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ORIGINAL ARTICLE

Preclinical toxicity and toxicokinetics of GTI-2040, a phosphorothioate oligonucleotide targeting ribonucleotide reductase R2

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Abstract
Purpose GTI-2040, a 20-mer phosphorothioate oligonucleotide, was designed to hybridize to the mRNA sequence of human ribonucleotide reductase R2. GTI-2040 has been shown to inhibit human cancer cell proliferation by down-regulation of R2 expression in vitro and to significantly inhibit tumor growth in xenograft models of human cancer in mice. As part of the safety evaluation for human clinical trials, the toxicity and toxicokinetics of GTI-2040 were determined in Sprague–Dawley rats and rhesus monkeys.
Methods GTI-2040 was administered to rats at 2, 10, and 50 mg/kg/day by bolus intravenous injection every second day for 21 days with a 21-day recovery. In monkeys, an acute study was performed with single, escalating doses of GTI-2040 ranging from 10 to 80 mg/kg given as a 24-h continuous intravenous infusion. As well, a 21-day, continuous intravenous infusion study with GTI-2040 was conducted in monkeys at 2, 10, and 50 mg/kg/day, with a 3-week recovery. Blood sampling was done to measure GTI-2040 plasma concentrations, metabolites, and pharmacokinetic parameters, and tissues were collected to assess the distribution of GTI-2040 and/or metabolites.
Results The toxicities of GTI-2040 in both rats and monkeys were typical for the phosphorothioate oligonucleotide class of compounds. In monkeys, there was a dose-related increase in GTI-2040 plasma levels with concomitant increase in complement activation and prolongation of activated partial thromboplastin time. In both rats and monkeys, the tissues having the highest concentrations of GTI-2040 (kidney, liver, spleen) had the largest dose-related toxic effects. Adverse effects were diminished or absent in the recovery animals.
Conclusions GTI-2040 was well tolerated when infused over 24 h at doses up to 80 mg/kg in monkeys. In rats and monkeys, GTI-2040 was reasonably well tolerated and showed reversible toxicities when administered at doses up to 50 mg/kg/day for 21 days. The no observed adverse effect dose level for GTI-2040 in both animal species was 2 mg/kg/day. There were no apparent sequence-specific effects related to the interaction of GTI-2040 with the R2 component of the mRNA expressing ribonucleotide reductase.

Keywords Antisense · Oligonucleotide · Ribonucleotide reductase · Toxicity

Introduction

Mammalian ribonucleotide reductase is an enzyme containing two dimeric protein components: R1 (also referred to as M1 and RRM1) and R2 (M2, RRM2). Both subunits are required to catalyze the reduction of ribonucleoside diphosphates to their corresponding deoxyribonucleotides, a rate-limiting step in DNA synthesis [1, 2]. Although the levels of the R1 protein do not appear to change substantially during the cell cycle, there is an S-phase-correlated increase in the R2 protein resulting from its de novo synthesis [3]. Deregulation of the R2 component of mammalian ribonucleotide reductase in tumor cells is capable of modifying the *ras*/MAPK pathway and can act as a novel determinant in tumor progression that cooperates with a variety of

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oncogenes to enhance malignant potential [4]. GTI-2040, a fully phosphorothioated 20-mer oligonucleotide (ODN), was selected as a lead compound from a series of related ODNs designed to hybridize in an antisense manner with the mRNA sequence responsible for the expression of human ribonucleotide reductase R2 [5]. GTI-2040 was shown to inhibit human cancer cell proliferation by the downregulation of R2 expression in vitro. The effects of GTI-2040 were both target specific and sequence specific as control ODNs were ineffective in downregulating R2 and a number of non-related target genes were unaffected by treatment with GTI-2040 in vitro. In vivo GTI-2040 was found to significantly inhibit tumor growth and metastasis in murine xenograft models of human cancer and dramatically increase survival in a murine model of human lymphoma [5]. As with in vitro studies, the antitumor effects observed with GTI-2040 were sequence specific, as mismatched and scrambled control ODNs were ineffective in in vivo models. Based on excellent preclinical antitumor efficacy, GTI-2040 was selected for further development as a potential therapeutic anticancer agent.

The purpose of this study was to determine the toxicity and toxicokinetics of GTI-2040 in rats and non-human primates as part of the safety evaluation for human clinical trials. Previous studies with other phosphorothioate oligonucleotides have demonstrated that the observed adverse reactions were generally unrelated to the target sequence [6–8]. In addition, systemic toxicities of phosphorothioate oligonucleotides have occurred at levels above the therapeutic level and are related to the plasma levels of the oligonucleotide [9, 10]. The results obtained in this study indicated that the adverse effects resulting from the treatment of rats and monkeys with GTI-2040 were typical of phosphorothioate oligonucleotides and, like other compounds in this class, the observed toxicities correlated with the plasma and tissue levels of GTI-2040 and its metabolites. Adverse effects were observed in the high-dose groups, corresponding to a dose that is 10 times higher than the therapeutic dose, suggesting that efficacy can be effectively achieved with minimal toxicity.

Materials and methods

Compound

GTI-2040 (5′-GGCTAAATCGCTCCACCAAG-3′) is a 20-mer phosphorothioate oligodeoxynucleotide complementary to a sequence in human ribonucleotide reductase R2 mRNA. GTI-2040 was synthesized by Boston BioSystems (Bedford, MA). Results of tests for molecular weight (MALDI-TOF MS with IS), sequence identity (MALDI-TOF MS with IS), purity (capillary gel electrophoresis, IE-HPLC), P–S purity (^{31}P NMR), DNA content for concentration (UV 260), water content, residual organic solvent from synthesis and purification (gas chromatography and capillary electrophoresis), sodium and heavy metal content (ICP/OES), pH (USP method), and endotoxin (USP method, LAL gel clot) were reported and were within specifications. Oligonucleotide was formulated in isotonic saline for intravenous administration to animals.

Cloning and sequencing of rat and monkey R2 cDNA

Cos-7 (monkey kidney) and Rat 2 (rat embryonic) cells (American Type Culture Collection ATCC) (Rockville, MD) were maintained according to ATCC recommendations. Cells were seeded in 100-mm tissue culture dishes at a density of 1.5–2 × 10^6 cells and grown to 70–80% confluency. Total cellular RNA was prepared using Trizol reagent according to the manufacturers' instructions (Invitrogen). R2 cDNAs were prepared by RT–PCR using two forward (upstream of the target sequence) and two reverse primers (downstream of the target sequence) in all four combinations. Primers were based on conserved regions in the alignment of the human and mouse R2 sequences. The * denotes nucleotide mismatches between human and mouse. The primer sequences were as follows:

5′ CCAAGGAC*ATTCAGCACTGGGAA 3′ (103 bp upstream of GTI-2040 target site)
5′ GTTCTGGCTTTCTTTGCAGC 3′ (44 bp upstream of GTI-2040 target site)
5′ GAA*TGTATGTTTTCCATGGCAA 3′ (67 bp downstream of GTI-2040 target site)
5′ CTTTT*ATGTAAGTGTCAATAAG 3′ (105 bp downstream of GTI-2040 target site)

Specific first-strand cDNA synthesis was performed with 5 μg of total RNA using Ready-To-Go You-Prime First-Strand Beads Kit (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) according to the manufacturer's recommendations. An aliquot of 15 μL of the cDNA was added to a standard PCR reaction mix containing 40 pmol of the upstream primer and 20 pmol of the downstream primer for PCR. The PCR products were resolved by electrophoresis on 1% agarose gels and purified by electroelution. All four combinations resulted in RT–PCR products. Four independent clones were sequenced for each species at the University of Maine DNA sequencing facility (Orono, Maine, USA).

