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A Phase I Pharmacodynamic Study of GTI-2040, an Antisense Oligonucleotide Against Ribonuclotide Reductase, in Acute Leukemias: A California Cancer Consortium Study

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Abstract

We performed a phase I study of GTI-2040, an antisense oligonucleotide against ribonucleotide reductase mRNA, on a novel dosing schedule of days 1-4 and 15-18 by continuous infusion to examine efficacy and tolerability in patients with leukemia. A dose of 11 mg/kg/d was safely reached. Dose limiting toxicities at the higher levels included elevated troponin I and liver function enzymes. There were no objective responses to GTI-2040 in this study; 7/24 patients were able to complete the predetermined 3 infusion cycles. Pharmacokinetic and pharmacodynamic studies were performed, indicating a trend towards increasing intracellular drug levels and decreasing RRM2 gene expression with increasing doses. This dose schedule may be considered if appropriate combinations are identified in preclinical studies.

Keywords

Phase I Trial; Acute Leukemia; GTI-2040; Intermittent Infusion; Ribonucleotide Reductase; Pharmacodynamics; Pharmacokinetics

Introduction

New agents for the treatment of AML, particularly AML in the older patient population, are desperately needed. One approach is to more efficiently target pathways whose inhibition with older agents has been shown to have clinical efficacy in AML. Ribonucleotide Reductase (RR) is one such target, a highly regulated enzyme critical for cell proliferation (1); it is responsible for the *de novo* conversion of ribonucleoside diphosphates to deoxyribonucleoside diphosphates, which are essential for DNA synthesis and repair (1, 2). RR consists of two subunits, M1 and M2. M2 is an M_r 88,000 dimer containing a tyrosine

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free radical and a non-heme iron (3). The enzyme is S-phase specific and rate limiting for the synthesis of DNA, and therefore plays an important role in the regulation of cell proliferation (4, 5), and RR is frequently over expressed in tumor cells. RR is a target of multiple agents used in the treatment of cancer, as the M1 component is targeted by a number of chemotherapeutic agents such as cytarabine, gemcitabine, fludarabine and clofarabine (6). Many of these agents have activity against various leukemias, supporting further development of agents against this target.

Hydroxyurea (HU), a drug used commonly in the palliation of AML, is an M2-specific inhibitor of RR; it quenches the tyrosine free radical and, as a result, inactivates the enzymatic activity(5). Currently, HU is used clinically as a chemotherapeutic agent in the treatment of AML, MDS, chronic myelogenous leukemia, cervical cancer, and head and neck cancer (7, 8). However, as a single agent, the utility HU is limited because of its short half-life (1.9-3.9 hour) due to its extremely polar nature resulting in rapid renal excretion. This low potency allows human tumors to rapidly develop resistance (9-13). Overexpression of M2 mRNA and RR protein has been demonstrated to be a mechanism of HU resistance (5, 14). It is well established that RR activity is closely correlated with DNA synthesis and cell proliferation (15, 16). Alterations in the levels of RR can have significant effects on the biological properties of cells such as tumor promotion and tumor progression (17, 18). *In vitro* studies have also shown that aggressive tumor proliferation is associated with amplification of RR gene expression (19, 20). Dysregulation of the expression of the M2 subunit of RR in tumor cells is also capable of modifying the Ras/MAPK pathway (21-25). The human RR M2 (hRRM2) subunit can also act as a tumor promoter, cooperating with a variety of oncogenes to enhance cellular transformation and malignant potential (24, 25). In murine cell lines, signal transduction factors (such as cyclic AMP and protein kinase C) also appear to be involved in the regulation of RR mRNA. GTI-2040 is a 20-mer oligonucleotide that is complementary to the M2 component of human ribonucleotide reductase mRNA (26). Previous agents with anti-RR activity acted via protein inhibition or protein inactivation. In theory, an antisense oligonucleotide with specificity to the M2 target, leading to decreased levels of RRM2, might be more potent than hydroxyurea and with less likelihood of evoking resistance, and may have synergistic activity with inhibitors that act via protein inhibition or inactivation.

The initial phase I trial of GTI-2040 was conducted in 36 patients with advanced solid tumors. GTI-2040 was administered as a 21-day continuous intravenous infusion (CIV) followed by 1 week of rest. Doses ranged from 18.5 mg/m^2/day to 222 mg/m^2/day, with dose-limiting hepatic toxicities and diarrhea experienced at the highest dose level. The recommended phase 2 dose (RP2D) of GTI-2040 on this schedule was 185 mg/m^2/day. No responses were noted in that study (27), and combination studies were launched with that dose schedule.

