the course of the dosing period. By Week 26, the group means were reduced up to 82% for both stimuli (Table 1, Figure 1).

A similar, progressive reduction in photopic 29 Hz b-wave amplitude was present in the group given RO6885247, reaching a reduction of -62% at Week 26.

A reduction in scotopic a and b-wave amplitude compared to C was observed in the drug-treated groups in almost all tests, starting at about Week 13. However, since the control group also showed amplitude reductions and the responses remained within the range of variability observed in the control, they were considered unrelated to drug administration in the study report; however, given the findings in monkeys and in this and previous rat studies, it seems more likely that these findings were also drug-related.

Table 1. Summary of Electroretinography

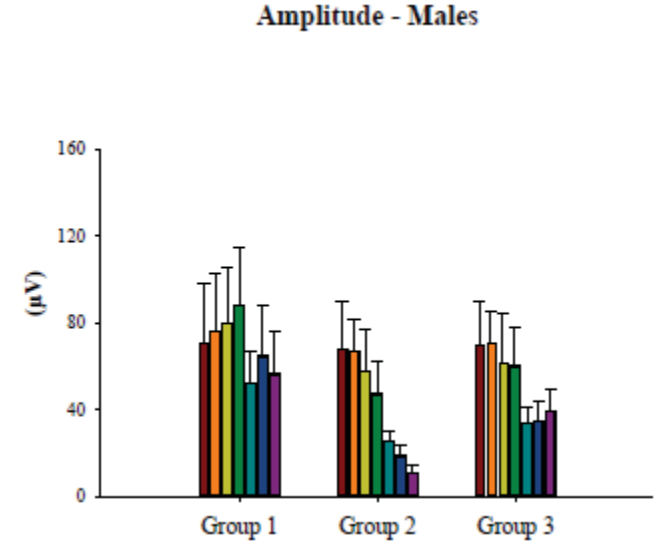
Photopic 29Hz Flicker - B-Wave Amplitude (µV)/Latency (ms)
Week 26
Males

Group 1 - Vehicle Control
Group 2 - RO7034067 7.5/5 mg/kg/day
Group 3 - RO6885247 18 mg/kg/day

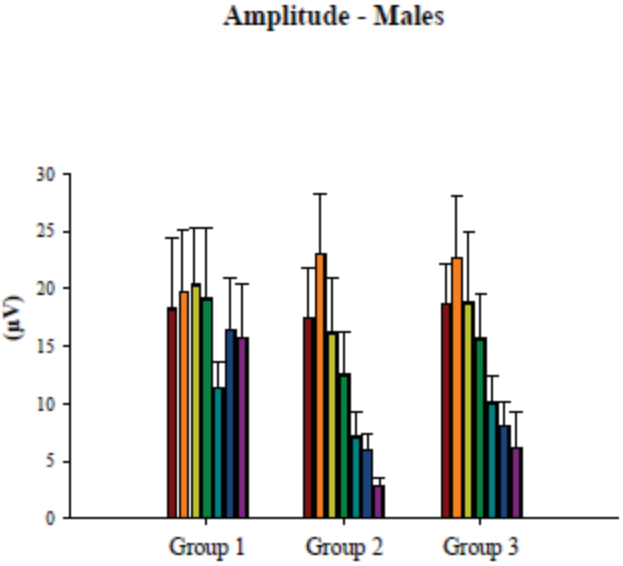
Group	Summary Information	Amplitude Combined Eyes	Latency Combined Eyes
1	Mean	15.66	37.75
	SD	4.68	1.06
	N	12	12
2	Mean	2.81	38.56
	SD	0.80	9.93
	N	8	8
3	Mean	6.13	39.60
	SD	3.23	11.85
	N	10	10

Figure 1. Summary of Electroretinography

Photopic 0.5Hz Flicker - B-Wave



Photopic 29Hz Flicker - B-Wave



Group 1 - Vehicle Control
Group 2 - RO7034067 7.5/5 mg/kg/day
Group 3 - RO6885247 18 mg/kg/day

Prestudy
Week 2
Week 4
Week 8
Week 13
Week 19
Week 26

Optical coherence tomography (OCT)

Reflectivity was observed in the vitreous in proximity to the retina and progressed over time. It was noted in all groups but with a slightly higher severity in the drug-treated groups by Week 26. It was thought that this may have contributed to the hazy appearance of the retina during the ophthalmology examination.

Gross Pathology

Drug-related gross findings consisted of an increased incidence of small testis and small thymus in animals sacrificed in Week 19, and increased incidence of small and soft testis, small epididymis and small thymus in animals sacrificed in Week 26. No histopathologic examination was performed, but these findings were considered drug-related since degeneration/atrophy of the seminiferous tubules in the testis, reduced sperm in the epididymis, and decreased lymphocytes in the thymus were seen in animals that died in this study and in previous studies. No macroscopic findings were observed in the eyes.

Histopathology

In animals that died or were euthanized prior to scheduled termination, the cause was attributed to moderate to marked decreased cellularity of the bone marrow of femur and sternum in combination with intestinal tract findings. Findings in the intestinal tract consisted of single cell necrosis of the crypts and the surface epithelium of duodenum, jejunum, ileum, cecum, colon, and rectum, which was associated with villous atrophy and regenerative crypt hyperplasia. In individual animals, drug-related single cell necrosis was also noted in the pharyngeal epithelium and the exocrine pancreas. Histopathological changes were noted in the testis and epididymis and consisted of degeneration/atrophy of the seminiferous tubules in the testis and degeneration and necrosis of the ductular epithelium and secondary decreased sperm with luminal cellular debris in the epididymis. Decreased lymphocytes were observed in the thymus (correlating with the gross finding of small thymus), spleen (correlating with small spleen), GALT, and mandibular and mesenteric lymph nodes.

Histopathology was conducted only on eyes, brain, adrenal gland, and pancreas. Drug-related findings were seen in the adrenal gland and consisted of minimal cortical pigmentation in animals sacrificed at Week 26 only. The pigmentation was characterized by brown granular pigment within the cells of the zona reticularis and fascicularis, consistent with lipofuscin/ceroid as revealed by the Schmorl's stain. Adrenocortical pigmentation was not associated with any degenerative lesion. No drug-related findings were present in retina, brain (including substantia nigra), or pancreas at scheduled euthanasia at Weeks 13, 19, and 26.

Toxicokinetic

TK data for RIS (and related drug RO6885247 and its major metabolite) are shown in Table 3. RIS concentrations in choroid were at least four orders of magnitude higher than comparable plasma Cmax, and retina concentrations were about one order of magnitude higher than comparable plasma Cmax.

Table 3.

Summary of TK results on Day 176

Treatment Duration	Animals/ Dose	Test Item	Occasion and Dose Level (mg/kg/day)	Cmax (ng/mL) M	AUC(0-24h) ((hr*ng)/mL) M
26 weeks	6	RO6885247	Day 1 18	269	5100
			Day 28 18	512	10600
			Day 176 18	473	10700
		RO6885241	Day 1 18	3.67	65.9
			Day 28 18	20.2	436
			Day 176 18	25.2	544
		RO7034067	Day1 7.5	731	5500
			Day 28 7.5	1270	14200
			Day 91* 5	1500	18000
			Day 127 5	997	16800
			Day 176 5	844	14500

* : dose reduction, dosing resumed again on Day 52/53.

Study title: RO7034067: 2 Week Oral (Gavage) Administration Toxicity Study in the Monkey with a 2 Week Treatment-Free Period

Study no.:	1066289
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	27 July 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 024780-026/ 97.66%

Key Study Findings

Daily oral (gavage) administration of RIS (0 (vehicle), 2, 4, or 6 mg/kg/day; 5 mL/kg) to young (112-141 weeks) cynomolgus monkeys (3/sex/grp + 2/sex/C & HD recovery) for 2 weeks resulted in clinical findings in the skin correlating with microscopic findings of parakeratosis, inflammation, erosion/ulcer and epidermis hyperplasia at the HD. In addition, there were histopathological changes in the larynx (epithelial degeneration, erosion/ulcer, inflammation, squamous cell metaplasia), thymus (atrophy), and testis (increases in multinucleate cells, germ cell degeneration) at the MD and/or HD. A single minimal focus of retinal dysplasia was seen in one eye of 1 HD male. The anatomical findings in the skin, larynx and thymus showed complete reversal, but reversal of the testicular effects could not be assessed due to sexual immaturity. The NOAEL (2 mg/kg/day) was associated with RIS exposures of 345 ng/mL (Cmax) and 2550 ng·hr/mL (AUC0-24) at Day 14 for both sexes combined.

Methods

Doses:	0 (vehicle), 2, 4, or 6 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group main + 2/sex/C and HD recovery
Age:	112-141 weeks
Weight:	2.21 to 4.04 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Group number	Group description	Dose level (mg/kg/day)†	Number of animals in group	
			Male	Female
1	Control	0	5	5
2	Low	2	3	3
3	Intermediate	4	3	3
4	High	6	5	5

† Dose levels given in terms of free base; content was 84.8% and a correction factor of 1.18 was used.

Dose selection was based on the results of a 2 week dose range-finding study in the cynomolgus monkey ([(b) (4)] Study Number 8306338) in which the HD of 6 mg/kg/day resulted in histopathological findings in the skin, eyelid, and jejunum. Toxicogenomic analysis was conducted in this study using mRNA isoform specific qPCR marker assays for transcript variants of the APLP2, MADD, STRN3, and FOXM1 genes in spleen, duodenum, and testis. For the mRNAs of APLP2, MADD, and STRN3, a clear treatment-related splicing modification was detected at the HD in all 3 tissues. FOXM1B/C isoforms were reduced by ~75% in the testis of 1 HD male. The changes in the secondary splice targets MADD and FOXM1 were associated with the histopathological changes in the GI tract and testis. Transcripts of the SMN1 gene remained unchanged in alternative splicing in response to treatment.

In the 39-week study in the cynomolgus monkey ([(b) (4)] Study Number 8313482 started on 16 February 2015), the HD of 7.5 mg/kg/day resulted in findings in the skin (reddening and shedding/peeling) and a low incidence of systemic clinical signs (subdued, semi-closed eyes). In the 39-week study, the dose was reduced to 5 mg/kg/day from Day 26, which was tolerated and associated with a low incidence and low severity of skin effects (shedding/peeling). Experience with several compounds with similar pharmacological properties and with similar secondary targets, suggested steep dose-effect relationships. Therefore, the HD of 6 mg/kg/day was not expected to result in non-tolerable side effects, since the skin effects were considered mild at 7.5 mg/kg/day and reversed upon discontinuation of treatment.

Observations and Results

Mortality

There were no deaths during the study.

Clinical Signs

Drug-related clinical signs were primarily located in the skin of MD and HD animals of both sexes beginning on Day 11. Observations of shedding/peeling skin in various locations were recorded in 1/3 MD males, 4/5 HD males, and 4/5 HD females. Reddening

of the face, head, muzzle and/or scrotum was seen beginning on Day 10 in all HD animals. Excessive grooming was also noted on Days 13 and 14 in 2/5 HD males and 1/5 HD females. Semi-closed eyes were observed beginning on Day 11 in all males and 4/5 HD females. Other signs seen less frequently at the HD included hunched posture, increased activity; itching skin, mouth rubbing, twitching body, and staggering. At the end of the recovery period, shedding/peeling skin was still present in 1 HD male and 1 HD female.

Body Weights

BW gain and BWs were unaffected by drug.

Food Consumption

There were no effects on food consumption.

Ophthalmic Examination

No drug-related ophthalmic findings were noted.

Hematology

There were no drug-related changes in hematology or coagulation parameters.

Clinical Chemistry

C-reactive protein concentrations were increased in 1 HD male on Day 10 (8.84 mg/L) and 1 MD male on Day 10 (9.63 mg/L). Since no other inflammatory marker was increased in these animals, the sponsor did not consider this increase drug-related. There were no other drug-related clinical chemistry changes.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Organ Weights

Relative thymus weight ratios were decreased in MD and HD males and in females at all doses. This correlated with thymic atrophy seen microscopically in at least 2 HD males. At the end of the recovery period, thymus weights remained lower in HD females but were without microscopic correlates.

Gross Pathology

Flaky skin/subcutis was seen at the terminal necropsy in most HD animals (3/3 males and 2/3 females) but was no longer present after the recovery period.

Histopathology

Parakeratosis and epidermal hyperplasia of the skin/subcutis (including the skin of the back, taken from the lumbosacral transition, and samples of macroscopic skin findings), occasionally accompanied by inflammation and erosion/ulcer, which correlated with the flaky skin seen macroscopically, were seen in HD animals of both sexes (Table 1).

According to the report:

> Parakeratosis was characterized by nucleated keratinocytes in the outer keratin layer of the epidermis, with generally increased thickness of keratin and occasional

single cell necrosis and vesicle formation in some animals. Inflammation was characterized by infiltration of mainly acute inflammatory cells in the dermis and epidermis. Erosion/ulcer was characterized by loss of epidermal layers, varying from loss of superficial layers to full thickness loss. Epidermal hyperplasia was characterized by increased thickness of the epidermis.

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Table 1. Incidence of selected findings: Skin/subcutis and Back skin – terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	2	4	6	0	2	4	6
Skin/subcutis**	No. examined:	3	3	3	3	3	3	3	3
Parakeratosis	Grade -	3	3	3	0	3	3	3	0
	1	0	0	0	3	0	0	0	2
	2	0	0	0	0	0	0	0	1
Inflammation	Grade -	3	2	3	0	3	3	2	1
	1	0	0	0	2	0	0	0	2
	2	0	1	0	1	0	0	1	0
Erosion/ulcer	Grade -	3	3	3	2	3	3	3	3
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	1	0	0	0	0
Hyperplasia, epidermis	Grade -	3	3	3	0	3	3	3	0
	1	0	0	0	0	0	0	0	1
	2	0	0	0	3	0	0	0	1
	3	0	0	0	0	0	0	0	1
Skin, back	No. examined:	3	3	3	3	3	3	3	3
Parakeratosis	Grade -	3	3	3	1	3	3	3	1
	1	0	0	0	2	0	0	0	2
Hyperplasia, epidermis	Grade -	3	3	3	1	3	3	3	0
	1	0	0	0	0	0	0	0	1
	2	0	0	0	2	0	0	0	2

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate.
** includes samples of macroscopic skin findings.

Epithelial degeneration and inflammation in the vocal folds of the larynx were noted at the MD and HD (Table 2). In addition, individual HD animals showed epithelial degeneration in the lateral ventricle and erosion/ulcer and squamous cell metaplasia in the lateral ventricle of the larynx. Epithelial degeneration in the vocal folds was characterized by single cell necrosis and occasional vesicle formation in the epithelium of the vocal folds. Epithelial degeneration in the lateral ventricle was characterized by single cell necrosis and reduced thickness of the epithelium. Erosion/ulcer was characterized by loss of epidermal layers, varying from loss of superficial layers to full thickness loss. Inflammation

was characterized by infiltration of mainly acute inflammatory cells in the epithelium and submucosa in the area of the vocal folds. Squamous cell metaplasia in the lateral ventricle was characterized by replacement of the normal respiratory epithelium with squamous epithelium.

Table 2. Incidence of selected findings: Larynx – terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	2	4	6	0	2	4	6
Larynx	No. examined:	3	3	3	3	3	3	3	3
Epithelial degeneration, vocal folds	Grade -	3	3	1	0	3	3	1	2
	1	0	0	1	2	0	0	2	1
	2	0	0	1	1	0	0	0	0
Epithelial degeneration, lateral ventricle	Grade -	3	3	3	2	3	3	3	3
	1	0	0	0	1	0	0	0	0
Erosion/ulcer	Grade -	3	3	3	2	3	3	3	3
	1	0	0	0	0	0	0	0	0
	2	0	0	0	1	0	0	0	0
Inflammation	Grade -	3	3	2	1	3	3	1	2
	1	0	0	1	2	0	0	2	1
Squamous cell metaplasia, lateral ventricle	Grade -	3	3	3	3	3	3	3	2
	1	0	0	0	0	0	0	0	1

Key: "-" = finding not present, 1 = minimal, 2 = slight.

Increases in multinucleate cells and germ cell degeneration were seen in the testis of 2/3 HD males (Table 3). Increased multinucleate cells was characterized by increased numbers of degenerate multinucleate cells in the tubule lumen. Degeneration of germ cells was characterized by single cell necrosis and vacuolation of germ cells in the seminiferous tubules. The presence of testis findings could not be properly assessed in MD males due to their sexual immaturity.

Table 3. Incidence of selected findings: Testis – terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	2	4	6	0	2	4	6
Testis	No. examined:	3	3	3	3	0	0	0	0
Increased multinucleate cells	Grade -	3	3	3	1	0	0	0	0
	1	0	0	0	0	0	0	0	0
	2	0	0	0	1	0	0	0	0
	3	0	0	0	1	0	0	0	0
Degeneration, germ cell	Grade -	3	3	3	1	0	0	0	0
	1	0	0	0	1	0	0	0	0
	2	0	0	0	1	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate.

Thymic atrophy was seen in 2/3 HD males (Animals 12M and 14M), correlating with the decreased organ weights seen in these animals (Table 4). Atrophy was characterized by a general loss of lymphocytes from both cortex and medulla, with a reduction in size of the cortex.

Table 4. Incidence of Atrophy: Thymus – terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	2	4	6	0	2	4	6
Thymus	No. examined:	3	3	3	3	3	3	3	3
Atrophy	Grade -	3	3	3	1	3	3	3	3
	1	0	0	0	0	0	0	0	0
	2	0	0	0	2	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight.

A single minimal focus of retinal dysplasia was seen in one eye of 1 HD male. The finding was in the mid- to peripheral retina and was characterized by loss of nuclei in the outer nuclear layer, the presence of nuclei in the photoreceptor layer, thinning of the photoreceptor layer and hypertrophy of the underlying retinal pigment epithelium. According to the report, it was consistent with occasional findings seen as background lesions in cynomolgus monkeys in the laboratory and was therefore considered to be a spontaneous change.

At the end of the recovery period there were no drug-related microscopic findings in the skin, larynx and thymus of animals from the HD group. The reversibility of the testis findings could not be assessed due to the sexual immaturity of the HD males.

Toxicokinetics

TK data for RIS are shown in Table 5.

Table 5. TK data for RIS in 2-week monkey toxicity study

Occasion	Dose (mg/kg/day)	C_{max} (ng/mL) M / F	$AUC_{(0-24h)}$ ((ng·h)/mL) M / F
Day 1	2	373 / 398	1610 / 2110
	4	969 / 1010	5790 / 6340
	6	1290 / 1610	10500/11000
Day 14	2	305 / 386	2650 / 2460
	4	798 / 1220	8650 / 8450
	6	1020 / 1390	12800 / 11500

Study Title: RO7034067: 39 Week Oral (Gavage) Administration Toxicity Study in the Monkey with a 22 Week Treatment-Free Period

Study no.:	8313482
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	16 February 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 024780-026/ 97.66%

Key Study Findings

Daily oral (gavage) administration of RIS (0, 1.5, 3, or 7.5/5 mg/kg/day; 5 mL/kg) to young (24-26 months) cynomolgus monkeys (3/sex/grp + 2/sex/C & HD recovery) for 39 weeks resulted in clinical signs in the skin of HD animals, which correlated with microscopic findings of epidermal hyperplasia. Hematology changes consisting of decreased RBC parameters, increased reticulocyte counts, decreased lymphocytes, and increased platelet counts were reversible. C-reactive protein concentrations were increased at the HD and had not reversed by the end of the recovery period. Dose-related functional (as measured by ERG) and morphological (retinal degeneration as observed by OCT, histopathology, and EM) changes in the eye were observed at the MD and HD. In addition, thymic atrophy was seen in HD females. Testicular effects could not be assessed due to immaturity. After the recovery period, retinal findings in HD animals (retinal degeneration characterized by multifocal disorganization of the outer nuclear and photoreceptor layers of the retina, loss of photoreceptor layer, multifocal hypertrophy of retinal pigment epithelial cells, thinning and disorganization of the inner nuclear layer) were similar to those seen at the end of treatment. The NOAEL (1.5 mg/kg/day) was associated with RIS exposures of 414/396 ng/mL (Cmax) and 1870/2060 ng·hr/mL (AUC0-24) during Week 39 in males/females.

Methods

Doses:	0 (vehicle), 1.5, 3, or 7.5/5 mg/kg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group main + 2/sex/C and HD recovery
Age:	24-26 months
Weight:	NA
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that impacted study quality or integrity

Group number	Group description	Dose level (mg/kg/day)	Animal numbers			
			Main group		Recovery group (treatment-free)	
			Male	Female	Male	Female
1	Control	0	3	3	2	2
2	Low	1.5	3	3	-	-
3	Intermediate	3.0	3	3	-	-
4	High	7.5/5 #	3	3	2	2

Animals were administered 7.5 mg/kg/day on Days 1 to 12. Following a respite period over Days 13 to 25, dosing resumed at 5 mg/kg/day from Day 26 onwards.

Due to clinical signs (shedding/peeling skin at various locations and reddening of the face), which had progressed to exceed what was considered maximal tolerability based on data from previous studies, dosing of 7.5 mg/kg/day was suspended on Day 13 and restarted at 5 mg/kg/day on Day 26.

Electroretinography and spectral domain ocular coherence tomography (sdOCT) were conducted throughout the dosing phase starting from Week 20, subsequent to identification of changes in the retina by histopathology in a chronic monkey toxicity study for RO6885247, another SMN2 splice modifier.

Dose selection was based on the results of a 2-week dose range-finding study in the cynomolgus monkey ((b) (4) Study Number 8306338) in which the HD of 6 mg/kg/day resulted in histopathological findings in the skin, eyelid, and jejunum that were considered non-adverse.

Observations and Results

Mortality

There were no deaths during the study.

Clinical Signs

Drug-related clinical signs primarily consisted of shedding/peeling skin at various locations (anus, body, eyelids, face, legs, and/or mouth) starting on Day 11 onwards in all HD animals. Reddening of the face was noted in HD monkeys beginning on Day 10 but had resolved by Day 19 after the suspension of dosing. Other signs included hair loss on the tail (HD) and thin appearance (all dose groups).

Body Weights

BW gain and BWs were unaffected by drug.

Food Consumption

There were no effects on food consumption.

Ophthalmic Examination

No drug-related ophthalmic findings were noted.

Electroretinography Evaluation

ERG amplitudes (scotopic and photopic) were dose-dependently depressed at Weeks 20 (first assessment time), 26, and 34. The effect was primarily seen at the MD and HD but appeared to also occur in LD animals under some test conditions, e.g., the scotopic -34 dB blue b-wave condition (Table 1).

Table 1. Mean Electroretinographic Measurement Data – Dosing Period

Short wavelength flashes (Scotopic -34 dB Blue Single Flash)
B Wave - Amplitude (µV)

Group/Sex	Dose Level (mg/kg/day)		Right Eye Dosing Week 20	Right Eye Dosing Week 26	Right Eye Dosing Week 34	Left Eye Dosing Week 20	Left Eye Dosing Week 26	Left Eye Dosing Week 34
1/M	0	Mean	103.7	125.3	97.3	90.5	113.2	105.2
		SD	10.25	17.77	16.81	15.41	14.11	26.34
		N	5	5	5	5	5	5
2/M	1.5	Mean	138.8	127.4	150.5	110.6	126.2	113.7
		SD	28.80	6.43	11.00	15.66	7.70	12.72
		N	3	3	3	3	3	3
3/M	3.0	Mean	106.5	103.6	117.9	85.7	119.8	113.4
		SD	30.67	1.56	18.84	16.05	14.57	24.88
		N	3	2	3	3	2	3
4/M	7.5/5.0	Mean	69.2	82.9	75.5	70.1	85.3	73.1
		SD	21.88	24.18	10.59	25.20	23.80	19.33
		N	5	5	5	5	5	5

Group/Sex	Dose Level (mg/kg/day)		Right Eye Dosing Week 20	Right Eye Dosing Week 26	Right Eye Dosing Week 34	Left Eye Dosing Week 20	Left Eye Dosing Week 26	Left Eye Dosing Week 34
1/F	0	Mean	112.0	131.7	100.6	101.2	130.6	100.4
		SD	31.67	19.15	22.10	18.65	21.15	20.08
		N	5	5	5	5	5	5
2/F	1.5	Mean	110.8	130.1	85.6	98.1	127.1	86.8
		SD	15.94	22.91	9.79	15.19	9.84	34.20
		N	3	3	3	3	3	3
3/F	3.0	Mean	70.3	124.5	77.1	74.9	130.8	68.2
		SD	8.90	11.04	25.39	15.30	15.80	14.19
		N	3	3	3	3	3	3
4/F	7.5/5.0	Mean	69.4	84.2	55.1	62.3	91.0	46.6
		SD	27.44	29.22	22.79	23.71	34.67	14.26
		N	5	5	5	5	5	5

Depressed ERG amplitudes, compared to C, continued to be seen during Weeks 8, 13, and 22 of the recovery phase in the 2/sex from the HD group that were evaluated (Table 2).

