Vorinostat Combined with High-Dose Gemcitabine, Busulfan, and Melphalan with Autologous Stem Cell Transplantation in Patients with Refractory Lymphomas

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INTRODUCTION

High-dose chemotherapy (HDC) with regimens such as carmustine/etoposide/cytarabine/melphalan (BEAM) with autologous stem cell transplantation (ASCT) is standard treatment of chemosensitive relapsed lymphomas. Early randomized trials showed benefit from HDC in chemosensitive relapsed diffuse large B cell lymphoma (DLCL) in the pre-rituximab era, as well as in Hodgkin’s lymphoma (HL) [1-3]. However, the more recent CORAL trial showed worse outcomes after BEAM ± rituximab in DLCL patients who relapsed after rituximab-cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) than previously seen in patients who relapsed after CHOP alone [4]. Furthermore, particularly poor outcomes were seen in patients with primary refractory or relapsed tumors with poor-risk features, such as a first complete remission (CR) < 12 months, secondary International Prognostic Index (IPI) at

More active high-dose regimens are needed for refractory/poor-risk relapsed lymphomas. We previously developed a regimen of infusional gemcitabine/busulfan/melphalan, exploiting the synergistic interaction. Its encouraging activity in refractory lymphomas led us to further enhance its use as a platform for epigenetic modulation. We previously observed increased cytotoxicity in refractory lymphoma cell lines when the histone deacetylase inhibitor vorinostat was added to gemcitabine/busulfan/melphalan, which prompted us to clinically study this four-drug combination. Patients ages 12 to 65 with refractory diffuse large B cell lymphoma (DLCL), Hodgkin (HL), or T lymphoma were eligible. Vorinostat was given at 200 mg/day to 1000 mg/day (days 0 to 3). Gemcitabine was infused continuously at 10 mg/m²/minute over 4.5 hours (days 0 and 8). Busulfan dosing targeted 4000 μM-minute/day (days 0 to 5). Melphalan was infused at 60 mg/m²/day (days 3 and 2). Patients with CD20+ tumors received rituximab (375 mg/m², days 1 and 8). We enrolled 78 patients: 52 DLCL, 20 HL, and 6 T lymphoma; median age 44 years (range, 15 to 65); median 3 prior chemotherapy lines (range, 2 to 7); and 48% of patients had positron emission tomography–positive tumors at high-dose chemotherapy (29% unresponsive). The vorinostat dose was safely escalated up to 1000 mg/day, with no treatment-related deaths. Toxicities included mucositis and dermatitis. Neutrophils and platelets engrafted promptly. At median follow-up of 25 (range, 16 to 41) months, event-free and overall survival were 61.5% and 73%, respectively (DLCL) and 45% and 80%, respectively (HL). In conclusion, vorinostat/gemcitabine/busulfan/melphalan is safe and highly active in refractory/poor-risk relapsed lymphomas, warranting further evaluation.

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KEY WORDS: Vorinostat, Phase 1 trial, High-dose chemotherapy, Autologous stem-cell transplantation, Lymphoma
relapse > 1, exposure to multiple salvage regimens, or the presence of active tumor at HDC [5-8]. Similar adverse prognostic factors have been described for HL [9,10]. Therefore, there is a clear need to develop more effective HDC regimens for these patient populations.

We previously developed a high-dose combination of gemcitabine/busulfan/melphalan (Gem/Bu/Mel), which showed a synergistic interaction based on gemcitabine inhibition of DNA damage repair [11]. Gemcitabine was infused at a fixed dose rate of 10 mg/m²/minute, optimizing formation of its active intracellular triphosphate metabolite [12,13]. Busulfan was administered intravenously with pharmacokinetic-guided dosing. Gem/Bu/Mel showed encouraging activity in lymphomas. An analysis of concurrent patient cohorts with refractory HL treated with Gem/Bu/Mel, or BEAM, showed superior outcomes in the Gem/Bu/Mel cohort despite a higher prevalence of poor-prognosis features [14].

