



Published in final edited form as:

Am J Surg Pathol. 2010 March ; 34(3): 327–340. doi:10.1097/PAS.0b013e3181cd3aeb.

B-cell Lymphomas with Concurrent *IGH-BCL2* and *MYC* Rearrangements Are Aggressive Neoplasms with Clinical and Pathologic Features Distinct from Burkitt Lymphoma and Diffuse Large B-cell Lymphoma

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Abstract

B-cell lymphomas with concurrent *IGH-BCL2* and *MYC* rearrangements, also known as “double-hit” lymphomas (DHL), are rare neoplasms characterized by highly aggressive clinical behavior, complex karyotypes, and a spectrum of pathological features overlapping with Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL) and B-lymphoblastic lymphoma/leukemia (B-LBL). The clinical and pathological spectrum of this rare entity, including comparison to other high-grade B-cell neoplasms, has not been well defined. We conducted a retrospective analysis of clinical and pathologic features of 20 cases of DHL seen at our institution during a 5-year period. In addition, we performed case-control comparisons of DHL with BL and International Prognostic Index (IPI)-matched DLBCL. The 11 men and 9 women had a median age of 63.5 years (range 32-91). Six patients had a history of grade 1-2 follicular lymphoma (FL); review of the prior biopsy specimens in 2 of 5 cases revealed blastoid morphology. Eighteen patients had Ann Arbor stage 3 or 4 disease and all had elevated serum lactate dehydrogenase (LDH) levels at presentation. Extranodal disease was present in 17/20 (85%), bone marrow involvement in 10/17 (59%) and central nervous system (CNS) disease in 5/11 (45%). Nineteen patients were treated

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#YBC is a Special Fellow in Clinical Research of the Leukemia & Lymphoma Society.

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with combination chemotherapy, of whom 18 received rituximab and 14 received CNS-directed therapy. Fourteen patients (70%) died within 8 months of diagnosis. Median overall survival in the DHL group (4.5 months) was inferior to both BL ($p=0.002$) and IPI-matched DLBCL ($p=0.04$) control patients. Twelve DHL cases (60%) were classified as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL, 7 cases (35%) as DLBCL, not otherwise specified, and 1 case as B-LBL. Distinguishing features from BL included expression of Bcl2 ($p<0.0001$), Mum1/IRF4 ($p=0.006$), Ki-67 $<95\%$ ($p<0.0001$), and absence of EBV-EBER ($p=0.006$). DHL commonly contained the t(8;22) rather than the t(8;14) seen in most BL controls ($p=0.001$), and exhibited a higher number of chromosomal aberrations ($p=0.0009$). DHL is a high-grade B-cell neoplasm with a poor prognosis, resistance to multi-agent chemotherapy, and clinical and pathological features distinct from other high-grade B-cell neoplasms. Familiarity with the morphologic and immunophenotypic spectrum of DHL is important in directing testing to detect concurrent *IGH-BCL2* and *MYC* rearrangements when a karyotype is unavailable. The aggressive clinical behavior and combination of genetic abnormalities seen in these cases may warrant categorization as a separate entity in future classifications and call for novel therapeutic approaches.

Keywords

MYC; *BCL2*; diffuse large B-cell lymphoma; Burkitt lymphoma; cytogenetics; high-grade B-cell lymphoma

Introduction

B-cell lymphomas commonly exhibit chromosomal translocations involving immunoglobulin genes (*IG*). The t(14;18)(q32;q21) results in the juxtaposition of *BCL2* with enhancer elements of the *IG* heavy chain (*IGH*). This translocation is a hallmark of follicular lymphoma (FL) and is also found in 20-30% of de novo diffuse large B-cell lymphoma (DLBCL) (16, 56). Translocations involving *MYC* at 8q24 and *IG* partners, including *IGH* and kappa (*IGK*) and lambda (*IGL*) light chains, are characteristic of Burkitt lymphoma (BL), and are also found in 5-15% of unselected cases of DLBCL (2, 17, 24). B-cell lymphomas with concurrent *IGH-BCL2* and *MYC* rearrangements are rare, and are characterized by highly aggressive clinical behavior, complex karyotypes, and a broad morphologic and immunophenotypic spectrum that overlaps with BL and DLBCL, and occasionally B-lymphoblastic lymphoma/leukemia (B-LBL) (30). Because these “double-hit” lymphomas (DHL) share pathologic features with other high-grade B-cell neoplasms, their diagnosis and classification may be difficult, and their rarity and poor response to therapy make selection of appropriate treatment challenging.

Approximately 200 cases of DHL have been reported in the literature, mostly as case reports and small series (3, 4, 6, 8-10, 12, 13, 15, 18-20, 22, 25-27, 29-33, 36, 39, 40, 43, 47, 49-54, 59). Most reported patients have *de novo* disease, while a minority have a history of grade 1-2 FL and develop DHL secondarily, presumably by acquisition of a *MYC* translocation (8, 12, 18, 19, 26, 27, 31, 49-51, 54, 59). Few studies have directly compared the pathologic features and clinical outcome of DHL with BL and DLBCL (37, 42), and only a single series has classified DHL using criteria of the 2008 World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues (18, 48).

We conducted a retrospective analysis of the clinical, morphologic, immunophenotypic, cytogenetic, and molecular genetic features of 20 cases of DHL seen at our institution to define further the clinical and pathologic spectrum of this rare entity and to classify cases according to the 2008 WHO Classification. In addition, we performed a case-control

comparison with BL cases seen over the same time period, in order to identify distinguishing clinicopathologic features that facilitate early and correct identification of DHL. We also performed a case-control comparison with a group of International Prognostic Index (IPI)-matched DLBCL cases treated at the same institution to elucidate clinical differences between DHL and DLBCL. Based on the distinctive clinicopathologic features of DHL that we identified, we outline circumstances in which additional testing by fluorescence *in situ* hybridization (FISH) or PCR on diagnostic tissue samples may be helpful to confirm or exclude a diagnosis of DHL if a conventional karyotype is unavailable.

Methods

Identification of Cases and Controls

The Partners HealthCare Institutional Review Board (IRB) granted approval for the study before its initiation. The files of the Massachusetts General Hospital (MGH) Pathology Department were searched for cases of B-cell lymphoma on which cytogenetic and/or FISH analysis had been performed and had revealed concurrent *IGH-BCL2* and *MYC* rearrangements. In some cases, tissue had been sent for cytogenetic analysis at the time of frozen section evaluation at the discretion of the frozen section pathologist, while in other cases lacking cytogenetic analysis, FISH to detect *IGH-BCL2* and *MYC* and gene rearrangements had been performed at the time of diagnosis because BL was a diagnostic consideration based on the morphology and/or immunophenotype. A total of 19 cases from 2004-2009 were identified. One additional case (case 10) identified from this time period contained a *MYC* rearrangement and 8 copies of an intact *BCL2* confirmed by paraffin FISH, and was included in the study because of the functional equivalence of multiple copies *BCL2* to t(14;18) (11, 35, 58). Pathology, flow cytometry and cytogenetics reports, and H&E- and immunohistochemical-stained slides of these cases were reviewed by 4 hematopathologists to confirm the diagnosis and to classify each case according to the 2008 WHO Classification. Medical records were reviewed to determine the IPI score (1), prior history of lymphoma, clinical presentation, therapeutic regimen, response to therapy, outcome and overall survival (OS).

