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ARTICLE

Blastoid high-grade B-cell lymphoma initially presenting in bone marrow: a diagnostic challenge

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The 2016 WHO classification introduced the category of high-grade B-cell lymphoma (HGBL), which includes one poorly understood subset, blastoid-HGBL. Establishing the diagnosis and distinguishing blastoid-HGBL from B-acute lymphoblastic leukemia (B-ALL) in bone marrow can be challenging. We assessed 31 cases of blastoid-HGBL diagnosed initially in bone marrow and compared this group to 36 cases of B-ALL using immunophenotyping, fluorescence in situ hybridization, and targeted next generation sequencing analysis. The 31 blastoid-HGBL cases included 14 HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangements (double hit lymphoma, DHL), 13 HGBL, not otherwise specified (NOS), and four cases with TdT expression that were difficult to classify. Compared with B-ALL, blastoid-HGBL cases more often showed increased intensity/bright expression of CD20, CD38, CD45, BCL-6, and MYC, and less frequent bright expression of CD10 and TdT. Cases of blastoid-HGBL also more frequently had *MYC* rearrangement, a complex karyotype and *TP53* mutation ($p < 0.01$). With the exception of CD34, no other single factor, including TdT, was sensitive or adequately specific to distinguish blastoid-HGBL from B-ALL. We developed a scoring system using six distinctive features between 16 cases of unequivocal blastoid HGBL and 22 cases of CD34-positive B-ALL, with a score of ≥3 defining blastoid-HGBL. The system was further validated by using 15 cases of surface light chain negative, and/or CD45 dim to negative blastoid-HGBL and 14 cases of CD34-negative B-ALL. The sensitivity, specificity, positive, and negative predictive value of this scoring system were 100%, 94%, 94%, and 100%, respectively. Using this system, the four cases with TdT expression were all classified as blastoid-HGBL: three were DHL and one was HGBL-NOS. In conclusion, blastoid-HGBL shows distinctive immunophenotypic, cytogenetic, and molecular features as compared with B-ALL. The proposed scoring system can be helpful for the classification of diagnostically challenging blastoid lymphoid tumors presenting initially in the bone marrow.

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INTRODUCTION

The World Health Organization (WHO) classification introduced the category of high-grade B-cell lymphoma (HGBL) in 2016. This heterogeneous disease category is associated with a poor prognosis and includes B-cell lymphomas that resemble, more or less, diffuse large B-cell lymphoma, Burkitt or Burkitt-like lymphoma and a smaller, poorly understood disease subset composed of blastoid B-cells. Previous studies have shown that blastoid B-cell lymphomas are also heterogeneous at the molecular level. About half of these cases carry *MYC, BCL2* and/or *BCL6* rearrangements, so-called double (or triple) hit lymphoma (DHL)[1]. Other blastoid cases may carry solitary *MYC* rearrangement or lack characteristic cytogenetic abnormalities and are included in the HGBL, not otherwise specified (NOS) category[2].

The morphologic features of blastoid-HGBL overlap greatly with other blastoid B-cell lymphomas, such as blastoid variant of mantle cell lymphoma, as well as truly blastic neoplasms such as B-acute lymphoblastic leukemia (B-ALL). As a result, the diagnosis and differential diagnosis of blastoid B-cell neoplasms can be challenging, especially when the neoplasm presents initially in the bone marrow, where B-ALL is a major consideration. Immunophenotypic studies, and sometimes cytogenetic and/or molecular studies, are helpful in this differential diagnosis. In the WHO classification, a diagnostic algorithm for blastoid-HGBL was provided. However, in our daily practice we do encounter some cases of blastoid B-cell lymphoma that are difficult to classify. It is known that blastoid B-cell lymphomas usually present with lymph node involvement, whereas most B-ALL do not involve lymph nodes. However, some blastoid B-cell neoplasms with a leukemic presentation either do not involve lymph nodes or nodal involvement is unknown at the time of bone marrow examination. Establishing the diagnosis of de novo blastoid-HGBL and distinguishing these neoplasms from B-ALL can be a dilemma, especially when the blastoid neoplasm presents initially in bone marrow at time of initial diagnosis.

In this study, we attempt to address the following issues. First, we assessed the immunophenotypic and molecular cytogenetic features of blastoid-HGBL presenting initially in the bone marrow. As a part of this evaluation, we compared cases of CD45 bright and surface light chain restricted unequivocal blastoid HGBL and unequivocal CD34+ B-ALL cases to determine features useful in differential diagnosis. Secondly, we developed and validated a scoring system with sufficient sensitivity and specificity to aid the

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pathologist in the differential diagnosis of blastoid B-cell neoplasms that initially present in the bone marrow.

MATERIALS AND METHODS

Patient selection

The archives of the Department of Hematopathology at The University of Texas MD Anderson Cancer Center were searched for B-cell lymphomas with blastoid morphology diagnosed between January 1, 2016 and December 31, 2020. Inclusion criteria were: (1) Lymphoma cells showed blastoid morphology in routinely prepared, hematoxylin, and eosin-stained tissue sections; (2) a B-cell immunophenotype was identified by flow cytometry and/or immunohistochemistry; (3) the neoplasm presented initially in the bone marrow and lymph nodes were not involved or lymph node status was unknown at the time of bone marrow examination. Exclusion criteria were: (1) neoplasms classified as Burkitt lymphoma, B-ALL, and blastoid variant of MCL (including cyclin D1-negative cases); (2) cases without cytogenetic or FISH studies performed to assess *MYC*, or cases without adequate material at the time of this study to evaluate for *MYC* rearrangement. Cases of B-ALL diagnosed in bone marrow during the same time period and roughly age-matched with the study group were selected as a comparison group. Corresponding medical records were reviewed to obtain clinical information.

