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Abstract
Immunohistochemical analyses of archival tumor specimens were used for pre-planned exploratory analyses of protocol-specified candidate biomarkers of bortezomib activity in 73 patients with relapsed/refractory mantle cell lymphoma in the phase 2 PINNACLE study. Consistent with other studies, elevated Ki-67 was a marker of poor prognosis, demonstrating significant associations with shorter time to progression and overall survival. Elevated NF-κB p65 and low PSMA5 expression demonstrated a trend for better response and were significantly associated with longer time to progression; elevated NF-κB p65 demonstrated a trend toward longer overall survival. This is consistent with myeloma clinical genomics research, suggesting biomarker relevance across tumor types. Elevated p27 was significantly associated with longer overall survival. Overall survival analyses by International Prognostic Index and Mantle Cell Lymphoma International Prognostic Index confirmed differential prognosis by both scores. These biomarkers data begin to illuminate bortezomib’s mechanism of action in lymphoma.

Keywords: Biomarkers, bortezomib, immunohistochemical analysis, mantle cell lymphoma, NF-κB p65

Introduction
Although the median overall survival (OS) of patients with mantle cell lymphoma (MCL) has more than doubled over the past two decades as a result of multiple factors, it remains in the range of 4–5 years. There is considerable heterogeneity in the disease and in individual patient outcomes [1], and while some of the more indolent cases may be managed with a ‘watch-and-wait’ approach [2], most patients receive immunotherapy and/or dose-intensive approaches. Dose-intensive regimens and high-dose therapy strategies have clearly improved progression-free survival; however, most patients eventually relapse and subsequently represent a therapeutic challenge as they often develop chemoresistance [3]. The proteasome inhibitor bortezomib (VELCADE®; Millennium Pharmaceuticals, Inc., and Johnson & Johnson Pharmaceutical Research & Development, L.L.C.) is approved in the United States for the treatment of multiple myeloma (MM), and also for patients with MCL following at least one prior therapy. The approval of bortezomib in MCL was based on the results of the phase 2 PINNACLE study in 155 patients with relapsed or refractory MCL [4]. In an updated analysis, the response rate was 32%, including 8% complete response/unconfirmed complete response (CR/CRu), and after a median follow-up of 26.4 months, median time to progression (TTP) and OS were 6.7 and 23.5 months.
respectively [5]. Median TTP and OS were prolonged, at 12.4 and 35.4 months, respectively, in responding patients [5].

Numerous cellular pathways are directly or indirectly regulated by the proteasome [6], and it remains unclear which are critical to the anti-tumor activity of bortezomib. In MM, genomic analyses of patient tumors strongly suggest that elevated nuclear factor-kB (NF-kB) and low-level expression of proteasome (proteasome subunit alpha 5 [PSMA5], the target of bortezomib) are associated with better outcome [7,8]. Possible mechanisms of action in MCL include inhibition of NF-kB activity [9] and cell-cycle disruption via stabilization of cyclin-dependent kinase inhibitors such as p21 and p27 [6,9].

Potential markers of prognostic subgroups in MCL have been identified, including the Ki-67 proliferation index [10,11] and the level of p27 [12,13]. Genomic studies have highlighted tumor proliferation status as a major driver of differential prognosis [14], while prognostic tools based on clinical data, such as the International Prognostic Index (IPI) [15] and the more recent MCL IPI (MIPI) [1], have also been validated. The present study comprised pre-planned exploratory analyses of protocol-specified candidate predictive biomarkers of bortezomib activity. The objectives were to test the use of archival tumor blocks for immunohistochemical (IHC) analyses of candidate tumor biomarkers and to better characterise patients with bortezomib-sensitive and bortezomib-insensitive disease in this trial. Additionally, in order to examine clinical prognostic tools in the context of bortezomib therapy, OS was evaluated among patients within the PINNACLE trial stratified according to IPI and MIPI.