BL control cases were identified by review of the MGH pathology files from 2000-2008; a total of 29 consecutive cases in which a diagnosis of “Burkitt lymphoma” or “atypical Burkitt lymphoma” had been rendered were identified. Pathology, flow cytometry and cytogenetics reports, and H&E- and immunohistochemical stained slides of these cases were reviewed by 4 hematopathologists for a consensus diagnosis of BL according to criteria of the 2008 WHO Classification (28). Four cases that did not meet morphologic, immunophenotypic, and/or cytogenetic criteria for BL were excluded, yielding a total of 25 BL control patients whose medical records were reviewed to determine site of involvement by lymphoma, serum lactate dehydrogenase (LDH) level at presentation, outcome and OS. This control group included 7 patients ≤ 20 years old, none of whom were known to be HIV-positive, 9 HIV-positive adults, and 9 HIV-negative adults.

DLBCL control cases were selected from an IRB-approved clinical database of patients diagnosed with DLBCL and treated at the MGH from 2000-2008. Two IPI-matched controls were randomly selected and matched to each DHL case, yielding a total of 40 DLBCL patients whose pathology reports were reviewed to confirm the diagnosis, and whose medical records were reviewed to determine site of involvement by lymphoma, LDH level at presentation, outcome and OS.

Immunophenotyping and In Situ Hybridization Studies

Formalin- or B+–fixed, paraffin-embedded tissues from diagnostic biopsies of DHL cases and BL control cases were characterized by immunohistochemistry using previously described methods (44) with some or all of the following antibodies, if not already performed at the time of diagnosis: CD20 (L26 epitope, prediluted, Ventana Medical Systems, Tucson, AZ), CD79a (prediluted, Ventana), Pax5 (prediluted, Ventana), Bcl6 (1:10 dilution, Dako, Carpinteria, CA), CD10 (prediluted, Ventana), Bcl2 (clone 124, prediluted, Ventana), Mum1/IRF4 (1:20 dilution, Dako), Ki-67 (clone 30-9, prediluted, Ventana), terminal deoxynucleotidyl transferase (TdT, prediluted, Ventana). One case that lacked Bcl2 protein expression using clone 124 (targeting amino acids 41-54) was subsequently analyzed using clone C-2 (targeting amino acids 1-205) (Santa Cruz Biotechnology, Santa Cruz, CA). *In situ* hybridization for EBV-encoded RNA (INFORM EBER Probe, Ventana) was performed as previously described (44).

In cases with material available for flow cytometry at the time of diagnosis, 4-color flow cytometry was performed using previously described methods (44) for the following antibodies: CD45-peridinin chlorophyll protein (PerCP), CD19-PerCP (SJ25C1), CD19-phycoerythrin (PE; 4G7), CD20-PerCP (L27), CD10-fluorescein isothiocyanate (FITC; W8E7), kappa light chain-FITC (TB28-2), lambda light chain-phycoerythrin (PE; 1-155-2), TdT-FITC (HT1, HT4, HT8, and HT9) (BD Biosciences, San Jose, CA), and CD10-PE (SS2/36) (Dako).

Cytogenetic and Molecular Genetic Analyses

GTG-banded metaphases were obtained from unstimulated overnight lymph node or bone marrow cultures according to standard cytogenetic protocols in 17 tumors (11 DHL, 6 BL) at the time of diagnosis. Chromosome analysis was performed at a level of 400 bands or greater and the karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2005 (46).

In DHL and BL control cases lacking a karyotype, FISH analyses to identify *MYC*, *BCL6* and either *BCL2* or *IGH-BCL2* rearrangements were attempted on interphase nuclei of paraffin-embedded tissue at the time of diagnosis. In case 11, *MYC* and *IGH-BCL2* FISH was performed on a cerebrospinal fluid (CSF) air-dried cytospin and in cases 12 and 17, FISH was performed on abnormal metaphases from tumor cells. All FISH probes were purchased from Abbott Molecular (Des Plaines, IL) and included the Vysis LSI *MYC* Dual Color Break Apart Rearrangement Probe to identify any rearrangement at 8q24, the Vysis LSI *BCL6* Dual Color Break Apart Rearrangement Probe to identify any rearrangement at 3q27, the Vysis LSI *BCL2* Dual Color Break Apart Rearrangement Probe to identify any rearrangement at 18q21, the Vysis *IGH/BCL2* Dual Color Dual Fusion Translocation Probe to identify t(14;18)(q32;q21), and the Vysis CEP 18 (D18Z1) SpectrumOrange Probe. For formalin-fixed, paraffin-embedded tissue samples, interphase FISH was performed using standard cytogenetic protocols either on whole nuclei extracted from 50µm tissue sections or on 4µm tissue sections cut and fixed onto slides (57). Fifty to 100 nuclei were scored for each sample, and a sample was considered positive for the rearrangement if >15% of nuclei exhibited a break-apart and/or fusion signal.

Statistical Analysis

All statistical analyses were performed using SAS version 9. Fisher's exact test was used for comparing proportions and Student's t-test was used for comparing means between 2 groups. Matching between the DHL and DLBCL groups was taken into account by using conditional logistic regression. OS was calculated from date of diagnosis to date of death or last follow-up. Distributions of OS were estimated using the method of Kaplan and Meier

and differences in OS were assessed by the stratified log-rank test. Cox regression was used to analyze the effect of continuous variables on OS (5). Significance was tested at the $\alpha=0.05$ level.

Results

Clinical Features

Clinical characteristics of DHL patients are summarized in Tables 1 and 2. The 20 patients included 11 men and 9 women with a median age of 63.5 years (range: 32-91). None were known to be HIV-positive. Six patients (30%) had a reported history of FL, all histologic grade 1-2 of 3, diagnosed 6 months to 12 years before their DHL diagnosis. Patients were treated with a variety of multiagent chemotherapy regimens that could be broadly divided into 2 categories: moderate intensity regimens (CHOP, R-CHOP and R-ICE) in 13 patients (70%) and high intensity regimens (CODOX-M/R-IVAC and R-EPOCH) in 6 patients (30%) (Table 1). All were treated with curative intent, except for patient 20 who received a single cycle of R-CHOP chemotherapy for palliation. Rituximab was included in 18 cases (90%) and 14 patients (70%) received CNS-directed therapy with intrathecal or high-dose systemic methotrexate. Treatment was not known in case 16. Eight patients (42%) achieved a CR, of whom 3 eventually died: 1 relapsed and died of disease, 1 had persistent neutropenia and died of infection, and 1 died of an unknown cause. After a median follow-up of 7.3 months (range: 1.5-26.6), 6 patients (30%) were alive (4 with no evidence of disease, 1 alive with disease who relapsed after 1 year in CR, and 1 diagnosed recently, undergoing chemotherapy) and 14 patients (70%) had died. Four patients remain alive in CR (median follow-up: 18.2 months, range: 8.9-26.6), all of whom were treated with R-CHOP. However, treatment regimen was not significantly correlated with response or OS (data not shown). The sole clinical variable that was significantly correlated with outcome was ECOG performance status (PS) of ≥ 2 ($p=0.04$). In addition, age at diagnosis was marginally significant in the univariate Cox regression analysis ($p=0.06$). The hazard ratio associated with older age (>60 years) was 2.7 (95% CI: 0.84-8.8).

Clinical comparison between the DHL patients and the 2 groups of control patients is summarized in Table 2. Patients in the DHL group had a median serum LDH level at presentation of 727 U/L, nearly twice that of either control group. An LDH cutoff of >630 U/L (>3 times the upper limit of normal in our laboratory) was found to be relatively specific for distinguishing DHL patients from those with BL (88% specificity, $p=0.006$) and DLBCL (82.5% specificity, $p=0.005$). DHL patients had a higher prevalence of bone marrow and CNS disease than either BL or DLBCL patients and a higher prevalence of extranodal disease compared with BL patients. OS for DHL patients was markedly worse than for BL ($p=0.002$) and DLBCL ($p=0.04$) patients (Fig. 1). The median survival time in the DHL group was only 4.5 months (95% CI: 4.0-7.9), and all observed deaths occurred within 8 months of diagnosis. In contrast, the BL group did not reach its median survival (median follow-up: 31.7 months) and the median survival time of the DLBCL group was 38.9 months (95% CI: 13.2-83.7).

