Systemic diffuse large B-cell lymphoma involving the central nervous system has high rates of defective antigen presentation and immune surveillance

Secondary central nervous system (CNS) involvement with diffuse large B-cell lymphoma (SCNSL), occurring either synchronously at diagnosis of systemic disease or at relapse, confers a dismal prognosis, and mechanisms of CNS dissemination are poorly understood.[1,2] We hypothesized that loss of immune surveillance mechanisms, similar to primary CNS lymphoma (PCNSL), may be frequent in SCNSL and represent a plausible mechanism for the establishment of CNS disease. We demonstrated that loss of antigen presentation mechanisms such as *B2M*, *CD58*, *CIITA* or *MHC* occurred in a similar high frequency between Epstein-Barr virus (EBV)-negative SCNSL and PCNSL (63% *vs*. 62%, respectively), which corresponded to lower antigen presentation scores than systemic-only diffuse large B-cell lymphoma (DLBCL).

The CNS is an immune-privileged site and PCNSL have distinct biological profiles: *MYD88*L265P and *CD79B* mutations, activated B-cell (ABC) phenotype, loss of *CDKN2A* and MHC involved in antigen presentation.[3,4] Such losses lead to the hypothesis that immune surveillance plays a critical role in CNS homeostasis, and that loss of such mechanisms may contribute to the establishment of CNS lymphoma. It is unclear whether this is true of secondary CNS dissemination of DLBCL.[5]

Pathobiological features of systemic DLBCL associated with CNS dissemination include ABC-DLBCL, MYC/BCL2 overexpression, and *MYD88*L265P/*CD79B* mutations, although the underlying reasons for this remain unclear.[6,7] Clinical scores that predict likelihood of SCNSL in DLBCL lack precision and omit biological characteristics.[5] Understanding SCNSL molecular mechanisms could lead to novel therapeutic combinations.

We aimed to comprehensively characterize immunological and molecular profiles of SCNSL to determine whether immune evasion is a plausible mechanism of this devastating complication.

Adult patients diagnosed in the rituximab era with confirmed systemic plus CNS DLBCL (i.e., SCNSL either synchronously at diagnosis or with confirmed CNS relapse) with adequate formalin-fixed paraffin embedded tissue were included in the study. Histological subtypes are included in Table 1. Clinical data collection has been previously described.[1] Systemic or CNS biopsies from diagnosis or relapse were accepted. Three Australian sites contributed tissue and data, identified through pharmacy records for patients receiving CNS-directed chemotherapy. The study was approved by the relative ethics committees (HREC/14/Austin425) and conducted in accordance with the principles of the Declaration of Helsinki. No formal patient consent was required.

Targeted massively parallel sequencing (MPS), copy number variant (CNV) analysis and gene expression profiling for cell of origin (COO) (Lymph2Cx) and immune gene signatures (IGS) via the nanoString Pan Cancer Immune panel were performed on the SCNSL cohort. MPS, COO and IGS results were compared to our published EBV-negative / HIV-negative PCNSL cohort,[8] and COO and IGS to 35 internally tested advanced stage, systemic-only HIV-negative DLBCL with a revised International Prognostic Index of 3-5 and no relapse with at least two years of follow up ('systemic-only' cohort).[9]

We identified 53 SCNSL patients, 41 of whom had DNA (N=37), RNA (N=36) or both (N=32) successfully extracted; biopsy details are included in Table 1. Figure 1 demonstrates SCNSL sequence variants for SCNSL. Thirty-five out of 37 had a driver mutation or CNV of interest; the remaining 2 were EBV-positive. All cases were HIV-negative.[8]

CD58, the ligand of CD2, demonstrated loss of function aberrations in 10/37 cases (27%), either through loss-of-function mutation (N=5) or copy number loss at the *CD58* locus on chromosome 1p13 (N=5). *B2M* was either mutated (N=5, 14%), deleted (N=2, 5%), or both (N=1, 3%) in 8 (22%) cases. Co-mutation with *CD58* was present in 2 cases.

