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Journal

Blood Advances, 7(13)

ISSN

2473-9529

Authors

Guney, Ekin
Lucas, Calixto-Hope G
Singh, Kunwar
et al.

Publication Date

2023-07-11

DOI

10.1182/bloodadvances.2022009521

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RESEARCH LETTER

blood advances

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TO THE EDITOR:

Molecular profiling identifies at least 3 distinct types of posttransplant lymphoproliferative disorder involving the CNS

Ekin Guney,[1,*] Calixto-Hope G. Lucas,[1,*] Kunwar Singh,[1] Melike Pekmezci,[1] Sebastian Fernandez-Pol,[2] Kanish Mirchia,[1] Angus Toland,[2] Hannes Vogel,[2] Serguei Bannykh,[3] Kristian T. Schafernak,[4] Sanda Alexandrescu,[5] Bret C. Mobley,[6] Suzanne Powell,[7,8] Christian J. Davidson,[9] Janna Neltner,[10] Daniel R. Boué,[11] Eyas Hattab,[12] Sean P. Ferris,[13] Robert S. Ohgami,[1,9] James L. Rubenstein,[14] Andrew W. Bollen,[1] Tarik Tihan,[1] Arie Perry,[1] David A. Solomon,[1] and Kwun Wah Wen[1]

[1]Department of Pathology, University of California, San Francisco, San Francisco, CA; [2]Department of Pathology, Stanford University Medical Center, Palo Alto, CA; [3]Department of Pathology & Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA; [4]Department of Pathology, Phoenix Children's Hospital, Phoenix, AZ; [5]Department of Pathology, Harvard Medical School, Boston Children's Hospital, Boston, MA; [6]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN; [7]Department of Pathology & Genomic Medicine, Houston Methodist Hospital, Houston, TX; [8]Department of Pathology & Genomic Medicine, Weill Cornell Medical College, New York City, NY; [9]Department of Pathology, University of Utah Health, Salt Lake City, UT; [10]Department of Pathology & Laboratory Medicine, University of Kentucky Chandler Medical Center, Lexington, KY; [11]Department of Pathology and Laboratory Medicine, Nationwide Children's Hospital and The Ohio State University, Columbus, OH; [12]Department of Pathology and Laboratory Medicine, University of Louisville School of Medicine, Louisville, KY; [13]Department of Pathology, University of Michigan Medical School, Ann Arbor, MI; and [14]Department of Medicine, University of California, San Francisco, San Francisco, CA

Posttransplant lymphoproliferative disorder (PTLD) is an immunodeficiency-associated lymphoproliferative disorder defined as occurring in the posttransplant setting. The risk factors for development of PTLD include Epstein-Barr virus (EBV) serostatus, type of organ transplant, intensity and type of immunosuppressive regimens, patient age, and presence of genetic polymorphisms (eg, HLA).[1-8] PTLD with involvement of the central nervous system (PTLD-CNS) is a rare posttransplant complication, often with poor prognosis. PTLD-CNS has most commonly been observed after renal transplantation and typically occurs later after transplant (mean, >9 years) when compared with its EBV^+ systemic counterpart.[8]

Prior studies of systemic PTLD demonstrated various molecular alterations in critical oncogenic pathways, such as clonal immunoglobulin gene rearrangement, somatic mutations of *RAS* and *TP53*, translocations of *MYC* and *BCL6*, and aberrant somatic hypermutation of proto-oncogenes including *BCL6*, *PIM1*, *PAX5*, and *MYC*.[9-18] Notably, PTLD cases showed a substantially lower frequency of oncogenic molecular aberrations compared with sporadic lymphomas in immunocompetent patients. In this setting of PTLD with minimal oncogenic alterations, EBV is thought to be the predominant driver of lymphoproliferation. However, the genetic landscape of PTLD involving the CNS is not well established, with the most comprehensive study including cases of PTLD-CNS demonstrating that EBV^+ and HIV^- cases had a low burden of somatic mutations with a lack of alterations in *MYD88*, *CD79B*, *PIM1*, and *CDKN2A/B* known to be frequent in adult-type *MYD88*-mutant primary CNS large B-cell lymphoma (PCNS-LBCL), as well as mutations in *TP53*, *NKFBIE*, and *GNA13* that we recently described as frequent in pediatric-type *MYD88*-wild-type PCNS-LBCL.[19-23]

Here, we sought to investigate the clinical, pathologic, and molecular characteristics of PTLD-CNS to better identify factors that may be useful in risk stratification of this patient population. Through a multi-institutional collaboration, we assembled a cohort of 24 patients with PTLD-CNS (Figure 1; supplemental Tables 1 and 2). Twenty-one patients had undergone solid organ transplantation, and 3 had undergone bone marrow transplantation. Patient #22 had severe combined immunodeficiency and Nijmegen breakage syndrome, and patient #8 had Down syndrome. The remaining patients did not have any

Submitted 12 December 2022; accepted 16 February 2023; prepublished online on *Blood Advances* First Edition 10 March 2023; final version published online 3 July 2023. https://doi.org/10.1182/bloodadvances.2022009521.