Morphologic and Immunophenotypic Characteristics and WHO Classification

Morphologic and immunophenotypic features are summarized in Tables 3 and 4. Diagnostic tissue samples included lymph nodes (13 cases), extranodal sites (8 cases), bone marrow (5 cases), and peripheral blood (3 cases). Cases were classified according to the 2008 WHO Classification as follows: 12 cases of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (BCLU) (60%) and 7 cases of DLBCL, not otherwise specified (DLBCL-NOS) (35%). Case 20 with a leukemic presentation and lymphoblastic morphology and immunophenotype was classified as B-LBL (Fig. 2).

All 12 cases classified as BCLU exhibited a diffuse growth pattern with abundant mitoses and frequent apoptosis (Table 3). Five of 12 (41.7%) contained a conspicuous starry-sky pattern and monomorphous cell population closely resembling BL (“Burkitt-like” morphology, Fig. 3A-B). In 4 of 12 cases (33.3%), there was some morphologic overlap with BL but cell size was variable and a proportion of the cells contained more irregular nuclei and more prominent nucleoli than classic BL; these cases had morphological features intermediate between BL and DLBCL, and therefore were classified as BCLU rather than DLBCL-NOS (“intermediate” morphology, Fig. 3C) (21, 28). Three of 12 BCLU cases (25%) contained small cells with finely dispersed chromatin resembling lymphoblasts (“blastoid” morphology, Fig. 3D); all 3 expressed strong CD20 and surface light chain and 2 cases tested for TdT were negative. Despite the high-grade morphology seen in cases classified as BCLU, the Ki-67 proliferation index (PI) ranged from 25-100% (median: 80%) (Fig. 4A).

All 7 cases classified as DLBCL-NOS exhibited morphological features outside of the spectrum of BL with predominantly large cells with oval or irregular nuclei; most had a conspicuous population of immunoblasts with prominent central nucleoli, while some contained mainly centroblasts with vesicular chromatin or cells with finely dispersed chromatin (Table 3, Fig. 3E-F). Some cases had frequent mitoses and single-cell necrosis, but all lacked a starry-sky pattern or areas of geographic necrosis. All had a Ki-67 PI of $\geq 80\%$ (median: 85%, range: 80-95%) (Fig. 4B). Patients with tumors classified as DLBCL-NOS showed a trend toward improved OS compared to other DHL patients ($p=0.18$) (Fig. 5).

Among the 6 patients with a prior history of grade 1-2 FL, tissue from the initial FL was available for review in 5 (Table 3). The prior diagnosis of grade 1-2 FL was confirmed by morphologic examination and immunohistochemistry in 3 cases (case 1 with Burkitt-like BCLU, case 8 with BCLU with intermediate morphology, and case 19 with DLBCL-NOS). None of them showed evidence of a *MYC* rearrangement and a *BCL2* rearrangement was present in 2 of 3 by FISH analysis of the initial lymphoma samples. In 2 cases (cases 10 and 11 with blastoid BCLU), review of the prior biopsies revealed morphological findings consistent with the blastoid variant of FL (38, 41, 55). In case 10 (Fig. 6A-C), a diagnosis of grade 1-2 FL had been made on a supraclavicular lymph node; 2 years later, DHL, with a *MYC* rearrangement and 8 copies of *BCL2*, developed in the testis. Subsequent FISH analysis of the supraclavicular lymph node revealed the same genetic abnormalities. In case 11 (Fig. 6D-F), the diagnosis of grade 1-2 FL had been made on 2 small retroperitoneal core needle biopsies; 6 months after the diagnosis, DHL developed in the central nervous system (CNS). Tissue from the prior biopsies was not available for FISH analysis to determine initial *MYC* status.

All 18 DHL cases tested for Bcl2 by immunohistochemistry were positive (Fig. 4C). Case 3 was Bcl2-negative with the commonly used antibody (clone 124), and positive with a different Bcl2 antibody (clone C-4) recognizing a longer amino acid segment of the protein (34). The majority of cases (18/20, 90%) had a germinal center B-cell like (GCB) immunophenotype (either CD10+, or Bcl6+, Mum1- if results of CD10 staining were not available), while only 2 (cases 2 and 15) had a non-GCB immunophenotype (CD10-, Bcl6+, Mum1+) (Fig. 4D-F) (14). Significant differences distinguishing DHL cases from BL controls included Bcl2 ($p<0.0001$) and Mum1/IRF4 ($p=0.006$) protein expression, Ki-67 PI $<95\%$ ($p<0.0001$), and EBER-negativity ($p=0.006$) (Table 4, Fig. 4A-F). Within the DHL group, Mum1/IRF4 expression or Ki-67 PI had no significant correlation with response to therapy, outcome or OS (data not shown).

Cytogenetic and Molecular Genetic Characteristics

Cytogenetic and molecular genetic features of DHL are summarized in Tables 4 and 5. Cytogenetic analysis, available in 11 cases, showed a complex karyotype in all (≥ 3 numerical or structural aberrations), with a higher median number of aberrations than BL controls ($p=0.0009$) (Fig. 4G). Among the DHL cases, the *MYC* partner was frequently *IGL* (82%) or unknown (18%), while in BL cases the *MYC* partner was usually *IGH* (83%) or rarely *IGL* (17%), a difference that was statistically significant ($p=0.001$). There was no significant correlation between number of karyotypic abnormalities and response to therapy, outcome or OS within the DHL group (data not shown). Besides the t(14;18) and t(8q24), there was no cytogenetic abnormality common to all DHL cases. However, certain numerical and structural aberrations were seen frequently, including trisomy 12 (6 cases) and trisomy 7 (5 cases). Two cases had i(17)(q10), resulting in loss of heterozygosity for *TP53* at 17p13.

Among the 9 cases lacking karyotypes, 8 had *MYC* and *IGH-BCL2* rearrangements detected by interphase FISH (Fig. 4H-I). In case 10, FISH identified a *MYC* rearrangement and 8 copies of an intact *BCL2*; further analysis with a chromosome 18 centromeric probe revealed multiple signals, confirming chromosome 18 polysomy. *BCL6* FISH analysis identified a rearrangement in a single case (case 4), confirming a 3q27 rearrangement suspected by karyotype (i.e. “triple-hit”) (Fig. 4G).

Discussion

The purpose of this retrospective analysis was to define further the clinicopathologic spectrum of DHL in a well-characterized group of patients, to classify them according to the 2008 WHO Classification, and to compare the clinical and pathologic characteristics of DHL with 2 other high-grade B-cell lymphomas with which DHL may show morphologic and immunophenotypic overlap, DLBCL and BL. Our findings confirm that DHL has an aggressive clinical presentation and a poor prognosis with currently available therapies (6, 18, 19, 26, 30, 31, 36, 42, 47, 49). Our findings also suggest that DHL has clinical features distinct from both BL and DLBCL. DHL patients had a higher incidence of marrow and CNS involvement than either BL or DLBCL, and DHL patients had higher LDH levels at presentation compared with BL and IPI-matched DLBCL controls, with a median 3-4 times the upper limit of normal (Table 2). Importantly, DHL patients had a median OS of only 4.5 months, vastly inferior to that of either BL or DLBCL, and 70% of patients died within 8 months of diagnosis (Fig. 1). No clinical parameter emerged as predictive of superior OS within the DHL group, although PS <2 and age ≤ 60 years may suggest a slightly better prognosis. Our DHL patients were treated with either moderate intensity regimens used in DLBCL or high intensity regimens used in BL (Table 1). Neither regimen was associated with a superior outcome or OS, suggesting that highly intensive chemotherapy successfully used in BL may not offer clear benefit in DHL. Despite the retrospective nature of our data, our findings are in line with those of other studies and suggest the need for innovative therapeutic approaches to treat this disease (30, 37). Due to the high risk of CNS involvement, inclusion of CNS-directed therapy should be considered. Treatment of the majority of patients in our series with rituximab may help explain the somewhat lower mortality rate seen in our study as compared to others with similar follow-up in which fewer patients received rituximab (18, 26, 31, 42).

