Featured Article

A Phase I and Pharmacokinetic Study of VNP40101M, a Novel Sulfonylhydrazine Alkylating Agent, in Patients with Refractory Leukemia

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Abstract

Purpose: VNP40101M is a novel sulfonylhydrazine alkylating agent with broad antitumor activity in animal models. As alkylating agents are important antileukemia drugs, a Phase I and pharmacokinetic study of VNP40101M was conducted in patients with refractory or relapsed leukemias or poor-risk myelodysplastic syndromes (MDS).

Experimental Design: VNP40101M was given as a single i.v. infusion over 15–70 min on day 1. Courses were repeated every 4 weeks according to antileukemic activity. The starting dose of 220 mg/m² was escalated by ∼33% in cohorts of 3–6 patients until a maximum-tolerated dose was established. One additional cohort was treated with the maximum-tolerated dose divided over days 1 and 8.

Results: Thirty-eight patients, including 28 with acute myeloid leukemia and 5 with MDS, received 52 courses of treatment. Nondose-limiting, reversible infusion-related toxicities were the most frequent adverse event, occurring in 24 (63%) patients on the first course. Dose escalation was terminated at 708 mg/m² for prolonged myelosuppression in 1 of 7 patients, and 600 mg/m² was selected as the recommended Phase II dose, with no significant extramedullary toxicity at this dose level. Two patients, 1 with MDS treated with 300 mg/m² and 1 with acute myeloid leukemia treated with 600 mg/m², achieved complete remission.

Conclusions: VNP40101M had significant antileukemic activity and minimal extramedullary toxicity in patients with relapsed or refractory disease.

Introduction

The prognosis for patients with relapsed or refractory leukemias is poor, and more effective agents are needed to improve complete remission (CR) rates and durations of response (1). Increasingly, promising new compounds with unique and targeted mechanisms of action are being introduced into the clinic (2, 3). However, current data suggest that many of these agents will be combined with standard cytotoxic agents in therapeutic antileukemia regimens. Thus, the development of potentially more active or better tolerated cytotoxic agents continues to be important in attempting to improve therapy for patients with leukemia.

DNA alkylators are among the most active cytotoxic agents in patients with leukemias (4–6). Alkylating agents have in common the ability to damage DNA and/or impair DNA replication, but among the class, there is a spectrum of antitumor activity and toxicity (7). These differences have been attributed to various individual biological properties, including the type of DNA damage, the specificity for attacking DNA versus other cellular components, the mechanisms by which the cell repairs the particular type of DNA damage, entry into and disposition of the drug within the tumor and normal cells, and relative susceptibility to tumor resistance mechanisms (7–10). In view of the diversity of mechanisms that influence their efficacy and/or safety, new alkylating agents with a distinct biological profile and potential advantages over currently available drugs merit consideration for clinical evaluation in patients with hematological malignancies.

VNP40101M [101M, 1,2-bis (methylsulfonyl)-1-(2-chloroethyl)-2-(methylamino) carbonylhydrazine (Fig. 1)] is a new sulfonylhydrazine alkylating agent that demonstrated potential advantages over existing alkylating agents in preclinical studies (11–18). VNP40101M first undergoes activation to yield 90CE [1,2-bis (methylsulfonyl)-1-(2-chloroethyl) hydrazine] and methylisocyanate. The 90CE rapidly produces an alkylating, chloroethylating species, which is similar to the chloroethylating species generated by 1,3-bis (2-chloroethyl)-1-nitrosourea (carmustine or BCNU; Ref. 15). In contrast to BCNU, VNP40101M does not generate a hydroxyethylating, vinylating, or aminoethylating species, and alkylolation is relatively specific to the OCH2 position of guanine (17, 19, 20). Partially as a result of the different alkylating and isocyanate species generated by each agent, VNP40101M produces more cross-links and fewer DNA single-strand nicks compared with BCNU in vitro studies (17).

In animal models, VNP40101M demonstrated broad antitumor activity and was shown to distribute across the blood-
brain barrier (18, 21). Activity was observed against cell lines derived from solid tumors and hematological malignancies, including leukemia cell lines selected for resistance to other alkylators. Doses \( \geq 20 \text{ mg/kg (60 mg/m}^2 \) \) administered by the i.p. route produced 100% long-term survival in 1-day-old i.p.-implanted L1210 leukemia and P388 leukemia (18). VNP40101M also produced long-term survival in mice implanted with L1210 cell lines that were selected for resistance to BCNU, cyclophosphamide, and melphalan, respectively. The doses that were effective in sensitive and resistant L1210-bearing mice (20–60 or 60–180 mg/m\(^2\)) were only modestly myelosuppressive when administered to nontumor-bearing mice.

