Targeted Mutational Profiling Reveals Clonal Relationships in Metachronous Occurrence of Classic Hodgkin and Mediastinal Large B-Cell Lymphomas

Kunwar Singh, MD, Lhara S. Lezama, MD,† Jason Kurzer, MD, PhD,* Jean Oak, MD, PhD,* Liora M. Schultz, MD,‡ Ann Walkush,‡ Tse-Chang Cheng, MD,† Everett H. Chen, MD,† William A. May, MD,§ Cheryl Chang,‖ Michael P. Link, MD,‡ Ranjana H. Advani, MD,‖ Carlos J. Suarez, MD, MSc,* and Yasodha Natkunam, MD, PhD**

Abstract: Classic Hodgkin lymphoma (CHL) patients may infrequently present with a prior or recurrent disease with discordant histology resembling non-Hodgkin lymphomas. These include primary mediastinal large B-cell lymphoma (PMBL), diffuse large B-cell lymphoma (DLBCL), or mediastinal gray-zone lymphoma (MGZL). Such patients are often refractory to standard therapy and their diagnosis is hampered by significant morphologic and immunophenotypic overlap and insufficient molecular data. Among 509 CHL patients seen at an academic medical center, 6 patients had a prior or subsequent diagnosis different from CHL. Paired tissue samples were evaluated by targeted mutational analysis using a 164-gene panel. Our findings show multiple shared variants indicative of a clonal relationship between the CHL and the PMBL, DLBCL, or MGZL diagnoses. Most frequent mutated genes included *TNFAIP3* (4 of 6, 66.7%), *STAT6* (3 or 6, 50%), *ARID1A* (3 of 6, 50%), and *XPO1* (3 of 5, 60%). Three patients showed the same oncogenic variant within the *XPO1* gene (E571K), and mutations in *TNFAIP3* and *B2M* were observed in 2 of the 5 patients with shared variants. In addition, differences in the mutation profile between the lymphoma pairs were also observed, which could represent clonal evolution. Mutational profiling could be of benefit in patients with recurrent/refractory disease with discordant histology, where the clonal relationship could be helpful to inform and guide therapeutic decisions. These findings provide further evidence of a true biological continuum surrounding CHL, PMBL, DLBCL, and MGZL and shed light on underlying genetic events and their clinical impact.

Key Words: Hodgkin lymphoma, large B-cell lymphoma, mediastinal gray-zone lymphoma, next-generation sequencing

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KEY POINTS

- Metachronous occurrence of classic Hodgkin lymphoma (CHL) shows phenotypic and genetic support for a biological continuum with mediastinal large B-cell lymphomas.
- Targeted mutational analysis provides evidence of clonal relationships despite discordant histology.
- Mutational profiling could be considered in patients with recurrent/refractory disease with discordant histology to optimize clinical management.

INTRODUCTION

The clinicopathologic overlap among classic Hodgkin lymphoma (CHL) and subtypes of large B-cell lymphomas, particularly those occurring in the mediastinum, is well recognized.[1] These lymphoma subtypes include primary mediastinal large B-cell lymphoma (PMBL), B-cell lymphoma, unclassifiable, with features intermediate between CHL and diffuse large B-cell lymphoma (DLBCL) (BCLU, also known as mediastinal gray-zone lymphoma [MGZL], as well as DLBCL, not otherwise specified). Similarities between CHL and PMBL have long been recognized, and these entities are hypothesized to form a biological continuum.[2–7] The cell of origin in both CHL and PMBL is presumed to be thymic B cells, and amplification of the *REL* locus on chromosome 2p and the *JAK2* locus on 9p are found in both entities.[8,9] Likewise, gene expression profiling studies also show shared fea-

From the *Department of Pathology, Stanford University School of Medicine; ‡Department of Pediatrics, Division of Pediatric Hematology/Oncology; ‖Department of Medicine, Division of Oncology, Stanford University School of Medicine, Stanford; †Kaiser Permanente; and §Department of Pediatrics, UCLA Medical Center, Los Angeles, CA.

C.J.S. and Y.N. contributed equally.

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Correspondence: Yasodha Natkunam, MD, PhD, Department of Pathology, L235 Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305 (e-mail: yaso@stanford.edu).

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tures, especially low expression of B-cell receptor components and the downstream signaling cascade and high expression of tumor necrosis factor family members and extracellular matrix elements.[10,11] Although the shared features provide support for common pathogenesis, there are clear clinicopathologic differences that significantly impact clinical decision-making.

