Bortezomib and gemcitabine in relapsed or refractory Hodgkin’s lymphoma


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Background: Given the significant activity and tolerability of gemcitabine in patients with relapsed Hodgkin’s lymphoma (HL), the critical role that nuclear factor kappa B (NF-κB) appears to play in the pathogenesis of this tumor, the ability of bortezomib to inhibit NF-κB activity, and laboratory studies suggesting synergistic antitumor effects of gemcitabine and bortezomib, we hypothesized that this combination would be efficacious in patients with relapsed or refractory HL.

Patients and methods: A total of 18 patients participated. Patients received 3-week cycles of bortezomib 1 mg/m² on days 1, 4, 8, and 11 plus gemcitabine 800 mg/m² on days 1 and 8.

Results: The overall response rate for all patients was 22% (95% confidence interval 3% to 42%). Three patients developed grade III transaminase elevation: one was removed from the study and two had doses of gemcitabine held. Almost all patients exhibited inhibition of proteasome activity with treatment.

Conclusions: The combination of gemcitabine and bortezomib is a less active and more toxic regimen in relapsed HL than other currently available treatments. It poses a risk of severe liver toxicity and should be pursued with caution in other types of cancer.

Key words: bortezomib, gemcitabine, hepatotoxicity, Hodgkin’s lymphoma, proteasome, relapsed

introduction

The majority of patients with Hodgkin’s lymphoma (HL) are cured with first-line therapy; however, approximately 35%–40% of patients with stages III–IV HL and risk factors relapse after first-line therapy [1, 2]. Current practice in patients with relapsed or refractory HL dictates the prompt use of second-line, salvage chemotherapy and autologous stem-cell transplant (ASCT), which cures a minority of patients. Therefore, new treatment options are needed to improve induction therapy for high-risk patients, as well as for salvage before and after ASCT.

A hallmark of malignant Reed–Sternberg cells in classical HL is constitutive activity of the transcription factor nuclear factor kappa B (NF-κB) [3, 4]. NF-κB activity is thought to mediate resistance to standard therapeutic approaches. Inhibition of NF-κB has been shown to induce apoptosis in cell lines and primary cultures in HL [5]. Thus, we postulated that strategies targeting NF-κB activity in patients with HL would have clinical utility.

Bortezomib (Velcade, Millennium Pharmaceuticals) is a proteasome inhibitor that has been shown to inhibit NF-κB activity [6–8]. It is currently approved in the United States for treatment of multiple myeloma and relapsed/refractory mantle cell lymphoma [9]. Bortezomib markedly enhances the apoptotic effects of radiation, chemotherapy, and mAb therapy [10, 11]. This enhancement of apoptosis is hypothesized to be partially due to its inhibitory effects on NF-κB. By inhibiting the proteasome, bortezomib causes stabilization of inhibitor of kappa B alpha, an NF-κB inhibitory protein, thereby reducing NF-κB activity.

Gemcitabine (Gemzar, Eli Lilly) is a deoxycitidine analogue, which requires intracellular phosphorylation to inhibit DNA synthesis [12]. Several studies have suggested significant activity in patients with newly diagnosed [13] or relapsed/refractory HL [14, 15]. A multicenter trial of 23 patients with relapsed or refractory HL demonstrated an overall response rate of 39%, with minimal nonhematopoietic toxicity [16]. The combination of gemcitabine and bortezomib has been shown to have more antitumor activity than either agent alone in multiple types of tumor cell lines and mouse xenografts [17–22]. Recently, a phase I study of bortezomib and gemcitabine was completed in patients with advanced solid tumors [23]. Dose-limiting toxic effects included thrombocytopenia and leukopenia, and the maximum tolerated dose of a 21-day cycle was determined to be gemcitabine 1000 mg/m² (days 1 and 8) and bortezomib 1.0 mg/m² (days 1, 4, 8, and 11).
Given the significant activity and tolerability of gemcitabine in patients with relapsed HL, the critical role that NF-κB appears to play in the pathogenesis and chemotherapy resistance of this tumor, and laboratory studies showing more antitumor activity of gemcitabine + bortezomib relative to either agent alone, we sought to determine whether this combination was efficacious in patients with relapsed or refractory HL. In addition, we sought to determine toxicity in this population and to correlate molecular effects on proteasome activity with clinical response.

