REGULAR ARTICLE

blood advances

Sinonasal DLBCL: molecular profiling identifies subtypes with distinctive prognosis and targetable genetic features

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Key Points

- ABC and GCB subtype sinonasal DLBCL are geno- and phenotypically distinct entities, exhibiting a contrasting disease course and prognosis.
- ABC subtype sinonasal DLBCL is part of a superordinate family of MCD lymphomas, presenting at immune-privileged and other extranodal sites.

Primary sinonasal diffuse large B-cell lymphoma (PSDLBCL) is a rare lymphoma with a variable prognosis and a unique relapse/dissemination pattern involving the central nervous system and skin. The underlying molecular mechanisms leading to this heterogeneity and progression pattern remain uncharted, hampering patient-tailored treatment. To investigate associated mechanisms, we analyzed clinical data and used immunohistochemistry, gene-expression profiling, cytogenetics, and next-generation sequencing in a cohort of 117 patients with PSDLBCL. The distribution in cell-of-origin (COO) was 68 (58%) activated B-cell (ABC), 44 (38%) germinal center B-cell (GCB), and 5 (4%) unclassifiable. COO was significantly associated with progression-free survival (PFS) and lymphoma-specific mortality (LSM) in both the overall cohort (5-year PFS: ABC, 43% vs GCB, 73%; LSM: ABC, 45% vs GCB, 14%) and in the subgroup of patients receiving immunochemotherapy (5-year PFS: ABC, 55% vs GCB, 85%; LSM: ABC, 28% vs GCB, 0%). ABC lymphomas were mainly MCD class, showing a high prevalence of *MYD88* (74%) and *CD79B (35%)* mutations compared with GCB lymphomas (*MYD88* 23%; *CD79B* 10%) ($P < .01$). The ABC subtype frequently displayed cMYC/BCL2 coexpression (76% vs 18% GCB; $P < .001$) and HLA-II loss (48% vs 10% GCB; $P < .001$). PD-L1 expression and copy-number alterations were rare. All lymphomas were Epstein-Barr virus-negative. Our data suggest molecular profiling as a potent tool for detecting prognostic subgroups in PSDLBCL, exposing links to known relapse/dissemination sites. The ABC subgroup's MCD genetic features, shared with lymphomas at other nonprofessional lymphoid sites, make them potential candidates for targeted B-cell and toll-like receptor signaling therapy.

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The next-generation sequencing data reported in this paper have been deposited in the Sequence Read Archive (accession number PRJNA1004445).

Other forms of data will be available upon reasonable request from the corresponding author, Patrick René Gerhard Eriksen (patrick.rene.gerhard.eriksen@regionh.dk).

The full-text version of this article contains a data supplement.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a common non-Hodgkin lymphoma known for its distinct phenotypic, genetic, and clinical heterogeneity; as a result, the World Health Organization classification recognizes multiple DLBCL subgroups based on molecular and clinical characteristics.[1-3] The introduction of gene expression profiling has allowed for the molecular subdivision of DLBCL by cell-of-origin (COO) into germinal center B cell (GCB) and activated B cell (ABC).[4,5] Although COO classification has proven useful in partly understanding the variability in clinical behavior and outcome of DLBCL, this phenotypic classifier incompletely accounts for the heterogeneous treatment response and disease outcome after treatment with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) and targeted therapies. Recent multiplatform genomic studies have revealed the existence of genetic subtypes of DLBCL that are characterized by genomic aberrations in class-specific hallmark genes.[6,7] These aberrations lead to the deregulation of distinctive biological pathways and are associated with COO, clinical behavior, and susceptibility to targeted therapy.[6-8] In the MCD/C5 classes, mutations in the toll-like receptor (TLR) adapter MYD88 and B-cell receptor (BCR)–associated protein CD79 drive constitutive NF-κB pathway activation, promoting tumor cell proliferation and survival. Interestingly, this genetic profile is strongly associated with DLBCL arising at extranodal sites, including primary DLBCL of the central nervous system (CNS), skin (leg-type), breast, testes, and vitreoretinal compartment.[9-15] All of these lymphomas bear a high risk of dissemination to other extranodal sites, particularly the CNS.[12,13,16-18]

Primary sinonasal DLBCL (PSDLBCL) is a rare extranodal lymphoma localized in the sinonasal mucosa, comprising 0.4% of all non-Hodgkin lymphomas.[19] The ectodermally-derived sinonasal mucosa is lined with respiratory epithelium and does not contain organized secondary lymphoid tissue, unlike the rest of the endodermally-derived upper respiratory tract.[19] It is associated with site-specific diseases, such as chronic rhinosinusitis and intestinal-type sinonasal adenocarcinoma[20,21] but also with hematological neoplasms, specifically extranodal natural killer (NK)/T-cell lymphoma (nasal-type), solitary extraosseous plasmacytoma, and sinonasal BCL.[22,23] In a previous comprehensive nationwide study of sinonasal BCL, we found that ~85% of all included BCLs with a primary presentation in the nasal cavity or paranasal sinuses were DLBCLs. Although most of these patients responded favorably to treatment with (immuno)chemotherapy, relapse/dissemination occurred in about a quarter. These cases preferentially affected the CNS and skin and were associated with high disease-related mortality; furthermore, an inverse relationship was observed, in which primary lymphomas originating from the CNS and skin relapsed to the sinonasal mucosa.[24] In this study, we interrogated the molecular mechanisms underlying the heterogeneous clinical course of PSDLBCL by exploring COO, oncoprotein overexpression, Epstein-Barr virus (EBV) status, immune evasion mechanisms, and the DLBCL genetic subtype.

Materials and methods

Patient selection and clinical data

All cases of PSDLBCL were from our established nationwide cohort (1980-2018).[24] We defined PSDLBCL as a single lesion in the nasal cavity or paranasal sinuses with or without regional lymph node involvement at the time of diagnosis and no prior lymphoma.

Clinical records were retrieved, and the following data were extracted: age, sex, symptoms, clinical findings, laterality, lymph node involvement, treatment modalities and response to therapy, relapse/dissemination pattern, survival, and cause of death. If data on the cause of death were unavailable from autopsy reports or medical records, the Danish Register of Causes of Death would substitute for missing data. Clinical data and diagnostic resources at the time of diagnosis determined systemic involvement and laterality at the time of diagnosis. The study was authorized by the Scientific Ethics Committee of the Capital Region of Denmark (file number H-16023080) and the Danish Data Protection Agency (file number P-2020-588). It was conducted in accordance with the Declaration of Helsinki.

