

Diffuse large B-cell lymphoma involving the central nervous system: biologic rationale for targeted therapy

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is an aggressive B-cell lymphoma curable even in advanced stages. DLBCL involving the central nervous system (CNS) is more difficult to cure and fewer treatment options exist. Primary CNS lymphoma (PCNSL) refers to aggressive lymphomas confined to the CNS, and are almost always DLBCL. Standard approaches for PCNSL use high-dose methotrexate-based combinations as induction therapy and younger patients often receive dose-intensive consolidation. However, dose-intensive therapies are not suitable for all patients, and older patients have fewer effective treatment options. Patients with relapsed or chemotherapy-refractory disease have a very poor prognosis. Secondary CNS lymphoma (SCNSL) describes aggressive lymphomas involving the CNS at initial presentation or relapses within the CNS after treatment for systemic DLBCL. Isolated CNS relapse is often managed as PCNSL, but patients with synchronous involvement of DLBCL in both the periphery and the CNS pose a unique clinical challenge. Insights into the molecular circuitry of DLBCL have identified distinct genetic subtypes including cases with a predilection for CNS invasion. PCNSL and subsets of SCNSL are characterized by chronically activated B-cell receptor and NFκB signaling along with genetic evidence of immune evasion which may be exploited therapeutically. Improved mechanistic understanding of targetable pathways underpinning CNS lymphomas has led to numerous clinical trials testing targeted agent combinations and immunotherapy approaches with promising early results. Biologically rational strategies may further improve the cure rate of CNS lymphomas, either by overcoming intrinsic or acquired treatment resistance and/or by being broadly applicable to patients of all ages.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is a spectrum of aggressive B-cell lymphomas with remarkable clinical and molecular heterogeneity. These tumors manifest in myriad clinical presentations, including involvement of the central nervous system (CNS). Importantly, DLBCL can be cured with immunochemotherapy, even in advanced stages and after relapse.

Primary DLBCL of the CNS (PCNSL) is a rare subtype of DLBCL that involves the brain, eyes, leptomeninges, or spinal cord without evidence of systemic involvement. It accounts for 6% of all new CNS tumors with an annual incidence of approximately 1,500 cases in the United States.¹ The incidence is rising in patients with advanced age, particularly those aged ≥75 years.^{2,3} Front-line treatment for PCNSL

varies across institutions, but patients deemed suitable for dose-intensive therapy receive one of several high-dose methotrexate (HD-MTX)-based regimens and consolidation with either autologous stem cell transplantation (ASCT), radiotherapy, or cytarabine-based chemotherapy.⁴⁻⁷ There is still no consensus on the optimal induction regimen, and treatment guidelines rely almost exclusively on phase II clinical trials.⁸ Furthermore, the cure rate may not be evident when surrogate markers for survival are used since late recurrences are not uncommon.^{9,10} Patients deemed unsuitable for HD-MTX, including those with advanced age or co-morbidities, are treated with reduced doses of chemotherapy with palliative intent.¹¹⁻¹³ Notably, patients with chemotherapy-refractory disease have a very poor prognosis, although it is unclear if these poor clinical results are due to intrinsic chemotherapy resistance, ineffective drug

delivery, or a combination of these factors.¹⁴ Whilst good results are seen in patients able to tolerate dose-intensive therapy, the rising proportion of older patients means that most patients with PCNSL will die of lymphoma, and novel treatment approaches are needed.

Secondary CNS lymphoma (SCNSL) is distinguished from PCNSL as either synchronous CNS and systemic involvement of lymphoma or an isolated CNS relapse. SCNSL is not a specific biologic entity, but most cases are DLBCL. The incidence of CNS involvement at diagnosis for DLBCL is only approximately 5%, but CNS progression may arise shortly after or during initial therapy;¹⁵ in a retrospective study of SCNSL, the majority (87%) occurred at the time of first relapse and 39% had synchronous systemic relapse.¹⁶ The prognosis of SCNSL is poor, so emphasis has been placed on prevention with intrathecal chemotherapy or HD-MTX prophylaxis delivered with front-line therapy in high-risk patients, but these strategies are not very effective and remain controversial.^{17,18} Furthermore, patients with CNS involvement are often unnecessarily excluded from clinical trials testing novel approaches, even when the agents are effective for CNS disease. Overall, there are few effective treatments for SCNSL, and this remains an unmet clinical need.

We highlight advances in our understanding of the molecular pathogenesis of primary and secondary DLBCL of the CNS, including aspects of biology that are shared with genetic subtypes of DLBCL and the oncogenic pathways that are emerging as therapeutic targets. We discuss the treatment landscape of CNS lymphomas, and emphasize targeted agents with the strongest biologic rationale.

Standard approach to diffuse large B-cell lymphomas involving the central nervous system

Treatment of primary diffuse large B-cell lymphoma of the central nervous system

The prognosis of PCNSL is significantly worse than systemic DLBCL. Although induction therapy achieves remission in approximately 50% of patients, without consolidation, the risk of relapse remains unacceptably high.⁹ Furthermore, both induction therapy and consolidation are associated with significant toxicities that are not tolerated by all patients. Clinical outcomes are worse in older patients, particularly in those who do not receive dose-intensive therapy.¹⁹ The most pressing clinical needs are to improve the efficacy of induction therapy to potentially obviate the need for consolidation and to develop effective alternative strategies for patients who are not suitable candidates for dose-intensive therapy.