Because of its morphologic and immunophenotypic spectrum, recognition and classification of DHL has been difficult (30). All of the BCLU cases in our series bore some morphologic resemblance to BL, but had an immunophenotype unusual for BL such as positivity for Bcl2 or Mum1/IRF4 or a relatively low PI. All DHL cases diagnosed as DLBCL-NOS had a Ki-67 PI $\geq 80\%$ and were morphologically distinct from BL, with conspicuous immunoblasts

in most cases. In our study, classification as DLBCL-NOS was associated with a marginally superior OS compared with non-DLBCL morphology (Fig. 5), in contrast to the findings of Johnson et al (18) who also applied the 2008 WHO Classification to their cases and found that DLBCL morphology conferred a statistically significant OS benefit. In addition, our data showing that DHL overall has a worse prognosis than either BL or IPI-matched DLBCL underscores the importance of recognizing the presence of both *MYC* and *IGH-BCL2* rearrangements for appropriate prognostic stratification. In particular, the fact that most DHL patients had a GCB phenotype but did so poorly suggests that pathologic prognosticators commonly used in DLBCL do not apply in DHL (14, 30).

In our series as with other published series of DHL, most patients presented with *de novo* disease, while a subset had a prior history of grade 1-2 FL from which the DHL was presumed to have transformed (18, 26, 30, 31, 49). Interestingly, review of the prior tumors in 2 cases revealed morphologic findings unusual for low-grade FL and associated with the blastoid variant of FL (38, 41, 55). Detection of a *MYC* rearrangement in the initial tumor from case 10 and the short time course from diagnosis of grade 1-2 FL to the development of DHL in case 11 suggest that both original tumors may have contained a DHL component. This is further supported by chromosomal studies of FL showing that secondary 8q24 abnormalities were found exclusively in patients with the blastoid variant of FL (38).

In our series, *MYC* was most frequently translocated with *IGL*. This is in contrast to some reports of DHL that show *MYC* to be more frequently translocated with *IGH* or a non-*IG* partner (18, 42) and to the majority of BL in which *IGH* is the most common *MYC* translocation partner (23). The pathogenetic significance of t(8;22) in DHL is not entirely known, but its presence implies that t(14;18) occurs primarily followed by t(8;22), since neither the expressed nor rearranged *IGH* is available to participate in a balanced translocation with *MYC*. The t(8;22) may play a diagnostic role by raising the possibility of DHL if early karyotypic analysis reveals a t(8;22) rather than a t(8;14). Careful review of the karyotype for the presence of t(14;18) or metaphase FISH to identify a cryptic *IGH-BCL2* rearrangement may be warranted.

Despite the importance of conventional cytogenetic analysis in confirming a diagnosis of DHL, nearly half of the cases in our series lacked a karyotype and we relied on FISH to make the diagnosis. In many pathology laboratories, including our own, not all lymphoma cases are routinely sent for cytogenetic analysis, either due to insufficient tissue or lack of recognition of the importance of such analysis to a particular case at the time of surgery. In addition, some laboratories may not have access to routine cytogenetic analysis. In contrast, FISH is readily available at most large and reference laboratories and can confirm the diagnosis of DHL without the need for cell culture. Since DHL is relatively rare, with estimates ranging from 3-5% of high-grade B-cell lymphoma (18, 42), sending all cases of high-grade B-cell lymphoma for FISH analysis may be unnecessary. We suggest that when a karyotype is not available, FISH for both *MYC* and *IGH-BCL2* should be limited to the following circumstances, in order to increase cost-effectiveness and minimize unnecessary testing:

- high-grade B-cell lymphoma patients who present with advanced stage disease with extranodal or CNS involvement or with an LDH exceeding 3 times the upper limit of normal
- all adult patients whose tumors have some morphologic resemblance to BL, particularly patients in whom adult BL is rare, such as HIV-negative or non-immunosuppressed patients
- tumors resembling BL morphologically with an atypical immunophenotype for BL, including positivity for Bcl2, Mum1/IRF4, and/or a Ki-67 PI <95%

- adult patients diagnosed with DLBCL-NOS with a Ki-67 \geq 80%
- patients with a history of low-grade FL who relapse with a high-grade B-cell neoplasm
- tumors resembling low-grade FL with unusual features, such as diffuse growth, absence of centrocyte-like cells, blastoid cytologic features, very high PI, a starry-sky pattern or focal necrosis

Finally, our findings suggest that DHL warrants separate categorization in future classifications due to the clinical and molecular genetic features that distinguish it from other high-grade B-cell neoplasms, perhaps reflecting a different underlying pathogenesis (17, 30, 45). The basis for the extremely aggressive clinical behavior of DHL is likely related to *MYC*-induced growth promotion combined with the anti-apoptotic effect conferred by *BCL2* overexpression (30). As in our series, the vast majority of reported cases exhibit a complex karyotype, pointing to a role for clonal evolution and further gene dysregulation. Most DHL cases studied using gene expression profiling have shown a molecular signature intermediate between DLBCL and BL (17), while fewer cases more closely resemble BL (7). Comparative genomic hybridization studies demonstrated that even such cases of gene expression-defined BL that do not fulfill WHO criteria for BL (so-called “discrepant BL” cases) show significant alterations from pediatric and adult BL and DLBCL (45). It is possible that DHL represents a neoplasm in which a primary genetic aberration and induction of a specific gene-expression profile was subsequently shifted in a different direction due to the acquisition of additional genetic changes (17). This may explain the morphologic and immunophenotypic heterogeneity, but shared molecular genetic and clinical features, seen in these cases.

In summary, we describe 20 patients with B-cell neoplasms with concurrent *IGH-BCL2* and *MYC* rearrangements. Our findings confirm these neoplasms to be clinically aggressive with a poor prognosis. High intensity regimens used to treat BL have yet to demonstrate a clear benefit in DHL patients. DHL may show morphologic and immunophenotypic overlap with BL or DLBCL, or less frequently with LBL or FL with blastoid morphology. Awareness of this pathologic spectrum is important in directing ancillary testing to detect *IGH-BCL2* and *MYC* rearrangements, particularly in laboratories where conventional cytogenetics is not routinely performed or available. Even with the use of the current WHO Classification containing intermediate categories, classification of DHL remains problematic. Despite its morphologic and immunophenotypic heterogeneity, the distinct clinical and molecular genetic features of DHL may warrant categorization as a separate entity in future classifications, as well as the development of novel treatment strategies.

Acknowledgments

The authors are grateful to Dr. A. John Iafrate of the MGH Diagnostic Molecular Pathology Laboratory for his assistance with interpretation of FISH results.