Lymphoma classification

All cases were classified according to the 2017 World Health Organization classification[11]; specifications of monoclonal antibodies used for immunohistochemistry can be found in supplemental Methods. Other monoclonal antibodies used were anti-HLA-DR (TAL 1B5, DAKO), anti-HLA-ABC (W6/32, DAKO), and anti-PD-L1/CD274 (22C3, DAKO); results were independently assessed by 2 hematopathologists (E.C.-L. and S.T.P.). EBV status was established by EBV-encoded RNA (EBER) in situ hybridization (EBER ISH). *BCL2*, *BCL6*, and *MYC* rearrangements were analyzed by fluorescence in situ hybridization using break-apart probes; see supplemental Methods. Probes covering CD274 and *PDCD1LG2* (PD-L1 and PD-L2) and *CEN 9* (centromere of chromosome 9) were used to assess *PD-L1* copy number alterations (CNAs); copy number gain was defined as target-to-control probe ratios of ≥2:1, whereas >5:1 was classified as amplification. The immunohistochemical COO classification was performed using the algorithm by Hans et al.[25] For ease of reading, non-GCB (Hans algorithm) and ABC (Lymph2Cx) will be termed "ABC" in Results and Discussion. For COO subtype classification in the survival analyses, we used a "composite" of both approaches, with NanoString overriding immunohistochemistry in case of a discrepant subtype assignment.

Gene expression profiling

Microdissection of the paraffin-embedded tumor was performed using hematoxylin and eosin– and CD20-stained slides, marking the tumor area; see supplemental Methods for further details on messenger RNA (mRNA) extraction, concentration measurements, and quality control. We determined COO by mRNA expression profiling using the NanoString Lymph2Cx Panel, which includes 15 target genes and 5 housekeeping genes; see supplemental Methods for analysis methods and subtype determination using the validated lymphoma subtyping signature.[26]

Targeted next-generation sequencing (NGS)

BLYMFv2 is a custom-made, validated Ion-Torrent–based AmpliSeq panel (Thermo Fisher Scientific) consisting of 3359 amplicons covering 128 BCL-relevant genes. It is an updated version of the already published LYMFv1 and BLYMF200 panels[27,28]; see supplemental Methods for detailed information. The unaligned sequence reads were aligned to the human reference genome (GRCh37/hg19) using TMAP 5.07 software (default parameters). Variants were called using TMAP 5.07 software with a minimum of 100 reads and a 10% variant allele frequency threshold. Identified

variants were annotated into 5 pathogenicity classes using Geneticist Assistant NGS Interpretive Workbench. Pathogenicity classes 4 and 5 were interpreted as pathogenic. Class 3 variants were assessed using risk classifiers and called pathogenic if the CADD-PHRED (Combined Annotation-Dependent Depletion-Phil's Read Editor) score was >25 and/or if ≥2 of the Sift, PolyPhen, Likelihood ratio-test, and MutationTaster scores indicated pathogenicity. See supplemental Methods and supplemental Figure 1 for number of variants allocated to each pathogenicity class. Manual calling of mutations in specific hotspots *MYD88*(L265P) and *CD79B*(Y196*) was performed, even in samples affected by deamination. By adding cytogenetic information to the sequencing data, we subclassified the samples using the publicly available algorithm provided by the National Institue of Health; only samples with >90% probability of a class would be allocated, alternatively being subclassified as "other." As an alternative to LymphGen, we used the newly published simplified LymphPlex algorithm containing 38 genes based on partition around medoid clustering[29] and the "two-step" approach by Pedrosa et al, which is based on the LymphGen classifier. In the first step of the "two-step" approach, classification is determined by the presence of highly specific mutations characteristic of each class. In case of a tie in the first step, the second step of the classification relies on mutations from a wider range of subclass-associated mutations[30]; the algorithms are available at https://kylinmu.shinyapps.io/LymphPlexR/ and https://github.com/Lymphoma-IDIPHISA/Two-step-classifier.

Statistics

Overall survival (OS), progression-free survival (PFS), and lymphoma-specific mortality (LSM) were estimated using the Kaplan-Meier and Aalen-Johansen estimators. OS was defined as time to death from any cause, whereas PFS was time to either relapse, progression, or death from any cause. Death from causes other than lymphoma was considered a competing risk. We used the log-rank test and Gray test to determine significant differences between curves. Categorical variables were tested for significance using Fisher exact test. All statistical analyses were performed using R ("survival," "ComplexHeatmap," and "cmprsk" packages, version 4.0.5, R core team). The significance level was set at 5%.

Results

Patient characteristics

Our study cohort comprised 117 patients with PSDLBCL.[24] The majority of lesions originated from the nasal cavity (n = 43 [37%]) and maxillary sinus (n = 37 [32%]), whereas only a few involved other sinuses, which included ethmoid (n = 9 [8%]), sphenoid (n = 3 [3%]), frontal sinuses (n = 3 [3%]), or unknown sinus (n = 7 [6%]). Fifteen patients (11%) had multiple sinonasal lesions, and 15 (11%) showed involvement of regional cervical lymph nodes at the time of diagnosis; when stratifying the cohort by Ann Arbor stage, no differences in clinical characteristics were detected (see supplemental Cohort; supplemental Table 1). For staging purposes, all patients apart from 1 had computed tomography or magnetic resonance imaging of the head and neck.

Impact of COO in PSDLBCL - COO subtype predicts dissemination, relapse, and survival

To assess the COO subtype and its prognostic value in our PSDLBCL cohort, we used Lymph2Cx (mRNA profiling) and Hans algorithm (immunohistochemistry-based profiling). mRNA analysis was feasible in 77 of 117 patients (66%), with 56% ABC, 36% GCB, and 8% "unclassifiable." Classification by Hans algorithm was possible in 112 of 117 patients (96%); 59% were classified as ABC and 41% as GCB. There was a high concordance (92%) between Lymph2Cx- and Hans-defined COO class, with only a few discordant cases. In our analyses of the impact of COO, mRNA profiling was prioritized over immunohistochemistry in cases with a discrepant subtype assignment. The combined classifiers identified 68 of 112 of lymphomas (61%) as ABC and 44 of 112 (39%) as GCB (Figure 1).