The first therapy that improved clinical outcomes was whole brain radiotherapy (WBRT), but all patients relapsed and

neurotoxicity was problematic.²⁰ Thus, the original role of chemotherapy was to reduce reliance on WBRT. Indeed, when HD-MTX was added to WBRT, many patients deferred radiotherapy for an extended period of time.²¹ In this way, HD-MTX became the *de facto* cornerstone chemotherapeutic agent for PCNSL due to its reliable CNS penetration, not based on a strong biologic rationale for efficacy in DLBCL. Combination chemotherapy regimens were subsequently developed to further reduce reliance on WBRT. A randomized phase II study demonstrated that high-dose cytarabine added to HD-MTX improved the complete response (CR) rate to 46% compared to 18% with HD-MTX alone.²² Rituximab has also been used to improve the response rates of induction therapy. A multicenter phase II study comparing HD-MTX-based induction regimens found that patients treated with the HD-MTX and cytarabine backbone plus both rituximab and thiotepa (MATRix) had a CR rate of 49% compared to 30% with rituximab added to HD-MTX backbone and 23% with HD-MTX backbone alone.⁷

Another strategy has been to incorporate high-dose chemotherapy with ASCT as a consolidation strategy.^{23,24} This strategy can be effective for long-term disease control, but results vary depending on the conditioning regimen used. A retrospective registry study showed that patients who received thiotepa/busulfan/cyclophosphamide (TBC) or thiotepa/carmustine (TT-BCNU) conditioning had superior 3-year progression-free survival (PFS) compared to those who received carmustine, etoposide, cytarabine, melphalan (BEAM) conditioning.²⁵ This improved efficacy, however, came at the cost of increased toxicity and non-relapse mortality with TBC. Indeed, a randomized phase II study in 140 patients with PCNSL aged <60 years compared thiotepa-based conditioning followed by ASCT to WBRT as a consolidation strategy and the 8-year event-free survival was 67% versus 39% ($P=0.03$) in favor of ASCT.⁵ Selected older patients without co-morbidities may be candidates for ASCT consolidation after careful consideration of the risks associated with this treatment.²⁶ Ongoing trials are addressing the feasibility and efficacy of this approach.²⁷ Taken together, these studies strongly suggest that HD-MTX-based induction regimens with ASCT consolidation improve clinical outcomes compared to WBRT consolidation in younger patients. Furthermore, the ability of these regimens to cure PCNSL without consolidation is limited, and there are few effective alternative treatment options.

Treatment of secondary diffuse large B-cell lymphoma of the central nervous system

Compared to systemic DLBCL, the outcomes for SCNSL are significantly worse with an expected survival of <6 months.^{16,28} Patients with synchronous CNS and systemic involvement are particularly challenging since therapy needs to be both effective for systemic DLBCL and penetrate the blood-brain barrier.

In a large retrospective series, the 2-year survival rate for

SCNSL was only 20%, but patients deemed eligible for dose-intensive therapy had a 2-year survival rate of 62%.¹⁶ Patients with isolated CNS disease may have a better prognosis; a series of 113 patients with isolated CNS relapse demonstrated that a subset of patients could survive for more than 2.5 years, but ultimately most patients died from lymphoma.²⁹ In patients with synchronous DLBCL involving the CNS and periphery, clinical outcomes are largely related to the ability to control the CNS disease.³⁰ A retrospective series of 80 patients with synchronous systemic DLBCL and CNS involvement demonstrated a 2-year OS of 49%. Patients treated with dose-intensive regimens had a superior 2-year PFS compared to those treated with less intensive therapy (50% vs. 31%, respectively; $P=0.006$). Notably, the 2-year OS of patients with relapse in the CNS was significantly worse than those with systemic relapse (13% vs. 36%, respectively; $P=0.02$).

The potential benefit of ASCT for SCNSL is also restricted to younger patients and the supporting data mostly come from retrospective case series.³¹⁻³⁴ A prospective multicenter phase II study of 79 patients with SCNSL tested alternating cycles of the MATRix regimen with rituximab, ifosfamide, carboplatin, etoposide (R-ICE) and responding patients received ASCT consolidation.³⁵ In this study, only 37 (47%) patients received an ASCT. Indeed, this strategy should be considered for suitable candidates, but is largely restricted to younger patients and those without co-morbidities.

Molecular biology of primary central nervous system lymphoma and secondary central nervous system lymphoma

Diffuse large B-cell lymphoma arises from the malignant transformation of B lymphocytes that are either engaged in, or exiting, the germinal center (GC) reaction. GC are transient microanatomic structures that form in secondary lymphoid tissue in response to antigenic stimulation, where GC B cells undergo somatic hypermutation of their immunoglobulin genes associated with intense competition for T cell prior to differentiation into plasma or memory B cells. As such, systemic DLBCL typically develops within lymphoid tissue, and tumor cells remain reliant on the lymph node microenvironment. This presents a biological challenge for CNS lymphomas, since GC are not found within the CNS, and the usual supportive microenvironment of the lymph node is absent. Whilst secondary central nervous system lymphoma (SCNSL) represents spread from cells that initiated as systemic DLBCL, the case of PCNSL is even more intriguing since these cases lack evidence of lymphoma outside of the CNS, and relapses are almost universally restricted to the CNS. The question of how and where these tumors initiate, and how they survive or

even depend upon the microenvironment of the CNS are key to understanding how biology might ultimately direct the therapy for CNS lymphomas. Two simplistic ‘pull or push’ hypotheses can be formed: firstly, the pull of some survival factor exclusive to the CNS, to which tumor cells are addicted, or secondly, the push from outside the CNS of some factor which tumor cells are unable to tolerate. Research over the last two decades, reinforced by recent genomic studies, suggest that both play a role in terms of neural-specific antigen within the CNS and host antitumor immunity outside the CNS. These studies highlight vulnerabilities that might be exploited for the biologically-directed therapy of CNS lymphomas. We will mainly consider CNS lymphomas that arise within immunocompetent patients, since these are a biologically homogeneous group, but also highlight important contrasts with the biology of CNS lymphomas in the immune deficient patient. We will focus on aspects of the biology that have the greatest therapeutic relevance.

