Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma

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Gene expression profiling of diffuse large B-cell lymphoma (DLBCL) has revealed distinct molecular subtypes that include germinal center B cell–like (GCB) and activated B cell–like (ABC) DLBCL. ABC DLBCL has a worse survival after upfront chemotherapy and is characterized by constitutive activation of the antiapoptotic nuclear factor–kappa B (NF-κB) pathway, which can inhibit chemotherapy. We hypothesized that inhibition of NF-κB might sensitize ABC but not GCB DLBCL to chemotherapy and improve outcome. As the proteasome inhibitor bortezomib can inhibit NF-κB through blocking IκBα degradation, we investigated bortezomib alone followed by bortezomib and doxorubicin-based chemotherapy in recurrent DLBCL. Tumor tissue was analyzed by gene expression profiling and/or immunohistochemistry to identify molecular DLBCL subtypes. As a control, we showed that relapsed/refractory ABC and GCB DLBCL have equally poor survivals after upfront chemotherapy. Bortezomib alone had no activity in DLBCL, but when combined with chemotherapy, it demonstrated a significantly higher response (83% vs 13%; $P < .001$) and median overall survival (10.8 vs 3.4 months; $P = .003$) in ABC compared with GCB DLBCL, respectively. These results suggest bortezomib enhances the activity of chemotherapy in ABC but not GCB DLBCL, and provide a rational therapeutic approach based on genetically distinct DLBCL subtypes. This trial is registered with http://www.ClinicalTrials.gov under identifier NCT00057902. (Blood. 2009;113:6069-6076)

Introduction

The diagnosis of diffuse large B-cell lymphoma (DLBCL) is currently made histologically, but molecular profiling has revealed marked heterogeneity within this diagnostic category.[1] Gene expression profiling led to a molecular classification of DLBCL into at least 3 distinct subtypes: germinal center B cell–like (GCB)–, activated B cell–like (ABC)–, and primary mediastinal B-cell lymphoma (PMBL).[2,3] GCB DLBCL appears to arise from germinal center B cells, whereas ABC DLBCL likely arises from postgerminal center B cells that are blocked during plasmacytic differentiation. Genetic analysis has revealed ABC and GCB DLBCL to be pathogenetically distinct diseases: ABC DLBCLs have frequent amplification of the oncogene *SPIB*, deletion of the INK4a/ARF tumor-suppressor locus and trisomy-3 with upregulation of FOXP1, while GCB DLBCLs instead have amplification of the oncogenic mir-17-92 microRNA cluster and deletion of the tumor-suppressor *PTEN* as recurrent events.[4] The NF-κB pathway is constitutively activated in most ABC DLBCL cases, which has been ascribed to activity of a signaling cascade involving CARD11, BCL10, and MALT1, leading to activation of IκB kinase.[5-7] Indeed, 10% of ABC DLBCL cases have somatic mutations in CARD11, a signaling scaffold protein, that cause it to constitutively engage the NF-κB pathway.[6] Inhibition of NF-κB in ABC DLBCL cell lines is toxic, in keeping with the ability of this pathway to inhibit apoptosis.[5,8] Notably, the antiapoptotic effects of NF-κB counteract the action of cytotoxic chemotherapy.[9]

Patients with the newly diagnosed ABC DLBCL subtype have a significantly worse survival than those with GCB DLBCL when treated with standard doxorubicin-based chemotherapy such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), with or without rituximab.[3,10] To date, no therapy has shown greater benefit in ABC DLBCL.[3,10,11] Given the constitutive activity of the NF-κB pathway in ABC DLBCL, we hypothesized that inhibition of NF-κB might sensitize ABC to chemotherapy and improve its clinical outcome compared with GCB DLBCL.[12,13] In vitro, bortezomib, a proteasome inhibitor, blocked degradation of phosphorylated IκBα and consequently inhibited NF-κB activity in ABC DLBCL cell lines (data not shown). We recognized bortezomib has multiple effects but reasoned that its targeted action on NF-κB could be clinically observed above other positive effects it might have in GCB DLBCL.[14,15] Therefore, we investigated if the addition of bortezomib to doxorubicin-based chemotherapy (dose-adjusted infusional etoposide, vincristine, doxorubicin, with cyclophosphamide and prednisone [DA-EPOCH]) would preferentially improve the survival of patients with the ABC DLBCL subtype.[16,17]

Methods

Patients

Eligible patients had relapsed or refractory DLBCL and had received doxorubicin-based treatment. They had an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or less, and adequate organ function (absolute neutrophils $\geq 1000/mm^3$, platelets $\geq 50\,000/mm^3$, and serum creatinine ≤ 1.5 mg/dL). Patients were HIV and hepatitis B surface antigen negative. The study was approved by institutional review boards (Roswell Park Cancer Institute and National Cancer Institute) and conducted in accordance with the Declaration of Helsinki. All patients were required to provide written informed consent.