Focal loss of the major histocompatibility complex (MHC) via focal deletion of chromosome 6p was present in 12 cases (32%) and loss or truncating mutation of the transcriptional regulator *CIITA* was found in a further 2 cases (5%). Twenty-two out of 35 (63%) EBV-negative SCNSL demonstrated loss of function in either *B2M*, *CD58*, *CIITA* or *MHC versus* 62% EBV-negative PCNSL (P=0.96).[8] Loss of antigen presentation was seen in 81% of relapsed SCNSL *versus* 43% synchronous SCNSL at diagnosis (P=0.04). Only 2 cases (5%) had 9p24 gains at the *CD274* / *PDCD1LG2* (PD-L1/PD-L2) loci (Table 1).

MYD88 L265P and/or *CD79B* mutations occurred in 14 (40%); *MYD88* in 11 (31%, L265P in 9), *CD79B* mutations in 8 (23%), and both in 5 cases. This contrasted with 74% of PCNSL harboring *MYD88* and/or *CD79B* mutations (SCNSL *vs*. PCNSL, P=0.005).

Other frequent B-cell receptor (BCR)-dependent NF-κB signaling pathway mutations included *CARD11* (23%), *TBL1XR1* (11%), *NFKBIE* (9%). Nine cases had CN loss of *TNFAIP3*, all of whom had co-operating gain-of-function mutations in BCR-dependent pathways. Mutation / deletion in ≥1 BCR

