Phase 1 and pharmacodynamic studies of G3139, a Bcl-2 antisense oligonucleotide, in combination with chemotherapy in refractory or relapsed acute leukemia


Overexpression of Bcl-2 is a potential mechanism for chemoresistance in acute leukemia and has been associated with unfavorable clinical outcome. We hypothesized that down-regulation of Bcl-2 would restore chemosensitivity in leukemic cells. To test this hypothesis, we performed a phase 1 study of G3139 (Genasense, Genta, Berkeley Heights, NJ), an 18-mer phosphorothioate Bcl-2 antisense, with fludarabine (FL), cytarabine (ARA-C), and granulocyte colony-stimulating factor (G-CSF) (FLAG) salvage chemotherapy in patients with refractory or relapsed acute leukemia. Twenty patients with refractory or relapsed acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) were enrolled. G3139 was delivered by continuous infusion on days 1 to 10. FLAG chemotherapy was administered on days 5 to 10. Common side effects of this combination included fever, nausea, emesis, electrolyte imbalance, and fluid retention that were not dose limiting. Plasma pharmacokinetics of G3139 demonstrated steady-state concentrations (Css) within 24 hours. Of the 20 patients, 9 (45%) had disease response, 6 (5 AML, 1 ALL) with complete remission (CR) and 3 (2 AML and 1 ALL) with no evidence of disease but failure to recover normal neutrophil and/or platelet counts or to remain in remission for at least 30 days (incomplete remission). Bcl-2 mRNA levels were down-regulated in 9 of the 12 (75%) evaluable patients. This study demonstrates that G3139 can be administered safely with FLAG chemotherapy and down-regulate its target, Bcl-2. The encouraging clinical and laboratory results justify the current plans for a phase 3 study in previously untreated high-risk AML (ie, age at least 60 years).

Introduction

Acute leukemia is a heterogeneous disease characterized by maturation arrest and uncontrolled proliferation of various hematopoietic precursor cells. Among a number of clinical and biologic factors shown to predict poor prognosis, cytogenetic aberrations, age, antecedent clonal hematologic disorders, and prior chemotherapy are the most important. Despite many advances made in the last 2 decades in the management of acute leukemia, only 20% to 30% of adult patients are cured of their disease following intensive chemotherapy treatment. Further, although cure can be achieved with allogeneic stem cell transplantation (SCT) in patients who fail initial treatment, many are not candidates for these aggressive strategies, underscoring the need for novel therapeutic approaches that could improve the current clinical results.

Most treatment failures in patients with acute leukemia are related to development of chemoresistance in malignant cells. Defects in pathways of apoptosis contribute significantly to inducing resistance to a variety of chemotherapeutic agents. Bcl-2 is a potent inhibitor of caspase- and noncaspase-mediated apoptosis. Indeed, overexpression of this protein was shown to result in resistance to a variety of apoptosis-inducing signals including radiation, steroids, and chemotherapy. In recent clinical studies, abnormal expression of Bcl-2 was proven to be predictive of poor response to treatment and adverse clinical outcome in patients with a variety of hematologic malignancies, including acute leukemia and non-Hodgkin lymphoma. Based upon these data, we and others, therefore, have hypothesized that down-regulation of Bcl-2 could ultimately induce a lower apoptotic threshold and restore chemosensitivity in chemoresistant leukemic cells.

To validate this strategy, we initiated a phase 1 study of G3139, an 18-mer phosphorothioate oligodeoxynucleotide (ODN) antisense designed to bind to the first 6 codons of the human Bcl-2 mRNA, in acute leukemia. Preclinical studies showed that when administered alone or in combination with chemotherapy, G3139 down-regulates Bcl-2 expression in vitro and in vivo, resulting in a significant increase in tumor cell apoptosis. G3139 clinical activity was also shown in trials of patients with non-Hodgkin lymphoma and melanoma. In the current study, we combined G3139 with escalated doses of the fludarabine (FL), cytarabine (ARA-C), and granulocyte colony-stimulating factor (G-CSF) combination (FLAG) in patients with refractory or relapsed acute leukemia.
leukemia. The primary objective of this trial was to determine the safety and tolerability of G3139 administered in the combination with an intensive chemotherapy regimen and to validate that the target Bcl-2 mRNA was down-regulated in most of the treated patients.