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Study design and treatment

This multicenter study enrolled 49 patients from 3 centers. Initial evaluation included a history and physical examination, standard blood tests, whole body computed tomography, and bone marrow biopsy. Patients with accessible tumor underwent a fresh tumor biopsy. The study was divided into 2 parts (A and B) as described in Figure 1A. Clinical end points were to assess the activity of bortezomib alone (part A) and in combination with DA-EPOCH (part B), and to assess the toxicity and maximum tolerated dose (MTD) of bortezomib with DA-EPOCH (DA-EPOCH-B).[17] The primary scientific objective was to investigate whether ABC compared with GCB DLBCL was more responsive to DA-EPOCH-B. Because a phase 2 randomized design was not clinically or technically practical at this early stage to address the scientific end points, we designed a novel therapeutic end point based on the relative efficacy of DA-EPOCH-B in ABC and GCB DLBCL. Based on studies that showed that survival with ABC compared with GCB DLBCL was significantly worse after initial standard treatment, we hypothesized that equivalent survival with ABC and GCB DLBCL after DA-EPOCH-B was consistent with preferential activity of bortezomib in ABC DLBCL.[3,10,11] However, because there were no data on the relative outcome of ABC and GCB DLBCL in relapsed or refractory patients, we performed a survival analysis by molecular subtype in a separate cohort of patients who progressed after standard R-CHOP treatment. Based on this analysis, as discussed below, we modified our end point to require superior outcome of ABC compared with GCB DLBCL as evidence for preferential activity of bortezomib in ABC DLBCL.

In part B, bortezomib was initially escalated to determine the MTD in combination with DA-EPOCH at dose levels of 0.5, 1.0, 1.5 and 1.7 mg/m^2 intravenously on days 1 and 4 of DA-EPOCH every 21 days. An accelerated phase 1 design with single patients per dose level was used until the first instance of first-course grade 3 or more (except hematologic) toxicity, grade 4 thrombocytopenia or neutropenia, or the second instance of first-course grade 2 toxicity (except hematologic, nausea/vomiting, fatigue, alopecia) was observed. Thereafter, 3 patients per dose level underwent escalation to a new dose level if 0/3 patients developed dose-limiting toxicity (DLT). If 1/3 patients developed DLT, an additional 3 patients were enrolled with no further DLT (ie, 1/6) before escalation could proceed. The safe tolerated dose of bortezomib was defined as that dose at which there were 0/6-1/6 episodes of DLT or the maximum dose (1.7 mg/m^2) was reached, whichever occurred first; this dose was used in an expanded phase for all subsequent patients. Bortezomib was reduced one dose level for grade 2 or more neurotoxicity. Responding or stable patients received up to 6 cycles of DA-EPOCH-B. Diseases were restaged every 2 cycles during treatment, and every 3, 4, and 6 months during years 1, 2, and 3, respectively, thereafter. Standard response criteria were applied.[19]

To assess the relative outcome of GCB and ABC DLBCL in patients whose disease relapsed or progressed after upfront treatment, we analyzed a dataset from a study of R-CHOP with gene expression profiling in previously untreated DLBCL, which was recently published by the Lymphoma/Leukemia Molecular Profiling Project (data available at www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token = rhojvaiwkcsaihq&acc = GSE10846, accession number GSE10846 [NCBI GEO]).[10]

Gene expression profiling and immunohistochemical analysis

Analysis of tissue for molecular subtype was performed in all patients after completion of the clinical trial. mRNA was extracted from frozen biopsy samples and profiled for gene expression on custom Affymetrix oligonucleotide microarrays (Santa Clara, CA), as described.[20] Immunohistochemistry was performed at the National Cancer Institute on achieved paraffin-embedded tissue as previously reported.[21] Sections were stained with monoclonal antibodies to Bcl-6 (clone PG-B6p), MUM-1 (clone MUM1p), and CD10 (clone 56C6 from Novocastra, Burlingame, CA). For Bcl-6 and MUM-1, cases were scored as positive if expression occurred in at least 30% of neoplastic cells. CD10 stained uniformly positive or negative in all cases. Classification of tumor biopsies into GCB or ABC (non-GCB) subtypes was determined by S.P. using the validated method of Hans et al and blinded to the gene expression profiling results.[18]

Statistical analysis

Survival of patients on part B was calculated from the start of DA-EPOCH-B treatment until death or the last follow-up, as appropriate, and survival of R-CHOP failures was calculated from disease progression or relapse until death or the last follow-up, as appropriate, using the Kaplan-Meier method. Gene expression classification was based on a Bayesian predictor generated as described with the exception that the predictor was trained on gene expression data from 78 ABC DLBCL and 82 GCB DLBCL samples using the 100 most differentially expressed genes.[20,22] Samples with greater than 0.85 probability of being ABC or GCB DLBCL were classified as such (Figure 1B); all others were declared unclassified.

