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ORIGINAL ARTICLE

Concordant bone marrow involvement of diffuse large B-cell lymphoma represents a distinct clinical and biological entity in the era of immunotherapy

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In diffuse large B-cell lymphoma (DLBCL), the clinical and biological significance of concordant and discordant bone marrow (BM) involvement have not been well investigated. We evaluated 712 *de novo* DLBCL patients with front-line rituximab-containing treatment, including 263 patients with positive and 449 with negative BM status. Compared with negative BM disease, concordant BM adversely impacted overall and progression-free survival, independent of the International Prognostic Index (IPI) and cell-of-origin classification. Once BM is concordantly involved, poor prognosis was not associated with the extent of BM involvement. Conversely, patients with discordant BM showed favorable overall survival similar to stage I–II DLBCL. A BM-adjusted IPI, using three parameters: concordant BM involvement, age >60 years, and performance status >1, improves the risk stratification for DLBCL with positive BM. Intensive immunochemotherapy seemingly rendered survival benefit for patients with concordant BM, as did rituximab maintenance for the discordant BM group. Frequently revealing adverse clinical and molecular characteristics, patients with concordant BM demonstrated gene expression signatures relevant to tumor cell proliferation, migration and immune escape. In conclusion, clinical and biological heterogeneity is seen in DLBCL with positive BM but concordant BM involvement represents a distinct subset with unfavorable gene signatures, high-risk clinicopathologic features and poor prognosis.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma and is highly heterogeneous in regard to clinical manifestations, biological features and prognosis. The introduction of rituximab (R) combined with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) has resulted in improved outcome in DLBCL patients; however, variable prognosis is present, and identifying distinct subsets for prognostication and therapeutic decisions is essential.^{1–3}

Approximately 11–34% of patients have bone marrow (BM) involvement when DLBCL is initially diagnosed.^{4–6} In most patients, BM is involved by large B-cell lymphoma (concordant disease), but a subset of patients have small cell low-grade lymphoma in the BM (discordant disease).^{4,7–10} BM involvement by lymphoma is clinically recognized as advanced disease and contributes to higher International Prognostic Index (IPI) scores. The IPI, a powerful clinical tool for risk-stratification of DLBCL, is

calculated by using five clinical parameters: age >60 years, Ann Arbor stage III–IV, Eastern Cooperative Oncology Group performance status (PS) >1, elevated serum lactate dehydrogenase (LDH), and involvement of more than one extranodal site.^{11,12} Of interest, some studies have reported that the impact of concordant vs discordant BM involvement on overall survival (OS) and progression-free survival (PFS) was different.^{4,7–10} Owing to the limited number of enrolled patients with positive BM and the dissimilarity of design and inclusion criteria in earlier studies, the consensus regarding the prognostic impact of concordant vs discordant BM is controversial and needs to be validated in a larger series. Furthermore, whether differences between concordant and discordant BM involvement can be optimized in an adjusted IPI for risk stratification is unknown.

In addition to the IPI, cell-of-origin (COO) classification defined by gene expression profiling (GEP) or immunohistochemistry surrogates has been widely adopted, which classifies DLBCL cases into germinal center B-cell-like (GCB) subtype and activated B-cell-

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like/non-GCB subtype, with the former associated with better survival.^{13–17} Meanwhile, several other biomarkers, including CD5, p53, MYC expression, MYC/BCL2 co-expression, TP53 mutation, BCL2 or MYC rearrangement and MYC/BCL2 double hit, have been implicated in mechanisms of disease and have been suggested as predictors of poor prognosis in DLBCL.^{18–24} Thus far, no large studies have explored the relationship between these high-risk pathologic features and the various types of BM involvement in DLBCL patients.

To address these equivocal questions and elucidate the role of BM involvement in DLBCL patients, we conducted a large study of *de novo* DLBCL treated with immunochemotherapy. We analyzed GEP and molecular analyses to better characterize the pathologic features of concordant BM involvement.

MATERIALS AND METHODS

Patients

The study cohort included 263 *de novo* DLBCL patients with positive BM involvement and 449 patients with negative BM. This study is part of the International DLBCL Consortium Program. All patients needed to meet the following prerequisites: they were treated with front-line rituximab-containing standard protocols; age 16 years or older with a confirmed diagnosis of DLBCL according to 2016 WHO criteria²⁵ on pathology review; they underwent a pretreatment bilateral or unilateral posterior superior iliac crest BM biopsy (with both biopsy and aspiration); they had available BM slides for a central review in case of positive for lymphoma in BM. Patients were excluded if they had primary central nervous system (CNS), cutaneous, or mediastinal DLBCL, HIV infection, an identified past history of lymphoma or another malignancy that was uncontrolled. The study were reviewed and approved by the institutional review boards of each participating center, and the overall study was approved by the institutional review board of the University of Texas MD Anderson Cancer Center.

Disease staging and treatment response of all patients were assessed by the Ann Arbor system²⁶ and the Revised International Working Group response criteria,²⁷ respectively. A modification of the Ann Arbor classification defined stage I–II as limited DLBCL and stage III–IV as advanced DLBCL.²⁸ The interpretation of TP53 mutations by gene sequencing, MYC and BCL2 rearrangements by fluorescence *in situ* hybridization analyses, and p53, CD5, BCL2 and MYC expression by immunohistochemistry staining were based on published data.^{17,20,22,23} A cut-off value for a high Ki-67 index was considered to be 70%. gene expression profiling of specimens involved by DLBCL was performed and the COO classification was analyzed by GEP and the immunohistochemistry algorithm based on Choi and Visco/Young's algorithm methods.¹⁷

Definition and classification of BM involvement

BM trephine biopsies, clot sections and aspirate smears from DLBCL patients who were reported to have positive BM were centrally reviewed. Utilizing morphology and immunohistochemistry, the types and extent of BM involvement were identified.^{9,29–31} Concordant BM disease was defined by the involved BM area consisting of mostly large non-cleaved DLBCL cells; discordant BM was defined by the involved BM consisting of mostly small and low-grade lymphoma cells. We defined extensive BM involvement at 25% and higher replacement of the medullary space; less than 25% BM infiltration defined as limited or focal BM involvement.³² When bilateral BM specimens were available, determination of the extent of BM involvement depended on analysis of the sample with the greater degree of infiltration by lymphoma.