Immunophenotypic analysis

We performed multicolor flow cytometry immunophenotypic analysis on bone marrow aspirate specimens collected in EDTA-anticogulant and processed within 6 h of collection as described previously[3]. Erythrocytes were lysed with ammonium chloride (Pharm LyseTM, BD Biosciences, San Diego, CA) at room temperature for 10 min using a standard lyse/wash technique after incubation with monoclonal antibodies for 10 min at 4 °C. The following antibodies were used: CD2, cytoplasmic CD3, surface CD3, CD4, CD5, CD7, CD10, CD13, CD14, CD15, CD19, CD20, CD22, CD23, CD25, CD33, CD34, CD36, CD38, CD41, CD45, CD49d, CD52, CD56, CD64, CD66c, cytoplasmic CD79a, CD81, CD117, CD123, cytoplasmic IgM, human leukocyte antigen (HLA)-DR, kappa, lambda, myeloperoxidase, and terminal deoxynucleotidyl transferase (TdT). All antibodies were purchased from BD Biosciences (BD Biosciences). Samples were acquired on FACSCanto II instruments (BD Biosciences) and analysis of flow cytometry data was performed using FCS Express software (De Novo Software, Los Angeles, CA).

Immunohistochemical analysis was performed using formalin-fixed, paraffin-embedded tissue sections as described previously[3]. The antibody panel included reagents specific for: CD3, CD20, CD30, CD79a, CD138, BCL6, and Ki-67 (Dako North America, Carpinteria, CA); CD5 and cyclin D1 (Thermo Scientific, Fremont, CA); SOX11 (Cell Marque, Rocklin, CA); CD34 and PAX5 (BD Biosciences); BCL2 and TdT (Leica Biosystems, Buffalo Grove, IL); and MYC (Ventana, Tucson, AZ). The positive cutoff was ≥30% for CD10 and BCL6; ≥40% for MYC; ≥50% for BCL2 and >10% for SOX11[4–6]. In situ hybridization for Epstein-Barr virus encoded small RNA (EBER) (Ventana) was also performed.

Conventional cytogenetic analysis and fluorescence in situ hybridization (FISH)

Conventional cytogenetic analysis was performed on G-banded metaphase cells prepared from unstimulated bone marrow aspirate cultures using standard techniques. Twenty metaphases were analyzed and the results were reported according to the International System for Human Cytogenetic Nomenclature[7]. Fluorescence in situ hybridization (FISH) was performed on bone marrow smears. A total of 200 interphase nuclei for each probe were analyzed. FISH probes used in this study included the following: LSI IGH/CCND1 dual-color, dual fusion translocation probe; LSI MYC as well as BCL6 dual-color, break-apart probes; LSI IGH/BCL2 dual-color, dual-fusion translocation probe (Vysis/Abbott Laboratories, Des Plaines, IL, USA).

Molecular analysis

Gene mutation analysis was performed using DNA extracted from bone marrow aspirate specimens in a subset of patients. Amplicon-based next generation sequencing (NGS) targeting the entire coding regions of a panel of 28 genes (earlier cases) or 81 genes (more recent cases) was performed using a MiSeq platform (Illumina, San Diego, CA) to detect somatic mutations and insertions/ deletions as previously described[8]. The 28-gene panel included: *ABL1, ASXL1, BRAF, DNMT3A, EGFR, EZH2, FLT3, GATA1, GATA2, HRAS, IDH1, IDH2, IKZF2, JAK2, KIT, KRAS, MDM2, KMT2A (MLL), MPL, MYD88, NOTCH1, NPM1, NRAS, PTPN11, RUNX1, TET2, TP53*, and *WT1*. The 81-genet panel included: *ANKRD26, ASXL1, ASXL2, BCOR, BCORL1, BRAF, BRINP3, CALR, CBLB, CBLC, CBL, CRLF2, CREBBP, CEBPA, CSF3R, CUX1, DDX41, DNMT3A, EED, ELANE, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GFI1, GNAS, HNRNPK, HRAS, IDH1, IDH2, IKZF1, IL2RG, IL7R, KRAS, JAK2, JAK3, KDM6A, KIT, KMT2A, MAP2K1, MPL, NF1, NOTCH1, NPM1, NRAS, PAX5, PHF6, PIGA, PML, PRPF40B, PTEN, PTPN11, RAD21, RARA, RUNX1, SETBP1, SF1, SF3A1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG1, STAG2, STAT3, STAT5A, STAT5B, SUZ12, TERC, TERT, TET2, TP53, U2AF1, U2AF2, WT1*, and *ZRSR2*. All coding exons for each gene were covered with an analytical sensitivity of 5% mutant reads in a background of wild-type reads. Established bioinformatics pipelines were used to identify somatic variants.

Statistical analysis

Overall survival (OS) was defined from the date of diagnosis to the date of last follow-up or death. Patient survival was analyzed by the Kaplan–Meier method and compared using the log rank test (GraphPad Prism 8 software). Differences in the blastoid HGBL and B-ALL groups were analyzed using the Fisher exact test. All p values were two-tailed and considered significant when <0.05.

RESULTS

Clinicopathologic features of blastoid high-grade B-cell lymphoma

Clinical features. The study cohort included 31 patients, 18 men, and 13 women, with a median age of 61 years (range, 30–85). Twenty-five (81%) patients presented with de novo blastoid B-cell lymphoma and six had a history of low-grade B-cell lymphoma, including four follicular lymphoma, one chronic lymphocytic leukemia/small lymphocytic lymphoma, and one with clonal B-cells but an unclear diagnosis. All patients presented with bone marrow involvement and therefore had stage 4 disease. The central nervous system was involved in 5 of 24 (21%) patients, who underwent cerebrospinal fluid analysis. Serum lactate dehydrogenase levels were high in all patients except one (97%). Twenty-one (68%) patients had extranodal sites of disease other than bone marrow detected immediately after diagnosis of the bone marrow specimen, with most sites of disease being detected by imaging studies. Other involved extranodal sites included: central nervous system, spleen, bones, pleural fluid, peritoneal fluid, pancreas, stomach, liver, kidneys, breasts, adrenal glands, and soft tissue. Twenty-six of 29 (90%) patients had a high-intermediate or high international prognostic index score.

