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

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JW and EH conceived of 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 graphics

JW prepared the manuscript

All authors reviewed the manuscript.

EH and CK contributed equally to the work

Conflicts of interest:

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 consulting/advisory/honoraria from Adaptive Biotechnologies, AstraZeneca, Servier.

JL none to disclose

FS none to disclose

MKG has received research funding from Beigene and Janssen

CG none to disclose

AM none to disclose

MB none to disclose

MBS none to disclose

AB none to disclose

CK advisory boards for Karyopharm, Roche, Beigene and BMS and has received research funding from Beigene and MSD

EH- Research funding to institution: Bristol Myers Squibb/Celgene, Merck KgA, Astra Zeneca, and F. Hoffmann-La Roche. Advisory board: F. Hoffmann-La Roche,* Antigene,* Bristol Myers Squibb, Astra Zeneca, Novartis,* Merck Sharpe Dohme,* and Gilead* (*paid to institution). Speaker engagement: Roche (institution), Astra Zeneca (institution), Janssen, and Regeneron. Consultancy: Specialized therapeutics.

Ethics and patient consent:

The study was ethically approved (HREC/14/Austin425) and conducted in accordance with the Declaration of Helsinki. No formal patient consent was required.

Clinical trial registration:

Not applicable

Permission to reproduce material from other sources

Not applicable

To the editor,

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 hypothesised 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 similar high frequency between 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, though 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 characterise 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. 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 ethically approved (HREC/14/Austin425).

Targeted massively parallel sequencing (MPS), copy number variant analysis and gene expression profiling for COO (Lymph2Cx) and immune gene signatures (IGS) via the nanoString Pan Cancer Immune panel was 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 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. 35/37 had a driver mutation or CNV of interest; the remaining two 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 two 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 two cases (5%). 22/35 (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 two 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 eight (23%), and both in five cases. This contrasted to 74% of PCNSL harbouring *MYD88* and/or *CD79B* mutations (SCNSL *versus* PCNSL; p=0.005).

Other frequent B-cell receptor (BCR)-dependent NF-κB signalling pathway mutations included *CARD11* (23%), *TBL1XR1* (11%), *NFKBIE* (9%). Nine cases had CN loss of *TNFAIP3*, and all of whom had cooperating gain-of-function mutations in BCR-dependent pathways. Mutation/deletion in ≥1 BCR 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 FISH (n=18) or by structural variant analysis from NGS data (n=22).[10] One case had neither available. Thus, *MYC* rearrangement was present in 8/40 cases (20%), and HGBL-DH (all *MYC/BCL2* rearranged) was identified in four cases.

COO was successfully assigned in 29/36 SCNSL, 29 systemic-only DLBCL and 32 PCNSL. 48% of SCNSL were GCB (n=14); 34% ABC (n=10) and 17% unclassified (n=5) (Tables 1 and 2). 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 versus 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 including differed between SCNSL, PCNSL and systemic-only cohorts (Figure 2). PCNSL and SCNSL had lower normalised 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 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 tumour infiltrating lymphocytes (TILs) and total T-cells was enriched in systemic-only DLBCL compared to both the 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 immune evasion through loss of antigen presentation is potentially important in CNS dissemination.

The most striking similarity 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 are 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, suggest antigen presentation may be a key tumour 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 overrepresented 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, PTL, 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 signalling downstream from the TLR.[14] 40% 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 harboured 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] contrasting the high rates of ABC COO in PCNSL.[8] Our relatively high rate of GCB-DLBCL was somewhat unexpected with most harbouring EZB-type mutations, and a proportion, *MYC* +/- *BCL2* rearrangements. In contrast to recent randomised studies,[6] our data suggest that GCB-DLBCL (especially EZB subtypes) remain at CNS disease risk, particularly with coexistent *MYC* rearrangements or antigen presentation/immune surveillance aberrations.

The location of tumour biopsies was a limitation in our study, with most samples coming from of non-CNS sites however most were synchronous or early relapse presentations and CNS biopsies are challenging.[7] There was some heterogeneity in the histology due to changes in diagnostic criteria, and we needed to compare with established mutational profiles in the systemic DLBCL cohort instead of performing our own mutational analysis due to tissue amounts. 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 poor prognostic disease.

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Table 1: Patient Characteristics of Secondary central nervous system lymphoma

Variable	**Value**		
Median age-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 value (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 WHO 2016 classification as B-cell lymphoma, unclassifiable, with features intermediate between Burkitt lymphoma and diffuse large B cell lymphoma. These cases had no blastoid features, so were included in the analysis.