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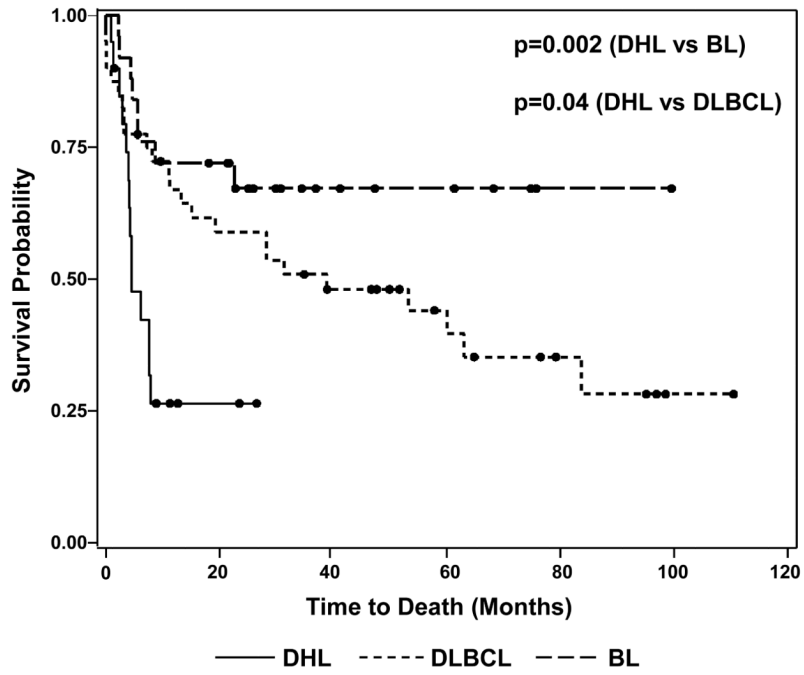


Figure 1. Kaplan-Meier Overall Survival Distributions for Double-Hit Lymphoma, Burkitt Lymphoma and IPI-Matched Diffuse Large B-cell Lymphoma Patients. Black circles denote patients who were alive at the time of last follow-up. DHL: double-hit lymphoma, BL: Burkitt lymphoma, DLBCL: diffuse large B-cell lymphoma.

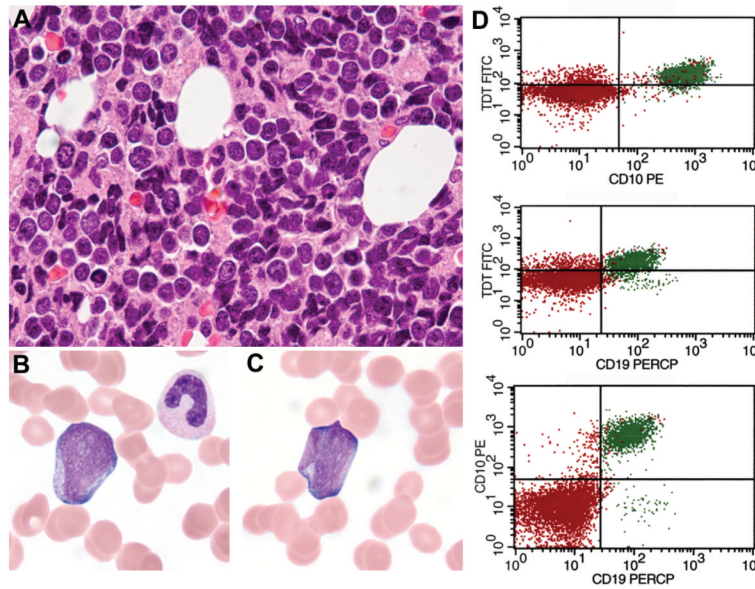


Figure 2.

B-Lymphoblastic Leukemia with *IGH-BCL2* and *MYC* Rearrangements

The bone marrow core biopsy from case 20 showed medium-sized cells with round nuclei and finely dispersed chromatin in a background of extensive cellular necrosis (A). The peripheral blood contained a significant population of blasts with round to irregular nuclei, prominent nucleoli, dispersed chromatin and deeply basophilic cytoplasm (B-C). Immunophenotyping of the circulating leukemic cells by flow cytometry showed a population of CD19+, CD10+, terminal deoxynucleotidyl transferase (TdT)+ B lymphoblasts (D) that were CD45dim+ and negative for CD20 and surface light chain (not shown).

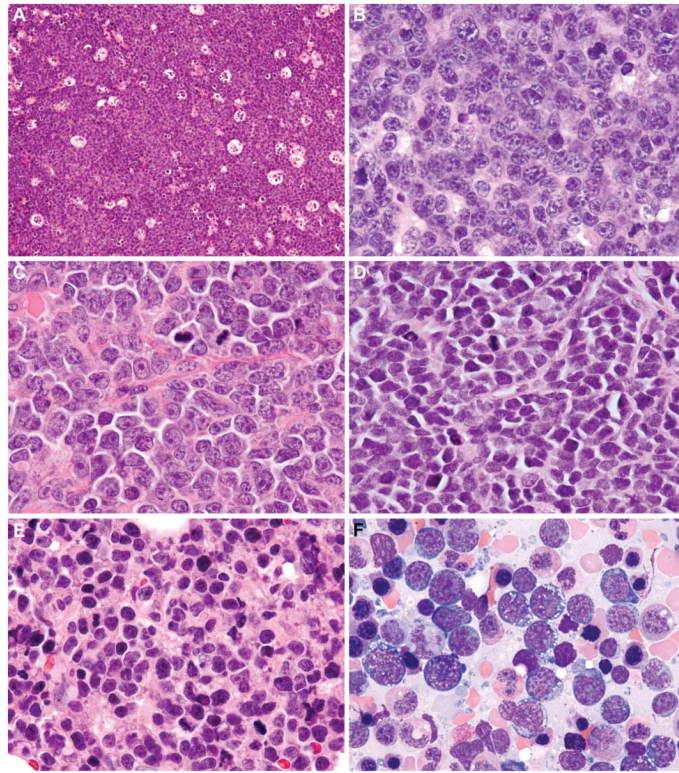


Figure 3.

Morphologic Spectrum of Double-Hit Lymphoma

Five double-hit lymphoma cases classified as B-cell lymphoma, unclassifiable (BCLU), with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) showed Burkitt-like morphological features, with a prominent starry-sky growth pattern at low magnification (A: cervical lymph node, case 3) and a population of monomorphic medium-sized cells with round to slightly irregular nuclei, finely clumped chromatin and multiple small nucleoli at high magnification (B: axillary lymph node, case 2). Four BCLU cases were morphologically intermediate between BL and DLBCL with greater cytomorphic variation, including medium-sized to slightly larger cells containing conspicuous nuclear irregularity and single prominent central nucleoli (C: abdominal wall subcutaneous soft tissue, case 9). Three BCLU cases exhibited blastoid cytologic features, with small cells containing finely dispersed chromatin and inconspicuous nucleoli (D: testicle, case 10). Cases classified as diffuse large B-cell lymphoma, not otherwise specified (DLBCL-NOS) contained predominantly large atypical cells with oval to irregular nuclei, including some with prominent central nucleoli (E: bone marrow biopsy, case 19). The corresponding aspirate smear contained a population of large cells with irregular nuclei, prominent nucleoli, and deeply basophilic cytoplasm with conspicuous vacuolation (F).