The diagnosis of CHL requires the presence of the typically rare neoplastic Hodgkin/Reed-Stenberg (HRS) cells within a tumor microenvironment enriched for immune cells.[1] Although HRS cells are derived from germinal center B cells, they exhibit defective antigen receptor signaling and B-cell expression program.[12–14] The resultant loss or downregulation of B-cell markers, in addition to CD30 and/or CD15 expression, are routinely used to distinguish CHL from large B-cell lymphomas occurring in the mediastinum such as PMBL, MGZL, as well as DLBCL, not otherwise specified. The clinical, morphologic and immunophenotypic spectrum across these lymphomas overlap greatly, and at least in some patients, their separation from one another is challenging.[2–7]

Diagnostic borderlines surrounding CHL, PMBL, MGZL, and DLBCL may also manifest at the time of disease recurrence. Leveraging our large clinical database of Hodgkin lymphoma patients, we identified 6 patients with CHL who had a prior or subsequent diagnosis in keeping with PMBL, MGZL, or DLBCL. Although rare, the diagnosis and clinical management of such patients are particularly problematic due to refractory disease that is typically unresponsive or only partially responsive to standard treatment regimens. The biological link, if any, between the initial and subsequent lymphomas with discordant histology is poorly understood. In this context, whether the changes manifest at recurrence are due to the evolution of underlying genetic aberrations, posttherapy selection of subclones, lymphoid plasticity, or other mechanisms, are unknown. Given that these lymphomas present in all age groups including children and young adults, and the optimal choice of therapy hinges on the clinicopathologic diagnosis, additional molecular tools are critically needed to render a precise diagnosis. To this end, we used targeted next-generation sequencing to analyze paired samples in all patients in our cohort and report our findings.

METHODS

Patient Samples

The Stanford Hospital Department of Pathology archive spanning the last 10 years was searched for cases of Hodgkin lymphomas. A total of 509 accessions were identified and further curated using the following inclusion criteria: (1) an unequivocal diagnosis of CHL with a prior or subsequent B-cell lymphoma diagnosis that differed from CHL; and (2) availability of sufficient tissue at initial diagnosis and at recurrence, for additional studies including targeted next-generation sequencing. Patients with low-grade B-cell lymphomas with or without transformation and those with known immunodeficiency were excluded. The study cohort consisted of 6 patients with a diagnosis of CHL at initial presentation (4 patients) or at recurrence (2 patients).

The second diagnoses included PMBL, MGZL, or DLBCL, and the majority of the cases involved the mediastinum (Table 1). The CHL diagnosis in 5 of the 6 patients represented the nodular sclerosis subtype (NS), and the sixth case (case #5, liver biopsy) was favored to represent the NS subtype due to the presence of background fibrosis. All cases were reviewed by 2 hematopathologists (K.S. and Y.N.), in addition to which other hematopathologists also reviewed select cases at the time of diagnosis and at multidisciplinary conferences. We have designated the relapse biopsy in case #3 as "DLBCL" primarily because this case occurred in the liver and did not involve the mediastinum; the term primary extra-MGZL has also been applied to such cases. The cases were diagnosed according to 2017 WHO classification.[1] Clinical information were extracted by retrospective chart review by the treating oncologists. Institutional review board approval was obtained for this study.

Immunohistochemistry and In Situ Hybridization

Immunohistochemistry was performed on 4-μm-thick formalin-fixed paraffin-embedded tissue sections on automated immunostainers (Ventana BenchMark Ultra System; Roche Diagnostics, Tucson, AZ and Leica Bond III; Leica Biosystems, Buffalo Grove, IL). Sections were stained with the following antibodies: CD30, CD15, CD20, CD79a, PAX5, OCT2, BOB1, CD3, CD5, CD10, BCL6, MUM1, cMYC, BCL2, and Ki-67. Staining was scored on a 4-part scale as follows: 0, negative, 1+ weak positive in <10% of cells; 2+, moderate staining in 10% to 30% of cells; 3+, strong staining is >30% of the cells. In situ hybridization for Epstein-Barr virus (EBV)–encoded RNA (EBER) was performed using the EBER1 DPN probe and visualized using the ISH iView system (Roche).

Molecular Studies

Targeted gene sequencing of the formalin-fixed paraffin-embedded samples was performed in the Stanford Molecular Genetic Pathology Laboratory using a clinically validated, laboratory-developed, targeted next-generation sequencing assay (HemeStamp). The assay involves acoustic shearing of isolated genomic DNA (M220 focused ultrasonicator, Covaris, Woburn, MA), followed by preparation of sequencing libraries (KK8232 KAPA LTP Library Preparation Kit Illumina Platforms; KAPABiosystems, Wilmington, MA), and hybridization-based target enrichment with custom-designed oligonucleotides (Roche NimbleGen, Madison, WI). Unique molecular identifiers are used to remove polymerase chain reaction duplicates. Partial or full coverage of 164 genes of recurrently mutated genes that are clinically relevant in hematolymphoid malignancies are included in the assay, which detects variants with a variant allele fraction as low as 1%. A complete list of the 164 genes is shown in Supplementary Table S2 (Supplemental Digital Content 1, http://links.lww.com/PAS/B422). A summary of

