Purpose: A phase I study was conducted to determine the dose-limiting toxicities (DLT) and maximum tolerated dose (MTD) for the combination of bortezomib and alvocidib in patients with B-cell malignancies (multiple myeloma, indolent lymphoma, and mantle cell lymphoma).

Experimental Design: Patients received bortezomib by intravenous push on days 1, 4, 8, and 11. Patients also received alvocidib on days 1 and 8 by 30-minute bolus infusion followed by a 4-hour continuous infusion. Treatment was on a 21-day cycle, with indefinite continuation for patients experiencing responses or stable disease. Dose escalation employed a standard 3 + 3 design until the MTD was identified on the basis of DLTs. Pharmacokinetic studies and pharmacodynamic studies were conducted.

Results: Sixteen patients were treated. The MTD was established as 1.3 mg/m² for bortezomib and 30 mg/m² for alvocidib (both the 30-minute bolus and 4-hour infusions). Common hematologic toxicities included leukopenia, lymphopenia, neutropenia, and thrombocytopenia. Common nonhematologic toxicities included fatigue and febrile neutropenia. DLTs included fatigue, febrile neutropenia, and elevated aspartate aminotransferase (AST) levels. Two complete responses (CR; 12%) and five partial responses (PR; 31%) were observed at the MTD (overall response rate = 44%). Pharmacokinetic results were typical for alvocidib and pharmacodynamic studies yielded variable results.

Conclusions: The combination of bortezomib and alvocidib is tolerable and an MTD has been established for the tested schedule. The regimen appears active in patients with relapsed and/or refractory multiple myeloma or non–Hodgkin’s lymphoma, justifying phase II studies to determine the activity of this regimen more definitively. 

Introduction

A variety of indolent to moderately aggressive B-cell neoplasms are generally responsive to, but not cured by, treatments that include conventional DNA- or microtubule-targeted cytotoxic agents such as alkylating agents, purine nucleoside analogues, and vinca alkaloids; corticosteroids; monoclonal antibodies; radiolabeled monoclonal antibodies; radiation; and new agents such as the proteasome inhibitor bortezomib. These neoplasms are also often responsive to myeloablative drug and/or radiation therapy followed by autologous or allogeneic stem cell infusion, with occasional patients achieving cures with this approach (1). Nonmyeloablative therapy followed by allogeneic stem cell infusion is also a promising investigational strategy (2). Nevertheless, while many such patients have a variety of therapeutic options, few of these are potentially curative.

The boronic anhydride proteasome inhibitor bortezomib (VELCADE) was the first of its class to enter the clinical arena (3). Several mechanisms have been invoked to explain its toxicity toward transformed cells, including inhibition of NF-κB, antiangiogenic effects, and upregulation of proapoptotic proteins, among others (4). The most
Translational Relevance

While multiple treatment options exist for indolent lymphoma to moderately aggressive B-cell neoplasms, curative approaches are largely lacking. Consistent with the concept that simultaneous interruption of cell survival signaling and cell-cycle regulatory pathways effectively induces transformed cell death, previous preclinical studies showed synergistic interactions between the CDK (cyclin-dependent kinase) inhibitor alvocidib and the proteasome inhibitor bortezomib in malignant hematopoietic cells. To test the clinical relevance of these observations, a phase I clinical trial was conducted to determine the dose-limiting toxicities (DLT) and maximum tolerated dose (MTD) of the combination of alvocidib, administered by a novel hybrid infusion schedule, and bortezomib in patients with various B-cell neoplasms. Correlative pharmacokinetic and pharmacodynamic studies were conducted in parallel. The completion of this study lays the foundation for successor phase II trials of alvocidib and bortezomib designed to test the activity of this novel strategy more definitively.

frequently employed bortezomib schedule is 1.3 mg/m² intravenous push (IVP) on days 1, 4, 8, and 11, with asthenia, gastrointestinal toxicity, anemia, and thrombocytopenia representing the most common toxicities. Bortezomib has been approved for use in patients with multiple myeloma (5, 6) and in patients with refractory mantle cell lymphoma (7).