Histopathologic features. Pathologic examination of bone marrow biopsy specimens showed hypercellular (~90%) bone marrow with numerous immature-appearing lymphoid cells in a diffuse or interstitial pattern. The neoplastic cells were predominantly medium-sized with irregular round nuclear contours, inconspicuous to conspicuous nucleoli, and scant cytoplasm. Mitotic figures were easily identified. Bone marrow aspirate smears showed a predominance of monomorphic medium-sized cells with scant basophilic cytoplasm, finely dispersed chromatin, round nuclei and inconspicuous nucleoli, and resembling lymphoblasts (Fig. 1).

Immunophenotypic features. The immunophenotypic features of the blastoid-HGBL cases are summarized in Table 1. All cases were evaluated by flow cytometry immunophenotypic analysis, and MYC, BCL2, and BCL6 were evaluated by immunohistochemistry. In all 31 cases, the blastoid lymphoid infiltrate was composed of B-cells, positive for pan B-cell markers (CD19, CD20, CD22, etc.). CD20 was positive in 23 (74%) cases, including five cases with dim expression (dimmer than normal B cells). CD22 was expressed in 27 (87%) cases including ten with dim expression. CD10 was expressed in 28 (90%) cases and only one showed bright intensity greater than hematogones. CD38 was expressed in 30 (97%) cases,

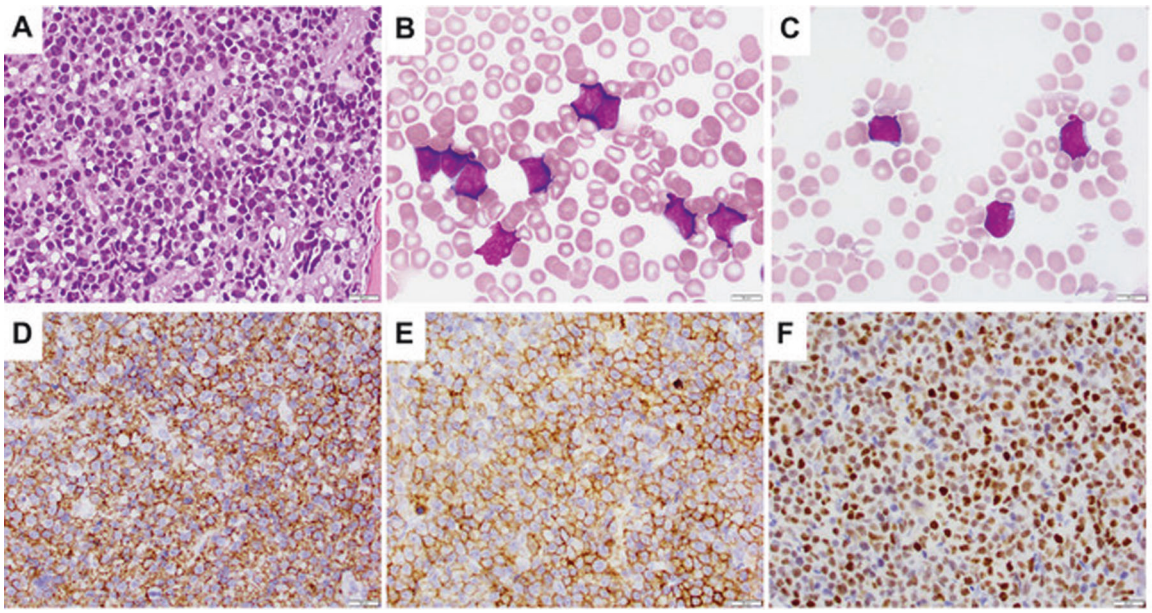

Fig. 1 A representing case of blastoid B cell lymphoma diagnosed in bone marrow. A Core biopsy showed a hypercellular marrow almost completely replaced by a diffuse infiltrate of medium-sized blastoid lymphoid cells. Blastoid cells in bone marrow aspirate smear (**B**) and peripheral smear (**C**). The blastoid cells showed diffuse expression of CD20 (**D**), CD10 (**E**) and BCL6 (**F**) (**A**, H&E stain, x500; **B** and **C**, Giemsa stain, x1000; **D**–**F**, immunohistochemistry, x500).

Table 1. Immunophenotypic comparision of blastoid high grade B cell lymphoma (Blastoid HGBL) and B lymphoblastic leukemia (B-ALL).

	CD45bri-light chain restricted blastoid HGBL *n* = 16% (Positive/Total)	**CD34+ B-ALL *n* = 22% (Positive/Total)**	***p***	**Total blastoid HGBL *n* = 31% (Positive/Total)**	**Total B-ALL *n* = 36% (Positive/Total)**	***p* (Total)**
CD45 intensity > Granulocytes	100 (16/16)	14 (3/22)	**0.0001**	74 (23/31)	17 (6/36)	**0.0001**
CD38 bright	75 (12/16)	14 (3/22)	**0.0002**	87 (26/30)	28 (10/36)	**0.0001**
CD10 bright	6 (1/16)	68 (15/22)	**0.0002**	3 (1/30)	50 (18/36)	**0.0001**
CD20 dim/negative	31 (5/16)	86 (18/21)	**0.0016**	43 (13/30)	92 (33/36)	**0.0001**
CD34+	0 (0/16)	100 (22/22)	**0.0001**	0 (0/30)	61 (22/36)	**0.0001**
TdT+	8 (1/12)	90 (18/20)	**0.0001**	14 (4/28)	94 (31/33)	**0.0001**
CD22 dim/negative	25 (4/16)	52 (11/21)	0.1757	47 (14/30)	44 (15/34)	1.000
BCL6 ≥ 30%	79 (11/14)	25 (3/12)	**0.0162**	74 (20/27)	25 (3/12)	**0.0061**
MYC ≥ 40%	92 (11/12)	17 (2/12)	**0.0006**	90 (25/27)	17 (2/12)	**0.0001**
BCL2 ≥ 50%	67 (10/15)	100 (6/6)	0.2621	70 (21/30)	100 (6/6)	0.3026
CD13 and/or CD33				0 (0/14)	48 (16/35)	**0.002**