It is possible that shortening the duration while increasing the daily dose may result in greater single agent activity and better tolerability through more profound inhibition of the target by the antisense oligonucleotide. For this reason, we studied a four day continuous infusion, given on days 1-4 and 15-18, starting with a dose of 5 mg/kg/day, which is approximately equivalent to the 185 mg/m^2 recommended dose established in the earlier

phase I, with dose escalation in the absence of dose-limiting toxicity. Correlative studies utilizing peripheral blasts allowed us correlate dose with biological activity.

Patients and Methods

Patient Selection

Eligible patients were age 18 or over with AML or ALL refractory to primary standard induction therapy; relapsed/refractory acute leukemia; CML in blast crisis at diagnosis or after failing aggressive induction chemotherapy (they could also continue on imatinib); acute leukemia secondary to preexisting hematologic condition or prior chemotherapy were eligible at diagnosis or after failing aggressive induction chemotherapy, advanced myelodysplastic syndrome (Int-1 and above); patients with de-novo acute leukemia (myeloid or nonmyeloid) who were not eligible for aggressive standard induction chemotherapy, and patients above age 60 with de-novo AML or ALL. At least 2 weeks must have elapsed between completion of most recent cytotoxic chemotherapy, or biologic therapy except for hydroxyurea which could be given up to 24 hours prior to treatment and in the interim period between treatments. Patients who had previously received an autologous or allogeneic stem cell transplant were eligible provided that the patient had recovered from transplant associated toxicities and had no active graft versus host disease above grade 2. Standard end organ function criteria were applied, such as direct serum bilirubin 1.5 mg/dl, SGOT and SGPT 3 times the institutional upper limits of normal, and excluding patients with a pretreatment calculated creatinine clearance (absolute value) of less than 60 ml/minute or a serum creatinine of $< 1.5 \times$ upper limit of normal. There were no minimum hematological parameter requirements prior to enrollment, as patients with AML and MDS are understood to have low ANC and platelet counts when the disease is active. Signed informed consent was obtained for all study participants and central registration occurred at the Data Coordinating Center at City of Hope. Protocol and consent forms were approved by the institutional review boards of the participating centers.

Treatment Plan—GTI-2040 was administered as a continuous infusion days 1-4 and days 15-18 on a 28 day cycle. Starting dose was 5 mg/kg/day, escalated by 2 mg/kg/day on a standard 3+3 schedule, with an additional six patients treated at the MTD as described in the Study Plan. The planned dosing levels are presented in Table 1. Patients were permitted to stay on study as long as there were no unacceptable toxicities and there was evidence of clinical benefit by the end of the third cycle. Intra-patient dose escalation was permitted to the next open dose level if CR was not reached in the first cycle and there was no significant toxicity. For subjects with high white blood cell counts after week 1 or 3 of any cycle, hydroxyurea was permitted on the intervening days. Allopurinol was started 24 hours before the first dose of GTI-2040 and continued until at least day 22 of the first cycle or as long as circulating blasts were present. For patients who achieved CR, two further cycles of GTI-2040 could be administered.

Evaluation of Response—Bone marrow aspiration and biopsies were performed as part of the on-study evaluation within two weeks prior to starting therapy. Patients were seen a minimum of once weekly while on study, with peripheral blood counts and chemistry was

monitored at least once weekly. Bone marrow studies were repeated between days 26 to 28 of each cycle. Clinical responses were measured according to International Working Group criteria (28).

Study Design—Patients completed study diaries which were reviewed as part of the toxicity assessment at each clinic visit. Adverse events (AEs) were graded using the NCI common toxicity criteria (CTCAE) version 3.0. Dose limiting toxicities (DLT) were defined as any grade 3 or higher non-hematologic toxicity possibly related to study drug, except for nausea, vomiting and diarrhea controllable by routine palliation, any electrolyte disturbance corrected with supplementation, or any grade 4 hematologic toxicity in the absence of circulating blasts not reversible to grade 3 or less, by 14 days after the end of a cycle, with the exception of platelets, which must return to grade II   50,000/mm^3. In the presence of residual leukemia seen upon aspiration, neutropenia and thrombocytopenia did not count as DLT. Dose escalation proceeded if none of the 3 patients in the cohort had a first cycle DLT. If one of the three patients in a cohort experienced a DLT, three more patients were accrued at that dose level. In the event that a second patient experienced a DLT, that is, 2 or more out of 6 patients, then patients were accrued to the next lower dose level. The maximally tolerated dose (MTD) was defined as the highest tested dose at which no more than one patient out of six experienced a DLT during the first cycle of treatment. Intra patient dose escalation to highest dose open for accrual was allowed if CR was not reached in the first cycle. If PR or clinical improvement was not reached after 3 cycles, patients were categorized as treatment failure and removed from the study.

