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Pharmacodynamics and Subchronic Toxicity in Mice and Monkeys of
ISIS 388626, a Second Generation Antisense Oligonucleotide that Targets
the Human Sodium Glucose Cotransporter 2 (SGLT2)

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Running title: Inhibition of SGLT2 by ISIS 388626 in Mice and Monkeys

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Number of text pages = 32

Number of Tables = 4

Number of Figures = 4

Number of References = 20

Number of words in Abstract = 245

Number of words in Introduction = 492

Number of words in Discussion = 1094

Abbreviations: ASO (antisense oligonucleotide); SGLT2 (sodium glucose cotransporter 2); MOE (2'-(2-methoxyethyl)-D-ribose); ISIS 388626 (2'- methoxyethyl - modified antisense oligonucleotide); Korea Institute of Toxicology (KIT); LLOQ (lower limit of quantitation)

Recommended section assignment: Drug Discovery and Translational Medicine

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Abstract

ISIS 388626, a 2'-methoxyethyl (2'-MOE) modified antisense oligonucleotide (ASO) that targets human sodium glucose cotransporter 2 (SGLT2) mRNA, is in clinical trials for the management of diabetes. SGLT2 plays a pivotal role in renal glucose reabsorption, and inhibition of SGLT2 is anticipated to reduce hyperglycemia in diabetic subjects by increasing urinary glucose elimination. In order to selectively inhibit SGLT2 in the kidney, ISIS 388626 was designed as a "shortmer" ASO, consisting of only 12 nucleotides with two, 2'-MOE-modified nucleotides at the termini. Mice and monkeys received up to 30 mg/kg/week ISIS 388626 via subcutaneous injection for 6 or 13 weeks. Dose-dependent decreases in renal SGLT2 mRNA expression were observed, which correlated with dose-related increases in glucosuria without concomitant hypoglycemia. There were no histologic changes in the kidney attributed to SGLT2 inhibition after 6 or 13 weeks of treatment. The remaining changes observed in these studies were typical of those produced in these species by administration of oligonucleotides, correlated with high doses of ISIS 388626, and were unrelated to inhibition of SGLT2 expression. The kidney contained the highest concentration of ISIS 388626, and dose-dependent basophilic granule accumulation in tubular epithelial cells of the kidney, which is evidence of oligonucleotide accumulation in these cells, was the only histologic change identified. No changes in kidney function were observed. These results revealed only readily reversible changes after administration of ISIS 388626, and support the continued investigation of the safety and efficacy of ISIS 388626 in human trials.

Introduction

The low-affinity/high-capacity sodium glucose co transporter SGLT2 plays a pivotal role in renal glucose re-absorption and is an attractive therapeutic target for the treatment of type 2 diabetes (Bailey, 2011). SGLT2 is expressed at the luminal (brush border) membrane of the S1 and S2 segments of the proximal renal tubule. SGLT2 is believed to mediate > 80% of renal glucose reabsorption (Kanai et al., 1994; Wood et al., 2003). In contrast, the high-affinity, low capacity glucose transporter SGLT1 is thought to be responsible for 10 to 20% of glucose reabsorption that occurs in the kidney. In cases of hyperglycemia, inhibition of SGLT2 results in glycosuria and a reduction in serum glucose concentrations. Since this mechanism is independent of either the amount of circulating insulin or changes in insulin sensitivity, it has the potential to be effective in all stages of type 2 diabetes, including those patients who are refractory to other anti-diabetic therapies. The reduction in plasma glucose concentrations after SGLT2 inhibition can also result in secondary improvements in insulin sensitivity and secretion, due to a reduction in glucotoxicity (Han et al., 2008, Fujimori et al., 2008). In addition, since this approach does not involve alteration of counter-regulatory mechanisms, it is not expected to cause hypoglycemia.

ISIS 388626 is a 2'-methoxyethyl (2'-MOE) modified 12-mer phosphorothioate oligonucleotide, structurally complementary to a portion of the coding region of human SGLT2 mRNA. The drug acts as an antisense oligonucleotide (ASO) inhibitor of human SGLT2 through hybridization with mRNA and is currently in Phase I clinical investigation for utility in the management of diabetes (Wancewicz et al., 2008). Unlike most second generation antisense oligonucleotides, which are 18 to 20-nucleotides in length, ISIS 388626 is only 12 nucleotides long, with two 2'-methoxyethyl (2'-MOE) modified nucleotides at the termini. The shorter

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length of this particular antisense oligonucleotide (12 vs. more typical 20-nucleotide length) is designed to more selectively target the proximal tubular epithelium (the intended target cell type) due to lower plasma protein binding and greater fractional clearance through glomerulus. This communication describes the conduct, results and interpretation of subchronic toxicology studies, performed in mice and cynomolgus monkeys, prior to the initiation of the human clinical studies of the drug. These studies also documented the pharmacokinetic and pharmacodynamic relationships.

Mice and cynomolgus monkeys were selected as the experimental models because of the large and accumulating data base for these species in toxicity evaluations of ASOs. In addition, ISIS 388626 is pharmacologically active in both mice and monkeys, so toxicities due to reduction of SGLT2 mRNA expression would be observed in these species as well as non-target-related effects characteristic of the ASOs.

The objectives of these studies in monkeys and rodents were to: 1) assess the toxicologic effects associated with a high degree of SGLT2 inhibition in mice, 2) identify potential target organ effects specific for repeated administrations of ISIS 388626 in mice and monkeys, and 3) assess the reversibility of treatment-induced clinical and histopathological changes in both species.

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Materials and Methods

Oligodeoxynucleotide characteristics and preparation

ISIS 388626 is the sodium salt of a 12-base phosphorothioate oligonucleotide with the sequence 5'-GGCATGAGCTUC-3' (molecular formula = $C_{132}H_{167}N_{45}O_{68}P_{11}S_{11}Na_{11}$; molecular weight = 4418.30 amu). Each of the eleven internucleotide linkages is a 3'-O to 5'-O phosphorothioate diester. Eight of the twelve sugar residues are 2-deoxy-D-ribose while the remaining four are 2'-(2-methoxyethyl)-D-ribose (MOE). The residues are arranged such that two MOE nucleosides at the 5' and 3'-ends of the molecule flank a gap of eight 2'-deoxynucleosides. Each of the three cytosine bases is methylated at the 5-position. ISIS 388626 targets exon 9 of human mRNA for SGLT2, and is homologous to SGLT2 mRNA in multiple species, including mouse, rat, monkey and human. The oligonucleotide was synthesized at Isis Pharmaceuticals, Inc., using a GE Healthcare, OligoProcess II synthesizer with solid-phase phosphoramidite chemistry (Beaucage and Iyer, 1992).

ISIS 388626 was formulated in phosphate buffered saline (PBS) at concentrations such that the appropriate amount of the test agent was delivered in a subcutaneous (sc) dose of 10 mL/kg body weight to mice and 4 mL/kg body weight to monkeys. In monkeys sc injection sites were rotated between the interior and exterior aspects of each thigh.