Study design

Three separate studies were conducted in rat and monkey species. The study conducted in rats was a repeat dose, 21-day intravenous toxicity study with a 21-day recovery.

A total of 68 male and 68 female Sprague–Dawley rats (Charles River Canada Inc., St. Constant, Quebec, Canada) were randomly assigned to one control and three treatment groups based on body weight. Body weights were recorded throughout the study. Group mean body weights ranged from 189.9 to 198.8 g (males) and 155.2 to 159.0 g (females) 1 day prior to treatment initiation. Rats were individually housed in stainless steel mesh-bottomed cages and were maintained in environmentally controlled rooms (12-h light/dark cycle; 22 ± 3°C; 30–70% relative humidity) with ad libitum access to water and food (PMI Certified Rodent Chow 5002) except when specific testing required a fasted state. The animals were administered GTI-2040 (or isotonic saline vehicle for control animals) by bolus i.v. injection (tail vein) every other day for 21 days (total, 11 doses) at 2, 10, or 50 mg/kg/dose in a volume of 1.67 mL/kg/dose. Group sizes were 10 rats/sex for toxicological evaluations, and the control and high-dose groups included five additional animals/sex for recovery assessment. In addition, the treatment groups included six satellite animals/sex to measure GTI-2040 levels in plasma and tissues for toxicokinetic evaluation. After completion of treatment, animals were euthanized with isoflurane anesthesia followed by exsanguination from the abdominal aorta according to the following schedule: Main study, Day 23 (2 days after the last dose); Recovery animals, Day 43 (3 weeks after the last dose); Toxicokinetic satellites, 4 h after the last dose.

Two intravenous toxicity studies were conducted in Rhesus monkeys: an acute intravenous toxicity study and a 21-day continuous intravenous infusion toxicity study with a 21-day recovery. For both studies, rhesus monkeys (SBi Animal Colony, Sierra Biomedical Inc., Sparks, Nevada USA) were randomly chosen and assigned to treatment groups based upon body weight [acute study: three males (body weight range, 4.0–5.6 kg) and two females (3.4–4.1 kg); 21-day study: 14 males (2.8–3.9 kg) and 14 females (2.8–3.6 kg)]. The age of the animals ranged from 2.9 to 5.4 years (acute study) and 2 to 4.5 years (21-day study). Monkeys were housed in individual stainless steel cages and were maintained in an environmentally controlled room (12-h light/dark cycle; 64–84°F) with ad libitum access to water. Food (Harlan Teklad Certified Primate Diet®) was provided daily in amounts appropriate for the size and age of the animals.

In the acute study, monkeys were administered GTI-2040 (or isotonic saline vehicle for control animals) by continuous intravenous infusion over a 24-h period at a rate of 0.4 mL/kg/h. Each animal received escalating doses of GTI-2040 at 10, 20, 40, and 80 mg/kg, with a minimum of 2 days between administration of each dose. Prior to the study, the animals were surgically implanted with telemetry transmitters for monitoring cardiovascular parameters. In addition, each animal had a central venous catheter (with subcutaneous access port) surgically implanted for dose administration. Electrocardiogram (ECG) and mean arterial pressure (MAP) were remotely monitored in each animal by telemetry for 30 s once prior to infusion, at approximately 3-h intervals during infusion and once following the end of infusion.

In the 21-day study, monkeys were administered GTI-2040 (or isotonic saline vehicle for control animals) by continuous intravenous infusion for 21 consecutive days at 2, 10, or 50 mg/kg/day. Catheters were surgically implanted into the femoral vein of each animal. After surgery, each animal was fitted with a jacket through which the catheter was passed into an attached tether such that the animals were freely moving in the cage. Following catheter implantation, all animals were infused with isotonic saline (0.9% sodium chloride for injection, U.S.P.) at a rate of 1.0 mL/h until treatment initiation to maintain catheter patency. Group sizes were 4 animals/sex in the vehicle control and high-dose groups and 3 animals/sex in the low- and mid-dose groups. ECGs were recorded from all animals prior to the study and in week 3 and from the recovery animals in week 6 after start of treatment. At the end of the 21-day infusion, 24 animals (3/sex/group) were euthanized (on Day 22) by exsanguination while under deep anesthesia induced with ketamine and Beuthanasia D solution. The remaining four animals (1/sex each from the vehicle control and high-dose groups) were retained for a 21-day recovery period and euthanized on Day 43. All animals were necropsied for gross pathology and histopathological evaluation.

Observations and sample collection

During the treatment period, all animals were observed twice daily for signs of toxicity. In addition, both animal body weights and individual food consumption (quantitatively for rats and qualitatively for the monkeys) were determined on a weekly basis. Ophthalmic examinations were performed on both rats and monkeys prior to each 21-day study, during the treatment phase and on the recovery animals in week 6 after treatment initiation.

Blood samples were collected for the evaluation of clinical pathology (serum chemistry, hematology, coagulation parameters), complement values (complement split product Bb and CH50; measured in monkey studies only), and toxicokinetics. Rat blood samples for clinical pathology evaluation were collected at the end of the treatment and recovery period. Blood samples were collected from the abdominal aorta and placed into tubes containing EDTA or citrate as anticoagulants. For toxicokinetic evaluation in rats, one serial (orbital sinus) and one terminal (abdominal aorta) blood sample was collected from each satellite and placed in heparinized tubes at 0.25, 1, 4, 10, and 48 h after the first injection and at 4 h after the end of the 21-day

treatment. Monkey blood samples were collected by femoral venipuncture for serum chemistry, hematology, and coagulation parameters as follows: acute study, prior to initiation of each infusion and at the end of each infusion period; 21-day study (after an overnight fast), prior to and after catheter implantation (prior to infusion). In addition, blood samples to evaluate hematology, coagulation parameters, and complement split product Bb in the 21-day study were collected in tubes containing EDTA on Day 20 of infusion and on Day 41 in the recovery animals. For toxicokinetic evaluation in monkeys, single blood samples were withdrawn from each monkey and placed into tubes containing EDTA within 2 min following the end of each 24-h infusion period (Days 2, 5, 9, and 12) (acute study) and at 8, 24, 48, 96 h, and on day 20 after start of infusion (21-day study).