On the basis of the promising preclinical data, the first Phase I study of VNP40101M was initiated in patients with solid tumors using a schedule of short i.v. infusion every 4–6 weeks.

Thrombocytopenia was the dose-limiting toxicity (DLT), and the maximum-tolerated dose (MTD) was 305 mg/m\(^2\) with platelet and neutrophil nadirs occurring at a median of 27 and 34 days, respectively. Of particular interest was the lack of significant nonhematological toxicity, suggesting that substantial dose escalation might be possible in patients with refractory leukemias. Thus, a Phase I study of VNP40101M was conducted in patients with refractory leukemia.

Patients and Methods

The study was reviewed and approved by the Institutional Review Board of the M. D. Anderson Cancer Center. All patients gave signed informed consent, indicating that they were aware of the investigational nature of this study.

Patient Eligibility. Patients with relapsed or refractory leukemias or with poor-risk myelodysplastic syndrome for which no standard therapy was anticipated to result in a durable remission were eligible for study entry. Active controlled infection or known central nervous system leukemia was allowed. The protocol did not include age restrictions. Other eligibility criteria included the following: Eastern Cooperative Oncology Group performance score of \( \leq 2 \); serum bilirubin of \( \leq 1.5 \text{ mg/dl} \); aspartate aminotransferase or alanine aminotransferase levels \( \leq 3 \) times upper limit of normal; and serum creatinine \( \leq 2.0 \text{ mg/dl} \). In the absence of rapidly progressive disease, the interval from prior treatment with myelosuppressive cytotoxic agents could not be \( < 2 \) weeks, and those requiring hydroxyurea for control of peripheral blood cell counts must have discontinued the hydroxyurea at least 48 h before treatment on study. All patients of childbearing potential agreed to use adequate contraception for the duration of the study. Pregnant or nursing patients were excluded and any woman of childbearing potential required a negative pregnancy test within the week before study entry. Additional ineligibility criteria included myocardial infarction within the previous 3 months, symptomatic coronary artery disease, arrhythmias not controlled by medication, uncontrolled congestive heart failure, concomitant standard or investigational antileukemia treatment while on study, and, because the formulation of VNP40101M contains 30% ethanol, concurrent treatment with disulfiram (Antabuse).

Treatment and Study Design. VNP40101M was supplied by Vion Pharmaceuticals, Inc. (New Haven, CT) as a clear, colorless, slightly viscous, sterile, nonaqueous solution for i.v. administration in 10-ml vials containing 100 mg of VNP40101M, 3 ml of anhydrous ethanol, 7 ml of polyethylene glycol 300, and 6 ml of citric acid. VNP40101M was stored under refrigeration, at 2°C to 8°C (36°F to 46°F), except when being prepared for injection. For administration to patients, VNP40101M was diluted in 5% dextrose injection and USP up to concentrations of 4 mg/ml in a final volume of 50–500 ml. For total doses up to 800 mg, the final infusion volume was 250 ml administered over 15–30 min, although some patients received the infusions over periods of up to 70 min. For total doses of 801–1600 mg, the final infusion volume was 500 ml administered over 30–60 min. Dilutions were prepared in glass or plastic containers not containing di-(ethylhexyl) phthalate. VNP40101M concentrations of 0.1–4 mg/ml in D5W are stable for 24 h at room temperature.

VNP40101M was administered by i.v. infusion using a polyethylene-lined administration set inserted into a peripheral or central vein. Treatment was given as an outpatient unless patients were hospitalized for other reasons. Management of neutropenia, neutropenic fever, thrombocytopenia, mucositis, or diarrhea occurring during the cycle followed standard institutional guidelines. Transient infusion-related reactions were observed at doses \( \geq 400 \text{ mg/m}^2 \), consisting of one or more of the following: facial flushing; headache; nausea; dizziness; and asymptomatic hypotension. Subsequently, patients were pretreated with antihistamines. For severe acute drug-related reactions, infusions were stopped, and supportive care administered until the reaction resolved. Subsequently, the infusion could be restarted and completed at 50% of the original infusion rate. If a severe reaction recurred at the lower dose rate, no additional treatment was given for that cycle.