An important factor affecting the activity of DNA-targeting agents is access to DNA. Changes in histone acetylation lead to changes in chromatin configuration. Inhibition of histone deacetylases weakens the histone-DNA bonds and decondenses chromatin [15]. In our prior preclinical experiments in resistant B and T lymphoma cell lines, a synergistic increase in cytotoxicity was observed when the histone deacetylases inhibitor vorinostat (suberanilohydroxamic acid) was added to gemcitabine/busulfan/melphalan combination [16]. We saw a significant increase in the γ-H2AX level and cleavage of poly-[ADP-ribose]-polymerase-1 (PARP-1), suggesting increased DNA damage response and apoptosis, respectively [17]. Furthermore, there was important sequence specificity, and concurrent treatment was more active than sequential drug exposures.

These intriguing preclinical observations motivated us to test vorinostat/Gem/Bu/Mel clinically. Prior studies have shown that the duration of histone hyperacetylation is proportional to vorinostat dose [18]. We hypothesized that vorinostat can be safely combined with Gem/Bu/Mel, based on dose-limiting toxicity (DLT), defined as any grade 4 nonhematological and noninfectious toxicity, or any grade 3 mucositis or skin toxicity lasting more than 3 days at peak severity. Secondary endpoints included event-free survival (EFS), overall survival (OS), response, and CR rates and to describe the toxicity profile. Dose finding used the continual reassessment method with a target DLT probability per cohort of 20% [22]. In stage 1 of the trial, doses were chosen adaptively for successive cohorts of minimum size 2, up to dose level 11 with a MPD of vorinostat of 1000 mg/day (Table 2). If the lowest dose level was found to be excessively toxic, the trial would move to stage 2 using a lower dose of gemcitabine. Toxicity scoring followed the NCI Common Toxicity Criteria, v3.0 [23].

**Correlative Studies of DNA Damage Repair and Apoptosis**

The phosphorylation status of histone H2AX (γ-H2AX) and the level of PARP-1 were determined in peripheral blood mononuclear cells from patients enrolled in this study at the highest dose of suberanilohydroxamic acid (1000 mg/day) and in separate trials of Gem/Bu/Mel and Bu/Mel, all of which followed a parallel schedule from day 8 to 2 using the same drug doses as in the present studies. Samples were collected at baseline, day −7 (1 hour after busulfan), and day −2 (1 hour after melphalan). The γ-H2AX level was measured by immunofluorescent staining and flow cytometry using anti-γ-H2AX antibody (EMD Millipore, Billerica, MA) [24]. Western blot assay used protein extract separation on polyacrylamide-SDS gels and blotting onto nitrocellulose membranes probed with anti–PARP-1 (Santa Cruz, Dallas, TX) and anti–β-actin (Sigma-Aldrich, St Louis, MO) antibodies. Chemiluminescence immunoblot analysis used Immobilon Western Chemiluminescent HRP Substrate (EMD Millipore). X-ray films were scanned with the EPSON Perfection V750 PRO and analyzed with UN-SCAN-IT software (Silk Scientific, Orem, Ut). PARP-1 bands were normalized to β-actin.
Dose Escalation

<table>
<thead>
<tr>
<th>Level</th>
<th>Vorinostat (mg/day)</th>
<th>Gemcitabine (mg/m²/day)</th>
<th>Busulfan (µg/min/day) or mg/m²/day</th>
<th>Melphalan (mg/m²/day)</th>
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<td>Loading Dose</td>
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Continuous infusion of gemcitabine administered at 10 mg/m²/minute.

Statistical Methods

Overall response (OR) and CR rates were calculated among patients with measurable disease at HDC following the usual criteria [25]. EFS was defined as the time from transplantation to either relapse or death, whichever occurred first, or last contact. OS was defined as the time from transplantation to death or last contact. Kaplan-Meier survival curves estimated unadjusted time-to-event distributions [26]. The log-rank test was used to compare EFS and OS between subgroups [27]. Categorical variables were compared by generalised Fisher’s exact test [28]. P values are 2 sided. All calculations used R ver 2.12.1 and OpenBUGS v3.1.2 rev668.