Alvocidib (flavopiridol) was the first cyclin-dependent kinase (CDK) inhibitor to enter the clinic (8). Like bortezomib, alvocidib also exerts pleiotropic actions. In addition to inhibition of proliferation, alvocidib acts as a transcriptional repressor through inhibition of the CDK9–cyclin T (pTEFb) transcription complex (9). This can lead to downregulation of various short-lived proteins, such as Mcl-1 and cyclin D1, that have been implicated in the survival and proliferation of multiple myeloma and mantle cell lymphoma (10, 11). In addition, alvocidib, by inhibiting IKK, can interrupt the NF-κB pathway (12), analogous to the effects of bortezomib. Other postulated mechanisms of alvocidib antineoplastic actions include binding to DNA duplexes (13), interference with STAT3–DNA complexes (14), and anti-angiogenic activities (15). Alvocidib has been administered by various schedules, including daily IVP × 5 days and by continuous 72-hour infusions (16), with secretory diarrhea and hypotension representing the DLTs. To date, single-agent activity in multiple myeloma and mantle cell lymphoma has been limited (17, 18), possibly a consequence of pharmacokinetic factors including extensive plasma protein binding. Recently, a pharmacokinetically designed alvocidib schedule has been designed in which 50% of the alvocidib dose is administered as a 30-minute infusion, followed by 50% dose as a 4-hour infusion (19). With this hybrid infusion schedule, significant responses have been observed in patients with refractory and/or high-risk chronic lymphocytic leukemia (CLL; ref. 20).

Accumulating evidence suggests that neoplastic cells may be particularly susceptible to a strategy in which cell survival signaling and cell-cycle-related pathways are simultaneously interrupted (21). In that context, preclinical findings showed that in malignant hematopoietic cells, alvocidib interacted synergistically with proteasome inhibitors to induce apoptosis (22, 23). This interaction involved multiple perturbations, including interruption of the NF-κB pathway, downregulation of NF-κB–dependent proteins [e.g., Bcl-xL, XIAP], and activation of the stress-related JNK (c-Jun N-terminal kinase) pathway (22). These findings, along with the established activity of bortezomib in multiple myeloma and mantle cell lymphoma, as well as emerging evidence of its activity in follicular lymphoma (24), raise the possibility that a combination strategy involving alvocidib might be efficacious in certain B-cell malignancies. To address this question, a phase I trial was initiated in which bortezomib was administered according to a standard day 1, 4, 8, and 11 schedule in conjunction with alvocidib administered by a hybrid infusional schedule on days 1 and 8 in patients with relapsed/refractory multiple myeloma, indolent lymphoma, or mantle cell lymphoma. The results of this trial show that the combined administration of alvocidib and bortezomib is tolerable in this patient population and identify the MTD for the regimen. They also show that the alvocidib/bortezomib regimen has activity in a highly refractory group of patients including several patients who had progressed following prior treatment with bortezomib.

Materials and Methods

Drug sources and formulation

Bortezomib (PS-341; NSC 681239) was supplied by the Pharmaceutical Management Branch of Cancer Therapy Evaluation Program (CTEP), National Cancer Institute (NCI). Each sterile single use vial contained 3.5 mg bortezomib as a lyophilized powder with 35 mg mannitol, USP (U.S. Pharmacopeia). The drug was reconstituted with 3.5 mL normal saline, USP, such that each mL of solution contained 1 mg bortezomib at a pH of 5 to 6. The drug was administered without further dilution by an IVP over 3 to 5 seconds.

Alvocidib (flavopiridol; NSC 649890) was provided by Sanofi-Aventis Pharmaceuticals, Inc. and distributed by the Pharmaceutical Management branch of CTEP, NCI. The drug was provided as a sterile yellow to greenish-colored 10 mg/mL solution in flint glass with elastomeric closures. Each vial contained 54.5 mg of HMR 1275, which is equivalent to 50 mg of the free base, acetic acid, and water for injection, with a pH of about 3. The drug
was diluted with 0.9% sodium chloride injection USP or 5% dextrose injection USP to final concentrations ranging from 0.109 to 1 mg/mL alvocidib (free base equivalent). The isoosmotic diluted solutions had a pH 3.5 to 4.1. A final concentration of 0.09 to 1 mg/mL is recommended to decrease the risk of thrombotic complications. The final solutions were administered intravenously as described in Treatment Plan later.

**Eligibility criteria**

The eligibility criteria were as follows: (a) recurrent or refractory B-cell neoplasms include follicle center lymphoma, follicular or diffuse; mantle cell lymphoma; marginal zone B-cell lymphoma, splenic, nodal or extranodal; lymphoplasmacytoid lymphoma/immunocytoma; plasma cell myeloma; plasmacytoma; plasma cell leukemia; or Waldenstrom’s macroglobulinemia; (b) age 18 years or older; (c) ECOG performance status of 1 or less; (d) no neuropathy grade 2 or more; (e) hemoglobin level of 8 g/dL or more; (f) absolute neutrophil count (ANC) of 1.5 × 10^9/L or more; (g) platelet count of 100 × 10^9/L or; (h) preserved kidney and liver function; (i) prior allogeneic stem cell transplantation was not; and (j) patients with history of central nervous system neoplasm or a primary central nervous system neoplasm were not eligible.