Patients were evaluated on the day of therapy and at least three times weekly and as clinically indicated while on protocol. At each evaluation, patients were assessed for toxicity, and complete blood count with platelets and differential, serum chemistries, and liver function tests were obtained. Patients were evaluated for response, including a bone marrow (BM) aspirate and biopsy \( \sim 4 \) weeks after each dose. Those without evidence of disease progression or severe or life-threatening drug-related toxicity were eligible to receive additional cycles of treatment every 4 weeks for up to six cycles. Patients with

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Footnote:

complete response were allowed to receive one to three cycles past the cycle where complete response has been documented.

A minimum of 3 patients were entered at each dose level. No new patients were entered at an escalated dose level until all patients at the current dose level had been observed for a minimum of 3 weeks. If none of the first 3 patients at a dose level experienced first cycle DLT, new patients were entered to the next higher dose level. If one of the first 3 patients in a dose level developed first cycle DLT, up to 3 more patients were required to start treatment at that same dose level. When ≥2 patients experienced first cycle DLT, no additional patients could be treated at that dose level. The MTD was defined as the highest dose level in which <2 patients of 6 developed first cycle DLT.

The starting dose was 220 mg/m² based on the highest safe dose evaluated in the ongoing solid tumor study and was escalated in ~33% increments for successive cohorts. When DLT were observed sufficient to conclude that a dose level was above the MTD, the next lower dose level was expanded to 6 patients as necessary, and if the MTD was not exceeded, an intermediate dose level between the dose above the MTD and the next lower dose level could be evaluated. After the MTD was determined, one additional cohort of 6 patients was enrolled in which the MTD was administered divided over days 1 and 8. Patients <19 years old were allowed to enter the study in cohorts of 1–3 to the dose level below the highest level being evaluated in patients ≥19 years old.

Individual patients developing less than grade 2 nonhematological toxicity could be escalated to the next higher dose level for subsequent cycles. Patients developing severe and life-threatening toxicity in which additional treatment posed a substantial risk for toxicity had treatment discontinued permanently. Patients developing a DLT but continuing on the study would have a dose reduction to the next lower dose level in the next cycle. Dose reductions for individual patients were permanent, and all toxicity for the previous cycle had to have returned to grade ≤1 before beginning a new cycle of therapy.

Toxicity was graded on a scale of 0–5 using the National Cancer Institute Common Toxicity Criteria, version 2.0 criteria. All patients who received any therapy on study were considered evaluable for toxicity. DLT was defined as any grade 3 nonhematological toxicity that does not resolve to grade ≤2 within 24 h, any grade 4 nonhematological toxicity, or myelosuppression manifest as BM hypoplasia for ≥42 days with a BM cellularity of ≤5% and no evidence of leukemia. Because certain DLTs (e.g., rapidly reversible grade 3 liver function test elevations) preclude dose escalation but are tolerable and manageable, with approval of the Institutional Review Board, accrual could continue to a dose level in which reversible and manageable DLTs were observed. This dose level could be defined as the MTD with the provision that all toxicities, including DLTs, were rapidly reversible and manageable.