Table 2. Mean Electroretinographic Measurement Data – Recovery Period

Short wavelength flashes (Scotopic -34 dB Blue Single Flash)
B Wave - Amplitude (µV)

			Right Eye			Left Eye		
Group/Sex	Dose Level (mg/kg/day)		Recovery Week 8	Recovery Week 13	Recovery Week 22	Recovery Week 8	Recovery Week 13	Recovery Week 22
1/M	0	Mean	126.0	140.4	91.5	131.6	125.5	90.8
		SD	19.16	25.95	13.51	18.88	1.91	4.31
		N	2	2	2	2	2	2
2/M	1.5	Mean	-	-	-	-	-	-
		SD	-	-	-	-	-	-
		N	-	-	-	-	-	-
3/M	3.0	Mean	-	-	-	-	-	-
		SD	-	-	-	-	-	-
		N	-	-	-	-	-	-
4/M	7.5/5.0	Mean	72.6	103.6	85.5	97.8	117.4	76.7
		SD	11.38	3.25	1.91	17.82	15.70	11.46
		N	2	2	2	2	2	2

			Right Eye			Left Eye		
Group/Sex	Dose Level (mg/kg/day)		Recovery Week 8	Recovery Week 13	Recovery Week 22	Recovery Week 8	Recovery Week 13	Recovery Week 22
1/F	0	Mean	89.3	119.7	93.9	88.7	134.9	82.4
		SD	28.57	27.44	30.69	36.63	0.99	3.25
		N	2	2	2	2	2	2
2/F	1.5	Mean	-	-	-	-	-	-
		SD	-	-	-	-	-	-
		N	-	-	-	-	-	-
3/F	3.0	Mean	-	-	-	-	-	-
		SD	-	-	-	-	-	-
		N	-	-	-	-	-	-
4/F	7.5/5.0	Mean	51.5	57.5	72.6	48.1	65.6	67.9
		SD	24.61	35.99	41.30	20.58	42.36	21.50
		N	2	2	2	2	2	2

There was an association between functional depression of the ERG and abnormal retinal structural changes observed with sdOCT. These were seen in MD and HD animals of both sexes at the earlier examination time (Week 22) and got progressively worse.

According to the sdOCT report:

> All MD and HD animals all showed some degree of disorganization and thinning of the inner segment/outer segment (IS/OS) and increased reflectivity and thinning of the outer nuclear layer (ONL) in the retinal periphery. Degenerative changes were most pronounced in the far periphery in the dosing phase. While some improvement was seen in layer integrity/organization in the far periphery during the recovery phase, the areas with extensive loss of cells did not change. Microcystoid macular degeneration (MMD), characterized by microcystoid spaces in the inner nuclear layer (INL), was seen in the INL temporal to the optic nerve in the most affected animals in the group given 7.5/5 mg/kg/day. MMD progressed throughout the dosing phase to include areas temporal to the fovea but remained relatively focal, appearing in patches, not diffuse throughout the retina. MMD spaces decreased significantly in the recovery phase. A few of the most affected animals given 7.5/5 mg/kg/day had what appeared to be small pockets of fluid under the inner limiting membrane (ILM) and in the ONL. These spaces gradually decreased throughout the recovery phase. Line scans taken adjacent to or through the optic nerve showed no evidence of neuroretinal rim tissue thinning or optic nerve cupping. Fundus autofluorescence (FAF) imaging showed areas of speckled hypofluorescence and hyperfluorescence solely in the periphery of the animals with the most pronounced degenerative changes. Dark zones generally correspond to loss of RPE and bright areas suggest impaired RPE cell function and potential progression. The areas of white speckled hyperfluorescence became less dense in the recovery phase and appeared to recede slightly. The structural findings noted on sdOCT correlated well with the functional ERG findings.

Table 3. OCT grading: dosing phase

8313482		Week 22 July 2015	Week 27 Aug 2015	Change in Aug	Week 35 Oct 2015	Change in Oct	
Male	Group	Grading	Grading				MMD
1	1	0	0		0		
2	1	0	0		0		
3	1	0	0		0		
4	1	0	0		0		
5	1	0	0		0		
6	2	0	0		0		
7	2	0	0		0		
8	2	0	0		0		
9	3	1	1		1		
10	3	1	1		1		
11	3	1	1		1		
12	4	2	2	sl worse	2		
13	4	2	2		2		
14	4	3	3	sl worse	3	worse	Yes
15	4	1	1		1		
16	4	1	1	sl worse	1		
Female	Group						
101	1	0	0		0		
102	1	0	0		0		
103	1	0	0		0		
104	1	0	0		0		
105	1	0	0		0		
106	2	0	0		0		
107	2	0	0		0		
108	2	0	0		0		
109	3	1	2	sl worse	2	sl worse, OD	
110	3	2	2	sl worse	2		
111	3	1	1		1		
112	4	2	2		2		
113	4	2	2	sl worse	3	worse, OS	Yes
114	4	3	3	sl worse	3	worse	Yes
115	4	3	3		3	worse	Yes
116	4	3	3	sl worse	3		Yes

MMD = microcystoid macular degeneration

According to the report, “the flash VEP [visual evoked potential] response was maintained, suggesting intact projection of macular retinal ganglion cell axons to thalamus and cerebral cortex in HD animals, despite retinal dysfunction noted by ERG and OCT.”

Hematology

Decreases in RBC parameters were seen at the MD and HD. Increases in absolute reticulocyte counts, seen at these doses as early as Week 4, were considered to indicate drug-related effects on erythropoiesis accompanied by a regenerative erythroid response.

No histological correlates in the bone marrow were reported, and the changes had reversed by Week 13 of the recovery phase. Decreased lymphocytes seen at the HD correlated with pathologic changes in the thymus (small thymus macroscopically, decreased thymus wt ratios, and thymic atrophy microscopically) and were reversible. Platelet counts were dose-dependently increased at the MD and HD (up to 100%, respectively) as early as Week 13 and continued until the end of the dosing phase. These were considered secondary to increased reticulocyte counts (reactive thrombocytosis); and were reversible by Week 13 of the recovery phase. There were no drug-related changes in coagulation parameters.

Clinical Chemistry

There were no clearly drug-related changes in clinical chemistry parameters. However, as seen in the 2-week study, C-reactive protein concentrations were increased in 1 HD male (Animal 0014) and 1 HD female (Animal 0112) as early as Week 13 and persisted until the end of the dosing period (Week 39). But because the changes occurred in the absence of a histological correlate, they were considered of uncertain relationship to RO7034067. In the male, these changes exhibited a trend towards reversibility but had not reversed by the end of the recovery phase.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Organ Weights

Relative thymus weights were decreased in MD and HD males and in HD females. This correlated with thymic atrophy seen microscopically. At the end of the recovery period, thymus weights remained lower in HD males but were without microscopic correlates.

Gross Pathology

Flaky skin/subcutis was recorded in 1 male and 1 female at the HD, and small thymus was recorded for 1 HD female, both of which correlated with findings seen microscopically.

Histopathology

Retinal degeneration was seen at the MD (1 F) and HD (2 M, 2 F) (Table 4). In 1 HD female (116F) the lesion was characterized by multifocal disorganization of the outer nuclear and photoreceptor layers of the retina, with some loss of the photoreceptor layer, multifocal hypertrophy of retinal pigment epithelial cells, some thinning, disorganization and vacuolation of the inner nuclear layer, and vacuolation of the ganglion cell layer. In the other HD animals (13M, 15M, and 113F) and MD female (110F), the retinal degeneration was minimal in grade and characterized by multifocal disorganization of the outer nuclear layer. All findings were seen mainly in the mid to peripheral area of the retina.

Transmission electron microscopic investigations confirmed the retinal degeneration, observed histopathologically in HD female 116F. Findings were present in the peripheral retina and revealed retinal pigment epithelium (RPE) hypertrophy, photoreceptor

degeneration, and nodular arrangement of cells observed within the outer nuclear layer, which was interpreted as retinal reactive gliosis.

Increased multifocal GFAP expression was observed in astrocytes and Müller cells of eye specimens from MD and HD animals and was interpreted as reactive gliosis in areas of retinal degeneration. Staining with the neuronal ion transporter marker Na-K-ATPase revealed less intense staining in areas of gliosis, indicating a decrease in neuronal membranes in areas of degeneration.

Table 4. Incidence of retinal degeneration – terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	1.5	3	7.5/5	0	1.5	3	7.5/5
Eye	No. examined:	3	3	3	3	3	3	3	3
Degeneration, retina	Grade -	3	3	3	1	3	3	2	1
	1	0	0	0	2	0	0	1	1
	2	0	0	0	0	0	0	0	1

Key: "-" = finding not present, 1 = minimal, 2 = slight,

After the recovery period, retinal findings in HD animals (retinal degeneration characterized by multifocal disorganization of the outer nuclear layer mainly in the mid to peripheral area of the retina in animals 12M, 14M, and 115F; retinal degeneration characterized by multifocal disorganization of the outer nuclear and photoreceptor layers of the retina, some loss of photoreceptor layer, multifocal hypertrophy of retinal pigment epithelial cells, and some thinning and disorganization of the inner nuclear layer in 114F) were similar to those seen at the end of treatment indicating that there was no reversal, except that vacuolation of the inner nuclear layer was no longer present.

Epidermal hyperplasia was seen in the skin (taken from the lumbosacral transition and including macroscopically altered skin) and eyelid of HD animals, which correlated with the findings of flaky skin seen macroscopically (Table 5). Epidermal hyperplasia was characterized by increased thickness of the epidermis, with occasional hyperkeratosis or parakeratosis.

Table 5. Incidence of epidermal hyperplasia - terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Dose (mg/kg/day)	0	1.5	3	7.5/5	0	1.5	3	7.5/5
Skin/subcutis	No. examined:	3	3	3	3	3	3	3	3
Hyperplasia, epidermis	Grade -	3	3	3	0	3	3	3	2
	1	0	0	0	3	0	0	0	1
Eyelid	No. examined:	3	3	3	3	3	3	3	3
Hyperplasia, epidermis	Grade -	3	3	3	0	3	3	3	0
	1	0	0	0	1	0	0	0	1
	2	0	0	0	2	0	0	0	2

Key: "-" = finding not present, 1 = minimal, 2 = slight,

After the recovery period, there were no drug-related microscopic findings in the skin/subcutis or eyelids.

At the end of the treatment period, the presence or absence of drug-related testicular findings could not be properly assessed because of the lack of maturity of the testes. After the recovery period, the testes of 1 HD male (14M) were mature but had multifocal segmental hypoplasia and segmental tubular dilatation. These were considered unusual findings but were attributed to recently attained sexual maturity rather than drug. It seems more likely that this was a drug effect.

Thymic atrophy in HD females correlated with the lower organ weights and macroscopic finding of small thymus. Atrophy was characterized by a general loss of lymphocytes from both cortex and medulla, with a reduction in size of the cortex. After the recovery period, there were no drug-related microscopic findings in the thymus.

Toxicokinetics

TK data for RIS in plasma are shown in Table 6. Tissue to plasma ratios were high for brain (Table 7), and levels in the eye persisted through the recovery period (Table 8).

Table 6. Plasma TK parameters for RO7034067 in cynomolgus monkeys

Occasion	Dose (mg/kg/day)	C_{max} (ng/mL) M / F	$AUC_{(0-24h)}$ ((ng·h)/mL) M / F
Day 12	7.5	1020 / 1070	11200 / 10200
Day 267	1.5	414 / 396	1870 / 2060
	3	1000 / 973	4880 / 4850
	5	701 / 1160	5880 / 6470

AUC = Area under the matrix concentration-time curve from 0 to 24 hours postdose; C_{max} = Maximum observed concentration; F = Female; M = Male.

Note: Group 4: A dose of 7.5 mg/kg/day was administered from Days 1 to 12; no dose was administered from Days 13 to 25, and a dose of 5 mg/kg/day was administered from Day 26 onwards. Day 267 corresponds to Day 242 for animals administered 5 mg/kg/day (Group 4).

Table 7. Terminal tissue-to-plasma concentration ratios of RO7034067

Group	Dose [mg/kg]	Week	Sex	Subject	CSF [ng/mL]	Brain Cortex [ng/g]	Brain Stem [ng/g]	Skin [ng/g]
G2	1.5	39	m	006	0.0469	0.513	0.399	5.40
G2	1.5	39	m	007	0.0469	0.415	0.573	1.59
G2	1.5	39	m	008	0.121	2.29	0.915	21.8
G2	1.5	39	f	106	0.0585	0.582	0.605	2.95
G2	1.5	39	f	107	0.0768	0.625	0.637	4.13
G2	1.5	39	f	108	0.135	1.01	0.963	23.4
			N		6	6	6	6
			Mean		**0.0809**	**0.906**	**0.682**	**9.87**
			SD		0.0384	0.707	0.216	9.93
			CV [%]		47.5	78.0	31.7	101
G3	3	39	m	009	0.0439	0.340	0.478	1.21
G3	3	39	m	010	0.0591	0.456	0.550	28.3
G3	3	39	m	011	0.0725	0.697	0.605	5.33
G3	3	39	f	109	0.0469	0.503	0.445	1.03
G3	3	39	f	110	0.112	1.11	1.27	2.68
G3	3	39	f	111	0.103	0.948	0.807	13.1
			N		6	6	6	6
			Mean		**0.0730**	**0.676**	**0.693**	**8.60**
			SD		0.0289	0.302	0.311	10.6
			CV [%]		39.6	44.7	44.9	124
G4	5	39	m	013	0.0486	0.364	0.460	1.40
G4	5	39	m	015	0.0701	0.556	0.673	3.08
G4	5	39	m	016	0.109	0.941	0.995	34.0
G4	5	39	f	112	0.0973	1.36	0.950	7.65
G4	5	39	f	113	0.108	0.856	1.11	4.59
G4	5	39	f	116	0.126	1.62	1.02	5.71
			N		6	6	6	6
			Mean		**0.0932**	**0.950**	**0.868**	**9.41**
			SD		0.0285	0.476	0.248	12.3
			CV [%]		30.6	50.1	28.6	130

Ratios calculated based on GLP data (plasma and CSF) and non-GLP data (brain and skin)

Table 8. Terminal concentrations of RO7034067 in ocular tissue

Group	Dose [mg/kg]	Week	Sex	Subject	Time [h]*	Choroid +RPE [ng/g]	Iris [ng/g]	Sclera [ng/g]	Retina [ng/g]	Cornea [ng/g]	Lens [ng/g]	Vitreous Humor 1 [ng/mL]	Vitreous Humor 2 [ng/mL]	Aqueous Humor [ng/mL]
G1	0	39	m	001	1.8	15.3	20.5	3.68	3.60	<2.50	<1.25	<0.500	<0.500	<0.500
G1	0	39	f	102	0.85	12.9	26.9	5.94	6.26	12.2	2.20	0.826	1.35**	<0.500
G1	0	61*	m	004	-	4.17	11.9	<0.750	11.3	<2.50	<1.25	<0.500	<0.500	<0.500
G1	0	61*	f	104	-	3.86	11.9	1.08	1.24	24.2	2.17	<0.500	<0.500	<0.500
G2	1.5	39	m	006	3.0	487000	1640000	23600	18100	2110	719	21.8	111	6.88
G2	1.5	39	f	107	1.8	939000	932000	27300	21100	767	330	21.7	68.5	2.91
			m&f	**Mean**		**713000**	**1290000**	**25500**	**19600**	**1440**	**525**	**21.8**	**89.8**	**4.90**
G3	3	39	m	009	5.3	1450000	1070000	48600	22000	1890	1090	86.9	343	12.7
G3	3	39	f	110	2.7	679000	1410000	47800	7090	2110	332	133	136	6.30
			m&f	**Mean**		**1060000**	**1240000**	**48200**	**14500**	**2000**	**711**	**110**	**240**	**9.50**
G4	5	39	m	013	6.2	2440000	1180000	96400	22500	4500	1170	141	459	66.9
G4	5	39	f	113	5.1	993000	1330000	52900	23300	4300	493	69.0	233	19.5
			m&f	**Mean**		**1720000**	**1260000**	**74700**	**22900**	**4400**	**832**	**105**	**346**	**43.2**
G4	5	61*	m	014	-	879000	585000	24800	477	47.5	53.0	2.10	16.9	0.964
G4	5	61*	f	115	-	273000	531000	14800	456	886	16.0	0.523	66.1	<0.500
			m&f	**Mean**		**576000**	**558000**	**19800**	**467**	**467**	**34.5**	**1.31**	**41.5**	**0.482**
Mean concentration Week 39 / Week 61 ratio						2.98	2.25	3.77	49.1	9.43	24.1	80.1	8.34	89.6

Week 39 / Week 61	End of treatment / Recovery Week 22
*	Time after last dose
**	Small section of choroid with vitreous humor
RPE	Retinal pigmented epithelium
Vitreous Humor_1:	Central vitreous
Vitreous Humor_2:	Peripheral vitreous close to retina

7 Genetic Toxicology

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

Study title: RO7034067: Bacterial reverse mutation test (Ames test)

Study no.:	2259M15
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Hoffmann-La Roche, Basel, Switzerland
Date of study initiation:	04 May 2015
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	RO7034067-000-011/97.66% and RO7034067-000-013/99%

Methods and Results

RO7034067 (78.3 to 5000 µg/plate) was evaluated for mutagenic activity in the Ames test (TA1535, TA97, TA98, TA100, and TA102) using the plate incorporation method in the absence and presence of rat S-9. Two different batches of RO7034067 were used for the experiments: RO7034067-000-011 with a purity of 97.66% and RO7034067-000-013 with a purity of ≥99%. Increases (up to 2.1X in TA 98) in revertant colony counts were observed with the less pure batch in the presence of S-9 (Table 1). However, the re-synthesized, purer batch (RO7034067-000-013) did not increase revertant colony counts in any of the tester strains in the absence or presence of metabolic activation (Table 2).

Table 1.

Study Name: RO7034067-000, 2259M15 GLP
Experiment: 2259M15/3
Assay Conditions: Plate incorporation assay

Study Code: 2259M15
Experimental Start Date: 19.05.2015
Date counted: 22.05.2015

Metabolic activation	Test article	Dose level per plate	Revertant colony counts (Mean ± SD)				
			TA 1535	TA 97	TA 98	TA 100	TA 102
Without activation	ascorbic acid Buffer		16 ± 5	136 ± 10	13 ± 2	107 ± 4	419 ± 10
	RO7034067-000	78.13 µg	16 ± 9	138 ± 13	16 ± 6	106 ± 7	428 ± 36
		156 µg	11 ± 1	134 ± 15	9 ± 3	105 ± 10	400 ± 29
		313 µg	17 ± 13	133 ± 25	14 ± 4	120 ± 13	439 ± 8
		625 µg	13 ± 3 f	127 ± 11 f	15 ± 3 f	113 ± 15 f	440 ± 10 f
		1250 µg	13 ± 2 f	128 ± 17 f	10 ± 5 f	117 ± 17 f	432 ± 19 f
		2500 µg	10 ± 3 f	102 ± 35 f	12 ± 1 f	96 ± 14 f o m	295 ± 58 f
		5000 µg	16 ± 4 f	28 ± 16 r f	12 ± 1 f	110 ± 9 f o m	166 ± 13 f
	(b) (4)	1 µg	675 ± 17			736 ± 37	
		0.2 µg					1917 ± 66
		1 µg		3046 ± 361			
		2 µg			255 ± 26		
With activation	ascorbic acid Buffer		14 ± 2	163 ± 10	17 ± 5	112 ± 10	506 ± 18
	RO7034067-000	78.13 µg	12 ± 4	192 ± 2	30 ± 7	120 ± 10	494 ± 40
		156 µg	7 ± 2	184 ± 22	23 ± 4	125 ± 8	559 ± 50
		313 µg	14 ± 4	183 ± 28	25 ± 5	142 ± 8	502 ± 43
		625 µg	15 ± 4 f	193 ± 9 f	26 ± 3	119 ± 11 f	444 ± 45 f
		1250 µg	14 ± 3 f	224 ± 28 f	33 ± 5 f	140 ± 4 f	414 ± 26 f
		2500 µg	7 ± 4 f	178 ± 7 f	29 ± 8 f	128 ± 21 f o	306 ± 21 f
	(b) (4)	5000 µg	10 ± 6 f	207 ± 19 f	36 ± 3 f	128 ± 4 f o	198 ± 11 t f
		10 µg					2220 ± 102
		4 µg	145 ± 19	1758 ± 95	1567 ± 100	2860 ± 66	

Key to Positive Controls

Key to Plate Postfix Codes

(b) (4)

Table 2.

Study Name: RO7034067-000, 2259M15 GLP
Experiment: 2259M15/4
Assay Conditions: Plate incorporation assay

Study Code: 2259M15
Experimental Start Date: 11.06.2015
Date counted: 15.06.2015

Metabolic activation	Test article	Dose level per plate	Revertant colony counts (Mean ± SD)				
			TA 1535	TA 97	TA 98	TA 100	TA 102
Without activation	ascorbic acid Buffer		18 ± 7	148 ± 8	14 ± 2	125 ± 9	459 ± 26
	RO7034067-000	156 µg	20 ± 9	136 ± 19	14 ± 2	129 ± 7	452 ± 57
		312 µg	15 ± 7	134 ± 1	16 ± 3	129 ± 8	492 ± 5
		625 µg	16 ± 4	134 ± 10	13 ± 2	126 ± 19	480 ± 10
		1250 µg	16 ± 7	132 ± 12	12 ± 1	142 ± 9 o m	452 ± 23
		2500 µg	19 ± 2	132 ± 14	15 ± 4	117 ± 12 o m	450 ± 152
		5000 µg	5 ± 2 w m	77 ± 38 w m	10 ± 5 w	120 ± 7 w m o	305 ± 164 c
	(b) (4)	1 µg	745 ± 94			1050 ± 5	
		0.2 µg					2087 ± 162
		1 µg		2353 ± 191			
		2 µg			201 ± 8		
With activation	ascorbic acid Buffer		15 ± 5	183 ± 16	21 ± 8	147 ± 6	562 ± 62
	RO7034067-000	156 µg	12 ± 1	164 ± 6	24 ± 4	156 ± 4	515 ± 56
		312 µg	10 ± 3	173 ± 24	21 ± 4	149 ± 21	582 ± 45
		625 µg	10 ± 2	168 ± 2	28 ± 3	153 ± 5	522 ± 24
		1250 µg	14 ± 5	231 ± 4	24 ± 5	183 ± 10	540 ± 26
		2500 µg	11 ± 2	251 ± 17	26 ± 3	182 ± 10	419 ± 30
	(b) (4)	5000 µg	14 ± 2 w	234 ± 33 w	27 ± 3 w m	138 ± 13 w m o	189 ± 59 w
		4 µg	127 ± 9	2042 ± 74	1607 ± 151	2641 ± 252	
		10 µg					3901 ± 184

Key to Positive Controls

Key to Plate Postfix Codes

(b) (4)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Results of the in vitro micronucleus test (MNT) with R07034067-000 using a microscale screening protocol with L5178Y tk+/- mouse lymphoma cells

Study no.:	1063026
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Hoffmann-La Roche, Basel, Switzerland
Date of study initiation:	22 June 2015
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	003/NA

Methods and Results

R07034067 (0.5 – 20 mg/mL) was evaluated for clastogenic and aneugenic activity using a screening version of the MNT in vitro (Non-GLP). Exponentially growing L5178Y tk+/- mouse lymphoma cells were exposed to the drug in absence of an exogenous metabolic activation system. Cell cultures were exposed to the drug for 24 h and harvested immediately and following an additional 24 h recovery period. Induction of micronucleated cells was observed starting at a concentration of 10 mg/mL (3.7% MN cells) and showed a maximum of 4.4% MN cells at the highest evaluated concentration of 20 mg/mL (Table

1). Micronucleation was associated with cytotoxicity (reduced relative cell counts [RCC]) at 10 (50% RCC) and 20 mg/mL (44% RCC). Following the 24 h recovery period, micronucleation started at 1 mg/mL in the absence of pronounced cytotoxicity and showed a maximum of 10% MN cells at 2 mg/mL associated with strong cytotoxicity as indicated by a 42% decrease in RCC (Table 2). Higher dose levels could not be evaluated due to excessive cytotoxicity.

Table 1.

Test item: RO7034067-000 Batch: 003
PPL: 2409S13

	S9: - treatment time: 24 h recovery: 0 h					
	concentr. (µg/ml)	MN cells (%)	RCC (%)	mitotic cells (%)	PD	
MMS	15.00	12.2	61	3.9	0.34	
1% DMSO	-	0.7	100	3.9	1.05	
RO7034067-000	0.50	1.0	101	4.5	1.06	1
RO7034067-000	1.00	0.5	94	8.4	0.97	1
RO7034067-000	2.00	1.8	74	6.6	0.62	1
RO7034067-000	5.00	1.2	55	11.1	0.18	1
RO7034067-000	10.00	3.7	50	11.0	0.04	1
RO7034067-000	20.00	4.4	44	13.2	-0.13	1

1 precipitation visible under the microscope

Table 2.