*E.G. and C.-H.G.L. contributed equally to this work.

The full clinical, pathologic, and molecular data are available in the supplemental Data available with the online version of this article. Further original data are available on request from the corresponding author, Kwun Wah Wen (kwun.wen@ucsf.edu).

Sequence data from the 14 PTLD-CNS lesions have been deposited at the Sequence Read Archive (SRA) under BioProject ID PRJNA950018 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA950018).

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

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A

Sporadic PCNS-LBCL like

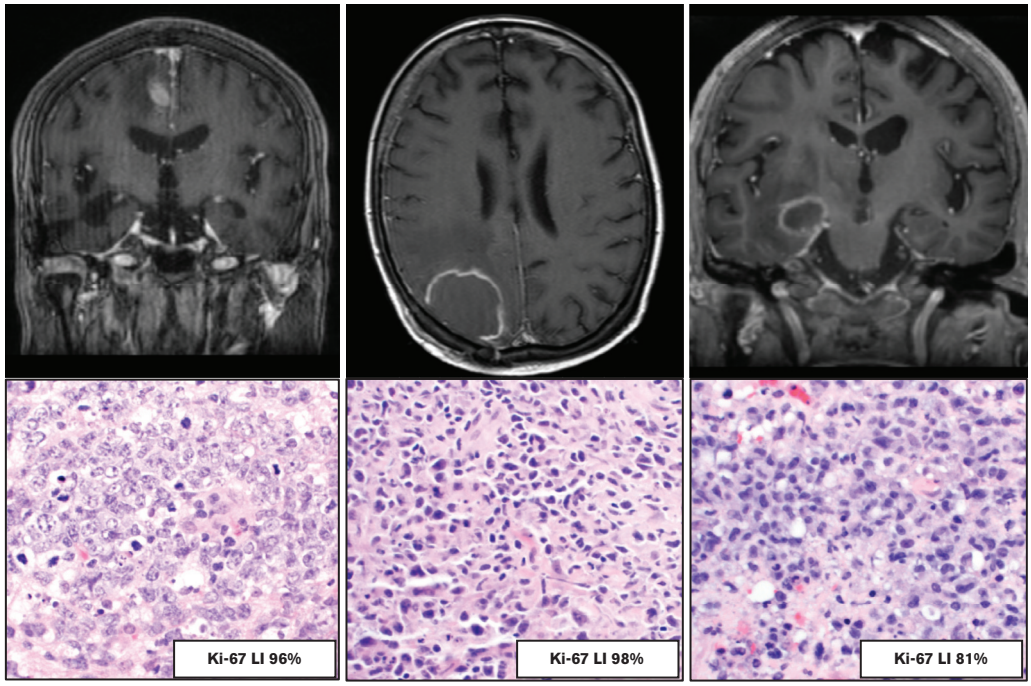
Patient #3, 77-year-old female
Posttransplant: Kidney
Involvement: Limited to CNS
Monomorphic, EBV-negative
MYD88, CD79B, KMT2D mutations

Systemic Ras-driven

Patient #4, 56-year-old female
Posttransplant: Kidney and liver
Involvement: Systemic and CNS
Monomorphic, EBV-negative
HRAS, NF1, TP53 mutations

EBV-driven CNS-limited

Patient #1, 68-year-old male
Posttransplant: Liver
Involvement: Limited to CNS
Monomorphic, EBV-positive
No pathogenic alterations