**Response Criteria.** CR in patients with acute myelogenous leukemia (AML), myelodysplastic syndrome, or acute lymphocytic leukemia (ALL) was defined as normalization of the blood and BM with <5% blasts, a granulocyte count > 1 × 10⁹/liter, a hemoglobin of at least 9 g/dl, and a platelet count above 100 × 10⁹/liter. Patients who met these criteria but still had 6–25% marrow blasts were considered to have a partial remission as long as the blast count had decreased by at least 50% from prestudy treatment with VNP40101M. CR with incomplete platelet recovery (CRp) was defined as for CR but with platelet counts remaining <100 × 10⁹/liter. Other responses were considered as failures and categorized as the following: (a) early death if death occurred within 2 weeks from start of therapy; (b) aplastic death if death occurred during therapy without evidence of hematological recovery and with too few cells to count; (c) died with response unknown if death occurred without BM or peripheral blood picture indicative of aplasia or progressive disease; (d) secondary resistance if treatment induced a reduction in marrow blast + promyelocytes to ≤10% unless the blast percentage fell under this parameter at the time of study entry; and (e) primary resistance if any marrow during the course did not show too few cells to count or blasts + promyelocytes ≤ 10%. For patients with chronic myeloid leukemia-blastic phase (CML-BP), return to chronic phase was considered a CR. This was defined as <15% blasts in the BM and peripheral blood, <30% blasts plus promyelocytes in BM and peripheral blood, and <20% basophils in spleen and liver. Hematological improvement was defined as meeting the criteria for CR but with persistence of thrombocytopenia <100 × 10⁹/liter and few immature peripheral cells. A partial hematological response was defined as meeting the criteria for CHR but allowed persistence, although ≥50% reduction of palpable splenomegaly and thrombocytosis (platelets > 450 × 10⁹/liter), or the presence of few immature peripheral cells. Extramedullary CML-BP was considered a partial remission if the measurable disease was reduced by ≥50% and a CR if there was complete resolution of all measurable disease. CR for chronic lymphocytic leukemia was defined by an absolute lymphocyte count < 4 × 10⁹/liter with hemoglobin ≥ 11 g/dl, an absolute neutrophil count ≥ 1.5 × 10⁹/liter, platelet count > 100 × 10⁹/liter, and BM with <30% lymphocytes; if lymphoid nodules were seen, response was deemed as nodular CR. In addition to the peripheral blood response, there must be disappearance of all palpable lymph nodes, spleen, and liver without the appearance of new lesions. A partial remission was defined by an absolute lymphocyte count reduced by >50%, hemoglobin > 11 g/dl or 50% improvement in deviation from normal, absolute neutrophil count ≥ 1.5 × 10⁹/liter or 50% improvement in deviation from normal and platelet count > 100 × 10⁹/liter or 50% improvement in deviation from normal. When compared with pretreatment measurements, a reduction >50% in the sum of the products of two perpendicular diameters of all measurable lesions without the appearance of new lesions was required. Progressive disease was defined by a ≥50% increase in absolute lymphocyte count over baseline in first course, or lowest prior thereafter, with a sustained level > 10 × 10⁹/liter, an increase in the product of two perpendicular diameters of a measured lesion by ≥50% over the size present at entry on study, or for patients who respond, the size at the time of maximum regression and/or the appearance of new areas of malignant disease. Deterioration in performance status or increasing symptoms did not constitute progression; however, their appearance initiated an evaluation for extent of disease.

**Pharmacokinetics.** Blood samples for the determination of VNP40101M pharmacokinetics were collected from the arm opposite the infusion, or if a central line was used for drug
administration, samples were collected from a peripheral vein or a separate port. Samples were collected during the first and, when possible, the second course of treatment as well as whenever there was a change in dose. The following time points were collected: immediately before the start of infusion; end of infusion; and 5, 10, 20, 40, 60, 120, and 240 min after the end of infusion. At each time point, ~10 ml of blood were collected in a Vacutainer tube containing heparin as an anticoagulant. The blood was mixed with the anticoagulant by inverting the tube four to six times, and the blood was then immediately acidified by injecting 0.25 ml of a 2.0 M citric acid solution. After the addition of citric acid, the tube was inverted four to six times and immediately placed on ice. Within 30 min after blood collection, the sample was centrifuged at 1500 × g for 10–20 min at 2°C to 8°C. After centrifugation, the plasma fraction was transferred into two separate labeled Nunc cryovials, immediately frozen on dry ice, and stored frozen at −20°C until analysis. In each cycle in which blood pharmacokinetic studies were performed, urine specimens were collected for the following time periods: before the infusion and 0–4 and 4–8 h after end of infusion. Urine from each time period was kept cold at −4°C and pooled, mixed well, weighed or measured, and pH measured. An aliquot of 50 ml of the pooled urine from each time period was saved in a labeled plastic storage vial and immediately acidified by adding 0.5 ml of a 2.0 M citric acid solution. The acidified urine sample was subsequently frozen and stored at −20°C until analysis. VNP40101M is stable under the conditions in which the urine samples were collected and stored (at least 2 h at room temperature, for at least 12 weeks at −20°C, and for at least 24 h at room temperature after dilution before analysis). Stability studies for 90CE in urine were not done.

