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Phase I Study of GTI-2040, an Antisense to Ribonucleotide Reductase, in Combination with High-Dose Cytarabine in Patients with Acute Myeloid Leukemia

Rebecca B. Klisovic¹, William Blum^{1,3}, Xiaohui Wei², Shujun Liu¹, Zhongfa Liu², Zhiliang Xie², Tamara Vukosavljevic¹, Cheryl Kefauver³, LeNguyen Huynh¹, Jiuxia Pang¹, James A. Zwiebel⁴, Steven Devine^{1,3}, John C. Byrd^{1,3}, Michael R. Grever^{1,3}, Kenneth Chan^{1,2}, and Guido Marcucci^{1,3}

¹ Division of Hematology and Oncology, Department of Medicine, The Ohio State University, Columbus, Ohio ² Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, Ohio ³ The Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio ⁴ Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland

Abstract

Purpose—Inhibition of ribonucleotide reductase reduces the availability of the endogenous pool of deoxycytidine and may increase cytarabine (AraC) cytotoxicity. We performed a phase I dose escalation trial of AraC combined with GTI-2040, a 20-mer antisense oligonucleotide shown in preclinical studies to decrease levels of the R2 subunit of ribonucleotide reductase, to determine the maximum tolerated dose in adults with relapsed/refractory acute myeloid leukemia.

Experimental Design—Twenty-three adults (ages 18–59 years) were enrolled in this dose escalation phase I trial, receiving high-dose AraC twice daily combined with infusional GTI-2040. An ELISA-based assay measured plasma and intracellular concentrations of GTI-2040. R2 protein changes were evaluated by immunoblotting in pretreatment and post-treatment bone marrow samples.

Results—The maximum tolerated dose was 5 mg/kg/d GTI-2040 (days 1–6) and 3 g/m²/dose AraC every 12 hours for 8 doses. Neurotoxicity was dose limiting. Eight patients (35%) achieved complete remission. Mean bone marrow intracellular concentration of GTI-2040 were higher at 120 hours than at 24 hours from the start of GTI-2040 ($P = 0.002$), suggesting intracellular drug accumulation over time. Reductions in bone marrow levels of R2 protein (>50%) were observed at 24 and 120 hours. Higher baseline R2 protein expression ($P = 0.03$) and reductions after 24 hours of GTI-2040 ($P = 0.04$) were associated with complete remission.

Conclusions—GTI-2040 and high-dose AraC were coadministered safely with successful reduction of the intended R2 target and encouraging clinical results. The clinical efficacy of this combination will be tested in an upcoming phase II study.

Nucleoside analogues constitute the backbone of several primary and salvage chemotherapy regimens for acute myeloid leukemia (AML; refs. 1,2). By mimicking endogenous

Requests for reprints: Guido Marcucci, Division of Hematology and Oncology, Department of Medicine, The Ohio State University, A437A Starling-Loving Hall, 320 West 10th Avenue, Columbus, OH 43210. Phone: 614-293-5738; Fax: 614-293-7525; guido.marcucci@osumc.edu.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

nucleosides, these compounds are incorporated into newly synthesized DNA and induce inhibition and chain termination of newly synthesized nucleic acid, thereby leading to apoptosis (3). Among pyrimidine analogues, cytarabine (AraC) differs from the endogenous cytidine counterpart by the presence of an arabinoside rather than a ribose sugar. This compound undergoes enzymatic phosphorylation into the active metabolite Ara-CTP before being incorporated in newly synthesized DNA. A direct correlation between intracellular levels of Ara-CTP and antileukemic effect has been identified, and different strategies to increase levels of the active metabolite have been investigated (4–7).

Inhibition of the ribonucleotide reductase (RNR) has the potential to increase AraC cytotoxicity. Composed of two subunits, R1 and R2, RNR is required for the reductive conversion of ribonucleotides to deoxynucleotides, a crucial rate-limiting step during DNA synthesis and repair (8,9). Overexpression of RNR, commonly found in malignant cells, increases the endogenous pool of deoxynucleoside triphosphates, a potential mechanism of chemoresistance to nucleoside analogues competing for DNA incorporation (10–15).