**Treatment plan**

This phase I trial was a nonrandomized, dose-escalation study to determine the MTD for the combination of alvocidib and bortezomib. The dose of bortezomib for all 3 dose levels was 1.3 mg/m^2. The total dose of alvocidib at dose level 1 was 40 mg/m^2 (20 mg/m^2 as a 30-minute bolus followed by a 20 mg/m^2 4-hour infusion); at dose level 2, 60 mg/m^2 (30 mg/m^2 as a 30-minute bolus followed by a 30 mg/m^2 4-hour infusion); and at dose level 3, 80 mg/m^2 (30 mg/m^2 as a 30-minute bolus followed by a 50 mg/m^2 4-hour infusion). Bortezomib was administered via an IVP over 3 to 5 seconds on days 1, 4, 8, and 11. Alvocidib was administered via intravenous infusion over 30 minutes (loading dose) followed by a continuous 4-hour infusion on days 1 and 8. The treatments were repeated at 3-week cycles.

Clinical issues unique to this schema included hyperacute tumor lysis syndrome (TLS) and cytokine release syndrome (20) and necessitated extensive attention to supportive care regimens to ensure appropriate monitoring and treatment of such sequelae. Prophylaxis, monitoring, and treatment of TLS during the first course (doses 1 and 2) of alvocidib were required. All patients were treated with dexamethasone (20 mg) on course 1, days 1 and 8 to prevent cytokine release syndrome.

Disease status was assessed after the first 6 weeks of treatment and every 6 to 8 weeks thereafter. Patients experiencing a response or stable disease were allowed to continue treatment indefinitely. Patients received full supportive care including herpes zoster prophylaxis.

**Dose levels, definition of DLT, and identification of MTD**

The patients were enrolled to dose levels in cohorts of 3 with dose level escalation on the basis of a 3 + 3 design. The dose levels were expanded to include 6 patients if a DLT was noted. The MTD was defined as the highest dose level at which fewer than 2 of 6 patients experienced a DLT. DLT was initially defined as any of the following which occurred during the first course of treatment and was determined to be possibly, probably, or definitely related to study treatment: (a) grade 3 or greater nonhematologic toxicities and (b) grade 4 hematologic toxicity. Late in the study, the DLT definition was amended to include instances in which both agents were omitted due to toxicity on at least 2 days of planned drug administration during course 1.

**Toxicity evaluation**

All adverse events were characterized in terms of attribution, severity, and study treatment relatedness according to the NCI Common Terminology Criteria for Adverse Events (CTCAE v3.0).

**Response evaluation**

The following response criteria were used: (a) Patients with lymphomas were evaluated using the NCI-sponsored Working Group Lymphoma Response Criteria (25). (b) Patients with plasma cell myeloma or plasmacytoma were evaluated according to European Group for Blood and Bone Marrow Transplant (EBMT) criteria (26). (c) Patients with plasma cell leukemia were evaluated according to the criteria of Vela-Ojeda and colleagues (27). (d) Patients with Waldenstrom’s macroglobulinemia were evaluated according to the criteria of the Second International Workshop on Waldenstrom’s macroglobulinemia (28).

**Alvocidib pharmacokinetic studies**

Venous blood samples (<10 mL) were obtained before and following treatment on cycle 1 day 1 and cycle 3 day 8 according to the following schedule: preinfusion, 30 minutes (end loading dose), 4.5 hours (end infusion dose), and 6, 8, 12, 24, and 48 hours. Blood samples were processed to plasma and frozen at −80°C prior to analysis by the study reference pharmacokinetic laboratory. Plasma samples were analyzed using a validated HPLC (high-performance liquid chromatography)–UV assay. Two-compartmental pharmacokinetics analysis was conducted using WinNonlin software (Pharsight).