High-performance liquid chromatography/mass spectrometry was used to analyze plasma and urine samples for VNP40101M concentration and plasma for 90CE concentrations. An Agilent Technologies 1100 series high-performance liquid chromatography system (Agilent Technologies, Palo Alto, CA) and a Finnigan Navigator mass spectrometer system (Thermo Finnigan, San Jose, CA) equipped with an electrospray ionization probe and a matrix flow lens were used. Chromatographic separation was achieved using a Phenomenex Prodigy ODS-3 column (5 μm, 250 × 2.0 mm; Phenomenex, Torrance, CA), using the following conditions: the mobile phase consisted of 10 mM acetic acid/acetonitrile (70:30 v/v); the flow rate was 0.2 ml/min; column temperature was 40°C; and retention times for VNP40101M and 90CE were 8.8 and 7.0 min, respectively. Finnigan MassLab software was used for data acquisition and processing. Plasma samples (0.5 ml) were deproteinized with 1.0 ml of acetonitrile. After microfuge centrifugation at 13,000 rpm for 5 min at room temperature, the resulting supernatant was concentrated to dryness under N2 at room temperature and reconstituted with 0.25 ml of a solvent consisting of 1% acetic acid solution/acetonitrile (70:30 v/v). The reconstituted sample (10 μl) was then injected into the high-performance liquid chromatography/mass spectrometry system with single ion recording at m/z 249. External calibration standards were prepared in pooled control human plasma (for VNP40101M and 90CE) or urine (VNP40101M) and processed identically to test samples. The validated assay has a nominal curve range of 0.026–2.6 and 0.025–2.5 μg/ml for VNP40101M and 90CE, respectively, in plasma and 0.10–2.5 μg/ml for VNP40101M in urine. Quality control samples of VNP40101M and 90CE in plasma and urine and VNP40101M in urine were prepared at various concentration levels, stored with test samples in a freezer, and analyzed with each sample batch.

Pharmacokinetic modeling and pharmacokinetic parameter calculations were conducted using WinNonlin software (Pharsight Corporation, Mountain View, CA) with compartmental as well as noncompartmental methods. A one compartment i.v.-infusion model was used for the analysis of VNP40101M, whereas a one compartment first order model was used for the analysis of 90CE. The following pharmacokinetic parameters were computed: area under the plasma concentration-time curve (AUC) from time 0 to the last data point; peak plasma concentration (Cmax); elimination half-life (T1/2); volume of distribution at steady state (Vss); and total body clearance (CL). Descriptive statistics (mean and SD) were calculated and used to characterize the pharmacokinetic parameters at each dose level. For urine samples, cumulative urinary recovery of unchanged drug (VNP40101M) was determined for the time period of 0 (start of the infusion) to 8 h after end of infusion.

Results

Patient Characteristics. The characteristics of 38 patients treated on study are shown in Table 1. Their median age was 53 years (range, 15–85 years), and performance status was 0 or 1 in 31 patients (82%). Twenty-eight (74%) patients had AML. Two (7%) were receiving a first salvage attempt, 1 patient after a first CR lasting <6 months and 1 with primary refractory disease. Nine (32%) patients with AML were being treated on study as second salvage, 8 (30%) as third salvage, and 9 (33%) as fourth or subsequent salvage therapy. Fourteen (37%) patients had diploid or −Y cytogenetics, 2 (5%) patients had the Philadelphia chromosome, and 5 (13%) had miscellaneous chromosome abnormalities. Seventeen (45%) patients had unfavorable cytogenetics: 3 (8%) 11q abnormality; 5 (13%) trisomy 8; and 9 (24%) with −5 and/or −7 abnormalities.

Toxicity. All patients were evaluable for toxicity and received a total of 52 courses of treatment, (range, 1–3 courses; median, 1 course). An infusion-related syndrome consisting of facial flushing, headache, nausea, dizziness, and/or hypotension was the most frequent adverse event with 19 (50%) and 5 (13%) patients having grade 1 and 2 toxicity, respectively. Any patient who experienced a combination of grade 1 and 2 infusion-related symptoms was considered to have a grade 2 toxicity overall. Acute infusion-related toxicities were observed most commonly at doses ≥ 400 mg/m2 were rapidly reversible and appeared to be diminished in intensity after institution of prophylactic antihistamines.