TABLE 1. Clinical Features

Patient No.	Age/Sex	Site of Biopsy	Diagnosis	Stage: IPS/IPI	Treatment	Outcome
1	21/female	Anterior mediastinum	CHL, NS	Stage IIEBX IPS 1 of 7	ABVD ×6 (PD)	DOD (28 mo)
		Left cervical lymph node	MGZL	IPI 1 of 5	R-ICE ×2 (PD) Bendamustine and brentuximab ×2 (PD) RT (40 Gy) and nivolumab ×7 (CR then PD) GND ×4 followed by autologous SCT (PD) Cyclosporine and brentuximab ×2	
2	29/male	Lung, left lower lobe	CHL, NS	Stage IVB IPS 3 of 7	ABVD ×4 (PD)	Alive, NED (88 mo)
		Anterior mediastinum	MGZL	Stage IV IPI 1 of 5	Dose-escalated BEACOPP ×4 (PD) RDHAP ×3 Autologous SCT, followed by allogeneic SCT	
3	21/male	Right cervical lymph node	CHL, NS	Stage IIAX IPS 1 of 7	ABVD ×2 (PD)	Alive, NED (20 mo)
		Right deep cervical lymph node	DLBCL	IPI 1 of 5	DA-EPOCH-R ×6 (CR) with consolidative radiotherapy (30 Gy)	
4	14/male	Left cervical lymph node, Anterior mediastinum	CHL, NS	Stage IVB IPS 4 of 7	ABVE-PC ×5 (PD) Radiotherapy (PD)	DOD (29 mo)
		Lung, right lobe	MGZL	Stage IVB IPI 1 of 5	Brentuximab and bendamustine ×2 (PD) Vinorelbine/gemcitabine (PD) ICE (PD) Nivolumab ×4 (transient response, then PD) CD19-CAR T cells (PD) Palliative brentuximab and hospice care	
5	49/female	Anterior mediastinum	MGZL	Stage IIIB IPI 2 of 5	CHOP ×6 (PR) Salvage 1: ICE ×2 (PD) Salvage 2: GemOx ×4 (PR) Autologous SCT (PR) Consolidative RT (PD)	Alive, NED (50 mo)
		Liver, periportal lymph nodes	CHL, favor NS	Stage IV IPS ~2 of 7	Nivolumab and brentuximab ×14 cycles Additional nivolumab ×3	
6	20/male	Anterior mediastinum	PMBL	Stage IVB IPI 3 of 5	Multiagent therapy for mature B-cell lymphomas of children plus rituximab (RD) Surveillance 7 mo off-therapy, early mediastinal mass	Alive, NED (27 mo)
		Anterior mediastinum	CHL, NS	Stage IVB IPS 1 of 7	Bendamustine and brentuximab with near CR followed by autologous SCT	

ABVD indicates adriamycin, bleomycin, vinblastine, dacabazine; ABVE, adriamycin, bleomycin, vincristine, etoposide; BEACOPP, bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, prednisone; CAR, chimeric antigen receptor; CHOP, cyclophosphamide, adriamycin, vincristine, prednisone; CR, complete response; DA-EPOCH, dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, hydroxyadriamycin; DOD, died of disease; GemOx, gemcitabine, oxaliplatin; GND, gemcitabine, navelbine, doxorubicin; ICE, ifodfamide, carboplatin, etoposide; IPI, International Prognostic Index; IPS, International Prognostic Score; NED, no evidence of disease; PD, progressive disease; PR, partial response; RD, recurrent disease; RDHAP, rituximab, dexamethasone, high-dose Ara-C, cisplatin; R-ICE, rituximab, ifosfamide, carboplatin, etoposide; RT, radiotherapy; SCT, stem cell transplant.

anticipated molecular abnormalities in CHL, PMBL, and MGZL are provided in Supplementary Table S3 (Supplemental Digital Content 1, http://links.lww.com/PAS/B422).

RESULTS

Clinical Features

The 6 patients in the study cohort ranged in age from 14 to 49 years (median, 21 y) and included 4 men and 2 women. Four patients were diagnosed with CHL at initial presentation, 3 of whom had a subsequent recurrence diagnosed as MGZL, and the fourth had a subsequent recurrence diagnosed as DLBCL. One patient had an initial diagnosis of MGZL and was subsequently diagnosed with CHL, and the remaining patient had an initial diagnosis of PMBL and was subsequently diagnosed with CHL. All CHL cases represented the NS subtype. The clinical findings are summarized in Table 1.

The 4 patients diagnosed with CHL at initial presentation showed progressive disease following standard treatment for CHL (ABVD or ABVE regimens [patients 1 to 4, Table 1]. After repeat biopsy showing discordant histology of MGZL or DLBCL were found, multiple different treatment modalities were employed including anthracycline-based chemotherapy or immunochemotherapy (patients 1 to 4), stem cell transplantation (patients 1 and 2), radiotherapy (patients 1 and 3), checkpoint blockade (patients 2 and 4) and chimeric antigen receptor T-cell therapy (patient 4). Two of these patients died of progressive disease, while 2 remain alive with no evidence of disease at 88 months (patient 2) and 20 months (patient 3), respectively.

The 2 additional patients who initially presented with MGZL, CD20-negative (patient 5), and PMBL (patient 6) had progressive disease and/or recurrence after multiple rounds of immunochemotherapy. Repeat biopsies showed divergent histologic and immunophenotypic features from their original diagnoses that met the criteria for CHL, at which time their treatment was modified, as shown in Table 1. Both patients remain alive with no evidence of disease at 50 months (patient 5) and 27 months (patient 6), respectively.