Of all patients with sufficient clinical data to evaluate disease relapse or progression, 33 of 104 (32%) relapsed. Relapses were significantly more common in the ABC (29/63 [46%]) vs GCB subtype (4/29 [14%]) lymphomas ($P < .01$; Figure 1). Survival analysis including all patients classified by COO, irrespective of the treatment regimen, revealed that the ABC subtype was a strong predictor of poor disease outcome; ABC cases showed a significantly decreased PFS and OS, as well as increased LSM. The 5-year PFS, OS, and LSM for ABC vs GCB subtypes were 43% vs 73% (log-rank test, $P < .01$), 47% vs 71% (log-rank test, $P = .01$), and 45% vs 14% (Gray test, $P < .001$), respectively. We found a similar impact of COO on survival in the subgroup of 52 patients treated with contemporary R-CHOP or R-CHOP–like immunochemotherapy. The 5-year PFS and OS for patients with ABC and GCB subtypes were 55% vs 85% (log-rank test, $P = .02$) and 59% vs 89% (log-rank test, $P = .05$), respectively, whereas the LSM was 28% vs 0% (Gray test, $P < .01$; Figure 2). To exclude the idea of treatment variation influencing the observed prognostic differences, we compared the treatment regimens between COO subtypes for the entire cohort (Table 1) and within the ABC subgroup for cases with or without relapse or progression (supplemental Cohort; supplemental Table 2). No significant differences were identified. Further insight into specific chemotherapy regimens and year of diagnosis by COO can be found in supplemental Cohort and supplemental Tables 3 and 4.

Molecular characterization of PSDLBCL: low frequency of BCL2, BCL6, and cMYC translocations and absence of EBV but high prevalence of BCL2/cMYC double expression and BCL6 overexpression in the ABC subtype

To explore the molecular basis of PSDLBCL and its position in the complex DLBCL landscape, we investigated several oncogenic drivers with an established role in the pathogenesis of specific DLBCL subtypes (Figure 1).

First, we assessed the presence of translocations of the oncogenes *BCL2*, *BCL6*, and *cMYC*, important in the subclassification of DLBCL into genetic subtypes[6] and, in the case of *BCL2*, potentially indicating transformed follicular lymphoma.[31] Using fluorescence in situ hybridization, we detected these translocations in only a minor percentage of the PSDLBCLs: *BCL2*, 4% (4/110); *BCL6*, 8% (9/110); and c*MYC,* 9% (10/112). Their presence was not significantly correlated to the COO (Figure 1).

Double expression of the oncoproteins BCL2 and cMYC, termed "double expression," is associated with an unfavorable prognosis and secondary involvement of the CNS.[32] Interestingly, double expression and concurrent BCL6 overexpression is frequently observed in extranodal lymphomas, including primary CNS DLBCL (~80%) and

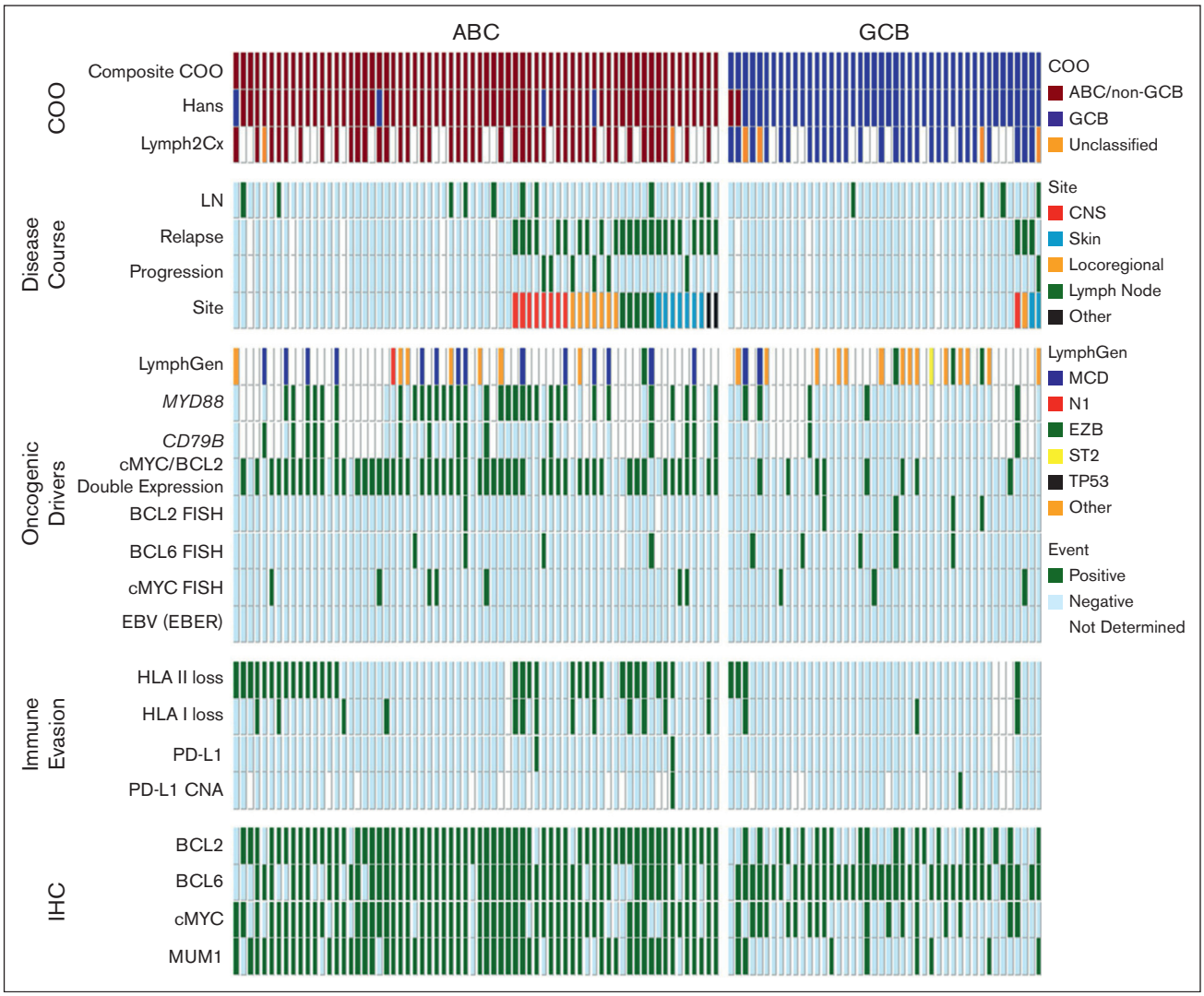

Figure 1. Diagram of 112 cases of PSDLBCL depicting COO, relapse/dissemination pattern, immunohistochemistry (IHC), cytogenetics, and mutations/ LymphGen. COO was determined hierarchically, with Lymph2Cx overriding Hans algorithm in cases of discordance. Cases with undetermined COO were excluded. LN, regional lymph node involvement.

primary cutaneous DLBCL (leg type) (~70%).[11-13,24,33-35] Our analysis revealed a high incidence of double expression in the ABC subtype: 77% (52/68) vs 18% (8/44) in the GCB subtype ($P < .001$) and high rates of BCL6 overexpression in both subtypes, 77% in ABC subtype vs 88% in GCB subtype (Figure 1).