Materials and methods

The design of PINNACLE (clinicaltrials.gov identifier: NCT00063713) has been reported previously [4]. Briefly, 155 patients with relapsed or refractory MCL received 3-week cycles of bortezomib 1.3 mg/m² on days 1, 4, 8, and 11 for up to 17 cycles or four cycles beyond initial reporting of CR/CRu, discontinuing for progressive disease (PD) or unacceptable toxicity. The protocol required 10 unstained tissue slides per patient from archival tumor blocks for pre-planned exploratory retrospective IHC analyses; tumor specimens comprised material that had been archived at diagnosis or earlier in a patient’s treatment course, prior to the start of PINNACLE. Samples were stained for IHC analyses of six protocol-specified candidate biomarkers that were either markers associated with poor prognosis in lymphoma and/or regulated by the ubiquitin–proteasome pathway, including Ki-67 [10,11,16–18], p27 [12], Skp2, and cyclin D1 [19], or markers reported in MM studies as showing an association between mRNA expression level and bortezomib efficacy, including PSMA5 and NF-κB p65 [8]. All IHC analyses were scored by a single pathologist (M.D.F.).

An indirect immunoperoxidase method was used to evaluate markers in formalin-fixed paraffin-embedded slides. For all assays except Skp2, the primary system was the Discovery® (Ventana Medical Systems, Tuscon, AZ) automated slide stainer using the DAB MAP system (Ventana 760-159). Assays for Ki-67 (Ventana 760-2910 clone K-2), PSMA5 (1:500, Biomol PW8215; BioMol International, Plymouth Meeting, PA), NF-κB p65 (1:500, Santa Cruz SC-372; Santa Cruz Biotechnology Inc., Santa Cruz, CA), and cyclin D1 (1:40, Neo-markers RM9104; Thermo Scientific, Fremont, CA) were pre-treated with cell conditioning solution 1 (Ventana 950-124), and p27 (1:250, Pharmingen 610241; BD Biosciences, San Jose, CA) was pre-treated with cell conditioning solution 2 (Ventana 950-123), for 20 min. Assays were followed by incubation with the respective antibodies: Ki-67 K-2, 30 min, 37°C; PSMA5, 1 h, room temperature; NF-κB p65 and p27, 1 h, 37°C; cyclin D1, 30 min, room temperature. The secondary system was the Vectastain Elite horse anti-mouse biotinylated system (Vector PK-6102; Vector Laboratory, Burlingame, CA) or goat anti-rabbit (Vector, PK-6101), depending on the antibody host.

For the Skp2 assay, antigen retrieval was performed in a 2100 retriever (PickCell Laboratories, Amsterdam, The Netherlands). Samples were buffered at pH 6 in a 0.01 M citrate buffer, heated to 120°C for 20 min, cooled to room temperature overnight, blocked for endogenous peroxidase with 3% hydrogen peroxide in methanol for 10 min, and incubated with Skp2 (1:75 Zymed 18-0307; Invitrogen Carlsbad, CA). DAB chromagen (Vector SK-4100) was then incubated for 10 min.

Statistical methods

Cut-points for Ki-67 (≤50%, >50%), p27 (<1+, ≥1+), PSMA5 (<3+, ≥3+), and NF-κB p65 (<2+, ≥2+) scores were derived from initial assessments of staining patterns and frequencies at which patients were allocated to the various score groups. These groups were used as categorical variables in all subsequent analyses. Each biomarker was compared with best response using Fisher’s exact test (univariate analysis), and by logistic regression modeling (multivariate analysis) adjusting for gender, age, time since diagnosis, prior therapies, prior high-intensity therapy, and refractory status. Best response to bortezomib was categorized into PD (bortezomib-refractory
Bortezomib biomarkers in mantle cell

Results

Patients

Archival samples for IHC analysis were collected from 77 patients; however, samples from four patients were invaluable for IHC analysis. Thus, samples were available from 73 patients (47% of the overall population in this multicenter study); a median of seven slides were obtained from each patient. Samples were derived from different pathological sites, including lymph node (49 patients), bone marrow (17 patients), and other/unknown sites (seven patients). The clinical characteristics of this patient population appeared similar to the overall study population (n = 155) [4], as summarized in Table I. Among these 73 patients, the efficacy of single-agent bortezomib also appeared similar to that seen in the overall study population (Table I).

Analyses of candidate biomarkers by immunohistochemistry

Of the 73 patients from whom evaluable slides were available, interpretable data for Ki-67, p27, PSMA5, and NF-κB p65 were obtained for 67, 41, 60, and 57 patients, respectively. The differences in sample size were primarily due to differences in failure rate for the different IHC assays (see ‘Materials and methods’). Table II summarizes associations between each candidate biomarker and best response, TTP, and OS. Figure 1 presents TTP and OS distributions by Ki-67, p27, PSMA5, and NF-κB p65 scores, with associated p-values by univariate analysis.