Test item: RO7034067-000 Batch: 003
PPL: 2409S13

	S9 - treatment time: 24 h recovery: 24 h				
	concentr. (µg/ml)	MN cells (%)	RCC day2 (%)	mitotic cells (%)	PD day2
MMS	15.00	22.1	75	-	1.02
1% DMSO	-	0.9	100	3.6	1.43
RO7034067-000	0.50	1.1	87	6.9	1.24
RO7034067-000	1.00	2.2	81	5.2	1.13
RO7034067-000	2.00	10.3	42	5.0	0.19
RO7034067-000	5.00		32		-0.22
RO7034067-000	10.00		28		-0.38
RO7034067-000	20.00		23		-0.68

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

Study title: RO7034067: Combined Comet assay (liver, jejunum) and Micronucleus test (bone marrow) in rat - Oral administration (gavage)

Study no:	2247M15
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Hoffmann-La Roche, Basel, Switzerland
Date of study initiation:	09 March 2015
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	(b) (4) 024780-026/ 97.66%

Methods and Results

Wistar rats (5 males/group) received oral (gavage) doses of RIS (b) (4) ascorbic acid/ (b) (4) 0.75, 1.5, 3, 6, 12.5, or 25 mg/kg/d) at 1, 24, and 48 hours prior to the collection of the bone marrow for the micronucleus test and the liver for the comet assay. Positive controls received ethyl methanesulfonate and cyclophosphamide for the comet assay and micronucleus test, respectively. There was no mortality or clinical signs. In the micronucleus test, statistically significant increases in the frequency of micronucleated polychromatic erythrocytes were seen at ≥ 6 mg/kg/d (Table 1). A reduction of more than 50% in the ratio of polychromatic to normochromatic erythrocytes was observed at the HD.

Table 1.

Test item	Dose (mg/kg/d)	Ratio PCE/NCE	MN-PCE (%)	p-values
Vehicle control	0	0.97	0.18	
RO7034067	0.75	1.37	0.05	*
RO7034067	1.5	1.20	0.21	n.s.
RO7034067	3	1.93	0.18	n.s.
RO7034067	6	0.75	0.30	*
RO7034067	12.5	1.35	0.78	**
RO7034067	25	0.41	0.41	**
CP/EMS	24/ 200	1.10	2.00	**

n.s.: not significant,*: $p < 0.05$ **: $p < 0.01$

In the comet assay, RIS-treated animals exhibited tail intensity and tail moment values for liver and jejunum that were comparable to the concurrent vehicle control and were within the range of negative control values. Visual examination of slides during evaluation did not reveal an increase in the number of hedgehog cells.

In conclusion, RIS induced chromosomal aberrations, as indicated by the bone marrow micronucleus test at doses of 6 mg/kg/d or greater, but was negative in the comet assay. The no-effect dose (3 mg/kg/d) was associated with Cmax and AUC values of 260 ng /mL and 3800 ng·h/mL.

8. Carcinogenicity

Study title: RO7034067: A 26 Week Oral Gavage Carcinogenicity Study in the 001178 RasH2 Mouse

Study no.:	8378427
Study report location:	4.2.3.4.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	20 December 2017
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 1702SA01/99.5%
CAC concurrence:	Yes, minutes dated December 6, 2017

Key Study Findings

Oral (gavage) administration of RIS (0 (vehicle control), 0 (water control), 1, 3, or 9 mg/kg/day) to Tg.RasH2 mice once daily for 26 weeks produced no drug-related clinical observations, body weight or food consumption alterations, or differences in survival, hematology, incidence of neoplasms, incidence of macroscopic findings, or incidence or severity of microscopic findings. The no effect level (9 mg/kg/day) was associated with Cmax and AUC values of 1230 ng/mL and 15600 ng.hr/mL in males and 1150 ng/mL and 11800 ng.hr/mL in females, respectively, on Day 179.

Adequacy of Carcinogenicity Study

Adequate

Appropriateness of Test Models

Appropriate

Evaluation of Tumor Findings

None drug-related. Positive controls as expected.

Methods

Doses:	1, 3, or 9 mg/kg/day
Frequency of dosing:	QD
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) ascorbic acid/ (b) (4)
Basis of dose selection:	4-week dose range-finding study in wild type RasH2 mice ((b) (4) Study 8360058)
Species/Strain:	RasH2 001178-T (hemizygous), CByB6F1-Tg(HRAS)2Jic (carcinogenicity animals) and RasH2 001178-W (wild type), CByB6F1-Tg(HRAS)2Jic (TK animals)
Number/Sex/Group:	25
Age:	8 to 9 weeks
Animal housing:	Females group-housed, males individually housed
Paradigm for dietary restriction:	none
Dual control employed:	Water, vehicle, N-methyl-N-nitrosourea (MNU)
Interim sacrifice:	no
Satellite groups:	6-30/sex/grp TK
Deviation from study protocol:	None that impacted study quality or integrity

Group	Subgroup	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Vehicle Control)[a]	1 (Carcinogenicity)	25	25	0	0
	2 (Toxicokinetic)	6	6		
2 (Water Control)[b]	1 (Carcinogenicity)	25	25	0	0
	2 (Toxicokinetic)	6	6		
3 (Low)	1 (Carcinogenicity)	25	25	1	0.1
	2 (Toxicokinetic)	30	30		
4 (Mid)	1 (Carcinogenicity)	25	25	3.0	0.3
	2 (Toxicokinetic)	30	30		
5 (High)	1 (Carcinogenicity)	25	25	9.0	0.9
	2 (Toxicokinetic)	30	30		
6 (Positive Control)[c]	1 (Carcinogenicity)	20	20	75	7.5

a Group 1 received vehicle control article only.
b Group 2 was administered water only.
c Group 6 animals were dosed with one intraperitoneal dose of positive control article on Day 1 of the dosing phase. These animals were included as positive controls to ensure animals supplied appropriately expressed oncogenes and responded to carcinogenic insult.

Doses were based on the results of a 4-week dose range-finding study of RIS (0, 3, 9, 20/16 mg/kg) in RasH2 wild-type mice (# 8360058) in which the HD of 20 mg/kg resulted in mortality and lowering to 16 mg/kg on Day 20. Degeneration of the intestinal mucosa and decreased bone marrow cellularity were detected in the early decedents (see review by Christopher Toscano dated 01/18/18 and Exec-CAC minutes dated 12/6/17).

Observations and Results

Mortality

No drug-related deaths occurred. Four vehicle or water control animals and 7 drug-treated animals were found dead, sacrificed in moribund condition, or died due to an accident. Survival rates for drug-treated toxicity animals (92 to 100%) were comparable to those of vehicle controls (88 to 100%) and water controls (96 to 100%), with no clear dose relationship among drug-treated groups (Table 1). The survival rate for positive controls was 35 and 25% for males and females, respectively.

Table 1. Summary of Fate Status

	RO7034067											
Sex	Males						Females					
Dose Level (mg/kg/day)	0[a]	0[b]	1	3.0	9.0	0[c]	0[a]	0[b]	1	3.0	9.0	0[c]
Number in Group	25	25	25	25	25	20	25	25	25	25	25	20
Terminal Sacrifice	25	25	25	24	25	7	22	24	24	24	23	5
Accidental	0	0	0	0	0	0	1	0	0	0	0	0
Found Dead	0	0	0	0	0	4	2	1	0	1	1	7
Moribund Sacrifice	0	0	0	1	0	9	0	0	1	0	1	8

a Group 1 was administered vehicle control article only.
b Group 2 was administered water only.
c Group 6 animals were administered one intraperitoneal dose of positive control article on Day 1 of the dosing phase.

Clinical Signs

There were no drug-related clinical observations.

Body Weights

There were no drug-related effects on BW or food consumption.

Hematology

Slightly increased reticulocyte counts were seen at all doses and increased eosinophil counts were seen MD and HD females.

Gross Pathology

No drug-related macroscopic findings were noted.

Histopathology

Neoplastic

No drug-related increase in the incidence of neoplasms occurred in RIS-treated groups (Table 2; also, see statistical review by Malick Mbodj).

Decreased survival in the positive control group was related primarily to the occurrence of fatal malignant neoplasms (malignant lymphoma, multiple sites), as well as rare

occurrences of carcinoma of the glandular stomach, benign neoplasms of the skin (keratoacanthoma), or neoplasm of the lung (bronchiolo-alveolar carcinoma).

Table 2. Incidence of Neoplastic Findings - All Sacrifices and Deaths

Sex	Male					Female				
Dose Level (mg/kg/day)	0[a]	0[b]	1	3.0	9.0	0[a]	0[b]	1	3.0	9.0
Number Examined	25	25	25	25	25	25	25	25	25	25
All sites combined: Vascular neoplasms										
B-Hemangioma	1	0	0	0	0	0	0	0	0	0
M-Hemangiosarcoma	1	0	1	2	2	0	4	3	0	1
Adrenal cortex										
B-Adenoma, subcapsular cell	0	0	0	0	0	1	0	0	0	0
Lung										
B-Adenoma, bronchio-alveolar	1	1	1	1	0	0	0	3	2	1
Harderian Gland										
B-Adenoma	0	0	0	1	0	1	0	0	0	1
M-Adenocarcinoma	0	0	0	0	1	0	0	0	0	1
Thymus										
B-Thymoma	1	0	0	1	0	5	2	2	3	2
M-Malignant thymoma	0	1	0	0	0	1	0	0	0	2
Liver										
B-Adenoma, hepatocellular	0	0	1	1	0	0	0	0	0	0
Skin/Subcutis										
B-Papilloma, squamous cell	1	0	0	0	0	0	0	0	0	0
B-Keratoacanthoma	0	0	0	0	0	0	0	1	0	0
Mandibular Salivary Gland										
M-Carcinoma	0	0	0	0	1	0	0	0	0	0
Stomach, Glandular										
M-Carcinoma	0	0	0	0	0	0	0	0	0	1
Stomach, Nonglandular										
B-Papilloma, squamous cell	0	0	0	0	0	0	0	0	1	0
Cavity, Other										
M-Carcinoma, squamous cell	0	0	0	0	0	0	0	0	1	0
HRS										
M-Malignant lymphoma	0	0	0	1	2	1	0	0	0	1

B = Benign; HRS = Hemolymphoreticular system; M = Malignant.
a Vehicle controls.
b Water controls.

Toxicokinetics

TK parameters for RIS and its major metabolite RO7112063 are shown in Table 3. Metabolite ratios (RO7112063/RO7034067) ranged between 5.43% and 18.4%.

Table 3. Mean TK results on Days 1, 87, and 179

Treatment Duration	Species/ Test System	Animals/ Dose	Day	Dose (mg/kg/day)	C_{max} (ng/mL) M/F	$AUC_{(0-24h)}$ ((ng.h)/mL) M/F
				RO7034067		
26 weeks	001178 RasH2 Mouse	30M/30F	1	1 (G3)	157/133	1180/1030
				3 (G4)	732/530	4680/3370
				9 (G5)	1550/1190	15000/8980
			87	1 (G3)	138/101	1190/853
				3 (G4)	359/317	3830/4050
				9 (G5)	1320/1140	11500/12700
			179	1 (G3)	170/119	1730/797
				3 (G4)	495/429	4850/3630
				9 (G5)	1230/1150	15600/11800
				RO7112063		
			1	1 (G3)	10.9/18.9	64.4/145
				3 (G4)	61.1/63.6	415/536
				9 (G5)	166/176	1570/1650
			87	1 (G3)	8.63/10.5	64.3/94.7
				3 (G4)	30.9/38.3	318/522
				9 (G5)	153/148	1100/1600
			179	1 (G3)	13.7/15.6	122/102
				3 (G4)	49.0/55.2	423/472
				9 (G5)	137/143	1690/1580

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not conducted.

9.2 Embryofetal Development

Study title: RO7034067: An Embryo-fetal Development Oral (Gavage) Study in the Rat

Study no.:	9001124
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 Jan 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot # (b) (4) 1702SA01, 99.5%

Key Study Findings

Oral (gavage) administration of RIS (0, 1, 3, or 7.5 mg/kg) to pregnant Wistar rats (22/group) throughout organogenesis (GDs 6-17) resulted in decreased maternal body weight gain (14%) over the dosing period (GDs 6-18). Fetal BWs were decreased (15%) and structural variations (supernumerary liver lobes, reduced ossification of the sternebrae and/or thoracic centrum) increased at the HD. There was no evidence of embryolethality or increased fetal malformations. The no-effect level for embryofetal toxicity was 3 mg/kg/day, corresponding to Cmax 319 ng/mL and AUC(0-24h) 4630 ng•h/mL on GD 15.

Methods

Doses:	0 (vehicle), 10, 30, 60 mg/kg
Frequency of dosing:	QD
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Wistar Hannover (Crl:WI[Han])
Number/Group:	22/group
Age:	82 to 86 days
Weight:	195 to 247 g
Satellite groups:	none
Deviation from study protocol:	None that impacted study quality or integrity

Experimental Design

Group No.	Test Material	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	No. of Main Study Animals	No. of Toxicokinetic Animals[a]
1	Vehicle Control (Reference Item)b	0	0	5	22	2
2	RO7034067	1	0.2	5	22	2
3	RO7034067	3	0.6	5	22	2
4	RO7034067	7.5	1.5	5	22	2

[a] Toxicokinetic animals were used for toxicokinetic evaluation only.
[b] 10 mM ascorbic acid/0.01 mg/mL sodium thiosulfate pentahydrate, pH 3.

RIS (0 (vehicle), 1, 3, 7.5 mg/kg/day) was administered to time-mated female Wistar rats once daily by oral gavage on GDs 6 to 17. TK animals were dosed on GDs 6 to 15. The following parameters and end points were evaluated: mortality, clinical signs, body weights, food consumption, maternal gross necropsy examinations, maternal reproductive (ovarian and uterine), fetal (body weight and external, visceral, and skeletal examinations), and TK parameters.

Dose selection was based on the results of a dose range-finding study (9001122) in which the same doses were administered to pregnant Wistar rats once daily by oral gavage on GDs 6 to 17 and resulted in decreased maternal BW gain (-13%) and fetal BW at the HD. There was no evidence of embryolethality or dysmorphogenesis at any dose.

Observations and Results

Mortality:

There was no maternal mortality.

Clinical signs:

There were no drug-related clinical signs.

Body weight and food consumption:

Decreased maternal BW gain was seen at the HD (14% over GDs 6 to 18 compared to C, SS; Table 1, Fig 1). The corrected body weight gains (i.e., minus gravid uterus weight) were comparable to control at this dose, indicating that the lower body weights were related to the lower fetal weights.

Table 1.

Summary of Maternal Body Weight Gains (g)

9001124

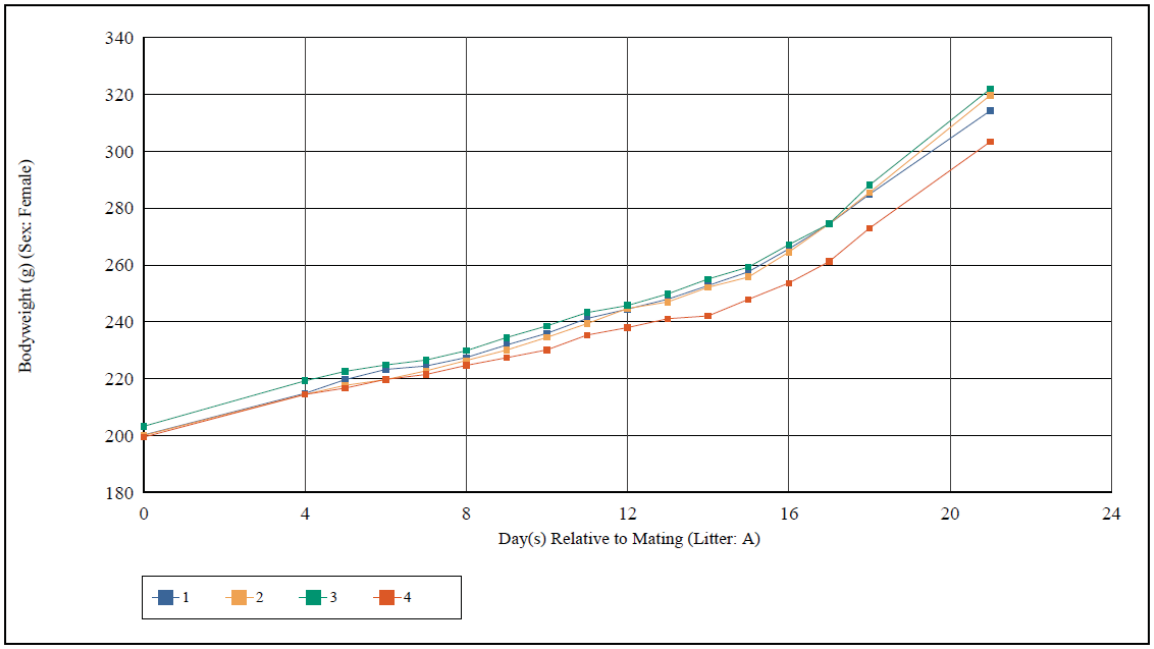
Bodyweight Gain (Interval)

Sex: Female		Day(s) Relative to Mating (Litter: A)			
		15 → 18	6 → 18	18 → 21	6 → 21
0 mg/kg /day Group 1	Mean	27.3	61.6	29.4	91.0
	SD	6.2	8.0	7.9	13.8
	N	21	21	21	21
		-	-	-	-
1 mg/kg /day Group 2	Mean	29.7	65.8	34.2	100.0
	SD	3.2	7.7	8.2	13.3
	N	20	20	20	20
		-	-	-	-
3 mg/kg /day Group 3	Mean	28.8	63.2	33.9	97.1
	SD	6.1	9.8	8.2	16.7
	N	20	20	20	20
		-	-	-	-
7.5 mg/kg /day Group 4	Mean	25.0	53.1*	30.5	83.6
	SD	7.4	11.3	7.4	16.6
	N	20	20	20	20
		-	-	-	-

Figure 1

Summary of Maternal Body Weights

9001124



Toxicokinetics

TK parameters for RIS and its major metabolite are shown in Table 2.

Table 2.

TK Results Summary

Treatment Duration	Animals/ /Dose	Occasions and Dose (mg/kg)	Mean Cmax (ng/mL) Females	Mean AUC(0-24h) ((hr*ng)/mL) Females
10 days	2	GD 15	RO7034067	
		1	143	1540
		3	319	4630
		7.5	962	10700
		GD 15	RO7112063	
		1	15.9	235
		3	41.0	576
		7.5	77.6	1210

Necropsy

There were no drug-related macroscopic findings at the terminal necropsy examination of the adult females.

Pregnancy and Litter Data:

The pregnancy rate was 96, 91, 91, and 91% in the C, LD, MD, and HD groups, respectively. There were no RIS-related effects on the number of corpora lutea, number of implantations, early/late resorptions, total number of resorptions, number of live and dead fetuses, sex ratio, or the pre/post-implantation losses (%).

Fetal Evaluations:

Fetal BWs were decreased (15%, SS) and fetal visceral and skeletal variations (supernumerary liver lobes, incomplete ossification of sternebrae and thoracic centrum) increased at the HD (Tables 3-4).

Table 3
Summary of Fetal Body Weights

9001124

Day(s): 21 Relative to Mating (Litter: A)

Sex: Female		0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4
Mean Fetal Weight (both) (g) [g]	Mean	5.459	5.333	5.331	4.622***
	SD	0.282	0.277	0.365	0.328
	N	21	20	20	20
	%Diff	.	-2.310	-2.344	-15.325
Mean Fetal Weight (M) (g) [g]	Mean	5.578	5.450	5.410	4.749***
	SD	0.301	0.332	0.294	0.363
	N	21	20	19	20
	%Diff	.	-2.300	-3.014	-14.864
Mean Fetal Weight (F) (g) [g]	Mean	5.309	5.197	5.199	4.476***
	SD	0.276	0.286	0.403	0.340
	N	21	20	20	20
	%Diff	.	-2.124	-2.084	-15.691

Table 4.
Summary of Fetal Abnormalities by Classification

9001124

Exam Type: Fresh Visceral BD Ab	0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4				
Number of Fetuses Examined:	108	99	105	105				
Number of Fetuses Evaluated:	215	203	210	208				
Number of Litters Examined:	21	20	20	20				
Number of Litters Evaluated:	21	20	20	20				
Variation								
Number of Fetuses	0	0	2	6				
Litter % of Fetuses [k]	0.00	0.00	2.08	5.10*				
Number of Litters	0	0	2	5				
All classifications								
Number of Fetuses	0	0	2	6				
Litter % of Fetuses [k]	0.00	0.00	2.08	5.10*				
Number of Litters	0	0	2	5				

Exam Type: Skeletal	0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4				
Number of Fetuses Examined:	107	104	105	103				
Number of Fetuses Evaluated:	215	203	210	208				
Number of Litters Examined:	21	20	20	20				
Number of Litters Evaluated:	21	20	20	20				
Variation								
Number of Fetuses	80	75	76	85				
Litter % of Fetuses [k]	75.01	72.38	72.26	82.40				
Number of Litters	21	19	20	20				
All classifications								
Number of Fetuses	80	75	76	85				
Litter % of Fetuses [k]	75.01	72.38	72.26	82.40				
Number of Litters	21	19	20	20				

Study title: RO7034067: An Embryo-fetal Development Oral (Gavage) Study in the Rabbit

Study no.:	9001125
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 Jan 2018
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	(b) (4) 1702SA01, 99.5%

Key Study Findings

Oral (gavage) administration of RIS (0, 1, 4, or 12 mg/kg) to pregnant NZW rabbits throughout organogenesis (GD 6-19) resulted in maternal toxicity at the HD as shown by lower food intake, weight loss, and abortion of 2 does. Embryofetal mortality (increased number of late resorptions) and increased incidences of fetal malformations (hydrocephaly) and visceral variations (absent accessory lung lobes, small gallbladder) were seen at the HD. The no-effect dose for adverse effects on embryofetal development (4 mg/kg/day) was associated with a maternal Cmax of 1500 ng/mL and AUC0-24h of 7990 ng•h/mL on GD15.

Methods

Doses:	0, 1, 4, 12 mg/kg/day
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) ascorbic aci (b) (4)
Species/Strain:	New Zealand White (Crl:KBL[NZW])
Number/Group:	22/group
Age:	5-6 months
Weight:	3.1 to 4.1 Kg
Satellite groups:	no
Study design:	Dosing GD 6-19, C-section GD 29
Deviation from study protocol:	None that impacted study quality or integrity

Experimental Design

Group No.	Test Material	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	No. of Main Study Animals[a]
1	Vehicle Control (Reference Item)[b]	0	0	5	22
2	RO7034067	1	0.2	5	22
3	RO7034067	4	0.8	5	22
4	RO7034067	12	2.4	5	22

[a] The last two animals of each group were used for Toxicokinetic evaluation.
[b] (b) (4) ascorbic acid, (b) (4)

RIS was administered to time-mated female rabbits once daily by oral gavage on GDs 6 to 19, inclusive. The following parameters and end points were evaluated: mortality, clinical signs, body weights, food consumption, hematology, maternal necropsy examinations, maternal reproductive (ovarian and uterine) parameters, fetal weights, fetal examinations (external visceral, and skeletal), and toxicokinetic parameters.

Doses were based on a preliminary study (9001123) in which oral (gavage) administration of RIS (0, 3, 6, or 12 mg/kg) to time-mated female NZW rabbits (6/group) on GDs 6 to 19 resulted in maternal toxicity at the HD characterized by reduced food consumption and bodyweight loss or reduced bodyweight gain. Increased embryofetal lethality was also observed at this dose level. No clear maternal toxicity was observed at the lower dose levels of 3 and 6 mg/kg/day. Increased incidences of malformed fetuses (primarily craniofacial and cardiac malformations) were noted in all groups given RIS compared to none in C.

Observations and Results

Mortality

Three HD does were sacrificed early (Table 1). Female Nos. 4603 and 4507 were euthanized on GD 22 and GD 24, respectively, after they had aborted and following prolonged periods of low food intake. Female No. 4509 was euthanized on GD 20, due to poor clinical condition and marked weight loss after a prolonged period of low food intake.

Clinical Signs

Decreased fecal output and decreased fecal size were increased at the HD from GD 8 onward. In addition, a few HD does showed thinness later in the study. Two does (Nos. 4507 and 4516) showed decreased activity between GDs 20 and 24, and 2 does (Nos. 4504 and 4515) showed salivation prior to dosing between GDs 17 and 19. In addition, red liquid material on the cage floor was noted for 4 HD does, the 2 that had aborted and 2 others (Nos. 4510 and 4514).

Body Weight

Decreased (SS) BW gain was seen at the HD (GDs 6-20 BW gain -0.12 kg vs. 0.21 kg in C; Table 1). Decreased (SS) BW was seen at the HD on GDs 19, 20, and 22, but BW was comparable to C on PND 29 (Fig. 1).

Table 1.