Antisense oligodeoxynucleotides are short DNA molecules that can interfere with gene expression by forming duplexes with complementary sequences of target mRNAs (16–18). GTI-2040 is a 20-mer phosphorothioate oligonucleotide antisense that is complementary to the R2 component of RNR. Thus, we hypothesized that treatment with GTI-2040 induces down-regulation of RNR and, when combined with high-dose AraC (HiDAC), results in enhanced AraC cytotoxicity by creating an imbalance in dCTP pools that favors DNA incorporation of Ara-CTP. To test this hypothesis, we conducted a phase I study of GTI-2040 in combination with HiDAC in AML. The primary study objectives were to describe the safety and tolerability of the regimen and validate R2 as an *in vivo* target for GTI-2040 in AML.

Patients and Methods

Eligibility criteria and study design

This was a National Cancer Institute/Cancer Therapy Evaluation Program–sponsored phase I dose escalation study of patients with refractory/relapsed AML ages 18 to 59 years. Performance status of ≤ 2 , left ventricular ejection fraction $\geq 50\%$, adequate hepatic (total bilirubin < 2 times the upper limit of normal and aspartate amino transferase/alanine amino transferase < 3 times the upper limit of normal), and adequate renal function (creatinine < 1.5 mg/dL) were required unless laboratory abnormalities were attributed to underlying disease. The protocol was reviewed and approved by the local institutional review board. In accordance with the Declaration of Helsinki, informed consent was obtained from all patients before study entry.

The dose escalation schema is detailed in Table 1. The starting dose of GTI-2040 was selected to be approximately 80% of the total dose of GTI-2040 administered safely during single agent solid tumor phase I studies (19). GTI-2040 infusion was followed by initiation of HiDAC to induce down-regulation of RNR before AraC exposure. We hypothesized that down-regulation of RNR could result in depletion of endogenous deoxyribonucleotides and preferential incorporation of AraC in the newly synthesized DNA of proliferating malignant blasts, thereby causing a high degree of cytotoxicity. The starting dose of GTI-2040 was chosen due to concern for potential added toxicity when combined with HiDAC. GTI-2040 was initially administered as a continuous i.v. infusion at 3.5 mg/kg/d (days 1–7), and AraC was infused over 2 h every 12 h at 2 g/m²/dose on days 2 to 7. The dose escalation schema was subsequently amended after one grade 3 neurotoxicity was seen on level 1 (Table 1).

All patients underwent neurologic examinations before each dose of AraC. Myeloid growth factors were allowed per American Society of Clinical Oncology guidelines (20). Adverse events were graded according to National Cancer Institute Common Toxicity Criteria (version 3.0, http://ctep.cancer.gov/reporting/ctc_v30.html). Dose-limiting toxicity was defined as grade 3 or 4 reversible nonhematologic toxicity or grade 3 or 4 hematologic toxicity at day 42 in patients with <5% blasts in the bone marrow attributable to GTI-2040. The dose level with two or more dose-limiting toxicities was defined as the maximally administered dose. The maximum tolerated dose (MTD) was defined as the dose level below the maximally administered dose. Disease response was defined according to published National Cancer Institute criteria (21).