The association of EBV with sinonasal DLBCL has never previously been investigated. To address this, we conducted EBER ISH analyses. Our findings revealed no evidence of EBV involvement.

NGS reveals marked differences in the mutational landscape of PSDLBCLs, with the ABC subtype exhibiting a high prevalence of MCD genetic features

Mutations affecting the *MYD88* and *CD79B* genes are important drivers of lymphomas presenting at immune-privileged sites, such as the CNS and testes, as well as at other extranodal sites, such as the skin (leg-type) and intravascular space.[11,12,36] These mutations activate the NF-κB pathway, promoting cell proliferation and survival.[6] Using NGS, we manually called the canonical hot spot mutations *MYD88* (L265P) and *CD79B* (Y196*). Among the 74 samples analyzed (43 ABC and 31 GCB), *MYD88* (L265P) mutations were more prevalent in ABC subtype lymphomas (74%) than GCB (24%; $P < .001$). Similarly, *CD79B* (Y196*) hot spot mutations were significantly associated with the ABC subtype (37% ABC vs 6% in the GCB subtype; $P = .03$). All cases, apart from 1 with a *CD79B* (Y196*) mutation, had a concurrent *MYD88* (L265P) mutation. Notably, 7 of 9 patients (78%) with secondary CNS involvement harbored an *MYD88* (L265P) mutation (Figure 1).

To further uncover the mutational landscape of PSDLBCL, we used a 128 lymphoma-related gene panel with stringent quality criteria (supplemental Cohort). We successfully sequenced 43 cases, 23 ABC and 20 GCB subtypes. The most prevalent mutations in the ABC subtype were *MYD88* (70%), *PIM1* (61%), and *CD79B* (39%). Other mutations of interest involved the tumor suppressor genes

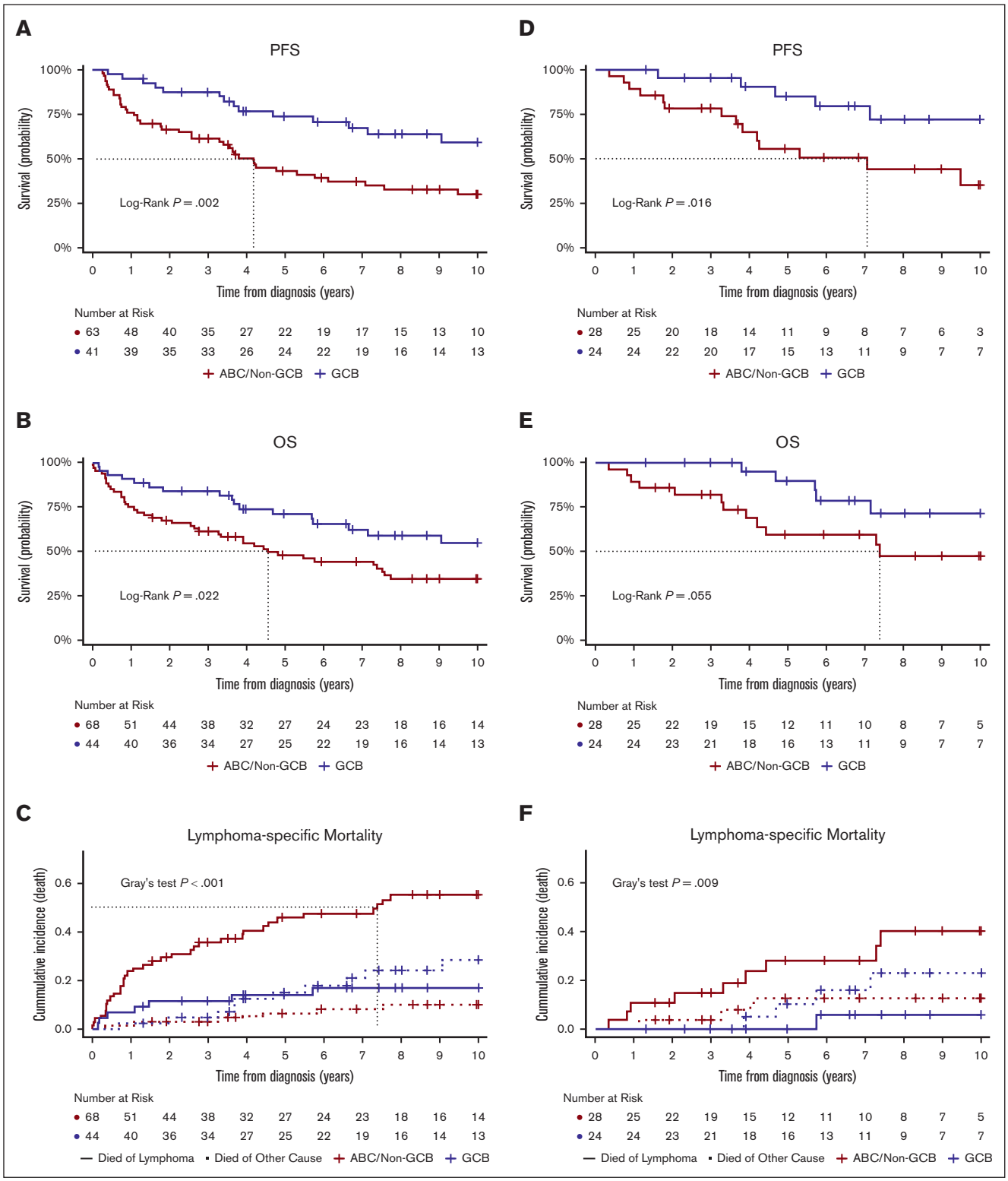

Figure 2. Survival curves for patients with PSDBLCL: an analysis of the total cohort and the subset of patients who received immunotherapy. Survival curves for patients with PSDLBCL stratified by COO; the left side represents the entire cohort (A-C) (OS, n = 112; PFS, n = 104), whereas the right side represents the subgroup treated with R-CHOP or R-CHOP–like regimens (D-F) (n = 52). The dotted vertical line represents median survival.