Bold *p* value indicate statistically significantly different.

with 26 cases showing bright intensity (87%) similar to hematogones. All cases were positive for CD45 with variable intensity, ranging from brighter than granulocytes in 23 (74%) cases, similar to granulocytes in 1 (3%) case, and dimmer than granulocytes in 7 (23%) cases. Surface light chain restriction, either kappa or lambda, was detected in 22 (71%) cases. TdT was tested in 25 cases by immunohistochemistry and three were positive, including one with diffuse variable expression, one with diffuse dim expression, and one in which only a subset of cells was positive. In 14 cases TdT was tested by flow cytometry and one was positive. Altogether, 4 of 28 (14%) cases of blastoid-HGBL were positive for TdT (Table 1). BCL6 was expressed in 20 of 27 (74%) cases evaluated (Fig. 1). All cases were negative for CD5, CD34, and cyclin D1. Fourteen cases of blastoid-HBCL were evaluated using the acute leukemia flow cytometry panel initially, and no case showed expression of myeloid antigens.

Molecular and cytogenetic features. FISH analysis for *MYC* was performed on all cases. *MYC* rearrangement (*MYC*-R) was detected in 26 (84%) cases (Table 2), including 11 *MYC/BCL2* double hit lymphoma, five *MYC/BCL2/BCL6* triple hit lymphoma, one *MYC/BCL6* double hit lymphoma, and nine cases with *MYC*-R only. Fives cases showed no *MYC*-R. The four cases with TdT expression included one *MYC/BCL2* double hit lymphoma, one *MYC/BCL6* double hit lymphoma, one *MYC/BCL2/BCL6* triple hit lymphoma, and one without *MYC*-R. All cases were negative for *CCND1* rearrangement.

Conventional karyotyping was performed in 24 cases and a complex karyotype (defined as at least have three structural and/or numerical abnormalities) was detected in 22 (92%) cases (Table 2). There was a significant correlation between karyotype complexity and *MYC* rearrangement ($p = 0.04$). All 19 cases of blastoid-HBCL with *MYC*-R assessed by conventional cytogenetics

had a complex karyotype. Of the five patients without *MYC*-R, three had a complex karyotype and two were diploid. A complex karyotype was detected in all ten cases of double/triple hit lymphoma and 12 of 14 HGBL-NOS cases tested. There was no significant difference in the frequency of a complex karyotype between HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangement versus HGBL-NOS ($p = 0.49$).

Targeted next generation sequencing (NGS) was performed in 15 cases of blastoid-HBCL. The most commonly mutated genes were *TP53* ($n = 7$; 47%), followed by *CREBBP* ($n = 5$; 33%) and nine cases of blastoid-HGBL had single gene mutations involving *ASXL1, ASXL2, ATM, CXCR4, EZH2, IDH2, MYD88*, and *TET2* (Table 2).

Treatment and prognosis. Thirty patients had treatment information available and all received combination chemotherapy: Twenty-nine (97%) patients received intensive induction chemotherapy, mainly rituximab, etoposide, prednisone, vincristine, and doxorubicin (R-EPOCH, $n = 19$) or rituximab, hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with methotrexate and cytarabine (R-Hyper-CVAD, $n = 10$). One (3%) patient received hydrea and cytarabine. The induction treatment regimen was similar in patients categorized as blastoid-HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangement versus blastoid HGBL-NOS ($p = 1.0$). After induction chemotherapy, 12 (39%) patients reached complete remission, six partial remission, ten had persistent or progression of disease, and two were lost follow-up. Four of 12 patients who went into complete remission relapsed. After a median follow-up of 15 months (range, 0.3–40.3 months), the median overall survival was 9.2 months. Patients with HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangement ($n = 17$) showed a similar overall survival compared to those with HGBL-NOS ($n = 14$) (Fig. 2A, 9.2 vs. 8.4 months respectively, $p = 0.41$). The median overall survival was also similar for patients with ($n = 26$) or without *MYC*-R ($n = 5$) (Fig. 2B, 9.2 vs. 13.4 months, respectively, $p = 0.79$).

Table 2. Molecular and cytogenetic comparision of blastoid high grade B cell lymphoma (Blastoid HGBL) and B lymphoblastic leukemia (B-ALL).

	Blastoid HBCL% (Positive/Total)	Total B-ALL% (Positive/Total)	*p*
MYC rearrangement	84 (26/31)	0 (0/35)	**0.0001**
Complex karyotype[a]	92 (22/24)	51 (18/35)	**0.0015**
TP53 mutation	47 (7/15)	13 (4/30)	**0.0256**
CREBBP	33 (5/15)	10 (3/30)	0.0947
NRAS	0 (0/15)	20 (6/30)	0.1602
KRAS	0 (0/15)	13 (4/30)	0.3024

Bold *p* value indicate statistically significantly different.
[a]Complex karyotype is defined by at least three structural and/or numerical abnormalities.

Comparison of blastoid-HGBL to B-ALL and establishing a scoring system for this differential diagnosis

The morphologic features were very similar between cases of blastoid-HGBL and B-ALL, either in core biopsy sections or aspirate smears (Figs. 1 and 3). However, the immunophenotype was significantly different between them (Table 1 and Figs. 1, 3, and 4). Of the 31 cases of blastoid-HGBL, 16 had surface light chain restriction and relatively bright CD45 compared with granulocytes. Among the 36 cases of B-ALL, 22 expressed CD34 (one with dim partial expression). Compared to the 22 cases of CD34+ B-ALL, the 16 cases of unequivocal blastoid-HGBL showed more frequent bright CD45 (intensity brighter than granulocytes) and brighter CD38, were more often positive for BCL6 and MYC, and less frequently showed bright CD10, dim or negative CD20, ot TdT expression ($p < 0.01$ for all). No statistically significant difference was noticed in CD22 and BCL2 expression between these groups ($p > 0.05$) (Table 1).