Pharmacokinetic Studies—The drug level measurements in this study were intended to document the plasma and intracellular concentrations of GTI-2040 during the infusion, as well as to determine whether intracellular drug levels are correlated with plasma concentrations obtained near the end of the infusion. Blood for pharmacokinetic studies were collected in cycle 1 prior to treatment and then prior to the end of the infusion on days 5 and 19. Plasma was separated from whole blood by centrifugation at $1500 \times g$ for 10 minutes and peripheral blood mononuclear cells (PBMC) were isolated using CPT Vacutainer® tubes (Becton Dickenson, Franklin Lakes, NJ). Plasma and intracellular levels of GTI-2040 were measured using a sensitive and specific ELISA assay developed at The Ohio State University as previously described (29).

Molecular Correlative Studies—To explore potential molecular pharmacodynamic biomarkers of response to GTI-2040, serial PBMC samples were also used for determination of RRM2 gene expression. Briefly, relative RRM2 mRNA levels in PBMCs were measured using real-time PCR as previously described (30) and potential associations between RRM2 expression and either administered GTI-2040 dose or plasma and intracellular concentrations were assessed.

Results

A total of 24 eligible patients with AML were enrolled in the study. Demographic data are listed in Table 2. Median age was 71 (range 37-80). Median number of prior regimens was 2,

range 0-9. Nine patients had prior anthracycline-based regimens, 9 patients had prior hypomethylating agents, and 2 patients had prior mylotarg.

Toxicity

No dose limiting toxicities were seen at the first dose levels. At dose level 4 (GTI-2040 11 mg/kg/day), a patient with a preexisting history of angina developed chest pain with troponin I elevations, within the first two days of starting treatment. This dose level was expanded to a total of 8 patients (6 evaluable) and dose escalations proceeded until two DLTs were seen at dose level 6 (15 mg/kg/day), a grade 3 liver function enzyme elevation, and an episode of Grade 3 hypotension. Three patients without a DLT were treated at 13 mg/kg/day and 6 patients were treated at 11 mg/kg/day with one grade 3 toxicity in that cohort, the aforementioned grade 4 troponin. Grade 3-4 treatment-related toxicities are summarized in Table 1. The 13 mg/kg/day dose level was not expanded due to drug supply issues.

Clinical Activity

There were no objective responses within the first 3 cycles of treatment with GTI-2040; nor was there clear evidence of a clinical benefit. Seven of the 24 patients were able to complete 3 full cycles of treatment, which was the defined number of treatment cycles allowed if PR or CR was not achieved, and went on to other treatment options.

Pharmacokinetic Studies

Plasma and PBMC concentrations of GTI-2040 determined on days 5 and 19 were averaged to determine the mean plasma and intracellular drug level in each patient (Table 3). With 6 evaluable patients at the safe dose of 11 mg/kg/day, median end of infusion plasma and PBMC GTI-2040 concentrations were 746 nM and 1.35 pmol/mg protein, respectively. With increasing dose, we observed a significant increase (p=0.001) in plasma concentration (Figure 1a). There was also an associated linear trend (p=0.07) for an increase in intracellular GTI-2040 in PBMCs with dose (Figure 1b), and a significant correlation (p<0.001) between plasma and PBMC concentrations (Figure 1c).

Molecular Correlative Studies

With increasing dose, there was a significant decrease in RRM2 expression in PBMCs (p<0.01) compared to baseline (Figure 2). There was also a trend (not statistically significant) correlating either plasma (p=0.16) and intracellular GTI-2040 levels (p=0.14) with RRM2 expression (data not shown). These data demonstrate that RRM2 was successfully targeted in a GTI-2040 dose-dependent manner, when the entire range of doses is considered. There are insufficient data to conclude that the effect was saturated at the highest doses, although the maximum inhibition observed in any patient was 82%.