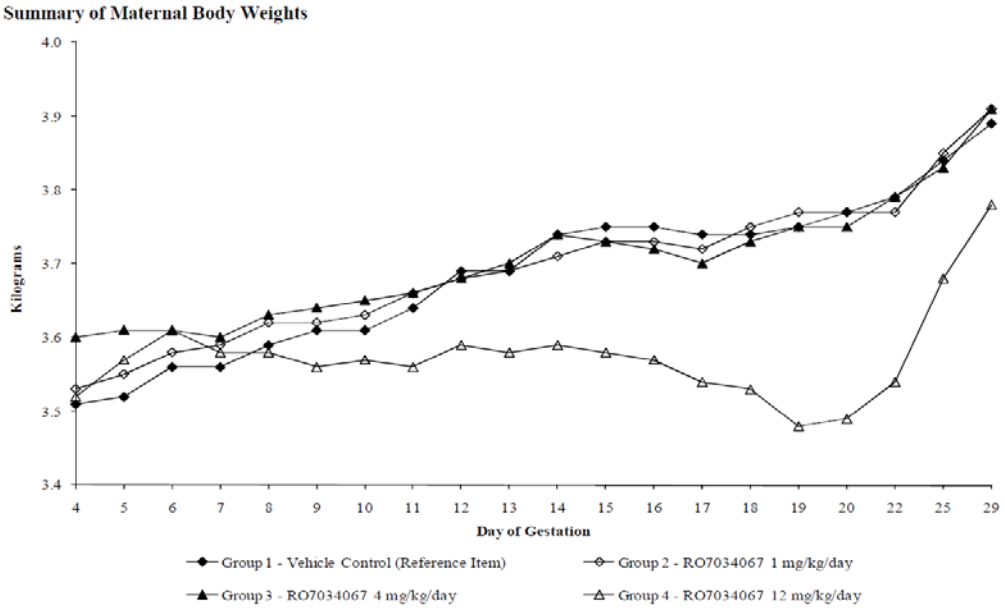
Summary of Maternal Body Weight Gains (kg)

Group 1 - Vehicle Control (Reference Item) Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 4 mg/kg/day Group 4 - RO7034067 12 mg/kg/day

Group / Sex		Day Change 18 - 20	Change 6 - 20	Change 20 - 29	Change 6 - 29
1F	Mean	0.03	0.21	0.13	0.33
	SD	0.05	0.12	0.16	0.20
	N	18	18	18	18
2F	Mean	0.02	0.19	0.15	0.33
	SD	0.08	0.12	0.15	0.19
	N	20	20	20	20
3F	Mean	0.02	0.14	0.16	0.30
	SD	0.07	0.16	0.13	0.19
	N	21	21	21	21
4F	Mean	-0.04e	-0.12c	0.29e	0.17
	SD	0.09	0.26	0.15	0.28
	N	18	18	18	18

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

Figure 1. Body weight in rabbit embryofetal development study



Toxicokinetics

TK parameters for RIS and its major metabolite are shown in Table 2.

Table 2. TK parameters in rabbits

TK Results Summary

Treatment Duration	Test System	Animals/ Dose	Occasions and Dose (mg/kg/day)	Mean Cmax (ng/mL)	Mean AUC(0-24h) ((h*ng)/mL)
10 Days	New	2	GD 15	RO7034067	
	Zealand		1	310	1880
	White		4	1500	7990
	Rabbit		12	4220	37900
				RO7112063	
			1	9.56	63.1
			4	57.0	303
			12	90.0	760

Necropsy

There were no drug-related macroscopic changes noted among adult females in any group.

Cesarean Section Data

One control female (No. 1521), examined terminally, had total resorption (Table 2). The numbers of corpora lutea and implantations and preimplantation loss were comparable among groups. The incidences of late resorptions and percent postimplantation loss were markedly increased at the HD when compared to control does with live litters (Table 3).

Table 2.

Summary of Maternal Performance

9001125

Day(s): 6 to 29 Relative to Mating (Litter: A)

Sex: Female		0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4
No. Pregnant	N-ve	4	2	1	1
	N+ve	18	20	21	21
	%	81.8	90.9	95.5	95.5
Fem. with Live Fetuses		17	20	21	18
Fem. with all dead or resorbed		1	0	0	3
Fem. Euthanized Preterminally	N+ve	0	0	0	3
Pregnant/Early PM	N+ve	0	0	0	3
Not Pregnant/Early PM	N+ve	0	0	0	0
Fem. Aborted	N+ve	0	0	0	2
Fem. Delivered	N+ve	0	0	0	0
Placenta exam Normal	N-ve	0	0	0	0
	N+ve	17	20	21	18

Table 3.

Summary of Ovarian and Uterine Findings: Excluding Dam with Total Resorption

9001125

Day(s): 29 Relative to Mating (Litter: A)

Sex: Female		0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4
Corpora Lutea-Left [k]	Mean	6.2	6.7	6.0	6.1
	SD	2.0	2.0	2.3	2.1
	N	17	20	21	18
	%Diff	-	7.5	-4.5	-2.0
Corpora Lutea-Right [k]	Mean	4.9	5.6	6.2	5.3
	SD	2.0	2.1	2.4	1.9
	N	17	20	21	18
	%Diff	-	14.7	27.8	8.1
Number of Corpora Lutea [k]	Mean	11.1	12.3	12.2	11.4
	SD	2.3	2.7	2.2	2.2
	N	17	20	21	18
	%Diff	.	10.6	9.6	2.4
Number of Implantations [k]	Mean	10.1	11.1	11.5	10.4
	SD	3.0	1.9	2.2	2.2
	N	17	20	21	18
	%Diff	.	9.2	13.4	3.2
% Pre-implantation Loss [k]	Mean	9.21	8.35	5.51	7.94
	SD	19.41	12.98	8.21	9.80
	N	17	20	21	18
	%Diff	.	-9.26	-40.15	-13.72
Number of Dead Fetuses [k]	Mean	0.0	0.0	0.0	0.1
	SD	0.0	0.0	0.0	0.2
	N	17	20	21	18
	%Diff	.	.	.	.
Number of Early Resorptions [k]	Mean	0.4	0.3	0.2	0.0
	SD	0.7	0.7	0.5	0.0
	N	17	20	21	18
	%Diff	.	-29.2	-46.0	-100.0
Number of Late Resorptions [k]	Mean	0.1	0.2	0.3	1.2
	SD	0.3	0.4	0.7	1.9
	N	17	20	21	18
	%Diff	.	27.5	183.3	891.7
% Post-implantation Loss (%) [k]	Mean	3.73	3.38	4.47	12.83
	SD	6.40	7.47	6.75	20.07
	N	17	20	21	18
	%Diff	.	-9.18	20.08	244.46

Fetal evaluations

Fetal BWs were decreased (5%, NS) in all treatment groups, but not in a dose-related manner. Fetal malformations and variations were increased in incidence at the HD. Apparent drug-related malformations included hydrocephaly in 4 fetuses (No. 4501/6, 4506/7, 4518/1 and 4516/4) from 4 separate litters showing domed skull (external) and dilated lateral ventricles of the brain (internal). One of these fetuses (No. 4516/4) was dead. This was in general agreement with the DRF study findings in which 1 HD fetus had a severe domed head and a small brain, 1 HD fetus had a domed head and severely dilated ventricles, and 1 LD (3 mg/kg) fetus had domed head, a small olfactory lobe, an

absent brain (only a small portion of cerebellum and medulla oblongata was present) and both eye lenses absent. Visceral variations (absent accessory lung lobes, small gallbladder) were also increased at the HD.

Table 4.

Summary of Fetal Abnormalities by Classification

9001125

Exam Type: External	0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4				
Number of Fetuses Examined:	164	213	230	167				
Number of Fetuses Evaluated:	164	213	230	167				
Number of Litters Examined:	17	20	21	18				
Number of Litters Evaluated:	17	20	21	18				
Variation								
Number of Fetuses	0	0	0	1				
Litter % of Fetuses [k]	0.00	0.00	0.00	0.51				
Number of Litters	0	0	0	1				
Malformation								
Number of Fetuses	2	2	3	5				
Litter % of Fetuses [k]	1.19	0.97	1.18	4.58				
Number of Litters	2	2	3	5				
All classifications								
Number of Fetuses	2	2	3	6				
Litter % of Fetuses [k]	1.19	0.97	1.18	5.09				
Number of Litters	2	2	3	6				
Exam Type: Fresh Visceral Th	0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4				
Number of Fetuses Examined:	163	211	230	166				
Number of Fetuses Evaluated:	164	213	230	167				
Number of Litters Examined:	17	20	21	18				
Number of Litters Evaluated:	17	20	21	18				
Variation								
Number of Fetuses	1	4	1	8				
Litter % of Fetuses [k]	0.53	2.04	0.37	4.74				
Number of Litters	1	3	1	6				
Malformation								
Number of Fetuses	3	0	3	2				
Litter % of Fetuses [k]	2.59	0.00	1.02	1.35				
Number of Litters	3	0	1	2				
All classifications								
Number of Fetuses	4	4	4	10				
Litter % of Fetuses [k]	3.13	2.04	1.39	6.09				
Number of Litters	4	3	2	7				
Exam Type: Fresh Visceral BD Ab	0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4				
Number of Fetuses Examined:	164	213	230	166				
Number of Fetuses Evaluated:	164	213	230	167				
Number of Litters Examined:	17	20	21	18				
Number of Litters Evaluated:	17	20	21	18				
Variation								
Number of Fetuses	6	11	5	13				
Litter % of Fetuses [k]	3.32	5.22	2.45	7.68				
Number of Litters	6	10	4	9				
Malformation								
Number of Fetuses	1	1	2	2				
Litter % of Fetuses [k]	0.53	0.50	0.73	1.67				
Number of Litters	1	1	2	2				
All classifications								
Number of Fetuses	7	11	7	15				
Litter % of Fetuses [k]	3.85	5.22	3.18	9.35				
Number of Litters	7	10	6	10				

Exam Type: Skeletal-Body Only	0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4				
Number of Fetuses Examined:	164	213	230	166				
Number of Fetuses Evaluated:	164	213	230	167				
Number of Litters Examined:	17	20	21	18				
Number of Litters Evaluated:	17	20	21	18				
Variation								
Number of Fetuses	130	166	193	150				
Litter % of Fetuses [k]	78.20	77.73	84.05	91.17				
Number of Litters	17	20	21	18				
Malformation								
Number of Fetuses	2	0	0	1				
Litter % of Fetuses [k]	1.07	0.00	0.00	0.56				
Number of Litters	1	0	0	1				
All classifications								
Number of Fetuses	130	166	193	150				
Litter % of Fetuses [k]	78.20	77.73	84.05	91.17				
Number of Litters	17	20	21	18				

Exam Type: FreshVisBrain	0 mg/kg /day Group 1	1 mg/kg /day Group 2	4 mg/kg /day Group 3	12 mg/kg /day Group 4				
Number of Fetuses Examined:	85	106	116	87				
Number of Fetuses Evaluated:	164	213	230	167				
Number of Litters Examined:	17	20	21	18				
Number of Litters Evaluated:	17	20	21	18				
Variation								
Number of Fetuses	1	0	0	0				
Litter % of Fetuses [k]	0.98	0.00	0.00	0.00				
Number of Litters	1	0	0	0				
Malformation								
Number of Fetuses	0	0	0	3				
Litter % of Fetuses [k]	0.00	0.00	0.00	5.56*				
Number of Litters	0	0	0	3				
All classifications								
Number of Fetuses	1	0	0	3				
Litter % of Fetuses [k]	0.98	0.00	0.00	5.56				
Number of Litters	1	0	0	3				

9.3 Pre- and Postnatal Development

Study title: RO7034067: An Oral (Gavage) Pre and Postnatal Development Study in the Rats

Study no.:	9001257
Study report location:	4 2 3 5 2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	18 Jun 2018
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	(b) (4) 1702SA01, 99.5%

Key Study Findings

When RIS (0, 0.75, 1.5, or 3 mg/kg/day) was orally administered to female Wistar rats throughout pregnancy and lactation, gestation was lengthened at the HD (1 HD dam was euthanized on GD 22 after showing signs of prolonged labor), but there were no other signs of maternal toxicity. There were no apparent effects on offspring survival or growth. Sexual maturation (vaginal opening) was delayed (SS) in HD female offspring, and when offspring were mated the numbers of corpora lutea, implantation sites, and live embryos were decreased at the HD. At necropsy of offspring, dose-related increases in total ovarian follicle number and ovarian follicles per section were noted. Locomotor activity tended to be increased in treated groups, but differences were not SS. (Increased locomotor activity was also seen in a preweaning JAS.) No apparent group differences were observed in auditory startle response and habituation or in performance in the Cincinnati maze. The no-effect dose for adverse effects on pre- and postnatal development in rats (1.5 mg/kg/day) was associated with maternal exposures of 2360 and 1880 ng.h/mL on GD 6 and PND 7, respectively.

Methods

Doses:	0, 0.75, 1.5, 3 mg/kg/day
Frequency of dosing:	QD
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) ascorbic (b) (4)
Species/Strain:	Crl:WI (Han) Wistar Hannover
Number/Group:	22/group
Satellite groups:	2/group TK
Study design:	PPND design, dosing GD6-PND20
Deviation from study protocol:	None

Experimental Design

Group No.	Test Material	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of F_0 Females	
					Main Study	TK Subset[a]
1	Vehicle Control (Reference Item)	0	0	5	22	2
2	RO7034067	0.75	0.15	5	22	2
3	RO7034067	1.5	0.3	5	22	2
4	RO7034067	3.0	0.6	5	22	2

[a] Toxicokinetic animals were used for toxicokinetic evaluation only.

RIS was administered to timed-mated female Wistar rats once daily by oral gavage from GD 6 until PND 20. Maternal end points were mortality, clinical signs, body weights, food consumption, toxicokinetic parameters (plasma), natural delivery and maternal performance, gross necropsy examinations including uterine and ovarian examination. Offspring assessments included survival, clinical signs, body weights, sexual maturation, behavioral performance (motor activity, auditory startle habituation, and Cincinnati water maze), TK parameters, reproductive performance (estrous cycles, mating and fertility, ovarian and uterine examination on Day 13 of gestation), ovaries weight, gross pathology, and primordial and primary follicle counts.

Doses were based on the results of a dose range-finding study (9001256) conducted by the same lab in which RIS (0, 1, 3, 7.5 mg/kg/day) administration to Wistar rats throughout pregnancy and early lactation (GD 6 - PND 6) resulted in the early sacrifice of 3/6 HD dams on GD 23/24 due to difficult parturition (maternal/fetal dystocia) and of 1 HD female on PND 1 after its whole litter had died. There were no drug-related clinical observations in surviving females and no effects on maternal BW or food consumption during gestation and lactation. There were 6, 5, 6, and 2 litters in C, LD, MD, and HD groups, respectively, available for assessment. There was a dose-related increase in the length of gestation at the MD and HD (22.2 and 22.7 days, respectively, vs. 21.7 days in C). The number of implants were similar across groups, but the live birth index was decreased at the MD (slight) and HD due to an increased incidence of pups found dead at birth. The pup PND 4 viability index was markedly decreased at the HD (58% compared to 100% in C).

Observations and Results

F0 Dams

Mortality

One HD female (No. 466) was euthanized on GD 22 after showing signs of prolonged labor (dystocia). At the uterine examination, there were 11 live fetuses. There were no macroscopic findings that could explain the dystocia in this animal.

Clinical signs

There were no drug-related clinical observations during either the gestation or lactation phases.

Body weight

Maternal BWs were unaffected by drug administration during the gestation and lactation periods.

Delivery data

The pregnancy rate and gestation index were at least 95% in all groups. Prolongation of the labor (dystocia) for 1 HD dam (No. 466) was likely related to drug. Gestation length was increased (SS) at the HD. Numbers of implantation sites, live and dead pups per litter at birth, and the live birth index were comparable across groups. There were no malformed pups at birth.

Necropsy

There were no drug-related macroscopic findings.

Toxicokinetics

Maternal plasma TK parameters for parent and major metabolite are shown in Table 1.

Table 1. Toxicokinetic Parameters in Dam Wistar Hannover Rat Plasma on Day 6 pc, Day 15 pc and Day 7 pp Following 0.75, 1.5 and 3.0 mg/kg/day Oral Administration of RO7034067

Analyte	Gender	Day	Dose (mg/kg/day)	Tmax (hr)	Cmax (ng/mL)	Cmax/D (ng/mL/ mg/kg/day)	AUC(0-24h) (hr*ng/mL)	AUC(0-24h)/D (hr*ng/mL/ mg/kg/day)	RAUC
RO7034067	Female	6 pc	0.75	3	103	137	1050	1400	NA
			1.5	1	218	145	2360	1570	NA
			3.0	1	514	171	5230	1740	NA
		15 pc	0.75	3	102	136	1020	1360	0.972
			1.5	3	249	166	3190	2130	1.35
			3.0	1	617	206	6710	2240	1.28
		7 pp	0.75	3	91.6	122	954	1270	0.912
			1.5	1	197	131	1880	1250	0.795
			3.0	1	549	183	4230	1410	0.808

NA = Not applicable.
RAUC = Day 15 pc or Day 7 pp AUC(0-24h)/Day 6 pc AUC(0-24h).

Analyte	Gender	Day	Dose (mg/kg/day)	Tmax (hr)	Cmax (ng/mL)	Cmax/D (ng/mL/ (mg/kg/day))	AUC(0-24h) (hr*ng/mL)	AUC(0-24h)/D (hr*ng/mL/ (mg/kg/day))	RAUC
RO7112063	Female	6 pc	0.75	3	19.6	26.1	188	251	NA
			1.5	7	34.1	22.7	504	336	NA
			3.0	3	60.0	20.0	804	268	NA
		15 pc	0.75	3	16.8	22.4	176	235	0.938
			1.5	7	39.2	26.1	624	416	1.24
			3.0	3	86.1	28.7	1020	338	1.26
		7 pp	0.75	3	11.5	15.3	112	149	0.596
			1.5	3	20.1	13.4	266	178	0.529
			3.0	3	51.6	17.2	490	163	0.609

NA = Not applicable.
RAUC = Day 15 pc or Day 7 pp AUC(0-24h)/Day 6 pc AUC(0-24h).

Offspring

Survival

There was no drug-related mortality.

Clinical signs

There were no drug-related clinical observations.

Body weight

Pup BWs were increased (SS) at birth in the HD group (probably due to increased gestation length) but were similar among groups thereafter.

Sexual maturation

Sexual maturation (vaginal opening) was delayed (SS) in HD female offspring (Table 2).

Table 2.

Summary of Vaginal Opening and Body Weight on Day of Development

F1 Generation Adults

Reproductive and Behavior Subset / Primordial and Primary Follicle Counts Subset (Day Post Partum)

Group 1 - Vehicle Control (Reference Item) Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day Group 4 - RO7034067 3.0 mg/kg/day

Group	Summary Information	Day of Development	Body Weights (g)
1	Mean	29.5	95.2
	SD	2.6	16.1
	N	31	31
2	Mean	30.2	101.1
	SD	2.3	14.6
	N	31	31
	% Diff (G1)	2	6
3	Mean	30.7	100.3
	SD	1.9	12.4
	N	31	31
	% Diff (G1)	4	5
4	Mean	31.5 E	103.5
	SD	2.2	11.4
	N	31	31
	% Diff (G1)	7	9

Significantly different from control group (Group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)
Significantly different from control group (Group 1) value (Day of development): D - P ≤ 0.05 E - P ≤ 0.01 F - P ≤ 0.001 (Dunn)

Neurobehavioral assessment

When locomotor activity was assessed (automated photobeam activity system) on PND 53, total fine and ambulatory movements tended to be increased in treated groups but differences were not SS (Table 3). Increased locomotor activity was also seen in a preweaning JAS (CR# 9000602). No apparent group differences were observed in auditory startle response and habituation on PND 55 (San Diego instruments system). Performance in the Cincinnati maze (both A and B paths tested) was comparable among groups when testing was conducted between PNDs 57 to 73.

Table 3. Motor activity

Male - Day 53 (±2) pp

Ambulation

Group	Statistics	Interval No. 1	2	3	4	5	6	Combined Intervals
1 - Vehicle control	LSMean	407.05	147.18	168.59	89.73	106.82	26.64	157.67
0 mg/kg/day	SELSM	35.055	17.887	21.211	20.254	19.583	14.956	10.711
	N	22	22	22	22	22	22	132
2 - RO7034067	LSMean	437.62	185.00	139.19	93.43	95.19	67.67	169.68
0.75 mg/kg/day	SELSM	35.880	18.308	21.710	20.730	20.044	15.308	10.963
	N	21	21	21	21	21	21	126
3 - RO7034067	LSMean	454.68	188.32	141.55	98.05	74.82	96.64	175.67
1.5 mg/kg/day	SELSM	35.055	17.887	21.211	20.254	19.583	14.956	10.711
	N	22	22	22	22	22	22	132
4 - RO7034067	LSMean	447.81	194.38	131.90	125.67	86.48	94.10	180.06
3.0 mg/kg/day	SELSM	35.880	18.308	21.710	20.730	20.044	15.308	10.963
	N	21	21	21	21	21	21	126

No significant pairwise comparison.

Female - Day 53 (±2) pp

Ambulation

Group	Statistics	Interval No. 1	2	3	4	5	6	Combined Intervals
1 - Vehicle control	LSMean	359.71	216.24	152.33	130.57	86.90	112.48	176.37
0 mg/kg/day	SELSM	21.573	18.363	12.672	22.018	21.542	21.913	11.946
	N	21	21	21	21	21	21	126
2 - RO7034067	LSMean	391.48	211.52	152.19	118.71	88.10	78.57	173.43
0.75 mg/kg/day	SELSM	29.816	18.230	24.685	18.789	18.183	15.907	9.907
	N	21	21	21	21	21	21	126
3 - RO7034067	LSMean	449.73	210.59	196.55	132.50	80.59	123.41	198.89
1.5 mg/kg/day	SELSM	39.903	20.772	33.484	21.257	20.819	23.737	16.292
	N	22	22	22	22	22	22	132
4 - RO7034067	LSMean	411.48	194.67	186.43	131.19	112.38	129.43	194.26
3.0 mg/kg/day	SELSM	24.677	18.673	24.099	20.194	28.665	16.530	10.156
	N	21	21	21	21	21	21	126

No significant pairwise comparison.

Reproduction (mating on PNDs 77 to 84)

No drug-related effects were noted on estrus cyclicity, mating and fertility indices, conception rate, or days to mating. However, the numbers of corpora lutea, implantation sites (SS at HD), and live embryos were decreased and preimplantation loss was increased (not D-R) at the MD and/or HD (Table 4). Other uterine parameters evaluated (number of dead embryos, early resorptions, and post-implantation loss) were comparable among groups.

Table 4.

Comparison of Selected Ovarian and Uterine Parameters

Group Dose Level	Number of Corpora Lutea	Number of Implantation Sites	Number of Live Embryos	Pre-implantation Loss (%)
HCD	13.3 (11.8 - 14.7)	12.0 (9.8 - 13.7)	11.3 (9.3 - 12.8)	10.0 (5.2 - 22.4)
1 0 mg/kg/day	14.0	13.5	12.7	3.9
2 0.75 mg/kg/day	13.7	13.3	12.8	3.0
3 1.5 mg/kg/day	13.7	12.4	11.9	9.0
4 3 mg/kg/day	13.1	**12.2***	11.7	6.4

HCD = Test Facility's historical control data mean (range); **Bold*** = Statistically significant ($p \leq 0.05$)

At necropsy, an increasing trend of total ovarian follicle number and mean ovarian follicles per section was noted with increasing dose (Table 5). The significance of this finding in relation to the reproductive effects noted above is unclear. No drug-related microscopic findings were noted at examination of the ovaries.

Table 5.

F_1 Mean Ovarian Follicle Counts per Group

Dose Group (mg/kg/day)	Group Size (N)	Total Ovarian Follicle Counts	STD	Mean Ovarian Follicle Counts per Section[a]	STD
0	10	134.3	36.9	13.4	3.69
0.75	10	143.0	40.1	14.3	4.01
1.5	9	156.7	70.6	15.7	7.06
3.0	10	182.8	56.8	18.3	5.68

[a] Mean of the average counts per section of ovary (i.e., from 10 sections from right and left ovaries combined).
STD = Standard Deviation

Toxicokinetics

Offspring plasma TK parameters for parent and major metabolite are shown in Table 6.

Table 6. TK parameters in on PND 7

Analyte	Gender	Day	Dose (mg/kg/day)	Tmax (hr)	Cmax (ng/mL)	SE Cmax (ng/mL)	Cmax/D (ng/mL/(mg/kg/day))	SE Cmax/D (ng/mL/(mg/kg/day))	AUC(0-24h) (hr*ng/mL)	SE AUC(0-24h) (hr*ng/mL)	AUC(0-24h)/D (hr*ng/mL/(mg/kg/day))	SE AUC (0-24h)/D (hr*ng/mL)
RO7034067	M+F	7 pp	0.75	7	5.07	0.759	6.76	1.01	NR	NR	NR	NR
			1.5	7	7.16	0.496	4.78	0.330	146	7.43	97.2	4.95
			3.0	7	20.0	0.857	6.66	0.286	381	11.7	127	3.90

NR = Not reported because there were less than 3 quantifiable concentrations at consecutive time points.
M+F = Sexes combined.