Patients with elevated tumor Ki-67 expression, a known poor prognostic factor in MCL, appeared less likely to experience clinical benefit with bortezomib and more frequently had bortezomib-refractory disease (PD as best response). The differential response between subgroups did not reach statistical significance, but p-values of 0.054 and 0.065 by univariate and multivariate analysis, respectively, indicated trends of interest. The median treatment duration was 6 and 2.5 cycles in patients with Ki-67 scores of ≤50% and >50%, respectively, reflecting the higher rate of PD in patients with Ki-67 scores of >50%. Time-to-event analyses showed that elevated Ki-67 expression (>50% of tumor cells) was significantly associated with shorter TTP (hazard ratio [HR] = 2.73, 95% confidence interval [CI]: 1.04, 7.20, p = 0.042 by multivariate analysis, Table II) and shorter OS (p = 0.004 by univariate analysis, Figure 1B; HR = 1.93, 95% CI: 0.90, 4.10, p = 0.09 by multivariate analysis, Table II).

Patients with tumors that had elevated PSMA5 expression or lower NF-κB p65 expression appeared less likely to experience clinical benefit with bortezomib. These groups more frequently had
Table II. Association between each individual biomarker and response to bortezomib, time to progression, and overall survival.

<table>
<thead>
<tr>
<th>Marker</th>
<th>N*</th>
<th>Cut-points</th>
<th>n</th>
<th>Bortezomib-refractory MCL</th>
<th>Median TTP</th>
<th>Median OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67</td>
<td>67</td>
<td>≤50% +ve</td>
<td>51</td>
<td>18%</td>
<td>6.9 months</td>
<td>24.9 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 50% +ve</td>
<td>16</td>
<td>50%</td>
<td>1.4 months</td>
<td>11.5 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uni, p = 0.054</td>
<td>Multi, p = 0.065</td>
<td></td>
<td>HR: 2.73</td>
<td>(95% CI: 1.04, 7.20)</td>
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<tr>
<td></td>
<td></td>
<td>HR: 0.208 by multivariate analysis, Table II.</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>p27</td>
<td>41</td>
<td>≤0.5+</td>
<td>10</td>
<td>33%</td>
<td>4.0 months</td>
<td>22.8 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;1+</td>
<td>31</td>
<td>26%</td>
<td>6.7 months</td>
<td>29.0 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uni, p = 0.686</td>
<td>Multi, p = 0.333</td>
<td></td>
<td>HR: 0.97</td>
<td>(95% CI: 0.31, 3.00)</td>
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<tr>
<td></td>
<td></td>
<td>HR: 0.178 by univariate analysis</td>
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<tr>
<td>PSMA5</td>
<td>60</td>
<td>≤2+</td>
<td>9</td>
<td>0%</td>
<td>6.7 months</td>
<td>21.0 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3+</td>
<td>51</td>
<td>24%</td>
<td>NA</td>
<td>33.7 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Uni, p = 0.178</td>
<td>Multi, p = 0.964</td>
<td></td>
<td>HR: 1.92</td>
<td>(95% CI: 0.31, 3.00)</td>
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<tr>
<td></td>
<td></td>
<td>HR: 0.090 by multivariate analysis</td>
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</tr>
<tr>
<td>NF-κB</td>
<td>57</td>
<td>≤1+</td>
<td>38</td>
<td>26%</td>
<td>6.1 months</td>
<td>23.5 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;2+</td>
<td>19</td>
<td>17%</td>
<td>7.4 months</td>
<td>29.9 months</td>
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<tr>
<td></td>
<td></td>
<td>Uni, p = 0.730</td>
<td>Multi, p = 0.136</td>
<td></td>
<td>HR: 0.36</td>
<td>(95% CI: 0.13, 1.00)</td>
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<tr>
<td></td>
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<td></td>
<td>Multi, p = 0.048</td>
<td></td>
<td>HR: 0.43</td>
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</tr>
<tr>
<td>p65</td>
<td>34</td>
<td>≤2+</td>
<td>5</td>
<td>0%</td>
<td>6.7 months</td>
<td>21.0 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3+</td>
<td>30</td>
<td>24%</td>
<td>NA</td>
<td>33.7 months</td>
</tr>
<tr>
<td></td>
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<td>Uni, p = 0.178</td>
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<td>HR: 1.92</td>
<td>(95% CI: 0.31, 3.00)</td>
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<tr>
<td></td>
<td></td>
<td>HR: 0.090 by multivariate analysis</td>
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</table>