Patients, materials, and methods

Eligibility criteria and study design

This phase 1 dose-escalation study included patients with refractory or relapsed acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) (Table 1). Patients were required to have received at least one induction treatment for acute leukemia and have unequivocal histologic evidence of recurrent or refractory disease. Prior treatment with high-dose chemotherapy supported with autologous or allogeneic SCT was allowed. No evidence of enhanced Bcl-2 expression by leukemic blasts in pretreatment bone marrow (BM) or blood from relapsed or refractory patients was required. Informed consent, approved by the Ohio State University Institutional Review Board (IRB), was obtained from all patients before entry onto the study.

G3139 (5′-TCTCCACGCTGCGCAT-3′) was delivered as a continuous intravenous infusion (CIVI) on days 1 to 10 through a separate line at a dose of 4 mg/kg/d for the first 4 cohorts of patients and at a dose of 7 mg/kg/d for the fifth cohort of patients. Both fludarabine (dose level 1:15 mg/m² intravenously over 0.5 hours) and cytarabine (dose level 1:1000 mg/m² intravenously over 4 hours) were given on days 6 to 10. Planned dose escalation was in approximately 25% increments to achieve the full dose of FLAG, which was administered to the fourth and fifth cohorts of patients (Table 2). G-CSF (filgrastim) was started at day 5 at a dose of 5 μg/kg/d in all cohorts until the absolute neutrophil count (ANC) was more than 3 × 10⁹/L (3000/μL) for 2 consecutive days or more than 10 × 10⁹/L (10 000/μL) for 1 day. Three patients were enrolled at the first 2 cohorts, 4 at cohorts 3 and 4, and 6 at cohort 5. Following enrollment of the first 14 patients, the protocol was amended to allow treatment with leukapheresis and hydroxyurea for uncontrolled blast proliferation prior to initiation of chemotherapy (ie, day 6). Adverse events were graded according to the National Cancer Institute (NCI) common toxicity criteria (CTC version 2.0; http://ctep.info.nih.gov).

Any grade 3 or 4 nonhematologic systemic toxicity was considered dose-limiting toxicity (DLT) if directly related to G3139. Complete remission (CR) was defined according to the NCI criteria as absence of leukemic blasts in BM and blood, BM cellularity of 20%, evidence of all 3 cell lineage maturation, neutrophil count at least 15 × 10⁹/L (1500/μL) and platelet count at least 100 × 10⁹/L (100 000/μL), and remission duration of at least 30 days. Incomplete remission (IR) was defined as the absence of leukemic blasts but failure to achieve CR according to the NCI criteria.

Patients were required to have received at least one induction treatment for acute leukemia and have unequivocal histologic evidence of recurrent or refractory disease. Prior treatment with high-dose chemotherapy supported with autologous or allogeneic SCT was allowed. No evidence of enhanced Bcl-2 expression by leukemic blasts in pretreatment bone marrow (BM) or blood from relapsed or refractory patients was required. Informed consent, approved by the Ohio State University Institutional Review Board (IRB), was obtained from all patients before entry onto the study.