Histologic and Immunophenotypic Features

Histologic and immunophenotypic characterization of the 6 cases at initial and recurrent presentations (12 biopsies) are summarized in Table 2; the markers tested are shown in Supplementary Table S1 (Supplemental Digital Content 1, http://links.lww.com/PAS/B422). Large atypical cells with HRS-like morphology were encountered in all 12 biopsies, albeit their frequency, growth characteristics, and the surrounding cells in the immune microenvironment were variable. All biopsies with a CHL diagnoses most frequently showed singly scattered HRS cells (Fig. 1), whereas the PMBL, DLBCL, and MGZL diagnoses showed predominantly clusters or sheet-like growth of the large cells (Fig. 2). A mixed inflammatory background (10 of 12) and dense fibrosis (8 of 12) were commonly encountered whereas necrosis was less common (5 of 12) and was typically found at the time of recurrent disease.

The immunophenotype of the 6 paired biopsies showed typical as well as unusual findings for the diagnoses rendered: CD30 was positive in 5 of 6 cases that were diagnosed as CHL. The single case that lacked CD30 (patient 3) showed characteristic CHL histology, robust CD15 expression and dim PAX5 in the absence of all other B-cell and T-cell markers tested. In addition, all 4 biopsies diagnosed as MGZL expressed variables CD20, CD30, CD15, CD45, as well as B-cell transcription factors, PAX5, OCT2, and BOB1 (Table 2). Among the 4 patients with MGZL, CD20 expression ranged from strong and diffuse (patient 2) to complete lack of expression (patient 5). Representative images are shown in Figures 1 and 2. A single case showed a light chain restricted B-cell lymphoma which was detected by flow cytometry (case 3 recurrence). In 3 other cases where flow cytometry was attempted, the results were inconclusive primarily due to lack of sampling of the relevant cells. All 12 biopsies lacked T-cell marker expression in the large atypical cells. In addition, EBV, as measured by EBER in situ hybridization, was negative in the large atypical cells of all biopsies; 2 posttherapy cases showed focal EBV reactivation limited to small background lymphoid cells (patients 1 and 4).

Molecular Genetic Features

The DNA mutation profiling performed highlighted the presence of shared variants within the paired CHL and PMBL, DLBCL or MGZL specimens (Table 3). All cases, except for patient 4, displayed multiple shared variants between the CHL specimen at diagnosis and the MGZL, PMBL, or DLBCL specimen at recurrence, excluding potentially germline sequence changes. The MGZL specimen of patient 4 showed a noise level higher than usual, a phenomenon typically associated with poor DNA quality that precluded the analysis of variants present at a low allele fraction ($< 6\%$).

Several recurrently mutated genes were identified either in the initial tumor or the recurrence. Most cases presented variants in *TNFAIP3* (4 of 6, 66.7%), a negative regulator of the nuclear factor-κB pathway. Other commonly mutated genes were *STAT6* (3 of 6, 50%), a member of the JAK-STAT pathway, *ARID1A* (3 of 6, 50%), whose gene product promotes the formation of the SWI/SNF chromatin remodeling complex and functions as a tumor suppressor, and *XPO1* (3 of 5, 60%), which participates in the export of nuclear proteins and RNA and behaves as an oncogene. Of note, 3 patients (2, 3, and 5) shared the same known oncogenic variant within the *XPO1* gene (E571K), and one of them in both the CHL and the subsequent DLBCL biopsies (patient 3). Finally, among the genes where variants were present in both the initial tumor and the recurrence across the 5 patients with shared variants, *TNFAIP3* (2 of 5, 40%) and *B2M* (2 of 5, 40%) where the only genes observed more than once (Table 3).

DISCUSSION

The concept of MGZL was formulated at a joint workshop on Hodgkin and gray-zone lymphomas,[2] following which a series of cases describing their clinicopathologic features was published.[3] MGZL was subsequently incorporated into the 2008 WHO classification.[15] Since its inclusion into the classification and with the refinement of diagnostic criteria in the last decade, a wider spectrum of lymphomas spanning the borderlines between CHL and DLBCL have been routinely diagnosed.[1] Among CHL, PMBL and MGZL, their derivation from thymic B cells and demographics typically affecting young adults are similar although there is varying predilection for specific sexes. Typical histologic and immunophenotypic features allow the appropriate subclassification of a large proportion of these patients; although many studies have emphasized the biological continuum imposed by the overlap in morphologic and genetic features across these lymphomas.[3–7] Therefore, a multidisciplinary approach is often essential when evaluating lymphomas affecting the mediastinum because of the importance of therapeutic decisions in the clinical management of these patients.

In the current study, we evaluated 6 patients with the metachronous occurrence of CHL with one of PMBL, DLBCL or MGZL as the second diagnosis. As expected, there was a significant overlap in clinicopathology features and immunophenotype. There was a highly variable expression of CD30, CD20, CD15, and CD45. None of these markers were singly able to define any of the entities in this spectrum including strong and diffuse expression of CD30 or