Analysis of plasma and intracellular levels of GTI-2040

Levels of GTI-2040 in plasma and lysates from bone marrow mononuclear cells (MNC) were measured using a novel hybridization-ligation-based ELISA assay developed and validated in our laboratory and shown to have a lower limit of quantification of 50 pmol/L and linearity from 50 to 10,000 pmol/L for GTI-2040 (22,23). Briefly, a capture oligodeoxynucleotide (5'-TAACTAGTGCTTGGTGGAGCGATTTAGCC-3' Biotin-3') was diluted in buffer [60 mmol/L phosphate buffer (pH 7.4), 1.0 mol/L NaCl, 5 mmol/L EDTA, and 0.2% Tween 20], heated at 95°C for 5 min, and mixed with sample. This was incubated at 42°C for 2 h, captured by binding to NeutrAvidin-coated 96-well plates (Pierce), and subsequently washed with buffer (TBS in 0.1% Tween 20). A detection oligodeoxynucleotide probe (5'-CACTAGTTA-3') with phosphate at the 5'-end and digoxigenin at the 3'-end was diluted in ligation buffer [66 mmol/L Tris-HCl (pH 7.6), 10 mmol/L MgCl₂, 10 mmol/L DTT, 1 mmol/L ATP] containing 5 units/mL T4 DNA ligase (Amersham Biosciences) and dispensed into wells followed by overnight incubation at 18°C. To remove excess probe bound to capture oligodeoxynucleotide, 60 units S1 nuclease (Invitrogen) in 3 mmol/L sodium acetate (pH 4.6), 1 mmol/L zinc acetate, 100 mmol/L NaCl, and 5% glycerol was added and incubated with samples for 2 h at 37°C. The plate was then washed with buffer. Next, anti-digoxigenin-alkaline phosphatase (1:2,500 with bovine serum albumin block buffer in TBS; Roche) was added. Following 30-min incubation at room temperature, the plate was washed with washing buffer. Attophos substrate (Promega) in diethanolamine buffer prepared as recommended by the manufacturer was added. Fluorescence intensity was measured at excitation 430/emission 560 (filter = 550 nm) using a Gemini XS plate reader (Molecular Devices).

For pharmacokinetic analysis, plasma samples were collected before treatment; at 2, 4, 6, 12, 24, 48, and 72 h from beginning of GTI-2040 infusion; at end of GTI-2040 infusion; and at 0.25, 0.5, 1, 2, 4, 6, 12, 24, and 48 h after drug discontinuation. WinNonlin (version 4.0; Pharsight) was used to fit the plasma concentration-time profiles and calculate the pharmacokinetic variables with appropriate model and model-independent methods.

For quantification of intracellular concentrations, bone marrow samples were collected 24 and 120 h following initiation of the GTI-2040. When adequate cell numbers and immunophenotype were allowed, CD34⁺ bone marrow MNCs were isolated using MACS CD34 selection columns (Miltenyi Biotec) according to the manufacturer. The effluent cells were ≥95% CD34⁺ cells using this approach as assessed by flow cytometry using FITC-conjugated CD34 antibody (Becton Dickinson) and the appropriate isotype-negative control. Pellets of bone marrow MNCs were treated with 0.1 μmol/L phosphorothioate 28-mer polycytidine. After addition of lysis buffer [10 mmol/L Tris-HCl (pH 8.5), 0.5 mmol/L EDTA, and 1% Triton X-100] and incubation on ice for 10 min, the cells were mechanically lysed. The homogenate was centrifuged, and supernatant was used in assays. The measured GTI-2040 levels were converted to intracellular concentration by dividing the GTI-2040 amount by the MNC volume as determined by Samba Image Analyzer 4000 (Imaging

Products International). The conversion factor was calculated as 0.035 μg protein equal to 1 μL cell volume or 2×10^6 cell number equal to 1 μL cell volume. Results were similar when instead of the cell volume the measured levels of GTI-2040 in protein lysates were normalized to total protein.

To separate cytoplasm from nucleus in subcellular compartment intracellular concentration analyses, viable bone marrow MNCs were incubated with 0.1 $\mu\text{mol/L}$ PS-dC28 for 2 min on ice and washed again with PBS. Nuclease preparation kit (Molecular Motif) was used to separate cytoplasm and nucleus according to the manufacturer's instruction. Lactate dehydrogenase concentration was measured in the nucleus fraction and in cytoplasm fraction to test for contamination of cytoplasm in the nucleus.