**patients and methods**

This trial has been registered in the clinicaltrials.gov database; identifier: NCT00262860. It was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

**patient selection**

Eligible patients had relapsed or refractory classical HL (histologically confirmed) after standard combination chemotherapy, age 18 years or older, Eastern Cooperative Oncology Group performance status of zero to two, measurable disease, adequate renal function, total bilirubin <2 × upper limit of normal, and transaminases <3 × upper limit of normal.

Exclusion criteria included history of non-Hodgkin’s lymphoma or other hematological malignancy, concomitant active malignancy requiring therapy, history of allogeneic stem-cell transplant, history of ASCT within the previous 6 months, and prior treatment with bortezomib or gemcitabine.

**treatment plan**

Patients were to receive at least two cycles of therapy with gemcitabine/bortezomib. Restaging evaluations for responses were carried out 1–2 weeks after the last infusion of the second cycle of therapy. Patients with disease progression were removed from the study. Patients with stable or responding disease could either proceed to ASCT or be given up to four additional cycles of therapy. Bortezomib (provided by Millenium Pharmaceuticals) was administered at a dose of 1 mg/m² on days 1, 4, 8, and 11 of a 21-day schedule. Gemcitabine was administered at a dose of 800 mg/m² on days 1 and 8 of the same 21-day schedule. All dosing was determined solely by body surface area as calculated from actual weight.

**patient evaluation**

All patients had pretreatment histories, physical examinations, pulmonary function tests, computed tomography (CT) scans of the chest/abdomen/pelvis, bone marrow aspirations, and biopsies. A subset of patients had 2-[fluorine-18]-fluoro-2-deoxy-D-glucose (FDG)–positron emission tomography (PET) scans carried out before and after therapy. Patients were seen during the study on days 1 and 8 of each cycle for physical examination, laboratory evaluation, and toxicity assessment. Common toxicity criteria (version 3) were used for all symptoms.

**toxicity**

There were no dose modifications of bortezomib and gemcitabine for hematological toxic effects. On day 1 of each cycle, therapy was held for absolute neutrophil count (ANC) < 1000/μl and platelet count < 100,000/μl; on day 8 of each cycle, therapy was held for ANC < 500/μl and platelet count < 75 000/μl. Bortezomib and gemcitabine were held for any grade III or IV non-hematological toxic effects, if possibly due to treatment. Once toxicity had reversed to grade 2 or better, treatment was resumed, with a dose reduction of bortezomib to 0.7 mg/m². If treatment was delayed by >3 weeks on any occasion, or >2 weeks on more than one occasion, the patient was removed from the study.

**response**

Response was evaluated after two cycles of therapy using the 1999 Cheson response criteria [24]. All responses were based on CT scans. For patients who had FDG–PET imaging, metabolic response was defined as a decrease in the standardized uptake value in target lesions (regions of abnormal FDG uptake on pretreatment FDG–PET images) to below three on post-treatment FDG–PET imaging. All PET scans were reviewed and interpreted by a single radiologist (SV).

**proteasome activity analysis**

Peripheral blood (~40 ml) was collected on cycle 1, day 1 prebortezomib and 2 h post-bortezomib treatment and 1–2 weeks after cycle 2, day 11. The samples were refrigerated at 4°C and processed within 36 h of collection. Frozen cell lysates were thawed and the proteasome activity in 10 μl was determined using a spectrofluorometric 20S proteasome assay kit (Millipore Corporation, Billerica, MA). Samples were run in triplicate on two separate days.

**statistical considerations**

The primary objective was to determine whether two cycles of the gemcitabine–bortezomib combination was superior to gemcitabine alone in this patient population, as previously reported [16]. We planned to enroll 24 subjects into a phase II pilot study to address this objective. Using the one-sided exact binomial test at the 0.05 level of significance, we would have rejected the null hypothesis (response rate is 39%), in favor of the alternative hypothesis that the response to the gemcitabine–bortezomib combination is greater, if we had observed 14 (58%) or more responses. Percent change in proteasome activity was compared among response groups using the Kruskal–Wallis statistic.

**results**

Eighteen patients were registered to the trial. The trial was stopped early by the Data Safety Monitoring Committee due to unexpected toxicity with no obvious improvement in the response rate compared with that previously reported with gemcitabine alone.