P values for survival analysis were calculated using the log-rank test. Proportions were compared using the Fisher exact test. All *P* values are 2-sided.

Results

Patient characteristics

All 49 enrolled patients were assessable for toxicity and response. Of these, 23 received part A and 44 received part B (Figure 1A). Patient characteristics are shown in Table 1. Overall, 34 (69%) had de novo DLBCL (non-PMBL subtypes), and the others had histologies distributed among PMBL, transformed follicular lymphoma, and other aggressive B-cell lymphomas. The clinical outcome of DA-EPOCH-B was assessed in 27 patients with de novo DLBCL who underwent successful molecular classification into GCB (n = 15) and ABC (n = 12) DLBCL subtypes. The clinical and prognostic characteristics of these subtypes were not statistically different from one another or those of the entire study cohort (Table 1). From a clinical perspective, however, 9 (75%) of the ABC and 6 (40%) of the GCB DLBCL ($P = .12$) were deemed to be too advanced to warrant single agent bortezomib and were enrolled directly in part B.

Treatment and toxicity

In part A, 23 patients received a median (range) of 2 (1-18) cycles. Treatment was well tolerated with 4 (5%) cycles each complicated by grade 3 thrombocytopenia and neutropenia. Forty-four patients in part B received a median (range) of 2 (1-6) cycles, and 34 (77%) received at least 2 cycles. Bortezomib was escalated 4 dose levels with 4 patients at 0.5, 2 at 1.0, 18 at 1.5, and 20 at 1.7 mg/m^2. The first 4 patients received dose level 1 due to concurrent enrollment in part B over 3 weeks. One patient received dose level 2 during the escalation phase and one during the expanded phase due to a dose reduction for neuropathy. Based on the phase 1 dose escalation, the bortezomib MTD was identified as 1.7 mg/m^2 and initially used in the expanded phase. However, when grade 4 autonomic neuropathy was observed in patients 18 and 20 after cycle 4 and 2, respectively, of the expanded phase, the MTD was reduced to 1.5 mg/m^2 for subsequent patients (Table 2). Overall, 5 (11%) patients developed autonomic neuropathy, 4 at 1.7 and 1 at 1.0 mg/m^2, necessitating bortezomib discontinuation in 4 of these patients.

We analyzed administered drug dose intensity in the 27 patients with ABC or GCB DLBCL in part B to assess if there were significant differences. We found no significant difference in the dose intensity for all cytotoxic agents (data not shown) or the mean plus or minus standard error for the bortezomib dose in patients with ABC (1.56 ± 0.058 mg/m^2) or GCB (1.57 ± 0.047 mg/m^2) DLBCL ($P = .913$).