Analyte	Gender	Day	Dose (mg/kg/day)	Tmax (hr)	Cmax (ng/mL)	SE Cmax (ng/mL)	Cmax/D (ng/mL/(mg/kg/day))	SE Cmax/D (ng/mL/(mg/kg/day))	AUC(0-24h) (hr*ng/mL)	SE AUC (0-24h) (hr*ng/mL)	AUC(0-24h)/D (hr*ng/mL/(mg/kg/day))	SE AUC (0-24h)/D (hr*ng/mL)
RO7112063	M+F	7 pp	0.75	7	1.71	0.0896	2.28	0.120	26.0	1.78	34.7	2.37
			1.5	7	2.40	0.102	1.60	0.0678	47.3	1.48	31.6	0.983
			3.0	7	5.32	0.589	1.77	0.196	99.8	6.54	33.3	2.18

M+F = sexes combined.

9.4 Juvenile Animal Toxicity Studies

Study title: A 13-Week Toxicity Study of RO7034067 by Oral Gavage in Juvenile Wistar Hannover Rats with an 8-Week Recovery Period

Study no.:	9000538
Study report location:	4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10 Jun 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 024780-026/ 84.8%

Key Study Findings

Juvenile Wistar rats (for numbers see experimental design) were administered RIS (0 (vehicle), 1, 3, or 7.5 mg/day) daily by oral gavage for 13 weeks, from PND 22 through PND 112. A positive control group was given a single dose of cyclophosphamide (20 mg/kg) on PND 112. There was no mortality and no effects on clinical signs, body weights, long bone growth, or sexual maturation. Neurobehavioral testing indicated possible (NS) effects on auditory startle and learning and memory in the HD group at the end of the treatment and recovery periods. Lymphocyte phenotyping showed dose-dependent increases in total, helper and cytotoxic T lymphocytes at the MD and HD males and decreases in the relative percentages of B cells in these groups at the end of the dosing period. Cytotoxic T cells remained elevated in HD animals at the end of the recovery period. Degeneration/necrosis of the seminiferous epithelium, with accompanying oligo/aspermia in the epididymis, was observed at the HD at the end of the dosing period and these testicular effects had not fully recovered after 8 weeks. Renal tubular dilatation was increased in incidence and severity in HD animals at the end of the treatment period but was seen in MD and HD animals after the recovery period. Malignant nephroblastoma was seen in a single HD male at the PND 113 necropsy. Single cell necrosis in the intestinal tract seen at the HD was shown to be reversible. At the end of the dosing period (PND 113), HD males and females at all doses showed increases (SS) in the number of micronucleated immature erythrocytes in the bone marrow. Values exceeded the vehicle control value by 12-fold for males and by 2.2 to 9.6-fold for females, and HD male and female values exceeded the cyclophosphamide positive control values. Decreased sperm concentrations and motility associated with an increased number of spermatozoa morphology abnormalities were found in the HD reproductive subset males at the completion of the mating period, but there was no evidence of impairment in male or female functional reproductive performance. The low-effect level (1 mg/kg/day), based on MN increases (SS) in females at all doses, was associated with AUC values of 600 and 511 ng*hr/mL on PND 22 in males and females, respectively.

Methods

Doses:	0 (vehicle), 1, 3, 7.5 mg/day
Frequency of dosing:	QD
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) ascorbic acid (b) (4)
Species/Strain:	Rat/ Wistar Hannover Crl:WI (Han)
Number/Sex/Group:	12 main, 6 recovery, 20 neurobehavioral/ reproductive, 4-8/sex/grp TK (see table below)
Age:	Postnatal Days 22 to 112

Experimental Design

Group No.	Test Item	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals	
					M	F
1	Vehicle	0	5	0	62	62
2	RO7034067	1	5	0.2	66	66
3	RO7034067	3	5	0.6	66	66
4	RO7034067	7.5	5	1.5	66	66
5	Positive Control (Cyclophosphamide)[a]	20	10	2	5	5

[a] Dosing once, by oral gavage, 24-hours before sampling for micronucleus assessment.

Experimental Design - Subset Allocation

Group No.	No. of Animals									
	Main Study (Subset A)[a]		Recovery (Subset B)[a]		Reproductive/ Behavioral Assessment (Subset C)[a, b]		Toxicokinetic (Subset D)[c]		Toxicokinetic/ Immunotoxicity (Subset E)[a, d, e]	
	M	F	M	F	M	F	M	F	M	F
1	12	12	6	6	20	20	4	4	10 + 10	10 + 10
2	12	12	6	6	20	20	8	8	10 + 10	10 + 10
3	12	12	6	6	20	20	8	8	10 + 10	10 + 10
4	12	12	6	6	20	20	8	8	10 + 10	10 + 10

[a] Dosing on Days 22 to 112 pp, inclusive, followed by a recovery period of 8 weeks, where applicable.
[b] 20 naïve females were assigned to each group as untreated partners to treated males; treated females were mated with proven breeder males.
[c] Dosing on Day 22 pp only; animals were used for toxicokinetic evaluations and bioanalysis only at Day 22 pp.
[d] Samples for immunotoxicology and/or bioanalysis at end of dosing period or end of recovery period (10 rats/sex/occasion).
[e] Samples for toxicokinetic evaluations at Days 79 and 112 pp (4 controls/sex and 8 rats/sex in Groups 2, 3, and 4 per occasion).

Dose selection was based on the results of a dose range-finding study in which RIS (0, 2.5, 5, or 10 mg/kg/day) was administered to neonatal/juvenile Wistar rats on PNDs 4 to 31 (Study # 9000537). Due to mortality seen at the HD and MD (on PNDs 8 and 11) and termination of these groups on PNDs 10/11 and 13/14, respectively, an additional group was added at 3.75 mg/kg. RIS was not tolerated at doses of 3.75 mg/kg/day and above, resulting in mortality, clinical signs, and reduced BW gain. Other findings included GI

toxicity (cryptal apoptosis/single cell necrosis) at 10 mg/kg and bone marrow myelosuppression (all cell lineages) at 3.75 and 5 mg/kg/day. The NOAEL for these findings with preweaning dosing was 2.5 mg/kg/day. In order to evaluate the effects on survival when pups were treated starting at weaning, additional animals were dosed on PNDs 22 to 36 at doses of 1, 3, and 9 mg/kg/day and no mortality or other adverse effects were observed. Exposures (AUC) at 2.5 and 3.75 mg/kg/day were 0.7- or 0.5-fold higher on PND 4 compared to PND 31, respectively. Based on limited data, exposures generally increased 2-fold between PNDs 22 and 36 at 1 and 3 mg/kg/day, respectively.

Observations and Results

Mortality

There was no unscheduled mortality.

Clinical Signs

There were no clinical observations attributed to RIS.

Body Weights

BW gain and BW were unaffected by drug.

Food Consumption

There were no effects on food consumption.

Bone growth

Femur lengths were comparable across groups.

Sexual maturation

No drug-related differences were noted.

Neurobehavioral assessment

FOB
There were no drug-related differences at the end of the dosing or recovery period.

Locomotor activity
There were no drug-related group differences at the end of the dosing or recovery period.

Auditory startle habituation
The auditory startle response (maximum startle) and ASR habituation (LTC) appeared to be decreased in HD males at the end of treatment (Table 2) and in HD animals of both sexes at the end of recovery, but SS was not reached (Table 3).

Table 2.

F1 Generation Adults - Subset C - Males
Day 108 (±1) Post Partum
Maximum Startle (voltage)

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Trial 1-10	11-20	21-30	31-40	41-50	Mean Level	Linear Time Contrast
1	Mean	1317.83	960.48	855.89	700.48	555.17	877.97	-1785.32
	SD	676.31	533.60	513.87	436.98	223.61	403.69	1356.73
	N	15	15	15	15	15	15	15
2	Mean	1437.06	1009.27	864.19	775.70	700.10	957.26	-1707.49
	SD	1011.36	786.62	668.18	704.32	408.30	672.17	1699.58
	N	15	15	15	15	15	15	15
3	Mean	1263.81	788.82	670.55	556.14	568.05	769.47	-1624.20
	SD	754.24	578.19	563.26	420.30	327.06	492.21	1346.24
	N	15	15	15	15	15	15	15
4	Mean	974.97	656.37	597.13	489.98	498.73	643.43	-1118.87
	SD	852.93	609.62	567.93	523.51	425.28	576.88	1121.31
	N	15	15	15	15	15	15	15

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

Table 3.

F1 Generation Adults - Subset B and E - Males
Recovery Period (Before Day 153 Post Partum)
Maximum Startle (voltage)

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Trial 1-10	11-20	21-30	31-40	41-50	Mean Level	Linear Time Contrast
1	Mean	1679.03	1318.05	1108.89	1015.89	864.18	1197.21	-1931.86
	SD	1013.28	1096.29	838.79	576.85	451.95	737.47	2004.34
	N	16	16	16	16	16	16	16
4	Mean	1462.46	1060.73	859.84	805.54	728.04	983.32	-1724.04
	SD	885.23	834.64	468.98	478.24	485.65	555.08	1956.98
	N	16	16	16	16	16	16	16

Significantly different from control group (Group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (*t*-test)
d - $P \leq 0.05$ e - $P \leq 0.01$ f - $P \leq 0.001$ (Wilcoxon)

F1 Generation Adults - Subset B and E - Females
Recovery Period (Before Day 153 Post Partum)
Maximum Startle (voltage)

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Trial 1-10	11-20	21-30	31-40	41-50	Mean Level	Linear Time Contrast
1	Mean	745.31	703.44	726.51	635.34	462.81	654.68	-633.12
	SD	311.96	250.76	522.05	533.94	188.81	315.26	654.51
	N	16	16	16	16	16	16	16
4	Mean	586.10	558.81	515.00	457.39	416.13	506.69	-441.35
	SD	217.63	224.75	213.43	143.51	154.56	159.02	372.26
	N	16	16	16	16	16	16	16

Significantly different from control group (Group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (*t*-test)
d - $P \leq 0.05$ e - $P \leq 0.01$ f - $P \leq 0.001$ (Wilcoxon)

Learning and memory (Cincinnati maze, only B path tested)

When tested at the end of the dosing period (PNDs 108-112), there were no clear effects on maze performance. However, when tested during the recovery period (before PND 153), there appeared to be a learning deficit (increased latencies and errors on last 3 trials; errors SS on trial 5) in HD males (Table 4).

Table 4. Cincinnati Water Maze

Latencies (sec)

F1 Generation Adults - Subset B and E
During Recovery Period - Path B
Males

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	301.0	247.9	210.3	158.9	98.0	83.7
	SD	0.0	79.8	105.9	102.1	107.9	97.9
	N	16	16	16	16	16	16
4	Mean	301.0	253.8	238.6	216.6	159.8	119.4
	SD	0.0	86.2	91.2	110.4	102.2	115.7
	N	16	16	16	16	16	16

Significantly different from control group (Group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (t-test)
d - $P \leq 0.05$ e - $P \leq 0.01$ f - $P \leq 0.001$ (Wilcoxon)

Errors

F1 Generation Adults - Subset B and E
During Recovery Period - Path B
Males

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	17.7	12.4	12.1	7.6	4.4	2.4
	SD	3.3	4.7	5.9	5.3	6.0	4.3
	N	16	16	16	16	16	16
4	Mean	16.8	11.9	12.4	10.1	8.6 d	6.2
	SD	3.6	4.4	4.3	4.7	7.6	8.1
	N	16	16	16	16	16	16

Significantly different from control group (Group 1) value: d - $P \leq 0.05$ e - $P \leq 0.01$ f - $P \leq 0.001$ (Wilcoxon)

Reproductive Performance

When animals were mated on PND 112, there were no clearly drug-related effects on estrous cycles, fertility indices (however, see below), or C-section parameters (numbers of corpora lutea, implantation sites, live embryos and resorptions, pre- and postimplantation loss).

Sperm Analysis

There was a decrease (SS) in sperm concentration (452 vs 621 million/g in C) and motility (52 vs 87% in controls) at the HD, associated with a decrease (SS) in cauda epididymis weight (0.19 vs 0.23 g in controls). In HD males No. 4031 and 4036, sperm count and motility could not be performed due to low amount of sperm production, which correlated with macroscopic findings observed in the testis and epididymis of these 2 males (also seen in main study HD male # 4003). Only male No. 4031 failed to produce a pregnancy. Morphologically, there was an increased percentage of abnormalities at the HD (21 vs 8% in C). These were related to an increased number of sperm with head separated from flagellum (33.8 vs 10.7 in C) and with misshapen head (3.2 vs 1.8 in C).

Clinical Pathology

Hematology

Increases in WBC (up to 56% compared to C), lymphocyte (60%), neutrophil (44%), and basophil (139%) counts were seen at the HD at PND 79 or 113. These were no longer seen after the recovery period. There were no group differences in coagulation parameters.

Clinical chemistry

Decreases in potassium (20%), total protein (6%), and albumin (8%) were seen at the HD on PNDs 79 and 113. There were no differences after the recovery period.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Ophthalmic examinations

When animals were examined between PNDs 21 and 24 and during the last week of the dosing period, there were no drug-related ophthalmic changes.

Bone Marrow Smear Evaluation

There were no drug-related differences in the myeloid:erythroid (M:E) ratios and or in the maturation or cellular morphology of the hematopoietic cell lines at the end of the dosing period.

TDAR

During the dosing and recovery periods, there were no drug-related changes in the anti-KLH IgM and anti-KLH IgG antibody responses following KLH immunization when compared to the vehicle control animals.

Immunophenotyping

Dose-dependent increases in the absolute counts of total, helper, and cytotoxic T lymphocytes, were observed in MD and HD males and females on PND 113 (Table 5). Decreases in the relative percentages of B cells in these groups (SS at HD) were considered secondary to the increases in the relative percentages of the T cells subsets, since the decreases were not clearly reflected in the absolute counts of B lymphocytes.

Table 5. Summary of phenotyping in blood values

Scheduled Termination - Subset A
Males

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

		Total Lymphocyte Count	Total T Lymphocytes (CD3+)		Helper T Lymphocytes (CD3+/CD4+)		Cytotoxic T Lymphocytes (CD3+/CD8a+)	
Group	Summary Information	(cells/µL)	Relative Percentage	Absolute Count (cells/µL)	Relative Percentage	Absolute Count (cells/µL)	Relative Percentage	Absolute Count (cells/µL)
1	Mean	5202.2	55.300	2828.592	41.922	2133.791	15.569	805.966
	SD	1261.2	8.583	632.043	7.557	438.432	3.636	280.441
	N	9	9	9	9	9	9	9
2	Mean	4761.0	55.612	2646.361	42.088	1982.964	16.108	790.975
	SD	1307.0	6.760	792.839	5.391	515.978	3.821	374.058
	N	10	10	10	10	10	10	10
	% Diff (G1)	-8	1	-6	0	-7	3	-2
3	Mean	5809.0	57.938	3355.811	42.694	2452.528	17.614	1044.339
	SD	1078.7	7.979	723.316	6.723	434.858	3.615	370.577
	N	10	10	10	10	10	10	10
	% Diff (G1)	12	5	19	2	15	13	30
4	Mean	6204.0	60.176	3718.492 A	44.814	2748.460 A	17.586	1101.017
	SD	1111.6	9.508	857.946	7.501	544.030	3.967	371.234
	N	10	10	10	10	10	10	10
	% Diff (G1)	19	9	31	7	29	13	37

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

Females

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day

Group	Summary Information	Total Lymphocyte Count (cells/µL)	Total T Lymphocytes (CD3+) Relative Percentage	Total T Lymphocytes (CD3+) Absolute Count (cells/µL)	Helper T Lymphocytes (CD3+/CD4+) Relative Percentage	Helper T Lymphocytes (CD3+/CD4+) Absolute Count (cells/µL)	Cytotoxic T Lymphocytes (CD3+/CD8a+) Relative Percentage	Cytotoxic T Lymphocytes (CD3+/CD8a+) Absolute Count (cells/µL)
1	Mean	4043.8	59.419	2422.856	45.421	1855.616	15.926	651.375
	SD	1108.2	8.455	748.554	6.641	539.155	2.936	258.708
	N	8	10	8	10	8	10	8
2	Mean	3844.0	57.823	2219.811	44.195	1689.556	15.782	616.163
	SD	907.5	7.923	668.812	6.294	504.767	3.145	216.963
	N	10	11	10	11	10	11	10
	% Diff (G1)	-5	-3	-8	-3	-9	-1	-5
3	Mean	4848.0	61.918	3065.405	45.752	2262.463	17.667	892.922
	SD	1137.4	8.519	1026.388	5.935	737.514	4.663	395.678
	N	10	11	10	11	10	11	10
	% Diff (G1)	20	4	27	1	22	11	37
4	Mean	5010.0	70.493 B	3532.894 A	51.232	2563.708	20.553 A	1035.504
	SD	1611.7	5.855	1210.238	6.783	880.043	3.037	407.172
	N	10	10	10	10	10	10	10
	% Diff (G1)	24	19	46	13	38	29	59

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

At the end of recovery, the increase in total T cells and T helper cell subset had recovered, but cytotoxic T cells remained somewhat elevated in HD males.

Necropsy

Organ weights

At the end of the dosing period, drug-related increases in absolute and relative kidney weights were seen in HD males and females. Microscopically, this appeared to correlate with an increased incidence of tubular dilatation compared to controls. Testis weights were slightly decreased (10%, NS) at the end of treatment. Following the 8-week recovery period, there were no drug-related organ weight differences.

Macroscopic

At the end of the dosing period, drug-related macroscopic findings in the testis and epididymis were seen in 1 HD male (No. 4003; also seen in 2 reproductive assessment subgroup HD males). In the testis, there were soft consistency and pale focus, correlating microscopically with sperm granulomas. In the epididymis, there was soft consistency correlating microscopically with oligo/aspermia. Dark discoloration was observed in the adrenal gland at all doses, but there were no corresponding weight changes or microscopic findings in the adrenals. There were no macroscopic findings considered drug-related after the recovery period.

Histopathology

Drug-related histopathology changes in testis, epididymis, and intestinal tract (duodenum, jejunum, ileum, cecum and colon) were seen at the HD. In the testis, degeneration/necrosis of the seminiferous epithelium, characterized by single cell necrosis of different cell types in the seminiferous epithelium and vacuolation of Sertoli cells, was found in 3/12 males (Table 6). In 2 of these, it was associated with oligo/aspermia in the epididymis. Additionally, male No. 4003 had bilateral spermatic granulomas with seminiferous tubule degeneration in the remaining testis, associated with severe oligo/aspermia in the epididymis. Following the recovery period, there was partial reversibility in the testis (seminiferous tubule degeneration/necrosis present in 1/6 HD recovery males) and full reversibility in the epididymis.

Table 6.

Summary of Microscopic Findings - Scheduled Euthanasia (Day 113 pp)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**1**	**3**	**7.5**	**0**	**1**	**3**	**7.5**
No. Animals Examined	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**
Testis (No. Examined)	12	12	12	12				
Degeneration/necrosis, seminiferous tubule	(-)[a]	(-)	(-)	(3)	n/a	n/a	n/a	n/a
Minimal	-	-	-	1	n/a	n/a	n/a	n/a
Mild	-	-	-	2	n/a	n/a	n/a	n/a
Sperm granuloma	(-)	(-)	(-)	(1)	n/a	n/a	n/a	n/a
Moderate	-	-	-	1	n/a	n/a	n/a	n/a
Epididymis (No. Examined)	12	12	12	12				
Oligo/aspermia	(-)	(-)	(-)	(3)	n/a	n/a	n/a	n/a
Moderate	-	-	-	2	n/a	n/a	n/a	n/a
Severe	-	-	-	1	n/a	n/a	n/a	n/a

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

In the intestinal tract, single cell necrosis in the crypts of the ileum, jejunum, duodenum, and colon was seen in HD males and females (Table 7). These findings in the GI tract were not present in HD animals examined at the end of the recovery period.

Table 7.

Summary of Microscopic Findings - Scheduled Euthanasia (Day 113 pp)

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	1	3	7.5	0	1	3	7.5
No. Animals Examined	12	12	12	12	12	12	12	12
Duodenum (No. Examined)	12	12	12	12	12	12	12	12
Single cell necrosis	(-)[a]	(-)	(-)	(1)	(-)	(-)	(-)	(-)
Minimal	-	-	-	1	-	-	-	-
Jejunum (No. Examined)	12	12	12	12	12	12	12	12
Single cell necrosis	(-)	(-)	(-)	(6)	(-)	(-)	(-)	(2)
Minimal	-	-	-	6	-	-	-	2
Ileum (No. Examined)	12	12	12	12	12	12	12	12
Single cell necrosis	(-)	(-)	(-)	(10)	(-)	(-)	(-)	(4)
Minimal	-	-	-	9	-	-	-	4
Mild	-	-	-	1	-	-	-	-
Cecum (No. Examined)	12	12	12	12	12	12	12	12
Single cell necrosis	(-)	(-)	(-)	(1)	(-)	(-)	(-)	(-)
Minimal	-	-	-	1	-	-	-	-
Colon (No. Examined)	12	12	12	12	12	12	12	12
Single cell necrosis	(-)	(-)	(-)	(2)	(-)	(-)	(-)	(2)
Minimal	-	-	-	2	-	-	-	2

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

Renal tubular dilatation was increased in incidence and severity in HD animals at the end of the treatment period and was only seen in MD and HD animals after the recovery period (Table 8). In addition, malignant nephroblastoma was seen in a single HD male.

Table 8.

Summary of Microscopic Gradings by Organ/Group/Sex, Main Study - Subset A
9000538

Removal Reason: TERMINAL EUTHANASIA	Male				Female			
	0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4	0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4
Number of Animals:	12	12	12	12	12	12	12	12
GLAND, THYROID								
Examined	12	0	0	12	12	0	0	12
No Visible Lesions	12	.	.	12	12	.	.	12
HEART								
Examined	12	0	0	12	12	0	0	12
No Visible Lesions	11	.	.	9	12	.	.	11
Infiltration, mononuclear cell	1	.	.	3	0	.	.	1
.... minimal	1	.	.	3	0	.	.	1
KIDNEY								
Examined	12	12	12	12	12	12	12	12
No Visible Lesions	7	8	7	2	9	9	7	3
Nephroblastoma, malignant	0	0	0	1	0	0	0	0
Dilatation; tubular	2	3	3	7	3	2	4	7
.... minimal	2	3	3	3	2	2	4	4
.... mild	0	0	0	4	1	0	0	3
Dilatation; pelvis	1	2	2	1	0	1	1	1
.... minimal	1	2	2	0	0	0	0	1
.... mild	0	0	0	1	0	1	1	0
Basophilia; tubular	2	0	1	1	0	0	0	1

Summary of Microscopic Gradings by Organ/Group/Sex, Recovery - Subset B
9000538

Removal Reason: RECOVERY EUTHANASIA	Male				Female			
	0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4	0 mg/kg /day Group 1	1 mg/kg /day Group 2	3 mg/kg /day Group 3	7.5 mg/kg /day Group 4
Number of Animals:	6	6	6	6	6	6	6	6
KIDNEY								
Examined	6	6	6	6	6	6	6	6
No Visible Lesions	4	4	4	3	6	6	4	3
Dilatation; tubular	0	0	0	2	0	0	1	3
.... minimal	0	0	0	2	0	0	1	3
Dilatation; pelvis	1	1	2	0	0	0	0	0
.... minimal	0	1	1	0	0	0	0	0
.... mild	1	0	1	0	0	0	0	0
Basophilia; tubular	1	1	1	1	0	0	0	0
.... minimal	1	1	1	1	0	0	0	0
Urolithiasis	0	0	0	0	0	0	1	0
.... minimal	0	0	0	0	0	0	1	0

Bone Marrow Micronucleus Assay

HD males and female at all doses showed increases (SS) in the number of micronucleated immature erythrocytes (MIE). Values exceeded the vehicle control value by 12-fold for males and by 2.2 to 9.6-fold for females, and HD male and female values exceeded the cyclophosphamide positive control values (Table 9).

Table 9.

Summary of Micronucleus Results

Sampling Time - Day 113 After the Final Administration
Males

Group 1 - Vehicle
Group 2 - RO7034067 1 mg/kg/day
Group 3 - RO7034067 3 mg/kg/day
Group 4 - RO7034067 7.5 mg/kg/day
Group 5 - Positive Control (Cyclophosphamide) 20 mg/kg [a]

Group	% IE/(IE+ME)	Incidence MIE *	% MIE	% MME
1	49.4	19	0.095	0.00
2	48.6	16	0.080	0.00
3	52.8	29	0.145	0.00
4	57.1	233 **	1.165	0.00
5	36.4	173 **	0.865	0.06

Group	% IE/(IE+ME)	Incidence MIE *	% MIE	% MME
1	49.3	14	0.070	0.07
2	42.3	31 **	0.155	0.00
3	48.6	32 **	0.160	0.00
4	48.0	134 **	0.670	0.07
5	27.1	107 **	0.535	0.00

%IE/(IE+ME)	Proportion of immature erythrocytes
%MIE	Percentage of micronucleated immature erythrocytes
%MME	Percentage of micronucleated mature erythrocytes
MIE	Number of micronucleated cells observed per 4000 immature erythrocytes examined
a	Dosed once, by oral gavage, 24 hours prior to sampling

Significant trend across dose-related increase (Groups 1 to 4): * - $P \leq 0.05$ (Cochran-Armitage)
Group 2,3,4 or 5 significantly different from control group (Group 1) value: ** - $P \leq 0.05$ (Fisher's exact)

Toxicokinetics

TK parameters for RIS are shown in Table 10.