Statistical analyses

Clinicopathologic features were compared between the groups by using the independent samples *t* test for continuous variables and the χ^2 test for categorical variables. Progression-free survival was calculated from the date of initial diagnosis to the time of recurrence, disease progression or death from any cause. Overall survival was measured from the date of initial diagnosis until death, regardless of the cause. Patients still alive were censored at the date of the last contact. Progression-free survival and OS were estimated by the Kaplan–Meier method and the log-rank test was used for comparison between groups. The Cox proportional hazard model

was used for multivariate analysis to assess the independent effects of prognostic variables on survival. The data were analyzed by SPSS V.22.0 for Windows (SPSS Inc., Chicago, IL, USA). A two-sided *P*-value of < 0.05 was considered statistically significant and multiple comparisons were assessed by Bonferroni correction.

RESULTS

Patient characteristics

A total of 712 patients with DLBCL were enrolled in this study. A total of 263 patients with BM positive for lymphoma consisted of 173 (65.8%) cases with concordant BM and 90 (34.2%) with discordant BM involvement. The remaining 449 patients had negative BM including 212 (47.2%) patients with advanced DLBCL (stage III–IV). Clinical and pathologic characteristics according to type of BM involvement were compared and summarized in Tables 1 and 2, respectively. Given that either concordant or discordant BM disease belongs to a category of advanced DLBCL, we considered advanced DLBCL but with negative BM as an independent group to exclude the influence of stage and highlight the role of BM involvement.

The median age and the proportion of elderly patients (> 60 years) were similar for those with negative BM, including patients with advanced DLBCL, as well as for patients with concordant or discordant BM involvement. The concordant BM group contained more females than males, which was significantly different from the other three groups. The concordant BM group was more likely to have aggressive clinical features such as elevated LDH, poor PS, B symptoms and high IPI scores than the negative BM group, even compared with advanced DLBCL patients with negative BM. In contrast, patients with discordant BM were less likely to have an elevated LDH or B symptoms. Compared with patients with negative BM (including advanced DLBCL patients), the concordant BM group was more likely to express CD5 and MYC, harbor MYC rearrangement, co-express MYC and BCL2 (double positive lymphoma; DPL), and to be MYC/BCL2 double-hit lymphoma. BCL2 overexpression was significantly more common in patients with concordant or discordant BM disease than in patients with negative BM. The proportion of GCB subtype was higher in the discordant BM group than in the negative BM group, concordant BM group or advanced DLBCL with negative BM (83.3% vs 53.4%, 51.6%, 46.3%, respectively; $P < 0.001$).

Positive BM involvement characteristics

Concordant BM disease was more likely to be associated with extensive BM involvement than discordant disease was (57.0 vs 11.1%, $P < 0.001$; Figure 1a). In all, 199 of 263 (75.7%) patients with BM positive for lymphoma underwent bilateral BM biopsies. In these patients, 125 (62.8%) were bilaterally positive and 74 (37.2%) were positive on a single side; patients with BM positive on a single side more often had limited BM involvement (75.7 vs 53.6% for bilateral involvement, $P = 0.019$; Figure 1a). In 90 patients with discordant BM involvement, precise histopathologic subtypes of BM samples of 78 patients were confirmed: 54 (69.2%) of 78 patients had low-grade follicular lymphoma, 14 (17.9%) small lymphocytic leukemia/chronic lymphocytic lymphoma and 10 (12.8%) marginal zone lymphoma. Representative images of BM involvement are shown in Figure 1 (b1–b8).

In this study, most of the patients were treated with front-line R-CHOP; 40 (23.1%) of 173 patients with concordant BM were initially treated with intensive regimens (R-Hyper-CVAD/MA (rituximab, hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with high-dose methotrexate and cytarabine) or R-DA-EPOCH (rituximab, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin); 14 (15.6%) of 90 patients with discordant BM lymphoma received rituximab maintenance for at least 1 year after R-CHOP

Table 1. Clinical characteristics of patients grouped by type of BM involvement

Characteristics	Negative BM (n = 449) No. (%)	Concordant BM (n = 173) No. (%)	Discordant BM (n = 90) No. (%)	P-value			Advanced DLBCL and negative BM (n = 212) No. (%)	P-value	
				Neg vs Con	Neg vs Dis	Con vs Dis		Adv-Neg vs Con	Adv-Neg vs Dis
<i>Age (years)</i>									
Median	62.6	63.0	63.0	0.841	0.715	0.838	62.0	0.950	0.814
Range	16–89	25–91	31–89				17–89		
<i>Sex</i>									
Male	267 (59.5)	81 (46.8)	54 (60.0)	0.004	0.925	0.042	123 (58.0)	0.029	0.749
Female	182 (40.5)	92 (53.2)	37 (40.0)				89 (42.0)		
<i>IPI factors</i>									
Age > 60 years	255 (56.8)	101 (58.4)	51 (56.7)	0.720	0.982	0.789	121 (57.1)	0.911	0.948
LDH elevated	240 (57.4)	138 (80.2)	34 (38.2)	< 0.001	0.001	< 0.001	134 (66.0)	0.003	< 0.001
Stages III–IV	212 (47.9)	173 (100.0)	90 (100.0)	< 0.001	< 0.001	1.000	212 (100.0)	1.000	1.000
ECOG PS ≥ 2	53 (12.8)	63 (36.6)	10 (11.2)	< 0.001	0.691	< 0.001	39 (19.7)	< 0.001	0.078
Extranodal sites > 1	63 (14.1)	115 (66.5)	55 (61.1)	< 0.001	< 0.001	0.388	61 (28.9)	< 0.001	< 0.001
<i>IPI score</i>									
Low (0–1)	173 (39.9)	6 (3.5)	11 (12.4)	< 0.001	< 0.001	< 0.001	21 (10.2)	< 0.001	0.861
Intermediate (2–3)	214 (49.3)	85 (49.4)	58 (65.1)				138 (67.4)		
High (4–5)	47 (10.8)	81 (47.1)	20 (22.5)				46 (22.4)		
Bulky mass ≥ 7 cm	108 (28.5)	50 (30.1)	28 (31.1)	0.700	0.623	0.869	60 (32.8)	0.586	0.781
B symptoms	141 (32.4)	100 (58.1)	18 (20.0)	< 0.001	0.02	< 0.001	97 (46.6)	0.028	< 0.001

Abbreviations: Adv-Neg, advanced DLBCL with negative BM involvement; BM, bone marrow; Con, concordant BM involvement; Dis, discordant BM involvement; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase; Neg, negative BM involvement; PS, performance status.

therapy. CNS prophylaxis (that is, intrathecal methotrexate and/or intravenous high-dose methotrexate with or without cytarabine) was given to 107 (61.8%) of patients with concordant BM and 13 (14.4%) of patients with discordant BM. As shown in Figure 1c, complete response was achieved in 75 patients (83.3%) with discordant BM lymphoma, which was a higher rate than that achieved in 116 patients (67%) with concordant BM ($P=0.005$). Nevertheless, there was no significant difference in the probability of disease relapse ($P=0.526$) and CNS relapse ($P=0.340$) between patients with discordant vs concordant BM involvement. In addition, compared to patients with negative BM, concordant BM disease had a similar CNS relapse rate (2.4 vs 1.6%, $P=0.503$); no significant difference of CNS relapse rate (4.5 vs 1.6%, $P=0.072$) was seen in patients with discordant BM involvement. 39 patients with concordant BM and 29 patients with discordant BM lymphoma underwent a second non-BM tissue biopsy when disease relapsed; the biopsy specimen was more likely to show low grade lymphoma in the discordant BM group than in the concordant BM group (37.9 vs 5.1%, $P < 0.001$).