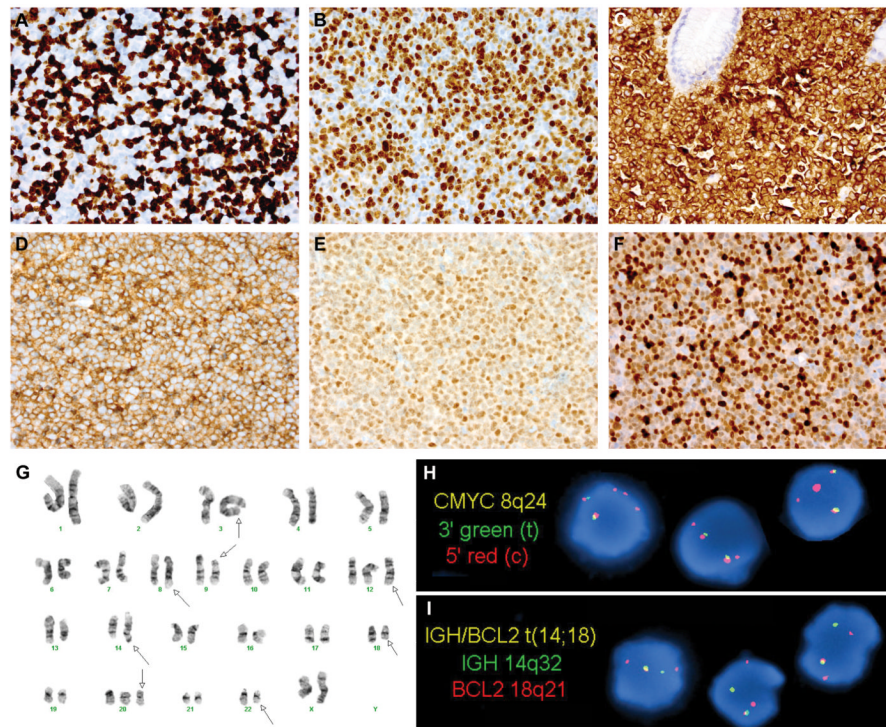


Figure 4.

Immunophenotypic, Cytogenetic and Molecular Genetic Features of Double-Hit Lymphoma
 Two examples of double-hit lymphoma (DHL) demonstrating a Ki-67 proliferation index (PI) of <95%: a retroperitoneal lymph node biopsy from case 5, classified as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCLU), with an overall Ki-67 PI of 60% (A); and an abdominal lymph node biopsy from case 14, classified as diffuse large B-cell lymphoma, not otherwise specified (DBLCL-NOS), with an overall Ki-67 PI of 80% (B). The vast majority of DHL cases expressed Bcl2 by immunohistochemistry for the commonly used antibody, clone 124 (C: stomach polyp, case 1). All DHL cases were of germinal center origin, expressing CD10, Bcl6, or both, as in this cervical lymph node biopsy from case 12 (D: CD10, E: Bcl6). Mum1 was expressed in 8/19 DHL cases, including this axillary lymph node biopsy from case 2 (F). Bone marrow cytogenetic analysis from case 4 showed a complex karyotype (G, arrows), as did all DHL cases. In addition to the t(8;22) and t(14;18), this case contained additional unknown material in the 3q27 region (G, arrow indicating chromosome 3). Subsequent fluorescence *in situ* hybridization (FISH) analysis confirmed a rearrangement involving *BCL6* at 3q27 (not shown). Interphase FISH analysis of paraffin-embedded stomach polyp tissue from case 1 confirmed both a *MYC* rearrangement (H: dual-color, split-apart probe) and an *IGH-BCL2* fusion (I: dual-color, dual fusion probe). The FISH patterns with both probes were consistent with unbalanced rearrangements (H-I). This patient's prior low-grade follicular lymphoma lacked both *MYC* and *BCL2* rearrangements (not shown), suggesting that both translocations were acquired at the time of DHL transformation.

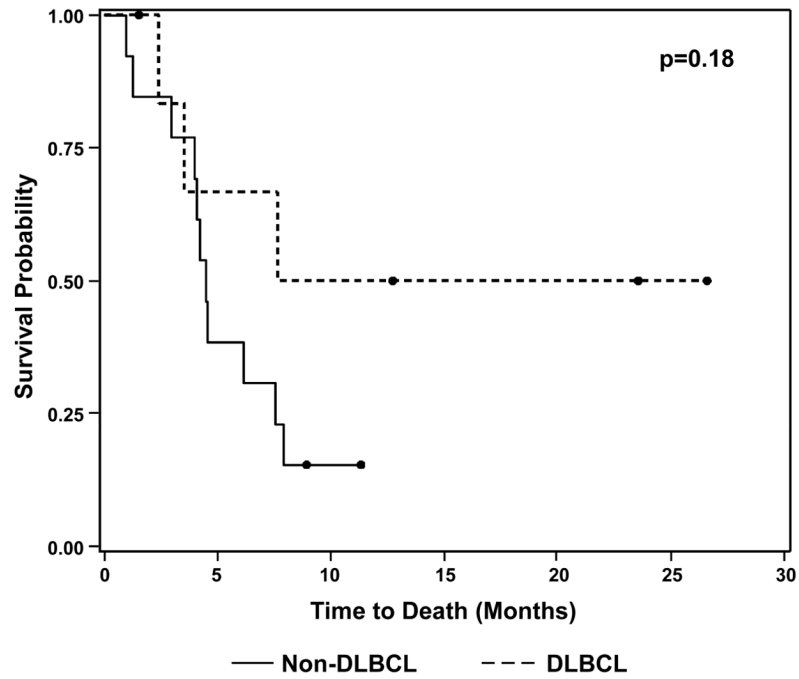


Figure 5.
Differences in Kaplan-Meier Overall Survival Distributions for Double-Hit Lymphoma Patients by 2008 WHO Classification
Black circles denote patients who were alive at the time of last follow-up.
DLBCL: diffuse large B-cell lymphoma, not otherwise specified.

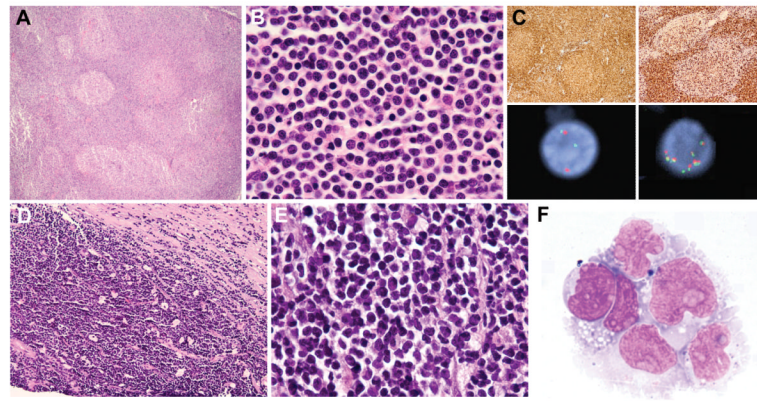


Figure 6.

Pre-Existing Low-Grade Follicular Lymphomas with Unusual Features

Examination of prior low-grade follicular lymphomas in 2 patients revealed findings unusual for this diagnosis. In case 10 with testicular double-hit lymphoma (DHL; illustrated in Fig. 3D), review of a supraclavicular lymph node biopsy from 2 years earlier showed architectural effacement by a follicular proliferation of B cells (A). Cells within and outside of follicles were small with blastoid features, including round nuclei, evenly dispersed chromatin and absent nucleoli (B); typical centrocytes were not identified. Both follicular and extrafollicular B cells were strongly CD20+, CD10+ and Bcl2+ (C, upper left). The follicular component weakly co-expressed Bcl6, but showed absent follicular dendritic cell staining (not shown). Ki-67 stain showed an unusually high proliferation index (PI) of approximately 40% within follicles and 90% outside of follicles (C, upper right). Fluorescence *in situ* hybridization (FISH) analysis with dual color break apart probes revealed a *MYC* rearrangement (C, lower left) and 8 copies of *BCL2* (C, lower right), the same genetic abnormalities seen in the subsequent DHL. In case 11, core needle biopsies of a retroperitoneal mass showed a CD10+, Bcl6+, Bcl2+ B-cell neoplasm with a diffuse growth pattern (absent staining for follicular dendritic cell antigens), a focal starry-sky pattern (D) and patchy necrosis. The cells were small to medium-sized; some were centrocyte-like, but the majority had oval nuclei with finely dispersed chromatin and absent nucleoli (E), prompting a diagnosis of diffuse grade 1-2 FL with blastoid features. Tissue from the initial biopsies was not available for cytogenetics or FISH analysis. Six months later, the patient relapsed with a CD10+ B-cell lymphoma involving the central nervous system (F), confirmed by FISH to have an *IGH-BCL2* fusion as well as a *MYC* rearrangement (FISH analysis not shown).