Both rat and monkey serum chemistry measurements included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase, total bilirubin, albumin, globulin, total protein, blood urea nitrogen (B.U.N.), creatinine, γ-glutamyl transferase (GGT), sodium, potassium, chloride, calcium, phosphorous, glucose, cholesterol, and triglycerides. Hematologic parameters examined included both total and differential leukocyte counts, erythrocyte counts, reticulocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, erythrocyte morphology, platelets, prothrombin time (PT), and activated partial thromboplastin time (APTT). Urine samples in the 21-day monkey study were obtained from the bladder at necropsy on Day 22 and from recovery animals on Day 43. Monkey urinalysis parameters examined included gross appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, and microscopic analysis. Complement (Bb) split product and CH50 values (acute study only) were measured in the plasma of monkey blood by Complement Laboratory, National Jewish Center of Immunology, Denver, Colorado.

Terminal procedures

At necropsy, a complete examination of all body cavities was conducted on animals in the 21-day studies. The following selected organs from both rats and monkeys were excised, trimmed of fat and connective tissue, and weighed: liver, kidney, lungs (monkeys only), spleen, thyroid, adrenals, brain, heart, pituitary, prostate, thymus, testes, epididymides (monkeys only), ovaries and uterus (rats only). Subsequently, the weighed tissues and the following other excised tissues and organs were preserved in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin for histopathological evaluation: aorta, salivary gland, tongue, esophagus, stomach, small intestine (duodenum, jejunum, ileum), large intestine (cecum, colon, rectum) pancreas, gallbladder (monkeys only), trachea, bone (femur and sternum), bone marrow (sternum), lymph nodes (mandibular and mesenteric), urinary bladder, epididymides, seminal vesicles, uterus (body/horn), cervix, vagina, skin/mammary gland, skeletal muscle, eyes with optic nerve, spinal cord (thoracic, cervical), infusion site (monkeys only), and gross lesions. At necropsy prior to formalin preservation, approximately 1 g of the following monkey tissues and the inguinal lymph nodes of the monkeys were flash-frozen in liquid nitrogen and used for toxicokinetic evaluation: heart, liver, lung, spleen, kidneys, and brain. Those tissues and bone marrow (femur) were also collected from satellite animals (rat study) and retained frozen for toxicokinetic evaluation.

Plasma and tissue analysis

Plasma (containing EDTA) and tissue samples were analyzed by capillary electrophoresis (CE) with UV detection at 260 nm (Beckman P/ACE System MDQ) after addition of an internal standard and a two-step (strong anion exchange and reverse-phase C-18 cartridges) solid-phase extraction procedure. The lower limit of quantitation for GTI-2040 was 0.125 μg/mL in plasma and 1 μg/g in tissues of both rats and monkeys. The method also provided an estimation of GTI-2040 metabolite concentrations in the samples by integration of all the peaks corresponding to the related shorter (N-1 to N-4) oligonucleotides in each sample electropherogram.

Pharmacokinetics and toxicokinetics

Non-compartmental pharmacokinetic analysis was carried out on the GTI-2040 plasma concentration data obtained from rats and monkeys (21-day continuous i.v. infusion). The parameters estimated by this analysis were the following: C_{max} and T_{max} (bolus injection); C_{ss}, steady-state concentration after infusion; Cl, plasma clearance (estimated as dose rate/C_{ss}); K_{el}, apparent elimination rate constant; $T_{1/2}$, apparent elimination half-life ($0.693/K_{el}$); and AUC_{0-t}, area under the plasma concentration/time curve calculated by the trapezoidal rule for time from 0 h to the time of last quantifiable concentration and from time 0 h to infinity (AUC_{0-inf}) by the relationship AUC_{0-t}, + C_t/K_{el}.

Statistical analysis

Statistical evaluation of numerical data was performed to identify any GTI-2040 treatment-related effects. Clinical pathology, organ weight and body weight parameters were evaluated using an appropriate one-way analysis of variance and a test for ordered response in the dose groups.

A standard one-way ANOVA using F-distribution to assess significance was used. If significant differences between the means were identified, Dunnett's test was used to determine which treatment groups differed significantly from controls.

Results

Target sequence conservation

The GTI-2040 target sequence should be conserved in the test animals in order to adequately address the question of whether there are sequence-specific adverse effects of this AS-ODN. Rat and monkey R2 cDNA sequences surrounding the GTI-2040 target site were amplified by RT–PCR and sequenced. The GTI-2040 target sequence from human R2 was completely conserved in both the rat and monkey R2 sequences (Fig. 1). Given that there is complete target sequence identity between ribonucleotide reductase R2 mRNA in human and that in rat and non-human primates, these species served as an appropriate model to evaluate adverse reactions related to both the inhibition of R2 expression and non-sequence-related toxicities. The GTI-2040 target sequence was also conserved in mouse R2 (data not shown).

Toxicology of GTI-2040 in rats

One male rat in the 50 mg/kg group was found dead on Day 21 of treatment. Although gross and histopathological evaluation did not reveal the cause of death, clinical signs (low activity, general weakness, reduced body temperature) and microscopic findings suggested that the mortality was related to GTI-2040 treatment. Clinical signs related to treatment with GTI-2040 were observed on Day 22 in three males in the 10 mg/kg dose level and included reduced activity and eyes partly closed. At 50 mg/kg, a reduction in body weight gain was seen for both male and female rats during the treatment and recovery periods (Table 1). A reduction in food consumption was also found in treatment and recovery animals in the same dose group. Most males and some females in the high-dose group presented the following clinical signs that were considered treatment related: general weakness (lying on the side), skin pallor, eyes partly closed, and soft feces. Reduced activity and eyes partly closed were also observed in a few male rats on Day 23 of the 10 mg/kg regimen. Changes in major organ weights were also observed in the mid- and high-dose groups (Table 1). Spleen weights of both males and females in the 50 and 10 mg/kg dose groups were significantly higher than those in controls, and this gross observation persisted in the recovery animals up to 21 days after the last dose. Changes in spleen weights correlated with microscopic lesions in the spleen. Statistically significant changes in organ weights were also found in the liver of animals in the mid- and high-dose groups, and kidney and uterus weights were increased in females at the 50 mg/kg dose. However, those differences in organ weights were small and were not considered biologically relevant. There were no hematology or clinical chemistry changes at 2 mg/kg that were considered to be related to treatment. Consistent, dose-related changes in hematology and clinical chemistry parameters with statistically significant differences relative to controls ($P < 0.01$) in mid- and high-dose animals were found in rats. Specifically, total white blood cell (WBC), lymphocyte counts, red cell size distribution width (RDW), and mean platelet volume increased, while total red blood cell counts, hemoglobin concentration, platelet counts, and hematocrit decreased with escalating dose. Coagulation parameters such as activated partial thromboplastin time (APTT) were unaffected by treatment (data not shown). At the end of the 21-day recovery period, the changes in the hematological parameters were less marked, particularly for the decreases in red blood cell and platelet counts (and associated indices). Clinical chemistry parameters such as blood urea nitrogen (B.U.N.), total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were moderately increased in mid- and high-dose groups, while alkaline phosphatase decreased with escalating dose in these groups. At the end of the recovery period for the high-dose group, AST and ALT were still elevated, similar to levels seen at 21 days. In general, most of the changes