Table 10.

Summary Mean (±SE) RO7034067 Toxicokinetic Parameters in Wistar Hannover Rat Plasma Following Oral Administration of RO7034067 on Day 22, Day 79 and Day 112 pp

Dose (mg/kg)	Day	Sex	T_{max} (hr)	C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	AUC(0-24) (hr*ng/mL)	AUC(0-24)D (hr*ng/mL/(mg/kg))	R_{AUC}
1	22*	Male	1	72.2 ± 29.8	46.0	600 ± 51.1	382	NA
	22*	Female	1	58.6 ± 12.0	37.3	511 ± 24.2	325	NA
	79	Male	3	147 ± 5.50	147	1610 ± 136	1610	4.21**
	79	Female	3	130 ± 5.00	130	1430 ± 208	1430	4.40**
	112	Male	3	170 ± 19.0	170	2010 ± 107	2010	5.26**
	112	Female	3	189 ± 13.5	189	1700 ± 123	1700	5.23**
3	22	Male	1	126 ± 19.5	41.8	989 ± 47.9	330	NA
	22	Female	3	82.5 ± 2.10	27.5	922 ± 80.2	307	NA
	79	Male	1	754 ± 50.0	251	7080 ± 595	2360	7.15
	79	Female	1	801 ± 20.0	267	7890 ± 777	2630	8.56
	112	Male	1	759 ± 47.0	253	7160 ± 952	2390	7.24
	112	Female	1	725 ± 22.0	242	7830 ± 453	2610	8.50
7.5	22	Male	3	309 ± 22.5	41.1	3150 ± 159	421	NA
	22	Female	3	385 ± 5.00	51.3	3490 ± 102	465	NA
	79	Male	1	1850 ± 240	247	18600 ± 1390	2480	5.90
	79	Female	3	1580 ± 55.0	210	19000 ± 445	2530	5.44
	112	Male	1	1500 ± 170	200	15900 ± 546	2120	5.03
	112	Female	3	1300 ± 70.0	173	17600 ± 724	2350	5.05

NA = Not Applicable
R_{AUC} = Day 79 or 112 AUC(0-24)/ Day 22 AUC(0-24)
* = Animal received 1.57 mg/kg ** = Dose normalized

Study title: RO7034067: A 4-Week Toxicity Study by Oral Gavage in Juvenile Wistar Hannover Rats with an 8-Week Recovery

Study no.:	9001258
Study report location:	4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	09 Jul 2018
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) 1702SA0/ 99.5%

Key Study Findings

Juvenile Wistar rats (for numbers see experimental design) were administered RIS (0 (vehicle), 0.75, 1.5, 2.5 mg/day) daily by oral gavage for 4 weeks, from PND 4 through PND 31. There was no mortality. Clinical signs consisted of suspected dehydration and thinness at the MD and HD. BW gain was reduced throughout the dosing period at the MD and HD; BW at end of the dosing period was 14 and 11% below C in HD M and F. BW remained significantly lower (9%) in HD males after the recovery period. Preputial separation was delayed (SS) at the MD and HD. Decreased tibia length was seen at the end of treatment and persisted up to the end of the recovery period at the MD and HD. Drug-related ophthalmic changes consisting of multiple small vacuoles in the anterior vitreous against the posterior capsule were seen at the HD. Neurobehavioral testing showed effects in the FOB at the end of the treatment period that partially recovered and possible (NS) effects on learning and memory in the Cincinnati water maze at the end of the recovery period. Impaired reproductive performance (mean day to mating, mating index, fertility index, and conception rate) was seen in HD females mated with proven breeder males. Decreases in the B lymphocyte relative percentages and absolute counts were observed in HD males and females at all doses on PND 49. Decreases in testis and epididymis weights were seen at the MD and HD at the end of the treatment and at all doses in the recovery groups. This correlated with degeneration of the seminiferous epithelium in the testis in MD and HD males at the end of dosing. Renal pelvic dilatation was increased in incidence in treated animals at the end of the treatment period and after the recovery period. Nephroblastomatosis was observed at all doses at the end of treatment, and nephroblastomas were present in the kidney of 1 MD and 1 HD female after the recovery period. The low-effect dose (0.75 mg/kg/day), based on immunophenotyping and testicular effects, was associated with AUC values of 680 and 686 ng*hr/mL for RIS and 163 and 173 ng*hr/mL for RO7112063 on PND 31 in males and females, respectively.

Methods

Doses: 0 (vehicle), 0.75, 1.5, 2.5 mg/day
Frequency of dosing: QD
Route of administration: Oral gavage
Dose volume: 5 mL/kg
Formulation/Vehicle: (b) (4) ascorbic acid (b) (4)
Species/Strain: Rat/ Wistar Hannover Crl:WI (Han)
Number/Sex/Group: 12 main, 20 neurobehavioral/ reproductive, 4-10/sex/grp/time TK (see table below)
Age: Postnatal Days 4 to 31

Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Number of Animals					
					Main Subset		Reproductive Subset		Toxicokinetic Study[a]	
					M	F	M	F[b]	M	F
1	Reference Item	0	5	0	12	12	20	20+20	4+4+4	4+4+4
2	RO7034067	0.75	5	0.15	12	12	20	20+20	8+10+10	8+10+10
3	RO7034067	1.5	5	0.3	12	12	20	20+20	8+10+10	8+10+10
4	RO7034067	2.5	5	0.5	12	12	20	20+20	8+10+10	8+10+10

Conc. = concentration; F = females; M = males
[a] N+N+N = numbers used for blood collection at Days 4, 12, and 31 pp occasions, respectively.
[b] 20 naïve females/group as mating partners for treated males

Endpoints included: mortality, clinical signs, body weights, food consumption, physical development (sexual maturation), long bone growth, ophthalmology, behavioral performance (functional observation battery, motor activity, auditory startle habituation, Cincinnati water maze), reproductive performance (estrous cycles, mating and fertility, ovarian and uterine examination on Day 13 of gestation), clinical pathology parameters (hematology, coagulation, clinical chemistry and urinalysis), toxicokinetic parameters, plasma protein binding, immunophenotyping, reproductive function including sperm assessment (concentration, motility, morphology), macroscopic findings, organ weights, and microscopic findings.

Dose selection was based on the results of the dose range-finding study in which RIS (0, 2.5, 5, or 10 mg/kg/day) was administered to neonatal/juvenile Wistar rats from PND 4 to 31 (Study # 9000537) and a previous GLP juvenile rat study (9000602) that was repeated due to an inadequate number of dose groups (2), lack of a mating assessment, and lack of a no-effect dose for testicular toxicity. In the DRF study, mortality was seen at the MD and HD. In the latter study, daily oral (gavage) administration of RIS (0, 1, or 2.5 mg/kg/day) to juvenile Wistar rats on PNDs 4 to 31 resulted in delayed (avg 5 days) male sexual maturation at the HD and neurobehavioral effects (decreased grip strength and increased hindlimb splay in FOB, increased locomotor activity, and impaired learning in Cincinnati maze at end of treatment and recovery), persistent decreases in bone length and testis weights, and degeneration of the seminiferous tubule epithelium (at end of treatment period but not after recovery) at both doses.

Observations and Results

Mortality

There was no unscheduled mortality.

Clinical Signs

Clinical observations of thinness and suspected dehydration at the MD and HD were attributed to RIS.

Body Weights

BW gain was decreased (SS) during the preweaning dosing period (PNDs 4 to 21) at the MD and HD in both sexes. BWs were reduced (SS) at weaning in MD and HD males (7 and 17% below C) and females (10 and 16%) and decreased BWs persisted to the end of the recovery period (PND 115) in HD males (9%) and through PND 56 in MD and HD females (5% in both groups).

Food Consumption

Food consumption was reduced (7%) in HD males between PNDs 21 and 31.

Bone growth

Tibia length was decreased (up to 9% below C, SS) in HD males and females during the preweaning period. During the postweaning period, tibia lengths were decreased in MD (up to 6%) and HD (up to 10%) males and females. Reduced (SS) tibia length persisted until the end of the recovery period in HD males and MD and HD females.

Sexual maturation

The day of preputial separation was delayed (SS) in MD and HD males (Table 1).

Table 1.

Summary of Preputial Separation and Body Weight on Day of Development

F1 Generation Adults
Day Post Partum

Group 1 - Reference Item
Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day
Group 4 - RO7034067 2.5 mg/kg/day

Group	Summary Information	Day of Development	Body Weight (g)
1	Mean	44.8	209.7
	SD	2.2	17.9
	N	20	20
2	Mean	45.0	203.3
	SD	1.7	15.6
	N	20	20
	% Diff (G1)	0	-3
3	Mean	46.2 D	205.8
	SD	1.2	14.6
	N	20	20
	% Diff (G1)	3	-2
4	Mean	47.2 F	198.4
	SD	2.6	18.8
	N	20	20
	% Diff (G1)	5	-5

Significantly different from control group (Group 1) value (Day of development): D - P ≤ 0.05 E - P ≤ 0.01 F- P ≤ 0.001 (Dunn)
Significantly different from control group (Group 1) value: A - P ≤ 0.05 B - P ≤ 0.01 C - P ≤ 0.001 (Dunnett)
D - P ≤ 0.05 E - P ≤ 0.01 F- P ≤ 0.001 (Dunn)

Ophthalmic examinations

Drug-related findings observed at the HD consisted of multiple small vacuoles in the anterior and peripheral vitreous against the posterior capsule (near the equator of the lens). A total of 12/128 eyes (approximately 9%) were affected on PND 21 or 23 but vacuoles were only seen in both eyes of 1 animal (No. 4032) on PND 28. Rats occasionally develop transient anterior vitreal vacuoles in the same area of the vitreous, and these are associated with regressing hyaloid vasculature (young rats). However, this rare background finding occurs as a single and larger vacuole compared to the small and multiple vacuoles observed in HD animals in this study. This finding was transient, only being observed for a few days. Before the mating period, none of the rats had anterior vitreal vacuoles, and all changes were considered background findings.

Neurobehavioral assessment

FOB

On PND 26, forelimb and hindlimb grip strength were dose-dependently decreased at the MD and HD (Table 2). At the end of recovery these parameters remained lower (NS) in HD males but were comparable among female groups. Hindlimb splay appeared to be increased (NS) in treated groups, but not in a dose-related manner.

Table 2.

Summary of Functional Observational Battery Quantitative: F1 Generation Adults - Reproductive Subset - Day 26 ± 1 Post Partum

9001258

Day: 26 Relative to Start Date (p)

Sex: Male		Reporting FOBs					
		Arena Rearing [G]	Arena Square Root [G]	Forelimb Grip Mean [G]	Hindlimb Grip Mean [G]	Hindlimb Splay (cm) [G]	Body Temperature (oC) [G]
0	Mean	10.1	3.2	271.78	119.56	4.8	37.93
mg/kg	SD	3.6	0.6	25.48	22.88	1.1	0.34
/day	N	15	15	15	15	15	15
Group 1		-	-	-	-	-	-
0.75	Mean	10.2	3.3	259.00	114.78	5.3	37.83
mg/kg	SD	2.2	0.3	29.09	21.82	1.1	0.31
/day	N	15	15	15	15	15	15
Group 2	%Diff	0.7	1.2	-4.70	-4.00	10.4	-0.25
1.5	Mean	9.1	3.1	255.00	112.04	5.2	37.93
mg/kg	SD	2.4	0.4	22.12	14.85	1.0	0.33
/day	N	15	15	15	15	15	15
Group 3	%Diff	-10.5	-4.7	-6.17	-6.28	8.3	0.00
2.5	Mean	9.1	3.1	226.00***	105.87	5.5	37.76
mg/kg	SD	2.2	0.3	36.82	15.68	1.3	0.38
/day	N	15	15	15	15	15	15
Group 4	%Diff	-10.5	-4.4	-16.84	-11.45	16.1	-0.44

Sex: Female		Reporting FOBs					
		Arena Rearing [G]	Arena Square Root [G]	Forelimb Grip Mean [G]	Hindlimb Grip Mean [G]	Hindlimb Splay (cm) [G]	Body Temperature (oC) [G]
0	Mean	11.4	3.4	257.89	121.87	4.3	38.18
mg/kg	SD	3.3	0.5	26.15	12.34	1.0	0.31
/day	N	15	15	15	15	15	15
Group 1		-	-	-	-	-	-
0.75	Mean	10.4	3.3	271.11	111.53	4.8	37.94
mg/kg	SD	2.0	0.3	36.07	17.84	1.1	0.37
/day	N	15	15	15	15	15	15
Group 2	%Diff	-8.8	-3.8	5.13	-8.48	10.6	-0.63
1.5	Mean	9.4	3.1	241.11	103.31**	5.0	38.04
mg/kg	SD	3.7	0.7	32.44	21.45	1.5	0.48
/day	N	15	15	15	15	15	15
Group 3	%Diff	-17.5	-10.2	-6.51	-15.23	15.7	-0.37
2.5	Mean	8.5	3.0	222.89*	105.58*	4.8	37.89
mg/kg	SD	3.2	0.6	35.21	13.11	1.2	0.44
/day	N	15	15	15	15	15	15
Group 4	%Diff	-25.1	-13.5	-13.57	-13.37	12.6	-0.77

Locomotor activity

There were no clearly drug-related group differences at the end of the dosing or recovery period.

Auditory startle habituation

There were no clearly drug-related group differences at the end of the dosing or recovery period.

Learning and memory (Cincinnati maze, only B path tested)

When tested near the end of the dosing period (PNDs 27-31), there were no clear effects on maze performance. However, when tested at the end of the recovery period, there appeared to be a learning deficit (increased latencies and errors on last 3 trials; NS) in HD males and females, as seen previously with postweaning dosing (Study no. 9000538) and in the previous study (9000602) with preweaning dosing.

Table 4. Cincinnati Water Maze

Latencies (sec)

Summary of Cincinnati Water Maze (sec)

F1 Generation Adults
End of Recovery Period
Males

Group 1 - Reference Item
Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day
Group 4 - RO7034067 2.5 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	284.9	208.5	157.9	92.0	54.0	25.8
	SD	46.7	102.4	91.2	78.8	72.5	16.0
	N	15	15	15	15	15	15
4	Mean	283.6	223.3	188.9	107.7	66.9	52.5
	SD	37.2	90.2	107.0	106.9	78.6	71.3
	N	15	15	15	15	15	15
	% Diff (G1)	0	7	20	17	24	104

Females

Group 1 - Reference Item
Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day
Group 4 - RO7034067 2.5 mg/kg/day

Group	Summary Information	Trial Number 1	2	3	4	5	6
1	Mean	287.7	206.5	179.9	105.1	64.9	49.1
	SD	26.4	88.9	93.1	94.3	76.7	73.1
	N	15	15	15	15	15	15
4	Mean	268.9	228.7	167.8	115.9	96.6	85.3
	SD	59.3	80.8	103.7	118.2	111.8	112.8
	N	15	15	15	15	15	15
	% Diff (G1)	-7	11	-7	10	49	74

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

Reproductive Performance

When animals were mated starting at PND 87, HD females mated with proven breeder males showed a decreased conception rate and fertility index (Table 5). There were no clear effects on male reproductive performance or C-section endpoints (numbers of corpora lutea, implantation sites, live/dead embryos, resorptions, pre- and postimplantation losses). No clearly drug-related differences were noted for sperm concentration (cauda epididymis weight and sperm count decreased 22 and 14%, respectively, at HD; NS), morphology, or motility in males from the reproductive subset.

Table 5.

F1 Generation Adults
Reproductive Subset Treated Females Mated with Proven Breeder Males

Group 1 - Reference Item
Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day
Group 4 - RO7034067 2.5 mg/kg/day

Group	Number Placed for Mating: Males	Number Placed for Mating: Females	Number of Males/Females Mating	Day to Mating Mean (SD) %Diff (G1)	Number of Males Producing Pregnancy	Number of Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
1	20	20	20	2.1 (1.1) N = 20	19	19	100.0	95.0	95.0
2	20	20	20	2.2 (1.1) N = 19 5	18	18	100.0	90.0	90.0
3	20	20	20	2.6 (2.5) N = 18 27	19	19	100.0	95.0	95.0
4	20	20	20	2.9 (2.7) N =20 41	15	15	100.0	75.0	75.0

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

Clinical Pathology

Hematology

There was a decrease in lymphocyte count (-29% compared to C) in HD males on PND 32. These were no longer seen after the recovery period. There were no group differences in coagulation parameters.

Clinical chemistry

There were no drug-related changes in clinical chemistry parameters.

Urinalysis

There were no drug-related changes in urinalysis parameters.

Immunophenotyping (conducted on PND 49)

A decrease (SS) in the relative percentage of B lymphocytes was seen in HD males and absolute counts were decreased in females at all doses (Table 6). An increase (SS) in the relative percentage of helper T lymphocytes was seen in HD males (not shown) on PND 49.

Table 6. Summary of phenotyping in blood values

Day 49 ± 3 Post Partum - Males

Group 1 - Reference Item
Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day
Group 4 - RO7034067 2.5 mg/kg/day

Group	Summary Information	Total Lymphocyte Count (cells/µL)	B Lymphocytes (CD3-/CD45RA+) Relative Percentage	B Lymphocytes (CD3-/CD45RA+) Absolute Count (cells/µL)	Natural-killer Cells (CD3-/CD161a+) Relative Percentage	Natural-killer Cells (CD3-/CD161a+) Absolute Count (cells/µL)
1	Mean	6015.0	37.283	2197.077	3.277	184.597
	SD	1163.1	7.894	513.277	1.517	49.122
	N	18	18	18	18	18
2	Mean	6526.9	33.778	2209.527	2.617	171.461
	SD	1361.2	4.882	570.813	0.556	53.730
	N	16	16	16	16	16
	% Diff (G1)	9	-9	1	-20	-7
3	Mean	6415.6	35.893	2306.924	2.671	170.259
	SD	876.8	7.073	586.256	0.498	33.378
	N	16	16	16	16	16
	% Diff (G1)	7	-4	5	-19	-8
4	Mean	5820.6	30.531 B	1783.483	2.791	162.340
	SD	824.7	5.764	458.868	0.639	42.063
	N	18	18	18	18	18
	% Diff (G1)	-3	-18	-19	-15	-12

Day 49 ± 3 Post Partum - Females

Group 1 - Reference Item
Group 2 - RO7034067 0.75 mg/kg/day
Group 3 - RO7034067 1.5 mg/kg/day
Group 4 - RO7034067 2.5 mg/kg/day

Group	Summary Information	Total Lymphocyte Count (cells/µL)	B Lymphocytes (CD3-/CD45RA+) Relative Percentage	B Lymphocytes (CD3-/CD45RA+) Absolute Count (cells/µL)	Natural-killer Cells (CD3-/CD161a+) Relative Percentage	Natural-killer Cells (CD3-/CD161a+) Absolute Count (cells/µL)
1	Mean	6062.5	33.499	2102.453	3.292	197.436
	SD	906.0	7.659	730.288	0.971	68.988
	N	16	17	16	17	16
2	Mean	5997.2	27.524	1644.762 A	2.823	169.667
	SD	1282.7	5.559	479.854	0.632	53.508
	N	18	18	18	18	18
	% Diff (G1)	-1	-18	-22	-14	-14
3	Mean	5708.1	29.140	1669.270	2.892	162.244
	SD	1229.4	6.199	523.569	0.667	39.850
	N	16	16	16	16	16
	% Diff (G1)	-6	-13	-21	-12	-18
4	Mean	5318.8	29.329	1491.551 B	2.916	153.278
	SD	1163.4	9.322	371.021	0.782	49.603
	N	17	17	17	17	17
	% Diff (G1)	-12	-12	-29	-11	-22

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

Necropsy

Organ weights

Dose-dependent decreases in testis and epididymidis weights were seen in main study males at the end of the treatment period (Table 7) and in reproductive subset males at the end of the recovery period (Table 8) at all doses.

Table 7.

Summary of Organ Weight Data – Main Study (Day 32 pp)

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (mg/kg/day)	**0.75**	**1.5**	**2.5**	**0.75**	**1.5**	**2.5**
No. Animals per Group	**12**	**12**	**12**	**12**	**12**	**12**
Epididymis (No. Weighed)[a]	(12)	(12)	(12)	NA	NA	NA
Absolute value	3.4	-0.7	**-22.5**			
% of body weight	-3.4	-3.8	**-15.5**			
% of brain weight	1.2	-4.4	**-24.0**			
Gland, Prostate (No. Weighed)	(12)	(12)	(12)	NA	NA	NA
Absolute value	6.4	5.2	-11.4			
% of body weight	-4.3	-0.5	-4.7			
% of brain weight	3.7	1.4	-13.1			
Testis (No. Weighed)	(12)	(12)	(12)	NA	NA	NA
Absolute value	10.6	-6.3	**-35.5**			
% of body weight	3.2	-9.8	**-30.0**			
% of brain weight	8.6	-9.6	**-36.8**			

[a] All values expressed as percent difference of control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $p \leq 0.05$; refer to data tables for actual significance levels and tests used. NA = not applicable.

Table 8.

Summary of Organ Weight Data – Reproductive Subset

	Males			Females		
Group	**2**	**3**	**4**	**2**	**3**	**4**
Dose (mg/kg/day)	**0.75**	**1.5**	**2.5**	**0.75**	**1.5**	**2.5**
No. Animals per Group	**20**	**20**	**20**	**19**	**18**	**20**
Epididymis (No. Weighed)[a]	(20)	(20)	(20)	NA	NA	NA
Absolute value	-5.6	**-9.9**	**-20.4**			
% of body weight	0.3	**-7.4**	**-12.4**			
% of brain weight	-3.8	**-9.7**	**-19.0**			
Testis (No. Weighed)	(20)	(20)	(20)	NA	NA	NA
Absolute value	**-8.4**	**-12.9**	**-19.5**			
% of body weight	-2.7	**-10.3**	**-11.2**			
% of brain weight	**-6.7**	**-12.6**	**-18.0**			

[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. NA = not applicable.

Macroscopic
At the end of the dosing period, small testes in 2 HD males correlating with lower testis weights and seminiferous tubule degeneration observed histologically. No drug-related gross findings were noted at the end of the recovery period.

Histopathology
Dose-dependent increases in the incidence of seminiferous tubule degeneration were seen at the end of the dosing period (Table 9). This was described as characterized mainly by a decreased density and/or loss of round spermatids compared to most control males. The seminiferous tubule degeneration correlated with decreased testicular weights and small size of the testes observed grossly.

Table 9.

Summary of Microscopic Findings – Main Study (Day 32 pp)

	Males				Females			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (mg/kg/day)	**0**	**0.75**	**1.5**	**2.5**	**0**	**0.75**	**1.5**	**2.5**
No. Animals Examined	**12**	**12**	**12**	**12**	**12**	**12**	**12**	**12**
Testis (No. Examined)	(12)	(12)	(12)	(12)				
Degeneration, seminiferous tubule	(2)[a]	(2)	(4)	(12)	NA	NA	NA	NA
Minimal	1	2	4	6				
Mild	1	0	0	6				

[a] Numbers in parentheses represent the number of animals with the finding. NA = not applicable.

Histopathology findings noted in the testis at the end of dosing were not observed at the end of the recovery period.

Renal pelvic dilatation was increased in incidence/severity in HD females at the end of the treatment period and in HD males and females after the recovery period (Table 10).

At the end of treatment, nephroblastomatosis was observed in the kidney of 1 male each at the LD and MD, 1 MD female, and 2 HD females. Nephroblastomatosis was unilateral in all animals and characterized by the presence of a single focus of dense basophilic cellularity (blastemal cells), located in the interstitial tissue of the inner cortex. These nephroblastomatosis foci were not seen macroscopically.

At the end of recovery, nephroblastomas were present in the kidney of 1 MD and 1 HD female (Table 10). These tumors correlated macroscopically with mass and/or nodule and were characterized by sheets of densely cellular blastoma cells with formation of immature glomerular and/or tubular structures, that expanded the interstitium and replaced the normal renal cortex. Nephroblastomatosis foci have the potential to progress toward hyperplasia and to nephroblastoma.

Table 10. Microscopic findings at end of recovery period

Removal Reason(s): TERMINAL EUTHANASIA	Male			
	0 mg/kg /day Group 1	0.75 mg/kg /day Group 2	1.5 mg/kg /day Group 3	2.5 mg/kg /day Group 4
Number of Animals:	20	20	20	20
KIDNEY (Continued...)				
Dilatation; pelvis	3	3	3	8
.... minimal	1	3	3	6
.... mild	2	0	0	1
.... moderate	0	0	0	1
Cyst	0	1	0	1
.... minimal	0	1	0	0
.... mild	0	0	0	1

Removal Reason(s): TERMINAL EUTHANASIA	Female			
	0 mg/kg /day Group 1	0.75 mg/kg /day Group 2	1.5 mg/kg /day Group 3	2.5 mg/kg /day Group 4
Number of Animals:	20	19	18	20
GLAND, THYROID				
Examined	20	1	0	20
No Visible Lesions	20	1	.	20
HEART				
Examined	20	0	0	20
No Visible Lesions	20	.	.	20
KIDNEY				
Examined	20	19	18	20
No Visible Lesions	18	15	14	10
Nephroblastoma, malignant	0	0	1	1
Dilatation; tubular	0	1	0	0
.... minimal	0	1	0	0
Dilatation; pelvis	2	2	2	6
.... minimal	2	0	2	4
.... mild	0	2	0	2
Cyst	0	0	0	2
.... minimal	0	0	0	2
Inflammation; pelvis	0	1	1	1
.... minimal	0	1	1	0
.... moderate	0	0	0	1

Toxicokinetics

TK parameters for RIS and its major metabolite RO7112063 are shown in Table 11.