Table 2. Dose levels and disease response

<table>
<thead>
<tr>
<th>Dose level</th>
<th>G3139, mg/kg/d, d 1 to 10</th>
<th>Fludarabine, mg/m²/d, d 6 to 10</th>
<th>Cytarabine, g/m²/d, d 6 to 10</th>
<th>G-CSF, μg/kg/d, d 5 to 10</th>
<th>No. of patients treated</th>
<th>CR, no.</th>
<th>IR, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>15</td>
<td>1.0</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>20</td>
<td>1.5</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>25</td>
<td>2.0</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>30</td>
<td>2.0</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>30</td>
<td>2.0</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

CR indicates complete remission; and IR, incomplete remission. See “Eligibility criteria and study design” for definition.
Pharmacokinetic analysis
An anion exchange high-performance liquid chromatography (HPLC) method was used to assess plasma concentration of G3139. A 39-mer ODN (Integrated DNA Technologies, IA) was used as an internal standard. Patient plasma samples collected and immediately frozen at 0, 4, 24, 48, 120, 125.25, 240, 240.25, and 240.5 hours from the start of the G3139 infusion were thawed at room temperature and thoroughly mixed by vortex. A 2.5-μg internal standard in 50 μL of 25 mM Tris (tri(hydroxymethyl)aminomethane) buffer (pH 8.5) was added to each of a set of 16 mm × 125 mm glass tubes, followed by an addition of 0.5 mL of a patient plasma sample or control plasma spiked with G3139 standards; 2 mL chloroform was used to precipitate the plasma proteins and remove the lipids. Following centrifugation at 1500g for 15 minutes at 6°C, 75 μL of the supernatant was injected for HPLC analysis, which was conducted on a BioRad HPLC system (Hercules, CA). Separation was achieved on a Phenomenex HiTrap Q 1 mL strong anion exchange column (Piscataway, NJ). The mobile phase A was composed of 25 mM Tris buffer and 30% dimethyl formamide (pH 8.5) in water, and the mobile phase B consisted of 1 M sodium bromide in mobile phase A. A linear gradient was used with the solvent composition of 20% B, 100% B, 20% B, and 20% B at times 0, 16, 20, 25, and 30 minutes, respectively. Using a flow-rate of 1 mL/min, the run time for each analysis was 30 minutes. ODNs were detected by UV at 267 nm. Chromatography was performed at room temperature.

Prior to the analysis of the samples, HPLC assay validation was performed. Linearity was observed over the concentration range of 0.5 to 50 μg/mL in 0.5 mL plasma, with the linear regression coefficients (r²) of more than 0.99 achieved routinely. The within-day coefficients of variation (CVs) were 8.3%, 3.9%, and 4.3% at the 1, 5, and 10 μg/mL level, respectively. The between-day CVs were 11.2%, 15.7%, and 6.0% at the 1, 10, and 25 μg/mL level, respectively. The limit of quantification was 0.5 μg/mL plasma. A calibration curve with a range of 0.5 to 25 μg/mL was performed with each analysis. Pharmacokinetic analysis of the G3139 data was performed with appropriate model and model-independent methods using WinNonlin (version 3.0; PharSight, Mountain View, CA).

Quantification of Bcl-2 RNA by real-time RT-PCR
AML cells from a bone marrow aspirate were obtained prior to G3139 treatment and following 5 days of G3139 infusion (before FLAG therapy was initiated) in all patients. Mononuclear cells were isolated from peripheral blood using density-gradient centrifugation (Ficoll-Paque Plus; Pharmacia) and viably cryopreserved. For the analysis, samples were thawed and run in batch to eliminate variation as a consequence of controls, reagents, personnel, or cDNA preparation. Total cellular RNA and cDNA were prepared as previously described.20 Each cDNA sample was used as a template in a polymerase chain reaction (PCR) amplification reaction run in duplicate on the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Quantification of Bcl-2 copy number in patient samples was obtained by comparing patient samples against a Bcl-2 standard curve as previously described.20 The result of the real-time reverse transcriptase (RT)–PCR assay for each sample was reported as specific copy numbers of transcripts per nanogram of RNA. The real-time RT-PCR assay was validated by amplifying limiting dilutions of Bcl-2 cDNA standards. Linearity was observed over the concentration range of 10 to 10² Bcl-2 copies per microliter of cDNA, with the linear regression coefficients (r²) of at least 0.98 achieved routinely. The within-day and between-day coefficients of variation were approximately 5% to 8% for each standard amplified. The lower limit of quantification was determined to be 10 Bcl-2 copies per microliter of cDNA.