Prognostic significance of BM involvement

At the time of analysis, the median follow-up duration was 51.1 months (range: 0.23–186.7). The survival of patients with concordant BM involvement were significantly worse than not only patients with negative BM (5-year OS: 42.3 vs 67.7%, $P < 0.001$; 5-year PFS: 37.2 vs 60.6%, $P < 0.001$) but also advanced DLBCL with negative BM (5-year OS: 42.3 vs 57.2%, $P=0.007$; 5-year PFS: 37.2 vs 51.5%, $P=0.002$) (Figures 2a and b). As for patients with discordant BM involvement, the 5-year OS (70.0 vs 77.6%, $P=0.510$) was comparable to patients with limited DLBCL (stage I–II), the 5-year PFS was similar to advanced DLBCL with negative BM (49.7 vs 51.5%, $P=0.665$) (Figure 2b).

Prognostic significance of BM involvement incorporating IPI and COO. Multivariate analysis incorporating the IPI and COO subtypes was performed to illustrate the independent prognostic significance of different types of BM involvement (Supplementary Table S1). Concordant BM lymphoma retained a negative prognostic impact on OS and PFS, independent of the IPI and COO subtypes (OS: RR = 1.396, $P=0.032$; PFS: RR = 1.497, $P=0.006$). In contrast, discordant BM lymphoma had no prognostic effect on OS and PFS in the multivariate analysis. When examining the outcome of patients with high IPI scores or in non-GCB patients, the prognostic value of concordant lymphoma in the BM was evident (Supplementary Figure S1).

Development of BM-adjusted IPI. The univariate analysis of baseline features in 263 patients with concordant or discordant BM involvement revealed that age > 60 years, PS > 1, elevated LDH, B symptoms, concordant BM involvement, high Ki-67 and non-GCB subtype adversely affected OS, whereas extranodal sites > 1 and bulky mass did not (Supplementary Figure S2). Following multivariate analysis, age > 60 years, PS > 1 and concordant BM involvement were independent predictors of OS (Table 3). Thereby, a BM-adjusted IPI for patients with positive BM was constructed by using three clinical parameters, each equal to one point; four risk categories were generated: low (0 point), low-intermediate (1 point), high-intermediate (2 points) and high (3 points). Based on this risk-stratification model, patients assigned to the low-risk group had relatively good outcomes (5-year OS: 77.3%), and high-risk patients experienced extremely poor outcomes (5-year OS: 16.5%; Figure 2c). The similar results were observed when we randomly divided 263 patients with positive BM into the training ($n=132$) and validation ($n=131$) sets (Supplementary Figure S3).

Table 2. Pathologic characteristics of patients grouped by type of BM involvement

Characteristics	Negative BM (n = 449) No. (%)	Concordant BM (n = 173) No. (%)	Discordant BM (n = 90) No. (%)	P-value			Advanced DLBCL and negative BM (n = 212) No. (%)	P-value	
				Neg vs Con	Neg vs Dis	Con vs Dis		Adv-Neg vs Con	Adv-Neg vs Dis
COO									
GCB	233 (53.4)	63 (51.6)	50 (83.3)	0.725	< 0.001	< 0.001	95 (46.3)	0.354	< 0.001
Non-GCB	203 (46.6)	59 (48.4)	10 (16.7)				110 (53.7)		
Ki-67 index									
≥ 70	283 (65.4)	91 (71.7)	30 (54.5)	0.185	0.115	0.025	143 (69.8)	0.713	0.034
< 70	150 (34.6)	36 (28.3)	25 (45.5)				62 (30.2)		
CD5 expression									
Positive	17 (4.0)	25 (19.1)	6 (9.5)	< 0.001	0.052	0.089	8 (3.9)	< 0.001	0.081
Negative	411 (96.0)	106 (80.9)	57 (90.5)				196 (96.1)		
BCL2 expression									
positive	247 (57.4)	92 (75.4)	40 (87.0)	< 0.001	< 0.001	0.104	127 (62.6)	0.017	0.001
negative	183 (42.6)	30 (24.6)	6 (13.0)				76 (37.4)		
MYC expression									
Positive	132 (31.3)	28 (58.3)	NA	0.001	NA	NA	70 (35.7)	0.004	NA
negative	290 (68.7)	20 (41.7)	NA				126 (64.3)		
BCL2/MYC DPL									
positive	85 (19.9)	19 (31.1)	0 (0.0)	0.045	0.266	0.139	47 (23.7)	0.246	0.214
negative	342 (80.1)	42 (68.9)	5 (100.0)				157 (79.3)		
BCL2 rearrangement									
positive	65 (18.5)	20 (28.6)	6 (26.1)	0.056	0.370	0.818	38 (22.8)	0.342	0.722
negative	286 (81.5)	50 (71.4)	17 (73.9)				129 (77.2)		
MYC rearrangement									
positive	31 (11.0)	21 (34.4)	1 (16.7)	< 0.001	0.662	0.377	17 (12.1)	< 0.001	0.736
negative	251 (89.0)	40 (65.6)	5 (83.3)				124 (87.9)		
BCL2/MYC DHL									
Positive	9 (2.6)	9 (12.9)	0 (0.0)	< 0.001	0.443	0.077	4 (2.4)	0.001	0.460
negative	336 (97.4)	61 (87.1)	22 (100.0)				161 (97.6)		
p53 expression									
positive	129 (35.1)	19 (45.2)	NA	0.193	NA	NA	64 (37.4)	0.352	NA
negative	239 (64.9)	23 (54.8)	NA				107 (62.6)		
TP53 mutation									
positive	79 (20.8)	10 (23.3)	NA	0.707	NA	NA	40 (22.9)	0.956	NA
negative	301 (79.2)	33 (76.7)	NA				135 (77.1)		

Abbreviations: Adv-Neg, advanced DLBCL with negative BM involvement; BM, bone marrow; Con, concordant BM involvement; COO, cell-of-origin; Dis, discordant BM involvement; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell-like; Neg, negative BM involvement; NA, not available.