TABLE 2. Histologic and Immunophenotypic Features

			Immunophenotypic and FISH Data*							
Patient No.	Diagnosis	Histology	CD30	CD15	PAX5/ OCT2/ BOB1	CD20	CD79a	EBER	Other IHC	FISH
1	CHL	Singly scattered large atypical cells associated with mixed inflammatory cells and dense fibrosis	3+	1+	0-1+	0	0	0	NA	NA
	MGZL	Effaced lymph node with sheets of large atypical cells; background rich in small lymphoid cells	3+	1+	0-2+	2+	2+	Focal+ in small cells	$CD23^+$ $CD19^-$ $CD22^-$	NA
2	CHL	Focal clusters of large atypical cells with prominent nucleoli associated with mixed inflammation	3+	0	0-2+	1+	0	0	$CD45^-$	NA
	MGZL	Sheets of large atypical cells with dense fibrosis and necrosis	3+	2+	3+	3+	0		$CD45^-$	NA
3	CHL	Scattered large atypical cells with prominent nucleoli, dense bands of fibrosis, and mixed inflammation	0	3+	1+	0	0	0	NA	NA
	DLBCL	Sheets of large highly pleomorphic cells, prominent nucleoli and associated apoptosis and necrosis	3+	0	3+	3+	3+	0	$MUM1^+$ $BCL2^+$ MYC^+ $CD23^+$	MYC^-
4	CHL	Effaced lymph node with scattered large atypical cells, mixed inflammation, dense fibrosis and necrosis	3+	0	0-2+	0	1+	0		NA
	MGZL	Consolidated nodular and diffuse proliferation of large atypical cells, prominent fibrosis, mixed inflammation and necrosis	3+	0	2-3+	2+	2+	Focal+ in small cells	ALK^-	MYC^- $BCL2^-$ $BCL6^-$ $IRF4^-$
5	MGZL	Effaced lymph node with clusters and sheets of large atypical cells associated with inflammatory cells	1-2+	0	1-3+	0	3+	0	$CD45^+$ $CD10^+$ $BCL6^+$ $MUM1^-$ $CD23^-$	NA
	CHL	Singly scattered large atypical cells with HRS morphology, inflammatory cells, bands of fibrosis and focal necrosis	3+	1+	0-1+	0	0	0	$CD45^-$	NA
6	PMBL	Sheers of large atypical cells associated with mixed inflammation and fibrosis	0	0	3+	3+	3+	0	$CD10^+$ $BCL6^+$ MYC^+ $BCL2^+$ $MUM1^+$	NA
	CHL	Partially effaced lymph node with singly scattered large atypical cells, mixed inflammation and fibrosis	3+	3+	1+	0	0	0	NA	MYC^- $BCL2^-$ $BCL6^-$ $CCND1^-$

Additional negative markers performed during the clinical workup are not shown. All T-cell markers tested including CD3, CD5, and CD43 were negative.
*Only the relevant markers are summarized.
FISH indicates fluorescent in situ hybridization; IHC, immunohistochemistry; LN, lymph node; NA, not available.

CD20 in designating CHL from PMBL, DLBCL, and MGZL. B-cell transcription factors PAX5, OCT2, and BOB1 were also highly variable, and although the absence of all 3 was not seen in cases diagnosed as PMBL or DLBCL, the high degree of variability in their protein expression profiles precluded definitive subclassification into a well-defined category. Markers that were helpful in subsequent workups included CD79a, CD23, and BCL6, which when expressed favored MGZL or PMBL over CHL. A diagnosis of large B-cell lymphoma was favored in the single case where a light chain restricted B-cell population was detected by flow cytometry.