Quantification of Bcl-2 protein by immunoblotting analysis
Expression of Bcl-2 was also assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting and quantified by chemiluminescence assay using standard techniques for patients with available material. Briefly, whole-cell lysates were prepared in batch from viably cryopreserved cells (at least 90% viability by trypan blue exclusion) by centrifuging 10⁷ phosphate-buffered saline (PBS)–washed cells. After vigorous mixing, this cell suspension was incubated at 4°C for 10 minutes and then centrifuged for 15 minutes at 12,000 rpm at 4°C. The supernatant was recovered and frozen at −80°C. Proteins were quantified in each supernatant utilizing the BCA Protein Assay (Pierce, Rockford, IL.). The samples were then separated on 12% polyacrylamide gels and the proteins transferred to a 0.45-μm nitrocellulose membrane (Schleicher and Schuell, Keene, NH) using an electroblot apparatus (BioRad). Even loading and transfer of the proteins was verified by staining the nitrocellulose membranes with Ponceau S stain (Sigma Chemicals, St Louis, MO). Membranes were blotted with 0.1 μg/mL monoclonal mouse anti-human Bcl-2 antibody clone 124 (Dako, Carpinteria, CA) or 0.1 μg/mL polyclonal goat anti-human actin antibody (Santa Cruz, Santa Cruz, CA). The blots were incubated with horseradish peroxidase–conjugated antimouse immunoglobulin G (IgG) (BioRad), antirabbit IgG (BioRad), or antigoat IgG (Santa Cruz). Membranes were developed with Pierce SuperSignal West Pico reagents (Pierce) for 1 to 5 minutes. Autoradiography was performed with Kodak X-OMAT film (Kodak, Rochester, NY). In addition to autoradiography, chemiluminescent signals from the protein bands were quantified directly from the nitrocellulose using the Chemi-Doc system (BioRad), with specific protein values calculated relative to the actin signal.

Statistical considerations
Descriptive statistics to include means, standard deviations, and frequencies were computed for all variables. Mean half-life was computed using a geometric mean. Patient groups were compared using t tests and analysis of variance for continuous data and the Fisher exact test or χ² tests for discrete data. Relationships between continuous variables, such as pharmacokinetics and clinical laboratory data, were performed using standard linear correlation and linear regression, as appropriate. When needed, log transforms were used to ensure normal distributions and linearity.

Results

Patient characteristics
Twenty patients were enrolled on this study (13 women and 7 men) (Tables 1 and 2). The median age was 56 years. Seventeen patients had AML, 5 with primary refractory disease, 8 in first relapse, and 4 in subsequent relapses. Three patients had ALL, 2 with refractory Philadelphia chromosome–positive disease and 1 with t(5;14)(q31; q32) relapsed disease and hypereosinophilia. Of the 20 patients, 9 received high-dose cytarabine (HiDAC) with previous treatments, 1 had autologous SCT, and 1 matched unrelated donor (MUD) SCT. The median time to relapse from the initial treatment for relapsed patients was 7 months (range, 3 to 21 months). The median number of previous treatments was 2.

Toxicity
Eighteen of the 20 patients developed pancytopenia following treatment with G3139 and FLAG. In the remaining 2 patients (unique patient numbers [UPNs] 10 and 14), rapidly increasing blast counts (more than 100 000/μL) requiring leukapheresis and hydroxyurea developed before the start of chemotherapy (day 6) and, therefore, they were taken off study. Hematologic toxicities were similar to those expected with FLAG alone.21 Median time for neutrophil recovery from start of chemotherapy (ie, day 6) was 23 days (range, 8 to 38 days); median time for platelet recovery (at least 50 × 10⁹/L [50 000/μL]) was 39 days (range, 21 to 56 days).