Cyclin D1 and Skp2 Expression uniformly moderate/high across all evaluable samples

No significant associations with response, TTP, or OS

*p-Values (two-sided) < 0.05 were considered statistically significant and are underlined and shown in bold type.

†Rate of PD as best response to bortezomib. Univariate analysis performed using Fisher’s exact test, multivariate analysis performed using linear logistic regression adjusting for gender, age at first dose, whether patient was refractory, time since diagnosis, and whether patient was on prior high-intensity therapy.

§Univariate analysis performed using log-rank test, multivariate analysis performed using Cox proportional hazard regression adjusting for same variables as in linear logistic regression.

HR, hazard ratio; multi, multivariate analysis; MCL, mantle cell lymphoma; NA, not applicable; OS, overall survival; PD, progressive disease; TTP, time to progression; uni, univariate analysis.

bortezomib-refractory disease (Table II). For example, among 38 patients with lower NF-κB p65 expression (≤1+), 26% had PD as best response, while 17% of the 19 patients with higher NF-κB p65 expression (≥2+) had PD as best response. This differential response between subgroups, as with Ki-67, did not reach statistical significance, but p-values indicated trends of interest (PSMA5: p = 0.178 by univariate analysis; NF-κB p65: p = 0.136 by multivariate analysis). The median treatment duration was 3 cycles and 4 cycles in patients with PSMA5 expression of ≤2+ and 3+, respectively, and 4.5 cycles and 6 cycles in patients with NF-κB p65 expression of ≤1+ and ≥2+, respectively.

The analyses of TTP by NF-κB p65 and PSMA5 expression showed similar statistical associations; for both, p-values were < 0.2 but > 0.05 in univariate analyses (Table II, Figure 1), and p-values were < 0.05 in multivariate analyses (Table II). Specifically, by multivariate analysis, elevated NF-κB p65 expression was significantly associated with longer TTP (HR = 0.36, 95% CI: 0.13, 1.00, p = 0.048, Table II), while patients with elevated tumor PSMA5 expression exhibited significantly shorter TTP (HR = 9.14, 95% CI: 1.01, 82.94, p = 0.049, Table II). Expression of p27 was not associated with TTP following bortezomib treatment (p > 0.5 in univariate and multivariate analyses, Table II). With respect to OS, by multivariate analysis, patients with tumors with elevated NF-κB p65 expression demonstrated a trend of interest toward prolonged OS (HR = 0.43, 95% CI: 0.19, 1.00, p = 0.051, Table II), and patients with tumors with elevated p27 expression exhibited significantly longer OS (HR = 0.32, 95% CI: 0.12, 0.89, p = 0.029, Table II). There was no trend toward shorter OS in patients with elevated tumor PSMA5 expression (HR = 1.92, 95% CI: 0.70, 5.28, p = 0.192 by multivariate analysis, Table II).

The expression of cyclin D1 and Skp2 was uniformly moderate or high across all evaluable samples, and thus no associations with response rate, TTP, or OS were identified (Table II).
Analyses by IPI and MIPI scores

Within the overall PINNACLE population, 147 of 155 patients were assessable for stratification by IPI and by MIPI. By IPI, 17 (12%), 96 (65%), and 34 (23%) patients had high-, high-/low-intermediate-, and low-risk disease, respectively. By MIPI, 53 (36%), 48 (33%), and 46 (31%) patients had high-, intermediate-, and low-risk MCL, respectively. There were some discrepancies in risk stratification between IPI and MIPI; for example, four patients with high-risk disease by IPI were classified as lower risk by MIPI, and eight patients with low-risk disease by IPI
were classified as higher risk by MIPI. OS distributions by IPI using combined high-intermediate and low-intermediate cohorts and by MIPI score are shown in Figures 2(A) and 2(B), respectively. In this dataset of relapsed or refractory MCL patients, the HR for the comparison of the intermediate-risk versus the high-risk cohort was 0.335 (95% CI: 0.187, 0.601) by IPI [Figure 2(A)] and 0.629 (95% CI: 0.384, 1.032) by MIPI [Figure 2(B)], and the HR for the comparison of the low-risk versus the high-risk cohort was 0.229 (95% CI: 0.111, 0.469) by IPI [Figure 2(A)] and 0.539 (95% CI: 0.319, 0.911) by MIPI [Figure 2(B)], suggesting that IPI identified the high-risk group of patients more effectively than MIPI.