**patient characteristics**

The median age upon entry was 36 years (range 19–62, Table 1). Three patients had progressed within 3 months of their most recent treatment (refractory disease). Fifteen patients had recurrent disease. Fifteen patients had prior induction with ABVD and three with Stanford V. Salvage regimens included ChiVPP, ESHAP, and DHAP. Ten patients had a history of prior radiation therapy and six had a history of ASCT.

**treatment**

Of 18 patients, two patients were unable to complete two cycles of therapy. One had liver toxicity and one had thrombocytopenia that precluded further treatment. Fifteen patients completed two cycles of therapy. One patient completed more than two cycles of therapy. Twelve of the patients completing at least two cycles did so without missed doses or delays. Four patients missed one dose; in all further analyses, these patients are considered to have completed two...
cycles of therapy. Of the patients missing one dose, two had cycle 2, day 8 doses of gemcitabine held due to liver toxicity, one had a cycle 2, day 8 dose of gemcitabine held due to neutropenia, and one had a cycle 1, day 11 dose of bortezomib held due to inclement weather.

**response**

Four patients out of the 18 enrolled [22%; confidence interval (CI) 3% to 42%] responded [one complete response (CR); three partial responses (PR)] to two cycles of therapy. Seven patients [three with PRs, three with stable disease (SD), and one with progressive disease (PD)] had FDG–PET/CT imaging carried out before and after therapy. Four of the seven patients (the three patients with PRs and one patient with SD) demonstrated a metabolic response. Among the patients with CR, PR, or SD, seven patients proceeded directly to stem-cell mobilizations and autologous transplant (SCT), four patients received additional salvage chemotherapy before SCT, and one patient received three further cycles of gemcitabine + bortezomib (Figure 1). Four patients with PD received salvage chemotherapy followed by SCT. Among the two patients who did not complete two cycles of therapy, one patient developed lung cancer and was started on appropriate therapy for this, and one patient received salvage chemotherapy followed by SCT. Peripheral blood stem-cell mobilization was effective in all but one patient undergoing SCT. That patient required bone marrow harvest. As of November 2007, 16 of 18 patients were still alive.

**adverse events**

The most common adverse events were neutropenia, leukopenia, thrombocytopenia, anemia, elevated transaminases, pain, and hypocalcemia (Table 2). Grade III/IV adverse events either clearly related to or probably related to therapy included the cytopenias and the elevated transaminases. In the patients with high-grade transaminase elevation, pretreatment transaminase levels were normal, hepatitis serologies were negative, and there was no report of preexisting liver disease or heavy alcohol use. Retrospective analysis of pretreatment imaging in these patients reveals that all had fatty infiltration of their livers. In total, five of 18 patients enrolled had fatty livers. The cause was likely obesity in four of the five (mean body mass index in fatty liver patients: 39.5 versus nonfatty liver patients: 24.7). Three of the five developed grade III transaminase elevation and two of five developed grade I transaminase elevation. None of the patients without fatty livers had therapy-limiting transaminase elevations.

**proteasome activity and correlation to clinical response**

Following the first dose of bortezomib, 15 of 17 patients had less proteasome activity compared with baseline (median change −50%, range −77% to +39%, Figure 2). After two cycles of therapy, most patients continued to have less proteasome activity compared with baseline (median change −57%, range −92% to +223%, Figure 2). To correlate changes in proteasome activity with clinical response, patients were grouped as responders (CR, PR, SD) or nonresponders (PD). There was no difference in proteasome response between groups after the first dose of bortezomib (median change in responders −50.3% versus nonresponders −56.9%, P = 0.49) or after two cycles of therapy (median change in responders −63.9% versus nonresponders +51.3%, P = 0.14). Two patients with PD had profoundly higher levels of proteasome activity after two cycles (Figure 2).