Rat sequence:
5' CCAAGGACATTCAGCACTGGGAATCCCTGAAACCCGAGGAGAGATATT
TTATATCCCATGTTCTGGCTTTCTTTGCAGCAAGCGATGGCATAGTAAAT
GAAAA**CTTGGTGGAGCGATTTAGCC**AAGAAGTTCAGATTACAGAAGC
CCGCTGTTTCTATGGCTTCCAAATTGCCATGGAAAACATACATTCT

Monkey sequence:
5' CCAAGGACATTCAGCACTGGGAATCCCTGAAGCCCGAGGAGAGATATT
TTATATCCCATGTTCTGGCTTTCTTTGCAGCAAGyGATGGCATAGTAAAT
GAAAA**CTTGGTGGAGCGATTTAGCC**AAGAAGTCCAGATTACAGAAGC
CCGCTGTTTCTATGGCTTCCAAATTGCCATGGAAAACATACATTCA

Note: "y" represents a nucleotide that was not readable

Fig. 1 Nucleotide sequences of rat and monkey ribonucleotide reductase R2 cDNAs showing the GTI-2040 target site from human R2 (*bold* and *underlined*) and adjacent sequences. Both rat and monkey R2 sequences demonstrate complete identity with the GTI-2040 target sequence from human R2

Table 1 Mean body and organ weights (±SD) in rats treated with GTI-2040 in the 21-day study

Parameter	Sex	Dose group					
		0 (Controls)		2 mg/kg	10 mg/kg	50 mg/kg	
		Day 21	Day 43	Day 21	Day 21	Day 21	Day 43
Body weight (g)	Male	303 ± 25	377 ± 3	297 ± 23	288 ± 23	275 ± 30*	297 ± 40*
	Female	189 ± 20	231 ± 17	184 ± 15	191 ± 12	171 ± 10**	192 ± 10*
Liver (g)	Male	9.2 ± 1.4	10.6 ± 0.68	9.4 ± 0.97	13.0 ± 2.5*	12.8 ± 2.5*	11.7 ± 2.0
	Female	5.5 ± 0.79	6.8 ± 0.76	6.3 ± 0.77	10.2 ± 2.1*	11.0 ± 3.2*	10.5 ± 1.5*
Kidney (g)	Male	2.31 ± 0.22	2.74 ± 0.10	2.31 ± 0.19	2.35 ± 0.21	2.17 ± 0.22	2.33 ± 0.21
	Female	1.49 ± 0.13	1.69 ± 0.18	1.51 ± 0.15	1.55 ± 0.078	1.78 ± 0.16*	1.81 ± 0.17
Spleen (g)	Male	0.68 ± 0.068	0.81 ± 0.14	0.83 ± 0.10	1.24 ± 0.27*	2.19 ± 0.64*	1.89 ± 0.89**
	Female	0.50 ± 0.066	0.55 ± 0.13	0.59 ± 0.093	1.01 ± 0.19*	1.60 ± 0.65*	1.16 ± 0.11*
Thymus (g)	Male	0.62 ± 0.18	0.42 ± 0.086	0.55 ± 0.11	0.52 ± 0.11	0.44 ± 0.079*	0.44 ± 0.048
	Female	0.37 ± 0.11	0.34 ± 0.035	0.36 ± 0.052	0.40 ± 0.074	0.36 ± 0.084	0.29 ± 0.079
Uterus (g)	Female	0.37 ± 0.072	0.41 ± 0.081	0.41 ± 0.067	0.46 ± 0.079	0.50 ± 0.096*	0.50 ± 0.039

Body weights shown are final fasted body weights recorded before euthanasia at the end of treatment (Day 21) and at the end of recovery (Day 43). Statistically significant differences are relative to controls (* $P < 0.01$; ** $P < 0.05$)

were small in magnitude (less than or equal to twice the control group means) and were not regarded as being of major toxicological significance. Treatment-related changes observed in other parameters such as mean glucose, electrolytes, protein fractions, and triglycerides were only a minor percentage of the control group means and were not considered to be biologically significant.

Generalized histiocytosis was a frequent histopathological treatment-related change that was seen in multiple organs in rats, both in the high-dose group (50 mg/kg) and to a lesser incidence and severity in the mid-dose group (10 mg/kg). This effect was not observed in the recovery animals 21 days after the last dose at 50 mg/kg. Other treatment-related findings were observed in the kidneys of mid- and high-dose groups included the following: tubular hyaline droplets, tubular karyomegaly, interstitial mononuclear cell infiltration, and cytoplasmic basophilic inclusions. In the liver, hepatic sinusoidal histiocytosis and erythrophagocytosis was observed in all treated groups, and a periportal mononuclear cell infiltration was observed as follows: 1 (male)/20 animals at the low dose; nine (seven males and two females)/20 at the mid dose; and 20/20 at the high dose. In general, treatment-related histopathological findings in the kidney and liver were minimal in the 10 mg/kg dose group and mild to moderate in the 50 mg/kg group. Mild to moderate perivascular and peribronchiolar mononuclear cell infiltration was seen in the lungs of animals treated at 50 mg/kg, often accompanied by slight edema. Lymphoid hyperplasia that was slight in the lymph nodes and mild to moderate in the spleen was observed mainly in the mid- and high-dose animals suggesting an effect of the test article, although such an effect can occur spontaneously. Minimal inflammation at the injection sites in some animals from all treated groups indicated a slight local response to the test article possibly associated with some extravasation with repeated tail vein injections. In the recovery animals at 21 days after the last high dose, the lymphoid hyperplasia, mononuclear cell infiltration, and sinusoidal histiocytosis in the liver, as well as the renal findings (except hyaline droplet formation), were still present, although the severity was slightly decreased. At the low dose, the only finding was a slight to moderate hepatic sinusoidal histiocytosis with erythrophagocytosis in most of the animals. Since the histiocytosis and erythrophagocytosis in the liver were reversible changes at the high-dose group and relatively benign in the low-dose group, 2 mg/kg (11.8 mg/m^2/day) was considered a no observed adverse effect level for GTI-2040 in the rat.

Toxicology of GTI-2040 in monkeys

In rhesus monkeys administered with single escalating doses of GTI-2040 by continuous intravenous infusion for 24 h, no treatment-related clinical signs or changes in other toxicity parameter evaluations (food consumption, body weight, ECGs, blood pressure, heart rate, serum chemistry, and hematology) were observed in the treated animals, with the exception of an increase in the coagulation parameter APTT at 80 mg/kg and a dose-related increase in plasma concentrations of complement split product Bb (Table 2). Similar to the acute regimen, administration of GTI-2040 to monkeys for 21 days by continuous intravenous infusion resulted in an increase in the coagulation parameter APTT at 50 mg/kg and a dose-related increase in the plasma concentrations of complement split product Bb (data not shown). A marginal increase in prothrombin time, exhibited

Table 2 Activated partial thromboplastin time (APTT) and complement values (Bb and CH50) in Rhesus monkeys treated with single, escalating doses of GTI-2040