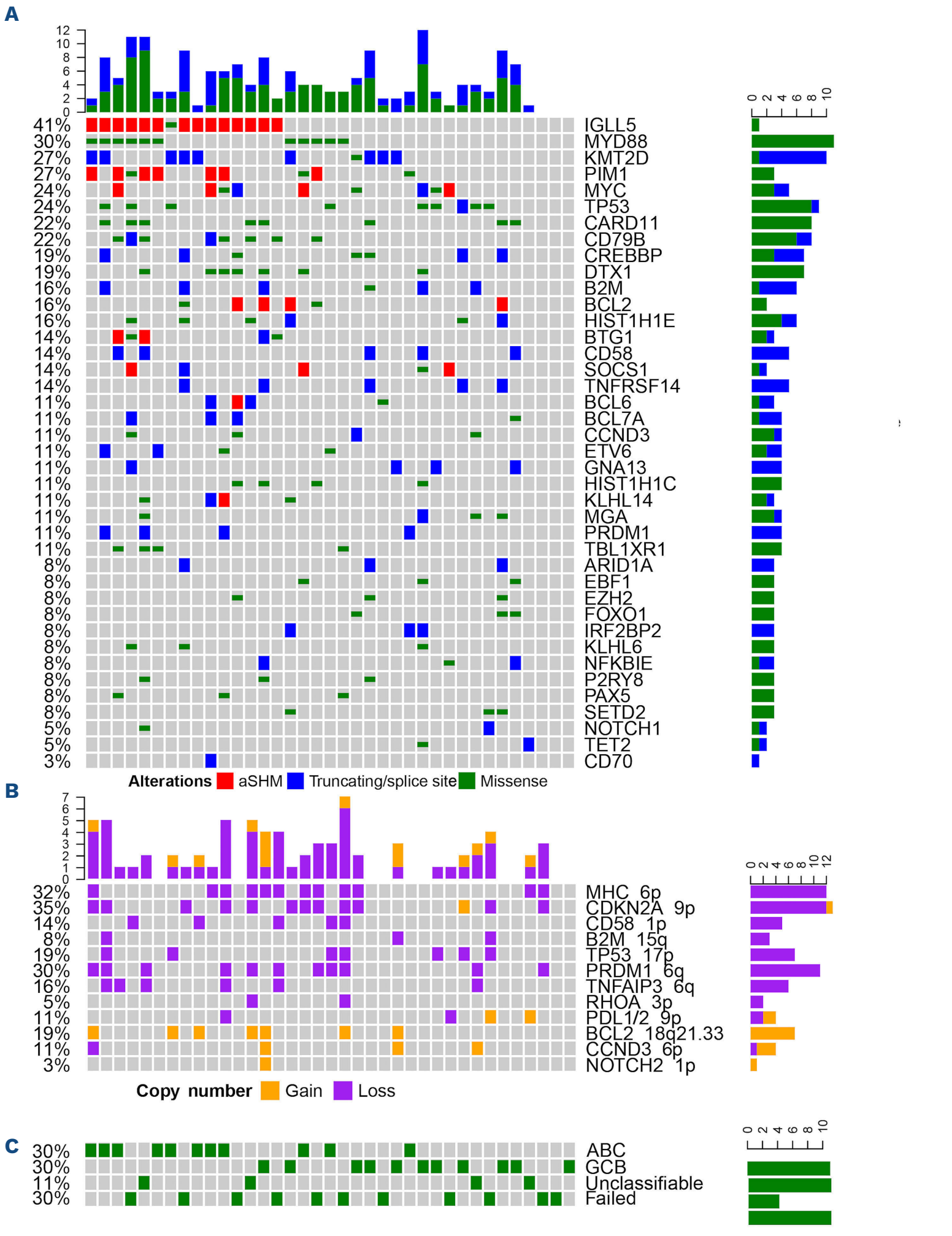

Figure 1. Genomic profile of secondary central nervous system lymphoma. (A) Pathological mutations (sequencing panel). (B) Copy number alterations. (C) Cell of origin (Lymph2Cx). Each column represents a single case sorted by mutational frequency in (A) with the same individual case directly underneath in (B) and (C). Types of mutation and copy number variant (CNV) are color-coded as shown. The 2 cases furthest right with no mutation or CNV of interest were Epstein-Barr virus-driven and were excluded from the analysis. aSHM: aberrant somatic hypermutation; ABC: activated B cell; GCB: germinal center B cell.

pathway genes was present in 23 cases (65%).

Aberrations of *TP53*, either loss-of-function mutation, deletion, or both, were present in 29%, evenly distributed between diagnosis and relapse (*P*=0.71). Loss of *CDKN2A* was also common (35%).

MYC rearrangements were assessed by fluorescent *in situ* hybridization (N=18) or by structural variant analysis from next-generation sequencing data (N=22).[10] One case had neither available. Thus, *MYC* rearrangement was present in 8/40 cases (20%), and high-grade B-cell lymphoma with rearrangements of *MYC* and *BCL2* was identified in 4 cases.

Cell of origin was successfully assigned in 29/36 SCNSL, 29 systemic-only DLBCL and 32 PCNSL. Forty-eight percent of SCNSL were GCB (N=14), 34% ABC (N=10), and 17% unclassified (N=5) (Table 1). Systemic only DLBCL COO was as follows: GCB 52%, ABC 31%, unclassified 17%, whereas PCNSL comprised 69% ABC, 19% GCB, and 12% unclassifiable (*P*=0.002 for PCNSL *vs*. SCNSL).

Tumor microenvironment digital gene expression was available in 35 SCNSL, 19 PCNSL, and 35 systemic-only DLBCL. Expression of individual immune effectors and checkpoints differed between SCNSL, PCNSL and systemic-only cohorts (Figure 2). PCNSL and SCNSL had lower normalized counts of CD4 and PD-L2 than systemic-only DLBCL. HLA-II gene expression was more severely compromised in PCNSL compared to SCNSL, though HLA-I expression

Table 1. Characteristics of patients with secondary central nervous system lymphoma.

Variable	N (%)
Median age in years (range)	58 (24-86)
Male gender	26 (63)
WHO 2016 diagnosis	
DLBCL-NOS	35 (85)
HGBL-DH (*MYC/BCL2*)	4 (10)
Other*	2 (5)
Synchronous CNS + systemic at diagnosis	23 (56)
Biopsy timepoint	
Diagnosis	36 (88)
Relapse	5 (12)
CNS relapse	18 (44)
Site of diagnostic biopsy	
CNS	7 (17)
Systemic	36 (83)
Non-CNS extranodal disease at diagnosis	35 (85)
Extranodal site of CNS risk (testes, uterus, breast, bone marrow, kidneys, adrenals)	25 (61)
Site of CNS involvement	
Parenchymal	26 (63)
Leptomeningeal	7 (17)
Both	8 (20)
CNS IPI ≥4 at diagnosis	23 (56)
COO, %	
GCB	48
ABC	34
Unclassified	17