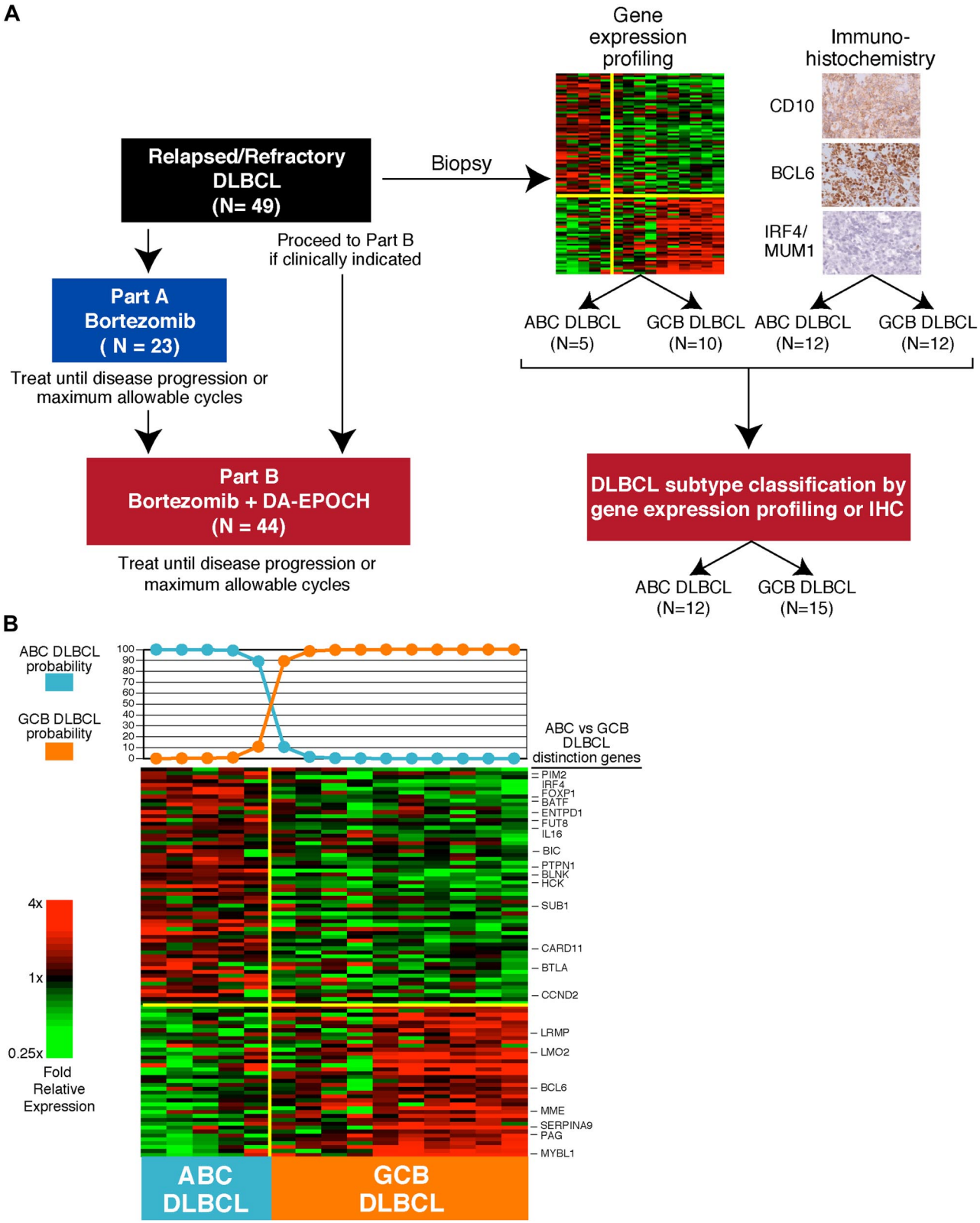

Figure 1. Study schema. (A) Clinical treatment paradigm. Patients initially received bortezomib alone at 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days (Part A) unless they had disease that the investigators judged to require immediate chemotherapy, as in cases of impending or ongoing organ compromise; these patients received only Part B. Patients with progressive disease in Part A later received bortezomib with DA-EPOCH (Part B). Molecular classification. Of 31 DLBCL cases analyzed by gene expression profiling, 16 were excluded due to ineligible subtype by classification or did not receive Part A, leaving 5 ABC and 10 GCB cases eligible for analysis of outcome. Of 24 paraffin-embedded tumor biopsies analyzed by immunohistochemistry, 12 of each were categorized as GCB and ABC (non-GCB) type.[18] By combining both methods, cases were identified as GCB in 15 and ABC in 12 and included in the analysis of outcome with Part B. (B) Gene expression profiling for 15 biopsy samples that were classified as GCB (10) or ABC (5) DLBCL. Relative mRNA expression levels for 100 genes that distinguish ABC and GCB DLBCL are depicted according to the color scale shown. The probability that a sample is ABC or GCB DLBCL based on the Bayesian gene expression-based classifier is shown at the top.

Table 1. Patient characteristics

Patient characteristics	All patients, n (%)	Molecular DLBCL subtypes by biomarker analysis on part B,* n (% of group)		P
		GCB DLBCL	ABC DLBCL	
Total patients	49 (100%)	15 (56%)	12 (44%)	
Median age, y (range)	54 (18-78)	57 (39-70)	58 (21-73)	.75
Stage				
III/IV	42 (86%)	12 (80%)	12 (100%)	.23
Lactate dehydrogenase				
> Normal	28 (57%)	12 (80%)	7 (58%)	.39
Performance status				
ECOG ≥ 2	9 (18%)	4 (27%)	2 (17%)	.66
International Prognostic Index				
High intermediate/high risk	24 (49%)	7 (47%)	7 (58%)	.70
Histology				
DLBCL (de novo)†	34 (69%)	15 (100%)	12 (100%)	
PMBL	4 (8%)	0	0	
Transformed	7 (14%)	0	0	
Other‡	4 (8%)	0	0	
Prior treatment				
Median CT regimens (range)	2 (1-7)	2 (1-5)	2 (1-7)	.78
Median months last therapy (range)	4 (1-69)	4 (1-37)	3 (1-18)	.84
Refractory to last CT regimen§	21 (43%)	5 (33%)	3 (25%)	.70
Prior BMT	10 (20%)	3 (20%)	4 (33%)	.66

DLBCL indicates diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; PMBL, primary mediastinal B-cell lymphoma; CT, chemotherapy; and BMT, autologous or allogeneic bone marrow/stem cell transplant.

*Molecular subtypes determined by microarray and/or immunohistochemistry.

†Excludes PMBL subtype.

‡Two DLBCL by histology were reclassified as Burkitt lymphoma by microarray in this group.

§No response to last combination chemotherapy regimen. *P* values for binary variables were calculated using a Fisher exact test, and numeric values (age, regimen number, and time from last treatment) were calculated using a Wilcoxon rank test.