Table 1. Clinical characteristics, treatment, and disease outcome of patients with PSDLBCL stratified by COO

	ABC/non-GCB (n = 68)	GCB (n = 44)	*P*	Total (N = 112)
Age				
Mean (SD)	73.7 (11.1)	70.8 (12.4)	.199	72.6 (11.7)
Median (min, max)	76.0 (45.0, 100)	71.5 (36.0, 91.0)		75.0 (36.0, 100)
Sex				
Female	27 (39.7%)	19 (43.2%)	.844	46 (41.1%)
Male	41 (60.3%)	25 (56.8%)		66 (58.9%)
Regional lymph node involvement				
Yes	10 (14.7%)	4 (9.1%)	.56	14 (12.5%)
No	58 (85.3%)	40 (90.9%)		98 (87.5%)
Performance status				
≥1	61 (89.7%)	41 (93.2%)	.737	102 (91.1%)
>1	7 (10.3%)	3 (6.8%)		10 (8.9%)
Chemotherapy				
Yes	54 (79.4%)	36 (81.8%)	1	90 (80.4%)
No	10 (14.7%)	6 (13.6%)		16 (14.3%)
Rituximab				
Yes	29 (42.6%)	24 (54.5%)	.248	53 (47.3%)
No	39 (57.4%)	20 (45.5%)		59 (52.7%)
CNS prophylaxis				
Yes	20 (29.4%)	18 (40.9%)	.226	38 (33.9%)
No	48 (70.6%)	26 (59.1%)		74 (66.1%)
Consolidative radiotherapy				
Yes	42 (61.8%)	26 (59.1%)	.844	68 (60.7%)
No	26 (38.2%)	18 (40.9%)		44 (39.3%)
Status				
Alive without disease	19 (27.9%)	24 (54.5%)	<.001	43 (38.4%)
Dead of disease	36 (52.9%)	7 (15.9%)		43 (38.4%)
Dead from other cause	13 (19.1%)	13 (29.5%)		26 (23.2%)

Demographical and clinical characteristics of patients with PSDLBCL and sufficient material for COO classification. Chemotherapy consisted of CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone) or CHOP-like regimes. CNS-prophylaxis mainly consisted of high-dose methotrexate either given intrathecally or systemically.
SD, standard deviation.

BTG2 and *BTG1*, with 35% and 30% of the ABC subtype harboring a mutation. Interestingly, *BTG2* mutations were primarily present in cases that relapsed or progressed: 6 of 8 cases of relapse or progression harbored a *BTG2* mutation (Figure 3). To contextualize the genetic composition of PSDLBCL, we explored the mutational rate of some of the most prevalent mutations and compared these with 4 major DLBCL cohorts.[7,8,31,37] This showed that the occurrence of mutations in the *MYD88*, *BTG1*, and *TBL1XR1* genes was significantly higher than that of the rates in ABC DLBCL in general; see Figure 3B and supplemental Cohort and Supplemental Figure 2.

To further classify PSDLBCL, we used the probabilistic LymphGen classifier, which groups lymphomas into genetic superfamilies based on their mutational and cytogenetic profiles. In the ABC subtype, 14 of 23 cases (60%) were classified as MCD. Among the analyzed GCB-subtype PSDLBCLs (n = 20), 15 were classified as "other," whereas a few were categorized as EZB, ST2, or MCD. MCD class samples in the GCB subgroup were "unclassifiable" by the Lymph2Cx classifier, implying more uncertainty about their definitive COO. The classification of lymphomas by the LymphPlex algorithm was consistent with the LymphGen model. However, there were minor discrepancies, because 2 further ABC subtype samples, otherwise classified as "other" by the LymphGen model, were allocated to the MCD group. The simpler "two-step" approach classified 16 of 23 with ABC-PSDLBCL (70%) as MCD, 3 of 23 (13%) as N1, and 1 of 23 (4%) as EZB. The "two-step" algorithm had difficulty classifying the mutational profile of GCB subtype PSDLBCL, similarly to the other more complex classifiers we applied. However, it classified slightly more cases as ST2 class. Furthermore, this approach classified 2 additional cases of GCB subtype PSDLBCL as MCD, solely based on the presence of *PIM1* mutations without *MYD88* or *CD79B* aberrations present (Figure 3). To provide a frame of reference, we examined the rate of MCD, categorized by the LymphGen classifier, in 173 nodal ABC DLBCLs in the publicly available data from the Phoenix phase 3 clinical trial by Wilson et al.[38] This revealed that 29 of 173 cases (16%) of nodal ABC DLBCL were classified as MCD, in contrast to the 14 of 23 cases (60%) seen in ABC subtype PSDLBCL ($P < .001$).