SCNSL = secondary central nervous system lymphoma

PCNSL = Primary CNS lymphoma

Figure 1. Genomic profile of secondary central nervous system lymphoma

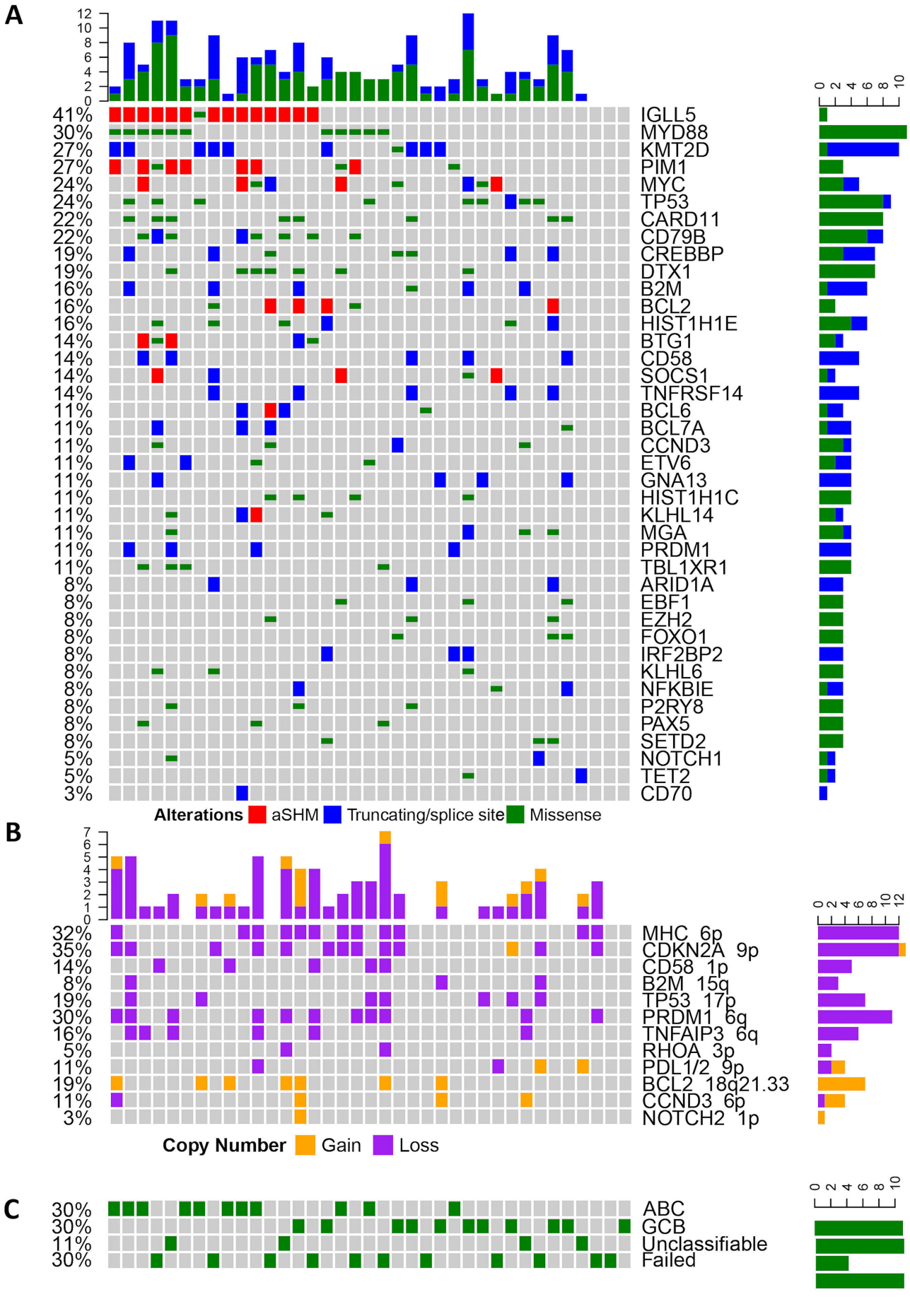
A. Pathological mutations (sequencing panel). B. Copy number alterations. C. Cell of origin (Lymph2Cx). Each column represents a single case sorted by mutational frequency in (A) with the same individual case directly underneath in (B) and (C). Types of mutation and CNV are colour coded as shown in the legend. The two cases furthest right with no mutation or CNV of interest were EBV-driven and were excluded.