B

	Sporadic PCNS-LBCL like	Systemic Ras-driven	EBV-driven CNS-limited	Entire cohort
Number of patients (n)	1	3	20	24
Sex (M:F)	0:1	2:1	12:8	14:10
Median age at PTLD diagnosis (years)	77	3	62	59
HIV positive	0	0	1	1
Type of Transplant				
Kidney	1	1	12	14
Liver	0	2	5	7
Heart	0	0	2	2
Bone Marrow	0	1	2	3
Small Bowel	0	1	0	1
Pancreas	0	0	1	1
Immunosupression after transplant				
Mycophenolic acid	0	0	16	16
Cyclosporine	0	0	4	4
Tacrolimus	1	2	10	13
Median time from transplant to PTLD diagnosis (years)	17.2	1.6	10.1	8.1
PTLD systemic involvement	0	3	0	3
Monomorphic morphology	1/1 (100%)	2/3 (66%)	15/20 (75%)	18/24 (75%)
Polymorphic morphology	0/1 (0%)	1/3 (33%)	5/20 (25%)	6/24 (25%)
EBV-positive	0/1 (0%)	2/3 (66%)	20/20 (100%)	22/24 (92%)
Median Ki-67 labeling index (%)	95.6	93.2	58.6	61
Treatment for PTLD				
Chemotherapy alone	1	2	13	16
Radiation plus chemotherapy	0	0	5	5
No treatment	0	0	1	1
Unknown	-	1	1	2
Median follow-up (months)	94.8	1.9	19.2	20.6
Median survival (months)	94.8	1.87	122	122

HIV: Human immunodeficiency virus; PTLD: Posttransplant lymphoproliferative disorder, EBV: Epstein-Barr virus.

Figure 1.

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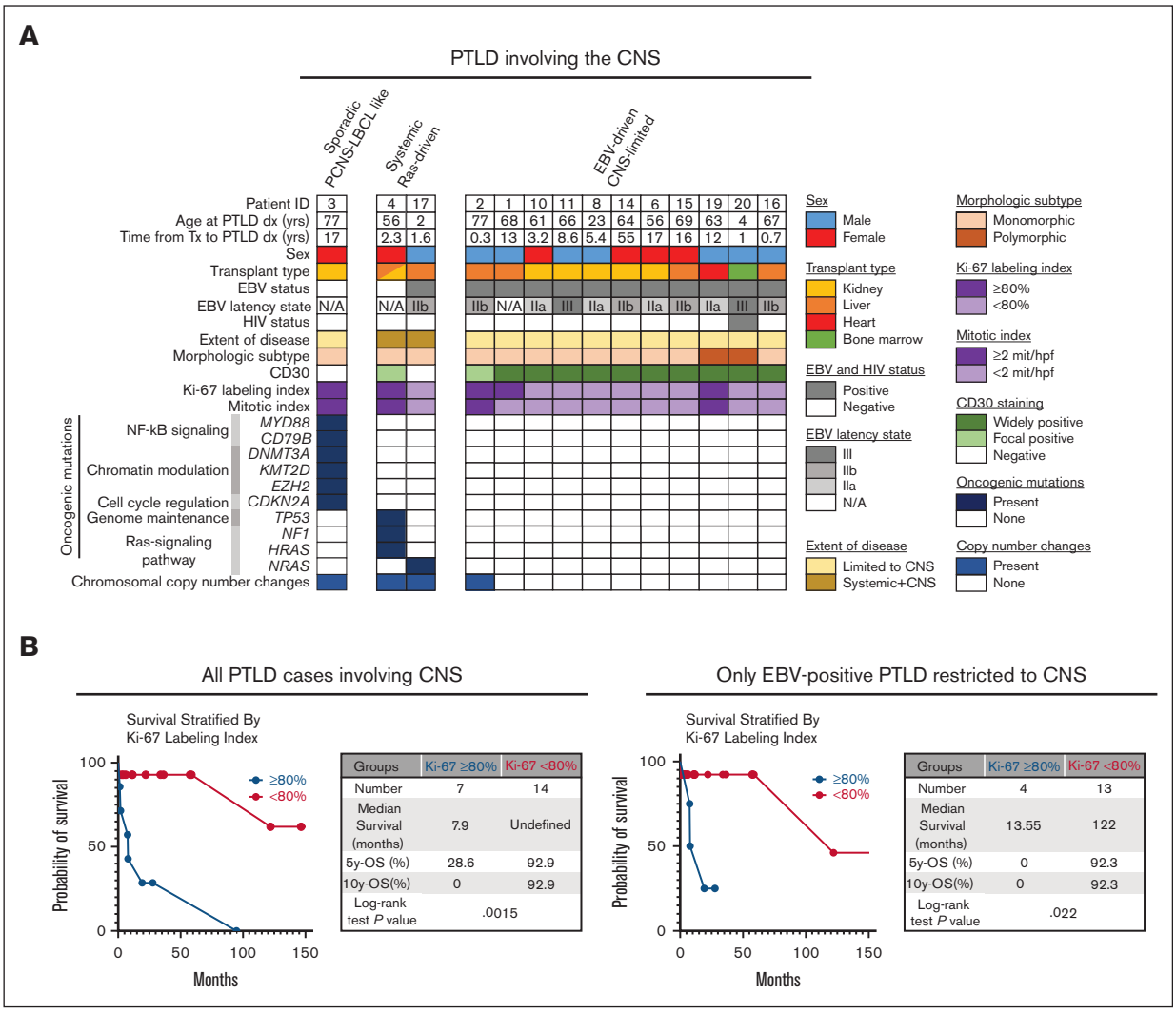