Common adverse effects included fever, nausea, emesis, hypocalcemia, hypophosphatemia, and fluid retention (Table 3). They were manageable and non–dose-limiting. Two patients developed dyspnea and hypoxia due to leukostasis at day 5 before the start of FLAG (UPNs 10 and 14). Eleven patients developed dyspnea and
infections and died on day 120 with no evidence of disease (NED).

ventilation, this patient eventually developed additional multiple system failure affecting the renal, hepatobiliary, and respiratory systems. Although her condition improved following admission to the intensive care unit, use of vasopressors, and mechanical ventilation, this patient eventually developed additional multiple infections and died on day 120 with no evidence of disease (NED).

4) on day 13 of the second treatment course that was complicated by multiorgan failure affecting the renal, hepatobiliary, and respiratory systems. Although her condition improved following admission to the intensive care unit, use of vasopressors, and mechanical ventilation, this patient eventually developed additional multiple infections and died on day 120 with no evidence of disease (NED).

4) on day 13 of the second treatment course that was complicated by multiorgan failure affecting the renal, hepatobiliary, and respiratory systems. Although her condition improved following admission to the intensive care unit, use of vasopressors, and mechanical ventilation, this patient eventually developed additional multiple infections and died on day 120 with no evidence of disease (NED).

4) on day 13 of the second treatment course that was complicated by multiorgan failure affecting the renal, hepatobiliary, and respiratory systems. Although her condition improved following admission to the intensive care unit, use of vasopressors, and mechanical ventilation, this patient eventually developed additional multiple infections and died on day 120 with no evidence of disease (NED).

none of the other patients receiving a second course of G3139 had dose-limiting toxicity.

Pharmacokinetics

Plasma pharmacokinetic profiles of 11 patients with a total of 12 CIVI courses at a 4-mg/kg G3139 dose, and 6 patients with a total of 7 CIVI courses at a 7-mg/kg G3139 dose, were monitored. The plots of plasma concentration–time profiles for patients receiving G3139 at 4 mg/kg and 7 mg/kg are shown in Figure 1A and B, respectively. Steady-state plasma concentrations (Css) were achieved rapidly and remained so until the end of CIVI. Following the end of infusion, G3139 plasma concentrations declined monoexponentially and became nondetectable (less than 0.5 ng/mL) within 4 hours. Css were less variable at 4 mg/kg than at 7 mg/kg (Figure 2). The composite plots for the mean plasma concentration–time profiles for these 2 doses of G3139 are shown in Figure 2. Using a one-compartment infusion model, the resultant relevant pharmacokinetic parameters were estimated and are shown in Table 4. The mean Css level for the 4-mg/kg dose was 3.19 ± 1.29 μg/mL (range, 1.59-5.69 μg/mL), which is significantly lower than the Css of 5.47 ± 2.16 μg/mL (range, 2.67-8.38 μg/mL) for the 7-mg/kg dose (P = .023). When normalized to dose, the Css values were 0.78 ± 0.33 and 0.78 ± 0.30 μg/mL for the 4- and 7-mg/kg doses, respectively. These results indicated that the Css levels were proportional and the pharmacokinetics linear for the 2 doses (ie, 4 and 7 mg/kg) administered. The linearity in pharmacokinetics was also reflected by dose-dependent differences in area under the curve (AUC) values (P < .05) and similar total clearance (4.35 ± 1.85 L/h at 4 mg/kg and 3.89 ± 1.48 L/h at 7 mg/kg, P > .5). The mean elimination constants for these 2 doses were 1.10 ± 0.55 and 1.32 ± 0.56 hours⁻¹, respectively, yielding the mean t½ values of 0.63 ± 0.33 hours (range, 0.36-1.80 hours) and

Figure 1. Pharmacokinetic analysis of 17 patients (total 18 courses) treated on 2 different doses of G3139 (4 and 7 mg/kg). (A) Logarithmic plasma concentration versus time profiles during and after infusion of 4 mg/kg G3139. (B) Logarithmic plasma concentration versus time profiles during and after infusion of 7 mg/kg G3139. Each dot represents a time point at which plasma was collected for pharmacokinetic analysis.
Pharmacokinetic parameters for G3139 given at 4 and 7 mg/kg CIVI