Dose (mg/kg/day)	APTT (s)		Complement Bb (μg/mL)		Complement CH50 (μg/mL)	
	Predose	Postdose	Predose	Postdose	Predose	Postdose
0 (Control)[c]	18.2 ± 1.0	21.2 ± 3.1	0.72 ± 0.20	0.74 ± 0.19	293 ± 19.6	278 ± 29.5
10	>200	21.7 ± 1.7	0.74 ± 0.15	0.80 ± 0.18	231 ± 63	243 ± 59
20	17.3[a]	16.7[b]	0.76 ± 0.10	1.15 ± 0.17	225 ± 44	228 ± 43
40	20.3 ± 1.8	23.8 ± 2.1	0.88 ± 0.10	1.46 ± 0.28	253 ± 45	238 ± 61
80	16.2 ± 0.5	34.6 ± 2.6	0.78 ± 0.10	1.61 ± 0.16	325 ± 43	231 ± 35

Data shown are mean values ± SD ($N = 3$)

Blood samples for evaluation of APTT and complement values were collected from all animals prior to initiation of each infusion (Days 1, 4, 8, and 11) and at the end of each infusion period (Days 2, 5, 9, and 12)

ND Not detected

[a] Mean, $N = 2$

[b] $N = 1$

[c] One ($N = 1$) control animal: mean ± SD of four pre- and postdose measurements on each parameter

Table 3 Mean body and organ weights in Rhesus monkeys treated with GTI-2040 by continuous intravenous infusion for 21 days

Parameter	Dose group[a]					
	0 (Controls)		2 mg/kg	10 mg/kg	50 mg/kg	
	Day 22	Day 43	Day 22	Day 22	Day 22	Day 43
Body weight (kg)	3.9 ± 0.9	4.1	3.8 ± 0.5	3.6 ± 0.6	3.4 ± 0.2	3.9
Liver (g)	95.6 ± 21.4	85	97.1 ± 23.1	114.9 ± 16.5	112.2 ± 9.4	100.8
Kidney (g)	19.4 ± 2.7	18.6	20.3 ± 3.9	25.3 ± 4.0*	29.5 ± 6.9*	22.3
Spleen (g)	5.95 ± 3.04	5.17	6.19 ± 3.66	12.2 ± 3.2*	10.0 ± 5.1	5.88
Lungs (g)	23.5 ± 2.5	19.8	25.2 ± 3.8	31.9 ± 5.5*	27.3 ± 7.0	23.7
Thymus (g)	2.52 ± 0.74	5.12	2.78 ± 1.14	1.28 ± 0.29*	1.12 ± 0.62*	2.50
Adrenals (g)	0.66 ± 0.092	0.86	0.73 ± 0.085	0.84 ± 0.078	1.01 ± 0.38	0.68

Body weight measurements shown are final weights obtained shortly before necropsy

* $P < 0.05$

[a] Mean ± SD: $N = 6$ animals/group, Day 22; $N = 2$ animals/group, Day 43. The groups comprised the same numbers of animals/sex

by most animals that showed APTT prolongation (data not shown), was not considered clinically significant. There were no apparent sex differences with respect to toxicological responses observed in the monkeys. Changes in specific organ weights were observed in the monkeys treated with GTI-2040 in the 21-day study. At 10 and 50 mg/kg/day, kidney and spleen size was enlarged while the thymus mass decreased in the animals (Table 3). Since kidney, spleen, and thymus weights in the high-dose recovery animals were similar to controls on Day 43, the changes in organ weights were considered reversible.

Several changes occurred in the hematology and clinical chemistry parameters in the monkeys dosed at 10 and 50 mg/kg/day in the 21-day study, although the changes observed were not considered toxicologically significant. Relative to controls and predose values, total WBC, lymphocytes, neutrophils, and monocytes were elevated in the mid- and high-dose groups, although a significant increase was only seen in neutrophil levels in the 10 mg/kg group on Day 20 (7.11 vs. 3.55 [X 10^3/mm^3] for control group; $P < 0.01$). Neutrophilia appeared to correlate with inflammatory reactions at the site of termination of the surgically implanted catheter, which tended to be more pronounced in animals from the GTI-2040-treated groups. A decrease in platelet counts was also seen in the mid-dose group on Day 20 (253 vs. 439 [X 10^3/mm^3] for control group; $P < 0.01$). Platelet counts were also moderately decreased in animals in the high-dose group, although this was not statistically significant. Of the clinical chemistry parameters, AST, ALT, LDH, and alkaline phosphatase levels were modestly elevated in the high-dose group on day 20 (data not shown). In the mid-dose group, the observed effect on these clinical chemistry parameters was only marginal. In general, the hematology and clinical chemistry parameters were at or

near predose or control levels in the high-dose recovery animals, indicating that most of the observed effects were reversible. Possible treatment-related changes in urinalysis parameters were limited to an increased incidence of positive results for occult blood and protein in the mid- and high-dose groups (data not shown).

Histopathology analysis was conducted on tissues from monkeys in the 21-day study following terminal sacrifice on day 22 and on day 43 in recovery animals. At terminal sacrifice, the main histologic finding related to GTI-2040 treatment was the presence of basophilic granular material in the cytoplasm of epithelial cells of the proximal tubules of the kidneys and reticuloendothelial cells (macrophages and Kupffer cells) in the lymph nodes and liver. The severity of the basophilic granulation in these tissues, observed in all animals treated with GTI-2040, was found to increase in a dose-related manner with a general minimal degree of granulation in the low-dose animals. Associated renal tubular degeneration was found in only a small percentage of the tubules of the mid-dose animals and graded mild and occasionally moderate in the tubules of animals in the high-dose group. In addition to accumulation of granular material, vacuolization was observed in macrophages (lymph nodes) and hepatocytes (liver) of mid- and high-dose animals. In the liver, hyperplasia of Kupffer cells was noted in some animals in the mid- and high-dose groups, likely a consequence of the accumulation of granular material. Basophilic granulation and/or vacuolization were also observed sporadically within macrophages from other organs of high-dose animals and in the choroid plexus of one mid-dose animal. In addition, a dose-related histiocytosis was found in the spleen. The histopathologic alterations observed in the kidneys, liver, spleen, and lymph nodes were associated with macroscopic alterations found in the tissues at necropsy. These included pale kidneys and liver, red foci in the kidneys, occasional enlarged lymph nodes, and mild spleen enlargement. The apparent treatment-related changes in organ weights (Table 3) were also consistent with these findings. In general, the histopathologic alterations in the kidney, liver, spleen, and lymph nodes were either absent or diminished in the recovery animals, indicating that those effects were reversible after treatment termination. Overall minimal and reversible toxicities were noted in monkeys in the 21-day study. The no observed adverse effect dose level for GTI-2040 in monkeys determined from this study was 2 mg/kg/day (24.6 mg/m^2/day).