**Enrichment of CD138^+ myeloma cells from bone marrow**

Bone marrow aspirates (5–10 mL) were obtained from patients with multiple myeloma. The aspirates from the patients receiving treatment were obtained at baseline prior to treatment and 24 hours after the first doses of alvocidib and bortezomib. CD138^+ multiple myeloma cells were enriched from the bone marrow aspirates using...
a magnetic cell sorter (MACS) and anti-CD138 antibody-coated magnetic microbeads (Miltenyi Biotec) as described previously (29). The CD138+ enriched fractions were collected and counted before aliquoting the cells. Three slides were made from each sample with 100,000 cells per slide and the remaining fraction was washed in PBS, pelleted, and stored frozen at −80°C for subsequent Western blot analysis.

**Protein extraction and Western blot analysis**

Frozen pellets of enriched CD138+ cells were resuspended in cell lysis buffer containing protease and phosphatase inhibitors (F. Hoffmann-La Roche Ltd.) and sonicated using a Misonix sonicator 3000. Total cellular protein was quantified using a Biorad protein assay. Protein (30–50 μg) was loaded and electrophoresed on a 4% to 12% NuPAGE gel (29). Primary antibodies included anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase) polyclonal antibody (Sigma-Aldrich) as a loading control for the analysis, anti-XIAP and anti-Mcl-1 (BD Biosciences), anti–NF-κB/p65NLS (nuclear localization signal; Millipore), and anti–phospho-JNK (pJNK; Cell signaling Technology, Inc.). Secondary antibodies were peroxidase labeled and affinity purified to rabbit and mouse IgG (KPL). Signals were detected and quantitative analysis was conducted as previously described (29). Two-dimensional spot densitometric images were obtained and analyzed with Alpha Ease FC software (Alpha Innotech/Cell Biosciences; ref. 29). Each protein band on a Western blot was assigned an average pixel value on a scale of 1 to 200 and adjusted to an arbitrary unit of 1 in pretreatment samples.

**Quantitative microscopy and fluorescence analysis**

RelA/p65 nuclear localization was assessed using a modification of a previously described immunohistochemical method (26). For quantitative microscopic image analysis, CD138+ enriched patient samples were centrifuged onto slides using a cytocentrifuge. Enriched CD138+ cells, obtained from a nonstudy myeloma patient, were treated ex vivo with 3 nmol/L bortezomib and used as controls for image analysis. The cells were fixed with 4% paraformaldehyde (EM Sciences) and stained for RelA/p65 expression with the monoclonal antibody MAB3026 (Millipore) and FITC (fluorescein isothiocyanate)-conjugated secondary antibody. MAB3026 recognizes the nuclear localization signal of the p65 subunit of the NF-κB heterodimer, corresponding to the activated form of NF-κB. Wide-field fluorescence microscopy was carried out with a fully automated, upright Zeiss Axio Imager. Z1 microscope (Carl Zeiss) with a 20×0.70NA dry objective and captured using an AxioCam MRM CCD camera and the AxioVision v4.6.0.2 Software Suite (30). Nuclear fluorescence was calculated as the pixel density of the fluorophore (FITC) conjugated to the secondary antibody. The parameters for the excitation wavelength are constantly fixed and hence the emission wavelength and fluorescence intensity are proportional to the amount of the bound secondary antibody. Fluorescence intensity was measured as the pixel density of the region of interest (ROI; in this case, the nuclear compartment), the boundary of which is defined by using a polyclonal anti-histone H4 antibody (Millipore) and TRITC (tetramethyl rhodamine isothiocyanate)-conjugated secondary antibody. The nuclear and total cellular amount of NF-κB in each plasma cell was internally controlled by histone H4 expression, with a minimum of 100 plasma cells assayed for each patient pre- and post-alvocidib/bortezomib exposure.

**Statistical analysis**

For the dose finding aspect of the study, Gehan’s 3 + 3 design as described above was used. To compare the pharmacokinetic measures across the dose levels, an ANOVA was applied. Post hoc 95% CIs were obtained. To adjust for multiple comparisons (for 5 different pharmacokinetic parameters), Bonferroni corrections were applied.

**Human investigation studies**

These studies were conducted after Institutional Review Board approval and in accordance with an assurance filed with and approved by the Department of Health and Human Services. Informed consent was obtained from each subject.

**Results**

**Patients**

A total of 16 patients, 11 male and 5 female, were enrolled on the study between September 2007 and April 2009 (Table 1). The median age of the patients was 62 years (range: 33–77). Nine patients had non–Hodgkin’s lymphoma (NHL; 6 of whom had mantle cell lymphoma), 6 had multiple myeloma, and 1 had an extramedullary plasmacytoma. The mean number of prior regimens was 2.5 (range: 1–6). Two patients had received prior autologous stem cell transplant (SCT). Four patients had received prior bortezomib.

