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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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.

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JL none to disclose

FS none to disclose

MKG has received research funding from Beigene and Janssen

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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


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 \(B2M, CD58, CITA\) 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-\(\kappa B\) 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 \(\geq1\) 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.\textsuperscript{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.\textsuperscript{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, PTCL, and other
lymphomas with CNS dissemination propensity DLBCL.\textsuperscript{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 rearrangements, 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.


Table 1: Patient Characteristics of Secondary central nervous system lymphoma

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

<table>
<thead>
<tr>
<th>Genomic factors</th>
<th>SCNSL</th>
<th>PCNSL</th>
<th>P value (SCNSL vs PCNSL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYD88/CD79B mutation</td>
<td>40%</td>
<td>74%</td>
<td>0.005</td>
</tr>
<tr>
<td>ABC COO</td>
<td>34%</td>
<td>69%</td>
<td>0.002</td>
</tr>
<tr>
<td>CD58 loss</td>
<td>27%</td>
<td>15%</td>
<td>0.17</td>
</tr>
<tr>
<td>CIITA loss</td>
<td>5%</td>
<td>16%</td>
<td>0.16</td>
</tr>
<tr>
<td>B2M loss</td>
<td>22%</td>
<td>16%</td>
<td>0.77</td>
</tr>
<tr>
<td>CN loss 6p</td>
<td>32%</td>
<td>40%</td>
<td>0.49</td>
</tr>
<tr>
<td>Any antigen presentation aberration</td>
<td>60%</td>
<td>62%</td>
<td>0.96</td>
</tr>
</tbody>
</table>
*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 Log₂ 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.