Pharmacokinetics and tissue distribution

Concentrations of plasma levels of GTI-2040 in the three toxicity studies are profiled in Fig. 2. Plasma levels of

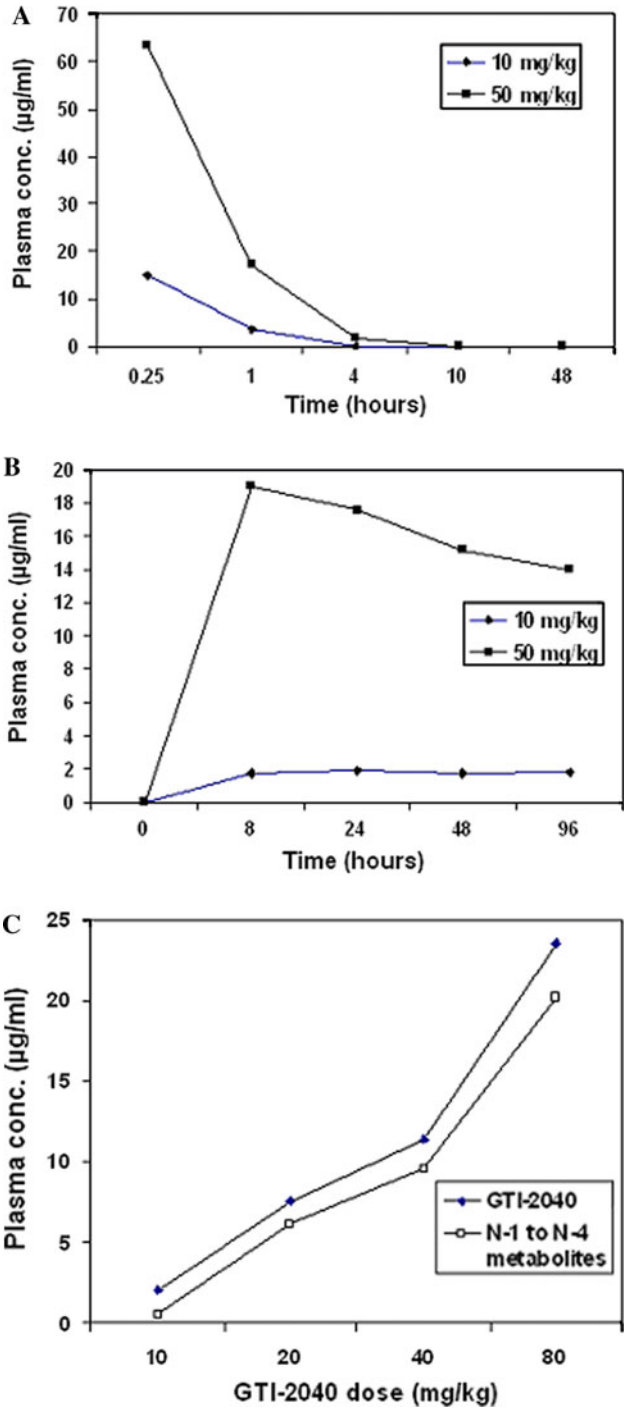

Fig. 2 Pharmacokinetic profiles for GTI-2040 measured in plasma in rats following bolus intravenous injections ever other day for 21 days (**a**) and in monkeys after continuous intravenous infusion for 21 days (**b**). Profiles for 10 and 50 mg/kg dose groups are shown. **c** Concentrations of intact oligonucleotide and total metabolites (N-1 to N-4) in plasma in monkeys in the acute intravenous toxicity study, following administration of single escalating doses of GTI-2040 (10, 20, 40, and 80 mg/kg). Timepoints for blood collection in each study are described in the text

Table 4 Mean pharmacokinetic parameters following iv administration of GTI-2040 at 2, 10, and 50 mg/kg/day by bolus injection every second day up to 21 days (rats) or continuous iv infusion for 21 days (monkeys)

Parameter	Rat			Monkey		
	Dose GTI-2040 (mg/kg/day)			Dose GTI-2040 (mg/kg/day)		
	2	10	50	2	10	50
C_{max} (μg/mL)	3.30	14.0	62.2	–	2.94	23.6
T_{max} (h)	0.25	0.25	0.25	–	325.33	248
K_{el} (1/h)	–	–	0.391	–	–	–
C_{ss} (μg/mL)	–	–	–	–	1.88	14.3
$T_{1/2}$ (h)	–	–	1.77	–	–	–
$AUC_{(0–t)}$ (μg h/mL)	–	–	68.2	–	–	–
$AUC_{(0–inf)}$ (μg h/mL)	–	–	72.7	–	–	–
Cl (mL/h/kg)	–	–	687	–	250	160
Accumulation ratio	–	–	1.90	–	–	–

Plasma GTI-2040 concentrations in rats determined at 0.25, 1, 4, 10, and 48 h after the first injection and at 8, 24, 48, 96 h after start of infusion in monkeys

intact oligonucleotide decreased quickly following iv bolus injection in rats (Fig. 2a). As expected, plasma levels of GTI-2040 and metabolites (N-1 to N-4) in the acute iv study in monkeys increased with escalating doses (Fig. 2c). Pharmacokinetic parameters were determined based on mean profiles of GTI-2040 plasma concentrations in the 21-day rat and monkey studies as part of toxicokinetic evaluations (Table 4). In rats, plasma GTI-2040 concentrations in the 2 mg/kg dose group were only detected at 0.25 h. The plasma half-life for the 50 mg/kg dose group based on the last three concentrations was determined to be 1.77 h. Clearance was calculated to be 687 mL/h/kg but is probably overestimated due to an underestimation of AUC. The accumulation ratio based on the concentration at 4 h after dosing for the 50 mg/kg dose level was 1.90.

In monkeys continuously infused with GTI-2040 for 21 days, the plasma concentrations of the parent oligonucleotide were below the limit of detection at the low-dose level (2 mg/kg/day). Plasma concentrations appeared to reach steady state by 8 h after dosing. At the mid and high doses, the mean C_{ss} levels were 1.88 and 14.3 μg/mL, respectively. Plasma clearance was 250 mL/h/kg for the 10 mg/kg group and 160 mL/h/kg for the 50 mg/kg group (Table 4).

Mean concentrations of GTI-2040 (rat and monkey) and GTI-2040 metabolites (monkey) in the selected tissues of animals treated in the 21-day studies are presented in Fig. 3. In both species, the highest tissue concentrations of GTI-2040 were in the kidney and liver, and the lowest levels were found in the brain, lung, and heart. A similar distribution pattern was observed with GTI-2040 metabolites in monkey tissues. In monkeys, lymph node concentrations of oligonucleotides (not measured in the rat) were relatively low in the 2 and 10 mg/kg/day dose groups but increased significantly in the high-dose group to concentrations comparable to those found in the spleen (Fig. 3b). In general, there were dose-related increases in GTI-2040 tissue concentrations in both species. These increases were less than proportional to dose in tissues containing the highest oligonucleotide concentrations (liver and kidney), approximately proportional to dose in spleen, and higher than proportional to dose in tissues with the lowest oligonucleotide levels (heart, lung, bone marrow, and lymph nodes).

Levels of GTI-2040 metabolites (N-1 to N-4) in monkey plasma were detected in the mid- and high-dose groups and were found to increase proportionately with the infusion dose, similar to the intact parent oligonucleotide (Fig. 3c).