Genomic factors	SCNSL , %	PCNSL, %	*P* (SCNSL *vs*. PCNSL)
MYD88/CD79B mutation	40	74	0.005**
ABC COO	34	69	0.002**
CD58 loss	27	15	0.17
CIITA loss	5	16	0.16
B2M loss	22	16	0.77
CN loss 6p	32	40	0.49
Any antigen presentation aberration	60	62	0.96

*Cases classified as "Other" were diagnosed prior to the World Health Organization (WHO) 2016 classification as B-cell lymphoma, unclassifiable, with features intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma (DLBCL). These cases had no blastoid features, so were included in the analysis. **Statistical significance. ABC: activated B cell; CNS: central nervous system; COO: cell of origin; GCB: germinal center B cell; HGBL-DH: high-grade B-cell lymphoma-double-hit; IPI: International Prognostic Index; NOS: not otherwise specified; PCNSL: primary CNS lymphoma; SCNSL: secondary CNS lymphoma.

was similar. Overall antigen presentation scores (calculated using nSolver pathway analysis) were lower in PCNSL cases compared to both systemic-only DLBCL (P<0.0001) and SCNSL (P=0.0052). However, SCNSL still had a lower score compared to systemic-only cases.

Systemic-only DLBCL demonstrated an increase in immune T-cell scores reflective of enhanced T-cell function and infiltration compared to both SCNSL and PCNSL. The digital gene score for tumor infiltrating lymphocytes (TIL) and total T cells was enriched in systemic-only DLBCL compared to both PCNSL (P=0.002) and SCNSL (P=0.0064). The findings of this comprehensive genomic analysis of DLBCL with SCNSL (the first to our knowledge) suggests that SCNSL is not a distinct biological entity with mutational profile and gene expression features common to both PCNSL and systemic-only DLBCL. However, we show