RESULTS

Patient Enrollment

Seventy-eight patients were enrolled between September 2011 and November 2013 (Table 3). Median age was 44 (range, 15 to 65). Diagnoses were DLCL (n = 52), HL (n = 20), and T non-Hodgkin lymphoma (T-NHL) (n = 6). Patients had received a median of 3 prior regimens (range, 2 to 7) and had extensive tumor involvement (median, 3 organs). Of 52 patients with DLCL, 26 had primary refractory tumors, 13 were in first relapse (6, 6, and 1 patients had a secondary IPI of 0 to 1, 2 to 3, and >3, respectively), and 13 had >1 prior relapse. Ten patients had double-hit DLCL. In the HL subgroup, 13 patients (65%) had a primary refractory tumor, and extranodal disease and bulky tumor at relapse/progressive disease (PD) were present in 12 and 11 patients, respectively; 6 patients had >1 prior relapse. Four of 6 patients with T-NHL had primary refractory disease. At HDC, 48% of all patients had PET-positive tumors and 26% of patients had unresponsive disease (PD in 17% of them).

Hematologic Recovery

The stem cell source was peripheral blood. Neutrophil and platelets engrafted promptly at medians of 10 days (range, 8 to 13) and 12 days (range, 8 to 55), respectively.

Regimen-Related Toxicities

Dose escalation of vorinostat proceeded from 200 mg/day (dose level 1) up to its MPD of 1000 mg/day (dose level 11), combined with Gem/Bu/Mel (Table 2). The MPD level was expanded to 22 patients to fully characterize its toxicity profile. No regimen-related deaths or grade 4 toxicities were seen in the study. The side effect profile of vorinostat/Gem/Bu/Mel was as follows.

Mucositis

Grade 2 and 3 mucositis were observed in 45% and 32% of patients, respectively, in levels 1 to 10, and in 48% and 38% of patients, respectively, at level 11. It started at median day +4 (range, day 0 to +7), lasting at maximal severity for a median of 3 (range, 1 to 9) days.

Dermatitis

Grade 1 and 2 erythematous rashes were common. Six patients had a grade 3 rash, 2 of them at level 11. All cases resolved spontaneously or with topical sunburn remedies or topical steroids.

Hepatic effects

Early self-limited transaminase elevation was frequent across all levels (6 patients grade 3, 21 patients grade 2), starting on median day –1 (range, day –7 to 9), peaking at a...
median 146 (range, 43 to 930) IU/L and resolving within 1 week. Transient hyperbilirubinemia at median 2.1 mg/dL, (range, 1.2 to 9.7) was seen in 51% patients in the first week after transplantation (12 patients grade 3, 19 patients grade 2), with no cases of veno-occlusive disease.

**Pulmonary effects**

DLCO decreased from before (median, 78.5% of predicted; range, 52% to 152%) to 1 to 3 months after transplantation (median, 71%; range, 31% to 150%) (P = .002). There were 3 symptomatic cases of steroid-responsive grade 2 pneumonia.

**Other toxicities**

Diarrhea was mild, with only 12 and 4 cases of grade 2 and grade 3 diarrhea, respectively. Two patients experienced grade 2 renal toxicity. No neurological or cardiac toxicities were observed. Specifically, there was no QTc prolongation from before HDC (median, 462; range 425 to 599 milliseconds) to day −2 (median, 451; range 418 to 497 milliseconds).

There was no correlation of prednimisone values of C-reactive protein, B-type natriuretic peptide, ferritin, haptoglobin, or troponin with toxicity (data not shown). Likewise, there was no significant effect of age on toxicity (data not shown).

**Infections**

Two patients had antibiotic-responsive methicillin-resistant *Staphylococcus aureus* pneumonia. The following documented infections resolved with antimicrobials: *Candidemia* (n = 1), *Stenotrophomonas* bacteremia/urinary tract infection (UTI) (n = 1), *S epidermidis* bacteremia (n = 1), *cytomegalovirus* pneumonia (n = 1), respiratory syncytial virus group A respiratory infections, Herpes simplex esophagitis (n = 1), *Enterococcus* UTI (n = 1), and *Pseudomonas aeruginosa* UTI (n = 1).

**Busulfan Pharmacokinetic Studies**

Busulfan pharmacokinetics were calculated in all patients. The overall mean of the variation of the calculated test-to-therapeutic clearance was 6.3% (95% confidence interval, −9.9% to 19.5%). Only 3 patients showed a busulfan clearance >20% different when the first and test doses were compared. For the remaining 75 patients, the clearance variation between the test and the first therapeutic doses was <20%. The mean (% coefficient of variation) population clearance, volume of distribution, and plasma half-life from the first therapeutic dose were 97 mL/minute/m² (16.3%), 24.2 L/m² (16%), and 2.9 hours (14.7%), respectively. These population pharmacokinetics do not differ from those previously estimated with Bu/Mel [21] or Gem/Bu/Mel [11] (data not shown).