Animals, husbandry, and experimental design

Two hundred six male and 98 female 8 week old CrljBgi:CD1(ICR) mice (Orient Bio Inc., Gyeonggi-Do, Korea) and 38 two to five year old cynomolgus monkeys of each sex (Guangxi Grandforest Scientific Primate Company, Ltd., Dayiling Ping Nan County Guangxi, China) were used for these studies. Animals of both species were individually housed under conditions of controlled light, temperature, humidity, and room air circulation. Monkeys were

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acclimated to laboratory conditions during which time they were accustomed to chair restraints which were employed during electrocardiography and physical examinations. Animal studies were conducted at the Korea Institute of Toxicology (KIT) in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and studies were approved by the KIT Institutional Animal Care and Use Committee prior to study initiation.

Main study and toxicokinetic mice and monkeys were randomized to the experimental groups detailed in Table 1 and administered ISIS 388626 or vehicle. The initial week of each study served as a “loading period” during which animals received sc doses (0, 1, 3, 10 or 30 mg/kg) every other day. Thereafter they were administered once weekly sc injections of 0, 1, 3, 10, or 30 mg/kg for 12 consecutive weeks.

Male mice designated for toxicokinetic analyses were similarly administered 3 or 10 mg/kg/week of ISIS 388626 until their utilization for assessment of plasma kinetics and tissue distribution on experimental days 1 or 42. A single group of monkeys (7 of each sex) was designated for toxicokinetic analyses. Each was administered 3 mg/kg every other day during the 7-day loading period then they were sacrificed (1/sex/interval) for tissue collection on experimental days 3, 9, 15, 23, 31, 39, and 55.

Toxicokinetic Analyses

Plasma concentrations of ISIS 388626 were determined at multiple time points after administration of the drug to mice and monkeys (detailed in Table 1). Plasma samples were analyzed using a hybridization enzyme linked immunosorbent assay (Yu et al., 2002). Tissues from both species were collected and analyzed for ISIS 388626 concentrations using a validated HPLC-MS/MS method (Murphy et al., 2005). Plasma sample analyses were conducted at KIT

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and tissue sample analyses were conducted at PPD Development (Richmond, VA). Plasma and tissue sample analyses were performed based on the principles and requirements described in 21 CFR part 58. The lower limits of quantitation (LLOQ) were 1.00 ng/mL, and 0.200 µg/g in plasma and tissue, respectively.

ISIS 388626 plasma concentration-time data were analyzed by non-compartmental (individual animal profiles) methods using the computer program: WinNonlin[®] Professional, Version 5.2 (Pharsight Corporation, Mountain View, CA). The maximum observed drug concentration (C_{\max}) and the time taken to reach C_{\max} (T_{\max}) were obtained directly from the concentration-time data. The plasma disposition half-life ($t_{1/2\alpha}$) associated with the initial absorption/distribution phase was calculated using a non-compartmental analysis extravascular model (WinNonlin[®]) for all the individual profiles in all treated animals, using the equation, $t_{1/2\alpha} = 0.693/\alpha$, where α is the rate constant associated with the initial absorption/distribution phase. The plasma disposition half-life ($t_{1/2\lambda_z}$) associated with the terminal elimination phase was calculated from recovery animals (Groups 4 and 5) using the equation, $t_{1/2\lambda_z} = 0.693/\lambda_z$, where λ_z is the rate constant associated with the terminal elimination phase. A minimum of three data points were used to define the rate constants (α or λ_z) and the correlation of determination values (rsq) had to be greater than 0.8 for the estimate to be accepted. Area under the plasma concentration-time curve from zero time (pre-dose) to 48 hr time point ($AUC_{0\rightarrow 48\text{hr}}$) or from zero time (pre-dose) to 168 hr (dosing interval, τ) ($AUC_{0\rightarrow 168\text{hr}}$) at steady-state was calculated using the linear trapezoidal rule.

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Pharmacologic demonstration

The effect of ISIS 388626 on expression of renal SGLT2 mRNA were assessed in kidney samples collected during interim (week 6), terminal (week 13) and recovery sacrifices in both species. Approximately 100 mg of kidney tissue was homogenized in guanidinium isothiocyanate. Total RNA was then centrifuged over a cesium chloride gradient and resuspended in RNase-free water. Total RNA was purified further using a RNeasy mini RNA preparation kit. After quantitation, the kidney tissue was subjected to qPCR analysis (VanGuilder et al., 2008; Nolan et al., 2006; Bustin, 2000). The Invitrogen-ABI StepOnePlus™ realtime PCR system was employed. The assay is based on a target-specific probe labeled with a fluorescent reporter and quencher dyes at opposite ends. The probe is hydrolyzed through the 5' exonuclease activity of the Taq DNA polymerase, leading to an increasing fluorescence emission of the reporter dye that can be detected during the reaction. SGLT2 mRNA was then normalized to total RNA as determined by RiboGreen fluorescence from the same RNA sample.

Determination of ED₅₀ and EC₅₀

ISIS 388626 pharmacodynamic (% inhibition of SGLT2 in kidney tissue) data were analyzed using the computer program Phoenix™ WinNonlin®, Version 6.1 (Pharsight Corporation, Mountain View, CA). The effective dose to achieve 50% inhibition of SGLT2 mRNA expression in the kidney (ED₅₀) and the effective concentration of ISIS 388626 in the kidney to achieve 50% inhibition of SGLT2 mRNA (EC₅₀) were calculated using the Inhibitory Effect Sigmoid E0 models (Model 107) with uniform weighting.

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Toxicology Assessments

Antemortem evaluative criteria for both species included survival, clinical signs, body weight and food consumption, ophthalmological examinations, clinical pathology (hematology, clinical chemistry, and urinalysis in both species and complement analysis in monkeys) and toxicokinetic analyses.

Monkeys also received electrocardiographic (ECG) and physical (heart rate and blood pressure) examinations prior to initiation of dosing, following the first dose, and after the completion of Weeks 6 and 13. ECG tracings were examined for qualitative abnormalities, QT- and RR-intervals were determined, and QTc values were derived.

The conduct and data collection for all routine observations and physical and clinical examinations was accomplished in compliance with the principles of Good Laboratory Practices according to the Standard Operating Procedures established at the performing laboratory.

Sacrifice, necropsy and tissue collection for microscopic examinations of main study mice and monkeys at the interim, terminal and recovery phases were conducted on Study Days 44, 93, and 182, respectively. Numbers of animals sacrificed on specific study days are shown in Table 1. Animals were fasted over night, taken to deep anesthesia with isoflurane or thiopental then sacrificed by exsanguination.

Grossly observable lesions, injection sites, and representative sections of approximately 45 to 50 organs/tissues were collected from each animal. Testes were fixed in Bouin's solution and eyes and optic nerves in Davidson's fixative. All other tissue samples were preserved in neutral buffered formalin. Specimens were routinely processed and the full range of tissues was examined microscopically.

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Results

Systemic exposure of mice and monkeys to ISIS 388626

Toxicokinetic analyses verified systemic exposures of mice and monkeys to ISIS 388626 (Tables 2 and 3) and revealed similar TK profiles in both species. There were no gender-related differences in monkeys, and data for that species were pooled across the sexes to simplify graphic and tabular representation.