Prognostic significance of the extent of BM involvement and treatment in patients with concordant vs discordant BM disease

Extensive BM involvement compared with limited BM lymphoma did not lead to worse OS ($P=0.969$) or PFS ($P=0.960$) in the concordant BM subset (Figure 3a). However, in patients with discordant BM disease, limited BM involvement seemingly indicated a better OS (71.7 vs 50.0%, $P=0.051$; Figure 3a). For concordant BM group, patients treated with intensive regimens had better 5-year OS (71.1 vs 34.5%, $P < 0.001$) and PFS (66.4 vs 28.0%, $P < 0.001$) than patients accepting R-CHOP (Figure 3b); after controlling two remaining BM-adjusted IPI factors (age and PS), the survival benefit from intensive chemotherapy still existed (Supplementary Table S2). In patients with discordant BM, rituximab maintenance brought a 5-year OS (92.3 vs 65.8%, $P=0.007$) and PFS (69.2 vs 46.1%, $P=0.038$) benefit in comparison with observation after R-CHOP (Figure 3b).

Prognostic significance of biomarkers in concordant vs negative BM groups

We compared the prognostic significance of these adverse biomarkers independently and in combination summarized in Table 2 in concordant vs negative BM groups and estimated the prognostic power of concordant BM lymphoma relative to these biomarkers. Non-GCB/ABC, high Ki-67, CD5, p53 expression and MYC/BCL2 DPL predicted significantly or borderline inferior survival in both patients with concordant and negative BM, whereas BCL2 or MYC expression, BCL2 or MYC rearrangement and TP53 mutation lost their prognostic power in concordant BM group. Concordant BM disease remained a significant negative predictor in each of above-mentioned biomarker-positive subsets except in MYC/BCL2 double-hit lymphoma (DHL) subgroup (Supplementary Figure S4).

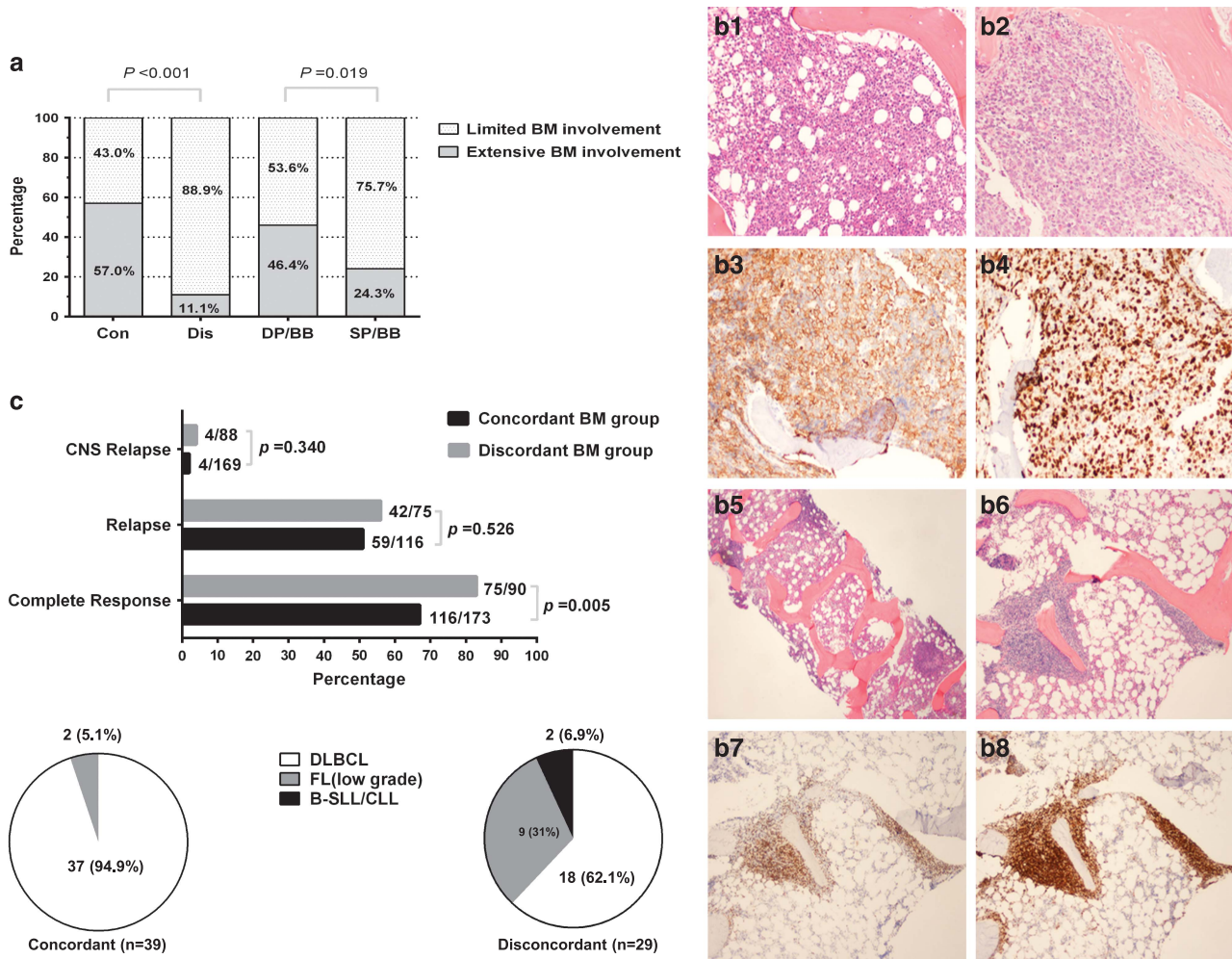


Figure 1. Characteristics of patients with BM positive for lymphoma. (a) Percentage of limited vs extensive BM involvement in concordant (Con) vs discordant (Dis) groups and double-positive BM involvement in bilateral BM biopsies (DP/BB) vs single-positive BM involvement in bilateral BM biopsies (SP/BB) groups. (b) A representative illustration of morphologic and immunophenotypic profiling of BM specimens in Con and Dis groups. Two DLBCL patients with 70% (b1) and 100% (b2) BM involvement; DLBCL cells are positive for CD20 (b3) with proliferation index Ki-67 (b4) at 80%; one FL case with paratrabecular involvement (b5, b6). FL cells are positive for PAX-5 (b7) and BCL2 (b8). Magnification $\times 20$ and $\times 40$. (c) Percentage of complete response, relapse and CNS relapse in Con vs Dis groups. Pie charts showing the proportions of different pathologic subtypes of second biopsy when relapse occurs in Con vs Dis groups. FL, follicular lymphoma; Con, concordant; Dis, discordant.