Discussion

GTI-2040 is an antisense molecule designed to target the M2 component of ribonucleotide reductase, an important target in AML therapy. Initial preclinical animal studies demonstrated preclinical activity and safety with minimal and reversible toxicities seen only

at very high doses (31). In the original studies, due to the short IV half life of the drug, GTI-2040 was given as a 21 day infusion, with hepatic and GI toxicities seen at the highest doses. The maximum tolerated dose in the original phase I study was 185 mg/m^2/day which approximates 5 mg/kg/day (32). At that dose level, when combined with high dose cytarabine, activity was seen in younger patients with AML, with eight patients achieving complete remission on a six day infusion schedule. Limited activity was seen in similar dose schedules in renal cell carcinoma when combined with capecitabine (33), prostate cancer when combined with docetaxel/prednisone (34), lung cancer when combined with docetaxel (35), or in a phase I study of solid tumors when combined with oxaliplatin/capecitabine (36). In theory, GTI-2040, as an oligonucleotide complementary to the R2 subunit of RR, should show enhanced suppression of RR translation based on achievable concentration until saturation is reached. This study tested the hypothesis that a shorter duration of infusion would allow higher doses to be achieved and thus achieve potentially better inhibition of the enzyme. If increased dose of the drug at this schedule could show enhanced diminution of RR activity, then perhaps efficacy of single agent or combination therapy might be enhanced. In order to test this hypothesis, pharmacologic studies including plasma and intracellular levels of drug, as well as pharmacodynamic studies of inhibition of the target in vivo were performed. Previous studies using agents that act against the M1 or RR by either protein inhibition or inactivation have gauged the efficacy of the agent by studying downstream effects, in particular DNTP pools (37, 38). As GTI-2040 is an antisense against the M2 component, we studied levels through real time PCR in order to quantitate the direct effects on RRM2 protein levels in correlation with plasma and intracellular GTI-2040 levels. While GTI-2040 has the ability to target RRM2 in a dose-dependent fashion, the highest dose obtained without an unacceptable level of dose-limiting toxicity did not decisively reduce RRM2 (e.g. 10% or less of pre-treatment expression) despite the intermittent schedule designed to deliver a higher dose.

In this dosing schedule as a single agent, we were able to attain levels of 13 mg/kg/day for 5 day infusions every two weeks. Interestingly, the total administered dose per cycle on the original 21 day continuous infusion schedule is very similar to the total administered at the 13 mg/kg/day dose level (105 mg/kg vs 104 mg/kg). At 15 mg/kg/day, grade 3 toxicities of increased AST/ALT and an episode of hypotension was seen. RRM2 mRNA levels were seen to decrease with dose in a linear fashion, with insufficient data to know if there is a plateau after 11 mg/kg/day. At these doses of GTI-2040, there was still no single agent activity against AML in this primarily older patient population. This lack of response in this patient population is supported by a parallel phase I study in which GTI-2040 administered from doses of 3.5-7 mg/kg/d over 6 days combined with high dose cytarabine failed to show any formal responses in older patients. While there were responses in the initial study involving younger patients, it is plausible that the physiologic role of RRM2 differs in AML involving older patients, as suggested by Klisovic, et al (39,40), and it is also possible that the single agent activity may prove discouraging even in younger populations.

In summary, higher daily doses of GTI-2040 are feasible and safe when delivered over the shorter infusion period of 5 days every other week. Furthermore, pharmacokinetic and pharmacodynamic data demonstrate there is a GTI-2040 dose-dependent increase in intracellular drug concentrations and a decrease in RRM2 gene expression within the range

of safely administered doses. Therefore, the combined results of the current study suggest that higher intermittent daily doses may be preferable to lower continuous exposures. While GTI-2040 as a single agent is not likely to be a meaningful clinical approach for older patients with AML, combination studies using other agents along with increased daily doses of GTI-2040 would be valid for disease states in which inhibition of RR-M2 may be efficacious, as single agent or in combination with other therapies.