Fourteen cases of B-ALL were CD34-negative, seven of which had bright CD38, and four cases had bright CD45 without CD10 expression, making these cases difficult to distinguish from blastoid-HGBL initially. Subsequent cytogenetic studies revealed six with 11q23/*KMT2A (MLL)* rearrangement and eight with t(1;19)(q23;p13.3) or *E2A/PBX1* translocation, supporting a diagnosis of B-ALL. Fifteen cases of blastoid-HBCL either lacked expression of surface light chain or/and had CD45 intensity similar to or less than granulocytes, and therefore 14 of them were evaluated by an acute leukemia flow cytometry panel initially. None of these cases showed myeloid antigen expression. This was in contrast to one or more myeloid markers (CD13 and/or CD33) expressed in 16 of 35 (48%) cases of B-ALL ($p = 0.002$). When all cases are included, the immunophenotypic differences between blastoid-HGBL and B-ALL was still significantly different (Table 1).

Blastoid-HGBL had a complex karyotype in 92% of cases tested, and 84% of these cases harbored *MYC*-R (Table 2). In contrast, of the 35 cases of B-ALL tested, a complex karyotype was detected in 18 cases (51%, $p = 0.0015$), and none showed *MYC* rearrangement ($p = 0.0001$). By targeted NGS study performed in a subset of blastoid-HGBL cases ($n = 15$) and most ($n = 30$) B-ALL cases, *TP53* mutation was more frequently observed in blastoid-HGBL than in B-ALL cases ($p = 0.03$); *NRAS* and *KRAS* mutations were only observed in B-ALL (Table 2), although the latter comparison did not reach statistical significance, possibly because of the limited number of cases tested in the blastoid-HGBL group.

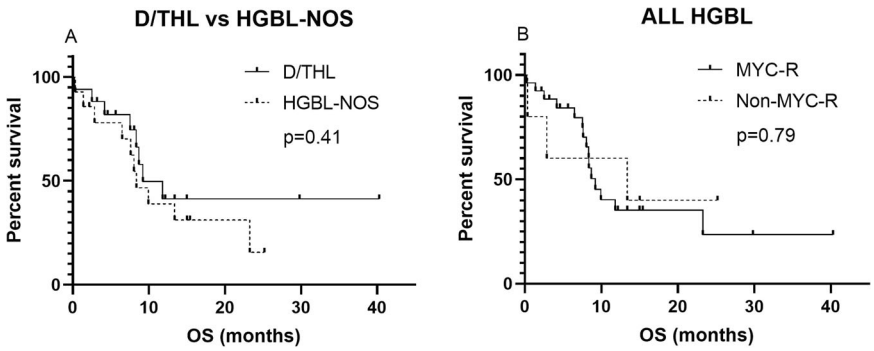

Fig. 2 Comparison of overall survival (OS). A OS between blastoid HGBL with *MYC*, *BCL2*, and/or *BCL6* rearrangements (D/THL, $n = 17$) and blastoid HGBL-NOS ($n = 14$). **B** OS between blastoid HGBL with *MYC-R* ($n = 26$) and those without *MYC-R* ($n = 5$).

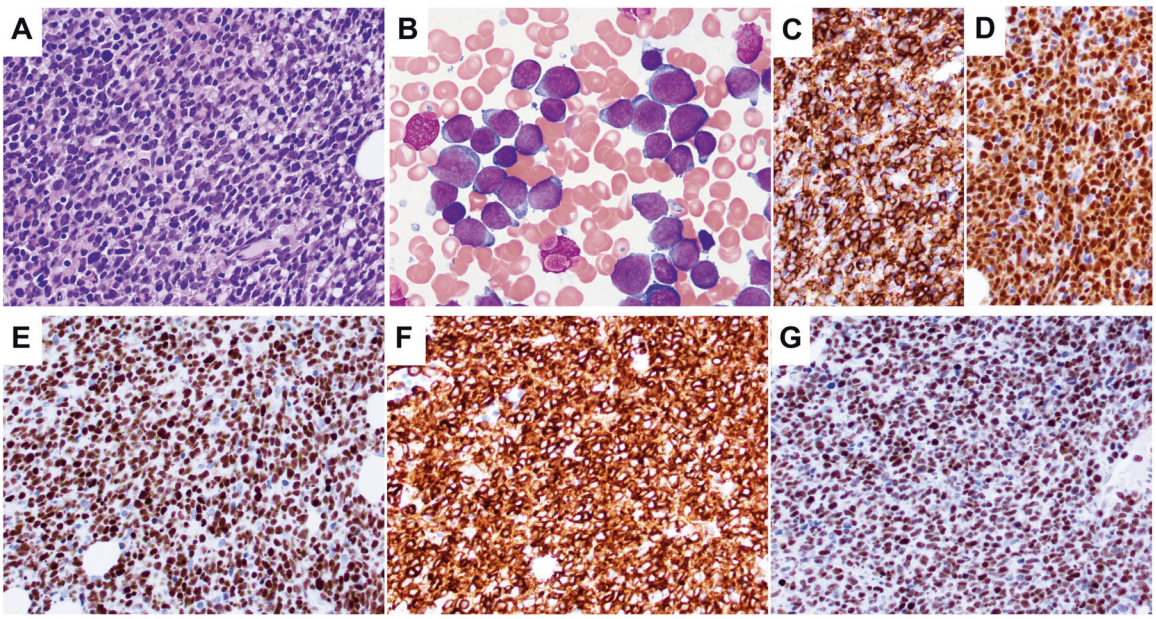

Fig. 3 A representative case of B acute lymphoblastic leukemia (B-ALL) in bone marrow. The B lymphoblasts demonstrating a similar morphology to blastoid lymphoma cells shown in Fig. 1 in core biopsy (**A**) and aspirate smear (**B**). The blasts showed diffuse strong expression of CD34 (**C**), TdT (**D**), PAX5 (**E**), and CD10 (**F**). While BCL6 is usually not expressed in B-ALL, it is unusually expressed in this case (**G**) (**A**, H&E stain, x500; **B**, Giemsa stain, x1000; **D**–**G**, immunohistochemistry, x500).