The prevalence of MIPI high-risk patients in the biomarker subgroups was assessed to discern whether this contributed to the differential outcomes observed according to biomarker expression. Among patients with Ki-67 expression of \( \geq 50\% \) and \( > 50\% \), 15/48 (31%) and 9/15 (60%) had high-risk MCL by MIPI; these data suggest a somewhat elevated proportion of high-risk patients by MIPI in the population with higher Ki-67 expression. No clear trends were evident regarding the prevalence of high-risk patients by MIPI according to p27, PSMA5, or NF-\( \kappa \)B p65 expression. Among patients with p27 expression of \( \leq 0.5 + \) and \( \geq 1 + \), 4/9 (44%) and 13/30 (43%) had high-risk MCL by MIPI, while 3/9 (33%) and 22/47 (47%) patients with PSMA5 expression of \( \leq 2 + \) and \( 3 + \), respectively, and 13/34 (38%) and 9/19 (47%) patients with NF-\( \kappa \)B p65 expression of \( \leq 1 + \) and \( > 2 + \), respectively, had high-risk MCL by MIPI. These data suggest that the overpopulation of high-risk patients by MIPI is not an explanation for the differential outcomes seen between these biomarker subgroups after treatment with bortezomib.

**Discussion**

Although the use of high-dose or dose-intensive therapies has clearly improved progression-free survival in MCL, most patients nevertheless relapse and subsequently represent a therapeutic challenge, often developing chemoresistance [3]. A small subset of patients can be cured with non-myeloablative allogeneic transplant, although this is not an option for most patients, given their typically advanced age (mid- to late 60s) at time of relapse. Both IHC [10,11,16–18] and genomic studies [14,23,24] have identified the proliferative index as a meaningful prognostic factor that can be used independent of or in concert with clinical factors [1,14]. Despite such progress, risk stratification in MCL remains challenging, and this, in combination with the availability of recently approved [25] and emerging therapies [3], makes the management of MCL patients particularly complex. Therefore, it is increasingly important that clinical studies include research aimed at identifying patients most likely to benefit from specific drugs or regimens, in addition to assessing traditional prognostic factors.

Importantly, our research demonstrates that IHC analyses of archival tumor specimens can be useful for exploration of candidate biomarkers in clinical trials. These pre-planned exploratory analyses of protocol-specified biomarkers identified MCL tumor types that may be particularly sensitive or insensitive to bortezomib, with \( p < 0.05 \) being considered a statistically significant association. To avoid overlooking any false-negative findings, biomarker results that were consistent with expectations and showed a \( p\)-value \( < 0.2 \) are reported as a trend of interest and worthy of further analysis; these trends may potentially be shown to be significant associations in subsequent non-Hodgkin lymphoma (NHL) studies (see below).

Elevated Ki-67 expression (\( > 50\% \) of tumor cells) was a poor prognostic factor in our study, which is consistent with other studies in MCL linking...
elevated proliferation index, and Ki-67 specifically, with poor outcomes \[10,11,16,17\]. The cut-point of > 50% for defining highest-risk Ki-67 staining in our study, and the proportion of patients (~ 25%) within this group, were somewhat higher than in some other studies of Ki-67 in MCL \[11,16,26\], but similar to another report \[10\]; this may reflect sampling differences or differences in patient populations between studies. Our Ki-67 data strongly suggest that the poor outcome seen with highly proliferative MCL is not overcome with bortezomib monotherapy. The development of additional drugs or drug combinations to treat this patient population is a critical priority. Importantly, the data from analyses of this validated prognostic marker indicate that the patient subset in the present study is adequate to detect associations with outcome.