Acknowledgments

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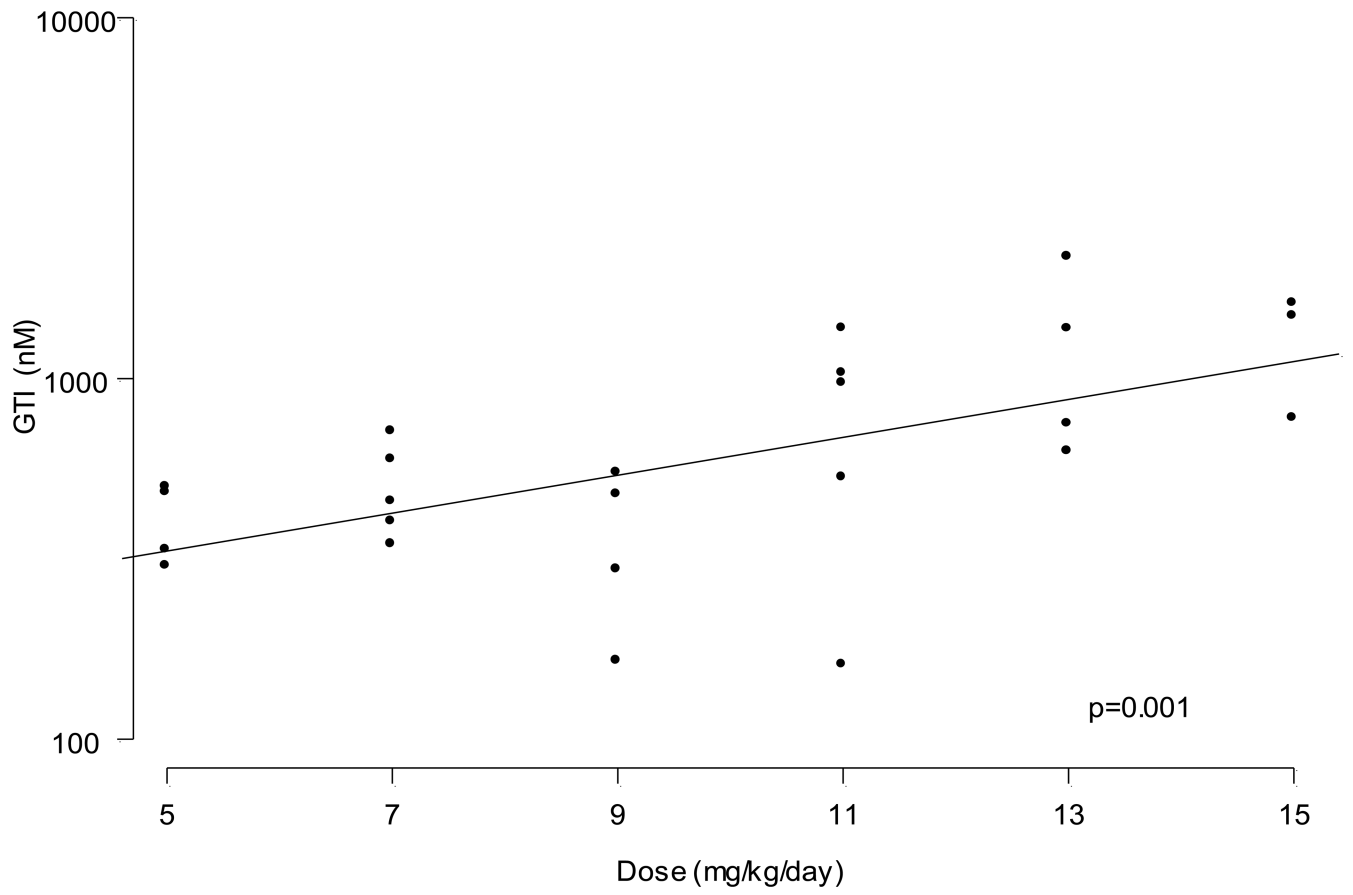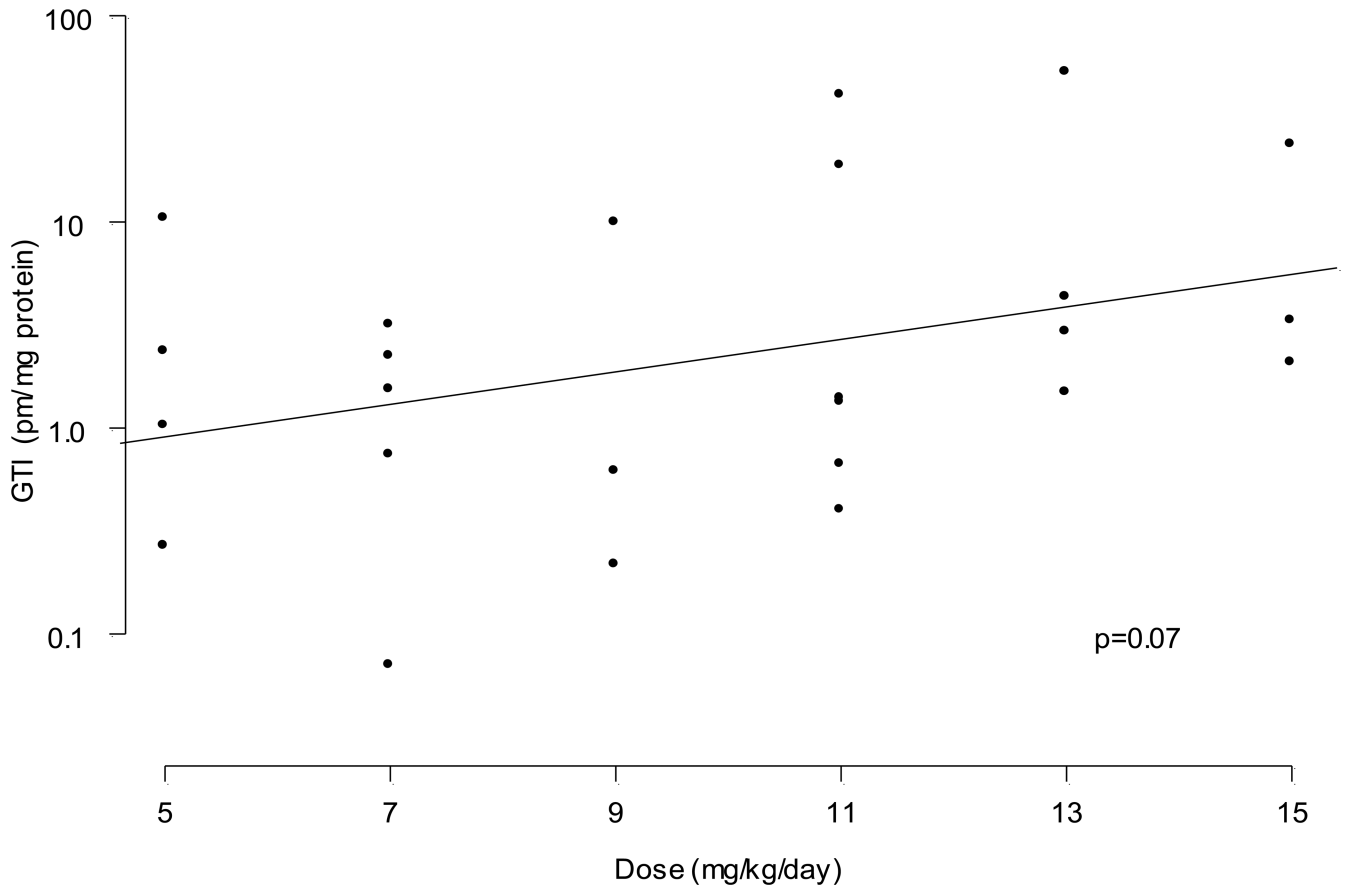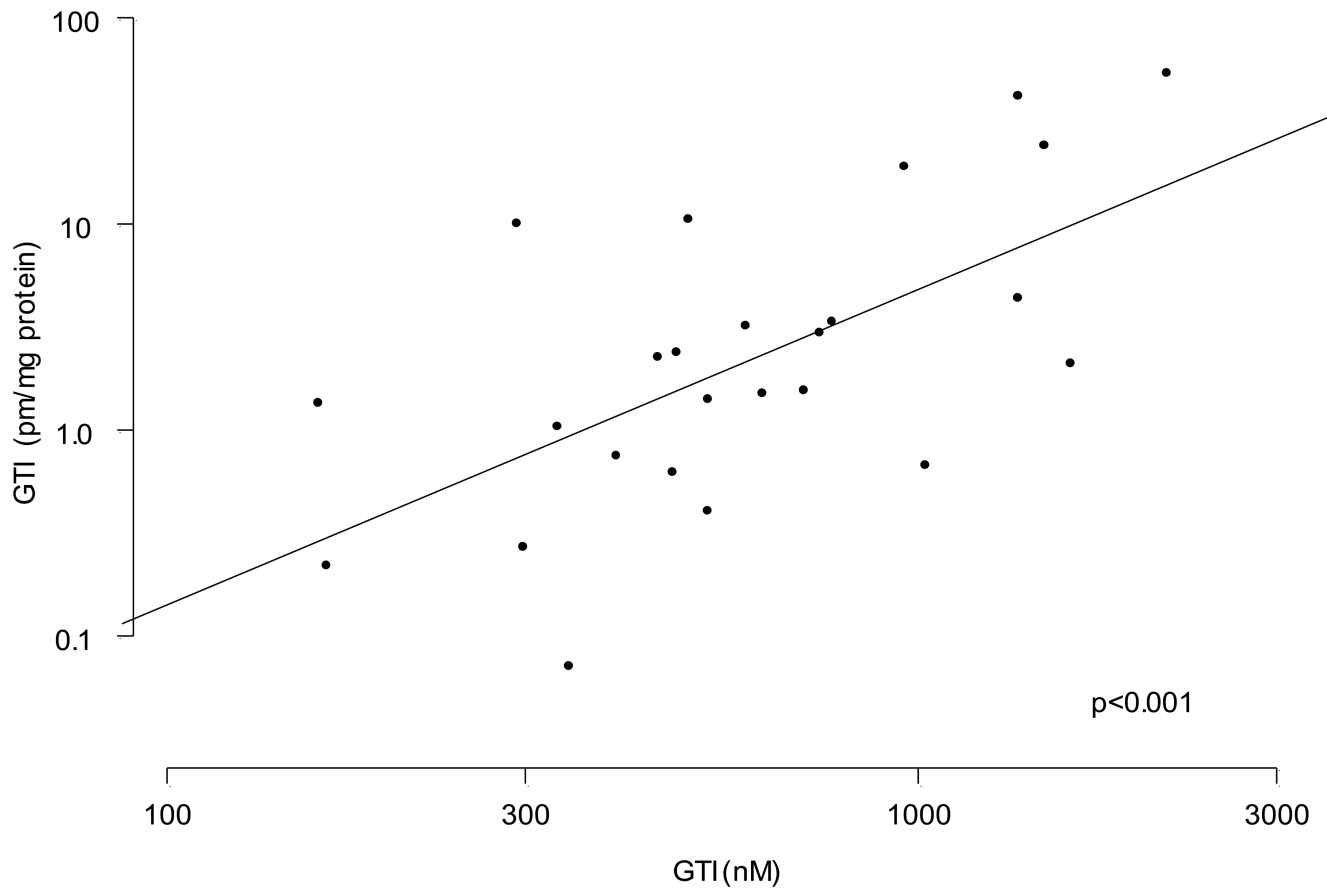
Figure 1.
Correlations of GTI-2040 Dose, Plasma Concentration, and Intra-cellular Concentration.
Figure 1a, GTI-2040 end-of-infusion plasma concentrations as a function of dose. Figure 1b,
intracellular GTI-2040 levels as a function of dose. Figure 1c, intracellular GTI-2040 levels
as a function of end-of-infusion GTI-2040 plasma concentrations.