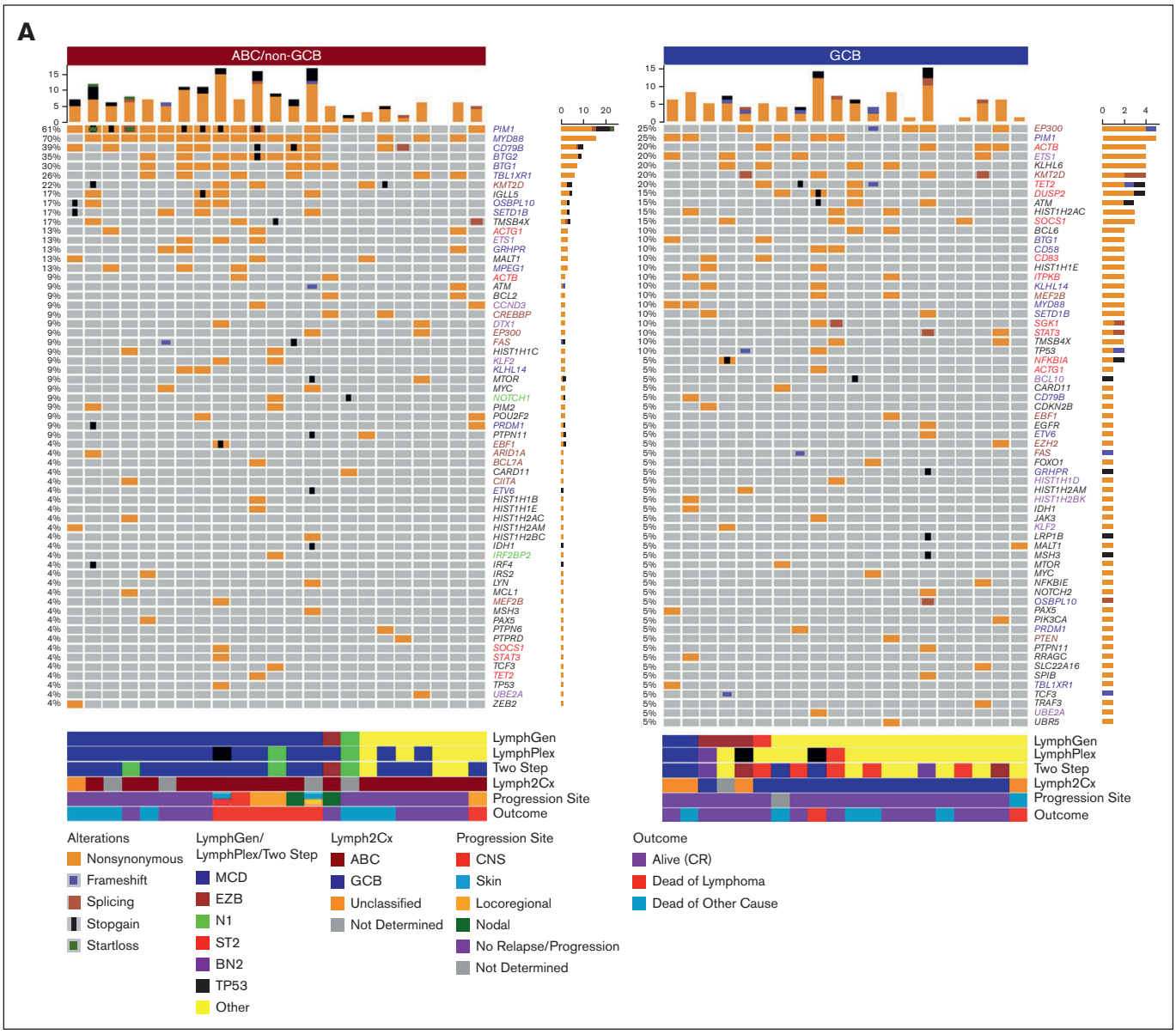

Figure 3. Comparative analysis of the mutational burden in ABC/GCB PSDLBCL and a look at ABC PSDLBCL's place in the mutational landscape of ABC DLBCL. (A) An oncoprint plot of PSDLBCL eligible for sequencing (n = 43; 23 ABC and 20 GCB). Each row represents a specific gene and alteration, and each column represents a case. The color of the gene name corresponds to the LymphGen class with which the gene is most associated. The category "TP53" is only applicable to the LymphPlex classification. Only truncation mutations to the proline (P), glutamate (E), serine (S), and threonine (T) or PEST domain of the NOTCH1 gene are considered to raise the probability of a sample belonging to the N1 class when using the LymphGen classifier. The top bar chart illustrates the number of mutation types per patient, whereas the bar chart on the right shows the number of alterations per gene. COO is a hierarchical composite with Lymph2Cx overriding Hans algorithm. The outcome, progression site, and LymphGen, LymphPlex, and two-step classifications are annotated at the bottom. (B) A comparison of mutations with a significantly higher rate in Lymph2Cx-varified ABC subtype PSDLCL as compared with ABC DLBCL from 4 extensive international cohorts.[7,8,31,37] (C) Pie charts showing the distribution of LymphGen class in ABC subtype PSDLBCL (n = 23) relative to purely nodal ABC DLBCL (n = 173).[38] *$P < .05$.

Immune evasion in PSDLBCL: frequent loss of HLA in the ABC subtype, whereas loss of PD-L1 expression is rare

Immune evasion is a major hurdle for effective anticancer therapy. Tumor cells use several strategies to evade T-cell and NK cell recognition, including major histocompatibility complex downregulation and activation of immune checkpoints,[39] in particular, programmed cell death 1 (PD-1) and its ligands PD-L1 and PD-L2.[12,40,41] Both strategies are used by hematological malignancies, including BCLs.[12,40,41] Assessment of HLA expression revealed that HLA-I loss was present in only a minority of the PSDLBCLs, and although there was a higher prevalence in ABC than in GCB subtype lymphomas, this difference was not