Primary central nervous system lymphoma shares molecular biology with specific diffuse large B-cell lymphoma subtypes

Diffuse large B-cell lymphoma can be classified by gene expression profiling into subtypes that resemble either GC B cells (GCB DLBCL) or *in vitro* activated B cells (ABC DLBCL), and, more recently, genetic subtypes have emerged.³⁶⁻³⁹ Early work using immunohistochemistry showed that the majority of PCNSL cases could be classified as ABC DLBCL,⁴⁰ a finding borne out by transcriptional analysis using Nanostring.⁴¹ Early studies also revealed that PCNSL was enriched for mutations associated with ABC DLBCL. These included mutation of *MYD88*, *CD79B*, *TBL1XR1*, *PIM1*, and deletions of the *CDKN2A* and HLA loci.⁴²⁻⁴⁶ Since the advent of next-generation sequencing technologies, more than 250 PCNSL exomes or whole genomes have now been published.⁴⁷⁻⁵² Four main conclusions,⁵¹ summarized from across all these studies, capture the unique biological aspects of PCNSL: 1) an extremely high frequency of *MYD88* (67-86%) and *CD79B* (61%) mutations, implicating corrupted toll-like receptor (TLR) and B-cell receptor (BCR) signaling as a mechanism of enhanced NF κ B activity; 2) frequent mutations that impede onward differentiation and lock tumor cells into a GC-like proliferative state, including mutation of *TBL1XR1*, *PRDM1* and translocation of *BCL6*; 3) the near universal loss of negative regulator of cell cycle, *CDKN2A*, with only rare mutation of *TP53*; and 4) the very high frequency of mutations that lead to immune evasion, including deletion of HLA loci, or mutation of *B2M* or *CD58* (Figure 1).

Intriguingly, this genomic profile is shared with other forms of extranodal lymphoma such as primary DLBCL of the testis, breast, and skin.^{39,53} Moreover, this genetic profile of PCNSL aligns precisely to a specific subtype of systemic DLBCL, variably termed C5, MCD or MYD88, identified

from DLBCL genetic clustering studies.³⁶⁻³⁹ The C5/MCD/MYD88 subtype is characterized by BCR/TLR activation and immune evasion, and is strongly enriched for cases with extranodal involvement. Where genetic classifiers such as LymphGen have been applied to cases of PCNSL, almost all classified cases are found to fall within the MCD subtype,³⁹ this suggests an overlap between PCNSL and systemic MCD DLBCL in terms of biology, but also therapeutically exploitable vulnerabilities.

Addiction to chronic active B-cell receptor signaling

The strikingly high frequency of *MYD88* and *CD79* mutations points strongly to oncogenic NFκB signaling as a critical driver of PCNSL. These mutations affect specific hotspots, resulting in a leucine to proline change at amino acid 265 in *MYD88* (L265P) and the exchange of tyrosine 196 in *CD79B*. The function of these hotspot mutations

is already well established from the study of ABC DLBCL. *MYD88* is an adapter protein that is activated downstream of the toll-like receptor TLR9. It supports the assembly of a signaling complex that also includes IRAK 1 and IRAK4, thereby promoting activation of NFκB, and further enhanced by the L265P mutation.⁵⁴ *CD79B* forms a heterodimer with *CD79A* to create the signaling component of the B-cell antigen receptor. Tyrosine 196 sits within the immunoreceptor tyrosine-based activation motif (ITAM) and its mutation interrupts a negative feedback pathway, contributing to a hyperactive signaling state termed chronic active BCR signaling.⁵⁵ For reasons that remain elusive, this state of chronic active BCR signaling appears to be almost always associated with the IgM isotype. Despite strong expression of the class switching enzyme activation-induced cytidine deaminase (AID), PCNSL show blocked class switch recombination while retaining expression of surface IgM.⁵⁶