ISIS 388626 was readily absorbed from subcutaneous administration sites with C_{\max} values being attained in 0.25 hr in mice and 0.6 to 1.5 hr in monkeys and then decreased in an apparent multi-exponential fashion with time. Systemic exposure, as exemplified by AUC values, increased with dose and in mice the magnitudes of increments between the 3 and 10 mg/kg doses were generally dose-proportional. In monkeys, increments between AUC values achieved after 1 and 3 mg/kg were consistently dose proportional as were increments between 10 and 30 mg/kg. Increments between 3 and 10 mg/kg were somewhat supraproportional to dose.

Elimination of ISIS 399626 from the plasma (evidenced by distribution half life, $T_{1/2}$) ranged from 1 to slightly longer than 3 hours and was mainly attributable to distribution to and storage in peripheral tissues, primarily the kidney (Yu et al., 2009). The estimated mean terminal elimination plasma half-life values ($t_{1/2\lambda_z}$) obtained from recovery animals were 15.3 and 22.2 days following 10 and 30 mg/kg/week dose for 13 weeks, respectively (Figure 2b).

Plasma ISIS 388626 concentration curves for the 2 species were similar (Figures 1 & 2). Consistent with TK evidence of systemic exposure in mice, Day 1 and Day 42 plasma concentration curves within doses were indistinguishable and there was no evidence of bioaccumulation of the drug in mouse plasma but there was some evidence of accumulation in

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monkey plasma on Days 42 and 91 relative to Day 1 at 48 hours. Although increments in C_{max} values in mice were somewhat supraproportional to dose on both Days 1 and 42 (Table 2), there was no evidence of bioaccumulation in plasma with concentrations on Days 1 and 42 being indistinguishable.

After subcutaneous injection, ISIS 388626 was primarily distributed to the kidneys in mice and monkeys. Liver contained measurable concentrations of ISIS 388626; however, the concentrations in liver were approximately 5 to 100-fold lower than the concentrations in the kidneys in both mice and monkeys (Table 4). Concentrations of intact ISIS 388626 in kidney of mice and monkeys were dose-dependent, but the increase was less than dose proportional over the dose range studied. Concentrations of ISIS 388626 in kidney of mice and monkeys increased approximately 3 to 4-fold over a 30-fold increase in dose, which suggests saturation of kidney uptake (Table 4). Meanwhile, concentrations of ISIS 388626 in liver of mice were approximately dose-proportional, and for monkeys were greater than dose-proportional over the dose range studied. Tissue concentrations of ISIS 388626 following 13 weeks of treatment were approximately 5 to 20-fold higher in monkeys than in mice at comparable mg/kg doses (Table 4).

Elimination of ISIS 388626 from tissues was slow. The tissue half-lives of intact ISIS 388626 (12-mer) were 6 and 7 days in monkey kidney and liver, respectively. Although slow, elimination of ISIS 388626 from tissues was also observed during the recovery period (Day 181). Concentrations of oligonucleotide in tissues of animals assigned to the recovery group were substantially lower than those measured in monkeys that had been necropsied 2 days after the last dose (on Day 93). For example, concentrations of ISIS 388626 remaining in kidney cortex and liver after the treatment free period were approximately 0.5% to 5% of the concentrations at the end of treatment.

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Pharmacologic effects

Both mice and monkeys exhibited dose-dependent decreases in renal SGLT2 mRNA expression reflecting the intended pharmacologic activity of the drug. SGLT2 mRNA was reduced by 74 to 97 % (26 to 3% of control) in mice and by approximately 30 to 90% (70 to 10% of control) in monkeys over the dose range tested (1 to 30 mg/kg/week; Figures 3a and 4a, respectively). The magnitudes of inhibition in each species were comparable after the 6- and 13-week treatment regimens. During the treatment-free recovery phase renal SGLT2 expression returned to approximately 70% and 50% of control at 30 mg/kg/wk in mice and monkeys, respectively (recovery data not shown). The ED₅₀ for SGLT2 mRNA inhibition was 0.12 mg/kg/wk (CV% = 60.22) in mice and 3.04 (CV% = 24.33) mg/kg/wk in monkey, while the EC₅₀ of ISIS 388626 in the kidney was 10.64 µg/g (CV% = 12.3) in mice and 242.27 µg/g (CV% = 9.73) in monkey (based on 13-week data). Thus, ISIS 388626 is approximately 25-fold more potent in the mouse than the monkey based on dose and approximately 23-fold more potent based on kidney ASO concentration.

Consistent with reduced renal SGLT2 expression/function, administration of ISIS 388626 caused glucosuria. Extensive urinalysis revealed no evidence of renal injury and the treatment-related glucosuria, expressed as urinary glucose/creatinine ratio, was dose-related and of similar magnitudes at both the 6- and 13-week observation times (Figures 3B and 4B). In monkeys maintained for the treatment-free period at 10 or 30 mg/kg/wk, the glucosuria persisted through week 4 but was essentially recovered by recovery week 13.

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Toxicology Assessments

Neither treatment associated clinical signs nor mortality occurred in mice or monkeys and ophthalmologic examinations revealed no changes attributable to administration of ISIS 388626. Mice administered 10 or 30 mg/kg/wk ISIS 388626 exhibited increased body weight gains relative to controls, with the magnitude of effect being greater in males than in females. From Week 3 until the end of the study, mean body weight gains for males dosed with ≥ 10 mg/kg/wk were significantly greater than those of controls (Supplemental Fig. 1). Increases in mean body weights were most likely secondary to increases in food consumption which was also more pronounced in males than in females (data not shown). Administration of ISIS 388626 was without effect on body weights or food consumption in monkeys.

Physical examinations of ISIS 388626 dosed monkeys revealed no ocular abnormalities, nor were there ISIS 388626-related changes in hemodynamic parameters or electrocardiographic activity. Negative data are not tabulated.

Administration of ≥ 10 mg/kg/week ISIS 388626 to mice elicited serum chemistry changes indicative of mild hepatotoxicity (Supplemental Table 1). These changes included 1.2 to 1.8-fold increases in the mean concentrations of serum alanine aminotransferase and aspartate aminotransferase, and total cholesterol. Changes in serum chemistry parameters were fully resolved after the treatment-free period. No treatment-associated changes in serum chemistry parameters were observed in ISIS 388626-dosed monkeys (negative data not shown).

Male and female mice administered ISIS 388626 for 13 weeks exhibited approximately 10 to 30% decreases in erythrocytic parameters (RBC, HGB, and HCT) with males being more affected than females (Supplemental Table 2). Both sexes exhibited 1.6 to 3.5-fold increases in leukocytic parameters (mean WBC and absolute neutrophil and large unstained cell counts). At

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the conclusion of the treatment-free recovery period the leukocytic changes were resolved although the erythrocytic changes persisted. Administration of ISIS 388626 was without effects on hematologic parameters in monkeys.