Analysis of molecular subtypes

To investigate the association between de novo GCB and ABC DLBCL and response to DA-EPOCH-B, fresh tumor biopsies were obtained where possible for gene expression profiling. Among 31 biopsies analyzed (1 failed array), GCB and ABC DLBCL subtypes were identified in 19 and 6 cases, respectively. The remaining 6 were identified as Burkitt lymphoma (2), PMBL (3), and unclassified (1), and they were excluded. Of the 19 GCB DLBCL cases, 7 were histologically transformed follicular lymphomas and were excluded. In addition, one ABC DLBCL and 2 de novo GCB DLBCL cases did not receive part B and were excluded.

Table 2. DA-EPOCH-B toxicity

Adverse event	Toxicity grade*		
	Grade 2, n (%)	Grade 3, n (%)	Grade 4, n (%)
Anemia	22 (18)	22 (18)	2 (2)
Neutropenia	5 (4)	12 (10)	39 (32)
Thrombocytopenia	17 (14)	30 (24)	19 (15)
Platelet transfusion		13 (11)	5 (41)
Fever and neutropenia†		25 (20)	
Nausea/vomiting	10 (8)	5 (4)	
Diarrhea	11 (9)	2 (2)	
Motor	1 (2)	1 (2)	
Sensory	4 (9)	6 (14)	
Neuropathic pain	1 (2)	2 (4)	
Autonomic	1 (2)	2 (4)	2 (4) (DLT)
Fatigue‡	11 (9)	7 (6)	

DLT indicates dose-limiting toxicity.

*Toxicity based on 123 cycles in 44 patients. Includes all dose levels.

†Toxicity incidence per cycle.

‡Toxicity incidence per patient.

In total, 15 eligible cases were classified by gene expression profiling as ABC DLBCL (n = 5) or GCB DLBCL (n = 10; Figure 1B). We also used immunohistochemistry to determine the molecular subtype of 24 archived tumor biopsies from patients with de novo DLBCL treated in part B, as described.[18] This effort identified 12 GCB DLBCL and 12 non-GCB cases, which we considered to be ABC DLBCL for this analysis. For 13 cases, both the immunochemical assay and gene expression profiling were available. There was concordance in DLBCL subtype classification for 12 of these cases, indicating the robustness of both techniques ($P < .001$). The only discordant case was classified as GCB DLBCL by immunohistochemistry but unclassified by gene expression profiling and was excluded from analysis. When the results from both classification methods were combined, 15 GCB DLBCL and 12 ABC DLBCL cases were included in the analysis of outcome with DA-EPOCH-B.

Clinical outcome after bortezomib and DA-EPOCH

Among 23 patients in part A, there was one partial response in a patient with transformed follicular lymphoma and 22 (96%) nonresponders. When considering all 44 patients in part B, 15 (34%) responded, including 8 with complete responses (Table 3). The subset of 27 patients with de novo DLBCL who were classified as GCB or ABC DLBCL had similar response rates compared with all patients treated in part B.

To address the scientific hypothesis that inhibition of NF-κB by bortezomib may increase the activity of chemotherapy, we analyzed response and overall survival in the GCB and ABC DLBCL patients who received DA-EPOCH-B. When considering all 27 patients, the overall response rate was 13% in GCB DLBCL compared with 83% in ABC DLBCL ($P < .001$; Table 3). It was

Table 3. DA-EPOCH-B overall response and by molecular subtype

Treatment group	n (%)	Response, n (%)			*P**
		Complete	Partial	None	
All patients	44	8 (18)	7 (16)	29 (66)	
DLBCL (de novo)†	31 (70)	7 (23)	6 (19)	18 (58)	.63
Molecular subtypes‡	27	6 (22)	6 (22)	15 (56)	
ABC DLBCL	12 (44)	5 (41.5)	5 (41.5)	2 (17)	
GCB DLBCL	15 (56)	1 (6.5)	1 (6.5)	13 (87)	< .001

*Fisher exact test.
†Excluding primary mediastinal B-cell lymphomas (PMBL).
‡All de novo DLBCL except PMBL with microarray or immunohistochemical determination of molecular subtype.

particularly striking that 41.5% of patients with ABC DLBCL achieved complete remission compared with only 6.5% of those with GCB DLBCL. To assess if the method by which a tumor was classified introduced a bias, we separately analyzed the responses in cases classified by either gene expression profiling or immunohistochemistry. Among 15 cases classified by gene expression profiling, the overall response rates in the GCB and ABC DLBCL subtypes were 20% and 100% ($P = .007$), respectively, and among 24 cases classified by immunohistochemistry, these response rates were 17% and 83% ($P = .003$). Hence, both molecular classification methods identified a similar difference in overall response rates between the molecular subtypes of DLBCL.