Gene expression signature of concordant BM involvement

To unfold the potential molecular basis underlying the aggressive clinical course of patients with concordant BM, we profiled and compared the GEP results of DLBCL with concordant vs negative BM involvement (Figure 4a). Thirty-four genes were differentially expressed, including 32 genes upregulated and only 2 genes downregulated in the concordant BM group (false discovery rate = 0.1; $P < 0.001$; Table 4a). Among the 32 upregulated genes, 10 genes were involved in cell metabolism; 6 participated in signaling pathways and cell cycle regulation; *RND2*, *GPR111*, *PPP1R9A* and *PRG4* encoded various proteins playing a key role in cellular adhesion or cytoskeletal reorganization; *GATAD1*, *GTF3A*, *ZRSR2*, *SRPX* and *MEOX2* were transcriptional regulators; and *TIMD4*, *CLEC4G* and *HP* linked with immune response. We further compared the GEP results between DLBCL in patients with concordant BM involvement and patients with advanced DLBCL without BM involvement (Figure 4b). Seventeen genes differentially expressed were picked out (false discovery rate = 0.15; $P < 0.001$; Table 4b); 12 (70.6%) of which were also identified in the former comparison.

We compared the GEP results between low (score: 1) and high BM-adjusted IPI (score: 2 or 3) subgroups and between MYC+ and MYC- subgroups with concordant BM involvement (Figure 4c). A total of 56 genes were differentially expressed in the former comparison and 26 differentially expressed genes were determined in the latter. Information about these genes is listed in Supplementary Tables S3 and S4.

DISCUSSION

In the current clinical practice, positive BM involvement (whether concordant or discordant) in DLBCL is generally recognized as high-risk advanced disease and is treated with the same immunochemotherapy regimen.^{33,34} This study, enrolling the largest number of *de novo* DLBCL patients with positive BM involvement to date, allowed valuable and effective analysis and confirmed that there is heterogeneity in the positive BM cohort.

It is well-known that patients with concordant BM involvement have a poor prognosis; however, the impact of discordant BM on the outcome of DLBCL patients remains controversial and even contradictory.^{7,8,35} Our study showed distinct observations: the OS

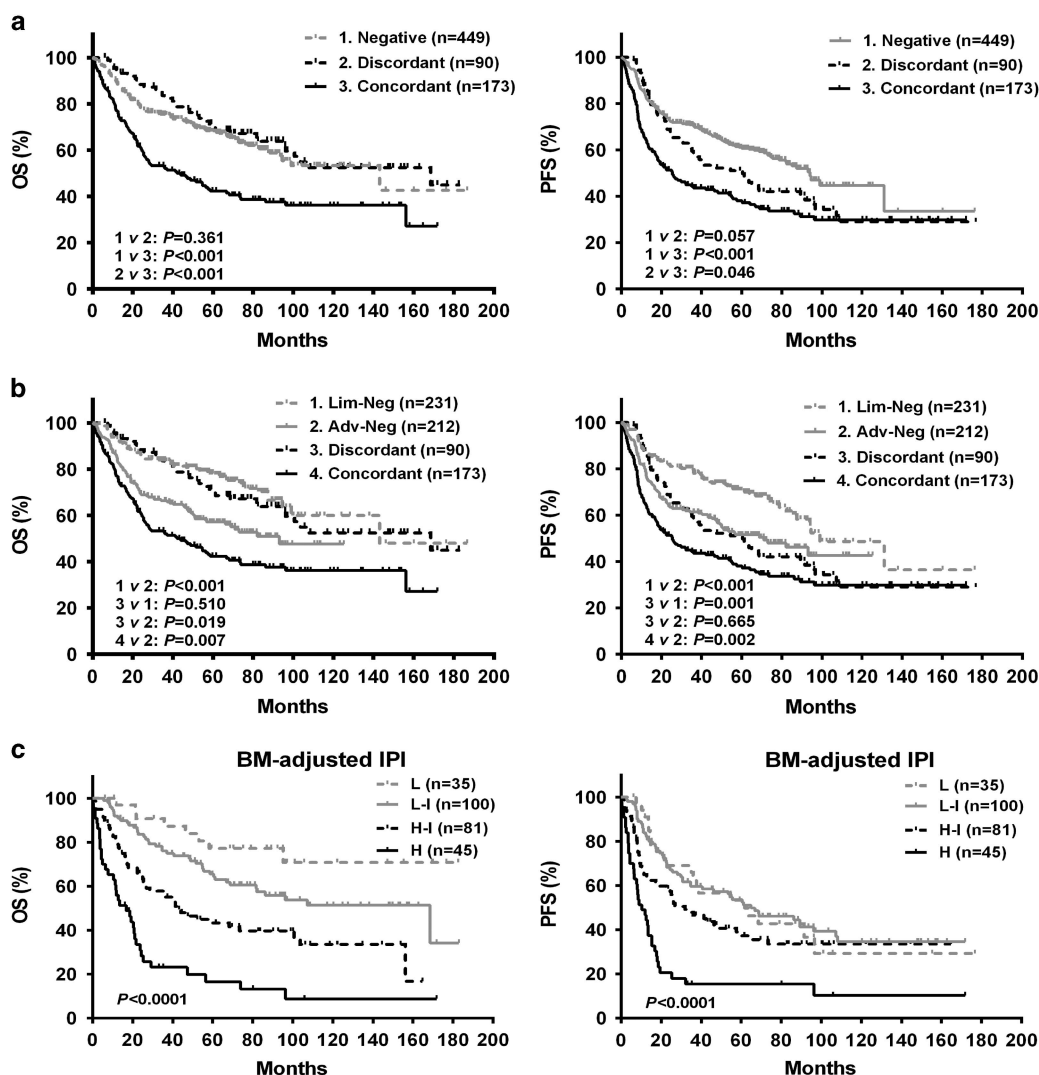


Figure 2. Survival curves for OS and PFS according to type of BM disease. **(a)** Comparisons of OS and PFS among negative, discordant and concordant BM involvement groups. **(b)** Comparisons of OS and PFS among limited DLBCL with negative BM involvement (Lim-Neg), advanced DLBCL with negative BM involvement (Adv-Neg), discordant BM and concordant BM groups. **(c)** The BM-adjusted IPI for risk stratification in patients with positive BM involvement. L, low risk; L-I, low-intermediate risk; H-I, high-intermediate risk; H, high risk.