Discussion

Ribonucleotide reductase is a well-recognized target for cancer therapy because of its clear association with malignancy and tumor progression [4]. A number of anticancer agents that target the R1 or R2 subunits of ribonucleotide reductase either have been approved or are being developed as investigational drugs [11]. GTI-2040 is an R2-targeted antisense drug candidate that is currently being tested in clinical trials in solid tumors and acute myeloid leukemia [12–14]. In preclinical studies, GTI-2040 showed strong antitumor activity against a wide range of human tumor xenografts with no apparent toxicity [5]. Here, we present the results of formal toxicity studies on GTI-2040 that were conducted to support initial clinical cancer trials. The treatment schedules for the toxicity studies were based on the dosing regimen used in preclinical efficacy studies, i.e., bolus injections every other day (for the rat study), as

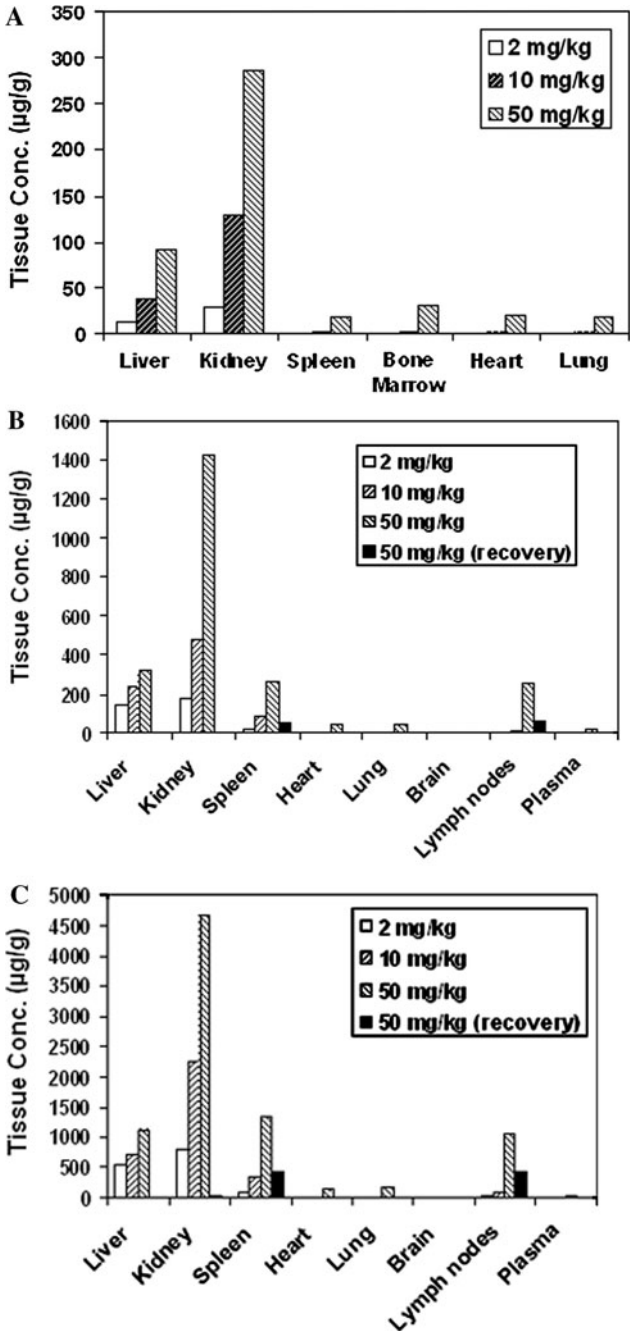

Fig. 3 Biodistribution and concentrations of GTI-2040 in rats and monkeys in the 21-day studies. Values shown are mean concentrations of GTI-2040 (µg/g tissue) from selected organs. Organ biodistribution data were not corrected for oligonucleotide content in blood within tissues. In the rat study (**a**), satellite animals (6 animals/sex) were dosed with GTI-2040 by tail vein injection at 2, 10, and 50 mg/kg/day every other day for 21 days. Tissues were harvested 4 h following the last treatment. Tissue concentrations of intact GTI-2040 (Panel **b**) and total metabolites (N-1 to N-4) (**c**) are shown in Rhesus monkeys treated with GTI-2040 by continuous intravenous infusion for 21 days at doses of 2, 10, and 50 mg/kg/day. Plasma concentrations of GTI-2040 and metabolites (µg/mL) at necropsy are also shown. Oligonucleotide levels were determined on Day 22 (end of infusion) and on Day 43 (end of 21-day recovery for 50 mg/kg group)

well as the 21-day dosing schedule intended for the Phase I trial (rat and monkey studies). An acute toxicity study in monkeys was conducted to assess the preliminary toxicity of escalating doses of GTI-2040 prior to a subchronic, 21-day infusion study. Intravenous infusion of GTI-2040 for 24 h at doses up to 80 mg/kg was well tolerated in rhesus monkeys, with no treatment-related clinical signs or changes in body weight, blood pressure, heart rate, serum chemistry, or hematology parameters. In 21-day toxicity studies, GTI-2040 showed reversible toxicities at the highest-dose level tested (50 mg/kg/day) with essentially no adverse reactions at the low-dose level (2 mg/kg/day) in both rats and monkeys. While some toxic effects were observed at 10 mg/kg/day, those adverse reactions were also reversible on the basis of the diminished effects observed in the recovery animals in the high-dose groups. In both the rat and monkey, there were no apparent gender-specific effects related to the GTI-2040 treatment. These findings are consistent with the adverse events seen with GTI-2040 in clinical trials. In a Phase I dose-escalation trial in patients with advanced solid tumors, toxicity of GTI-2040 administered by continuous intravenous infusion for 21 days every 4 weeks was minimal, with few grade 3 or grade 4 adverse events [12]. The recommended dose of GTI-2040 determined from the Phase I trial (185 mg/m^2/day) was based on reversible hepatic toxicity (elevated hepatic transaminases and bilirubin levels) seen in two patients treated at the next highest dose level [12]. Subsequent trials have shown that GTI-2040 can be administered safely at 185 mg/m^2/day in combination with chemotherapy agents [13, 14].

Except for one male rat in the high-dose group that died on Day 21 of treatment, there were no other animal deaths associated with the GTI-2040 administration. Mice and rats have been shown to be significantly more sensitive to phosphorothioate ODNs when compared to non-human primates. As a result, the toxicity observed at the high dose was not unusual for this class of compounds and morbidity at high doses is not unusual [15]. It is not clear from these studies whether the death was a result of exaggerated immuno-stimulatory effects or the result of hepatic toxicity, as has been suggested for other ODN compounds [6, 8, 15]. In both the acute and 21-day studies in monkeys, there were no treatment-related changes in body weights or food consumption and no abnormalities in the ECG recordings and ophthalmic parameters. By contrast, body weight gain was significantly reduced in rats administered GTI-2040 at the high dose (Table 1). These gross clinical observations indicated that rats were more sensitive to GTI-2040 treatment than monkeys. Since the mRNA target sequence for the R2 component of ribonucleotide reductase is equally conserved in both rats and monkeys, the apparent higher