Quantification of R2 expression

For protein analysis, lysates of bone marrow MNCs before treatment, 24 h, and 120 h following GTI-2040 initiation were prepared in cell lysis buffer (Cell Signaling Technology). Standard SDS-PAGE was done as described previously (24). Blots were probed for R2 (Santa Cruz Biotechnology) and glyceraldehyde-3-phosphate dehydrogenase (Santa Cruz Biotechnology). Immunoblots were scanned and quantified using ImageQuant software (Molecular Dynamics), and R2 protein levels were normalized to glyceraldehyde-3-phosphate dehydrogenase.

Statistical considerations

Descriptive statistics (mean, SD, and frequency) were computed for all variables. When large variability in the biological samples was noted, median values were calculated. Groups were compared using Mann-Whitney *U* tests and ANOVA for continuous data. Paired data were analyzed using the Wilcoxon matched-pairs signed rank test.

Results

Patient characteristics

Twenty-three patients with AML were enrolled. Demographic and pretreatment features are detailed in Table 2. Twelve patients had refractory disease, defined as disease unresponsive to initial chemotherapy, and 11 had relapsed disease, defined as disease that recurs after treatment with conventional chemotherapy. Prior complete remission (CR) durations for 6 of the 11 relapsed patients were less than 6 months (median CR duration, 5.2 months; range, 1–18 months). Thirteen patients had intermediate-risk and 10 had poor-risk cytogenetics according to the Cancer and Leukemia Group B classification for overall survival (25). Ten had received prior HiDAC and 3 patients had undergone autologous stem cell transplants (SCT).

Toxicity

Compiled grades 3 and 4 nonhematologic toxicities observed are listed in Table 3. Three patients (one in dose level 1 and two in dose level 6A) developed ataxia and dysarthria consistent with previously described HiDAC toxicity (26–28). Symptoms were reversible within 48 hours after treatment discontinuation. Because the first neurotoxicity event was observed at dose level 1, the protocol was amended to allow a more gradual dose escalation compared with the original schema. Two additional neurotoxicity events deemed to be dose-limiting toxicity were observed at dose level 6A. The MTD, defined as the doses of the combination administered one level (that is, 5A) below that of maximally administered dose (that is, 6A), was therefore declared to be GTI-2040 at 5 mg/kg/d for 6 days combined with 3 g/m²/dose AraC administered every 12 hours beginning on day 2 for eight doses. Overall, the incidence of central nervous system toxicity (13%) in this trial was not dissimilar from

that reported with HiDAC alone (26–28). One death occurred during the first 30 days due to *Enterobacter* sepsis on day 13. This death was not considered a dose-limiting toxicity but attributed to the underlying disease and neutropenia. Other toxicities were reversible and observed previously with HiDAC alone. For patients who achieved CR, the median time to neutrophil recovery (neutrophils $\geq 1 \times 10^9/\text{L}$) was 32 days (range, 22–41 days), and the median time to platelet recovery (platelets $\geq 50 \times 10^9/\text{L}$) was 32 days (range, 19–47 days).

Disease response

Of the 23 patients enrolled, 8 patients achieved CR. Details of the responding patients are highlighted in Table 4. Of the 8 responders, 3 occurred in primary refractory AML and 5 in relapsed AML. Of the 18 patients with refractory disease or first CR durations of less than 6 months, 4 patients achieved CR. Four patients achieving CR had unfavorable cytogenetics. Three responding patients had received prior HiDAC as consolidation therapy. Five of the 8 responders were successfully able to proceed to myeloablative allogeneic SCT. Reasons for not proceeding to allogeneic SCT in other responders included relapse before SCT could be done ($n = 1$, relapse at 1 month), disseminated fungal infection ($n = 1$, relapse at 3 months), and no donor available ($n = 1$, relapse at 2 months). An additional 2 patients had significant cytoreduction (5–10% bone marrow blasts after treatment without blood count recovery), allowing them to proceed to myeloablative allogeneic SCT.

