Beyond high-dose methotrexate and brain radiotherapy: novel targets and agents for primary CNS lymphoma

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Received 23 May 2013; revised 14 August 2013; accepted 16 August 2013

Background: While there has been significant progress in outcomes for patients diagnosed with primary central nervous system (CNS) lymphoma (PCNSL), survival rates will likely plateau with the current armamentarium of agents used to treat these patients. Moreover, given that PCNSL increasingly impacts an older population, a significant proportion of patients are not eligible for intensive therapies such as high-dose chemotherapy or whole-brain radiation. There is a need for the development of novel agents, which target key survival pathways in order to continue to make progress in this disease.

Patients and methods: We reviewed the key molecular pathways and genomic aberrations in PCNSL in order to identify candidate targets. We focused on molecules and pathways that have been identified and confirmed by more than one investigator or methodology.

Results: While PCNSL tumors usually express a BCL6+, MUM1+ ‘activated, germinal center’ immunophenotype, they exhibit multiple shared genetic properties with ABC-type diffuse large B-cell lymphomas. Candidate targets and pathways include NFkB, the B-cell receptor, the JAK/STAT pathway, IRF4, BCL-6 as well as PIM kinases. Elements of the tumor microenvironment that may be exploited therapeutically include chemokine pathways, as well as macrophage and T-cell responses.

Conclusions: There is a significant need for developing novel therapies in PCNSL, given that an increasing proportion of patients are not eligible for high-dose chemotherapy and brain radiation is associated with detrimental cognitive side-effects. We provide an overview of potential drug targets and novel agents that may be integrated with existing strategies in order to make further progress in this disease.

Key words: B-cell receptor, novel agents, primary CNS lymphoma, protein kinases, tumor microenvironment

introduction

Since the 1960s, the cornerstone of therapy for primary central nervous system (CNS) lymphomas (PCNSL) has been whole-brain radiotherapy. The use of whole-brain irradiation for this radiosensitive tumor has historically been of great value in the production of immediate responses to patients who otherwise faced a rapidly deteriorating course caused by an unusual type of brain tumor rarely encountered in clinical practice. Through the 1960s, physicians had no prospective data to guide management of patients diagnosed with this neoplasm, historically known as reticulum cell sarcoma or microglioma [1, 2], and the results were consistent, and the median survival for PCNSL was on the order of 12 months [3]. However, treatment strategies for primary and secondary CNS lymphomas began to improve in the late 1970s when studies carried out by Canellos et al. demonstrated the remarkable efficacy of systemic high-dose methotrexate plus leukovorin rescue in the treatment of recurrent CNS lymphomas [4, 5]. It is now recognized that large-cell lymphoma within the brain microenvironment has, for biological reasons that are unclear, approximately twofold greater sensitivity to high-dose methotrexate compared with systemic lymphomas of the same histology [6].

DeAngelis et al. pioneered a combination regimen consisting of high-dose systemic methotrexate plus CNS penetrating agents such as procarbazine followed by whole-brain irradiation and high-dose cytarabine, and demonstrated long-term survival in a subset of patients [7, 8]. Because of this encouraging efficacy, combined-modality therapy became a widely adopted approach for patients with PCNSL [9, 10]. Ultimately, however, hematologists and oncologists who managed brain tumor patients inevitably encountered the profoundly deleterious neurocognitive effects of whole-brain irradiation, particularly evident in CNS lymphoma patients who often lived longer than other patients.
who received whole-brain radiotherapy. Early studies of the profound neurotoxicity of whole-brain radiotherapy in CNS lymphoma patients, particularly evident in those >60, began to be reported in the literature in the late 1980s [7]. Because the median age of PCNSL patients in most series is approximately 56, and because age-related treatment-induced neurotoxicity is likely a continuous variable, it has been appreciated that a very large proportion of PCNSL patients are at high risk for clinically significant delayed radiation injury from standard-dose whole-brain irradiation [11, 12]. For this reason, in parallel, a reductionist approach has been to maximize the potential efficacy of repeat cycles of high-dose methotrexate as monotherapy, without consolidative brain irradiation [13, 14]. In some clinical series, this approach appeared to yield rates of long-term survival comparable with that achieved with combined modality therapy [15]. Given that the incidence of PCNSL is increasing in patients aged >65 years [16], a population most vulnerable to treatment-related toxicities, high-dose methotrexate as monotherapy, which is generally well-tolerated, has been prescribed for many years with significant efficacy both at induction and at relapse in older patients [17]. Moreover, an important, randomized trial carried out by Thiel et al. demonstrated that the omission of standard dose whole-brain radiotherapy as consolidation after methotrexate-based induction chemotherapy had no effect on overall survival [18]. It should be noted, however, that reduced-dose whole-brain radiotherapy at consolidation has also shown favorable results for newly diagnosed PCNSL patients, [19] without documented evidence of neurotoxicity, and that this novel approach to combined-modality therapy is the basis for further evaluation in an active radiation therapy oncology group protocol.