<table>
<thead>
<tr>
<th>Patient UNP</th>
<th>No. of courses</th>
<th>AUC0-∞, µg × h/mL</th>
<th>Css, µg/mL</th>
<th>CL, L/h</th>
<th>K0, h⁻¹</th>
<th>t1/2, h</th>
<th>V, L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mg/kg, average ± SD (range)</td>
<td>12</td>
<td>856 ± 361</td>
<td>3.19 ± 1.29</td>
<td>4.35 ± 1.85</td>
<td>1.10 ± 0.55</td>
<td>0.63 ± 0.33 (0.36-1.80)</td>
<td>7.45 ± 4.91</td>
</tr>
<tr>
<td>7 mg/kg, average ± SD (range)</td>
<td>6</td>
<td>1546 ± 659</td>
<td>5.47 ± 2.16</td>
<td>3.89 ± 1.48</td>
<td>1.32 ± 0.56</td>
<td>0.52 ± 0.23 (0.33-1.13)</td>
<td>4.70 ± 4.27</td>
</tr>
<tr>
<td>Total*</td>
<td>18</td>
<td>—</td>
<td>—</td>
<td>4.20 ± 1.71</td>
<td>1.18 ± 0.54</td>
<td>0.59 ± 0.27</td>
<td>—</td>
</tr>
</tbody>
</table>

All parameters are mean ± SD.
AUC0-∞ indicates area under the plasma concentration-time curve calculated by linear trapezoid rule with actual data plus extrapolated data; Css, plasma steady-state concentration; CL, the total body clearance; K0, elimination rate constant; t1/2, plasma elimination phase half-life with harmonic mean half-life and its SD; V, volume of distribution calculated by model fitting; —, not applicable.

*Because all pharmacokinetic parameters are linear with dose, the appropriate mean values were computed for all patients.

Discussion

Herein, we describe the first published experience of G3139 combined with intensive chemotherapy in the treatment of acute leukemia.22-24 Our study pursued this Bcl-2 antisense strategy to test the safety of this approach and to assess modulation of the target Bcl-2 in patients with acute leukemia who failed previous therapy. To test the feasibility of this approach and to determine whether the actual target was altered in vivo, we performed a phase 1, biologic validation study that combined G3139 with FLAG salvage chemotherapy in patients with refractory or relapsed acute leukemia. This study demonstrated that the Bcl-2 antisense G3139 can be administered safely with FLAG chemotherapy in previously treated acute leukemia and that target Bcl-2 mRNA decreases in 75% of the evaluated patients. Concurrent with this observation, we noted a 35% CR rate in the relapsed patients receiving this regimen. While the primary objective of this study was neither to validate modulation of the target Bcl-2 nor therapeutic efficacy, the results of this trial provide justification for future investigation of G3139 in acute leukemia.

This paper also provides the first detailed pharmacologic report of G3139 administered by intravenous infusion in patients with leukemia. A linear relation was noticed between the dose of the
G3139 administration prior to chemotherapy initiation. These patients from the study, prompted a protocol amendment to allow sive leukocytosis and leukostasis, which caused removal of these 2 t1/2 reported by these authors may be due to the sustained release infusion for 14 or 21 days in patients with advanced cancer.25 The longer t1/2 values for the current study with intravenous infusion were significantly shorter than the mean t1/2 of 7.46 ± 4.32 hours reported by Waters et al using subcutaneous infusion.17 The longer t1/2 reported by these authors may be due to the sustained release effect of G3139 from the subcutaneous infusion site. Our results are closer to those reported by Morris et al, who used an intravenous infusion for 14 or 21 days in patients with advanced cancer.25

No dose-limiting toxicity was observed in any of the cohorts examined. While 2 patients had rapidly increasing blast counts following G3139 start, this is unlikely related to G3139, because both of these individuals had rapidly proliferating disease prior to beginning therapy (Figure 3).28 However, the occurrence of progressive leukocytosis and leukostasis, which caused removal of these 2 patients from the study, prompted a protocol amendment to allow treatment with hydroxyurea and/or leukapheresis during the time of G3139 administration prior to chemotherapy initiation. These measures should be applied in future studies to make feasible a therapeutic approach that allows delays in administration of cytotoxic chemotherapy in order to “prime” the leukemic cells with the antisense.