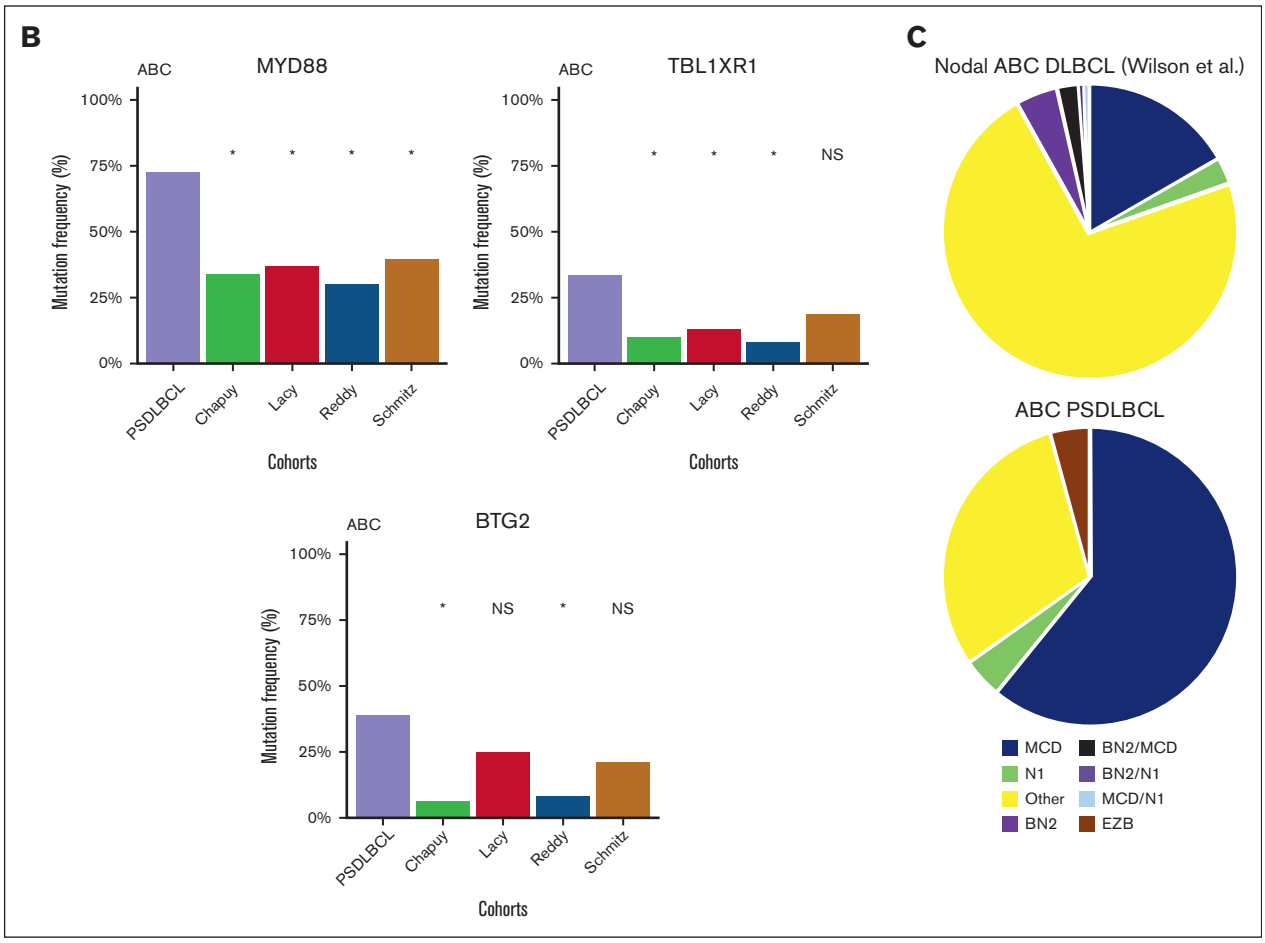

Figure 3 (continued)

statistically significant; 13 of 67 (19%) of ABC subtype vs 3 of 41 (7%) of GCB subtype ($P = .10$). Noticeably, when comparing cases with and without relapse or progression among ABC subtype lymphomas, those with relapse or progression had a 31% HLA-I loss rate (9/29), whereas those without had 9% (4/33; $P = .05$). Moreover, HLA-II loss was highly prevalent in ABC subtype PSDLBCL (32/67 [48%]) but much less common in GCB subtype tumors (4/41 [9%]; $P < .001$; Figure 1).

In addition, we also explored the possible role of the PD-1/PD-L1 checkpoint by analyzing PD-L1 protein expression and *PD-L1/2* CNAs. Both PD-L1 expression and CNAs were rare, only evident in 2% of the PSDLBCLs (Figure 1).

Discussion

Our large nationwide PSDLBCL cohort allowed for us to comprehensively investigate the molecular mechanisms underlying the highly variable clinical outcome of the disease.[24] We demonstrated that PSDLBCL can be stratified into 2 COO-defined subtypes with distinctive biological and clinical behavior: GCB subtype with an excellent prognosis and ABC subtype prone to relapse or progression, resulting in substantial disease-specific mortality despite treatment with immunochemotherapy. Most of the ABC subtype PSDLBCL displayed MCD-class molecular features, specifically a high prevalence of *MYD88* and *CD79B* mutations, frequent cMYC/BCL2 double expression, BCL6 overexpression, as well as loss of HLA-I and -II. Interestingly, these molecular features are shared with lymphomas at other nonprofessional lymphoid sites, including CNS, testes, and skin (leg-type), all of which are typical sites of relapse or dissemination for PSDLBCL. In addition, these locations are often the primary sites for lymphomas that relapse to the sinonasal mucosa.[18]

COO subclass strongly correlates to specific extranodal locations.[12,27,36] Our study is the first to demonstrate the prognostic value of COO subclassification for PSDLBCL. Consistent with our results, a previous study also found sinonasal tract DLBCL to comprise ABC as well as GCB subtype tumors, with ABC as the predominant COO; however, this study did not report survival data.[42] Our data show that the ABC subtype represents a distinct disease entity different from GCB subtype PSDLBCL, exhibiting a much higher mortality rate both in the overall cohort as well as the subset of patients receiving immunochemotherapy. Moreover, the ABC subtype encompassed nearly all cases of relapse or progression (Figure 1). Our findings align with the substantial evidence showing that the prognosis of ABC subtype DLBCL treated with standard immunochemotherapy is unfavorable compared with that of GCB subtype tumors.[4,5,43-46] Furthermore, relapse or dissemination was mainly to sites strongly associated with the ABC gene

expression profile, for example, CNS and skin (leg-type).[12,47] Additionally, our study showcased the remarkable interchangeability between Hans algorithm and Lymph2Xc for the accurate COO classification of these extranodal lymphomas.

Our mutational analysis revealed that most ABC subtype lymphomas display MCD-class genetic features: 74% contained *MYD88* and 34% *CD79B* mutations (Figure 1). Similar percentages were obtained in the subset of comprehensively sequenced patients; 70% contained *MYD88* and 39% *CD79B* mutations (Figure 3). These gain-of-function mutations enhance TLR and BCR pathway activity leading to a multiprotein supercomplex formed by MYD88, TLR9, and the BCR. The My-T-BCR supercomplex and mTOR come together in endolysosomes, in which they actively promote both prosurvival NF-κB and mTOR signaling.[48] In addition, ABC lymphomas showed enrichment for mutations in *PIM1* and 2 cell cycle–regulatory genes, *BTG1* (30%) and *BTG2* (35%).[45] *BTG2* mutation is strongly linked to the MCD class and has previously been reported to carry a poor prognosis in primary testicular lymphoma.[6,49] In line with this, we also found a high percentage of relapse or progression cases harboring this mutation. Furthermore, by placing PSDLBCL within the broader framework of DLBCL, we demonstrated that ABC PSDLBCL exhibits a distinct mutational profile compared with the ABC subtype of DLBCL as a whole. Additionally, an analysis of nodal ABC DLBCLs emphasized that ABC subtype PSDLBCL is a distinct entity with its own mutational profile. Particularly noteworthy is the elevated rate of TBL1XR1 aberrations in PSDLBCL, supporting the hypothesis of an aberrant memory B cell as a probable COO for the MCD subclass DLBCLs, as proposed by Venturutti and Melnick.[50]

Subclassification by the LymphPlex algorithm and the two-step approach replicated the high prevalence of MCD class tumors identified by the LymphGen algorithm. In fact, these alternative approaches labeled a few additional cases as MCD (Figure 3), reflecting the significance of these mutations in the alternative models. Due to the stochastic distribution of mutations in GCB subtype PSDLBCLs, subclassification was challenging (Figure 3).