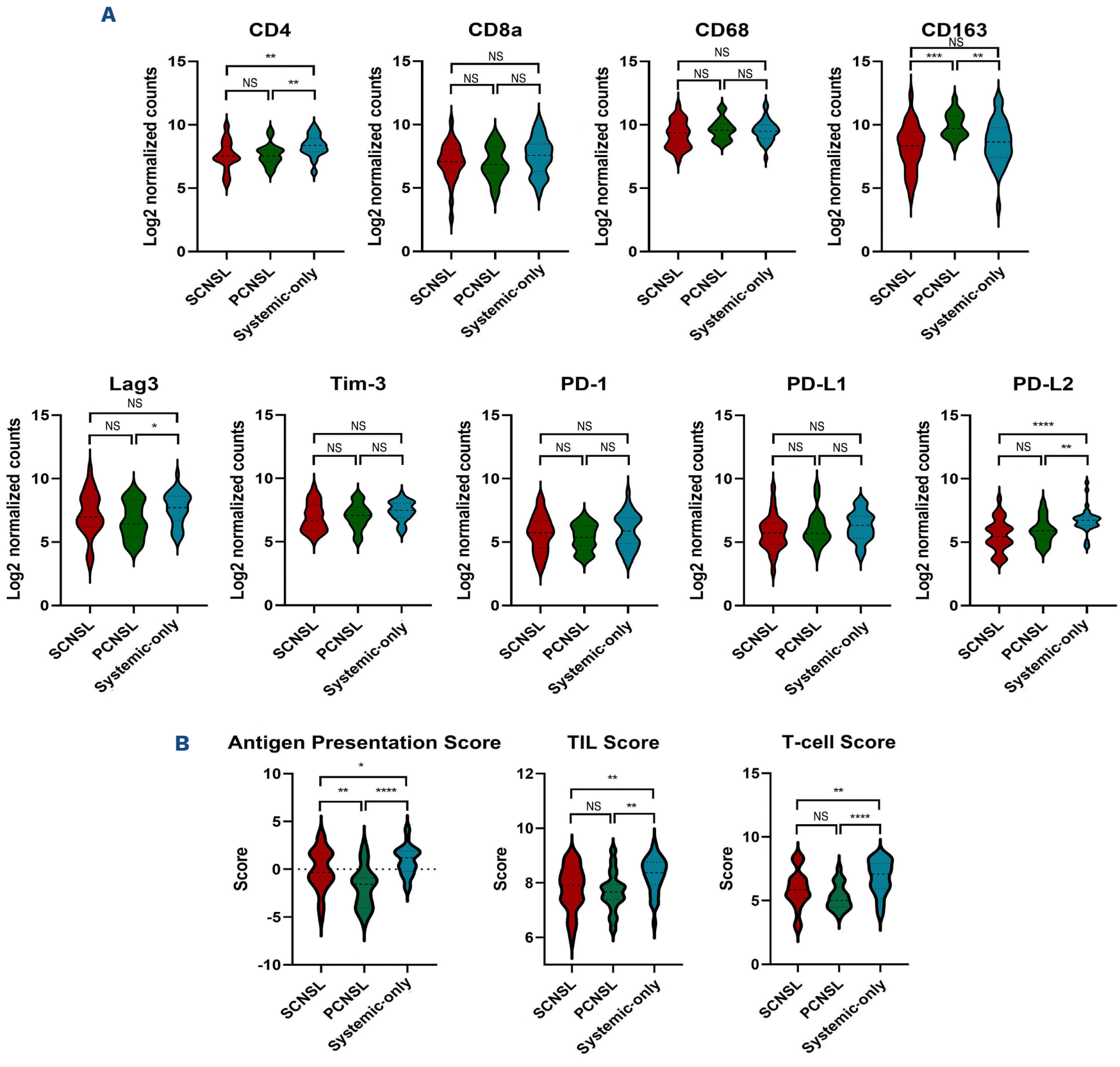

Figure 2. Gene expression of immune effectors and checkpoints. (A) Demonstrates the expression of immune effectors and checkpoints comparing the three tumor types: secondary central nervous system lymphoma (SCNSL), primary central nervous system lymphoma (PCNSL), and systemic-only. Statistical comparison of the Log_2 normalized counts has been made for SCNSL *versus* PCNSL, SCNSL *versus* systemic-only, and PCNSL *versus* systemic-only. (B) Immune function scores reflecting the aggregate expression of genes involved in (left) antigen presentation, (center) tumor infiltrating lymphocytes (TIL), and (right) T cells across SCNSL, PCNSL and systemic-only diffuse large B-cell lymphoma. NS: not statistically significant (i.e., P>0.05); *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

immune evasion through loss of antigen presentation is potentially important in CNS dissemination.

The most striking similarities between SCNSL and PCNSL were antigen presentation and immune surveillance mechanism aberrations, including functional loss of *MHC*, *CD58*, *CIITA* and *B2M*. Loss of these genes is well described in DLBCL involving immune-privileged sites.[3] However, such losses appear infrequent in systemic DLBCL compared with our SCNSL cohort. Conversely, the loss of antigen presentation mechanisms is profound in both SCNSL and PCNSL, although more severe in the latter, and suggests antigen presentation may be a key tumor surveillance mechanism within immune-privileged sites. The loss of these mechanisms may, in part, contribute to the inherent chemotherapy refractoriness of SCNSL that we and others have demonstrated.[1,11]

Poor prognostic DLBCL mutations such as *MYD88*, *CD79B*, *TP53* and *MYC* rearrangements are over-represented in SCNSL. *BCL6* and *NOTCH2* aberrations were infrequent, suggesting those with the BN2 subtype may have lower CNS dissemination potential; the vast majority of our ABC-DLBCL had *MYD88* and/or *CD79B* variants seen in MCD subtype, common in PCNSL, primary testicular lymphoma, and other lymphomas with CNS dissemination propensity DLBCL.[12,13]

The establishment of *MYD88*L265P-mutated lymphoma within immune-privileged tissues may be permitted despite the lack of antigen stimulation through toll-like receptor (TLR) mechanisms at these sites, given that activating *MYD88* mutations drive NFκB signaling downstream from the TLR.[14] Forty percent of our SCNSL shared a similar genomic profile to PCNSL, supporting exploration of similar novel therapeutic strategies.