The patients received a median of 4 courses of study treatment, with a range of 2 to 6 courses administered per patient. Six patients were treated at dose level 1, 6 patients were treated at dose level 2, and 4 patients were treated at dose level 3.

**Toxicities**

The treatment was well tolerated with toxicities that were transient and/or manageable (Table 2). Myelosuppression, particularly neutropenia, lymphopenia, and thrombocytopenia, was common. Of the 16 patients, 5 were treated for elevated potassium, although none of them met the laboratory or clinical criteria for TLS. Four of the patients were treated for potassium values of 4.3 to 4.9 mEq/L within the first 6 hours after the initial alvocidib administration. All of the patients responded to treatment and had no further evidence of impending TLS.
One patient received dexamethasone on cycle 1, day 2 for presumed grade 2 cytokine release syndrome. Three patients were admitted to the hospital with febrile neutropenia. Among nonhematologic toxicities, fatigue was the most common. One patient experienced grade 2 neuropathy in cycle 4 which required dose modification. Three patients developed grade 3 painful neuropathy (1 in cycle 2, 2 in cycle 3). Of these 4 patients, 1 had previously received bortezomib. Finally, 3 patients experienced grade 3 diarrhea in cycle 2. In one of these patients, the diarrhea did not recur following alvocidib dose reduction. All patients received prophylaxis with acyclovir and there was no outbreak of herpes zoster in patients enrolled on this study.

### Disease response

Although this study was not powered to assess response, 2 CRs (12%) and 5 PRs (31%) were observed among the 16 patients who received alvocidib/bortezomib treatment and were evaluable for response (overall response rate = 44%; Tables 3 and 4). The CRs and PRs were approximately equally divided between patients with NHL and multiple myeloma. Both of the CRs were achieved at the MTD. Notably, of the 4 patients previously treated with bortezomib (2 with NHL, 2 with multiple myeloma), 1 achieved a PR, 1 had stable disease (SD), and 2 (NHL) had progressive disease (PD).

Two particularly noteworthy responses were observed. A 56-year-old African-American female was diagnosed with multiple myeloma, IgA type. At the time of initial diagnosis, the patient had 70% plasma cells in the marrow with complex cytogenetic abnormalities (Supplementary Table S2) and extramedullary (sacral) and lytic bone lesions (hip). Prior therapies included localized radiation to extramedullary and bony lesions; thalidomide and pulse dexamethasone × 6 months; and tandem autologous SCT. Posttransplant, the patient achieved a CR with normal cytogenetics. Approximately 3 years after

---

**Table 1. Patient enrollment and characteristics**

<table>
<thead>
<tr>
<th>Nature</th>
<th>Hematologic toxicities (events/patients)</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>3/1</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>10/7</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>7/4</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>9/8</td>
<td>3/3</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>5/4</td>
<td>4/2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nature</th>
<th>Nonhematologic toxicities (events/patients)</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>3/3</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Elevated AST</td>
<td>1/1</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>6/5</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>3/3</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>0/0</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>0/0</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>2/2</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Lung infection (normal ANC)</td>
<td>1/1</td>
<td>0/0</td>
<td></td>
</tr>
<tr>
<td>Pain-neuralgia/peripheral nerve</td>
<td>3/3</td>
<td>0/0</td>
<td></td>
</tr>
</tbody>
</table>

*Only those toxicities deemed possibly, probably, or definitely related to the treatment are included in the table.*
transplant, the patient experienced a relapse of her multiple myeloma with 100% plasma cells in the bone marrow with additional complex cytogenetic abnormalities (Supplementary Table S2). After 2 cycles of study treatment (alvocidib/bortezomib), the patient had 2% plasma cells in the bone marrow. A CR was confirmed following 2 additional treatment cycles with normal cytogenetics. The patient received a total of 5 cycles of study treatment and proceeded to allogeneic SCT. The patient remained in a pathologic CR (CRp) for 9 months post-transplant.

A 42-year-old female was diagnosed with an extramedullary plasmacytoma. Prior therapies included VAD (vincristine, doxorubicin, and dexamethasone) CR and RICE (rituximab, ifosfamide, carboplatin, and etoposide) × 2 (PD), with extensive pleural effusions. After 2 cycles of study treatment, measurable disease decreased by 96.7% (Supplementary Fig. S1) and the patient experienced significant symptomatic improvement. In addition, there was near-complete resolution of her pleural effusions. The patient received a third cycle of study treatment, and subsequently underwent an autologous SCT.