Given that >95% of CNS lymphomas are B-cell neoplasms which express CD20 [20], beginning with its FDA approval in 1997, there has been significant interest in the potential utility of rituximab in the treatment and/or prophylaxis of CNS lymphomas. Because of the poor penetration of intravenous rituximab within the leptomeningeal compartment [21] and in areas of CNS lymphoma protected by the blood–brain barrier, one experimental approach which has been explored in two multicenter phase I trials has been via intrathecal delivery, both as monotherapy and in combination with methotrexate. These studies have provided evidence for the safety and activity of intraventricular rituximab, not only in the CSF but also in lesions up to 2 cms in size within the brain parenchyma and intracranial compartments [22, 23]. In addition, since 2001 there have been several prospective studies of intravenous rituximab in combination with methotrexate as induction treatment of PCNSL, yielding promising results, although randomized data evaluating the impact of rituximab as part of induction therapy have not yet been presented [19, 24]. Single-agent activity of intravenous rituximab in the treatment of recurrent CNS lymphoma has also been demonstrated [25]. Combination intraventricular plus intravenous rituximab for recurrent CNS lymphoma is currently under evaluation in the phase I setting (NCT01542918).

Another innovative approach that has been extensively studied in the treatment of CNS lymphomas has been the pharmacologic disruption of the blood–brain barrier to facilitate enhanced delivery of chemotherapy agents such as methotrexate [26]. Access to this treatment, however, is limited to specialized centers with technical expertise, and even in the best of hands, iatrogenic blood–brain barrier disruption may be associated with a high peri-procedural rate of seizures that affects up to a third of patients. Also, the therapeutic efficacy of this approach does not appear to be superior to regimens that employ conventional intravenous delivery of drugs [27]. Nevertheless, lessons learned from this pioneering approach may be informative in the consideration of enhanced delivery of biological therapies such as rituximab and other agents [28].

Prospective analyses of high-dose chemotherapeutic consolidation, using both myeloablative and non-myeloablative approaches, have been pursued for >15 years for patients with CNS and intraocular lymphomas [24, 29–31]. There is now published evidence for the efficacy of this approach in the cooperative group setting [27]. However, high-dose chemotherapeutic strategies obviously have limited utility for older patients with PCNSL (age >70), a subgroup at high risk for severe complications of chemotherapy and whole-brain radiotherapy.

Despite these advances, it has become clear that there exists a plateau in the anticipated progression-free survival for newly diagnosed patients with PCNSL. While >50% of patients typically achieve a complete response and Kaplan–Meier survival curves in recent studies suggest an ~40%–50% rate of long-term progression-free survival, in nearly every clinical series and trial, between 20% and 30% of patients succumb to tumor progression within the first 6 months of treatment, suggestive of the existence of a biologically distinct form of PCNSL that exhibits primary drug resistance [24, 32, 33].

Given that the majority of clinical interventions in PCNSL currently being evaluated are based upon combinations of older chemotherapy agents, such as methotrexate, thiotaque and cytarabine, there is a critical need to develop and implement novel, biologically based strategies in order to realize progress for the other 50% of patients, not cured with current genotoxic and immunotherapeutic strategies. The goal of this review is to provide an outline of current knowledge regarding the biological properties of CNS lymphomas that may be exploited to develop new therapies and to provide a roadmap for their implementation. While a relatively large number of reports describe molecules and genomic aberrations that may be targeted, we will focus on pathways that have been identified in more than one study and replicated by more than one methodology.

**Histopathology and significance of the PCNSL immunophenotype**

The majority of PCNSL cases (>95%) are diffuse large B-cell lymphomas (DLBCL) which exhibit a uniform activated B-cell like immunophenotype with ~95% staining positive for MUM-1, between 50% and 80% positive for BCL-6 and ~10% positive for CD10 [34, 35]. These findings, combined with evidence that PCNSL tumors exhibit ongoing immunoglobulin gene somatic hypermutation, [36], suggest that PCNSL DLBCL displays an activated germinal center B-cell origin. Based upon the fact that PCNSL tumors require distinct therapeutic protocols and display unique transcriptional features by gene expression profiling [37–40], PCNSL is recognized as a distinct histologic subtype by current WHO classification [41].