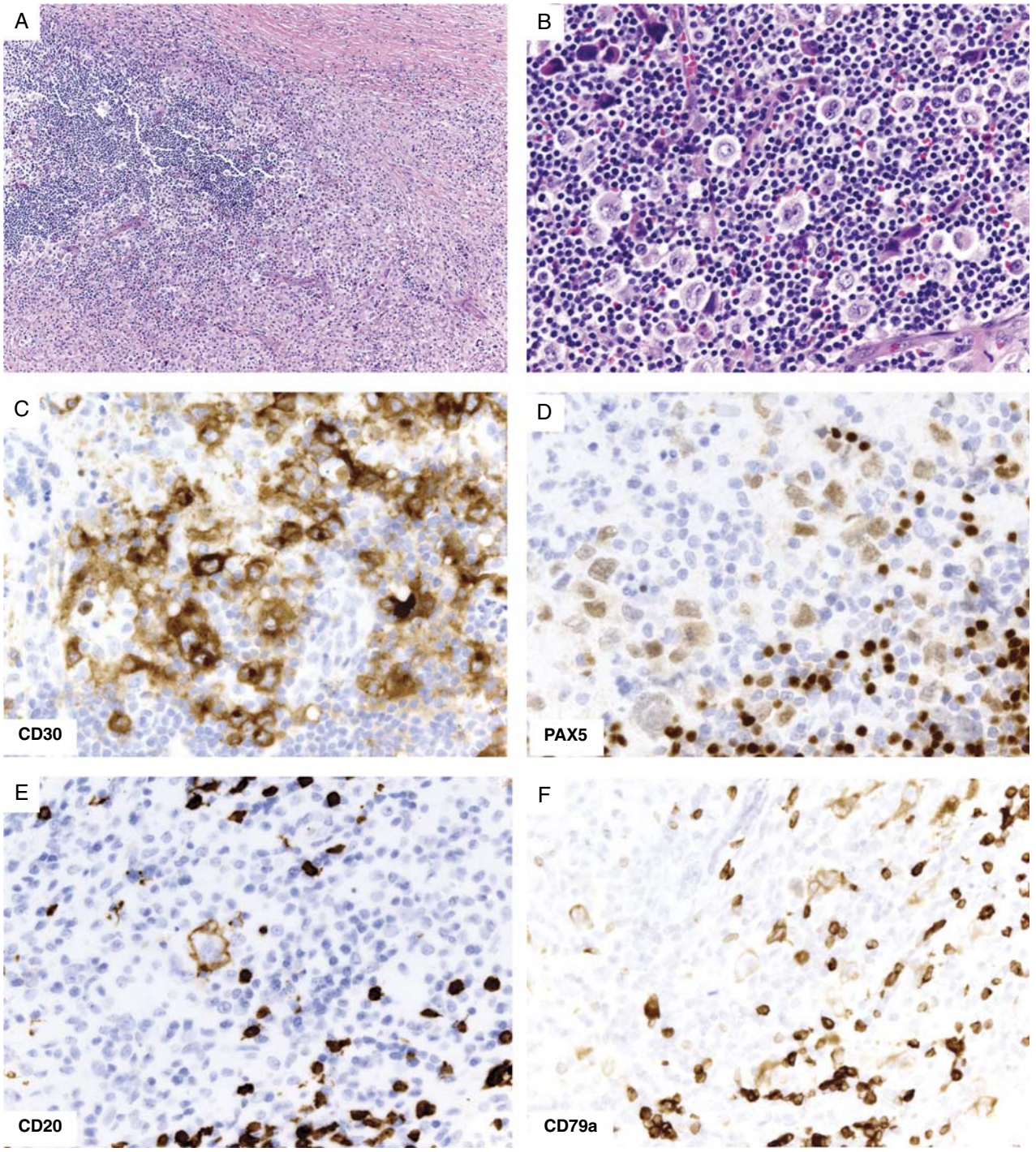

FIGURE 1. Initial presentation of CHL in a patient with subsequent recurrence of MGZL. Representative images of the cervical lymph node at initial presentation in case 4 show an atypical nodular lymphoid proliferation separated by dense bands of fibrosis (A), within which there are large atypical cells with HRS cell morphology and an associated immune microenvironment (B). The large cells are positive for CD30 (C) and dim PAX5 (D), with very rare cells positive for CD20 (E) and CD79a (F).

Metachronous occurrence of CHL with discordant histology has been previously documented in the literature.[16] In our cohort, we aimed to highlight the metachronous occurrence of CHL with MGZL, PMBL or DLBCL which addresses the possibility that there is plasticity in the neoplastic cells which are postulated to arise from common B-cell precursors. Previous studies have shown that even though there are many similarities among CHL, PMBL, DLBCL, or MGZL, each of these entities have unique characteristics based on clinical,