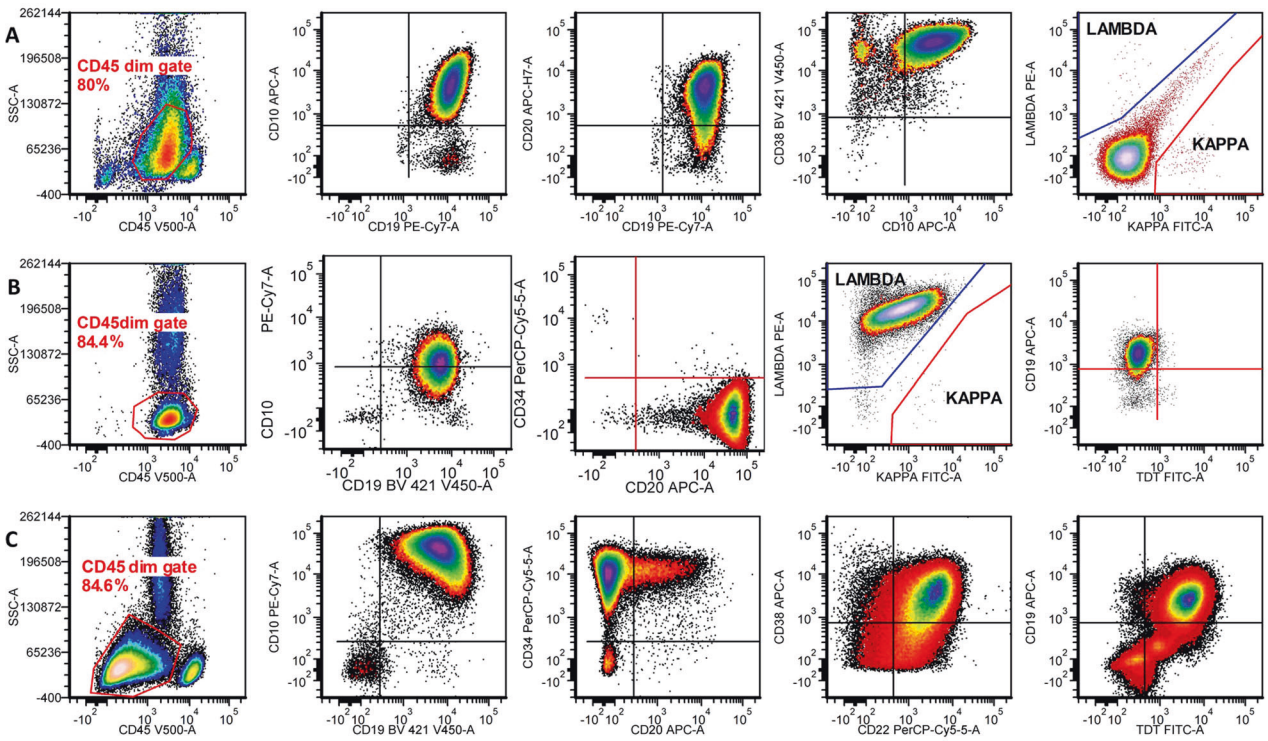

Fig. 4 Flow cytometry immunophenotyping comparison between blastoid HGBL and B-ALL in bone marrow. Row **A** and **B**: blastoid HGBL showed expression of dim CD45 with intensity ≥granulocytes, dim CD10, relatively bright CD20, bright CD38, with surface light chain restriction or lack of light chain expression, but negative for CD34 and TdT, while (Row **C**) B-ALL, in contrast, demonstrated decreased CD45, bright CD10, not bright CD38, with CD34 and TdT expression, but no CD20 or surface light chain (not shown) expression.

From the data described above and shown in Tables 1 and 2, a scoring system was developed by using the most significantly distinctive immunophenotypic and cytogenetic features between the diagnostically most unequivocal blastoid-HGBL ($n = 16$) and CD34+ B-ALL cases ($n = 22$), including the intensity of expression of CD10, CD20, CD38, and CD45, presence of TdT and MYC expression. The presence of CD45 intensity higher than granulocytes, bright CD38 expression similar to hematogones, lack of

Table 3. A scoring system to aid distinguish blastoid B cell lymphoma from B-ALL.

Parameter	Presence	Absence
CD45 intensity > Granulocytes	1	0
CD38 bright (≈hematogones)	1	0
CD10 not bright (<hematogones)	1	0
CD20 intensity ≥ mature B cells	1	0
TdT negative	1	0
MYC rearrangement/MYC IHC ≥40%	1	0

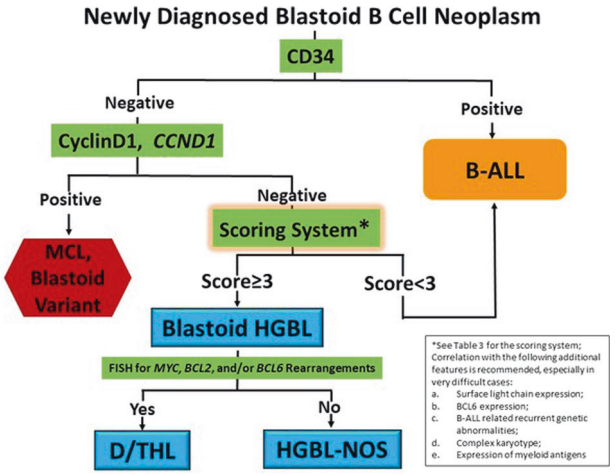

Fig. 5 Recommended algorithm for the differential diagnosis of blastoid B cell neoplasm (HGBL high-grade B-cell lymphoma, B-ALL B acute lymphoblastic leukemia, MCL mantle cell lymphoma, D/THL HGBL with *MYC*, *BCL2*, and/or *BCL6* rearrangements, or double/triple hit lymphoma).