An analysis of overall survival also revealed a significant difference between patients with GCB and ABC DLBCL. Among all 31 patients with de novo DLBCL, the median potential follow-up was 49 months and the overall median survival was 8 months (Figure 2A). Among these patients, the 27 who were classified as GCB or ABC DLBCL had a significant difference in median overall survival of 3.4 and 10.8 months, respectively ($P = .003$) (Figure 2B). We also separately analyzed overall survival in the cases analyzed by the 2 classification methods. GCB and ABC DLBCL cases analyzed by gene expression profiling had a similar difference in overall survival, with median overall survival times of 5 and 10.8 months, respectively, although the

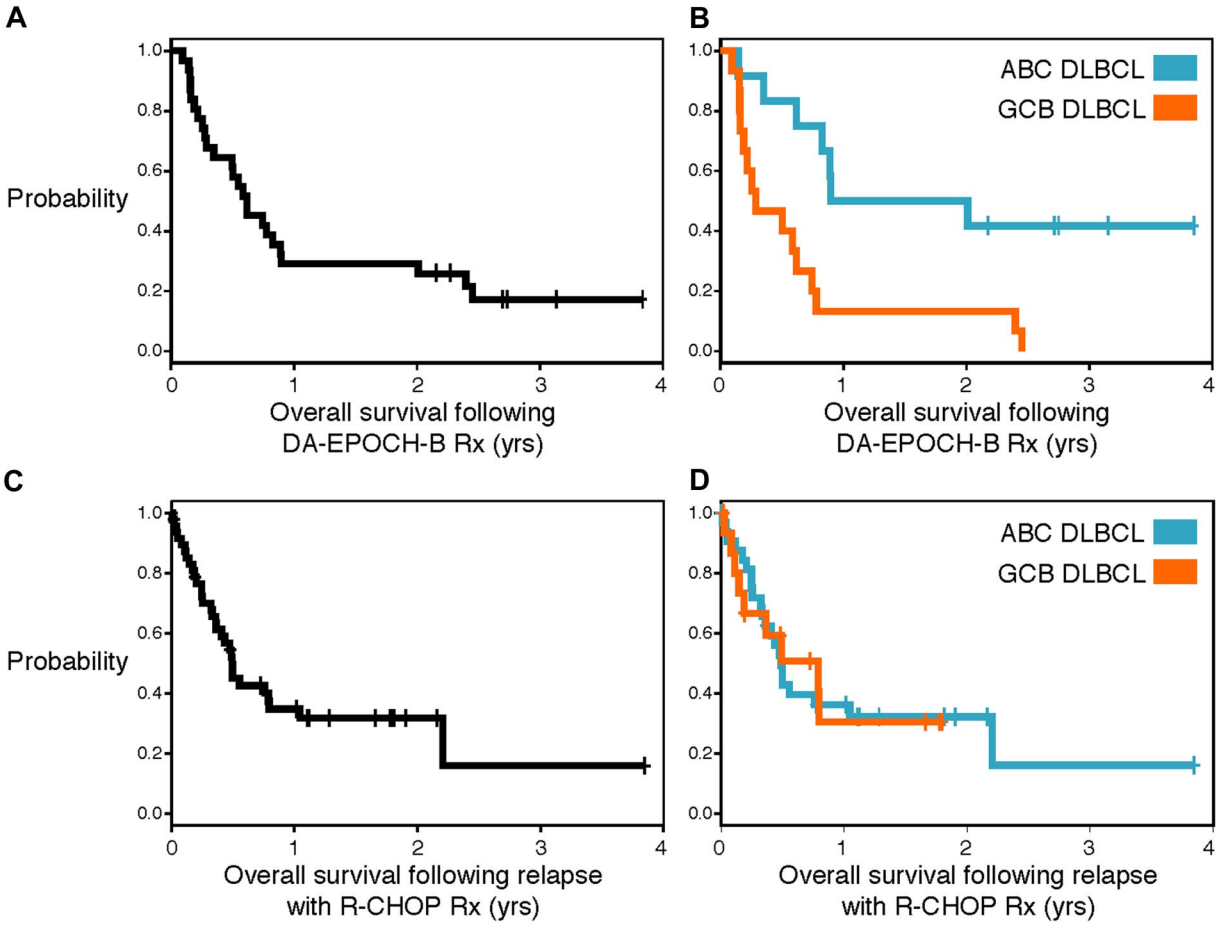

Figure 2. Overall survival in patients with DLBCL. (A) Overall survival of 31 patients with de novo DLBCL who received DA-EPOCH-B. With a median potential follow-up of 49 months, the median survival was 8 months. B. (B) Overall survival of 27 patients with ABC or GCB DLBCL who received DA-EPOCH-B showed a median survival of 10.8 and 3.4 months, respectively ($P = .0026$). (C) Overall survival of 54 patients with de novo DLBCL whose disease progressed after R-CHOP. With median potential follow-up of 22.5 months for survivors, the median survival was 5.6 months. (D) Overall survival of 50 patients with ABC or GCB DLBCL whose disease progressed after R-CHOP showed a median survival of 5.8 and 9.5 months, respectively ($P = .93$).

difference did not reach statistical significance ($P = .2$). Similarly, among cases analyzed by immunohistochemistry, GCB and ABC DLBCL cases had median survivals of 3.4 and 10.8 months, respectively ($P = .006$). These results indicate that both analytic methods produced similar results.