Table 3. Prognostic factors of OS according to multivariate selection in patients with positive BM involvement

Positive BM involvement (n = 263)	RR	95% CI	P-value	Score
Age > 60 years	2.376	1.432–3.943	0.001	1
Elevated LDH	1.239	0.667–2.303	0.498	
ECOG PS ≥ 2	1.757	1.027–3.005	0.040	1
B symptoms	1.549	0.914–2.626	0.104	
Concordant BMI	1.997	1.079–3.696	0.028	1
Non-GCB	0.925	0.553–1.546	0.766	
High Ki-67	1.469	0.810–2.665	0.206	

Abbreviations: BM, bone marrow; BMI, body mass index; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell-like; LDH, lactate dehydrogenase; OS, overall survival; PS, performance status; RR, relative risk.

of patients with discordant BM involvement unexpectedly was comparable to that of patients with limited DLBCL; however, PFS remained poor, similar to that of advanced DLBCL with negative BM. Moreover, we found that concordant BM involvement demonstrated even poorer OS and PFS than did advanced DLBCL

but with negative BM. These findings further emphasize the worse outcome of DLBCL patients with concordant BM and the different prognosis of patients with concordant vs discordant BM involvement. In addition, we noted that the difference in PFS curves between discordant and concordant BM groups seemingly disappeared at 10 years later; based on the second biopsy pathology, patients with discordant BM were more likely to have indolent or low-grade lymphoma recurrence when BM was involved. These information are useful to help clinicians to design the BM status-based disease surveillance strategies.

Compared with the negative BM group, which has the largest proportion of low IPI scores, concordant BM large B-cell lymphoma is associated with a higher frequency of high IPI scores; patients with discordant BM involvement more often have intermediate IPI scores. This difference is attributed to groups' differing probabilities of having poor-risk clinical features encompassed by the IPI. Along with the IPI, the COO classification is widely accepted as a biological predictor in clinical practice. The results of our study showed that there is a similar proportion of the non-GCB subtype between concordant and negative BM groups and a significantly greater proportion of the GCB subtype in the discordant BM group; these findings

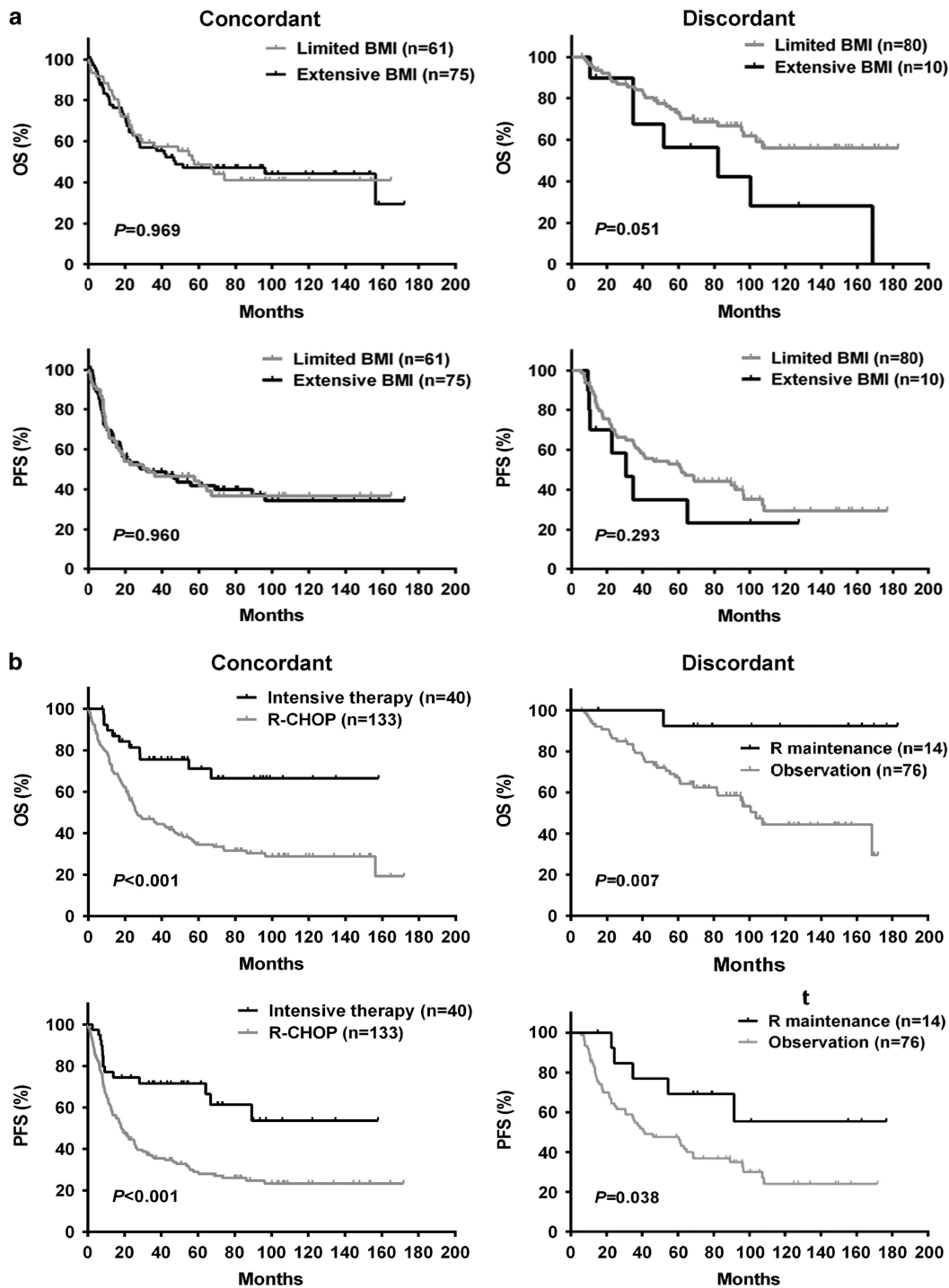


Figure 3. Survival curves for OS and PFS according to the extent of BM involvement and treatment in concordant and discordant BM groups. Limited BMI indicates limited BM involvement; Extensive BMI, extensive BM involvement; R maintenance, rituximab maintenance after induction therapy; Observation, observation after induction therapy.

are not in agreement with those of Park *et al.*⁷ of Korea, who observed a greater frequency of the non-GCB subtype in patients with concordant BM involvement. The discrepant results may be explained by the variation in ethnic groups and the size of the two studies. Although different types of BM involvement have a significant correlation with different IPI scores and COO subtypes, concordant BM involvement provides additional prognostic information beyond that conferred by the IPI and COO classification.