Pharmacokinetic studies
Pharmacokinetic parameters for alvocidib were calculated on the basis of a 2-compartmental analysis for 13 of the 16 patients on the study (Table 5). Of the patients for which adequate pharmacokinetic data were available, 5 had data for 2 cycles (C1D1 and C3D8) and 4 were eligible for intercycle pharmacokinetic analysis (C1D1 vs. C3D8). There were no statistically significant correlations between cycles for exposure, Cmax, or clearance. No statistically significant differences across the dose levels among the patients between cycles were observed for any of the pharmacokinetic parameters. The only statistically significant correlations for this schedule were between C1D1 loading dose and Cmax (P = 0.007), and C1D1 total dose and area under curve (AUC; P = 0.001), which suggested linear pharmacokinetics. The lack of correlation between total dose and clearance further suggested linear pharmacokinetics.

Pharmacodynamic studies
Attempts were made to determine the feasibility of monitoring candidate pharmacodynamic response determinants by Western blot analysis in patients with multiple myeloma for whom sufficient material was available. A sufficient number of bone marrow–derived CD138+ cells for analysis was obtained from 3 patients, 2 of whom had stable disease (patients 1 and 2), and one who experienced a partial response (patient 3). Changes in pharmacodynamic markers prior to treatment and 24 hours after the first doses of alvocidib and bortezomib were quite variable and clear response patterns were not readily apparent (Fig. 1). For example, XIAP expression increased in cells from one patient with stable disease, but declined substantially in cells from the other stable patient and to a lesser extent in cells from the patient who experienced a PR. A small increase in JNK phosphorylation was observed in cells from 1 patient (SD), but modest or moderate declines were observed in the remaining 2 patients. In separate studies involving cells from additional patients, immunohistochemical staining of cells for pJNK also yielded variable results (data not shown). Mcl-1 expression increased in cells obtained from one patient but either did not change or declined slightly in the others. Nuclear localization of p65/RelA, an indicator of NF-κB activation, was evaluable in 2 specimens, and declined markedly in cells obtained from

Table 3. Treatment response by schema and diagnosis

<table>
<thead>
<tr>
<th>Response</th>
<th>NHL (n = 9)</th>
<th>Multiple myelomaa (n = 7)</th>
<th>All (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission (CR)</td>
<td>1b</td>
<td>1c</td>
<td>2</td>
</tr>
<tr>
<td>Partial remission (PR)</td>
<td>2b</td>
<td>3c,d</td>
<td>5</td>
</tr>
<tr>
<td>CR + PR (%)</td>
<td>3 (33)</td>
<td>4 (57)</td>
<td>7 (44)</td>
</tr>
</tbody>
</table>

aIncludes 1 patient with extramedullary plasmacytoma.
bIncludes 1 patient with mantle cell lymphoma.
cIncludes 1 patient with prior autologous SCT.
dIncludes 1 patient previously treated with bortezomib.

Table 4. Treatment response by dose level, diagnosis, response

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Diagnosis</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NHL-4</td>
<td>SD, PD, a SD, PD</td>
</tr>
<tr>
<td>MM-2</td>
<td>SD, SD</td>
<td>SD</td>
</tr>
<tr>
<td>2</td>
<td>NHL-4</td>
<td>PR, a PD, a CR, a PD</td>
</tr>
<tr>
<td>MM-2</td>
<td>CR, a PR</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NHL-1</td>
<td>PR</td>
</tr>
<tr>
<td>MM-3</td>
<td>PR, SD, c PR</td>
<td></td>
</tr>
</tbody>
</table>

aMantle cell lymphoma.
bExtramedullary plasmacytoma.
cPreviously treated with bortezomib.
methods. Observed for the 1 sample (patient 3) analyzed by both intensity (i.e., minimal change posttreatment) was RelA by Western blot and digitized confocal fluorescence (Supplementary Fig. S2). Notably, concordance of results for nuclear were detected in all samples posttreatment (Supplementary analysis. Minimal changes in nuclear RelA localization was conducted. One of these studies was conducted on cells analyzed by Western blot (patient 3), whereas the other 2 (patients 4 and 5) had insufficient cells for Western blot analysis. Minimal changes in nuclear RelA localization were detected in all samples posttreatment (Supplementary Fig. S2). Notably, concordance of results for nuclear RelA by Western blot and digitized confocal fluorescence intensity (i.e., minimal change posttreatment) was observed for the 1 sample (patient 3) analyzed by both methods.