Grade 3 and 4 adverse events are summarized in Table 2. There were no grade 4 drug-related nonhematological adverse events, and the incidence of grade 3 events was low. Three (8%) patients had grade 3 nausea and/or vomiting independent of
infusion-related nausea. One (3%) patient had grade 3 leg cramps. This patient also experienced grade 3 nausea/vomiting (included above), which, along with the leg cramps, occurred on day 1 of study drug administration; however, these events persisted longer than the typical infusion-related symptoms. One (3%) patient had an increase in transaminases from a baseline grade 1 to a grade 3; however, this event coincided with an episode of *Clostridium difficile* colitis. One (3%) patient developed grade 3 neutropenic colitis.

Most nonhematological adverse events were infrequent and mild in severity. The most common events were nausea and/or vomiting (11 [29%], all grade 1), fatigue (7 [18%], all grade 1), diarrhea (7 [18%]) grade 1 diarrhea, and 1 (3%) grade 2), and stomatitis [3 (8%) grade 1 and 1 (3%) grade 2]. A grade 2 bilirubin elevation was observed in 1 (3%) patient, which was possibly related to VNP40101M. Three (8%) patients had grade 1 arthralgias, shoulder pain, or bone pain of uncertain relationship to drug administration, and 1 (3%) patient had central nervous system symptoms consisting of temporary loss of hand grip strength (grade 2) and ataxia (grade 1). One (3%) patient had grade 1 conjunctivitis. There was no clear relationship between dose and occurrence of nonhematological adverse events, with the exception of the infusion-related symptoms.

Six (16%) patients developed prolonged myelosuppression (>28 days) where the cytopoenias were not attributable to disease progression or overt persistence of disease and were associated with hypoplastic BMs in patients with normocellular or hypercellular BMs at time of study entry. At the 708 mg/m² dose level, a patient in the first cohort of three experienced prolonged myelosuppression (>42 days) with a hypocellular marrow and no evident leukemia. This was the first DLT observed on the study, and thus, a total of 7 patients was treated at this dose level. An additional patient who received 708 mg/m² on the second course (532 mg/m² on course one) also experienced grade 3 prolonged myelosuppression (>42 days). The 532 mg/m² dose level, which had previously accrued 3 adults and 1 pediatric patient, was thus expanded to a total of 8 patients. The larger cohort sizes were necessary to have a sufficient number of patients assessable for at least 42 days to ensure that the incidence of myelosuppression as a DLT was not being underestimated. No additional DLTs were observed at the 532 and 708 mg/m² dose levels; nevertheless, additional dose escalation > 708 mg/m² was not attempted. An intermediate dose level of 600 mg/m² accrued 8 patients with one observed episode of grade 3 nausea and vomiting and no DLT. In an

<table>
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<td>Diagnosis</td>
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<td>7</td>
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<td>No. of patients</td>
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additional cohort of 6 patients treated with 300 mg/m² on a day 1 and 8 schedule, 1 developed a grade 3 neutropenic colitis.

Patients were eligible for therapy with baseline infection if the infections were considered to be controlled by antimicrobial therapy at time of study entry. At start of therapy, 3 patients had fever of unknown origin, 4 patients had controlled pneumonias diagnosed by chest radiograph, 1 had controlled urinary tract infection, and 1 had a symptomatic pleural effusion, which was possibly infection related. This latter patient had a persistent pleural effusion suggestive of an empyema while on study and also developed symptoms of an oral soft tissue infection. Two patients with baseline pneumonia had persistent or unchanged pneumonia in the first course of therapy, although one of these patients also developed symptoms of an oral soft tissue infection. Two additional patients with onset of pneumonia, 1 had positive nasal swabs for respiratory syncytial virus, 1 also had Gram-negative rod sepsis, and 1 had *pseudomonas* infection of the central venous line. One additional patient had an infection of the blood, urine, and stool with *pseudomonas aeruginosa*.

**Response.** All patients were evaluable for response. Overall responses are shown in Table 3. Two patients (1 with refractory anemia with excess blasts at the 300 mg/m² dose level and 1 with AML at the 600 mg/m²) achieved CR. The patient with RAEB, a 68-year-old male with trisomy 8 blast karyotype, responded to the first course of therapy and received a second course of VNP40101M at 400 mg/m², followed by a course of idarubicin and cytarabine, as consolidation therapy. This CR duration was of 8 months. A 23-year-old male, in whom the AML had aberrant expression of T-lymphocyte markers with diploid karyotype, had an initial CR after idarubicin and cytarabine, did not respond to clofarabine at relapse, and required two courses of VNP4010M, both 600 mg/m², to achieve CR. An additional patient with AML who presented with extramedullary oral soft tissue leukaemia had resolution of this disease without evident remission in the peripheral blood or BM. A Kaplan-Meier curve of overall survival is presented in Fig. 2. Median survival for all patients was ~9 weeks.