There was no prolongation of APTT or alternative complement pathway activation following administration of ISIS 388626 (negative data not shown). The lack of effect on APTT or alternative complement pathway activation, which are commonly observed effects in monkeys treated with phosphorothioate oligonucleotides at doses ≥ 12 mg/kg/week (Sheehan et al., 2001, Henry et al., 1997b and Henry, et al., 2008), is likely due to the lesser degree of plasma protein binding as a result of the relatively short length of ISIS 388626 (12-nucleotides in length) compared to other phosphorothioate ASO which are typically 20-nucleotides long (Yu et al., 2007).

Postmortem Assessment

Macroscopic examination of mice revealed no changes attributable to administration of ISIS 388626. One male and one female monkey administered 30 mg/kg/wk ISIS 388626 exhibited pale discoloration of the kidneys at the terminal sacrifice, but high-dose monkeys sacrificed after the treatment-free recovery period did not exhibit this change.

Administration of ≥ 10 mg/kg/wk to mice caused a slight (1.1 to 1.3-fold) increase in mean kidney and spleen weights in both sexes (Supplemental Table 3). These small changes were corroborated by similar changes in relative (to brain and body) organ weight parameters. After the 13-week recovery period mean kidney and spleen weights in dosed mice were indistinguishable from controls. ISIS 388626 was without effect on absolute or relative organ weights in monkeys.

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Treatment-related microscopic changes in interim (6 weeks) sacrificed mice and monkeys were limited to animals administered ≥ 10 mg/kg/wk and reflected uptake and accumulation of the oligonucleotide at peripheral organ storage depots (Henry, et al., 2008). An interim change in mice, hepatic centrilobular hypertrophy, noted in 1/3 males dosed with 10 mg/kg/wk and 3/3 males administered 30 mg/kg/wk, was characterized by enlarged hepatocytes in the centrilobular zone. Treatment-related microscopic changes in monkeys (Supplemental Table 5) administered 30 mg/kg/wk ISIS 388626 for 6 weeks included Kupffer cell accumulation of basophilic granules (2/2 animals of each sex), histiocytic hypertrophy and vacuolation of lymph nodes (2/2 of each sex) and basophilic granules deposited in renal tubular epithelium (1/2 females).

Thirteen consecutive weeks of treatment of mice with 10 or 30 mg/kg/wk ISIS 388626 resulted in additional deposition of basophilic granules in kidney proximal tubular epithelial cells, and liver Kupffer cells, and centrilobular hypertrophy was again observed at ≥ 10 mg/kg/week (Supplemental Table 4). Additional microscopic changes, indicative of a proinflammatory effect, included minimal to moderate splenic extramedullary hematopoiesis in all groups (with a slight increase in severity with increasing doses), minimal to slight sinus histiocytosis and lymphoid hyperplasia in multiple lymph nodes (mandibular, inguinal and mesenteric) at doses ≥ 10 mg/kg/week, with slightly higher incidence and/or severity in males. Minimal lymphohistiocytic infiltration was observed in multiple organs and tissues (kidney, liver, heart, spleen, tongue, lung, esophagus, uterus, vagina, urinary bladder, skin/mammary gland and injection site).

Terminally sacrificed monkeys exhibited only microscopic changes reflective of uptake and accumulation of the oligonucleotide in peripheral tissues. Changes were qualitatively and quantitatively similar in both sexes and were confined to the kidneys, liver, lymph nodes and

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urinary bladder. Changes in terminally sacrificed monkey kidneys were minimal to moderate basophilic granular accumulations in the cytoplasm of tubular epithelial cells in the outer cortex at ≥ 10 mg/kg/week. Hepatic changes were limited to minimal to slight accumulation of basophilic granules in Kupffer cells with hyperplasia and/or hypertrophy of Kupffer cells at ≥ 10 mg/kg/week. In the mandibular, mesenteric and inguinal lymph nodes, minimal to moderate hypertrophy of histiocytes (in one or more animals at 10 and 30 mg/kg/week ISIS 388626) and minimal basophilic granule accumulation and vacuolation in the cytoplasm of histiocyte (in one or more animals at 10 and 30 mg/kg/week ISIS 388626) were observed. Minimal submucosal histiocytic hypertrophy was observed in urinary bladder at 30 mg/kg/week ISIS 388626.

After the recovery period, treatment-related histopathologic changes in mice were partially resolved as evidenced by decreased incidences and/or severities after the 13 week treatment-free period. Minimal changes in the animals recovering from the 30 mg/kg/wk regimen included basophilic granules in the kidneys and liver, sinus histiocytosis in the mandibular and mesenteric lymph nodes, and lymphohistiocytic infiltration in kidney, liver, esophagus, skin/mammary gland and injection sites. In monkeys all ISIS 388626-related microscopic changes were resolved after the treatment-free period.

Discussion

Subcutaneous administrations of ISIS 388626 to mice and monkeys resulted in extensive and dose-related systemic exposure to the drug. Absorption from subcutaneous injection sites was prompt with maximal plasma concentrations being achieved within 15- (mice) to 90- (monkeys) minutes. Plasma concentration curves for both species reflect rapid elimination from plasma. There was no evidence of accumulation of ISIS 388626 in plasma during the once weekly dosage regimen. However, the long residence time in tissues, kidneys in particular for this particular target, was quite long ($T_{1/2}$ = approx. 7 days), and supports the infrequent weekly dose regimen used in these studies to produce a sustained inhibition of SGLT2 mRNA expression.

Rapid elimination of drug from the plasma, featuring distribution to peripheral organs with little urinary excretion, is characteristic of both first and second generation antisense oligonucleotides (Henry, et al., 2001 and 2008). The target for ISIS 388626 (SGLT2) resides in the kidney rather than the liver where most targets for 2'-MOE oligonucleotides have been located. Therefore, ISIS 388626 was designed as a short oligonucleotide (12 nucleotides long vs. 20 nucleotides for most ASO) in order to optimize distribution to the renal proximal tubular epithelium. At the conclusion of the 13-week dosing period in this study the greatest concentrations of ISIS 388626 were found in the renal cortex of both species with much lower concentrations in liver and spleen. Still lesser concentrations of ISIS 388626 were found in essentially every organ examined and no drug was measured in the brain. As is the case with 20-mer ASO, urinary excretion accounted for only a small percentage of the administered dose (ranged from 12 to 26% over the dose range of 1 to 30 mg/kg in monkeys) within the first 48-hours of administration (Yu et al., 2009).

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The desired pharmacologic effect of ISIS 388626, inhibition of the expression of SGLT2 in renal proximal tubules, was demonstrated by mRNA expression analysis which revealed a dose-relatedness in both species. After 13 weeks of treatment, SGLT2 expression was reduced by approximately 80 to 97 % (20 to 3% of control) in mice, with an ED₅₀ of <1.0 mg/kg/wk and was reduced by approximately 30 to 90% (70 to 10% of control) in monkeys, with an ED₅₀ of approximately 3.0 mg/kg/wk. The reduction of SGLT2 mRNA in both mouse and monkey was accompanied by dose-related increases in urinary excretion of glucose. In mice, urine glucose was increased by approximately 14 to 130-fold over control at 1 to 30 mg/kg/week, and in monkey urine glucose was increased by approximately 7 to 125-fold over control at 3 to 30 mg/kg/week.