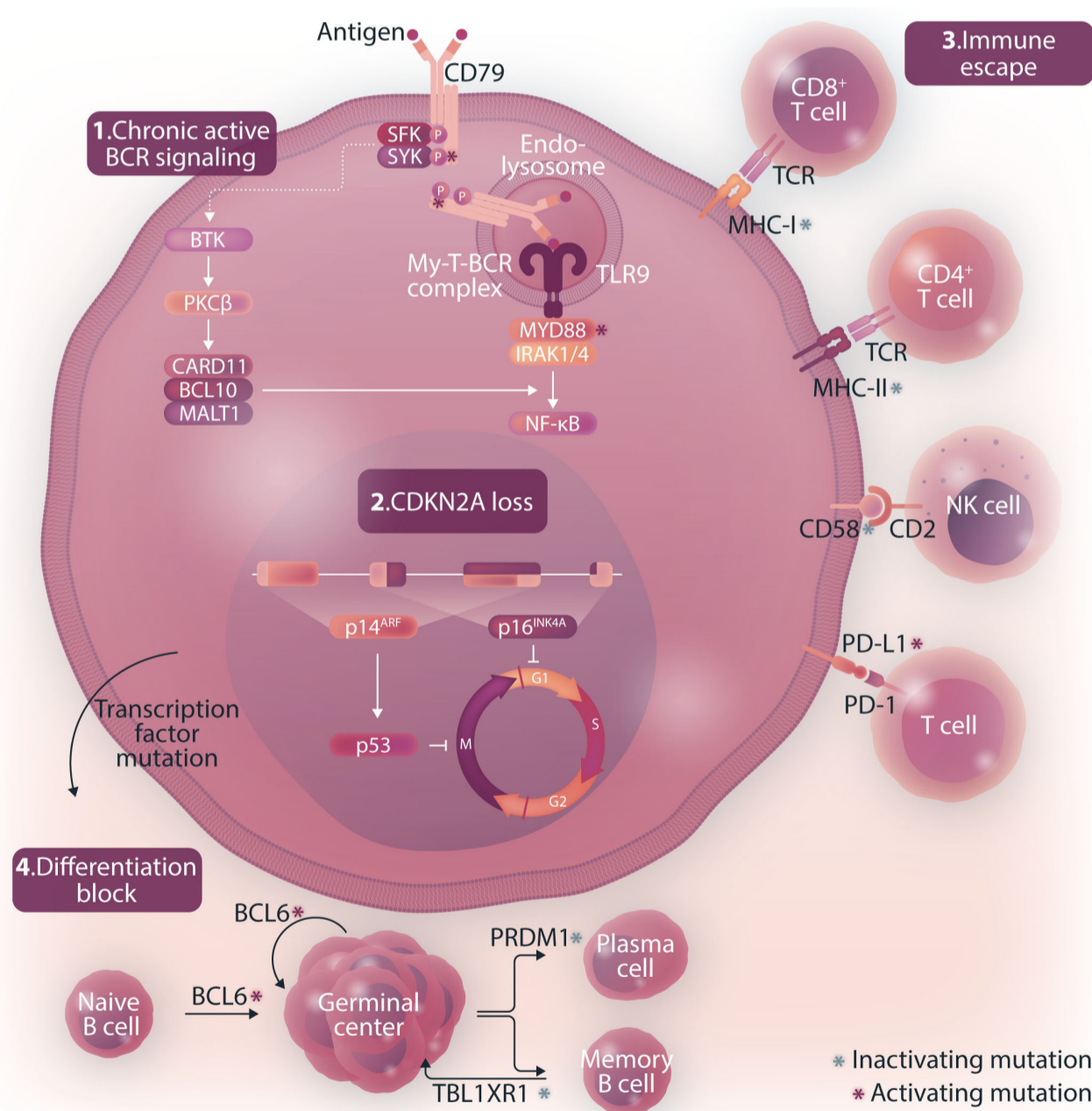


Figure 1. Four biological cornerstones of PCNSL biology. 1) Chronic active BCR signaling driven by neural-specific autoantigen, activating mutations of *MYD88* and *CD79B*, and formation of the MYD88-TLR9-BCR (My-T-BCR) complex. 2) Cell cycle activation secondary to deletion of the *CDKN2A* locus. 3) Escape from host immunity, resulting from loss of HLA expression, mutation of *CD58* or enhanced expression of immune checkpoint ligands. 4) Transcription factor mutations (*BCL6*, *PRDM1* and *TBL1XR1*) that block exit from, or promote re-entry into, the germinal center phenotype of proliferation and ongoing hypermutation. NK cell: natural killer cell.

MYD88, TLR9 and the IgM BCR have been found to associate into a multiprotein, oncogenic supercomplex known as the My-T-BCR, which drives activation of NF κ B in DLBCL. Consistent with the frequent double mutation of MYD88 and CD79B, the oncogenic My-T-BCR complex was strongly detected in biopsies of PCNSL, suggesting it is a critical driver of oncogenic NF κ B activity in PCNSL.⁵⁷ Importantly, the formation of this complex depends upon an active BCR signal and can be blocked by pharmacological inhibitors of BCR signaling such as ibrutinib, highlighting a potential therapeutic vulnerability in PCNSL.

In ABC DLBCL, CD79B ITAM mutations are frequently associated with a BCR signal that is initiated by engagement of autoantigen. Mouse models show how combined CD79B and MYD88 mutation allows autoreactive B cells to escape deletion.⁵⁸ Together with the strict tissue tropism seen in PCNSL, this has opened questions as to whether PCNSL might receive chronic BCR stimulation from a neural-specific autoantigen. In support of this, PCNSL shows strong bias towards usage of the immunoglobulin variable gene segment VH4-34, which encodes polyreactive or autoreactive immunoglobulin.⁵⁹⁻⁶¹ Moreover, PCNSL immunoglobulin genes show heavy somatic hypermutation that is biased towards codon-altering but non-destructive mutations, a pattern suggesting antigen-driven selection.⁶¹ Production of recombinant antibody based upon PCNSL immunoglobulin V gene sequences confirm that these antibodies are polyreactive and bind CNS proteins. Moreover, comparison of tumor sequences with the predicted naïve BCR showed that somatic hypermutation further increased self-reactivity, suggesting that immunoglobulin variants are selected according to their interaction with neural autoantigen.⁶² Protein microarrays have identified putative interacting neural antigens. Whilst PCNSL-derived recombinant antibodies failed to recognize common autoantigens, they showed reactivity to a large range of CNS-specific antigens.⁶³

Overall, these studies reveal the reliance of PCNSL on chronic activated signaling through the BCR. This may provide a partial explanation for the unique CNS tropism of PCNSL, but it also reveals an important tumor vulnerability that might be exploited by pharmacological inhibitors of the BCR pathway.