Pharmacokinetic studies

Pharmacokinetic profiles were monitored in 22 of 23 patients. Plots of mean GTI-2040 plasma concentration-time profiles are shown in Fig. 1A. Steady-state concentrations (C_{ss}) were achieved within 4 hours and remained stable until the end of continuous i.v. infusion. GTI-2040 was measurable up to 48 hours postinfusion and a biexponential GTI-2040 plasma concentration-time profile was observed (Fig. 1A). The pharmacokinetic profiles were fitted to a two-compartment infusion model and the relevant variables were computed with the exception of 2 patients in the 3.5 mg/kg/d group, where limited blood sampling only allowed for one-compartment modeling (Table 5). When pharmacokinetic variables were then pooled according to the antisense dose, there were no significant differences in dose-normalized pharmacokinetic variables (dose-normalized area under concentration-time curve, plasma steady-state concentration, and plasma elimination half-life) among different dose levels or between CR and nonresponder patients (data not shown).

Because target down-regulation and subsequent clinical benefit may depend on achievement of robust intracellular drug levels, we examined GTI-2040 uptake by measuring intracellular concentrations in unselected bone marrow collected from treated patients at 24 and 120 hours following initiation of GTI-2040. The median intracellular concentration of GTI-2040 in bone marrow was 24 nmol/L ($n = 14$ patients; range, 0.6–36 nmol/L) at 24 hours and 59 nmol/L ($n = 14$ patients; range, 5–576 nmol/L) at 120 hours. Bone marrow intracellular concentrations at 120 hours were significantly higher than intracellular concentrations at 24 hours of drug exposure (Wilcoxon matched-pairs signed rank test, $P = 0.002$), suggesting intracellular accumulation of the drug over time (Fig. 1B). There was no significant difference in GTI-2040 intracellular concentration between CR and nonresponder patients (median intracellular concentration, 56.9 nmol/L in CR versus 55.1 nmol/L in nonresponders; $P = 0.78$) at 120 hours (data not shown).

From four patients with CD34⁺ leukemic blasts and adequate cell numbers, paired CD34⁺ selected and unselected bone marrow samples were collected and compared. At 120 hours, there was a trend for higher intracellular concentration of GTI-2040 in the CD34⁺ bone marrow MNCs compared with nonselected bone marrow MNCs ($P = 0.20$). The median value of GTI-2040 intracellular concentration in CD34⁺ MNCs was 61 nmol/L (range, 9–

118 nmol/L) versus 29 nmol/L (range, 3–36 nmol/L) in unmanipulated MNCs, suggesting a preferential uptake of the antisense by the leukemic cells (data not shown).

Given our previous findings that the efficacy of antisense compounds directly correlates with high nuclear to cytoplasmic ratio of intracellular concentration (22), subcellular distributions of GTI-2040 in cytoplasm and nucleus were examined in bone marrow cells obtained from eight patients. Cytoplasm to nucleus contamination was found to be minimal during the cell fractionation procedure with <10% of the lactate dehydrogenase content found in the nucleus fraction. In seven patients, median intracellular concentration in the nucleus was 3.8 nmol/L (range, 1.0–9.2 nmol/L) and the median intracellular concentration in cytoplasm was 2.5 nmol/L (range, 0.20–19.2 nmol/L). As shown in Fig. 1C, the ratio of nuclear to cytoplasmic intracellular concentration GTI-2040 was greater in patients achieving CR ($n = 3$; mean, 2.9; range, 2.1–5.0) compared with nonresponders ($n = 5$; mean, 0.69; range, 0.28–3.6; $P = 0.07$), suggesting a trend for association of CR with GTI-2040 nuclear localization.