Table 11.

Day 31 pp Toxicokinetc Results Summary

Treatment Duration	Test System	Occasions and Dose (mg/kg/day)	Mean Cmax (ng/mL) M/F	Mean AUC(0-24h) (hr*ng)/mL) M/F
Once daily from Days 4 to 31 pp	Wistar Hannover Rat	Day 31 pp	RO7034067	
		0.75 (G2)	50.9/51.2	680/668
		1.5 (G3)	107/131	1570/1880
		2.5 (G4)	140/141	1950/2190
		Day 31 pp	RO7112063	
		0.75 (G2)	12.0/12.1	163/173
		1.5 (G3)	31.6/30.9	439/406
		2.5 (G4)	32.2/32.3	457/485

10. Special Toxicity Studies

Study title: In vitro evaluation of the concentration-effect relation of RO6885247, RO6885241, RO7034067, and RO7112063 on FOXM1 mRNA splicing and their potential to induce cell cycle modification in human and monkey induced pluripotent stem cells

Report no.:	1067264
Study report location:	4.2.3.7.3
Conducting laboratory and location:	Roche Innovation Center Research Basel, Switzerland
Date of study report:	10 December 2015
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	NA

Key Study Findings

In vitro testing for FOXM1 mRNA splicing and cell cycle modification was conducted with induced pluripotent stem cells from humans and cynomolgus monkeys. Treatment with RIS (RO7034067) for 24 h at concentrations from 0.64nM to ~10 µM resulted in a concentration dependent down-regulation of FOXM1B/C transcript variants in human and monkey cells (Table 1 and Table 2). IC50 values for FOXM1B/C down-regulation by RIS were 114 nM and 155 nM in human and monkey cells, respectively. The major metabolite, RO7112063, showed no effect on FOXM1B/C expression levels in iPSCs up to a concentration of 2 µM; at 10 µM, the metabolite appeared to down-regulate FOXM1B/C expression by 20% for human and by 42% for monkey iPSCs, which was SS (Table 1 and Table 2).

The cell cycle analysis showed a concentration-dependent induction of mitotic arrest in monkey and human cells for RIS but not for RO7112063 (data for another SMN2 splicing modifier, RO6885247, and its metabolite also shown). In monkey iPSCs, RIS induced a concentration-dependent cell cycle arrest in G2 phase (Table 3). RO7112063 did not induce cell cycle arrest in monkey iPSCs at the concentrations tested. In human iPSCs, RIS induced cell cycle arrest in S phase in a concentration-dependent manner. RO7112063 did not appear to exhibit an effect on cell cycle at the concentrations tested, although a slight increase was detected for cells in G2 phase at 2000 nM (Table 4). Treatment with 30 nM rapamycin resulted as expected in an arrest of human and monkey cells in G1 phase.

Down-regulation of FOXM1B/C seems to be associated with cell cycle-related findings in both human and cynomolgus monkey cells, which would be consistent with the proposed role of FOXM1 in mediating cell cycle transitions. IC50 values derived from the concentration-response curves were comparable between human and monkey cells.

Table 1. Relative mRNA expression data on human iPSCs

RO7034067				RO7112063			
concentration nM	Mean FOXM1B/C	SD	SEM	concentration nM	Mean FOXM1B/C	SD	SEM
0	1.016	0.231	0.133	0	1.004	0.111	0.064
0.64	1.115	0.084	0.048	0.64	1.322	0.058	0.033
3.2	1.032	0.094	0.054	3.2	1.246	0.075	0.044
16	0.898	0.023	0.013	16	1.193	0.084	0.048
80	0.748	0.073	0.042	80	1.353	0.193	0.111
400	0.178	0.017	0.010	400	1.282	0.063	0.036
2000	0.185	0.020	0.011	2000	1.579	0.174	0.101
10000	0.102	0.016	0.009	10000	0.798	0.060	0.035

Table 2. Relative mRNA expression in Cynomolgus monkey iPSCs

RO7034067				RO7112063			
concentration nM	Mean FOXM1B/C	SD	SEM	concentration nM	Mean FOXM1B/C	SD	SEM
0	1.000	0.035	0.020	0	1.000	0.010	0.006
0.64	0.967	0.097	0.056	0.64	1.008	0.043	0.025
3.2	0.990	0.129	0.075	3.2	0.938	0.174	0.101
16	0.963	0.120	0.069	16	0.990	0.144	0.083
80	0.704	0.049	0.028	80	1.162	0.150	0.087
400	0.176	0.024	0.014	400	1.247	0.345	0.199
2000	0.023	0.001	0.001	2000	1.077	0.069	0.040
10000	0.037	0.001	0.001	10000	0.585	0.014	0.008

Table 3. Human cell cycle data: percentage of events in G1, S and G2 phases

	mean			SD			p-value		
	G1	S	G2	G1	S	G2	G1	S	G2
no treatment	37.2	19.6	43.2	1.3	0.6	1.5			
DMSO	35.1	15.4	49.6	0.5	0.4	0.1			
RO6885247 80nM	35.1	15.8	49.1	1.2	1.1	2.3	9.4E-01	5.7E-01	7.4E-01
RO6885247 400nM	34.2	15.7	50.2	1.6	1.4	2.9	4.3E-01	7.6E-01	7.5E-01
RO6885247 2000nM	32.4	15.3	52.3	1.7	0.6	2.3	1.1E-01	8.2E-01	1.7E-01
Rapamycin 30nM	52.8	10.0	37.2	0.8	0.4	1.0	2.1E-05	8.3E-05	2.2E-03
no treatment	37.2	19.6	43.2	1.3	0.6	1.5			
DMSO	35.1	15.4	49.6	0.5	0.4	0.1			
RO6885241 80nM	31.2	15.3	53.4	0.2	0.3	0.1	1.6E-03	9.1E-01	1.3E-07
RO6885241 400nM	28.4	15.4	56.1	0.6	0.6	0.1	1.8E-04	8.8E-01	1.6E-08
RO6885241 2000nM	25.9	14.1	60.0	1.1	0.3	1.1	1.3E-03	1.6E-02	3.7E-03
Rapamycin 30nM	52.8	10.0	37.2	0.8	0.4	1.0	2.1E-05	8.3E-05	2.2E-03
no treatment	32.1	13.4	54.5	1.4	0.5	1.8			
DMSO	36.2	14.6	49.1	1.3	1.6	2.1			
RO7034067 80nM	33.8	14.0	52.2	2.2	0.7	2.9	1.9E-01	5.9E-01	2.1E-01
RO7034067 400nM	30.3	14.0	55.8	0.8	0.3	0.7	5.2E-03	5.6E-01	2.3E-02
RO7034067 2000nM	25.3	13.4	61.3	0.7	0.5	0.5	1.2E-03	3.1E-01	7.3E-03
Rapamycin 30nM	41.7	12.7	45.6	2.2	0.5	1.8	3.0E-02	1.7E-01	8.9E-02
no treatment	41.3	14.2	44.5	1.9	0.1	2.0			
DMSO	42.2	12.9	44.9	0.6	0.3	0.3			
RO7112063 80nM	43.8	13.5	42.7	0.9	0.6	0.4	6.7E-02	2.1E-01	1.9E-03
RO7112063 400nM	44.6	13.6	41.8	0.1	0.6	0.6	1.5E-02	1.7E-01	5.7E-03
RO7112063 2000nM	42.2	13.9	43.9	2.6	0.8	3.4	9.8E-01	1.4E-01	6.7E-01
Rapamycin 30nM	54.2	12.5	33.3	0.4	0.3	0.7	1.3E-05	1.2E-01	1.7E-04

Table 4. Cynomolgus monkey cell cycle data: percentage in G1, S, and G2 phases

	mean			SD			p-value		
	G1	S	G2	G1	S	G2	G1	S	G2
no treatment	44.7	23.9	31.4	1.9	0.3	2.2			
DMSO	45.7	24.3	30.1	1.9	0.8	1.7			
RO6885247 80nM	46.7	23.0	30.3	1.8	0.8	1.7	5.4E-01	1.2E-01	8.7E-01
RO6885247 400nM	49.6	23.5	26.9	1.1	0.9	1.6	4.8E-02	3.5E-01	7.4E-02
RO6885247 2000nM	48.5	24.0	27.5	1.0	1.6	2.3	1.0E-01	8.1E-01	2.0E-01
Rapamycin 30nM	58.3	11.2	30.5	0.7	0.4	0.8	3.2E-03	1.1E-04	7.2E-01
no treatment	44.7	23.9	31.4	1.9	0.3	2.2			
DMSO	45.7	24.3	30.1	1.9	0.8	1.7			
RO6885241 80nM	49.3	23.4	27.3	0.6	0.5	0.3	7.0E-02	1.9E-01	1.0E-01
RO6885241 400nM	48.9	24.5	26.6	0.8	0.8	0.3	8.1E-02	6.9E-01	6.7E-02
RO6885241 2000nM	43.5	28.0	28.5	1.5	0.8	2.3	2.0E-01	4.3E-03	4.0E-01
Rapamycin 30nM	58.3	11.2	30.5	0.7	0.4	0.8	3.2E-03	1.1E-04	7.2E-01
no treatment	44.7	23.9	31.4	1.9	0.3	2.2			
DMSO	45.7	24.3	30.1	1.9	0.8	1.7			
RO7034067 80nM	44.6	26.2	29.2	1.1	1.1	2.2	4.5E-01	7.5E-02	6.2E-01
RO7034067 400nM	40.8	27.8	31.4	3.8	1.5	5.3	1.4E-01	3.5E-02	7.1E-01
RO7034067 2000nM	37.6	33.1	29.3	1.5	1.0	1.3	5.2E-03	3.8E-04	5.5E-01
Rapamycin 30nM	58.3	11.2	30.5	0.7	0.4	0.8	3.2E-03	1.1E-04	7.2E-01
no treatment	44.7	23.9	31.4	1.9	0.3	2.2			
DMSO	45.7	24.3	30.1	1.9	0.8	1.7			
RO7112063 80nM	43.0	24.6	32.4	0.4	0.9	1.3	1.3E-01	6.5E-01	1.4E-01
RO7112063 400nM	42.7	24.3	33.0	1.8	0.3	1.9	1.2E-01	9.0E-01	1.2E-01
RO7112063 2000nM	42.2	23.4	34.4	1.3	0.2	1.2	6.5E-02	1.9E-01	2.6E-02
Rapamycin 30nM	58.3	11.2	30.5	0.7	0.4	0.8	3.2E-03	1.1E-04	7.2E-01

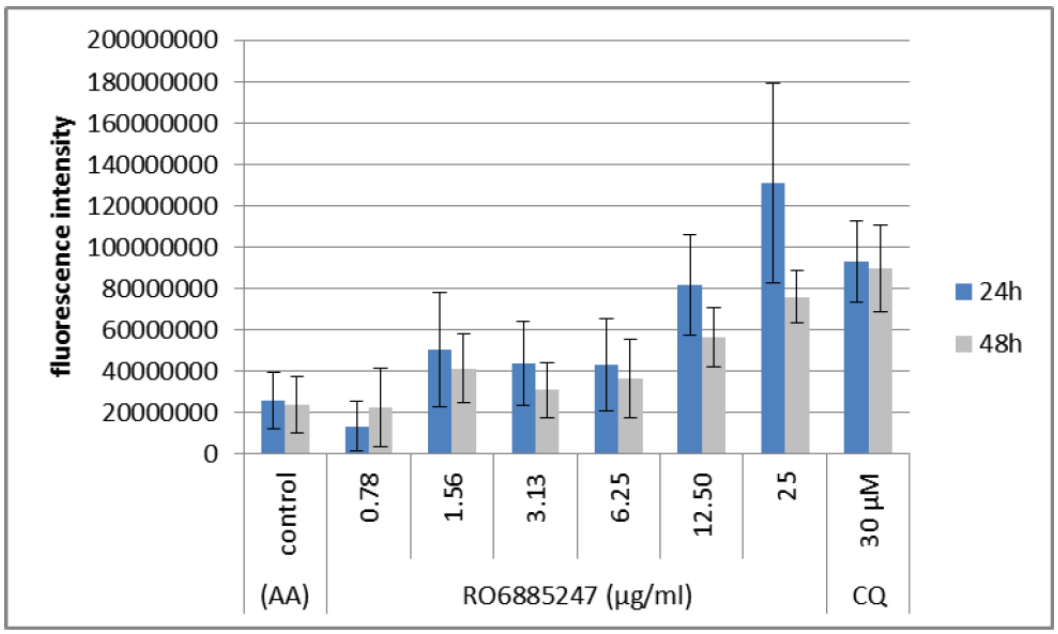
Study title: In-vitro investigations to assess potential of lead RO6885247 and its metabolite M1 RO6885241 to impair RPE cell function

Report no.:	1066985
Study report location:	4.2.3.7.3
Conducting laboratory and location:	Roche Innovation Center Research Basel, Switzerland
Date of study report:	12 January 2016
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	NA

Key Study Findings

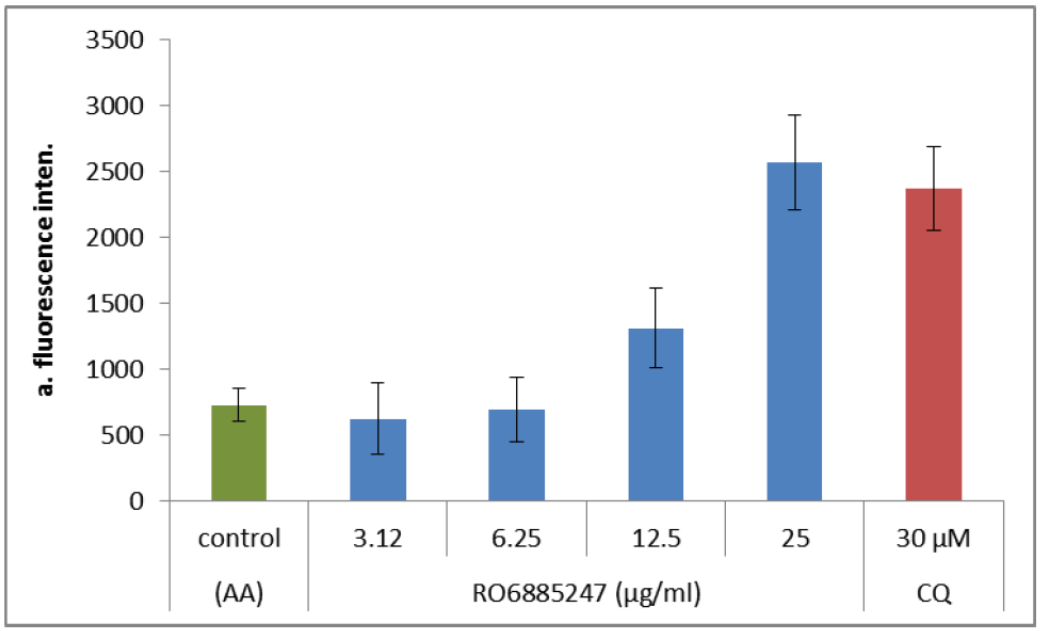
The goal of this study was to explore a mechanism of action for the retinal toxicity observed in a 39-week toxicity study in cynomolgus monkeys with another SMN2 splicing modifier, RO6885247 (described in Ratni et al. 2018). Retinal findings similar to those seen with RIS resulted in the discontinuation of clinical development of RO6885247. Human retinal pigment epithelial (RPE) cells (ARPE19) were treated with RO6885247 and its metabolite RO6885241 and key RPE cell functions were analyzed: phagocytosis, oxidative stress, lysosomal function, cellular autophagy. Treatment of cells for up to 48 hours revealed an increase in lysosomal mass (mainly for RO6885247, Figure 1) and an accumulation of autophagosomes (Figure 2). Phagocytosis as well as oxidative stress levels of ARPE19 cells remained unchanged at this time point. Taken together, these results were thought to indicate that lysosomal malfunction and an impairment of autophagy induced by RO6885247 and to less extend by RO6885241 may lead to a dysfunction of cellular degradation processes in RPE cells. Similar results were seen with RISA (Report 1066986 below). This dysfunction was thought to most likely be due to physicochemical properties, since pharmacologically inactive compounds with physico-chemical properties comparable to RO7034067 and RO6885247 were said to induce similar phenotypes in this RPE cell line in vitro. These processes have been shown to relate mechanistically to the retinal toxicity of chloroquine in vivo in animals and humans. Therefore, it was thought that the effect on lysosomal function may be associated with the peripheral photoreceptor degeneration observed in monkeys with RO6885247 and RIS.

Figure 1. Impairment of lysosomal function by RO6885247



Lysotracker staining of confluent human retinal pigmented epithelium cells after treatment for 24h and 48h with RO6885247: The mean ± SD was obtained from eight replicate values. Chloroquine treatment was used as positive control for lysosomal impairment.

Figure 2. Impairment of autophagy by RO6885247



LC3B staining to detect autophagososmes in ARPE19 cells 48h after treatment with RO6885247.The mean ± SD was obtained from at least eight replicate values of two independent experiments. Chloroquine treatment was used as a positive control for autophagosome accumulation.

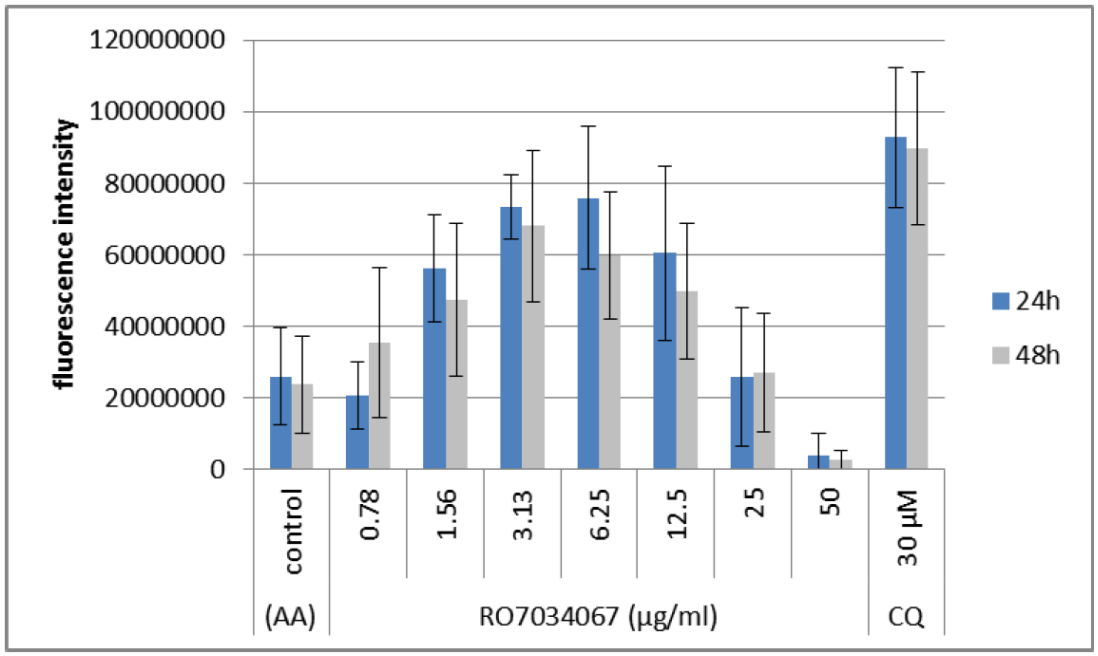
Study title: In-vitro investigations to assess potential of RO7034067, SMN splicing modifier, to impair RPE cell function

Report no.:	1066986
Study report location:	4.2.3.7.3
Conducting laboratory and location:	Roche Innovation Center Research Basel, Switzerland
Date of study report:	12 January 2016
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	NA

Key Study Findings

As in the previous mechanistic study (1066985, above), human retinal pigment epithelial cells (ARPE19) were treated with RIS and key RPE cell functions including phagocytosis, resistance to oxidative stress, lysosomal function and autophagy were analyzed. Data showed that RIS induced an increase in lysosomal mass with a bell-shaped dose-response curve and a maximum response at around 3 µg/ml to a similar extent after 24h and 48h (Figure 1). The increase in lysosomal mass was comparable to that caused by chloroquine, a positive control which is known to cause lysosomal dysfunction in RPE in vitro and the retina in vivo.

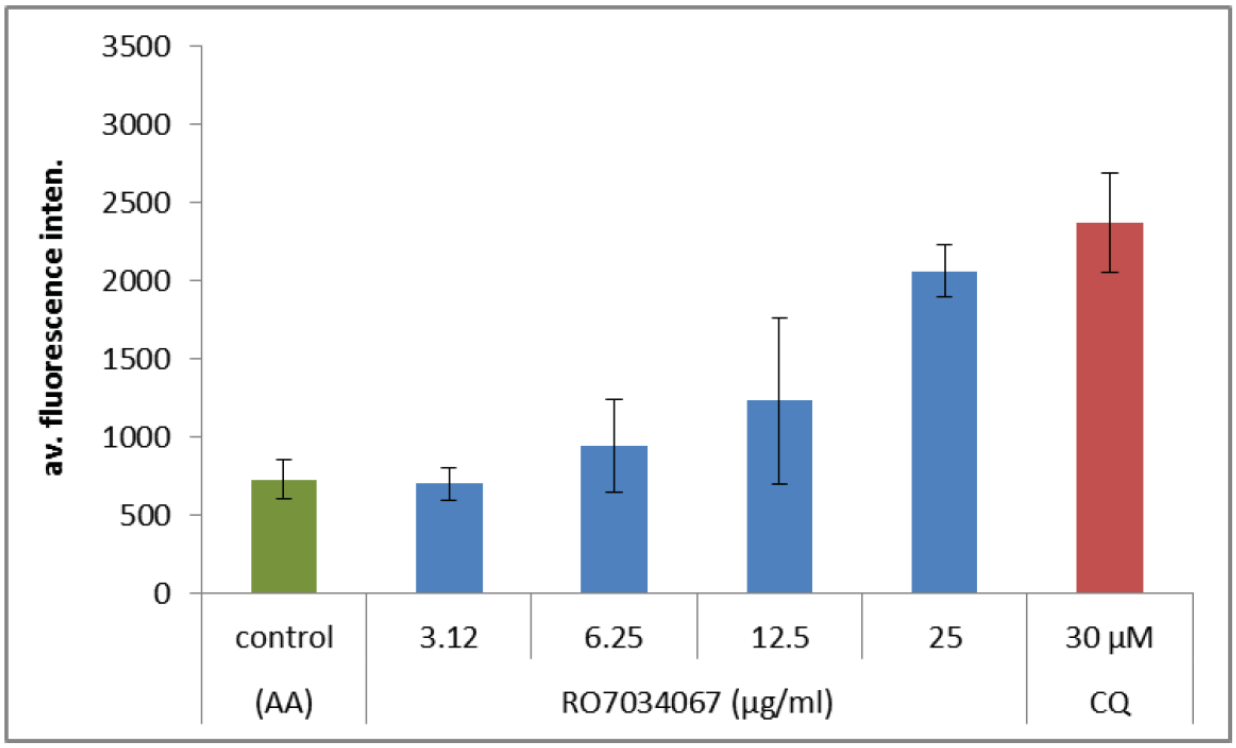
Figure 1. Impairment of lysosomal function by RO7034067



Lysotracker staining of confluent human ARPE19 cells after treatment for 24h and 48h with RO7034067: The mean ± SD was obtained from eight replicate values of two independent experiments. Chloroquine treatment was used as positive control for lysosomal impairment.

Analysis of autophagy in ARPE19 cells by immune staining for LC3B, at marker and effector of cellular autophagy which labels autophagosomes showed a concentration-dependent accumulation of LC3B-labeled autophagosomes (Figure 2). At a concentration of 25 µg/ml of RO7034067, the level of accumulated autophagosomes was comparable to that of chloroquine, a known inhibitor of autophagic flux leading to an accumulation of autophagosomes.

Figure 2. Impairment of autophagy by RO7034067



LC3B staining to detect autophagososmes in ARPE19 cells 48h after treatment with RO7034067.The mean ± SD was obtained from at least eight replicate values of two independent experiments. Chloroquine treatment was used as a positive control for autophagosome accumulation.