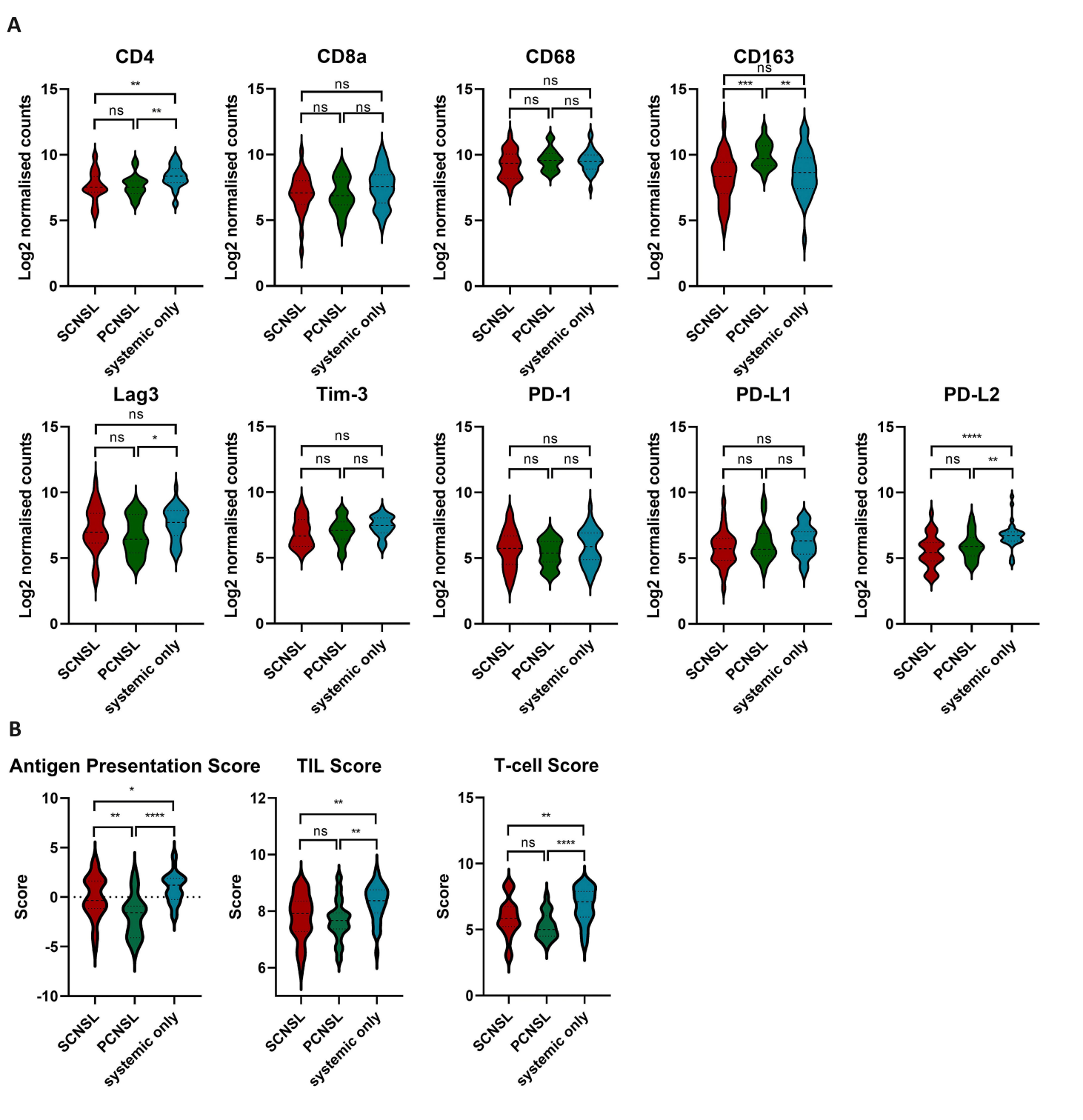
Figure 2 A-B. Gene expression of immune effectors and checkpoints

SCNSL: secondary central nervous system lymphoma. PCNSL: Primary central nervous system lymphoma. NS: not statistically significant (i.e. $p>0.05$). *: $p<0.05$. **: $p<0.01$. ***: $p<0.001$. ****: $p<0.0001$. COO: cell of origin. ABC: activated B cell. GCB: germinal centre B cell. N/A: not applicable. TIL = tumour infiltrating lymphocytes.

A: demonstrates the expression of immune effectors and checkpoints comparing the three tumour types: SCNSL, PCNSL and systemic only. Statistical comparison of the Log_2 normalised counts has been made for SCNSL vs PCNSL, SCNSL vs systemic only and PCNSL vs systemic-only.

B: Immune function scores reflecting the aggregate expression of genes involved in antigen presentation (A), tumour infiltrating lymphocytes (B) and T cells (C) across SCNSL, PCNSL and systemic only DLBCL.


A
41% IGLL5
30% MYD88
27% KMT2D
27% PIM1
24% MYC
24% TP53
22% CARD11
22% CD79B
19% CREBBP
19% DTX1
16% B2M
16% BCL2
16% HIST1H1E
14% BTG1
14% CD58
14% SOCS1
14% TNFRSF14
11% BCL6
11% BCL7A
11% CCND3
11% ETV6
11% GNA13
11% HIST1H1C
11% KLHL14
11% MGA
11% PRDM1
11% TBL1XR1
8% ARID1A
8% EBF1
8% EZH2
8% FOXO1
8% IRF2BP2
8% KLHL6
8% NFKBIE
8% P2RY8
8% PAX5
8% SETD2
5% NOTCH1
5% TET2
3% CD70
Alterations aSHM Truncating/splice site Missense
B
32% MHC_6p
35% CDKN2A_9p
14% CD58_1p
8% B2M_15q
19% TP53_17p
30% PRDM1_6q
16% TNFAIP3_6q
5% RHOA_3p
11% PDL1/2_9p
19% BCL2_18q21.33
11% CCND3_6p
3% NOTCH2_1p
Copy Number Gain Loss
C
30% ABC
30% GCB
11% Unclassifiable
30% Failed


A
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CD8a
CD68
CD163
Lag3
Tim-3
PD-1
PD-L1
PD-L2
Log2 normalised counts
SCNSL
PCNSL
systemic only
ns
B
Antigen Presentation Score
TIL Score
T-cell Score
Score