Table 1

Clinical Characteristics of Double-Hit Lymphoma Patients

Case	Age/ Sex	Stage	LDH (U/L)*	IP1 score	Disease site(s) at presentation	Prior history of lymphoma	Therapy #	BM disease	CNS disease	Response	Outcome
1	52/M	4	2142	3	Stomach	Yes	R-ICE, M, Auto-SCT	+	+	NR	DOD
2	68/M	3	1646	3	Axillary LN	No	CHOP	ND	ND	NR	DOD
3	66/F	4	419	5	Cervical LN	No	CODOX-M/ R-IVAC	-	-	NR	DOD
4	75/F	4	6413	5	BM, PB	No	R-CHOP, M	+	+	PR	DOD
5	51/M	4	2995	3	Retroperitoneum, BM, PB	No	R-EPOCH, M	+	-	CR	Relapsed, AWD
6	58/M	4	270	4	Retroperitoneum	No	CODOX-M/ R-IVAC	+	-	CR	Relapsed, DOD
7	76/F	3	390	4	Perirenal mass	No	R-CHOP, M	+	-	NR	DOD
8	68/F	4	335	5	Retroperitoneum	Yes	R-CHOP	ND	ND	CR	Died, infection
9	64/M	4	380	5	Retroperitoneum, abdomen	No	R-CHOP	-	-	NR	DOD
10	32/M	4	1382	4	Testicle	Yes	R-CHOP, M	+	ND	CR	Died, cause unknown
11	63/M	4	218	4	Retroperitoneum, CNS	Yes	R-EPOCH, M	-	+	NR	DOD
12	66/F	4	255	3	Cervical LN, BM	Yes	R-CHOP, M	+	ND	CR	AND
13	41/F	2	1564	2	Retroperitoneum	No	CODOX-M/ R-IVAC	-	-	PR	DOD
14	52/M	3	339	2	Abdomen	No	R-CHOP	-	ND	CR	AND
15	42/F	4	656	4	Inguinal LN	No	R-CHOP, M	-	ND	CR	AND
16	91/F	NK	NK	2 [^]	Right neck soft tissue	No	NK	ND	ND	NR	DOD
17	62/M	4	798	3	Retroperitoneum	No	R-CHOP, M	+	+	NR	DOD
18	63/F	4	NK	3 [^]	Left leg soft tissue	Yes	R-CHOP, M, Auto-SCT	+	ND	CR	AND
19	75/M	4	3640	3	BM, PB	Yes	R-EPOCH, M	+	-	Therapy ongoing	AWD
20	69/M	4	10,840	4	BM, PB	No	R-CHOP (palliative)	+	ND	NR	DOD

AND: alive, no evidence of disease, AWD: alive with disease BM: bone marrow, CNS: central nervous system, CR: complete response, DOD: died of disease, F: female, IPI: International Prognostic Index, LDH: serum lactate dehydrogenase, LN: lymph node, M: male, ND: not done, NK: not known, NR: no response, PB: peripheral blood, PR: partial response.

* Serum lactate dehydrogenase (LDH) normal range: 110-210 U/L.

Moderate intensity regimens included R-CHOP (rituximab, cyclophosphamide, adriamycin, vincristine, prednisone), CHOP and R-ICE (rituximab, ifosfamide, carboplatin, etoposide); high intensity regimens included CODOX-M/R-IVAC (cyclophosphamide, vincristine, adriamycin, methotrexate, intrathecal cytarabine, intrathecal methotrexate/rituximab, ifosfamide, etoposide, cytarabine, intrathecal methotrexate) and R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, adriamycin). M indicates CNS-directed therapy with intrathecal or high-dose systemic methotrexate. Auto-SCT indicates autologous stem cell transplantation, which was undertaken in first remission or after the completion of chemotherapy.

^ Minimum IPI score calculated based on available data.

Table 2
 Comparison of Clinical Characteristics of Double-Hit Lymphoma, Burkitt Lymphoma and Diffuse Large B-cell Lymphoma Patients

	DHL (n=20)	BL (n=25)	DLBCL (n=40)	p-value (DHL vs BL)	p-value (DHL vs DLBCL)
Median age (range)	63.5 (32-91)	41 (6-73)	71 (47-86)	<0.0001	0.03
Males (%)	11 (55)	19 (76)	21 (52.5)	NS	NS
Pre-existing FL (%)	3 confirmed (15%)	NA	6 (15%)	NA	NS
HIV-positive (%)	0/6 (0)	9/18 (50)	1/15 (7)	0.05	NS
Median LDH at presentation* (range)	727 U/L (218 - 10,840)	365 U/L (112-4862)	366 U/L (162- 5871)	0.07	0.02
Extranodal disease (%)	17 (85)	13 (52)	37 (90)	0.03	NS
BM involvement (%)	10/17 (59)	7/21 (33)	6/26 (23)	NS	0.04
CNS involvement (%)	5/11 (45)	7/25 (28)	6/17 (35)	NS	NS
Alive at last follow-up (%)	6 (30)	17 (68)	16 (40)	0.02	NS
Median OS in months (95% CI)	4.5 (4.0-7.9)	Not reached	38.9 (13.2- 83.7)	0.002	0.04

BL: Burkitt lymphoma, BM: bone marrow, CHL: classical Hodgkin lymphoma, CI: confidence interval, CNS: central nervous system, DHL: double-hit lymphoma, DLBCL: diffuse large B-cell lymphoma, FL: grade 1-2 follicular lymphoma, HIV: human immunodeficiency virus, LDH: lactate dehydrogenase, NA: not applicable, NS: not significant, OS: overall survival.

* Serum lactate dehydrogenase (LDH) normal range: 110-210 U/L.

Table 3
Morphologic, Immunophenotypic and In Situ Hybridization Characteristics of Double-Hit Lymphoma Cases

Case	WHO Classification	Morphology	Features of pre-existing lymphoma (if applicable)	Surface light chain*	CD20	Pax5/CD79a	CD10	Bcl6	Bcl2 [^]	Mum1	TdT	Ki-67 (%)	EBER
1	BCLU	Burkitt-like	FL, grade 1/3 (confirmed by morphology and FISH)	Absent	ND	+/+	+	-	+	+	-	100	-
2	BCLU	Burkitt-like	-	Lambda	+	+/+	-	+	+	+	-	80	-
3	BCLU	Burkitt-like	-	Absent	+	+/+	+	+	^	-	-	100	-
4	BCLU	Burkitt-like	-	Lambda	+	+/+	+	+	+	+	+	65	-
5	BCLU	Burkitt-like	-	Kappa	+	ND/+	+	+	+	-	ND	60	ND
6	BCLU	Intermediate	-	Lambda	+	+/+	+	+	+	-	-	90	-
7	BCLU	Intermediate	-	Lambda	+	+/+	+	+	+	+	-	100	-
8	BCLU	Intermediate	FL, grade 1-2 (confirmed by morphology and FISH)	Lambda	+	ND/ND	+	+	ND	ND	ND	25	-
9	BCLU	Intermediate	-	Lambda	+	ND/+	+	+	+	-	-	95	-
10	BCLU	Blastoid	GC-BCL, MYC+, BCL2x8, with high Ki-67 PI and blastoid morphology	Kappa	+	ND/ND	+	ND	+	-	-	80	-
11	BCLU	Blastoid	GC-BCL with diffuse growth and blastoid morphology (FISH ND)	Kappa	+	ND/+	+	+	+	-	ND	80	-
12	BCLU	Blastoid	CHL (by report)	Lambda	+	+/ND	+	+	+	-	-	70	-
13	DLBCL	DLBCL	-	Lambda	+	+/+	+	+	+	+	+	90	-
14	DLBCL	DLBCL	-	Lambda	+	+/+	+	+	+	-	-	80	-
15	DLBCL	DLBCL	-	ND	+	+/ND	-	+	+	+	+	80	-
16	DLBCL	DLBCL	-	Lambda	+	+/+	+	+	+	-	ND	85	-
17	DLBCL	DLBCL	-	Absent	+	ND/ND	+	+	+	+	+	90	-
18	DLBCL	DLBCL	FL, grade 1-2 (by report)	ND	+	+/ND	ND	+	+	-	-	95	-
19	DLBCL	DLBCL	FL, grade 1-2 (confirmed by morphology and FISH)	Absent	+	+/ND	+	+	+	+	-	80	-
20	B-LBL	B-LBL	-	Absent	-	+/+	+	-	+	+	+	50	-