Despite reduction of renal SGLT2 expression and the resulting glucosuria, clinical pathology analyses revealed no evidence of hypoglycemia. It is likely that hepatic metabolic processes maintained euglycemia by compensating for the renal elimination of glucose. However, neither biochemical nor microscopic changes suggestive of ongoing gluconeogenesis and/or glycogenolysis were observed in either species.

Administration of supra pharmacological doses of ISIS 388626 for 13 consecutive weeks elicited no grossly observable signs of systemic toxicity in either mice or monkeys. Increased body weight gains by mice administered 10 or 30 mg/kg/wk were likely related to increased food consumption which could have been precipitated by a period of relative hypoglycemia that may have been undetected during clinical chemistry analyses.

The lack of effect on APTT or alternative complement pathway activation in the monkey (Sheehan et al., 2001 and Henry et al., 1997b) likely reflects reduced plasma protein binding as

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a result of the relatively short length of ISIS 388626 (12-nucleotides in length) compared to other phosphorothioate ASO which are typically 20-nucleotides long (Yu et al., 2007).

Small and reversible hematologic changes observed in mice were typical of those observed in rodents administered large doses of oligonucleotides and were unrelated to inhibition of SGLT2 expression. Similar hematologic changes were not observed in monkeys despite extensive inhibition of SGLT2 expression. Proinflammatory effects, including increased spleen weight and multiorgan cell infiltrates, are commonly observed in mice administered high doses of traditional 20-nucleotide ASO (Henry et al., 2001 and 2008). While these findings were also observed in mice with ISIS 388626, they occurred with a lower incidence and severity compared to most 2'-MOE ASO thus reflecting a lower degree of proinflammatory effect for this relatively shorter length oligonucleotide. In monkeys, there was essentially no evidence of the proinflammatory effects, including no inflammatory cell infiltrates in the injection site skin. The reversibility of serum chemistry changes was proven during the treatment-free period. That these serum chemistry changes are rodent specific and unrelated to inhibition of SGLT2 expression was substantiated by their absence in monkeys that had been administered the drug and were exhibiting the pharmacologic effect. Although proinflammatory effects are not as prevalent in monkeys treated with ASO, slight increases in spleen weight and cell infiltrates at the subcutaneous injection site are typically observed at high doses (Henry et al., 2008). The absence of these findings in this study provides further evidence that ISIS 388626 is less pro-inflammatory than the typical 20-nucleotide ASO.

Tissue depots of ISIS 388626 and other antisense oligonucleotides are visualized microscopically as basophilic granules which are most prominent in kidney, liver and spleen (Henry et al., 2008). Specifically it is the distribution of oligonucleotide to the phagocytically

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active cells in these organs, such as the proximal convoluted renal tubules and hepatic Kupffer cells, where the drug is processed into endosomes and lysosomes. Upon staining the tissues with hematoxylin, oligonucleotide in the lysosome are visualized microscopically as basophilic granules (Monteith et al., 1999; Henry et al., 2008). Basophilic granules are common non-specific effects of 2'-MOE ASO that reflect uptake of oligonucleotide and are not considered toxicologically relevant. The extent of basophilic granulation was clearly dose related and resolved after cessation of dosing.

The results presented here indicate that it is possible, through minor modifications such as shortening the length of the ASO (12-mer), to optimize distribution to the kidney in order to specifically inhibit target expression in the kidney. ISIS 388626 provides a good example of an ASO targeting a human target mRNA that has cross species activity, and that also works in the kidney. Robust, dose-dependent mRNA target reduction was observed and was also reflected in the intended phenotypic change (glucosuria). Good tolerability of the short oligo was also achieved, with lesser degree of proinflammatory effects and complement activation than typical 20-nucleotide MOE. Furthermore, the subchronic safety assessment in mice and monkeys, combined with the nonclinical pharmacology assessments, provides sufficient justification for progression to human clinical studies to evaluate the safety and efficacy of ISIS 388626 for clinical application.

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Acknowledgements

We thank Dan Norris for assisting with the ED₅₀/EC₅₀ calculations.

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Authorship Contributions

Participated in research design: Zanardi, Yu and Henry.

Conducted experiments: Han, Jeong and Chakravarty.

Contributed new reagents or analytic tools: N/A

Performed data analysis: Zanardi, Rime, Yu and Chakravarty.

Wrote or contributed to the writing of the manuscript: Zanardi, Yu and Henry.

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Footnotes

Financial support for this project was provided by Isis Pharmaceuticals, Inc.

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Figure Legends

Figure 1. Mean plasma concentrations of ISIS 388626 in male mice following the first (Day 1) and ninth (Day 42) subcutaneous administration of the drug. Error bars represent standard deviation (n= 3).

Figure 2. Mean plasma concentrations of ISIS 388626 in monkeys (sexes combined) for up to 48 hours following subcutaneous administration at 10 mg/kg after single and multiple doses (A), and in recovery animals for up to 84 days following the last dose at 10 or 30 mg/kg/week ISIS 388626 via s.c. administrations (B) . Error bars represent standard deviation (n= 14 at Days 1 and 42, and n=10 on Day 91 for A and n=4 for B).

Figure 3. SGLT2 mRNA reduction in kidney (A) and urine glucose excretion (B) in male and female (sexes combined) mice administered ISIS 388626 for up to 13-weeks then afforded a 13-week recovery period. Error bars represent standard deviation (n=6 for A and n = 3 for B).

Figure 4. SGLT2 mRNA reduction in kidney (A) and urine glucose excretion (B) in male and female (sexes combined) cynomolgus monkeys administered ISIS 388626 for up to 13-weeks then afforded a 13-week recovery period. Error bars represent standard deviation (n=4 at 6 weeks and 6 at 13 weeks for A, and n= 14 at 6 weeks, 10 at 13 weeks, and 4 at recovery for B).

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TABLE 1

Protocol outline and animal disposition for 13-week toxicity studies of ISIS 388626 in male and female CD-1 mice and Cynomolgus monkeys

Group	Number M/F	Dose (mg/kg, sc)	Dose Regimen ¹	Number of animals sacrificed (M/F)		
				Interim	Termination	Recovery
Mice - Main Study						
1	22/22	0	q2d/q1w	6/6	10/10	6/6
2	16/16	1	q2d/q1w	6/6	10/10	
3	22/22	3	q2d/q1w	6/6	10/10	6/6
4	16/16	10	q2d/q1w	6/6	10/10	
5	22/22	30	q2d/q1w	6/6	10/10	6/6
Mice - Toxicokinetics						
6	54/0	3	q2d/q1w	3/grp at 15, 30 min, 1, 2, 4, 8, 24, and 48 hours after dosing on Days 1 and 42		
7	54/0	10	q2d/q1w			
Monkeys - Main Study						
1	7/7	0	q2d/q1w	2/2	3/3	2/2
2	5/5	1	q2d/q1w	2/2	3/3	0
3	5/5	3	q2d/q1w	2/2	3/3	0
4	7/7	10	q2d/q1w	2/2	3/3	2/2
5	7/7	30	q2d/q1w	2/2	3/3	2/2
Monkeys - Toxicokinetics						
6	7/7	3	q2d for 1 week	1/sex/interval on Days 3, 9, 15, 23, 31, 39, and 55		

1. ISIS 388626 was administered by sc injection every other day (q2d) for the first 7 days (Days 1,3,5 and 7) then once weekly (q1w) for the remainder of the study.