Pharmacodynamic studies

To evaluate changes in the R2 protein target, immunoblotting was done on bone marrow samples collected before treatment and at 24 and 120 hours after initiation of GTI-2040 exposure. Fourteen patients had samples assessable for these time points. A >50% reduction in R2 protein at 24 and 120 hours was seen in two and five patients, respectively. When comparing CR patients ($n = 6$) and nonresponder patients ($n = 8$), the former had a significantly higher baseline R2 protein level than the latter (median, 0.99 versus 0.32 R2/glyceraldehyde-3-phosphate dehydrogenase arbitrary units; $P = 0.03$; Fig. 1D). Furthermore, CR patients had reductions in R2 protein at 24 hours compared with increased levels in nonresponder patients (median, 88% versus 191%; $P = 0.04$) and 120 hours (median, 48% versus 157%; $P = 0.11$). These data suggest an association of higher pretreatment levels of R2 and greater target down-regulation with achievement of clinical response.

Discussion

In the current study, we report the first trial in refractory/relapsed AML with combination of GTI-2040 and HiDAC. This study was based on the rationale that the cytotoxic activity of AraC is enhanced by GTI-2040-induced RNR down-regulation and that the combination of GTI-2040 and AraC may overcome RNR-mediated chemoresistance. The results of the present phase I dose escalating trial show that this combination can be administered safely with a toxicity profile similar to that observed with HiDAC alone. The MTD of this combination was declared to be 5 mg/kg/d GTI-2040 on days 1 to 6 and 3 g/m²/dose AraC every 12 hours for eight doses in patients ages <60 years. Reversible cerebellar toxicity occurred in 3 of 23 (13%) patients. Because this incidence is similar to that reported previously for HiDAC (10–18%; refs. 26–28), the contribution of the antisense to this toxicity remains difficult to assess.

Although clinical response was not a primary endpoint of this trial, we noted encouraging results as 8 of the 23 (35%) patients treated achieved CR. Notably, this population included unfavorable patients with at least one of the following adverse characteristics: primary refractory disease, short CR duration, unfavorable cytogenetics, and prior HiDAC exposure. Seven responders (five with CR and two with significant cytoreduction) were able to successfully undergo myeloablative allogeneic SCT, suggesting that it is feasible to use this regimen as a salvage strategy without additional toxicity and/or preclusion to myeloablative regimens. The clinical response to this regimen and the ability of patients to proceed safely with allogeneic SCT is being tested definitively in a phase II trial.

Extensive pharmacokinetic and pharmacodynamic analyses were conducted in parallel with the clinical trial. Interestingly, no difference in intracellular concentrations between CR and nonresponder patients was noted, underscoring that optimal pharmacologic activity of antisense compounds may not depend only on absolute intracellular concentrations. It is possible, instead, that efficient antisense target down-regulation may also relate to drug levels achievable in distinct subcellular compartments (that is, nucleus versus cytoplasm; ref. 22). In support of this hypothesis, we showed a trend for higher antisense localization in the nuclear compartment in CR patients was observed. Future studies measuring intracellular concentrations in these different subcellular fractions to identify specific patterns of intracellular distribution of the parent drug and its metabolites that correlate with disease response could corroborate this initial observation.

Analysis of CD34⁺ bone marrow MNCs allowed performance of pharmacokinetic studies directly on leukemic cells, thereby avoiding the potential interference by contaminating non-leukemic cells (that is, normal neutrophils or lymphocytes). Although the small sample number precludes definitive conclusions, we were able to show a trend for preferential accumulation of GTI-2040 in the CD34⁺ compartment, suggesting successful delivery and accumulation of the anti-sense in the relevant malignant cell population.