Considering the independent prognostic significance of concordant BM involvement, current IPI systems, including the standard IPI, the revised IPI and the NCCN-IPI, have an inevitable defect, especially for DLBCL patients with a positive BM, for whom they equalize concordant and discordant BM involvement. Consequently, we used multivariate analysis in the context of patients with positive BM lymphoma to develop a BM-adjusted IPI, which includes three independent prognostic factors: concordant BM involvement, age > 60 years and PS > 1; extranodal sites > 1

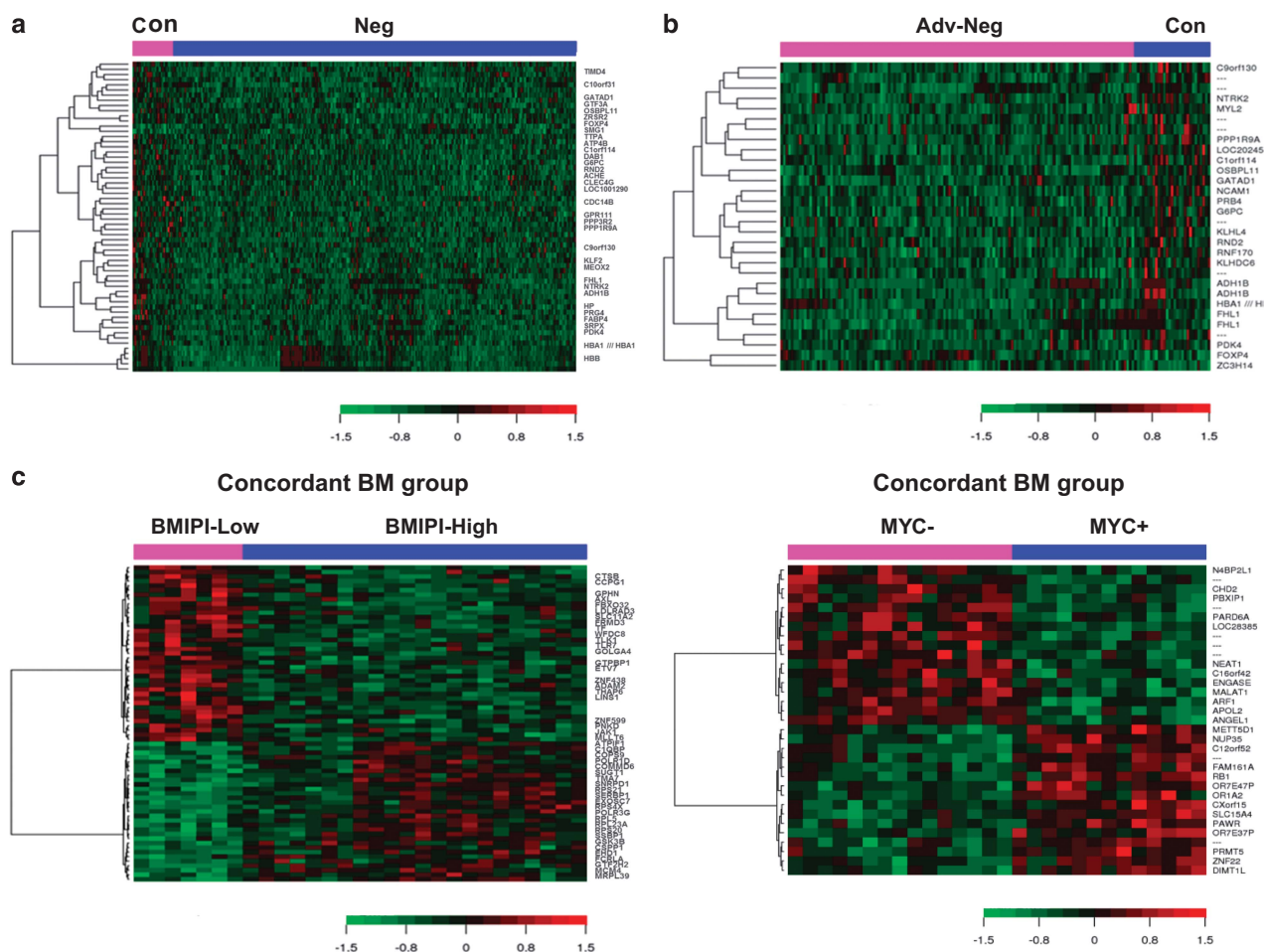


Figure 4. GEP heatmap in concordant BM involvement group. **(a)** GEP comparison between concordant and negative BM involvement groups. **(b)** GEP comparison between concordant BM group and advanced DLBCL with negative BM involvement group. **(c)** GEP comparison between high and low BM-adjusted IPI subsets in concordant BM group. GEP comparison between MYC⁺ and MYC⁻ subsets in concordant BM group. Con indicates concordant BM involvement; Neg, negative BM involvement; Adv-Neg, advanced DLBCL with negative BM involvement; BMIPI-Low, low BM-adjusted IPI; BMIPI-High, high BM-adjusted IPI.

and elevated LDH that are included in the current IPI systems are excluded because they have no independent prognostic significance. The BM-adjusted IPI is easy to use, is reasonable for DLBCL patients with positive BM involvement, and is potent in distinguishing the four risk groups.

In this study, extensive BM involvement was more often seen with concordant than with discordant BM disease and limited BM involvement was more likely in patients with unilateral involvement by lymphoma. Similar results were reported by Chuang *et al.*⁴ in the pre-rituximab era, despite the different cut-off values for distinguishing extensive from limited BM involvement. Interestingly, when we explored the prognostic significance of the extent of BM involvement in the concordant BM group, patients with limited BM involvement had the same poor survival as did those with extensive BM disease; however, in the discordant BM group, patients with limited BM involvement had a significant trend for longer survival than did those with extensive BM involvement. These phenomena illustrate that (1) the extent of BM involvement should be considered as one of the key points of differential diagnosis between concordant and discordant BM disease; (2) the importance of bilateral BM biopsies, as reported by Juneja *et al.*,³⁶ should be emphasized once again because unilateral biopsies might 'miss' positive BM involvement in some patients, in particular those with limited BM involvement; and (3) the prognostic significance of the extent of BM involvement in different types of BM disease may be divergent.