The MCD subtype provides an intriguing genetic link between PSDLBCL and other primary extranodal lymphomas, including CNS, skin (leg-type), testes, and breast, especially in conjunction with our previous findings on the site-specific relapse or progression of sinonasal DLBCL and sites relapsing to the sinonasal membrane.[24] In addition to the genetic subtype, the ABC subtype exhibited several molecular characteristics shared with the most associated sites of relapse and dissemination, including high levels of BCL2/cMYC double expression and BCL6 overexpression. Our findings suggest a superordinate family of lymphomas with shared phenotypic and genotypic attributes and a predilection for specific anatomical locations. Indeed, when compared with PSDLBCL, the MCD frequency in a publicly available purely nodal ABC data set was much lower (Figure 3C). Our findings hold significant value for future patients with PSDLBCL because substantial strides have been made in the targeted treatment of DLBCL based on genetic subtypes. Recent studies have reported a high response rate in young patients (age <60 years) with MCD DLBCLs treated with the Bruton tyrosine kinase inhibitor ibrutinib.[38]

Upon tumor expansion, cells become targets of immune surveillance by T cells, necessitating the activation of immune evasion strategies to survive. Consistent with this scenario, the majority of primary CNS and testicular lymphomas display loss of HLA-I expression, impairing antigen presentation to cytotoxic T cells but also subjecting themselves to surveillance of "missing self" by NK cells.[51] However, NK cells are somewhat restricted from entering these locations under normal physiological conditions, making tumors less exposed to NK cell surveillance.[52] HLA-I loss was relatively uncommon in ABC subtype PSDLBCL (19%) and even more so in GCB tumors (7%). This comparatively low frequency of HLA-I loss may result from selective pressure by NK cells, which seem to play an important role in nasal immunity.[53] Nevertheless, our data suggest that immune surveillance by cytotoxic T cells plays a significant role in the control of PSDLBCL, because ABC subtype cases with HLA-I loss showed a significantly increased risk of relapse or progression.

HLA-II was lost in nearly half (48%) of the ABC subtype, whereas it was less frequent (9%) in GCB subtype tumors. Several studies have found approximately half of all primary CNS and testicular lymphomas to have a loss of HLA-II,[12,54] yet again indicating a similarity between ABC subtype PSDLBCL and lymphomas originating from these locations. Compared with HLA-I loss, the consequences of HLA-II loss for immune recognition are less clear-cut. However, because B cells are professional antigen-presenting cells, impaired antigen presentation to helper and regulatory T cells most likely plays a central role in T-cell infiltration of tumors and may explain some of the variance in prognosis between COOs.[55,56]

In addition to HLA loss, we addressed the possible activation of the PD-1/PD-L1 immune checkpoint, which suppresses T-cell activity in the tumor microenvironment of various hematological malignancies such as Hodgkin lymphoma and primary mediastinal BCL.[41] Although an initial study reported a high prevalence of PD-L1 expression and *PDL1/2* CNAs in primary CNS and testicular lymphoma,[57] subsequent studies did not confirm this result; conversely finding PD-L1 expression and CNAs to be uncommon.[12,58-60] Furthermore, a phase 2 clinical trial from 2021 investigating patients with relapsed or refractory primary CNS lymphoma treated with nivolumab yielded disappointing results (unpublished data; registered at www.clinicaltrials.gov as #NCT02857426). In our PSDLBCL cohort, PD-L1 expression and *PD-L1/2* CNAs were rare (2%), implying that activation of this checkpoint is unlikely. These findings corroborate with literature data on the PD-1/PD-L1 checkpoint activation in MCD lymphomas at other anatomical sites and show that immune evasion in MCD lymphomas, including ABC subtype PSDLBCL, involves HLA loss rather than activation of the PD-1/PD-L1 checkpoint.

DLBCLs related to chronic inflammation are often EBV associated, and chronic inflammation of the sinonasal membrane is very common in the general public.[3,21] Moreover, EBV is a hallmark of extranodal NK/T-cell lymphoma (nasal type), prototypically localized in the sinonasal mucosa.[3,22] This made it relevant to test the association of EBV in PSDLBCL. EBER ISH assessment revealed no evidence for EBV involvement in PSDLBCL, excluding EBV as a driver in PSDLBCL.

In conclusion, molecular profiling of this large nationwide cohort of PSDLBCLs allowed for us to identify 2 separate COO-defined subtypes with distinctive tumor biology and prognosis among these rare extranodal lymphomas. Interestingly, the predominance of

the MCD class in the ABC group designates these PSDLBCLs as members of an expanded family of lymphomas, characterized by presenting at "nonprofessional" lymphoid sites and sharing genetic characteristics, immune-evasion strategies, oncoprotein expression, lack of translocations, and clinical features, including a typical progression pattern. The fact that PSDLBCLs have MCD features has potentially important clinical implications because it labels these lymphomas as candidates for therapies targeting BCR and TLR signaling.

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Authorship

Contribution: P.R.G.E. established the cohort and extracted data, conducted COO analyses and statistical calculations, created diagrams and figures, and wrote the manuscript. F.d.G. performed NGS and analyzed data; E.C.-L. validated diagnoses and analyzed markers of immune evasion; P.d.N.B. designed the research and provided data; R.d.G. conducted NGS and genetic subclassification; LC.M. and A.D.M. performed analyses; M.M. performed experiments; J.S.P.V. provided supervision of analyses and wrote the manuscript; C.v.B. designed the study and supervised; S.T.P. designed the research and analysis, supervised, conducted data analysis, interpretation of analyses, and wrote the manuscript; and S.H. designed the study and supervised.

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