We also observed a previously unreported finding that patients who eventually achieved CR had higher levels of baseline R2 expression than nonresponders. These data therefore suggest that baseline R2 expression should be further explored as a possible predictor for clinical response to GTI-2040, and perhaps it could serve as a marker for treatment stratification to GTI-2040/AraC salvage therapy in patients with relapsed/refractory disease. Additionally, CR patients had a significant reduction in the R2 target following antisense treatment compared with pretreatment baseline. In contrast, nonresponders showed an increase in the target expression following GTI-2040 administration compared with pretreatment baseline. The latter is a phenomenon that we had already reported with other antisense compounds (24). Although mechanistically target up-regulation following antisense treatment remains to be fully elucidated, we postulated that it may be caused by a negative feedback loop that activates transcription in a target gene not functioning to its maximum capacity in malignant cells exposed to antisense compounds (29,30). While realizing that many other factors may play a role in disease response, the findings reported here are intriguing and support pharmacologic activity of GTI-2040 via down-regulation of the RNR target. Given the limitations intrinsic to the phase I trial design (that is, small numbers of patients treated at different dose levels), future studies are necessary to confirm and extend these observations.

During the design and completion of this study, there was no validated method available for measurement of the deoxynucleoside triphosphate pools. Although GTI-2040 should modulate the deoxynucleoside triphosphate pool based on the intended mechanism of action, we are unaware of any published data in this regard. Since then, we have recently, in a separate preclinical study, shown the GTI-2040 decreases dCTP pool 1- to 2-fold in K562 cells following treatment with 5 μ mol/L for 24 hours using our newly developed liquid chromatography-tandem mass spectrometry method (31). Limited sample material prohibits completion of these evaluations in the phase I study reported in this article; however, these studies will be incorporated into future clinical trials.

Finally, one could raise the question of whether inhibition of RNR can be achieved using other, less costly agents such as hydroxyurea rather than antisense compounds. Although laboratory data support the possible role of hydroxyurea in inhibiting RNR by directly reducing the tyrosyl radical of R2 to a normal tyrosine residue via one-electron transfer from the drug (32,33), the effectiveness of this approach is likely to be limited by the hydroxyurea

low affinity for RNR, very high hydrophilicity, short half-life, and early development of mechanisms of resistance (34). The latter is mediated by R2 overexpression via gene amplification and/or alteration in gene transcriptional regulation (35,36), R2 mutations (37), and increased expression of ferritin-encoding genes that leads to increase in iron levels and reactivation of previously drug-inhibited R2 protein (38).

Based on the results from this current study, we conclude that GTI-2040 in combination with HiDAC is feasible and is active against its target R2. The combination holds promise in younger patients with refractory/relapsed AML. While encouraging, our clinical and pharmacodynamic results require confirmation, and a phase II trial in younger patients with refractory or relapsed AML is under way.

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Fig. 1.

A, mean $\pm$ SD plasma GTI-2040 concentration versus time in patients. B, intracellular concentration GTI-2040 after 24 and 120 hours of GTI-2040 exposure ($n = 14$; $P = 0.002$, Wilcoxon matched-pairs signed rank test). C, mean $\pm$ SE ratio of nuclear to cytoplasmic GTI-2040 in CR ($n = 3$) and nonresponder ($n = 5$) patients ($P = 0.07$). D, mean $\pm$ SE R2 protein expression in patients achieving CR and nonresponse. CR patients had higher levels of R2 at baseline compared with patients with nonresponder ($P = 0.03$).

Table 1

Dose escalation of GTI2040 in combination with HiDAC

Dose level	GTI2040 continuous i.v. infusion days 1–6 (A [*]) (mg/kg/d)	AraC i.v. q12 h		Cumulative dose AraC (g/m ²)	No. treated
		Dose (g/m ² /dose)	Treatment days		
1	3.5 (days 1–7)	2.0	2–7	24	3
1A [*]	3.5	2.5	2, -, 4, -, 6	15	3
2A [*]	5.0	2.5	2, -, 4, -, 6	15	3
3A [*]	5.0	3.0	2, -, 4, -, 6	18	3
4A [*]	3.5	3.0	2, 3, 4, -, 6	24	3
5A [*]	5.0	3.0	2, 3, 4, -, 6	24	3
6A [*]	5.0	3.0	2, 3, 4, 5, 6	30	5

^{*} Amended after grade 3 reversible cerebellar toxicity on dose level 1.