**discussion**

Our study did not demonstrate synergy between gemcitabine and bortezomib in vivo. The response rate to the combination of gemcitabine + bortezomib (22%) was not clearly better than to gemcitabine alone in a similar patient population [16]. It is possible that the assessment of clinical response after two cycles of therapy was too early to detect a significant proportion of patients who would have eventually responded.
However, recent data from the CALGB showed that with the combination of gemcitabine, vinorelbine, and pegylated liposomal doxorubicin, clinical responses were seen after two cycles of therapy [25]. Second, it is possible that the close timing of bortezomib and gemcitabine administration in our study was suboptimal. We chose this timing as previously described [23]. There are laboratory studies suggesting that the combination of gemcitabine and bortezomib may work optimally when gemcitabine treatment precedes bortezomib [18]. Third, it is possible that the lower dose of gemcitabine used in our study (800 mg/m²) negatively affected the response rate relative to the higher dose used by Santoro et al. [16] (1250 mg/m²).

Recently, Strauss et al. [26] found that in patients with relapsed lymphoma treated with bortezomib, in vitro sensitivity of primary patient lymphoma cultures to bortezomib and plasma tumor necrosis factor-alpha levels correlated with clinical response. We investigated whether proteasome inhibition would correlate with clinical response. Despite a majority of patients exhibiting proteasome inhibition with treatment, clinical responses were only observed in a minority of patients. Since our measurements were in peripheral blood cells, it is possible that the proteasome was not adequately inhibited in the tumor itself. Unfortunately, molecular analysis of Reed–Sternberg cells is complicated by the paucity of these cells in patients with HL.

It is also possible that NF-κB activity is unaffected by proteasome inhibition in Reed–Sternberg cells. Consistent with this idea are recent publications from the CALGB [27], and by Trelle et al. [28], that demonstrate no single-agent activity of bortezomib against HL.

The most surprising finding in this study was the high incidence of severe transaminase elevation. Previous clinical studies looking at the combination of gemcitabine and bortezomib have not reported the frequency of high-grade transaminase elevations seen in our study [22, 29]. Since the combination of these two agents is under active clinical investigation in different cancers, it is imperative that others be aware of this potentially dangerous side-effect. Fortunately, liver function normalized in all patients without additional intervention. It is of note that the only patients who had severe transaminase elevations in this study also had fatty infiltration.

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Table 2. Adverse events (N = 18 patients)

<table>
<thead>
<tr>
<th>Event</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Total</th>
<th>% Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>56</td>
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<tr>
<td>Leukopenia</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>56</td>
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<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Elevated transaminases</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain/cramps</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache/migraine</td>
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<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>1</td>
<td>1</td>
<td>6</td>
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</tr>
<tr>
<td>DVT near line</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worsening leg ulcer</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>6</td>
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<td>8</td>
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<td></td>
</tr>
<tr>
<td>Pain (various)</td>
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<td>4</td>
<td>7</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>6</td>
<td>6</td>
<td>33</td>
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<td></td>
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</tr>
</tbody>
</table>

All grade 3 and 4 events are presented, as well as grade 1 and 2 events occurring in ≥30% of the patients.

*Events graded using National Cancer Institute Common Toxicity Criteria version.

This patient was diabetic.

This patient had appendicitis.
of their livers. It is possible that the fatty infiltration *per se* predisposed the liver to gemcitabine/bortezomib-induced hepatotoxicity. Alternatively, since most of the patients with fatty livers were obese and received higher absolute doses of these agents, it is possible that the higher absolute doses contributed to the hepatotoxicity. Given the 17% to 33% estimated prevalence of nonalcoholic fatty liver disease (NAFLD) in Americans [30], it will be important to further explore whether a true relationship exists between cancer therapy-induced hepatotoxicity and NAFLD in the future.

We conclude that the combination of gemcitabine and bortezomib should not be pursued in HL, and caution should be exercised when combining these agents in other diseases due to potential for severe liver toxicity. As other studies have suggested no significant activity of bortezomib in HL, attempts to target the NF-κB pathway should utilize other agents, including novel proteasome inhibitors.

**funding**

Millenium Pharmaceuticals (Cambridge, MA) and Eli Lilly (Indianapolis, IN) to JWF; National Cancer Institute (CA-102216) to JWF.

**references**


**Figure 2.** Proteasome activity following gemcitabine and bortezomib. Percent proteasome activity, relative to baseline activity, at day 1 (blood drawn 2 h post-bortezomib, pre-gemcitabine) and ~2 weeks after two complete cycles of therapy. Proteasome inhibition is presented for each of *N* = 17 patients with measured proteasome activity, and line patterns are coded for response after two cycles. PD, progressive disease; SD, stable disease; PR, partial response; CRu, complete response unconfirmed.