Heightened sensitivity to antitumor immunity

Despite their location in an immune-privileged site, PCNSL cells remain under strong selective pressure from the immune system. Although the need to escape immune recognition is shared with systemic DLBCL, one of the most striking features of PCNSL is the almost universal presence of genetic alterations that promote immune evasion.⁶⁴ This prominent immune vulnerability may be secondary to the high expression of the DNA mutator enzyme AID in PCNSL, which leads to ongoing aberrant somatic hypermutation and an increasing neoantigen burden.^{64,65}

Multiple studies show that most PCNSL tumors have lost expression of both MHC class I and class II.⁶⁶⁻⁶⁸ This results from deletions within the HLA locus on chromosome 6p, truncating mutations of individual HLA genes and in *B2M*.^{52,53,65,69,70} Inactivating mutations of *TAP1* and *TAP2* are additional mechanisms that limit immunogenic peptide display on MHC.^{39,65} Loss of MHC renders tumor cells invisible to cytotoxic T cells that typically infiltrate the PCNSL tumor microenvironment.⁶⁷ However, MHC I loss may render tumor cells susceptible to destruction by natural killer (NK) cells. CD58 is a cell surface antigen and ligand for the receptor CD2, expressed on T cells and NK cells. CD58-CD2 ligation is essential for tumor lysis by T or NK cells, and CD58 is commonly inactivated in DLBCL that has lost MHC I expression.⁷¹ Indeed, CD58 is mutated in over a third of PCNSL cases.⁶⁵ Mutations of CD70 and CD80 represent other mechanisms used by PCNSL to avoid cytotoxic T-cell killing.^{48,64,65}

An alternative immune evasion strategy is the expression of immune suppressive ligands such as PDL1 and PDL2, inducing an exhaustion phenotype in T cells. Copy number gains of 9p24.1, which contains the *PDL1* and *PDL2* loci, and structural variants of both loci leading to increased surface expression of PDL1 and PDL2, were reported in PCNSL.⁵³ However, the impact of immune checkpoint ligands in PCNSL remains controversial as increased surface expression on tumor cells was not identified in other studies.^{50,68} Regardless of this mechanism, single cell transcriptomic analysis has revealed clear signatures of T-cell exhaustion within the PCNSL microenvironment.⁷²

Overall, it appears that almost every case of PCNSL is associated with genetic evidence of immune evasion, highlighting a critical vulnerability. Whilst this raises enticing therapeutic possibilities, greater understanding is still required to define precisely which of these genetic alterations might render cells more, or perhaps less, sensitive to immunotherapeutic approaches.

The enigmatic origins of primary central nervous system lymphoma

A fascinating enigma is the precise origin of PCNSL. DLBCL arises from a B cell engaged in or exiting the GC. In ABC DLBCL, a simplistic model of malignant transformation includes NF κ B activation to drive proliferation and survival, combined differentiation block, precluding cells from exiting the GC phenotype. Superficially, this fits with the genetics of PCNSL, including chronic BCR activation leading to oncogenic NF κ B activation, and blocked plasma cell differentiation as a result of frequent *BCL6* translocation, or genetic loss of *PRDM1*.^{53,65,73} Moreover, high levels of somatic hypermutation in the immunoglobulin genes support this post-GC origin of PCNSL.⁶² However, there is scant evidence for GC formation within the CNS, suggesting that this step may occur in peripheral lymphoid tissue, or alternatively reflects AID induction through a GC-indepen-

dent process. A slightly different model has been proposed prompted by the high frequency of mutations in *TBL1XR1*. Mouse models show how mutant *TBL1XR1* drives B cells towards a memory B (MB) fate.⁷⁴ *TBL1XR1* mutant MB cells are unable to differentiate to plasma cells, but, instead, undergo repeated cycles of re-entry into the GC reaction upon subsequent antigen exposure. Mouse models show how MB differentiation is also promoted by *MYD88* mutation. Of particular relevance to PCNSL, these mice develop increased self-reactive MB cells that can be stimulated to reactivate and proliferate with minimal T-cell co-stimulation.⁷⁵ Therefore, the genetics of PCNSL suggest a model where mutant, polyreactive MB engage neural-specific autoantigen outside a formal GC environment, which drives cellular activation, proliferation, up-regulated AID expression, and subsequent somatic hypermutation, leading to enhanced autoantigen recognition and malignant transformation in the CNS.⁷⁶ The point at which B cells enter the CNS during this process of malignant transformation remains unclear. However, it is notable that *MYD88* mutant, non-tumor cells were detected in peripheral blood in PCNSL patients, suggesting this is an early initiating mutation that occurs outside the CNS.⁴⁸ The distinction is of significance as the early transformed cell may represent a common precursor cell (CPC) that seeds relapse and may be the source of late relapses.

Comparison to secondary central nervous system lymphoma and immune deficient central nervous system lymphomas