bright CD10 expression (<hematogones), expression of CD20 which was not dim (≥mature B cells), TdT negativity, and presence of MYC protein expression each were assigned a score of 1 (Table 3). A score of ≥3 supports a diagnosis of blastoid HGBL, whereas a score <3 supports B-ALL. When we replace MYC expression with the presence of *MYC*-R, the significance of the comparison was the same, and therefore MYC expression and *MYC*-R were used as one parameter here, depending on the data available. Using this scoring system, all 16 cases of blastoid-HGBL were classified as such, and 21 of 22 CD34+ B-ALL cases were classified as B-ALL; one cases of CD34+ B-ALL had an equivocal score of 3. This scoring system was further tested using the more equivocal cases of blastoid-HGBL ($n = 15$) and CD34-negative B-ALL ($n = 14$); again all 15 cases of equivocal HGBL were classified as blastoid-HGBL, and 13 of 14 cases of CD34-negative B-ALL had a score of <3. One case of B-ALL had an equivocal score of 3. Combining all cases, this scoring system reached a sensitivity of 100%, specificity of 94%, positive predictive value of 94%, and negative predictive value of 100% for establishing the diagnosis of blastoid-HGBL. By using this scoring system, the four cases with TdT expression had scores of 3, 3, 4 and 4 respectively, supporting the classification of blastoid-HGBL.

Based on this scoring system, we propose a new algorithm for the differential diagnosis of blastoid B cell neoplasms that incorporates this scoring system to be used in the context of CD34-negative cases (Fig. 5).

DISCUSSION

In 2016, a new category designated high-grade B-cell lymphoma (HGBL) emerged in the WHO classification. This group is heterogeneous and includes HGBL with *MYC, BCL2*, and/or *BCL6* rearrangements (so-called double/triple hit lymphoma) and HGBL, not otherwise specified (NOS). A small subset of HGBL cases has lymphoma cells showing blastoid morphology, designated as blastoid-HGBL in this study. The differential diagnosis of blastoid-HGBL can be challenging, but is very important as the treatment and prognosis may be substantially different. The major differential diagnosis is with B acute lymphoblastic leukemia (B-ALL), and data on this topic are limited in the literature. A second entity in the differential diagnosis is blastoid variant of mantle cell lymphoma, which is usually positive for CD5, cyclin D1 and SOX11 and most often carries t(11;14)(q13;q32)/*CCND1-IGH*. Therefore, the differential diagnosis between blastoid-HGBL and B-ALL is most likely to be problematic for the practicing pathologist. This differential diagnosis may be especially challenge in patients, who present initially with a leukemic presentation and do not have lymph node involvement or the status of lymph node is unknown at the time of the bone marrow examination. As shown in this cohort, 14 of 31 cases of blastoid-HGBL were worked up initially for acute leukemia, which illustrated the difficulty of the differential diagnosis.

In this study, we showed that despite similar morphologic features, blastoid-HGBL cases show distinctive immunophenotypic and molecular cytogenetic features compared with B-ALL. B-ALL is an immature B-cell neoplasm with immature morphologic features and an immature immunophenotype. These neoplasms usually express TdT, CD34 (whole population or partial), bright CD10, CD19, CD22, dim to negative CD45, and cytoplasmic CD79a and are negative for CD20 and surface immunoglobulin light chain. Characteristically, CD38 often shows a decreased level compared with normal B lineage precursors (hematogones). Myeloid-associated antigens, such as CD13 and CD33, may also be aberrantly expressed. By immunohistochemistry, B-lymphoblasts generally lack of BCL6 expression[9]. B-ALL only rarely harbor a *MYC* translocation and uncommonly have a complex karyotype. Of note, we included 14 cases of rare CD34-negative B-ALL with either *KMT2A* (*MLL*) rearrangement or t(1;19) here, which significantly elevated the percentage of B-ALL with a complex karyotype. In contrast, despite of the immature morphology of blastoid-HGBL cases, these neoplasms share a mature B-cell immunophenotype[10], and typically are negative for immature markers such as CD34 and TdT. Most cases of blastoid-HGBL express surface light chain and bright CD45, similar to lymphocytes by flow cytometry, and usually express BCL6. Blastoid-HGBL more often carry *MYC* translocation and have a complex karyotype. These distinctive features are helpful to distinguish blastoid-HGBL from B-ALL[9,11].

Based on the above distinctive features many blastoid B-cell neoplasms can be correctly classified using the 2016 WHO algorithm, which mainly relies on TdT expression. However, there are some cases that are difficult to classify. In a recent study, Ok and colleagues reported 13 patients with aggressive B-cell lymphoma with TdT expression[12]. Two cases had blastoid morphology and were positive for CD10, CD19, and TdT and negative for CD20 and surface immunoglobulin. Based on morphology and immunophenotype, a diagnosis of B-ALL was favored. However, the clinical history of follicular lymphoma and presence of *MYC* and *BCL2* rearrangements made the diagnosis of HGBL/double hit lymphoma more likely. *MYC* and *BCL2* and/or *BCL6* rearrangements are very rare in B-ALL. Occasional double hit B-ALL cases have been reported in the literature, but often these cases represent secondary progression from follicular lymphoma[13–16], and therefore some of these cases could have been blastoid-HGBL with TdT expression. In another study, four of five blastoid B-cell neoplasms were CD34 negative but TdT positive. However, these cases had both bone marrow and nodal involvement and three carried double hit changes[14], features more in favor of blastoid-HGBL than B-ALL. In the current study,

four blastoid-HGBL cases had variable TdT expression and hybrid features favoring either lymphoma or B-ALL. The features favoring lymphoma included double/triple hit genetics, expression of BCL6 and MYC, and bright expression of CD38 and/or CD45. These same cases lacked CD20 or surface light chain and had variable CD45 expression (dim or negative), features more in keeping with B-ALL. On the other hand, a subset of B-ALL cases can lack CD34 and/or TdT expression. In this cohort, 40% B-ALL cases were negative for CD34 and 6% lacked TdT. These findings suggest that an algorithm may be useful for the correct classification of blastoid B-cell neoplasms in the bone marrow.