Table 5. Two-compartmental pharmacokinetic parameters by dose level

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose level</th>
<th>1 (n = 8)²</th>
<th>2 (n = 6)</th>
<th>3 (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC, h ng/mL</td>
<td></td>
<td>1.561 ± 311b</td>
<td>2.500 ± 813</td>
<td>1.865 ± 723</td>
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<tr>
<td>K10, L/h</td>
<td></td>
<td>0.803 ± 0.237</td>
<td>1.04 ± 0.438</td>
<td>0.569 ± 0.169</td>
</tr>
<tr>
<td>V1, mL/m²</td>
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<td>18,512 ± 8,687</td>
<td>14,888 ± 7,685</td>
<td>31,380 ± 7,368</td>
</tr>
<tr>
<td>V2, mL/m²</td>
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<td>66,367 ± 69,511</td>
<td>111,642 ± 130,871</td>
<td>48,448 ± 23,397</td>
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<tr>
<td>CL, mL/h/m²</td>
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<td>13,243 ± 2,540</td>
<td>12,825 ± 3,267</td>
<td>18,137 ± 8,097</td>
</tr>
</tbody>
</table>

²n, represents the number of doses per dose level.
²Data are presented as the mean ± SD.

a patient with stable disease but did not change in cells obtained from the patient who achieved a PR.

For 3 additional specimens, obtained from patients all of whom achieved PRs, quantitative immunohistochemical analysis of p65/RelA nuclear localization was conducted. One of these studies was conducted on cells analyzed by Western blot (patient 3), whereas the other 2 (patients 4 and 5) had insufficient cells for Western blot analysis. Minimal changes in nuclear RelA localization were detected in all samples posttreatment (Supplementary Fig. S2). Notably, concordance of results for nuclear RelA by Western blot and digitized confocal fluorescence intensity (i.e., minimal change posttreatment) was observed for the 1 sample (patient 3) analyzed by both methods.

Discussion

The results of this study indicate that a standard dose and schedule of bortezomib (1.3 mg/m² on days 1, 4, 8, and 11) in combination with alvocidib, given by a novel, pharmacokinetically directed hybrid infusional schedule, can be safely and tolerably administered to patients with indolent lymphomas or multiple myeloma. The MTD recommended for phase II study is bortezomib at 1.3 mg/m² and alvocidib at 30 mg/m² for a 30-minute infusion followed by alvocidib at 30 mg/m² for a 4-hour infusion. This alvocidib dose/schedule is similar to that recently employed in a phase II single-agent trial in patients with CLl which showed high response rates in patients with genetically high-risk disease (20). Notably, the alvocidib/bortezomib regimen displayed significant activity (overall response rate = 44%) in a generally heavily pretreated population of patients including several who had previously received bortezomib. Collectively, these findings suggest that this treatment strategy warrants further exploration in this patient population.

Myelosuppression was a frequent hematologic toxicity and fatigue was the most common nonhematologic toxicity encountered during the study (Table 2). Four patients developed neuropathy (1 grade 2, 3 grade 3). All patients received herpes zoster prophylaxis and no incidents of herpes zoster were observed. Although these toxicities are similar to those reported for bortezomib treatment alone, the small sample size precludes drawing definitive conclusions regarding whether or not the addition of alvocidib to the treatment regimen exacerbates known bortezomib-related toxicities. Furthermore, no serious and unexpected toxicities were associated with this treatment regimen. Importantly, no evidence of hyperacute TLS was observed in the present trial. In previous studies in patients with CLl, a subset of patients developed TLS requiring aggressive therapy, including dialysis (19, 20). Although this was most frequently encountered with alvocidib doses of 50 mg/m² or greater, some patients receiving doses of 30 mg/m² experienced TLS, precluding escalation of the infusion to the 50 mg/m² level (20). It is possible...
that TLS may be relatively specific for patients with CLL and/or patients who have high peripheral blood counts or very bulky disease. Nevertheless, given the potential consequences of TLS, continued close monitoring of patients in an appropriate treatment setting is recommended until the risk of this event is more clearly defined in patients with indolent lymphoma or multiple myeloma.

