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(see figure). Because cases of EBV-associated PCNSL often arise in immunocompromised hosts, a second stratification based on host HIV status was also performed, although biologic differences were less evident. Unsurprisingly, EBV⁻ PCNSL typically had a gene expression phenotype resembling ABC DLBCL, including mutations of MYD88^{L265P}, CD79B, and/or PIM1 in ~80% of cases. Immune evasion was also a prominent genetic feature, as mutations or deletions of HLA-A, HLA-B, or HLA-C were seen in most cases. These genetic features have been reported in PCNSL as well as genetic subtypes of DLBCL with secondary involvement of the central nervous system (SCNSL). These findings underscore the notion that hallmark features of EBV⁻ PCNSL include constitutive NF-κB activation as well as chronic active B-cell receptor and My-T-BCR signaling that is shared with specific systemic DLBCL subtypes.² Conversely, EBV-associated PCNSL was frequently unclassified by gene expression, did not demonstrate cardinal genetic drivers of ABC DLBCL, and exhibited a genomic landscape with few identifiable mutations or copy number abnormalities. Interestingly, EBV-associated PCNSL exhibited a tolerogenic tumor microenvironment, including overexpression of genes associated with T-cell immune checkpoints (PD-L1, PD-L2, LAG-3, TIM-3), macrophages (CD68, CD163), and the antiviral cytokine tumor necrosis factor-α (TNF-α). Paradoxically, the lack of genetic alterations involving HLA class I or II suggests that antigen processing is preserved in these tumors despite their

IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Gandhi et al, page 1468

Sorting biologic subtypes of primary CNS lymphoma

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In this issue of *Blood*, Gandhi et al studied 91 cases of primary diffuse large B-cell lymphoma (DLBCL) of the central nervous system (CNS) (PCNSL) and compared the biologic features of tumors associated with Epstein-Barr virus (EBV) to tumors that are EBV⁻ using digital gene expression signatures and customized hybrid-capture targeted sequencing panels.¹

The authors identified strikingly distinct genetic and immunologic features based on EBV status, including differences in gene expression, genetic drivers, signatures within the tumor microenvironment, and oncogenic signaling pathway additions

	Genetic alterations	Gene expression	Function	Microenvironment	Potential therapy
EBV-negative PCNSL	MYD88 ^{L265P} CD79A/CD79B mut./amp. PIM1 mut. CDKN2A mut./del. HLA-A–HLA-B–HLA-C loss TBL1XR1, PRDM1, TOX KLHL14, ETV6	ABC DLBCL NF-κB high IRF4 high JAK/STAT Proliferation	MY-T-BCR signaling Loss of antigen presentation	Immunosuppressive	BTK inhibitors IMiDs PI3K inhibitors CAR T
EBV-associated PCNSL	MYD88 ^{WT} CD79B ^{WT} PIM1 ^{WT} • Low mutational burden • Few copy number alterations	Macrophage high Immune checkpoint high	Antigen processing intact	Immunosuppressive	Adoptive immunotherapy Immune checkpoint inhibitors CAR T

Primary CNS lymphoma can be subdivided into EBV⁻ and EBV-associated subtypes based on unique genetic alterations, gene expression signatures, and functional and microenvironmental differences. These biologic differences suggest that potential therapeutic strategies should be tailored within subtypes. ABC, activated B cell; amp, amplifications; BCR, B-cell receptor; BTK, Bruton tyrosine kinase; CAR T, chimeric antigen receptor T cell; del, deletions; IMiD, immunomodulatory imide drug; My-T-BCR, MYD88-TLR9-BCR supercomplex; mut, mutation; PI3K, phosphoinositide 3-kinase.

relationship with an immunocompromised state. The authors found virtually no overlap between subtypes, strongly suggesting that these are indeed distinct biologic entities that may benefit from alternative treatment approaches (see figure).