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TABLE 2

Mean toxicokinetic parameters in mice dosed with ISIS 388626 on Day1 and Day 42

Subcutaneous Dose (mg/kg)	C_{max} ($\mu\text{g/mL}$)	T_{max} (hr)	AUC_{0-∞} ($\mu\text{g}\cdot\text{hr/mL}$)	T_{1/2} (hr)
Day 1				
3	1.52 ± 0.17	0.25	4.25	1.30
10	7.14 ± 1.0	0.25	11.8	1.56
Day 42				
3	1.81 ± 0.09	0.25	4.75	1.26
10	6.80 ± 4.2	0.25	15.1	1.0

Note. Data are means of n=3 ± SD.
T_{1/2}=distribution half life.

TABLE 3

Mean toxicokinetic parameters for ISIS 388626 in cynomolgus monkeys (sexes combined) on Days 1, 42, and 91

Group	Dose Level (mg/kg)	Study Day	T _{max} (hr)	C _{max} (µg/mL)	AUC _{0→48hr} (µg*hr/mL)	AUC _{0→168hr} (µg*hr/mL)	t _{1/2α} (hr)	t _{1/2λz} (day)
2	1	1	0.6 ± 0.2	1.74 ± 0.27	5.92 ± 0.41	NM	1.91 ± 0.25	NM
		42	1.1 ± 0.7	2.36 ± 0.77	7.13 ± 1.72	7.09 ± 1.42	2.03 ± 0.23	NM
		91	0.8 ± 0.3	1.83 ± 0.24	6.96 ± 0.35	NM	2.18 ± 0.11	NM
3	3	1	0.7 ± 0.2	5.66 ± 0.85	20 ± 2.66	NM	1.59 ± 0.18	NM
		42	1 ± 0.4	5.55 ± 0.87	24.4 ± 4.28	24 ± 4.18	2.14 ± 0.32	NM
		91	0.9 ± 0.2	5.04 ± 0.57	23.5 ± 4.89	23.9 ± 5	2.09 ± 0.16	NM
4	10	1	0.9 ± 0.5	20.6 ± 5.3	101 ± 21.2	NM	2.16 ± 0.36	NM
		42	1.3 ± 0.6	19.1 ± 4.7	102 ± 17.5	109 ± 21.2	2.23 ± 0.2	NM
		91	1.5 ± 0.5	17.2 ± 4	112 ± 25.5	118 ± 26.4	2.58 ± 0.31	15.3 ± 6.7
5	30	1	1.5 ± 1.4	44.6 ± 11.5	299 ± 74.9	NM	2.9 ± 0.63	NM
		42	1.3 ± 0.6	50.9 ± 13.2	332 ± 56.5	346 ± 56.2	2.9 ± 0.63	NM
		91	1.4 ± 0.5	61.7 ± 24.9	499 ± 210	577 ± 272	3.33 ± 0.51	22.2 ± 16.4
6	3	1	1 ± 0.5	6.06 ± 1.5	24.9 ± 3.19	NM	2.11 ± 0.27	NM
		7	0.8 ± 0.2	6.24 ± 1.01	27.1 ± 4.79	NM	1.87 ± 0.2	NM

Note: t_{1/2α} = general estimate of plasma half-life associated with the initial (major) distribution phase.

t_{1/2λz} = the plasma disposition half-life associated with the terminal elimination phase was calculated from recovery animals (Groups 4 and 5).

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TABLE 4

ISIS 388626 Concentrations in Tissues Measured Approximately 2 Days after the Last Dose (on Day 93) Following 13 Weeks of Repeated Dose Administration and 13 weeks of Recovery (Day 182) in Mice and Monkeys

ISIS 388626 Concentration ($\mu\text{g/g}$)^a in Tissue

Dose	Mouse			Monkey	
	Day	Kidney	Liver	Kidney	Liver
1 mg/kg	93	21.5 \pm 10.9	0.37 \pm 0.057	152 \pm 73	2.19 \pm 0.54
3 mg/kg	93	32.2 \pm 12.8	0.82 \pm 0.156	236 \pm 91	10.3 \pm 3.3
	182	BLQ	BLQ	ND	ND
10 mg/kg	93	43.6 \pm 13.2	2.56 \pm 0.58	414 \pm 134	62 \pm 20.7
	182	ND	ND	2.69 \pm 1.33	0.333 \pm 0.391
30 mg/kg	93	58.6 \pm 24.3	5.97 \pm 1.81	658 \pm 243	162 \pm 58
	182	BLQ	BLQ	4.18 \pm 2.28	7.81 \pm 6.04

^a Values are presented as mean \pm standard deviation (n = 6 on Day 93 and n = 4 on Day 182)

BLQ = below the lower limit of quantitation (LLOQ = 0.2 $\mu\text{g/g}$). Mean concentrations that were below the lower limit of quantitation were presented as BLQ.