Besides more often having poor-risk clinical features, the concordant BM group has an increased probability for some unfavorable molecular characteristics, which may contribute to the dismal outcome of patients with concordant BM. Interestingly, concordant BM disease remained an adverse predictor in almost all of biomarker-positive subsets, whereas only part of frequently used biomarkers retained their prognostic significance for patients with concordant BM, as did for patients with negative BM. These findings suggest that concordant BM involvement may not be entirely surrogate for these molecular features, and other possible mechanisms to induce concordant BM involvement may underscore the adverse biological events that should not be neglected. Through the GEP analysis in this study, some differential expression genes were revealed in the concordant BM group: high expression level of metabolism-related genes and transcription-activating genes may reflect a high proliferation potency of lymphoma cells; although genes associated with cellular adhesion or migration have not often been described in lymphoma, such a signature in the metastases of solid tumors is well-established;³⁷ *TIMD4* is a member of the T-cell immunoglobulin and mucin domain gene family, which plays a critical role in immunoregulation, and a high level of *TIMD4* has been reported as a negative regulator of antitumor immunity.³⁸ Therefore, a potential molecular basis underlying the development of concordant BM disease may be related to tumor proliferation, cellular adhesion or migration, and immune tolerance or escape.

Table 4. Genes differentially expressed in Con vs Neg and Con vs Adv-Neg

Gene functional categories	Upregulated genes		Downregulated genes	
	No. of genes	Representative genes	No. of genes	Representative genes
<i>(a) Con vs Neg</i>				
Metabolism	10	<i>ATP4B, TTPA, OSBPL11, G6PC, ADH1B, FABP4, PDK4, ACHE, HBA1/HBA2, HBB</i>	0	
Signaling pathways, cell cycle regulation	6	<i>DAB1, PPP3R2, NTRK2, CDC14B, KLF2, FHL1</i>	0	
Extracellular matrix, adhesion and cytoskeletal organization	4	<i>RND2, GPR111, PPP1R9A, PRG4</i>	0	
Transcriptional regulation	5	<i>GATAD1, GTF3A, ZRSR2, SRPX, MEOX2</i>	2	<i>FOXP4, SMG1</i>
Immune response	3	<i>TIMD4, CLEC4G, HP</i>	0	
Others (including ncRNA)	4	<i>C10orf31, C9orf130, LOC100129029, C1orf114</i>	0	
<i>(b) Con vs Adv-Neg</i>				
Metabolism	4	<i>OSBPL11^a, G6PC^a, ADH1B^a, PDK4^a, HBA1/HBA2^a</i>	0	
Signaling pathways, cell cycle regulation	3	<i>RNF170, NTRK2^a, FHL1^a</i>	0	
Extracellular matrix, adhesion and cytoskeletal organization	6	<i>MYL2, NCAM1, KLHL4, PPP1R9A^a, RND2^a, KLHDC6</i>	0	
Transcriptional regulation	1	<i>GATAD1^a</i>	2	<i>FOXP4^a, ZC3H14</i>
Immune response	0		0	
Others (including ncRNA)	3	<i>PRB4, C9orf130^a, C1orf114^a</i>	0	

Abbreviations: Adv-Neg, advanced DLBCL with negative BM involvement; Con, concordant BM involvement; Neg, negative BM involvement; ncRNA, non-coding RNA. ^aGenes differentially expressed in both Con vs Neg and Con vs Adv-Neg.

Kremer *et al.*³⁹ reported that a clonal relationship between the low-grade infiltrates in the BM and DLBCL was confirmed in the two-thirds of patients with discordant BM disease, with the remaining one-third exhibiting different clones as shown by comparatively analyzing *IGH* and *BCL2* rearrangements. In our study, most patients with discordant BM disease had low-grade follicular lymphoma in their BM samples, and displayed the hallmark of DLBCL transformed from follicular lymphoma that the proportions of GCB subtype and *BCL2* overexpression are significantly higher than in pure *de novo* DLBCL.⁴⁰ These data further support the hypothesis that discordant BM disease is considered as either an occult indolent B-cell lymphoma from which DLBCL transformed or coincident indolent B-cell lymphoma in DLBCL.

Some authors have recommended intensive immunochemotherapy as the initial therapy for high-risk DLBCL, and some specific dose-intensive approaches such as R-Hyper-CVAD/MA or R-DA-EPOCH could partly conquer the inferior prognosis caused by *MYC* translocation, *MYC/BCL2* co-expression and *MYC/BCL2* double hit.^{41–44} We found that intensive regimens seemingly resulted in longer survival than did R-CHOP for patients with concordant BM. Also, likely due to the above hypothesis for discordant BM involvement, the survival of this group appeared to be prolonged by rituximab maintenance, just as this strategy improved outcome in advanced indolent B-cell lymphoma.^{45,46} It may be appropriate for patients with different types of BM disease to accept precision treatment strategies, whereas this assumption needs further study in future prospective clinical trials.

Although former studies have reported positive BM involvement associated with an increased likelihood of CNS relapse in DLBCL, universal application of CNS prophylaxis is not justified.⁴⁷ Sehn *et al.*¹⁰ reported that without routine CNS prophylaxis, concordant BM involvement was a significant risk factor for CNS relapse and there was a trend toward a slightly higher rate of CNS relapse in patients with discordant BM. However, our study showed different observation in which the frequency of CNS relapse was not escalated in patients with concordant BM disease, most of whom had received CNS prophylaxis. Therefore, the difference in the two studies suggests CNS prophylaxis may reduce the likelihood of CNS relapse.

In conclusion, this retrospective study provides a comprehensive summary of clinical and biological features in DLBCL with BM involvement in the rituximab era. Positive BM infiltration in DLBCL represents a heterogeneous group of disorders. The BM-adjusted IPI for patients with positive BM is an effective, valuable and potent prognostic model for risk stratification. Our findings suggest that DLBCL with concordant BM involvement constitutes a distinct subset with unfavorable gene expression signatures, high-risk clinicopathologic features and poor prognosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conception, design and writing: ZY, KHY. Research performance: ZY, LD, ZYX-M, GCM, AT, YL, KHY. Provision of study thought, materials, key reagents and technology: ZY, LD, ZYX-M, GCM, PJ, AT, CV, GB, JW, KD, AO, EDH, JH, MP, AJMF, MBM, JNW, MAP, JF, YL, YS, RZO, HK, LJM, YL, JC, KHY. Collection and assembly of data under approved IRB and Material Transfer Agreement: ZY, LD, ZYX-M, PJ, AT, CV, GB, JW, KD, AO, EDH, JH, MP, AJMF, MBM, JNW, MAP, JF, YL, YS, YL, KHY.

Data analysis and interpretation: ZY, LD, ZYX-M, GCM, LJM, YL, JC, KHY. Manuscript editing: ZY, LD, ZYX-M, AT, LJM, YL, JC, KHY. Final approval of manuscript: All authors.

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