Figure 2.
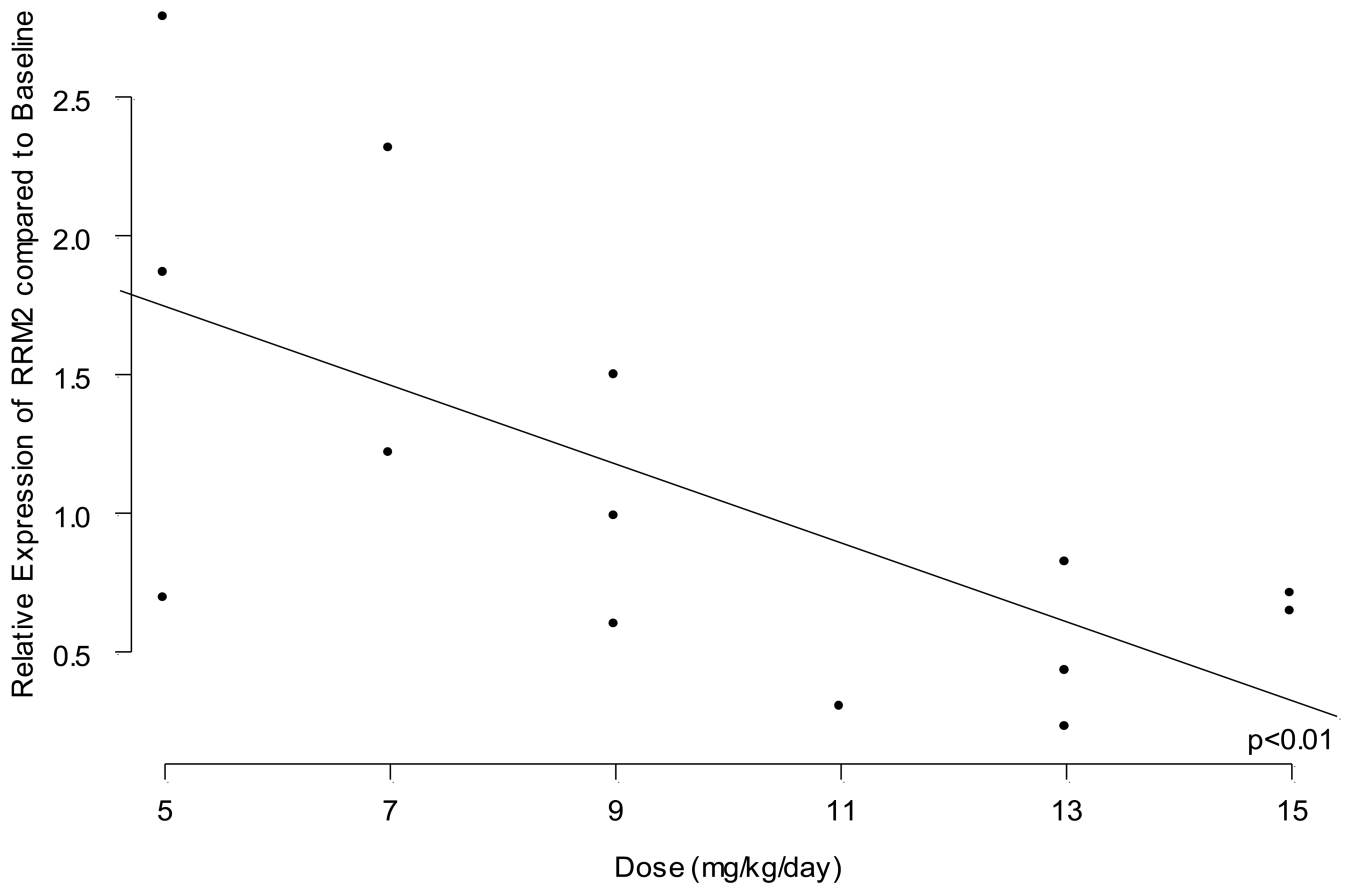Correlation of Ribonucleotide Reductase Subunit M2 levels (RRM2) with GTI-2040 Dose.