ND = not determined

FIGURE 01

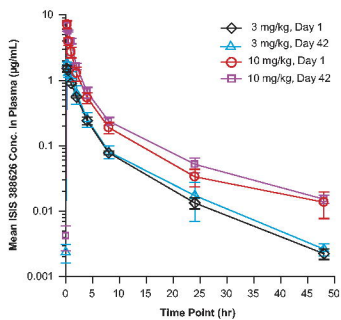


FIGURE 02

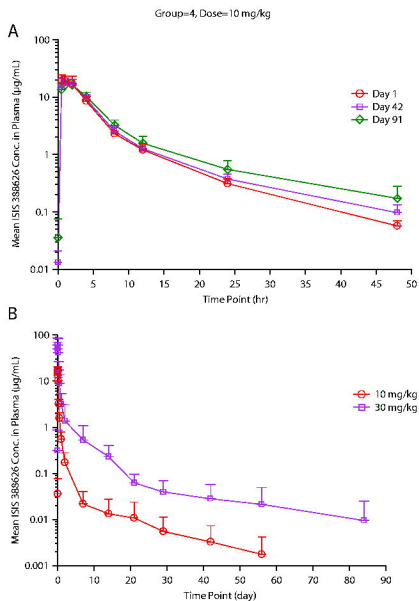


FIGURE 03

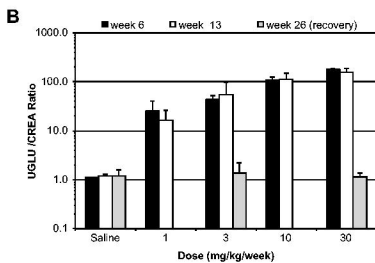
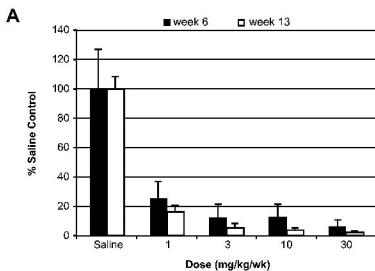
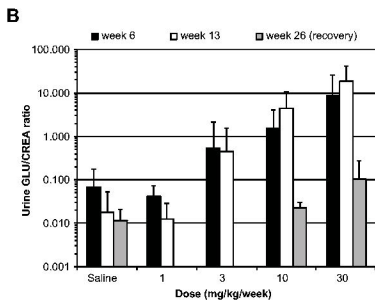
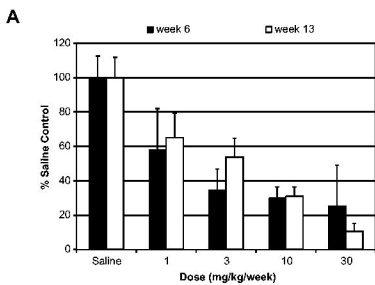


FIGURE 04



DATA SUPPLEMENT

(Supplementary Tables 1-5 and Supplemental Figure 1)

The Journal of Pharmacology and Experimental Therapeutics

Pharmacodynamics and Subchronic Toxicity in Mice and Monkeys of ISIS 388626, a Second Generation Antisense Oligonucleotide that Targets the Human Sodium Glucose Cotransporter 2 (SGLT2)

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Isis Pharmaceuticals, Carlsbad, California (T.A.Z., R.Z.Y., K.C., S.P.H.) and Korea Institute of Toxicology, Daejeon, Republic of Korea (S.C.H., E.J.J., S.R.)

SUPPLEMENTAL TABLE 1

Mean serum chemistry values in mice dosed with ISIS 388626 for up to 13 weeks

Parameter	Interim Values (Week 6)						Terminal Values (Week 13)						Recovery Values			
	Vehicle		10 mg/kg/week		30 mg/kg/week		Vehicle		10 mg/kg/week		30 mg/kg/week		Vehicle		30 mg/kg/week	
	Controls						Controls						Control			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
AST (U/L)	66.7 (15.7)	108 (62.6)	79.9 (18.2)	145 (21.2)	116^b (24.1)	132 (44.5)	61.1 (12.5)	83.7 (30.0)	66.3 (9.9)	91.8 (17.4)	94.1^b (14.3)	108.7 (19.3)	75.9 (24.5)	97.1 (26.3)	60.9 (8.6)	82.6 (23.1)
ALT (U/L)	29.6 (4.0)	36.7 (19.7)	31.2 (9.9)	40.3 (5.7)	48.6^b (15.1)	47.7 (14.1)	27.5 (10.7)	33.5 (26.8)	32.0 (6.5)	33.9 (9.3)	49.8^b (13.9)	41.4^b (13.1)	30.4 (5.5)	32.3 (8.1)	31.3 (9.2)	31.1 (9.2)
TCHO (mg/dL)	137 (18.6)	92.3 (20.4)	155 (19.6)	124 (25.9)	162.3 (26.8)	155^b (27.6)	143 (21.4)	92.8 (22.1)	158 (24.0)	128^a (24.9)	172^a (23.5)	141^a (33.0)	121 (15.4)	100 (17.2)	123 (33.8)	97.7 (29.0)
GLU (mg/dL)	168 (55.5)	156 (36.4)	143 (25.9)	133 (16.0)	154 (21.4)	175 (27.5)	177 (25.21)	144 (34.7)	195 (32.3)	151 (21.4)	199 (28.3)	166 (36.5)	251 (107)	146 (28.1)	241 (15.5)	181 (13.4)

Note: Doses were administered every-other day in the first week (q2d) followed by once weekly thereafter for a total of 13 weeks.

Numbers in parentheses are SD of the means.

^a $p \leq 0.05$, ^b $p < 0.01$ relative to control

SUPPLEMENTAL TABLE 2

Mean hematology values in mice dosed with ISIS 388626 for 13 weeks

Parameter (Unit)	Sex	Terminal Values				Recovery Values	
		Vehicle Controls	3 mg/kg/week	10 mg/kg/week	30 mg/kg/week	Vehicle Control	30 mg/kg/week
RBC 10 ⁶ /μl	M	9.4 (0.8)	9.4 (0.6)	8.6 (0.8)	6.8 ^b (0.7)	9.4 (0.5)	8.0 ^b (0.8)
	F	9.6 (0.6)	9.9 (0.6)	9.7 (0.5)	8.7 ^b (0.8)	9.7 (0.4)	9.1 (0.6)
HGB g/dL	M	14.9 (1.1)	14.8 (0.8)	13.1 ^b (1.1)	10.3 ^b (1.1)	14.4 (0.7)	12.1 ^b (1.2)
	F	15.5 (0.6)	15.8 (0.9)	15.7 (0.7)	14.0 (1.5)	15.3 (0.5)	14.4 (0.5)
HCT %	M	49.0 (3.9)	48.9 (2.7)	43.4 ^b (3.4)	33.6 ^b (3.9)	48.6 (2.0)	39.6 ^b (3.4)
	F	49.6 (3.2)	50.1 (3.1)	49.9 (2.4)	44.7 ^b (4.5)	48.5 (1.7)	45.7 (1.9)
WBC 10 ³ /μl	M	5.7 (1.8)	5.5 (1.3)	10.1 ^b (2.1)	10.1 ^b (1.6)	4.7 (1.3)	5.7 (1.8)
	F	3.2 (1.4)	5.7 ^a (2.5)	7.5 ^b (3.1)	7.0 ^b (1.4)	6.5 (1.2)	5.9 (1.8)
NEUA 10 ³ /μl	M	1.6 (0.7)	1.9 (0.7)	2.6 ^b (0.8)	2.6 ^a (0.6)	1.3 (0.2)	1.2 (0.4)
	F	0.6 (0.2)	1.2 (0.3)	1.6 ^b (0.6)	1.5 ^b (0.5)	1.7 (0.4)	1.5 (0.5)
LUCA 10 ³ /μl	M	0.05 (0.04)	0.04 (0.03)	0.13 ^b (0.05)	0.15 ^b (0.06)	.04 (0.01)	0.05 (0.03)
	F	0.02 (0.01)	0.03 (0.02)	0.07 ^b (0.05)	0.06 ^b (0.02)	0.04 (0.01)	0.02 (0.03)

Note: Doses were administered every-other day in the first week (q2d) followed by once weekly thereafter for a total of 13 weeks. Numbers in parentheses are SD of the means.