Additional biomarkers were specified in the PINNACLE protocol based upon either a presumed important mechanism of action of bortezomib (p27, NF-κB p65), or data from clinical studies in relapsed MM indicating better outcomes for specific patient subgroups (elevated NF-κB p65, lower PSMA5 expression) after therapy with single-agent bortezomib but not high-dose dexamethasone \[7,8\]. In this exploratory study in patients with relapsed MCL, elevated tumor NF-κB p65 or lower PSMA5 expression were also associated with better outcomes after single-agent bortezomib. Similar to the MM study \[7\], this effect was particularly noticeable for TTP (Figure 1), while there was only a trend toward improvement in short-term response in these groups (Table II). The magnitude of effect with both response and TTP for Ki-67 expression was similar to that seen for NF-κB p65 and PSMA5 expression in this dataset, suggesting that additional analyses may bear out the validity of these initial observations for these new biomarkers. Additionally, higher expression of p27, which, like Ki-67, is a prognostic factor linked with cell cycle progression, was associated with longer OS, further emphasizing the link between proliferation and poor prognosis in MCL. It will be important to replicate the PSMA5 and NF-κB p65 observations in additional MCL datasets and to explore whether they are prognostic in MCL or predictive of bortezomib outcome specifically.

Our findings suggest that candidate biomarkers derived from clinical genomics research \[8\] can be used in IHC analyses, and that markers of efficacy in one tumor type (MM) may be relevant in other malignancies (MCL). Notably, activation of the NF-κB pathway has been demonstrated in MCL cell lines and patient biopsy cells \[9,27,28\], and inhibition of NF-κB activity with bortezomib has been shown to result in apoptosis of various cancer cell types including MCL \[9\]. Importantly, Kato et al. recently demonstrated that a subset of clinical MCL specimens contain specific DNA mutations that activate NF-κB pathway signaling \[29\], and that cell lines with these mutations are dependent upon NF-κB activation for survival \[29,30\]. Our findings suggest that NF-κB pathway inhibition with bortezomib may result in greater anti-tumor activity and improved outcome in patients with tumors that have elevated activity of this important pathway. This concept is also supported by clinical findings from Dunleavy et al. in recurrent diffuse large B-cell lymphoma (DLBCL), another aggressive subtype of NHL. In this study, bortezomib plus chemotherapy resulted in significantly higher response rates and improved survival, specifically in patients with activated B-cell-like DLBCL, compared with the limited clinical activity observed in patients with germinal center B-cell-like DLBCL \[31\]. Activated B-cell-like DLBCL is characterized by mutational activation of the NF-κB pathway, whereas the germinal center B-cell-like subtype is not NF-κB-dependent \[29–31\].

The confirmatory analyses of OS by IPI and MIPI stratification in the overall PINNACLE population demonstrated that differential prognosis in patients according to risk group was retained following single-agent bortezomib using both indexes. While these clinical prognostic tools do not account for the poor prognosis seen with the various biomarker subgroups, these findings nevertheless provide additional validation of the dataset from the PINNACLE study.

The molecular aspects of the present study were limited by both the number of patients for whom unstained slides were submitted and the number of slides available per patient. Additionally, data were not collected on the timing of biopsy relative to the start of bortezomib therapy, and samples were derived from different pathological sites, factors that may possibly have affected our results. This illustrates the need for clinical trials incorporating correlative studies and the efficient collection of patient samples for such studies. Although our results from PINNACLE, the largest study of single-agent bortezomib in relapsed/refractory MCL to date, are only hypothesis-generating, they do highlight the importance of acquiring patient samples at the time of study entry, given the potential biological changes that may take place over time; in future studies this approach would be useful in further clarifying the biomarkers associated with response and progression with bortezomib therapy. The above limitations also constrained both the number of hypotheses that could be tested and the statistical power to detect clinical associations. Nevertheless, our findings will help inform the selection of IHC biomarkers to be studied in other clinical trials of bortezomib in NHL, including ongoing phase 3 trials.
in MCL (NCT00722137) and follicular lymphoma (NCT00312845), and ongoing IHC research in other trials of bortezomib in NHL and other cancers should help to refine and extend these analyses. Overall, greater access to patient pathology samples will be important in optimizing ongoing and future drug development in MCL.

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References