**Tumor Responses**

The OR and CR rate among 28 patients with DLCL and measurable disease were 96% and 73%, respectively. The OR and CR rate among 14 patients in PD at the time of HDC were 100% and 64%, respectively.

Eight of 10 patients with measurable HL (5 of them in PD at HDC) had a CR. Two of 2 patients with measurable T-NHL (both in PD at HDC) experienced a CR.

**Post-HDC Treatment**

Seventeen patients (9 DLCL, 6 NHL, 1 T-NHL, 1 plasmablastic lymphoma [PL]) with pretransplantation bulky (>5 cm) PET-positive lesions received involved site radiotherapy to 30.6 to 42 Gy starting 1 to 2 months after transplantation, with good tolerance. Irradiated sites were mediastinum (n = 8), axillary/supraclavicular lymph nodes (n = 3), chest wall (n = 2), thigh (n = 2), hip (n = 1), and splenectomy bed (n = 1).

**Patient Outcomes**

Median follow-up is 25 months (range, 16 to 41). Only 1 relapse has occurred beyond 1 year after HDC. The EFS and OS rates of the DLCL group were 61.5% and 73%, respectively (Figure 1A). The fraction of patients alive in CR within the different DLCL subgroups are 6 of 8 primary mediastinal, 6 of 10 double hit, 4 of 4 T cell rich, 2 of 2 plasmablastic, and 2 of 2 primary central nervous system. As per cell-of-origin type, 10 of 15 patients in the germinal center and 8 of 12 patients in the activated B cell categories remain in CR.

The EFS and OS rates among HL patients were 45% and 80%, respectively (Figure 1B). Lastly, 5 of 6 patients with T-NHL were alive in CR at 16 to 29 months after HDC.

As expected, patients with PET-positive tumors had worse EFS than those with PET-negative tumors at HDC, both in the DLCL (50% versus 81%, P = .01) (Figure 2A) and HL groups (27% versus 73%, P = .04) (Figure 2B).

**DNA Damage Response and Apoptosis Studies**

The levels of γ-H2AX increased after Bu/Mel (n = 19) from baseline to day −2 by a median 1.4-fold (Figure 3A). γ-H2AX increased after Bu/Mel (n = 20).
most relapses in these high-risk populations typically occur early after transplantation [4-10]. Given the limitations of this study, intrinsic to early clinical trials, these results should be considered preliminary. Determining whether vorinostat/Gem/Bu/Mel is superior to BEAM for any type of lymphoma will need a randomized study.

To our knowledge, this is the first report of the combination of an epigenetically active agent with HDC. This study builds on our prior clinical work with Gem/Bu/Mel, a regimen designed to exploit the synergistic inhibition exerted by gemcitabine on DNA damage repair [11]. The use of peripheral blood progenitor cells circumvents the increased myelotoxicity of infusional gemcitabine, allowing us to capitalize on its increased antitumor activity, compared to shorter infusions of this drug. Our prior observations of high activity of Gem/Bu/Mel in lymphoid malignancies prompted us to use this regimen as a platform for epigenetic HDC modulation. We previously observed that vorinostat augmented the in vitro cytotoxicity of gemcitabine, busulfan, and melphalan in resistant lymphoma B cell and T cell lines [16]. This effect could be, at least in part, mediated through activation of the DNA damage response signaling pathway, as shown by increased γ-H2AX [16]. The present observations in patient samples are also indicative of increased genomic injury that may, among other responses, lead to apoptosis. In our previous preclinical experiments, apoptosis was detected by cleavage of PARP-1. Interestingly, patients enrolled in this study experienced a decrease or disappearance of PARP-1, rather than its cleavage. Because PARP-1 is a DNA-repair protein [29], our present observations indicate a serious compromise of the ability of tumor cells to repair drug-induced damaged DNA. Because vorinostat has multiple effects on protein acetylation besides histone acetylation, other potentiating mechanisms of the effect of Gem/Bu/Mel are also possible.