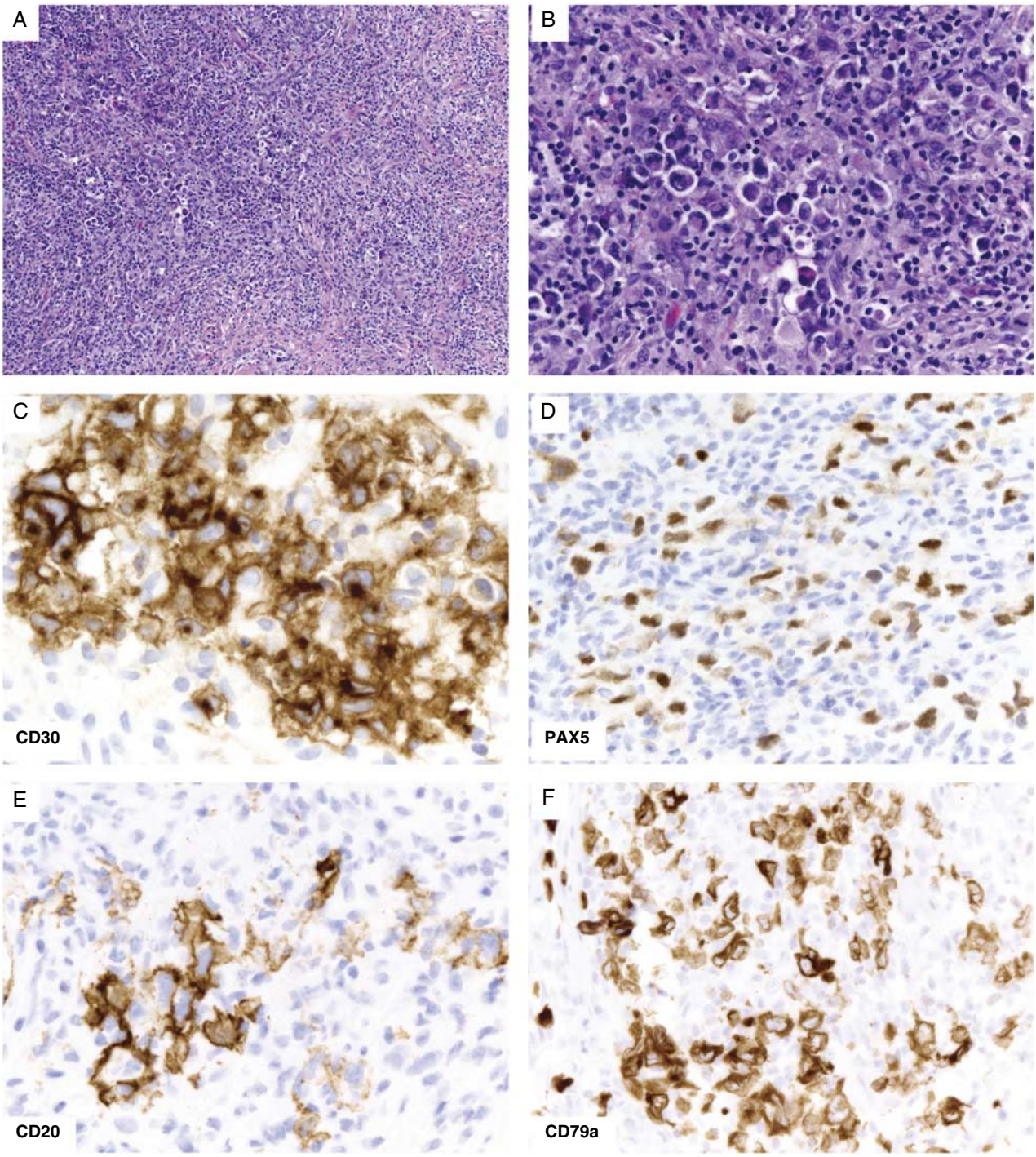

FIGURE 2. MGZL presenting in a patient with prior CHL. Biopsy of the lung at the time of disease recurrence in case 4 shows a sheet-like growth pattern (A), with a confluence of large, atypical cells (B); Immunohistochemistry shows that the large, atypical cells are positive for CD30 (C), bright PAX5 (D), CD20 (E), and CD79a (F), indicative of an intact B-cell program.

histologic, phenotypic and genetic characteristics.[3–10] The 6 cases in our study met the criteria for one of these specific diagnoses at initial presentation and at recurrence; however, the 2 diagnoses were different. The 4 patients who were initially diagnosed with CHL were subjected to further workup with additional B-cell markers such as CD79, CD19, CD22, CD23, PAX5, OCT2, and BOB1 at the time of recurrence. Although the recurrences showed robust expression of B-cell markers, the initial CHL biopsies lacked these markers,

TABLE 3. Genomic Alterations in paired lymphoma samples

Gene	Variant	Initial Diagnosis	Diagnosis at Recurrence	Predicted Biological Effect
Patient 1		CHL	MGZL	
TNFAIP3	L147fs	12.67%	34.70%	Oncogenic
CREBBP	H67D	41%	25.79%	VUS
NFKBIE	D288G	4.15%	16.39%	VUS
ARID1A	P757S		6%	VUS
BCOR	W509X		25.49%	Oncogenic
CARD11	V819I		4.75%	VUS
PRDM1	A175T		18.09%	VUS
PTEN	E150K		8.54%	VUS
SRSF2	D76N		9.86%	VUS
IRF8	A197V*	46.12%	46.49%	VUS
U2AF1	R119H*	41.16%	44.27%	VUS
Patient 2		CHL	MGZL	
STAT6	N417Y	1.19%	10.56%	VUS
PIM1	I66M	1.01%	6.42%	VUS
PIM1	P33A	1.25%	5.30%	VUS
XPO1	E571K†		5.87%	Likely oncogenic
TNFAIP3	Q490X		9.07%	Oncogenic
ARID1A	P1747S		8.05%	VUS
EP300	A783V*	57.17%	43.59%	VUS
IRF8	A201V*	52.59%	53.04%	VUS
Patient 3		CHL	PMBL/ DLBCL	
B2M	c.67+1G > A (p.?)	29.64%	3.02%	Likely oncogenic
PAX5	D2E	24.65%	45.32%	VUS
XPO1	E571K†	13.39%	1.45%	Likely oncogenic
MYC	V92F	10.08%	2.57%	VUS
TNFAIP3	Q110X	46.79%		Oncogenic
GNA13	G60D	27.32%		VUS
STAT6	W515R	22.66%		VUS
ARID1A	Y316fs	12.88%		Likely oncogenic
TNFAIP3	S573fs		3.16%	Oncogenic
CREBBP	A206V*	48.75%	48.72%	VUS
Patient 4		CHL	MGZL	
FBXW7	E50D		6.37%	VUS
SETD2	L222I*	48.91%	56.45%	VUS
Patient 5		MGZL	CHL	
TNFAIP3	Q143	4.85%	1.14%	Oncogenic
TNFAIP3	P567fs	5.28%	1.01%	Oncogenic
TNFAIP3	T108A	5.77%	1.25%	VUS
TNFAIP3	R123T	5.03%	1.31%	VUS
TNFAIP3	K131M	4.34%	1.12%	VUS
PIM1	E142D	4.03%		VUS
SOCS1	152_153del	5.06%		VUS
SOCS1	R127fs	4.50%		Likely oncogenic
JAK3	K550N	9.78%		VUS
STAT6	N417S	6.88%		VUS
PIM1	L184V	5.06%		VUS
EZH2	Y646N	4.32%		Oncogenic
XPO1	E571K†	4.14%		Likely oncogenic
ETV6	R49C*	51.53%	50.93%	VUS
PRDM1	G771D*	46.94%	43.92%	VUS
Patient 6		PMBL	CHL	
TP53	R249K	64.64%	1.45%	Likely oncogenic
B2M	c.2T > C (p.?)	65.37%	1.41%	Likely oncogenic
GNA13	I149V*	41.57%	49.75%	VUS

*Potentially germline variants—variants observed in both the primary and the recurrence specimens with a persistent allele fraction in the germline heterozygous range (around 50%) or germline homozygous range (around 100%).