sensitivity of rats to GTI-2040 treatment was not likely due to interaction with the target mRNA. GTI-2040 contains a CpG dinucleotide motif that is often associated with enhanced immuno-stimulatory effects. The sequence context of the CpG in GTI-2040 is not optimal for either NK cell stimulation (CpG A) or B-cell mitogen (CpG B) in rodents, and as a result immune stimulation was not expected through these mechanisms [16, 17]. Furthermore, the sequence context of the CpG in GTI-2040 is more similar to a canonical human stimulatory CpG motif [16, 17]. However, this is not in an optimal context for immune stimulation in non-human primates, which requires a TpT 3′ to the CpG and a TC at the 5′ end for optimal stimulation [18]. In addition to the sequence-specific CpG-mediated effect, previous studies have shown that immune stimulation is characteristic of the phosphorothioate oligodeoxynucleotide backbone and that rodents are particularly sensitive to this effect [16–19]. In the present study, a significant dose-related increase in spleen weight was found in rats, consistent with immune stimulation (Table 1). While spleen weight was also higher in monkeys in the mid- and high-dose groups (Fig. 3), the effect was more variable and less pronounced than in rats. Histopathological observations in rats indicated a dose-related lymphoid hyperplasia. In rat spleen, this finding was often accompanied by increased hematopoiesis, mainly erythroid, which was possibly related to anemia in some animals. Although histiocytosis was found in the spleen of monkeys in the high-dose group, the concentrations of GTI-2040 in spleen were more than ten times higher in the monkey (Fig. 3b) compared to the rat (Fig. 3a). In rodents, immune stimulation has been manifested as an increase in spleen weight, lymphoid hyperplasia, and multiorgan leukocytic infiltrate [20, 21]. Thus, the apparent higher level of toxic response to GTI-2040 exposure at 50 mg/kg/day in rats compared to monkeys was likely due to a general characteristic of this class of compounds, namely immune stimulation to which rodents have shown a particular sensitivity [22, 23].

In monkeys, exposure to GTI-2040 both at acute and at 21-day treatment regimens produced dose-dependent effects on coagulation, hematologic and hemodynamic parameters characteristic of phosophorothioate oligonucleotides [19, 20, 24, 25]. As shown in Table 2, a small dose-dependent effect on the intrinsic coagulation pathway, prolongation of APTT, was seen with concomitant rise in GTI-2040 plasma concentrations (Fig. 2c). Henry et al. [26] have suggested that the inhibition of coagulation by phosphorothioate oligonucleotides such as GTI-2040 may be due to direct interaction with plasma proteins such as thrombin and other clotting factors. The effect of GTI-2040 exposure on hemodynamic parameters was exemplified by a dose-dependent complement Bb activation that correlated with rising plasma levels of GTI-2040 in both the acute and 21-day studies (Table 2 and data not shown). Like the prolongation of coagulation time, this hemodynamic effect has been observed with other phosophorothioate oligonucleotides and appears to be a typical effect of this class of compounds [26, 27]. The mechanism of this alternative complement pathway activation has been postulated to involve direct interaction of phosphorothioate oligonucleotides with Factor H [24]. Taken together, the coagulation, hematologic and hemodynamic effects observed in monkeys treated with GTI-2040 were typical reactions found with other phosphorothioate oligonucleotides and were not related to an interaction with the mRNA sequence for the R2 component of ribonucleotide reductase.

In both rat and monkey, the highest tissue concentrations of GTI-2040 were found in the kidneys, followed by the liver and spleen, while the lowest levels were in the brain (Fig. 3). In general, the levels of GTI-2040 in tissues increased with escalating dose. In monkey tissues, the increases in the oligonucleotide concentrations were less than proportional to dose suggesting a saturation of those tissues at elevated doses. This apparent tissue saturation with escalating doses was supported in the 21-day monkey study by the calculated plasma clearance of GTI-2040, which was higher at a dose of 10 mg/kg/day (Cl, 250 mL/h/kg) than at 50 mg/kg/day (Cl, 160 mL/h/kg) (Table 4). In tissues such as heart, lung, bone marrow, and lymph nodes, the tissue uptake of GTI-2040, albeit low, was greater than proportional to dose. The findings of the histopathological examinations of the kidney, liver, and spleen of both rats and monkeys were consistent with the high tissue concentrations of GTI-2040. Dose-related findings included hepatic sinusoidal histiocytosis, renal tubular karyomegaly, and periportal mononuclear cell infiltration in the rat and renal tubular degeneration, hyperplasia of Kupffer cells, and accumulation of granular material in the hepatocytes of the monkeys. The basophilic granulation observed in renal proximal tubule epithelial cells and in Kupffer cells has been reported previously with other phosphorothioate oligonucleotides and is believed to represent the accumulation of ODNs [28, 29].

Metabolites of GTI-2040 were found in all tissues that contained intact GTI-2040 as well as in plasma. As with other antisense oligonucleotides, metabolism of GTI-2040 is likely due to nucleases rather than cytochrome P 450-mediated metabolism [30]. Characterization of GTI-2040 metabolites using liquid chromatography tandem mass spectrometry (LC–MS/MS) has shown that GTI-2040 is metabolized progressively from the 3′ end by the 3′–5′-exonuclease Phosphodiesterase I but is highly resistant to 5′–3′-exonuclease [31]. Metabolites of GTI-2040 shortened from the 3′end have been identified in enzyme reactions using a variety of biological matrices in vitro, including mouse liver and kidney homogenates as well as

blood and human liver microsomes [31]. In addition, five GTI-2040 metabolites (3′N-1 to 3′N-5) have been identified in plasma from acute myeloid leukemia patients treated with GTI-2040 by intravenous infusion [31]. Therefore, although the identities of individual metabolites in the present study were not determined, it is likely that they were 3′ chain-shortened oligomers of GTI-2040 generated by 3′ to 5′-exonuclease activity.

The tissue distribution of GTI-2040 in the rat and monkey was typical of other phosphorothioate oligonucleotides [32–34]. An investigation into the cellular distribution of phosphorothioate oligonucleotides in normal rodent tissues indicated that proximal tubule cells in the kidney and Kupffer cells and endothelial cells in the liver were among the most heavily labeled with oligonucleotides [28]. Thus, the accumulation of phosphorothioate oligonucleotides such as GTI-2040 in the kidney and liver and the concomitant reactions observed in those tissues were likely due to a non-sequence-specific mechanism of action [29]. Overall, the treatment-related toxic effects found for GTI-2040 were absent or diminished in the recovery animals of the high-dose groups.

In conclusion, GTI-2040 exposure in rats and monkeys elicited typical phosphorothioate oligonucleotide responses including dose-dependent changes in coagulation time and effects on target tissues such as kidney, liver, and spleen. The adverse reactions in both species were correlated with the plasma and tissue levels of GTI-2040 (and related oligonucleotide metabolites). In general, the observed toxic reactions were reversible on the basis of the diminished or absent effects in the recovery animals. Characteristic of the toxicity seen with this class of drug, monkeys appeared to be less sensitive to GTI-2040 exposure than rats. While the 10 mg/kg/day dose showed some transient effects, the no observed adverse effect level (NOAEL) for GTI-2040 in both species was 2 mg/kg/day. In general, there were no effects that could be attributed to the interaction of GTI-2040 with the R2 mRNA.

Conflict of interest All authors are or have been employees of Lorus Therapeutics Inc., and therefore are receiving or have received salaries and own or have owned stock options and/or shares in the Company.

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