^a $p \leq 0.05$; ^b $p \leq 0.01$, relative to controls

SUPPLEMENTAL TABLE 3

Absolute terminal organ weights in mice administered ISIS 388626 for 13 weeks

Organ	Sex	ISIS 388626				
		Control	1.0 mg/kg/week	3.0 mg/kg/week	10 mg/kg/week	30 mg/kg/week
Spleen	M	0.098 ± 0.016	0.087 ± 0.018	0.086 ± 0.017	0.126 ± 0.039	0.111 ± 0.018
	F	0.092 ± 0.011	0.104 ± 0.031	0.110 ± 0.016	0.115^a ± 0.019	0.116^b ± 0.009
Kidney	M	0.70 ± 0.012	0.61 ± 0.09	0.60 ± 0.08	0.80^a ± 0.09	0.80^a ± 0.08
	F	0.42 ± 0.02	0.42 ± 0.05	0.45 ± 0.03	0.45 ± 0.04	0.47^a ± 0.05

Note. Each data represents the mean ± SD of 10 animals.

^a $p < 0.05$; ^b $p \leq 0.01$ relative to controls.

SUPPLEMENTAL TABLE 4

Treatment-related, Terminal Microscopic Changes in Mice Administered ISIS 388626 for 13-Weeks

Tissue Observation	Vehicle		Incidence and Severity of Change							
	Control		ISIS 388626							
	Incidence	Severity	1.0 mg/kg/week		3.0 mg/kg/week		10 mg/kg/week		30 mg/kg/week	
			Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Males										
<u>Multiple organs</u>										
Lymphohistiocytic infiltration	0	--	0	--	0	--	4	+	5	+
<u>Lymph nodes</u>										
Sinus histiocytosis, Lymphoid hyperplasia	0	--	0	--	0	--	3	+, ++	3	+, ++
<u>Liver</u>										
Kupffer cell, Basophilic granules	0	--	0	--	0	--	4	+	7	+
Hepatocyte, centrilobular hypertrophy	0	--	0	--	0	--	5	+	4	+, ++
<u>Kidney</u>										
Proximal tubular epithelium Basophilic granules	0	--	0	--	0	--	6	+, ++	7	+, ++, +++

<u>Spleen</u>										
Extramedullary hematopoiesis	5	+, ++	4	+, ++	6	+, ++	6	+, ++	6	+, ++, +++
Females										
<u>Multiple organs</u>										
Lymphohistiocytic infiltration	0	--	0	--	0	--	1	+	4	+
<u>Lymph nodes</u>										
Sinus histiocytosis, Lymphoid hyperplasia	0	--	0	--	0	--	2	+	3	+
<u>Liver</u>										
Kupffer cell Basophilic granules	0	--	0	--	0	--	7	+	7	+
<u>Kidney</u>										
Proximal tubular epithelium Basophilic granules	0	--	0	--	0	--	7	+, ++	7	+, ++
<u>Spleen</u>										
Extramedullary hematopoiesis	5	+	6	+, +++	4	+, ++	6	+, ++, +++	5	+, ++

Note. Incidences of changes are expressed as the number of tissues indicated/10 animals examined. Severity of change: +, ++, +++ = minimal, slight, moderate, respectively.

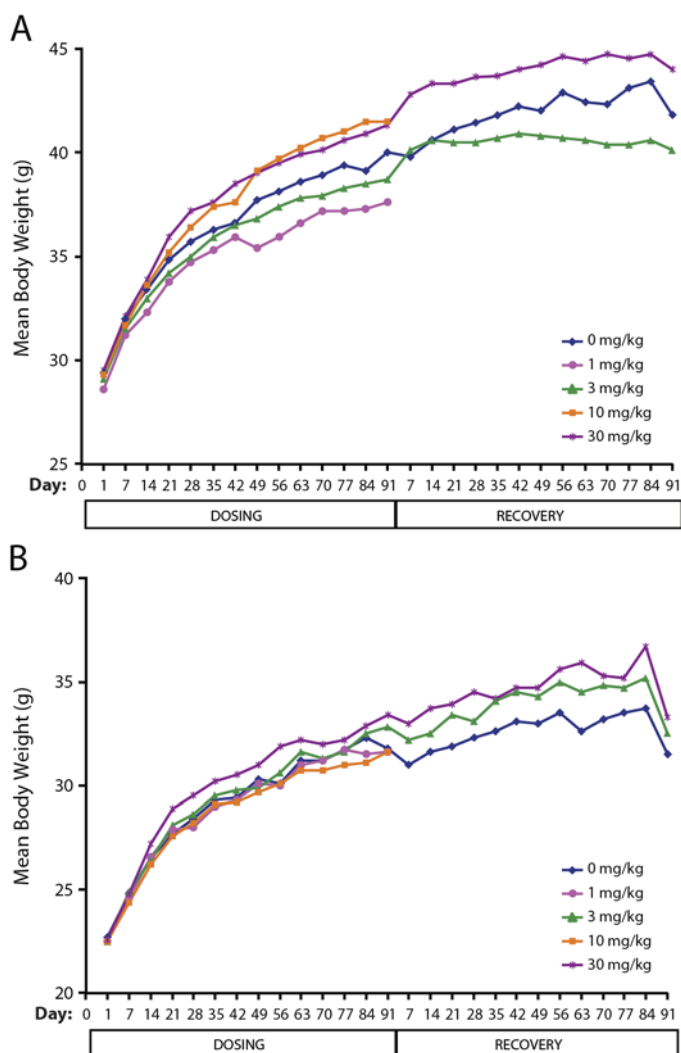
SUPPLEMENTAL TABLE 5

Interim, terminal and recovery microscopic changes observed in
monkeys treated with ISIS 388626

Group No.	Control			1.0 mg/kg/wk			3 mg/kg/wk			10 mg/kg/wk			30 mg/kg/wk		
Observation period ¹	I	T	R	I	T	R	I	T	R	I	T	R	I	T	R
MALES (No. Examined)	2	3	2	2	3	0	2	3	0	2	3	2	2	3	2
KIDNEY															
Basophilic granules, tubular epithelium										3			3		
URINARY BLADDER															
Hypertrophy, histiocytes, submucosa,														1	
LIVER															
Basophilic granules, Kupffer cell										3			2	3	
LYMPH NODES															
Basophilic granules, histiocyte														1	
Hypertrophy, histiocyte										1			2	2	
Vacuolation, histiocytes													2	2	
FEMALES (No. Examined)	2	3	2	2	3	0	2	3	0	2	3	2	2	3	2
KIDNEY															
Basophilic granules, tubular epithelium										2			1	3	
URINARY BLADDER															
Hypertrophy, histiocytes, submucosa														2	
LIVER															
Basophilic granules, Kupffer cell										3			2	3	
LYMPH NODES															
Basophilic granules, histiocyte										2				3	
Hypertrophy, histiocyte										3			2	3	
Vacuolation, histiocytes										1			1	2	

Note: Observation days: I= Interim sacrifice, Day 44; T= Terminal sacrifice, Day 93; R = Recovery sacrifice, Day 182.

SUPPLEMENTAL FIGURE 1



Supplemental Figure 1. Mean body weights of male (A) and female (B) mice administered ISIS 388626 for up to 13-weeks. Mean body weights of males and females at the high dose of 30 mg/kg/wk were significantly greater than those of respective controls. Error bars represent standard deviation (n= 16 or 22 through Day 42, n= 10 or 16 Days 49 through 91, and n= 6 during recovery).