Table 1

Summary of Grade 3 and 4 Toxicities with Possible/Probably/Definite Attribution by Dose Level

Adverse Event	Dose Level											
	5 mg/kg/day (n=3)		7 mg/kg/day (n=3)		9 mg/kg/day (n=3)		11 mg/kg/day (n=8)		13 mg/kg/day (n=4)		15 mg/kg/day (n=3)	
	Gr 3	Gr 4	Gr 3	Gr 4	Gr 3	Gr 4	Gr 3	Gr 4	Gr 3	Gr 4	Gr 3	Gr 4
Cardiac ischemia / infarction / troponin								1(13%)				
Fatigue							1(13%)					
Hemoglobin					1(33%)				1(25%)		1(33%)	1(33%)
Hypotension											1(33%)	
Infection (pneumonia)											1(33%)	
Leukocytes											1(33%)	
Nausea	1(33%)											
Neutrophils/granulocytes (ANC/AGC)												1(33%)
Phosphate, serum-low											1(33%)	
Platelets					1(33%)					2(50%)		2(67%)
Somnolence/depressed level of consciousness							1(13%)					
Transaminases (elevated ALT and/or AST)											2(67%)	

Table 2

Patient demographic and clinical characteristics

Patient Characteristic	GTI-2040 (n=24)
Gender, *n (%)*	
Male	9 (38)
Female	15 (62)
Age, years	
Median (Range)	70.7 (36.8 – 80.1)
Race, *n*	
Caucasian	24
Ethnicity, *n (%)*	
Hispanic	3 (12)
Non-Hispanic or Unknown	21 (88)
Histology, *n (%)*	
Acute megakaryolblastic leukemia	3 (12)
Acute myeloid leukemia	13 (54)
Myelodysplastic syndrome	1 (4)
Precursor B-cell lymphoblastic leukemia	1 (4)
Precursor cell lymphoblastic leukemia	5 (21)
Therapy-related acute myeloid leukemia	1 (4)
Prior treatments received (alone, or in combination), *n (%)*	
Radiation	1 (4)
Allogeneic transplant	1 (4)
Prior drug therapy	21 (88)
Number of Regimens: Median (range)	2 (0-9)
Prior anthracycline	9 (38)
Prior hypomethylation agents	9 (38)
Prior mylotarg	2 (8)
Karnofsky performance status, *n (%)*	
70%	2 (8)
80%	10 (42)
90%	9 (38)
100%	3 (12)
Off treatment reason, *n (%)*	
Progression, resistant disease or early death	21 (88)
Toxicity	1 (4)
Patient refusal	1 (4)
Overall poor condition	1 (4)

Table 3

GTI-2040 Pharmacokinetic summary

Dose Level (mg/kg/day)	Plasma Css[*]- (nM)	PBMC Css[*][^] (pmol/mg prot.)
5	N=4 407 (300, 498)	N=4 1.68 (0.27,10.32)
7	N=5 453 (345, 709)	N=5 1.52 (0.07, 3.14)
9	N=4 384 (164, 545)	N=3 0.61 (0.22, 9.83)
11	N=6 746 (160, 1368)	N=6 1.35 (0.40, 40.81)
13	N=4 1055 (624, 2157)	N=4 3.59 (1.48, 52.80)
15	N=3 1481 (772, 1606)	N=3 3.29 (2.06, 23.53)

[*] Css – mean end-of-infusion value days 5 and 19 in each patient

[^] Median (range)