Whilst PCNSL in the immunocompetent patient represents a comparatively homogeneous entity as described above, SCNSL and PCNSL arising in the context of immune deficiency show important biological differences. Gandhi *et al.* studied the genetics of PCNSL in immune deficient patients, most associated with Epstein-Barr virus (EBV) infection.⁷⁰ In contrast to the genetic profile of immune competent patients, almost all cases lacked mutation of *MYD88* and *CD79B*. Moreover, most cases retained surface HLA expression. There was evidence of an induced immune tolerant microenvironment with increased macrophage infiltration and increased expression of PDL1 and PDL2 on the surface of microenvironmental cells. These findings suggest that EBV may itself drive NF κ B activation and immune evasion without the need for the genetic alterations seen in immune competent PCNSL. SCNSL also shows important distinctions. Whilst PCNSL is almost always classified as the ABC subtype of DLBCL, SCNSL covers a more diverse spectrum of biology comprising an equal division of ABC and GCB subtypes.⁷⁷ Systemic DLBCL of the MCD genetic subtype is associated with extranodal involvement, including sites such as kidney and adrenal, considered to be of high risk for CNS recurrence. MCD DLBCL with secondary CNS involvement may share considerable overlap with PCNSL. However, GCB DLBCL with CNS involvement represents a

different biological entity. For example, whilst *MYC* and *BCL2* proteins are highly expressed, translocation of these loci is rarely seen in PCNSL. In contrast, translocation of *MYC* and/or *BCL2* is enriched in GCB DLBCL involving the CNS. Indeed, *MYC* translocated lymphoma, including high-grade B-cell lymphomas with double-hit and Burkitt lymphoma, have an increased risk of CNS involvement. The important implication of all these contrasts is that biologically directed, therapeutic vulnerabilities of CNS lymphomas may differ depending on whether tumors arise as true PCNSL or, alternatively, in the context of immune deficiency or secondary to system disease.

Multiple studies have collectively made tremendous progress in dissecting the biology of CNS lymphomas. They paint a picture of PCNSL as a homogeneous biological entity, which, like systemic MCD DLBCL, is characterized by chronically activated BCR and NF κ B signaling combined with a pronounced requirement for immune evasion. This phenotype presents a specific set of therapeutic vulnerabilities, many of which are already being tested in clinical trials. However, there are important biological differences between true PCNSL and other forms of CNS lymphoma that may require a different treatment approach.

Rational targeted agents for central nervous system lymphomas

The critical question then becomes whether the improved mechanistic understanding of the therapeutic vulnerabilities of CNS lymphomas can be translated to improve clinical outcomes. To this end, rational targeted agents and combinations have been tested in prospective trials (Tables 1 and 2). Notably, targeted agents have been given on indefinite treatment schedules which may increase the toxicity over time. To reduce treatment duration and improve efficacy, many ongoing clinical trials are testing novel combinations with fixed duration treatment schedules (Table 3). Clinical studies in systemic DLBCL first demonstrated that the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib inhibits chronic active BCR signaling and has selective activity in ABC DLBCL tumors that harbor both *MYD88*^{L265P} and *CD79B* mutations.⁷⁸ Given that this genetic profile is common in PCNSL and subsets of SCNSL, BTK is a highly rational therapeutic target in CNS lymphomas, and multiple inhibitors of BTK are being tested.⁷⁹⁻⁸¹

Another class of rational targeted agents are the immunomodulatory drugs, lenalidomide and pomalidomide, which exert direct cytotoxic effects, recruitment of NK cells, up-regulation of CD80 and CD40, impairment of inflammatory cytokines, and effects on the tumor microenvironment.⁸² Lenalidomide down-regulates the transcription factor interferon regulatory factor 4 (IRF4) and augments interferon β production to kill ABC DLBCL cell lines *in vitro*.⁸³ Furthermore, pre-clinical models have demonstrated synergy between lenalidomide and ibrutinib to inhibit NF κ B signaling in ABC DLBCL cell lines.⁸⁴

A third rational strategy involves various forms of immu-

Table 1. Prospective studies of novel targeted agents as monotherapy in central nervous system lymphomas.

Novel mechanism	Novel agent	Design	Study population	Response rate, %	Response duration	Reference
Inhibitor of BTK	Ibrutinib	Phase I	PCNSL, relapsed (N=13)	ORR: 77 CR: 38	Median PFS 4.6 mths	Grommes <i>et al.</i> ⁸⁷
			SCNSL, relapsed (N=7)	ORR: 71 CR/CRu: 57	Median PFS 7.4 mths	
	Ibrutinib	Phase II	PCNSL, relapsed (N=52)	ORR: 52 CR/CRu: 19	Median PFS 4.8 mths	Soussain <i>et al.</i> ⁸⁸
	Tirabrutinib	Phase II	PCNSL, relapsed (N=44)	ORR: 64 CR/CRu: 34	Median PFS 2.9 mths	Narita <i>et al.</i> ⁷⁹
Immunomodulatory	Lenalidomide	Phase I	PCNSL, relapsed (N=7) SCNSL, relapsed (N=7)	ORR: 86 CR: 14 ORR: 57 CR/CRu: 29	PFS not reported	Rubenstein <i>et al.</i> ⁸⁹
Inhibitor of mTOR	Temsirolimus	Phase II	PCNSL, relapsed (N=37)	ORR: 54 CR/CRu: 22	Median PFS 2.1 mths	Korfel <i>et al.</i> ⁸⁶

CNS: central nervous system; PCNSL: primary CNS lymphoma; SCNSL: secondary CNS lymphoma; BTK: Bruton's tyrosine kinase; mTOR: mammalian target of rapamycin; PI3K: phosphoinositide 3-kinase; N: number; mths: months; ORR: overall response rate; CR: complete response; CRu: unconfirmed CR; PFS: progression-free survival.

Table 2. Prospective studies of novel targeted agents as combination therapy in central nervous system lymphomas.