Clinical outcome by molecular subtype after R-CHOP failure

Multiple studies show ABC DLBCL has a worse outcome than GCB DLBCL after initial treatment with R-CHOP or DA-EPOCH-R, but there is no data in the relapsed setting.[3,10,11] Because our study design was based on a comparison of the relative outcomes of ABC and GCB DLBCL in relapsed patients, we felt an important control was to assess survival by molecular subtype of patients who relapsed or progressed after upfront therapy. Hence, we analyzed data from a recently published study of R-CHOP with molecular subtyping in previously untreated patients with DLBCL.[10] In this study of 233 patients, 211 had recorded dates for progression and survival and were included in the analysis. Of these, 180 were classified as ABC (81) or GCB (99) DLBCL, and 31 were unclassified. Overall, 54 patients progressed with a median potential follow-up of 22.5 months. When considering all patients with progression, the median survival was 5.6 months, and among those with ABC or GCB DLBCL, the median survival was 5.8 and 9.5 months, respectively (Figure 2C,D). These results show that after treatment failure, ABC DLBCL has a similarly poor outcome as GCB DLBCL.

Discussion

Gene expression profiling of DLBCL has revealed that this diagnostic category includes morphologically similar tumors that belong to molecularly distinct subtypes with different oncogenic mechanisms and prognoses.[3] Activation of the NF-κB signaling pathway is a distinguishing feature of ABC DLBCL and may partially explain its inferior prognosis when treated with doxorubicin-containing chemotherapy, as NF-κB signaling can block the apoptotic response to chemotherapeutic agents.[5,8] Based on the fact that the proteasome inhibitor bortezomib can inhibit NF-κB signaling, we undertook the present study to assess whether bortezomib would enhance the activity of DA-EPOCH in ABC DLBCL compared with GCB DLBCL.[23] Our analysis of response and survival supports our hypothesis that bortezomib synergizes with chemotherapy to improve the outcome for patients with ABC DLBCL.

When we initiated this study, there was limited information on the efficacy of bortezomib in DLBCL and no information on its safely tolerated dose with doxorubicin-based regimens such as DA-EPOCH or CHOP. To investigate the first of these issues, 23 patients initially received bortezomib alone (part A), and we found bortezomib to be inactive. Forty-four patients who had no response in part A or were too ill to justify bortezomib alone received combination bortezomib and DA-EPOCH (part B). Based on dose limiting severe autonomic neuropathy in 5 patients, we identified bortezomib 1.5 mg/m^2 as the MTD in this study. However, due to the development of painful peripheral neuropathy in a subsequent study at this dose, we currently recommend bortezomib 1.3 mg/m^2 in combination with regimens such as DA-EPOCH or CHOP (W.H.W., unpublished observations, March 2006).

The scientific question of this study was to assess whether patients with ABC DLBCL would preferentially benefit from the addition of bortezomib to doxorubicin-based treatment, and we observed that they did. In designing the study, we recognized that a randomized design of DA-EPOCH plus or minus bortezomib with stratification by GCB and ABC DLBCL subtypes would be ideal but also impractical, given the unknown safety and utility of bortezomib with chemotherapy, the large required sample size, and the need for rapid molecular characterization. Hence, we chose to compare the relative efficacy of DA-EPOCH-B in the GCB and ABC DLBCL subtypes based on the clinical observation that ABC DLBCL has never shown a favorable outcome compared with GCB DLBCL. Indeed, doxorubicin-based chemotherapy, such as CHOP and DA-EPOCH with or without rituximab, is less effective in newly diagnosed ABC DLBCL compared with GCB DLBCL.[3,10,11,24] Furthermore, we showed that relapsed or refractory ABC DLBCL after R-CHOP does not have a favorable survival with a relative risk of death during follow-up of 1.04 (95% CI: 0.447-2.25; $P = .929$) compared with GCB DLBCL. Thus, the significantly better outcome of ABC DLBCL that we observed cannot be readily explained by greater efficacy of doxorubicin-based chemotherapy alone in relapsed ABC DLBCL. Furthermore, the high response rate we observed in ABC DLBCL, including 41.5% complete response, would also be highly unexpected in this group that was heavily pretreated with DA-EPOCH.[16] It is also interesting to note that the median survival of patients with relapsed/refractory ABC and GCB DLBCL treated with DA-EPOCH-B (8 months) is somewhat longer than those whose disease progressed after R-CHOP (5.6 months), despite the former having had significantly more treatment and lead time bias. While recognizing the inherent pitfalls in such comparisons, these findings raise some additional evidence for the potential benefit of bortezomib in ABC DLBCL. On the other hand, our results suggest that bortezomib is not helpful in GCB DLBCL.