Notably, all cases were DLBCL, which encompasses a spectrum of diverse entities that can present in virtually any organ. Indeed, certain DLBCL subtypes, including PCNSL, are categorized exclusively by anatomic location without regard for distinct biologic features. Nevertheless, it is becoming increasingly evident that PCNSL often shares the genetic profile of specific systemic DLBCL subtypes. The heterogeneity of DLBCL was initially appreciated from gene-expression profiling where subtypes were identified according to their cell of origin: germinal center B cell (GCB), ABC, and unclassified.³ This classification identifies subtypes arising from B cells at different developmental stages with distinct oncogenic mechanisms, reliance on different survival pathways, and differential outcomes. The current World Health Organization classification system recognizes ABC DLBCL and GCB DLBCL as distinct molecular subtypes.

More recently, comprehensive multiplatform genomic approaches have described genetic DLBCL subtypes based on enrichment of driver mutations and signaling pathways.^{4,6} In many cases, genetic subtypes not only share genetic features and microenvironment gene expression signatures but also display differential response to chemotherapy and targeted agents. Critically, the MCD subtype of DLBCL shares genetic alterations with PCNSL, has an overall poor prognosis with standard therapy, and demonstrates CNS tropism.⁴ The current study by Gandhi et al further supports the conclusion that EBV⁻ PCNSL and MCD DLBCL share a similar etiology: both have frequent mutations in *MYD88*^{L265P}, *CD79B/A*, *HLA-A*, *HLA-B*, *HLA-C*, *PIM1*, *TBL1XR1*, and *TOX*, although previous studies in MCD have also observed enrichment for alterations in *KLHL14* and *ETV6* that were not included in the targeted gene panel of the current study.^{2,6} The overlapping molecular profile suggests these lymphomas share common oncogenic signaling dependencies initiated by the BCR, are highly immunogenic, and likely have a similar cell of origin. Therapeutically, BCR inhibitors that

target the proximal BCR kinase, BTK, are most effective in this genetic background and are being actively studied in both PCNSL and SCNSL.^{7,8} Similarly, the recently described cluster 5 (C5) also includes cases that are characterized by *MYD88*^{L265P} and *CD79B* mutations, gain of *18q*, and *PIM1* mutations closely mirroring the genetic features of MCD.⁵ These results suggest that comprehensive tumor profiling is required to fully exploit the differences between lymphoma subtypes and improve clinical outcomes. The results from Gandhi et al demonstrate that PCNSL is no exception.

The question then arises, can EBV replicate the oncogenic functions of this suite of oncogenic alterations, or do EBV-associated CNS lymphomas rely on distinct mechanisms of transformation that promote oncogenic growth and immune evasion? The current study suggests the latter in that EBV-associated PCNSL did not present with an ABC gene expression pattern, and MCD-associated mutations did not cooccur. This is striking because NF- κ B cooperating mutations are found in other EBV-associated lymphomas. What then are the cooperating genetic alterations underpinning EBV-associated CNS lymphomas? Does the nature of the immunodeficiency, whether acquired or congenital, influence the underlying tumor biology? These answers will rely on future studies because these tumors are exceptionally rare and the limitations of the current study include a targeted sequencing panel approach as well as a reliance on formalin-fixed paraffin-embedded specimens, which are suboptimal for comprehensive tumor profiling. However, some intriguing clues were uncovered, as EBV-associated PCNSL was enriched for macrophage immune signatures, high TNF- α expression, and high expression of immune checkpoints PD-L1/PD-L2, LAG3, and TIM3. This interesting finding suggests convergent evolution across CNS lymphomas that involves the need to escape immune surveillance despite the presence within an immune-privileged site. It also suggests a therapeutic vulnerability that may be exploited with immunotherapy approaches, including immune checkpoint inhibitors, chimeric antigen receptor T-cell therapy, or adoptive T-cell therapy with EBV-specific T cells designed to overcome immunosuppressive mechanisms.^{9,10} Clinical trials testing these hypotheses are clearly warranted, as additional rational targets are being discovered.

In summary, the study by Gandhi et al reinforces the notion that DLBCL encompasses a spectrum of diseases, and comprehensive tumor profiling is needed to fully characterize biologic subtypes of PCNSL that may respond differently to novel treatments. Clinical trials in CNS lymphomas should incorporate this molecular framework, and future genomic studies should aim to further define exploitable weaknesses of these difficult-to-treat tumors.

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

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