Table 2Patient clinical characteristics ($N = 23$)

Characteristic	
Age, y	
Median	47
Range	21–59
Sex	
Female, %	48
Hemoglobin, g/dL	
Median	9.1
Range	7.0–15.4
Platelets, $\times 10^9/L$	
Median	48
Range	3–220
WBC count, $\times 10^9/L$	
Median	4.3
Range	0.4–137
Peripheral blood blasts, %	
Median	17
Range	0–99
Bone marrow blasts, %	
Median	55
Range	9–90
Cytogenetics, n	
Favorable	0
Intermediate	13
Adverse	10
Disease status, n	
Refractory	12
Relapsed	11
CR duration (relapse patients), n (mo)	
0–6	6
>6	5
No. prior therapies	
Median	1
Range	1–3
Prior therapy, n	
HiDAC	10
Antisense	0

Table 3Compiled grades 3 and 4 nonhematologic toxicities ($N = 23$)

Event	Grade 3	Grade 4
Constitutional		
Fatigue	7	0
Dermatologic		
Rash/erythema	1	0
Gastrointestinal		
Anorexia	6	0
Diarrhea	2	0
Dysphagia	1	0
Esophagitis	1	0
Mucositis	1	0
Nausea	3	0
Infection		
Febrile neutropenia	10	0
Infection w/neutropenia	12	0
Metabolic		
Aspartate amino transferase	3	0
Musculoskeletal		
Myositis	1	0
Neurology		
Anxiety	1	0
Ataxia	3*	0
Somnolence	1	0
Pain		
Headache	1	0
Pleura	2	0
Pulmonary		
Hypoxia	1	0
Pneumonitis/infiltrates	5	0

* Dose-limiting toxicity.

Table 4
Clinical and cytogenetic characteristics in patients achieving clinical response

Dose level	Disease status before enrollment	Prior CR duration (mo)	Karyotype	No. prior therapies	Prior HiDAC	Poststudy treatment
1	Refractory	NA	-7	1	No	Relapse at 2 mo
1	Relapsed	9	Normal	1	No	Allogeneic SCT
1A	Relapsed	16	Normal	3	Yes	Allogeneic SCT
2A	Relapsed	5	Complex	1	No	Relapse at 3 mo
3A	Refractory	NA	inv(3)	1	No	Allogeneic SCT
3A	Refractory	NA	Complex	1	No	Relapse at 1 mo
5A	Relapsed	18	Normal	2	Yes	Allogeneic SCT
6A	Relapsed	18	Normal	3	Yes	Allogeneic SCT

Table 5Relevant pharmacokinetic variables of GTI-2040 (mean $\pm$ SD)

Pharmacokinetic variable	3.5 mg/kg/d (<i>n</i> = 9)	5.0 mg/kg/d (<i>n</i> = 13)
C_{ss} (nmol/L)	217 $\pm$ 80.9	292 $\pm$ 87.0
$AUC_{0-\infty}$ (μ mol/L $\times$ h)	29.2 $\pm$ 11.9	41.5 $\pm$ 15.7
CL (L/h)	13.9 $\pm$ 4.50	11.3 $\pm$ 2.91
V_{ss} (L)	21.1 $\pm$ 7.25	26.2 $\pm$ 19.4
$t_{1/2\alpha}$ (h)	0.711 $\pm$ 0.370	0.723 $\pm$ 0.297
$t_{1/2\beta}$ (h)	31.1 $\pm$ 18.9*	46.4 $\pm$ 21.3

Abbreviations: C_{ss} , plasma steady-state concentration; $AUC_{0-\infty}$, area under concentration-time curve; CL, total body clearance; V_{ss} , volume of distribution at steady state; $t_{1/2\alpha}$, plasma distribution phase half-life; $t_{1/2\beta}$, plasma elimination phase half-life.

*
n = 7 patients.