We performed several analyses to help exclude alternative explanations for our results. It was particularly important to confirm that the patients with GCB and ABC DLBCL had similar prognostic features. In this regard, we assessed multiple clinical features as well as performing a detailed analyses of prior treatment and found no significant differences between each of the 2 groups or between them and the entire study group. It is interesting, however, that more patients with ABC DLBCL were judged to be too ill to participate in part A, a determination made independent of molecular subtype, indicating that they had more clinically advanced disease. While this difference might be clinically expected to favor the GCB DLBCL group, we wanted to make sure it did not lead to increased bortezomib dose intensity in the ABC DLBCL group due to less prior bortezomib exposure and potentially lower neurotoxicity. However, we found no difference between the 2 groups in the dose intensity of bortezomib or DA-EPOCH chemotherapy.

We attempted to obtain fresh tumor biopsies in all patients in this study, but this was limited by clinical factors. Hence, to increase the number of patients in each group, archived paraffin embedded biopsies were analyzed by immunohistochemistry for assignment of molecular subtype.[18] The accuracy of this method raised concerns, given that it only had a positive predictive value of 73% to 87% based on gene expression profiling and had been reported as unreliable by other investigators.[18,25] We also considered the possibility that molecular subtype assignment may not be accurate in older biopsies, but felt this was unlikely because it reflects the cell of origin. To address these concerns, we performed immunohistochemistry on all achieved paraffin embedded biopsies and compared the results to gene expression

profiles, when available, on fresh tumor biopsies. In all cases except one, there was full concordance between the analytical methods, indicating the robustness of immunohistochemistry in our hands and the stability of the molecular subtype assignment in the older biopsies. Moreover, we showed that among patients classified by either method, those with ABC DLBCL had a superior overall response and survival after DA-EPOCH-B treatment.

Our study was predicated on 2 hypotheses relating to the constitutive activity of the antiapoptotic NF-κB pathway in ABC DLBCL. First, because cell line models of ABC DLBCL are killed upon inhibition of the NF-κB pathway, it was possible that bortezomib treatment alone might have preferential activity in ABC DLBCL compared with GCB DLBCL.[5-8] However, patients in our study with either ABC or GCB DLBCL failed to respond to bortezomib as a single agent. One possible explanation is that ABC DLBCL cells in vivo receive additional antiapoptotic signals from the tumor microenvironment that are not affected by proteasome inhibition. Alternatively, it is possible that the degree of proteasome inhibition that was achieved in our trial did not inhibit the NF-κB pathway to the extent necessary to induce apoptosis. The second hypothesis that we entertained was that bortezomib might synergize with chemotherapy in patients with ABC DLBCL given that the NF-κB pathway is a potent inhibitor of apoptosis induced by chemotherapeutic agents.[9] Our results are consistent with this hypothesis, although we cannot definitively establish that the activity of bortezomib plus DA-EPOCH in ABC DLBCL was due to NF-κB inhibition.

To date, clinical trials of novel therapeutic strategies in DLBCL have not included gene expression profiling and therefore cannot determine whether the therapy might have preferential activity in a particular DLBCL subtype. Our study provides provocative evidence for the utility of bortezomib in combination with chemotherapy for the treatment of ABC DLBCL—the subtype least curable by the current standard of care, R-CHOP—and indeed, may pave the way for the development of novel therapeutic strategies that could ultimately lead to improved curability of this subtype.[26] In our view, these results provide the clinical evidence necessary to justify a randomized comparison of R-CHOP with and without bortezomib in untreated patients with ABC DLBCL, which is presently under development. These results also provide clinical evidence for the importance of an ongoing phase 2 study of CHOP-R and bortezomib in previously untreated DLBCL.[27] They also raise the concern that bortezomib is not useful in GCB DLBCL and should be used with caution in this subtype. Because of the profound genetic differences between the DLBCL subtypes, we imagine that additional targeted therapies may have preferential activity in one or the other DLBCL subtype.[4] Our results highlight the importance of pairing molecular characterization and clinical outcome in DLBCL for the rational development of targeted agents in this disease.

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Authorship

Contribution: K.D. participated in the protocol writing, execution, analysis, and writing of this study; S.P. participated in the analysis of pathologic tissue; M.S.C. and J.E.J. participated in the study execution; S.S.D. participated in the molecular analysis of tissue; G.W. participated in the statistical analysis of data; N.G. and M.S. participated in the execution and data management; E.S.J. participated in the tissue analysis; L.M.S. participated in the conceptualization, molecular analysis, study analysis, and writing of this study; W.H.W. participated in the conceptualization, protocol writing, execution, analysis, and writing of this study; and all authors had access to the primary data and approved the manuscript.

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