The scoring system we developed in this study uses six features that are significantly different between blastoid-HGBL and B-ALL (Table 3). This system showed excellent sensitivity, specificity and positive and negative predictive values, even in challenging cases, such as TdT positive cases. This scoring system mainly adopted immunophenotypic parameters because the results of these parameters are usually available within 1–2 days of the biopsy, which is more helpful for a timely diagnosis and to start an appropriate therapy in clinical practice. In this scoring system, *MYC*-R and MYC expression are interchangeable making its use easier when FISH results are not available. This is not difficult to understand as MYC protein expression correlates with *MYC*-R in most cases[17,18]. Two of the 36 B-ALL cases had a score of 3, one was CD34+ and the other CD34 negative, which was more problematic. Obviously, for such rare cases correlation with additional immunophenotypic, molecular, and cytogenetic features is needed to reach a correct classification. As our results have shown, the presence of surface light chain restriction, BCL6 expression, *TP53* mutation, and the absence of myeloid antigen expression and *KRAS* and *NRAS* mutation all support a diagnosis of blastoid-HGBL, whereas the converse and presence of B-ALL associated translocations support a diagnosis of B-ALL. In the CD34 negative B-ALL case with a score of 3, *KMT2A (MLL)* rearrangement, *KRAS* mutation, and CD33 expression were detected which supported a diagnosis of B-ALL. By using this scoring system, the four cases with TdT expression were classified as blastoid-HGBL. Three blastoid-HGBL cases did not express CD20 and surface light chain and had been diagnosed as B-ALL elsewhere originally; all three were classified as blastoid-HGBL using the scoring system, and further confirmed by the molecular cytogenetic features. We therefore suggest an algorithm including our scoring system that is useful for the correct classification of all blastoid B cell neoplasms. This algorithm is especially helpful for those challenging cases that are difficult to classify by using the WHO TdT based algorithm. Although the scoring system we developed is based on bone marrow cases, it seems likely useful for blastoid cases involving extramedullary sites.

The prognostic value of blastoid morphology has rarely been evaluated, and the prognosis of blastoid-HGBL with a bone marrow presentation is unclear. In a retrospective study of 24 cases, blastoid B-cell lymphomas had a very short survival of 1.1 years; and overall survival was similar between those with or without *MYC*-R ($p = 0.92$)[19]. Moore et al.[20] reported 25 cases of blastoid large B-cell lymphoma, of which 64% were double/triple hit lymphomas, and the prognosis of these patients was worse than those with non-blastoid morphology. In this study, we focused on blastoid-HBCL presenting initially in the bone marrow. Overall, the blastoid-HBCL patients in our cohort had a very short survival of 9.2 months. The presence or absence *MYC*-R had no impact on survival (Fig. 2B). Although the treatment regimens were not uniform, the induction therapy was similar for patients with *MYC* and *BCL2* and/or *BCL6* rearrangements ($n = 17$) and those with HGBL-NOS ($n = 14$) and they also showed no significant difference in survival (Fig. 2A).

So why is blastoid HBCL so aggressive? Relevant data are very limited in the literature. From the current study, the reasons include but are not limited to a high frequency of *MYC* rearrangement, complex karyotype, and/or *TP53* mutation or p53 overexpression. Only a few studies in the literature have examined the genetic heterogeneity of B-cell neoplasms with blastoid morphology, and all studies showed frequent *MYC*-R and a complex karyotype[19,20]. Similarly, in this study, the blastoid-HBCL cases more frequently had *MYC*-R, a complex karyotype and *TP53* mutation than B-ALL cases. The aggressiveness of blastoid morphology, regardless of *MYC* or double hit genetics, in B-cell lymphomas is also related to these factors. It is well known that HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangements has an aggressive clinical course[21–24]. *MYC*-R also occurs in about one third of the HGBL-NOS cases[25,26]. Two independent studies have shown that patients with *MYC* single-hit lymphoma have a poor survival, similar to the patients with double hit lymphoma[27,28]. Previous studies have shown *TP53* mutation or overexpression is associated with a poor prognosis in diffuse large B cell lymphoma and TP53 and MYC may have synergistic effect[29,30]. Li et al. further showed that P53 overexpression is a surrogate of *TP53* mutation, and is more frequently associated with *MYC* SHL than with double hit lymphoma, contributing to the poor prognosis of SHL[27]. In this cohort, blastoid HGBL-NOS cases also had a high frequency of *MYC*-R (58%). In addition, the frequency of a complex karyotype is similar between the HGBL-double hit lymphoma and HGBL-NOS subgroups ($p = 0.48$), as is the frequency of *TP53* mutation ($p = 1.00$). These features likely explain the similar overall survival of between patients with HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangements and HGBL-NOS, as well as blastoid-HGBL with or without *MYC*-R (Fig. 2).

In summary, cases of blastoid-HGBL show distinctive immunophenotypic, cytogenetic, and molecular features compared to B-ALL. Nevertheless, the diagnosis of a blastoid B-cell lymphomas initially presenting in the bone marrow can be very challenging. We have therefore proposed an algorithm using our scoring system and related molecular cytogenetic features (Fig. 5). This algorithm is helpful for the classification of diagnostically challenging blastoid lymphoid neoplasms in the bone marrow. Blastoid-HBCL with bone marrow presentation had an aggressive clinical course and a poor prognosis, regardless of *MYC*-R or double hit genetics.

DATA AVAILABILITY

All data has been presented in the manuscript, Tables and Figures.

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AUTHOR CONTRIBUTIONS

S.L. designed the study, collected data, and wrote the manuscript; M.K. collected data and wrote the manuscript; L.J.M. designed the study and wrote the manuscript; P.L., J.X., M.J.Y., G.T. and C.C.Y. collected data and wrote the manuscript; W.W., L.Q., R.N.M. and C.B.R. wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the institutional review board at The University of Texas MD Anderson Cancer Center.

ADDITIONAL INFORMATION

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