In order to investigate if the observed impairment of lysosomal function and autophagy was due to primary or secondary pharmacological effects, 2 pharmacologically inactive compounds with comparable pharmacophores were tested. The two compounds, RO6889953 and RO6893915, were inactive at the primary splicing target SMN2 and, based on the structure activity relationship for the splicing target, were assumed to also be inactive at other secondary splice targets. These compounds share physicochemical characteristics with RIS and while both induced impairment of lysosomal function, they were less potent than RIS and only one (RO6893915) induced accumulation of autophagosomes.

Study title: RO7034067: mechanistic investigations on micronucleus induction in cultured cells (TK6, L5178Y)

Report no.:	2301M15
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Roche Innovation Center Research Basel, Switzerland
Date of study report:	31 August 2016
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4)-024639--060-K1, 99%

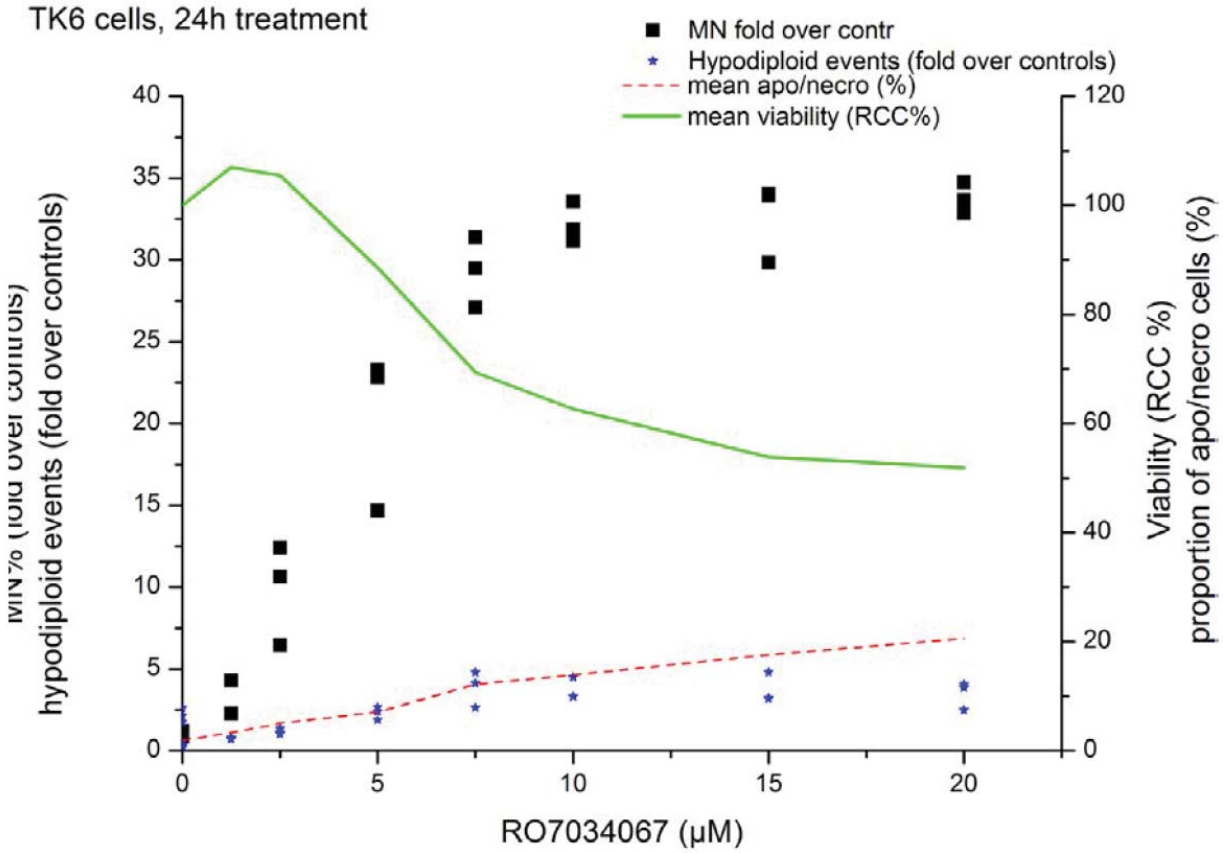
Key Study Findings

A series of in vitro studies were performed to investigate the mechanism of micronucleus (MN) formation. Human TK6 lymphoblastoid cells were treated with RIS under various conditions (with/without metabolic activation, with/without a recovery period) and analyzed for perturbations of the cell cycle. The following biomarkers were examined: γH2AX, which indicates primarily DNA double-strand breaks, cleaved poly (ADP-ribose)-polymerase (PARP), which indicates apoptosis, phospho-H3, which indicates mitotic cells, and DNA content, which is indicative of polyploidy and cell cycle phase. Cytotoxicity was observed in all experiments, starting at concentrations of approx. 5-10 µM in the absence of metabolic activation and approx. 50 µM in the presence of metabolic activation. Following 24h treatment in the absence of metabolic activation, a substantial, reproducible, and concentration-dependent increase in the proportion of apoptotic cells (cleaved PARP positive cells) was observed, starting at concentrations of approx. 5 µM and a shift in γH2AX fluorescence intensity was observed at cytotoxic concentrations (> 20 µM). Increases in the frequency of mitotic (phospho-H3 positive) and polyploid cells were noted at non-cytotoxic concentrations in some experiments. Cytotoxicity, PARP-cleavage, and weak polyploidy were also observed following treatment for 3 h in the presence of metabolic activation with subsequent wash-off and recovery for 21 h starting at approx. 50 µM. A clear, reproducible increase in the proportion of cells with ≤ 2n DNA content (sub G-1 peak), indicative of apoptosis induction, was observed following 24 h treatment in the absence of metabolic activation. The proportions of cells in G1/G0, S and G2/M phase decreased with increasing concentration.

When human TK6 lymphoblastoid cells and L5178Y mouse lymphoma cells were treated with RIS under various conditions and subsequently analyzed for MN induction, there was a marked increase in MN frequency as seen as in previous in vitro and in vivo studies. When measured directly after cessation of treatment, up to 29% of cells (an approx. 33-fold increase over concurrent controls) were micronucleated at a concentration of 20 µM (Figure 1). A concurrently observed increase in hypodiploid events (up to 25-fold) was thought to be indicative of an aneugenic effect, based on the responses of the clastogen MMS and known aneugen nocodazole. MN induction was suppressed by pretreatment of the cells with a caspase inhibitor, which was thought to be indicative of an apoptotic process; however, induction of MN by the clastogen MMS was also decreased. Micronucleation was observed at earlier timepoints in human TK6 cells than in L5178Y mouse lymphoma cells.

The results of these studies were thought to support an indirect, possibly aneugenic mechanism rather than a directly DNA-reactive, clastogenic mechanism at concentrations compatible with cell survival.

Figure 1. TK6 cells treated for 24h with RO7034067 in triplicate cultures



Micronucleus frequency and number of hypodiploid are shown as black squares and blue stars for individual cultures, viability and proportion of apoptotic/necrotic cells as green and red-dashed lines for mean of cultures.

11 Integrated Summary and Safety Evaluation

Risdiplam (RIS) is thought to mediate a specific effect on SMN2 exon 7 inclusion by a related but different mechanism than that of the ASO nusinersen. At therapeutically relevant concentrations (~121 nM), RIS appears to modify alternative splicing in vitro with high specificity for SMN2; however, secondary splice targets (STRN3 and SLC25A17) were identified in vitro even at these concentrations. At higher concentrations (5-fold the EC90, 605 nM), RIS was shown to significantly influence alternative splicing for at least 11 additional genes. Of those 13 off-target genes, FOXM1 and MADD were identified as likely contributors to toxicities seen in the nonclinical studies. While the intended pharmacological target, the SMN2 gene, exists only in humans, other mRNA splice targets such as FOXM1 and MADD are present in the toxicology species. The functions of these genes, such as FOXM1, which encodes a cell cycle regulator, and MADD, involved in apoptosis, were consistent with responses seen in in vitro and in vivo toxicity studies in the form of (stage-specific) cell cycle arrest, micronucleus induction, and apoptosis (Ratni et al., J Med Chem. 2018; 61(15):6501-6517).

FOXM1, forkhead box protein M1, encodes a cell cycle regulator that participates in a wide range of biological processes including cell proliferation, cell cycle progression, cell differentiation, DNA damage repair, tissue homeostasis, angiogenesis, and apoptosis (Zhao et al., PLoS One. 2014; 9(11): e113478). It is highly expressed in rapidly dividing cells, such as those found in the gastrointestinal tract, male germ cells, skin, and blood cell progenitors in the bone marrow. In humans and cynomolgus monkey, the transcriptionally inactive FOXM1A variant contains exon 9 and the transcriptionally active FOXM1B/C variants lack exon 9. Increased abundance of the FOXM1A isoform, together with decreased abundance of FOXM1B/C isoforms may disturb and inhibit cell cycle progression if splicing changes are at a biologically significant level. Knock-down of FOXM1 or a change in its splice variants can lead to mitotic arrest and apoptosis, depending on the stage of the cell cycle (Ratni et al., 2018). This was thought to be the likely mechanism for much of the toxicity observed in the animal studies of RIS. The monkey was considered to be the most relevant species for safety assessment due to the similarity of RIS-induced changes in splicing in secondary target genes, as observed in SMA patient derived fibroblasts, in stimulated monkey and human monocytes and iPSCs, and in monkey spleen tissue in vivo. When the effect of RIS on the expression level of FOXM1 mRNA variants (FOXM1b/c) were examined in human and cynomolgus monkey induced pluripotent stem cells (iPSCs), a similar potency was observed (IC50s of 114 nM and 155 nM in human and monkey cells, respectively), indicating that for any toxic effects mediated through FOXM1, the monkey results would have direct relevance to humans.

In support of the proposed mechanism involving off-target alternative splicing, many of the toxicity findings were observed in organs with rapid cell turnover. These included: micronucleus (MN) induction; decreased bone marrow cellularity; histopathological changes in gastrointestinal tract epithelia (increased apoptosis/single cell necrosis) and lamina propria (vacuolation) and exocrine pancreas epithelia (single cell necrosis); parakeratosis/hyperplasia/degeneration of the skin, tongue, and larynx epithelia with associated inflammation; and degeneration of germ cells in the testis. These effects

appeared early after initiation of dosing and, while the skin and GI epithelial effects were reversible, testicular toxicity persisted after chronic dosing in adults and after exposure of juvenile animals during postnatal development.

In addition to these effects seen in rats and monkeys, histopathological findings in the retina were prominent in the monkey. These were noted primarily in the chronic study; retinal dysplasia in a single HD animal in the 2-week monkey study was of uncertain relation to drug. In the chronic monkey study, multifocal peripheral retina degeneration in the photoreceptor layer and microcystic spaces in the inner retinal layers were detected using optical coherence tomography (OCT) at the first period animals were examined during Week 22. All MD and HD animals showed some degree of disorganization and thinning of the inner segment/outer segment (IS/OS) and increased reflectivity and thinning of the outer nuclear layer (ONL) in the retinal periphery. Degenerative changes were most pronounced in the far periphery in the dosing phase. While some improvement was seen in layer integrity/organization in the far periphery during the recovery phase, the areas with extensive loss of cells did not change. Microcystoid macular degeneration (MMD), characterized by microcystoid spaces in the inner nuclear layer (INL), was seen in the INL temporal to the optic nerve in the most affected animals at the HD (7.5/5 mg/kg/day). The OCT observations were associated with a depressed b-wave, which was more obvious in a scotopic (rod-driven) than a photopic (cone-driven) light setting in the electroretinogram (ERG). ERG changes were also apparent at the earliest examination time (Week 20), so the latency to functional and morphological changes is unknown. Histologically, the most affected layers were the ONL and photoreceptor layer with additional vacuolation of the INL. Retinal degeneration with peripheral photoreceptor loss remained present after a 22-week recovery phase. A no-effect dose for the retinal findings (1.5 mg/kg/day) was associated with RIS exposures (AUC0-24 h) of 1870 and 2060 ng·hr/mL during Week 39 in males and females, respectively. This exposure is the same as that targeted in SMA patients. The same retinal changes had been seen with a previous SMN2 splice modifier being developed by Roche. Mechanistic studies indicated that the retinal toxicity could be independent of the pharmacological action as splice modifiers, possibly involving effects on lysosomal function and autophagy.

Rats appeared to be less susceptible to retinal toxicity; however, functional ERG abnormalities were observed with chronic RIS administration in both albino and pigmented rats. In the 26-week study in Wistar rats, a dose-related reduction in scotopic b-wave amplitudes was seen in drug-treated females compared to control at Weeks 13 and 26. At Week 26, group mean b-waves were reduced by 34-40% at the HD and by 32-39% at the MD, compared to C for each stimulus. A similar trend of reduced amplitudes in drug-treated groups was observed for photopic responses in the females. These findings were dismissed in the study report based on the observed variability and the absence of any clinical signs related to loss of visual function, correlating ophthalmology or histopathological findings, or similar effect in males. However, given the consistent, dose-related pattern across stimulus conditions, it seems likely that the effect was drug-

related. No clear ERG changes were observed in males, and no clear differences among groups of either sex were observed at the end of the recovery phase.

Because retinal toxicity was thought to involve the strong melanin binding of RIS seen in monkeys, a non-GLP investigative study was conducted in pigmented (Brown Norway) rats. Ocular findings were observed starting at Week 19 in 9/18 animals given RIS (RO7034067) and at Week 8 or 9 in all animals given the related splicing modifier RO6885247 (clinical development was discontinued as a result of retinal toxicity observed in a chronic 39-week monkey toxicity study [described in Ratni et al. 2018]). This change was described as a diffuse retinal haziness, which became more prominent over time but was graded 'slight' in most eyes, while other ocular structures appeared normal. Retinal vessel attenuation was observed in 3 RIS-treated animals at Week 26 (only 1 dose level evaluated, 7.5/5 mg/kg/day). In the ERG evaluations, photopic b-wave amplitudes showed a progressive reduction in amplitude over the course of the dosing period, starting as early as Week 8. By Week 26, the group means were reduced up to 82% for both the 0.5 Hz and 29 Hz stimuli. A similar, progressive reduction in photopic 29 Hz b-wave amplitude was present in the group given RO6885247, reaching a reduction of 62% at Week 26. A reduction in scotopic a- and b-wave amplitude compared to C was also observed in the drug-treated groups in almost all tests, starting at about Week 13. However, since the control group also showed amplitude reductions and the responses remained within the range of the control variability, they were considered unrelated to drug administration in the study report; however, given the findings in monkeys and in the previous rat study, it seems much more likely that these ERG changes were drug-related.

RIS was negative for genotoxicity in an in vitro Ames test but positive for clastogenicity (micronucleus (MN) formation) in vitro in mouse lymphoma cells and in vivo in a combined bone marrow micronucleus test and comet assay in rat. A pronounced effect on MN formation was also seen the adult and juvenile rat toxicity studies. The no-effect dose for MN induction in the 4-week adult rat study (1 mg/kg) was associated with an exposure (1540/1650 ng.h/mL M/F) lower than those anticipated clinically (2000 ng.h/mL) and the low-effect dose in the 13-week juvenile rat study (1 mg/kg) produced exposures (600/511 ng*hr/mL M/F) well below the clinical. A 6-month carcinogenicity study in RasH2 mice showed no tumorigenic effects at oral doses up to 9 mg/kg/day, which was associated with exposures (AUC) of 15600 and 11800 ng.hr/mL in males and females, respectively. However, in the 4-week rat JAS, nephroblastomatosis was observed at all doses at the end of treatment and nephroblastomas were present in the kidney of 1 MD and 1 HD female after the recovery period; and in the 13-week rat JAS, a malignant nephroblastoma was observed in a single HD male. The sponsor considered this renal tumor and its precursor lesion, which were only seen in treated rats in the JASs, to be of spontaneous origin "based on the embryonal origin of these lesions, the location in the deep cortex as reported in the spontaneous cases, the beginning of the dosing that occurred after birth, and the absence of lesion in high dose males." However, nephroblastematosis and neuroblastoma in rats are reportedly noted most often in females (Elmore et al., Toxicol Pathol 42:12–44, 2014); and, while neuroblastomas can be induced transplacentally when N-ethylnitrosourea is given on GD 18 (Hard GC, Carcinogenesis 6:551-1558,1985), they have also reportedly been induced by dimethylbenzanthracene in

ovariectomized adult females (Jasmin and Riopelle, Cancer Res 30:321-326,1970). A series of in vitro studies performed to investigate the mechanism of micronucleus (MN) formation were thought to support an indirect, possibly aneugenic mechanism rather than a directly DNA-reactive, clastogenic mechanism. A 2-year rat carcinogenicity study is being conducted postmarketing.

A standard fertility and early embryonic development study was not conducted. The sponsor's justification was that assessment of fertility was integrated into the juvenile rat toxicity studies and reproductive organ histopathology was examined in the adult and juvenile toxicity studies. These studies demonstrated clear reproductive toxicity in both sexes. Male reproductive organ histopathology was seen in adult rats (degeneration/atrophy of the seminiferous tubules in the testis, degeneration and necrosis of the ductular epithelium of the epididymis), young monkeys (increased multinucleate cells and germ cell degeneration in the testis), and juvenile rats (delayed sexual maturation, degeneration/necrosis of the seminiferous tubule epithelium, oligo/aspermia in the epididymis, spermatic granulomas, decreased sperm concentration and motility, increased sperm morphology abnormalities). Female reproductive toxicity was seen in adult (estrous cycle arrest) and juvenile (decreased conception rate and fertility index) rats, and delayed female sexual maturation and impaired reproductive performance (increased preimplantation loss) were seen in the female offspring in the rat PPND development study.

In the rat embryofetal development study, fetal BWs were decreased (15%) and structural variations (supernumerary liver lobes, reduced ossification of the sternebrae and/or thoracic centrum) increased at a dose (7.5 mg/kg/day) that was not maternally toxic. There was no evidence of increased embryolethality or fetal malformations. This was surprising given the drug interactions with FOXM1 and MADD and the developmental effects seen in rabbits; however, the highest exposures (Cmax and AUC) evaluated in rats were only about 1/4 those tested in rabbits, and RIS effects on rat Foxm1 were equivocal. The no-effect dose for embryofetal toxicity (3 mg/kg/day) was associated with a maternal RIS exposure (AUC) of 4630 ng•h/mL on GD 15. This exposure is approximately 2X that expected in humans at the recommended clinical dose.

Embryofetal mortality (increased number of late resorptions) and increased incidences of fetal malformations (hydrocephaly) and variations were seen in the rabbit embryofetal development study at the HD (12 mg/kg), which was also maternally toxic. The no-effect dose for adverse effects on embryofetal development (4 mg/kg/day) was associated with a maternal AUC of 7990 ng•h/mL on GD 15.

In the pre- and postnatal development study in rats, in which the highest dose tested (3 mg/kg) was limited by apparent effects on parturition (dystocia), gestation was lengthened at the HD (1 HD dam was euthanized on GD 22 after showing signs of prolonged labor) but there were no other signs of maternal toxicity. The effect on gestation length was thought to possibly be due to COX2 inhibition. Sexual maturation (vaginal opening) was delayed (SS) in HD female offspring, and when offspring were mated the numbers of corpora lutea, implantation sites, and live embryos were decreased at the HD. At

necropsy of offspring, dose-related increases in total ovarian follicle number and ovarian follicles per section were noted. The significance of this finding is unclear in view of the other reproductive effects. There were no apparent effects on offspring growth, survival, or behavior (with adequate neurobehavioral testing). The no-effect dose for adverse effects on pre- and postnatal development in rats (1.5 mg/kg/day) was associated with maternal exposures of 2360 and 1880 ng.h/mL on GD 6 and PND 7, respectively.

Due to the increased sensitivity of preweaning rats in a dose range-finding study, separate juvenile rat studies were conducted to cover the pre- and postweaning periods of juvenile development. In a 4-week study in which oral doses up to 2.5 mg/day were administered from PND 4 through PND 31, decreased BW gain was seen at the MD and HD and BW remained significantly lower (9%) in HD males after the recovery period. Preputial separation was delayed (SS) at the MD and HD. Decreased tibia length was seen at the end of treatment and persisted up to the end of the recovery period at the MD and HD. Drug-related ophthalmic changes consisting of multiple small vacuoles in the anterior vitreous against the posterior capsule were seen at the HD. Neurobehavioral testing showed effects in the FOB at the end of the treatment period that partially recovered and possible (NS) effects on learning and memory at the end of the recovery period (only B path tested in Cincinnati maze, making test less sensitive). Impaired reproductive performance (decreases in day to mating, mating index, fertility index, and conception rate) was seen in HD females mated with proven breeder males. Decreases in the B lymphocyte relative percentages and absolute counts were observed in HD males and females at all doses on PND 49. Decreases in testis and epididymis weights were seen at the MD and HD at the end of treatment and at all doses in the recovery groups. Histopathology findings at the end of the dosing period included degeneration of the testis seminiferous epithelium at the MD and HD. This was not evident after the 8-week recovery period. However, the persistence of absolute and relative testis weight decrements suggests possible long-term injury. Testicular weight is a sensitive toxicity endpoint for detecting perturbations in rapidly dividing cells, physiology, or hormones and is considered valid for establishing a no-effect level even in the absence of a morphologic correlate (Michael et al., Toxicol Pathol 35:742-750, 2007). Nephroblastomatosis was observed at all doses at the end of treatment and nephroblastomas were present in the kidney of 1 MD and 1 HD female after the recovery period (see above). The low-effect dose (0.75 mg/kg/day), based on immunophenotyping and testicular weight effects, was associated with AUC values of 680 and 686 ng*hr/mL for RIS and 163 and 173 ng*hr/mL for RO7112063 on PND 31 in males and females, respectively.

The results of this preweaning juvenile rat study were consistent with those of a previous GLP juvenile rat study (9000602) that was repeated due to an inadequate number of dose groups (2), lack of a mating assessment, and absence of a no-effect dose for testicular toxicity. In the earlier study, daily oral (gavage) administration of RIS (0, 1, or 2.5 mg/kg/day) to juvenile Wistar rats on PNDs 4 to 31 resulted in delayed (avg 5 days) male sexual maturation at the HD and decreased growth (BW and bone length), neurobehavioral impairment (decreased grip strength and increased hindlimb splay in

FOB, increased activity, and learning deficit in the Cincinnati maze), decreased testis weights, and degeneration of the seminiferous tubule epithelium at both doses.

In the 13-week juvenile rat study with oral administration of doses up to 7.5 mg/kg from PND 22 through PND 112, there was no mortality and no effects on clinical signs, body weights, long bone growth, or sexual maturation. Neurobehavioral testing indicated possible (NS) effects on auditory startle and learning and memory (only B path tested in Cincinnati maze) in the HD group at the end of the treatment and recovery periods. Lymphocyte phenotyping showed dose-dependent increases in total, helper and cytotoxic T lymphocytes and decreases in the relative percentages of B cells at the end of the dosing period. Cytotoxic T cells remained elevated in HD animals at the end of the recovery period. Degeneration/necrosis of the seminiferous epithelium with accompanying oligo/aspermia in the epididymis was observed at the HD at the end of the dosing period and these testicular effects had not fully recovered after 8 weeks. The juvenile and peripubertal periods are critical for male reproductive development, so toxicant exposure during these periods may result in permanent damage (Erthal et al, Reprod Toxicol 96:17-26, 2020). Renal tubular dilatation was increased in incidence and severity in HD animals at the end of the treatment period and was only seen in MD and HD animals after the recovery period. A nephroblastoma was observed in a single HD male at the end of the dosing period. At the end of the dosing period, HD males and females at all doses showed increases (SS) in the number of micronucleated immature erythrocytes in the bone marrow. Values exceeded the vehicle control value by 12-fold for males and up to 10-fold for females, and HD male and female values exceeded the cyclophosphamide positive control values. Decreased sperm concentrations and motility and increased morphological abnormalities were found in the reproductive subset males at the completion of the mating period, but there was no evidence of impairment in male or female reproductive performance. The low-effect level (1 mg/kg/day) for genotoxicity was associated with AUC values of 600 and 511 ng*hr/mL on PND 22 in males and females, respectively.

In rats, monkeys, and humans, parent drug was the primary drug-related component in plasma. One metabolite identified in vitro in all species, the N-hydroxy metabolite M1, was identified as a major circulating metabolite in healthy volunteers and patients (median M1/parent ratio 0.334 in SMA patients). M1 was detected in mouse, rat, rabbit, and monkey plasma, but at lower levels relative to parent than in humans. M1 was not pharmacologically active (SMN2 or FOXM1 splicing) at concentrations resulting from therapeutic doses of RIS. All other metabolites were present at trace levels in plasma of nonclinical species. Human exposure to M1 appears to have been generally covered in the pivotal toxicology studies. M1 exposure slightly greater than the estimated human exposure (667 ng.h/mL based on approximately 1/3 the parent AUC of 2000 ng.h/mL) at the dose used in pivotal clinical trials in rats and mice, while the exposures at the NOAEL in monkeys and rabbits were ~50% of the human exposure. Based on the available data, it appears that M1 exposures equivalent to (rabbit) or exceeding that in humans were achieved at the higher doses in most of the pivotal studies, but not in the rat PPND or 4-week juvenile rat study (AUC of 457/485 ng.h/mL at HD of 2.5 mg/kg).

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