Figure 2. Integration of genomic profiling and clinicopathologic features identifies 3 distinct types of PTLD-CNS. (A) Oncoprint of genomic profiling results and clinical data from the PTLD-CNS cohort. Stratification was performed according to EBV status by in situ hybridization, presence or absence of systemic extra-CNS involvement, and pathogenic molecular alterations involving RAS family oncogenes (*HRAS* and *NRAS*) and known lymphoma oncogenes and tumor suppressor genes (*MYD88*, *CD79B*, *KMT2D*, and *CDKN2A*). (B) Kaplan-Meier survival analysis of patients with PTLD-CNS stratified by Ki-67 LI using a cutoff of 80%.

known innate immunodeficiency or tumor predisposition syndromes. The median time from organ transplant to development of PTLD-CNS was 8.1 years. Of 24 patients, 21 patients had PTLD with exclusive CNS involvement, whereas the other 3 patients also had PTLD at systemic sites (2 gastrointestinal and 1 nodal involvement) outside the CNS. The median age at time of PTLD-CNS diagnosis was 59 years (range, 2-77 years). Eighteen PTLD cases demonstrated monomorphic morphologic features resembling LBCL, whereas 6 PTLD cases demonstrated polymorphic morphology with variable composition of small-to-large sized B cells admixed with T cells and plasma cells (Figure 1A; supplemental Tables 3 and 4). Patients were treated with various combinations of dexamethasone, high-dose methotrexate, and rituximab, along with other accompanying agents in a subset. Five patients were additionally treated with radiotherapy. The median survival of all patients with PTLD-CNS was 122 months, and the 5-year and 10-year overall survival rates were 67.5% and 50.6%, respectively. The University of California San Francisco Institutional Review Board approved this study, which was conducted in accordance with the Declaration of Helsinki.

Targeted next-generation DNA sequencing and genome-wide copy number analysis were performed (Figure 2; supplemental Tables 5 and 6; supplemental Methods). Based on integration of the molecular features, EBV status, and presence or absence of

Figure 1. Clinical and pathologic features of the 3 identified types of PTLD involving the CNS. (A) Preoperative T1-weighted postcontrast magnetic resonance imaging and hematoxylin and eosin–stained sections from representative patients with sporadic PCNS-LBCL–like, systemic Ras-driven, and EBV-driven CNS-limited PTLD-CNS showing intraparenchymal mass lesions with contrast enhancement and significant edema in the surrounding white matter. Ki-67 LI is shown. (B) Summary of clinical and pathologic features of the multiinstitutional cohort of patients with PTLD-CNS.

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systemic extra-CNS involvement, we identified 3 distinct types of PTLD-CNS, which we term "sporadic PCNS-LBCL like," "systemic Ras-driven," and "EBV-driven CNS-limited."

Sporadic PCNS-LBCL like: The PTLD in patient #3 was EBV^- and showed an activating *MYD88* hotspot missense mutation, a *CD79B* mutation, a *CDKN2A* homozygous deletion, and deleterious mutations in chromatin modulators including *DNMT3A*, *EZH2*, and *KMT2D*. The molecular signature was overall similar to sporadic EBV^- PCNS-LBCL in immunocompetent older adults. This patient died at 94.8 months after diagnosis.

Systemic Ras-driven: In 2 patients (#4 and #17) with both systemic and CNS involvement, there were activating mutations in RAS oncogenes. In patient #4, the PTLD was EBV^- and demonstrated an *HRAS* p.Q61K hotspot mutation, along with *NF1* and *TP53* inactivating mutations. This patient died at 1.9 months after PTLD-CNS diagnosis. In patient #17, the PTLD was EBV^+ and demonstrated an *NRAS* p.Q61K hotspot mutation. This patient was alive at last follow-up at 146.6 months.

EBV-driven CNS-limited: In 11 patients, the PTLD was EBV^+ and restricted to the CNS. There was no clinical or radiographic evidence of systemic involvement. Evaluation by next-generation sequencing did not demonstrate oncogenic alterations in any of the evaluated genes, and only 1 of the 11 cases demonstrated clonal chromosomal gains/losses, whereas the other 10 cases showed normal diploid genomes.