Toxicity did not appear to correlate with the G3139 dose administered or the AUC and Css achieved. Nevertheless, among the 6 patients treated at dose level 5 with 7 mg/kg G3139, UPN 17 developed MRSA sepsis complicated by multiorgan failure and died at day 80 of the second treatment course. This patient was a 51-year-old woman with a past medical history significant for hemolytic anemia and splenectomy. Prior to G3139 and FLAG, she had received high-dose chemotherapy with autologous SCT as part of consolidation treatment for AML. Although her multiorgan failure syndrome clearly occurred following a Gram-positive sepsis, with prior splenectomy being a likely aggravating factor for this overwhelming infection, her G3139 AUC and Css were the highest among all patients studied. In previous animal and clinical studies, no unusual toxicity was reported in normal organs following Bcl-2 down-regulation.15,17 Nevertheless, the PK results in UPN 7 raise the question as to whether the high G3139 plasma concentration could have contributed to the severity of the multiorgan failure syndrome and suggest cautious use of this compound in patients who have previously received myeloablative doses of chemotherapy until larger studies adequately address if both the pharmacology and/or safety of Bcl-2 antisense is similar in this clinical population.

In our study, the specific role of G3139 in inducing 45% overall disease response could not be discriminated from the antileukemic activity of FLAG. Response to FLAG alone has been reported to be

![Figure 3. Blood absolute blast counts during days 1 to 10 of G3139 infusion.](image)

The blood absolute blast counts for each patient are plotted with regard to days of treatment. Patients UPN 10 and UPN 14 were taken off the study before initiation of fludarabine/cytarabine at day 6 because of highly proliferative disease and symptoms of leukostasis requiring immediate treatment with leukapheresis and cytoreductive agents (ie, hydroxyurea). The total white blood cell (WBC) count trend was similar to that of the blast counts represented in the figure.

![Figure 4. Bcl-2 protein quantification by immunoblotting.](image)

Bcl-2 levels in UPN 1 before treatment and at days 5 (prior to FLAG), 11 (at completion of G3139 and FLAG treatment), and 28 (at time of neutrophil and platelet count recovery) are shown. This patient achieved a complete response to G3139 and FLAG (Table 5).

Table 5. Disease response and changes in Bcl-2 mRNA and proteins at day 5 of the G3139 CIVI

<table>
<thead>
<tr>
<th>UPN</th>
<th>Response to G3139 plus FLAG</th>
<th>Baseline BM blast percentage</th>
<th>BM Bcl-2 copies per nanogram of RNA Day 1</th>
<th>Day 5</th>
<th>% changes in Bcl-2 mRNA level by day 5 of G3139 CIVI</th>
<th>% changes in Bcl-2 protein level by day 5 of G3139 CIVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CR</td>
<td>99</td>
<td>10</td>
<td>210</td>
<td>+38.4</td>
<td>+6.0</td>
</tr>
<tr>
<td>3</td>
<td>NR</td>
<td>71</td>
<td>246</td>
<td>1956</td>
<td>-12.9</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>CR</td>
<td>62</td>
<td>4</td>
<td>126</td>
<td>-54.7</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>IR</td>
<td>94</td>
<td>2</td>
<td>199</td>
<td>-75.7</td>
<td>-71.2</td>
</tr>
<tr>
<td>7</td>
<td>CR</td>
<td>21</td>
<td>5</td>
<td>212</td>
<td>-36.7*</td>
<td>-79.4</td>
</tr>
<tr>
<td>8</td>
<td>IR</td>
<td>7</td>
<td>8</td>
<td>186</td>
<td>+21.1</td>
<td>+33.1</td>
</tr>
<tr>
<td>10</td>
<td>NR</td>
<td>94</td>
<td>2</td>
<td>737</td>
<td>+33.6</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>NR</td>
<td>80</td>
<td>23</td>
<td>299</td>
<td>-55.9</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>NR</td>
<td>25</td>
<td>5</td>
<td>749</td>
<td>-42.3</td>
<td>+38.7</td>
</tr>
<tr>
<td>18</td>
<td>NR</td>
<td>12</td>
<td>3</td>
<td>843</td>
<td>-40.9</td>
<td>NA</td>
</tr>
<tr>
<td>19</td>
<td>NR</td>
<td>58</td>
<td>26</td>
<td>902</td>
<td>-34.1</td>
<td>NA</td>
</tr>
<tr>
<td>20</td>
<td>NR</td>
<td>90</td>
<td>18</td>
<td>914</td>
<td>-6.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