**Pharmacokinetics.** Pharmacokinetic evaluation was performed in 32 patients who consented to participation in the optional pharmacokinetic assessment and who received a total of 40 treatment courses. VNP40101M was quantifiable in plasma for up to 4 h after dosing. Table 4 summarizes the mean pharmacokinetic parameters for VNP40101M at each dose level. Mean VNP40101M AUC and $C_{max}$ versus dose are plotted in Fig. 3, A and B.

Mean peak concentrations/dose level occurring at end of infusion ranged from 14.6 to 62.3 μM (1 mg/liter = 3.257 μM). Peak levels were affected by the variable infusion durations. The mean elimination $T_{1/2}$ was ~29 min. A representative VNP40101M plasma concentration versus time plot for 1 patient/dose level is illustrated in Fig. 4. In urine, VNP40101M was detected during the 8 h after drug administration. Cumulative urinary recovery averaged 1.70% of the administered dose.

90CE is the initial activation product of VNP40101M and has an *in vitro* $T_{1/2}$ of ~30 s. Table 5 summarizes the mean pharmacokinetic parameters for 90CE at each VNP40101M dose level in plasma.
dose level. At low doses, the concentrations of 90CE were often below the detection limit. At higher doses, 90CE was typically quantifiable in patients' plasma for up to 2 h after drug administration. 90CE AUC was ~1.5–2.7% of the VNP40101M AUC.

**Discussion**

On this Phase I study of the new alkylating agent VNP40101M in patients with refractory leukemia, the DLT was myelosuppression, occurring in 1 of 7 patients treated at 708 mg/m² as a single i.v. infusion. The recommended dose for Phase II studies is 600 mg/m² as a single infusion or divided over days 1 and 8. Nonhematological toxicity consisted primarily of a grade 1–2 infusion-related syndrome that was easily managed with antihistamine pretreatment and supportive care. Severe extramedullary adverse events were unusual, and their relationship to drug administration was uncertain. Two patients, 1 with RAEB and 1 with AML, also achieved CR. Myelosuppression as a DLT and occurring at doses not associated with significant extramedullary toxicity are desirable properties in a cytotoxic chemotherapeutic agent for patients with hematological malignancies. Therefore, the data in this study encourage both additional single agent and VNP40101M-based combination studies.

The pharmacokinetic studies were generally consistent with findings in the solid tumor Phase I study. VNP40101M
AUC was similar in the two studies at overlapping doses and increased linearly with dose but with substantial interpatient variability. In the current study, the mean elimination $T_{1/2}$ was 29 min (versus 15 min in the solid tumor study) and similar to the activation rate of VNP40101M in human plasma at $37^\circ$C. 90CE, the initial activation product of VNP40101M, was detected in plasma at low concentrations, with an AUC $\sim1.5$–2.7% of VNP40101M. The low levels of 90CE are consistent with its very short $T_{1/2}$ of $\sim30$ s in vitro.

Dose escalation on this study was planned in a traditional manner with a minimum of 3 patients to be entered at each dose level. No new patients were to receive an escalated dose until all patients at the current dose level had been observed for a minimum of 3 weeks. However, as it became evident that myelosuppression was likely to be dose limiting, this period of observation was found to be inadequate. Evaluation of myelosuppression as a potential DLT also presented problems with regard to the number of patients that were required for safety assessment of each dose level because, as often occurs on a Phase I study in patients with refractory leukemia, there is a high early failure rate with overt, sometimes rapid, disease progression, and assessment of drug-related myelosuppression must be distinguished from leukemia and/or prior therapy-related marrow effects. On this study, 22 of 38 patients (58%) had baseline grade 3 or 4 myelosuppression because of leukemia and/or prior antileukemia therapy. Thus, at the higher dose levels, we treated more than the minimal number of patients indicated by the study design to assess a reasonable number of patients for a sufficient period of follow-up to determine the incidence of myelosuppression as a DLT. Our experience in this study raises the concern that current Phase I study designs for patients with leukemia are not optimal for assessing myelosuppression as a DLT, although this is a potentially desirable property in effective antileukemia cytotoxic agents.