Next, we sought to determine associations of any relevant clinical, pathologic, and molecular features with clinical outcomes for our PTLD-CNS cohort using Cox proportional hazards univariate models (supplemental Table 7). Kaplan-Meier stratification of the entire PTLD-CNS cohort based on a Ki-67 labeling index (LI) of ≥80% or mitotic index of ≥2 per high-power field, determined as optimal cutoff values by the Liu method (supplemental Table 8), demonstrated an association with worse survival (log-rank test, $P = .0015$ and $P = .0092$, respectively) (Figure 2B; supplemental Figure 1). This association of worse survival with Ki-67 LI of ≥80% was also found in the subgroup of patients with EBV^+ PTLD with exclusive CNS involvement (log-rank test, $P = .0092$), as well as the subgroup of patients with PTLD-CNS with monomorphic morphology (log-rank test, $P = .02$) (supplemental Figure 2A).

Overall, through integration of molecular and clinicopathologic features, we have demonstrated that PTLD involving the CNS is a heterogeneous disease category comprising at least 3 distinct biologic types. The sporadic PCNS-LBCL–like type demonstrated a molecular profile with *MYD88* and *CD79B* mutations similar to sporadic EBV^- adult-type *MYD88*-mutant primary CNS-LBCL,[22] which suggests that the pathogenesis is similar to that of conventional EBV^- PCNS-LBCL in immunocompetent adults. The systemic Ras-driven type was defined by systemic extra-CNS involvement and activating RAS family oncogene mutations, similar to those reported in systemic PTLD.[9,10] We speculate that PTLD-CNS with concurrent systemic involvement and activating RAS mutations is biologically similar to systemic PTLD with RAS mutations and may represent CNS spread of systemic Ras-driven PTLD. The lack of oncogenic alterations in EBV-driven CNS-limited cases distinguished them from the sporadic PCNS-LBCL–like and systemic Ras-driven types. Our findings support that PTLD-CNS is a molecularly heterogeneous category of lesions, and perhaps true CNS-restricted PTLDs are devoid of oncogenic molecular alterations and driven primarily by EBV reactivation. Future studies of additional cases are needed to confirm these findings of at least 3 distinct types of PTLD-CNS.

Various clinical risk factors such as multiorgan involvement, older patient age, earlier onset after transplantation, lack of response to reduced immunosuppression, and CNS involvement are known to be associated with worse prognosis for PTLD.[24,25] Here, we found that high proliferative activity defined by a Ki-67 LI of ≥80% conferred worse survival in patients with PTLD-CNS. Confirmation of the prognostic utility of proliferative activity in future independent studies is important for prospective clinical risk stratification of PTLD-CNS.

Acknowledgments: The authors thank the staff of the UCSF Histology Laboratory for assistance with immunohistochemical staining and the UCSF Clinical Cancer Genomics Laboratory for assistance with genetic profiling.

This study was supported by the Clinical Research Endowment Grant and Rebecca Frankel Award from the Department of Pathology, University of California, San Francisco (K.W.W.); NIH Director's Early Independence Award DP5 OD021403 (D.A.S.); and Leukemia and Lymphoma Society and NIH R01CA13983 and R01CA239462 grants (J.L.R.).

Contribution: All authors had access to clinical, pathologic, and molecular data and contributed to this study as follows: E.G., C.-H.G.L., D.A.S., and K.W.W. designed the study and contributed to specimen collection, clinical and pathologic data collection, pathologic review, data analysis, statistical analysis, and wrote the manuscript; D.A.S. and K.W.W. supervised the study; M.P. contributed to statistical analysis; R.S.O. contributed to pathologic review; K.S. performed the digital Ki-67 quantification; S.F.-P., K.M., A.T., H.V., S.P., C.J.D., J.N., D.R.B., E.H., S.P.F., R.S.O., J.L.R., A.W.B., T.T., and A.P. contributed to specimen collection, data collection, and pathologic review; and all authors critically reviewed the manuscript and approved its submission.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: E.G., 0000-0002-7050-0831; K.S., 0000-0002-9024-5751; M.P., 0000-0003-2548-8359; S.F.-P., 0000-0002-0428-0199; K.M., 0000-0002-7371-7059; S.A., 0000-0001-9246-4184; S.P.F., 0000-0001-5407-7212; T.T., 0000-0003-4551-2555; A.P., 0000-0002-8300-7261; D.A.S., 0000-0003-4571-7999; K.W.W., 0000-0002-8251-902X.

Correspondence: Kwun Wah Wen, Department of Pathology, University of California, San Francisco, 505 Parnassus Ave, M545, Box 0102, San Francisco, CA 94117; email: kwun.wen@ucsf.edu.

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