†Shared recurring variants.

VUS indicate variant of unknown significance.

substantiating the 2 disparate diagnoses rendered in each patient. Similarly, in the 2 cases with MGZL and PMBL that subsequently presented with CHL, the findings were aligned with 2 different diagnoses despite subsequent additional workup. These findings underscore the morphologic and immunophenotypic continuum that is inherent in the spectrum of diagnoses across CHL, PMBL, DLBCL, or MGZL. Thus, we sought genomic evidence to address whether these entities show a biological relationship.

Recent studies investigating the genomic landscape of mediastinal lymphomas show shared pathogenetic mechanisms.[17–29] These studies, however, individually address genomic alterations including mutational signatures in PMBL and/or CHL. In contrast, we present a unique subset of cases that have a metachronous occurrence of CHL with one of PMBL, DLBCL or MGZL, where targeted genomic sequencing was performed in each lymphoma pair at initial diagnosis and at recurrence. Such a comparative analysis of mutational signatures is likely to provide a granular view of a possible clonal relationship between the 2 lymphomas. Effectively, our results show that in 5 cases, there were multiple shared variants between the CHL and the PMBL, DLBCL or MGZL pair. The only exception was case 4, where the MGZL biopsy yielded poor DNA quality in the MGZL specimen precluded a robust comparative analysis. These findings suggest that the metachronous neoplastic processes are genetically related and may represent evolution of a common B-cell precursor. The etiology for this divergence, however, is unclear and could be due to selective pressure caused by treatment, random neoplastic evolution, or other unknown etiologies. Similarly, although the changes in Variant allele frequency between variants in the lymphoma pairs, including the apparent acquisition and loss of some variants, suggest clonal evolution, variations in neoplastic content could also account for some of these differences; in particular, CHL is known to be composed of many reactive cells and only a few neoplastic cells. In addition, across the 6 cases, several recurrently mutated genes including *TNFAIP3*, *STAT6*, *ARID1A*, and *XPO1* were identified. Three patients shared the same *XPO1* gene (E571K) variant in the MGZL or DLBCL although only one patient showed this variant also in the CHL bi-

opsy. *TNFAIP3* and *B2M* were observed more than once among variants shared between initial and recurrent lymphomas.

Neoplastic cells are known to hijack signaling pathways and transcription factor networks to promote lymphomagenesis. HRS cells of CHL are described to be dependent on the aberrant and constitutive activation of various signaling pathways including but not limited to the nuclear factor-κB and JAK/STAT pathways.[19] While these findings are present in our cohort, the mutations were seen at low allele frequency. Furthermore, enrichment of these mutations affecting similar pathways are also key to the pathogenesis of PMBL. Thus, as displayed in our cohort, there were additional genetic alterations present in the recurrences, and our analysis provides support of clonal relationships by demonstrating constitutive deregulation of shared mutational alterations that play a central role in B-cell activation and differentiation. Of note, the interactions of these genetic lesions and the immune microenvironment may be key to understanding the effect of immune evasion and adequate response to treatment.[19]

The optimal clinical management of CHL and PMBL are well established; however, for MGZL, treatment modalities vary between CHL-like and DLBCL-like combinations.[6] In our metachronous cohort, patients 1 to 4 were initially treated with regimens employed for CHL, which were changed in favor of treatments directed at large B-cell lymphomas after the second biopsies showing discordant histology. In the last 2 patients who presented with MGZL and PMBL before developing CHL at recurrence, DLBCL-like regimens were changed to CHL-like regimens. Establishing a clonal relationship between the pairs of lymphomas may lead to escalation of therapy rather than a completely separate therapeutic option. For example, if a patient with a prior CHL is then diagnosed with DLBCL and there is no clonal relationship between the lymphomas, RCHOP-based therapy is likely to be selected. If a clonal relationship is found, however, more aggressive therapy could be considered. Given the difficulty in establishing a histopathologic diagnosis as well as clinical management of patients with discordant histology at relapse, targeted sequencing to evaluate mutational signatures could be of benefit in making decisions regarding choice of therapy.

In summary, our results show that in addition to the overlap in morphology, immunophenotype, and gene expression profiles, there is a genetic relationship among the metachronous occurrence of CHL and MGZL, PMBL or DLBCL. Targeted mutational analysis by next-generation sequencing of paired samples provided evidence of a clonal relationship despite discordant histology. These findings offer genetic support for a biological continuum among these entities. Mutational profiling may be of help in optimizing clinical management in patients in frontline and recurrent/refractory disease. Given the limited sample size of our study, further investigation is warranted to fully understand the genetic events and their clinical impact in CHL patients with discordant histologies.

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