Although only 1 patient treated at 708 mg/m$^2$ in the first cycle had very protracted myelosuppression without clear evidence of marrow leukemia, we chose not to pursue additional dose escalation and, indeed, felt that even this level of severe myelosuppression may be unacceptable without the possibility for stem cell rescue. The 600 mg/m$^2$ dose recommended for future single agent studies is approximately twice the MTD established in the solid tumor Phase I study, confirming that substantial dose escalation was possible in advanced leukemia patients. The lack of extramedullary toxicity at the highest dose of 708 mg/m$^2$ suggests that additional dose increases may be possible in preparative regimens for stem cell transplantation.

<table>
<thead>
<tr>
<th># of patients analyzed</th>
<th># of courses</th>
<th>Dose (mg/m$^2$)</th>
<th>Peak plasma concentration (mg/liter) (mean value in $\mu$M)</th>
<th>Area under the curve (min × mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4</td>
<td>220</td>
<td>0.13 $\pm$ 0.07 (0.52 $\mu$M)</td>
<td>6.8 $\pm$ 2.3</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>300$^a$</td>
<td>0.13 $\pm$ 0.05 (0.52 $\mu$M)</td>
<td>9.8 $\pm$ 2.9</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>400</td>
<td>0.15 $\pm$ 0.01 (0.60 $\mu$M)</td>
<td>11.5 $\pm$ 3.3</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>532</td>
<td>0.33 $\pm$ 0.30 (1.3 $\mu$M)</td>
<td>23.1 $\pm$ 19.8</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>600</td>
<td>0.22 $\pm$ 0.22 (0.9 $\mu$M)</td>
<td>23.0 $\pm$ 18.4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>708</td>
<td>0.31 $\pm$ 0.01 (1.2 $\mu$M)</td>
<td>19.3 $\pm$ 3.1</td>
</tr>
</tbody>
</table>

$^a$ Includes patients treated on days 1 and 8 schedule.
For future studies of VNP40101M as a single agent or in combination in refractory leukemia, knowledge of key mechanisms of resistance may be important in developing approaches to select patients and improve antitumor activity. Evidence that initial resistance is mediated by the cellular enzyme O\textsuperscript{6}-alkylguanine DNA alkyltransferase (AGT), which removes initial O\textsuperscript{6}guanine DNA alkyltransferase (AGT), which removes initial O\textsuperscript{6}—eliminating any potential therapeutic gain from the addition of O\textsuperscript{6}-benzylguanine (22, 25, 26). Similar studies of O\textsuperscript{6}-alkylguanine in combination with an alkylating agent such as VNP40101M could be considered for patients with leukemia where greater levels of marrow suppression are acceptable. Recently, temozolomide, a methylating agent, was shown to have activity in relapsed leukemia, and other studies have reported that standard regimens of temozolomide reduce AGT in tumor cells (27, 28). Resistance to temozolomide has been associated with both AGT overexpression and lack of DNA mismatch repair (29, 30). Combinations of temozolomide followed by VNP40101M warrant investigation because both have activity in relapsed leukemia, temozolomide may deplete AGT and sensitize leukemia cells to VNP40101M, neither agent has substantial extramedullary toxicity, and mismatch repair deficiency is not known to play a major role in VNP40101M resistance.

Data generated in murine models of L1210 leukemia suggest that VNP40101M or a related sulfonilylhydrazine in combination with a ribonucleotide reductase inhibitor or with cytarabine will have greater antitumor activity than the single agents (31). The mechanism for the enhanced antitumor activity of the combinations is not known but likely involves inhibition of repair following VNP40101M-induced DNA damage. On the basis of the data from the currently reported study, a Phase I study of VNP40101M combined with cytarabine is being conducted in patients with refractory hematological malignancies. In addition, a Phase II study of VNP40101M as a single agent in patients with AML in first relapse or elderly patients who are considered unfit for standard induction therapy will also be initiated. As a component of the new studies, pretreatment leukemia samples will be assessed for expression of AGT and other DNA repair proteins that may be associated with resistance or sensitivity to VNP40101M (32, 33). Studies for patients with lymphoproliferative disorders are also being developed.

References


A Phase I and Pharmacokinetic Study of VNP40101M, a Novel Sulfonylhydrazine Alkylating Agent, in Patients with Refractory Leukemia

Francis Giles, Deborah Thomas, Guillermo Garcia-Manero, et al.