BCLU: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-LBL: B-lymphoblastic lymphoma/leukemia, CHL: classical Hodgkin lymphoma, DLBCL: diffuse large B-cell lymphoma, not otherwise specified, EBER: Epstein-Barr virus encoded RNA, FISH: fluorescence in situ hybridization, FL: follicular lymphoma, GC-BCL: germinal center-phenotype B-cell lymphoma, ND: not done, PI: proliferation index, TdT: terminal deoxynucleotidyl-transferase, WHO: World Health Organization, wk: weak, +: positive, -: negative.

* Surface light chain expression was determined by flow cytometry in each case.

^ All cases tested were Bcl2+ with clone 124 (targeting amino acids 41-54) except for case 3, which was subsequently tested using clone C-2 (targeting amino acids 1-205) and found to be positive.

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Table 4

Comparison of Pathologic Characteristics between Double-Hit Lymphoma Cases and Burkitt Lymphoma Controls

	DHL (n=20)	BL (n=25)	p-value
Light chain expression*	5/18 (28)	2/13 (15)	NS
Absent (%)	3/18 (17)	6/13 (46)	
Kappa (%)	10/18 (56)	5/13 (38)	
Lambda (%)			
CD10 expression (%)	17/19 (89)	21/23 (91)	NS
Bcl6 expression (%)	17/19 (89)	22/24 (92)	NS
CD10 or Bcl6 expression (%)	20/20 (100)	24/24 (100)	NS
Bcl2 expression (%)	18/18 [#] (100)	1/24 [^] (4)	<0.0001
Mum1 expression (%)	8/19 (42)	1/24 (4)	0.006
Ki-67 proliferation index <95% (%)	15/20 (75)	0/24 (0)	<0.0001
EBER expression (%)	0/19 (0)	8/24 ^{**} (33)	0.006
Complex karyotype (3 or more abnormalities) (%)	11/11 (100)	3/6 (50)	0.03
Median number of cytogenetic abnormalities (range)	9 (5-20)	2.5 (2-6)	0.0009
<i>MYC</i> partner	0/11 (0)	5/6 (83)	0.001
<i>IGH</i> (%)	9/11 (82)	1/6 (17)	
<i>IGL</i> (%)	2/11 (18)	0/6 (0)	
Unknown (%)			

BL: Burkitt lymphoma, DHL: double-hit lymphoma, EBER: Epstein-Barr virus encoded RNA, IGH: immunoglobulin heavy chain gene, IGL: immunoglobulin lambda light chain gene.

* Surface light chain expression was determined by flow cytometry in all DHL and BL cases.

[#]The single DHL case that was negative for Bcl2 expression with clone 124 but positive with clone C-2 (case 3) had a t(14;18) by karyotype.

[^]The single Bcl2+ BL case showed no BCL2 rearrangement by FISH.

^{**} Among 8 EBER+ BL cases, 4 arose in HIV+ adults.

Table 5

Cytogenetic and Molecular Genetic Characteristics of Double-Hit Lymphomas

Case	Karyotype	Fluorescence in situ hybridization		
		<i>IGH-BCL2</i>	<i>MYC</i>	<i>BCL6</i>
1	ND	+	+	-
2	51,XY,t(2;9)(q21;p24),+3,add(5)(p15.3),add(7)(q32), add(8)(q24) ,+11,+12, t(14;18)(q32;q21) ,+15,+18,add(19)(q13.1),-22,+2mar[2]	ND	+	Trisomy
3	46,XX,add(3)(q2?5),add(6)(q1?2),del(6)(q1?5q2?3), t(8;22)(q24;q11) , t(14;18)(q32;q21)[7]	ND	+	-
4	48,XX,add(3)(q2?7), t(8;22)(q24;q11) ,del(9)(p11),+12,add(12)(q22), t(14;18)(q32;q21) ,+20[10]/47,XX,add(3), t(8;22) ,del(9),+12,der(12)t(1;12)(q12;q24), t(14;18)[3]/47,XX,add(3),t(8;22) ,del(9),+12,add(12),der(12),t(14;18)[2]	ND	ND	+
5	ND	+	+	ND
6	ND	+	+	-
7	48,XX,add(7)(q32), add(8)(q24) ,i(11)(q10),dup(12)(q13q24), t(14;18)(q32;q21) ,+21,+1-2mar[cp3]	+	+	-
8	45,X,-X,add(1)(p36),del(6)(q15),del(7)(q22), t(8;22)(q24;q11.2) ,t(9;15)(p13;q24), t(14;18)(q32;q21)[cp14]	ND	ND	ND
9	47,XY,add(2)(p13),+7, t(8;22)(q24;q11) ,der(13)t(3;13)(q2?6;q21), t(14;18)(q32;q21) ,add(17)(p13)[10]	+	+	Trisomy
10	ND	<i>BCL2</i> x8	+	-
11	ND	+	+	ND
12	96<4n>XXXX,+X,add(1)(p36),-3,-4,i(6)(p10), t(8;22)(q24;q11)x2 , der(8)t(8;22)(q24;q11) ,+10,-11,del(11)(q2?2)x2,+12,der(12)ins(12;?)(q1?5;?)x3, ins(14;18)(q32;q21q21)x2 ,i(17)(q10),+20,-21,-21,+r,+mar1x2[10]	+	+	Trisomy
13	ND	+	+	-
14	ND	+	+	-
15	52,add(X)(p22.1),del(X)(q22),+X,add(1)(p3?4),add(1)(p3?6),add(4)(q21),+5,del(6)(q13q23),+7,der(8)t(1;8)(p3?4;p2?3), t(8;22)(q24;q11) ,add(11)(q2?3),+12, der(14)t(14;18)(q32;q21) ,add(15)(q22),add(18)(q21),add(19)(q13),+21,[cp6]/53,idem,+add(4)(q21)[cp7]	+	+	ND
16	ND	+	+	-
17	50,X,-Y,+X,del(2)(p21),add(5)(q35),+6,+7, der(8)t(8;22)(q24;q11) ,del(9)(p22),del(10)(q22q24),+11,-13, t(14;18)(q32;q21) ,i(17)(q10), der(22)t(8;22)(q24;q11)t(1;22)(q11;p11)x2 ,+mar[19]	ND	+	ND
18	ND	+	+	-
19	52,XY,+X,+7, t(8;22)(q24;q11) ,+11,+12,+13, t(14;18)(q32;q21) ,+ der(18)t(14;18)(q32;q21)[9]/53,idem,+5[8]	ND	ND	ND
20	50,XY,der(2)t(1;2)(q21;q3?7),+7, t(8;22)(q24;q11) ,+9,der(9;17)(q10;q10),+12, t(14;18)(q32;q21) ,+18[12]	ND	ND	ND

ND: not done, +: rearranged, -: no abnormalities detected by FISH.