BM indicates bone marrow; CR, complete remission; NR, no response; NA, not available; and IR, incomplete remission (see “Eligibility criteria and study design” for definition).

*Percentage of change at day 5 of G3139 CIVI.
between 30% and 80% depending upon the characteristics of the treated patients. In a recent multicenter phase 2 trial, Jackson and colleagues reported a CR rate of 81% for AML patients with late relapse (at least 6 months) and of 30% for patients with early relapse (less than 6 months) or refractory disease. In the latter group of patients, age negatively impacted on the probability of achieving remission, with only 11% of the patients aged 60 years or more attaining CR. Patients with a previous history of autologous or allogeneic SCT were also excluded. Comparison of these clinical results with our current trial is obviously not possible. It is interesting, however, that in our study 5 patients (UPNs 1, 4, 5, 7, and 8) achieved disease response (3 CR and 2 IR) despite not receiving the full dose of FLAG. Of the 5, 2 had primary refractory disease (UPNs 1 and 7) and 1 early relapse (UPN 5) (ie, 3 months). UNP 1, for example, achieved CR at dose level 1, with only 50% of the FLAG dose delivered, despite failing 3 previous different regimens. Further, 6 of our responders had previously failed treatment with HiDAC or autologous SCT, and 4 had poor-prognostic cytogenetics. Two patients (UPNs 4 and 11) had prolonged remission (more than 12 months) depending upon the characteristics of the patients. 21,29-33 In a recent multicenter phase 2 trial, Jackson and colleagues reported a CR rate of 81% for AML patients with high-risk disease. This could ultimately result in measuring unpredictably high levels of the target and in the impression of a total lack of activity of the antisense. Implementation of methodologic modifications in future studies such as selection of blasts from other normal mononuclear cells, preparation of protein lysates before cryopreservation, and examination of other antiapoptotic proteins such as Mcl-1 and Bcl-xL that might also prevent apoptosis in AML blasts could allow a better correlation between clinical response and G3139-induced changes in Bcl-2 expression.

Based upon the results from the current study, we conclude that combination of G3139 with intensive chemotherapy is feasible in refractory/relapsed acute leukemia. The biologic data derived from this study suggest that G3139 is active against its target Bcl-2 mRNA. Further, the achievement of Css within 24 hours from the start of G3139 suggested that the prechemotherapy infusion period might be shortened with respect to the 5 days used in the current study. Finally, in our trial, a traditional maximum tolerated dose (MTD) was not achieved with the current intensive regimen. However, because no safety data are available for the use of G3139 in conjunction with anthracyclines (ie, daunorubicin and idarubicin)—important components in many induction regimens for untreated AML—rather than pursuing dose escalation, we believe that the 7-mg/kg dose of G3139 should be reexplored in conjunction with these agents. Once completed, these preliminary studies will be used as a platform for now-planned phase 3 trials of G3139 with chemotherapy in high-risk AML (ie, patients at least 60 years old) to definitively assess the contribution of the Bcl-2 antisense strategy to chemotherapy activity in acute leukemia.

References