Previous reports of vorinostat combined with standard-dose chemotherapy in patients with various tumors had shown more prolonged myelosuppression but no significant increase of nonhematological toxicities [30-33]. The side effects of vorinostat/Gem/Bu/Mel in our study were manageable, including mucositis, dermatitis, and clinically silent elevation of liver function tests. This toxicity profile is similar to that we previously described with Gem/Bu/Mel [11]. Buglio et al. reported an in vitro dose response effect of vorinostat in lymphoma cell lines from 1 μM up to 5 μM [34]. By simple extrapolation from this range of in vitro levels, we estimated the dose escalation of vorinostat from 200 μM to a MPD of 10 μM [34]. This study builds on our prior clinical work with Gem/Bu/Mel, a regimen designed to exploit the synergistic inhibition exerted by gemcitabine on DNA damage repair [11]. The present observations in patient samples are also indicative of increased genomic injury that may, among other responses, lead to apoptosis. In our previous preclinical experiments, apoptosis was detected by cleavage of PARP-1. Interestingly, patients enrolled in this study experienced a decrease or disappearance of PARP-1, rather than its cleavage. Because PARP-1 is a DNA-repair protein [29], our present observations indicate a serious compromise of the ability of tumor cells to repair drug-induced damaged DNA. Because vorinostat has multiple effects on protein acetylation besides histone acetylation, other potentiating mechanisms of the effect of Gem/Bu/Mel are also possible.

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We did not observe DLTs at the MPD of vorinostat. It would be useful to identify predictive markers of toxicity. Previous reports have correlated high pretransplantation values of C-reactive protein, ferritin, or brain natriuretic peptide (BNP) with severe transplantation-related toxicity [35-37]. Likewise, haptoglobin levels have been inversely associated with gemcitabine hematological toxicity [38]. In contrast, we could not establish any correlation between those markers, or any patient characteristic, and toxicity. It is possible that polymorphic genetic variation of relevant enzymes involved in the metabolism of Gem/Bu/Mel might predict toxicity.

**DISCUSSION**

Our study shows that vorinostat can be safely added to Gem/Bu/Mel. The schedule includes a daily dose of vorinostat, at its MPD of 1000 mg/day, preceding full doses of Gem/Bu/Mel.

Confirming our preclinical studies, we saw high activity of vorinostat/Gem/Bu/Mel among patients with heavily pretreated and refractory lymphoma, mostly DLCL and HL. At a median follow-up of 25 months, the EFS rates in these 2 groups are 64% and 45%, respectively. Although longer follow-up is needed, these outcomes are encouraging, as increased on day 2 after Gem/Bu/Mel (n = 16) and vorinostat/Gem/Bu/Mel (n = 18) by 5.8-fold and 5.1-fold, respectively (P < .05 for each of these 2 groups compared to Bu/Mel, P = not significant between them). These results suggest activation of DNA damage response in patients who received Gem/Bu/Mel ± vorinostat, consistent with our previous in vitro data.

The levels of PARP-1 of Bu/Mel patients did not change significantly on day 17 (median, 1.2), and did only slightly on day 2 (median, .5) (Figure 3B). A steeper day 7 drop was observed after Gem/Bu/Mel (median, .5) and vorinostat/Gem/Bu/Mel (median, .4). PARP-1 was undetectable on day 2 in some Gem/Bu/Mel patients (median, 1) and in almost all vorinostat/Gem/Bu/Mel patients (median, 0). The relative baseline/day 2 PARP-1 decrease was more pronounced after vorinostat/Gem/Bu/Mel compared with after Gem/Bu/Mel (P = .05) or Bu/Mel (P = .003).

**Figure 2.** Event-free survival according to PET status at transplantation. (A) DLCL subgroup. (B) HL subgroup.
Finally, busulfan pharmacokinetics were similar to those we previously estimated with Gem/Bu/Mel [11], indicating no pharmacokinetic interaction between busulfan and vorinostat.

In conclusion, vorinostat up to 1000 mg daily can be safely combined with Gem/Bu/Mel with ASCT. This regimen induced high CR rates, promising early outcomes, and encouraging correlative in vitro data from patients with refractory or poor prognosis relapsed Hodgkin’s and NHL. Further investigation of this combination is warranted.

ACKNOWLEDGMENTS

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Conflict of interest statement: There are no potential conflicts of interest.

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