Novel mechanism	Targeted agent	Design	Study population	Response rate, %	Response duration	Reference
Inhibitor of BTK	Ibrutinib + TEDD-R	Phase I	PCNSL (N=18)	ORR: 94 CR/CRu: 86	2-year PFS 66%	Lionakis <i>et al.</i> ⁹¹
			PCNSL (N=9)	ORR: 89 CR/CRu: 67	Median PFS not reached	Grommes <i>et al.</i> ⁹³
	SCNSL (N=6)	ORR: 67 CR/CRu: 33	Median PFS not reported			
Immunomodulatory	Lenalidomide + rituximab	Phase II	PCNSL (N=50)	ORR: 32 CR/CRu: 29	Median PFS 7.5 mths	Soussain <i>et al.</i> ⁹⁴
	Pomalidomide + dexamethasone	Phase I	PCNSL (N=25)	ORR: 48 CR/CRu: 32	Median PFS 5.3 mths	Tun <i>et al.</i> ⁹⁵

N: number; BTK: Bruton's tyrosine kinase; TEDD-R: temozolomide, etoposide, doxil, dexamethasone, rituximab; PCNSL: primary CNS lymphoma; SCNSL: secondary CNS lymphoma; ORR: overall response rate; CR: complete response; CRu: unconfirmed complete response; PFS: progression-free survival; HD-MTX-R: high-dose methotrexate and rituximab; mths: months.

notherapy, including immune checkpoint inhibitors and chimeric antigen receptor (CAR) T cells. Given the increased surface expression of PDL1 and PDL2 in PCNSL, targeting the PD-1 receptor has a biologic basis. CAR T-cell therapy is also rational for CNS lymphomas since this approach has efficacy in chemotherapy-refractory systemic DLBCL, but it remains unknown as to whether the underlying genetic basis promoting immune evasion in CNS lymphomas will limit the efficacy of these approaches.

Targeted agents as monotherapy for central nervous system lymphomas

A fundamental limitation of monotherapy for CNS lymphomas is rapid onset of drug resistance, as observed in systemic DLBCL.⁸⁵ Indeed, despite remarkably high initial response rates, the responses after BTK monotherapy are often incomplete and of short duration.⁸⁶ In a phase I study, escalating doses of ibrutinib monotherapy were given to 20 patients with relapsed or refractory PCNSL

(N=13) and SCNSL (N=7).⁸⁷ The clinical activity of ibrutinib was excellent and 10 (77%) PCNSL patients responded, including 5 (38%) who achieved a CR. Yet the durability of response was short and the median PFS was 4.6 months. The clinical activity of ibrutinib for SCNSL was also tested and 5 (71%) patients responded, including 4 (57%) CR, but the median PFS was 7.4 months. A multicenter phase II study tested ibrutinib in 52 patients with relapsed or refractory PCNSL and 27 (52%) patients responded, including 10 (19%) CR, but the median PFS was 3.3 months.⁸⁸ Taken together, these data show that ibrutinib is highly active in PCNSL, and probably in subsets of SCNSL, but resistance to monotherapy develops quickly.

In a phase I study, 14 patients with relapsed or refractory CNS lymphoma received lenalidomide at escalating doses and 6 (86%) patients with PCNSL responded, including one (14%) CR.⁸⁹ In SCNSL, 4 (57%) responses were observed, including 2 (29%) CR. The duration of responses was variable, but included 4 responses lasting >12 months.

Table 3. Ongoing clinical trials testing novel targeted agents or combinations in central nervous system lymphomas.

Novel mechanism	Targeted agent combination	Design	Study population	Target enrollment, N	Trial reference
Inhibitor of BTK	Zanubrutinib	Phase II	PCNSL and SCNSL, relapsed	20	NCT05117814
Inhibitor of BTK + chemotherapy	Ibrutinib + TEDD-R	Phase I	PCNSL, relapsed and untreated	40	NCT02203526
	Ibrutinib + TEDD-R	Phase II	SCNSL, relapsed and untreated	32	NCT03964090
	1) Ibrutinib + MER 2) Lenalidomide + MER 3) MER	Randomized phase II	PCNSL, relapsed	120	NCT04129710
	Orelabrutinib + MR	Phase II	PCNSL, untreated	Not reported	Ma <i>et al.</i> ⁸¹
	Tirabrutinib + MTR or R-MVP	Phase II	PCNSL, untreated	112	NCT04947319
	Inhibitor of BTK + Immunomodulator + chemotherapy	Zanubrutinib + lenalidomide + MTR	Phase II	PCNSL, untreated	40
Inhibitor of BTK + Inhibitor of PI3K	Ibrutinib + copanlisib	Phase I	PCNSL, relapsed	45	NCT03581942
Inhibitor of BTK + Immunomodulator	Ibrutinib + lenalidomide + rituximab	Phase Ib	PCNSL and SCNSL, relapsed	40	NCT03703167
Inhibitor of BTK + Immunomodulator + Inhibitor of BCL2	Venetoclax, ibrutinib, prednisone, obinutuzumab, revlimid	Phase I	PCNSL and SCNSL, relapsed and untreated	10	NCT05211336
Inhibitor of PD-1	Nivolumab	Phase II	PCNSL, relapsed	65	NCT02857426
	Pembrolizumab	Phase II	PCNSL and SCNSL, relapsed	52	NCT03255018
Inhibitor of BTK + Inhibitor of PD-1	Ibrutinib + nivolumab	Phase II	PCNSL and SCNSL, relapsed	40	NCT03770416
	Acalabrutinib + durvalumab	Phase I	PCNSL and SCNSL, relapsed	21	NCT04462328
Immunomodulator + Inhibitor of PD-1	Pomalidomide + nivolumab	Phase I	PCNSL, relapsed	23	NCT03798314

N: number; BTK: Bruton's tyrosine kinase; TEDD-R: temozolomide, etoposide, doxil, dexamethasone, rituximab; PCNSL: primary central nervous system lymphoma; SCNSL: secondary CNS lymphoma; MER: high-dose methotrexate, etoposide, rituximab; MR: high-dose methotrexate, rituximab; MTR: high-dose methotrexate, temozolomide, rituximab; R-MVP: high-dose methotrexate, rituximab, procarbazine, vincristine; PI3K: phosphoinositide 3-kinase; PD-1: programmed death ligand.