Focal gains in *CD274* / *PDCD1LG2* (PD-L1/PD-L2) described in PCNSL,[4,8] were uncommon in SCNSL. PCNSL and SCNSL harbored lower PD-L1/PD-L2 gene expression compared to systemic-only DLBCL, indicating superior immune fitness in CNS-sparing DLBCL.

SCNSL COO was similar to systemic DLBCL,[15] in contrast to the high rates of ABC COO in PCNSL.[8] Our relatively high rate of GCB-DLBCL was somewhat unexpected, with most harboring EZB-type mutations, and a proportion having *MYC* +/- *BCL2* rearrangements. In contrast to recent randomized studies,[6] our data suggest that GCB-DLBCL (especially EZB subtypes) remain at risk of CNS disease, particularly with co-existent *MYC* rearrangements or antigen presentation / immune surveillance aberrations.

The location of tumor biopsies was a limitation in our study, with most samples coming from non-CNS sites. However, most were synchronous or early relapse presentations, and CNS biopsies are technically challenging and can cause significant morbidity.[7] There was some heterogeneity in the histology due to changes in diagnostic criteria, and we needed to make comparisons with established mutational profiles in the systemic DLBCL cohort instead of performing our own mutational analysis due to the amounts of tissue available. Nonetheless, this is the largest SCNSL cohort to be comprehensively mapped for molecular and immune biology.

In summary, 40% of SCNSL carry a PCNSL-like profile and a substantial proportion demonstrate *MYC* rearrangments, with the remainder being genomically heterogeneous. SCNSL demonstrates similar loss of antigen presentation and immune surveillance genes to PCNSL, suggesting a potential mechanism for CNS dissemination. Our findings require validation in larger prospective cohorts, and evaluation of relevant immune-targeting therapies should be a focus for the next generation of clinical trials for this disease that carries such a poor prognosis.

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https://doi.org/10.3324/haematol.2023.284600

Received: November 1, 2023.
Accepted: March 13, 2024.
Early view: March 21, 2024.

Disclosures

JW has received honoraria from Janssen, Abbvie, MDI, Beigene, educational subsidies and consulting fees from Abbvie, and has served on the advisory board for Alexion, Abbvie, MDI, MSD, Otsuka. PB has received consulting / advisory fees, and honoraria from Adaptive Biotechnologies, AstraZeneca, Servier. MKG has received research funding from Beigene and Janssen. CK has sat on the

advisory boards for Karyopharm, Roche, Beigene and BMS, and has received research funding from Beigene and MSD. EH has received research funding to the institution from Bristol Myers Squibb/ Celgene, Merck KgA, Astra Zeneca, and F. Hoffmann-La Roche, has sat on the advisory board of F. Hoffmann-La Roche,* Antigene,* Bristol Myers Squibb, Astra Zeneca, Novartis,* Merck Sharpe Dohme,* and Gilead* (*paid to the institution), has fulfilled speaker engagements for Roche (to the institution), Astra Zeneca (to the institution), Janssen, and Regeneron, and provided consultancy services to Specialized Therapeutics. All of the other authors have no conflicts of interest to disclose.

Contributions

JW and EH conceived and supervised the study. JW designed the study protocol, collected clinical data and arranged sample collection. AM collected clinical data. PB and JL performed the genomic sequencing and CNV analysis. JL and JW curated the variants. CG provided genomic data for the unselected DLBCL cohort. CK, FS, MB and MBS performed the GEP and provided the PCNSL comparator data set. JW, CK and AB performed the statistical analysis and generated the graphics. JW prepared the manuscript. All authors reviewed the manuscript.

Acknowledgments

We acknowledge the Australian Lymphoma Alliance for research collaboration. This work was previously presented as a poster at the EHA 25th Annual Congress, June 2020.

Funding

The authors thank the Christine and Bruce Wilson Centre for Lymphoma Genomics for research funding. Seed funding was also provided by the Austin Health medical research fund.

Data-sharing statement

De-identified individual participant data that underlie the reported results will be made available three months after publication for a period of five years after the publication date, and can be provided upon reasonable request to the corresponding author.

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