Although the primary endpoint of this phase I study was not efficacy, 2 CRs (12%) and 5 PRs (31%) were observed for the 16 evaluable patients, with an overall response rate of 44%. Of the 7 multiple myeloma patients, there were 1 CR (14%) and 3 PRs (43%), with an overall response rate of 57%. Notably, 1 patient with multiple myeloma who had previously received bortezomib had an objective response to the flavopiridol/bortezomib regimen. Of the 9 patients with NHL, all 3 responders had mantle cell lymphoma. Given the established single-agent activity of bortezomib in this setting, that is, approximately 33% (31), the possibility that these patients would have responded to bortezomib alone cannot be excluded. Responses to single-agent bortezomib in patients with refractory/refractory relapsed MM are approximately 35% (32). Finally, response rates of patients with refractory/refractory relapsed indolent NHL [including follicular, marginal zone, and (SLL) small lymphocytic lymphoma] to single-agent bortezomib are approximately 13.3% (33). It is clear that the limited number of patients entered in this trial do not permit firm conclusions to be drawn regarding the activity of this regimen in specific disease entities, or the relative efficacy of the alvocidib/bortezomib regimen compared with bortezomib alone. Nevertheless, the responses obtained, particularly in patients with multiple myeloma, are encouraging and support further investigation of this approach to determine whether this strategy may be of benefit for patients with advanced disease, particularly those who have received prior bortezomib therapy.

Pharmacokinetic studies were conducted on samples obtained from 13 of the 16 patients enrolled on the study. These studies revealed statistically significant correlations between the loading dose and the $C_{\text{max}}$ and between the total dose and the AUC. The former is consistent with results obtained with bolus schedules (34). The lack of correlation between dose and clearance suggests linear pharmacokinetics and is also in accord with findings obtained in studies involving bolus administration. Finally, in this relatively small patient population, the hybrid schedule did not clearly increase exposure to or maximal plasma alvocidib concentrations compared with results previously obtained with bolus administration (34). The clinical implications of these pharmacokinetic observations remain to be determined in a larger population.

Due to the small sample size and variable response pattern of the pharmacodynamic markers, no generalizations can be made concerning correlations (or lack thereof) between pre- and posttreatment changes in the expression of various stress and apoptotic regulatory proteins and clinical outcomes in this phase I trial. In human leukemia cells, coadministration of alvocidib and bortezomib in vitro led to NF-$k$B inactivation, downregulation of multiple NF-$k$B–dependent proteins (e.g., XIAP, and Bcl-xL) as well as the pTEFb-dependent protein McI-1, and activation of the JNK-related stress pathway (22, 23). The failure to observe such anticipated changes consistently in patient-derived CD138$^+$ myeloma cells pre- and posttreatment could reflect cell-type-specific differences between the responses of myeloma versus leukemia cells to this regimen, methodologic artifacts (i.e., due to freezing and storage of pellets), the purity of the CD138$^+$ cells obtained in the enrichment process, the failure to achieve sufficiently high concentrations of alvocidib and/or bortezomib in vivo, or a combination of these factors. In this context, the relative merits of Western blot analysis versus quantitative fluorescence analysis also remain to be determined. The latter strategy may be more feasible under circumstances in which only a limited number of tumor cells are available. In any event, correlations between candidate pharmacodynamic markers and clinical outcomes will best be determined in the setting of successor phase II trials involving a substantially larger number of patients as well as uniform drug doses.

In conclusion, this phase I study has determined the MTD for combination alvocidib/bortezomib therapy and has shown this schedule to be tolerable in patients with refractory/refractory relapsed multiple myeloma, follicular lymphoma, or mantle cell lymphoma. The observed hematologic and nonhematologic toxicities are similar to those previously observed in trials involving bortezomib therapy alone. Importantly, the alvocidib/bortezomib regimen resulted in 2 CRs and 5 PRs in a heavily pretreated patient population. In view of the small number of patients studied, however, a phase II study will be required to determine whether the addition of alvocidib to bortezomib offers the potential for improved efficacy compared with historical results with bortezomib alone (5, 7, 33). Finally, a residual question is whether employing the hybrid infusional schedule of alvocidib in conjunction with bortezomib offers advantages over a more standard bolus administration schedule in this patient population. Although the former regimen has shown impressive activity in patients with high-risk CLL (20), it remains to be determined whether it will exhibit similar activity in B-cell malignancies other than CLL, or whether it is optimally designed to enhance bortezomib efficacy. To address this issue, a companion phase I trial has been initiated in an identical patient population in which bortezomib given on days 1, 4, 8, and 11 is administered in combination with escalating doses of alvocidib given as a 1-hour infusion, also on days 1, 4, 8, and 11. It is anticipated that results of this trial will help determine which of these regimens should be evaluated in the phase II setting.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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