A retrospective series of 4 patients with relapsed and refractory CNS lymphoma treated with the immune checkpoint inhibitor nivolumab demonstrated clinical responses in all patients.⁹⁰ Two ongoing phase II studies of PD-1 inhibitors as monotherapy are ongoing and have yet to publish clinical results.

Combination targeted therapy for central nervous system lymphomas

In order to overcome the drug resistance that rapidly occurs after monotherapy, prospective clinical trials

have tested combination regimens for CNS lymphoma. In a phase I study, patients with relapsed or refractory PCNSL (N=13) and untreated PCNSL (N=5) were treated with escalating doses of ibrutinib monotherapy along with temozolomide, etoposide, doxil, dexamethasone, and rituximab (TEDDi-R), and most patients achieved a CR, including patients refractory to HD-MTX-based regimens.⁹¹ A follow-up report on this small series suggests that remissions were often durable.⁹² A major limitation of this regimen was the risk of developing aspergillosis infections when no antifungal prophylaxis was given. This regimen

has been revised to include anti-fungal prophylaxis and is being studied in both PCNSL and SCNSL (clinicaltrials.gov identifiers NCT02203526 and NCT03964090).

Ibrutinib has also been safely added to HD-MTX and rituximab in CNS lymphomas.⁹³ In a phase Ib study, 15 patients with relapsed PCNSL (N=9), relapsed SCNSL (N=3), and untreated SCNSL (N=3) received HD-MTX, rituximab, and ibrutinib. No unexpected toxicities were observed and no cases of aspergillosis were reported. In PCNSL, 8 (89%) patients responded, including 6 (67%) CR. The durability of response to the regimen was unclear as 4 of the responding patients received ASCT consolidation. In 4 cases without consolidation, 3 remained in remission >10 months. In SCNSL, 4 (67%) patients responded and 2 (33%) achieved a CR. Two responding patients progressed after a remission of <6 months, one received an ASCT, and one had an ongoing remission of 6 months. Numerous combination regimens using ibrutinib and second-generation BTK inhibitors, including zanubrutinib, acalabrutinib, tirabrutinib and orelabrutinib, are now being tested in ongoing clinical trials.

Lenalidomide and pomalidomide have both been tested as combination therapy for relapsed or refractory PCNSL.^{94,95} In a multicenter phase II study, 50 patients with relapsed or refractory PCNSL received lenalidomide and rituximab for 8 cycles and responding patients continued lenalidomide monotherapy for another 12 cycles.⁹⁴ The overall response rate was 32%, including 13 (29%) CR, but the median PFS was only 7.8 months. In a phase I study, escalating doses of pomalidomide were tested in combination with dexamethasone for 2 cycles followed by pomalidomide monotherapy until disease progression.⁹⁵ In 25 evaluable patients, the overall response rate was 48% including 8 (32%) CR. The median PFS for this study was 5.3 months. Taken together, these results support immunomodulatory agents as clinically active and biologically rational targeted agents for CNS lymphomas that are likely to be most effective as part of combination therapy.

CAR T-cell therapies targeting CD19 can overcome chemotherapy resistance in systemic DLBCL, and have been tested in small studies for CNS lymphoma.^{96,97} One concern about CAR T-cell therapy for CNS lymphomas is related to a potential increased incidence in immune cell-associated neurotoxicity syndrome (ICANS). In a pilot study of 12 patients with PCNSL, tisagenlecleucel resulted in a response in 7 (58%) patients, including 6 (50%) CR. Notably, only one patient had grade 3 ICANS. In another pilot study of axicabtagene ciloleucel in 6 patients with PCNSL and 3 patients with SCNSL, a CR was achieved by 6 (86%) patients with 3 months of follow-up and only one case of grade 3 or higher ICANS. These results suggest that effector CAR T cells cross the blood brain barrier and can induce remissions in CNS lymphoma. Indeed, a meta-analysis of 128 patients with CNS lymphoma suggested that the safety and efficacy of anti-CD19 CAR T-cell therapy is similar to that observed

in systemic DLBCL.⁹⁸ These strategies have only very short follow-up and many questions remain regarding persistence of CAR T cells in the CSF, mechanisms of treatment failure, and if biologic differences between PCNSL and SCNSL will impact treatment efficacy.

Fundamental questions and future directions

Primary and secondary DLBCL involving the CNS pose unique clinical challenges regarding delivery of therapy into tumor tissue, treatment-related toxicities, and the diversity of the underlying biology. Recent insights regarding the molecular biology of PCNSL and DLBCL subsets with CNS predilection, along with an expanding list of effective targeted agents, have introduced important fundamental questions that can only be answered with prospective clinical trials. What are the safest and most effective targeted agents for CNS lymphoma, and which combinations are tolerable in patients of all ages? Will predictive biomarkers based on the underlying biology be identified, and will they be reliable enough to select therapy? Will technological advances such as next-generation sequencing of cell-free DNA in the plasma and/or CSF help in clinical decision-making?⁹⁹ Well-designed clinical trials with strong translational molecular endpoints will be essential to address these questions and ultimately improve the cure rate of CNS lymphoma.

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No conflicts of interest to disclose.

Contributions

MR and DJH contributed equally to this manuscript.

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Data-sharing statement

Relevant primary source scientific publications are cited in the References section.

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