Given their unique immunophenotype, unlike systemic DLBCL, outcome prediction based upon the Hans algorithm is not
reproducible in PCNSL [42]. The prognostic determination based upon individual markers from this algorithm in PCNSL is either indeterminate, e.g. MUM-1 expression is not prognostic, or of variable significance, e.g. BCL6 is associated with favorable prognosis in some studies but not others [34, 35, 43–45]. A significant factor that may contribute to the heterogenous prognostic relevance of BCL6 is that the vast majority of these studies are retrospective in nature, evaluated small numbers of patients and involved different therapeutic regimens, including rituximab and radiation. Notably, the first prospective analysis of prognostic biomarkers conducted in the setting of a clinical trial was recently carried out with CALGB 50202; sufficient biopsy material was available for immunohistochemical staining in 59% of diagnostic specimens from patients who participated in this phase II investigation. In this setting, high BCL6 expression (immunoreactivity in >30% of neoplastic nuclei) was detected in 59% of cases, consistent with previous reports; however, high BCL6 was shown to correlate as a continuous variable with inferior progression-free and overall survival, with the most significant cut-point for dichotomizing BCL6 expression determined to be 60% [27].

The prognostic significance of BCL6 expression in PCNSL, a disease generally associated with overall poor prognosis, may be of secondary importance, given that there is general agreement that high relative expression of BCL6 can be detected in the majority of PCNSL cases, and that this proto-oncogene promotes aberrant proliferation, genomic instability and lymphoma survival in DLBCL [46]. Therefore, independent of its prognostic significance, its overall high expression suggests a potential role for BCL6 antagonists in the next generation of therapies for PCNSL, potentially in combination with other genotoxic strategies [47].

Another immunophenotypic marker in PCNSL, which also has significant therapeutic implications, is MUM1 (Interferon Regulatory Factor 4, IRF4). In normal germinal center B cells, expression of MUM1 and BCL6 is mutually exclusive with MUM1 expression highest in late stages of B-cell differentiation. MUM1 may contribute to the pathogenesis of B-cell malignancies such as B-cell lymphomas and multiple myeloma via transcriptional upregulation of MYC as well as other genes. Given the evidence that the IMiD class of biological agents, lenalidomide and pomalidomide, mediate therapeutic efficacy via down-modulation of MUM1/IRF-4, in a cerebro-enumerator-dependent manner [48–50], there is a reason to believe that these drugs may have significant activity in PCNSL. Indeed, there are reports of single-agent activity of lenalidomide in recurrent, refractory intraocular DLBCL as well as recurrent blastoid variant mantle cell lymphoma in the CNS [51, 52]. Based upon these data, and given the evidence of synergy between lenalidomide and rituximab [53], a phase II trial of lenalidomide plus intraventricular and intravenous rituximab in recurrent/refractory CNS and Intraocular Lymphoma is now in progress (NCT01542918). In addition, a trial of pomalidomide in CNS lymphoma is currently active (NCT01722305).

molecular genetics and transcriptional profile of PCNSL

Determination of the genetic features that underlie the molecular pathogenesis of PCNSL is significantly more difficult than for systemic DLBCL, largely because of the limited biological material remaining after diagnostic evaluation. Most pathologic specimens are obtained from stereotactic biopsies or via cytologic or flow-cytometric analysis of cerebrospinal fluid. Because the majority of studies which describe the molecular features of PCNSL are based on small studies and many have yielded conflicting results, here we focus on molecules and pathways that have been identified and confirmed by more than one investigator or methodology. The most frequent genomic aberrations identified in PCNSL are focal losses on chromosome 6p21 (HLA locus), as well as deletions on chromosome 6q21–6q25 [54–56]. Homozygous deletions as well as silencing by DNA methylation of CDKN2A, a cell cycle regulator, are detected in ~45% of cases and have been correlated with adverse prognosis [57, 58]. A number of candidate genes are linked to chromosome 6q, including PRDM1, a tumor suppressor which regulates B-cell differentiation [59], PTPRK, a protein tyrosine phosphatase involved in the regulation of cell adhesion [60], and A20 (TNFAIP3), a key negative regulator of NFκB signaling, located on 6q23 [61]. A variety of evidence supports aberrant activation of the NFκB pathway in PCNSL [62], including increased DNA copy number for MALT1 [57] and activating mutations of CARD11 [63] and MyD88 (toll-like receptor pathway). However, while the oncoenergically activating exchange of leucine to proline at position 265 of MyD88 is the most common mutation yet identified in PCNSL, detected in between 38% (11/29) to 50% (7/14) of patients, depending on the series, its occurrence did not correlate with adverse prognosis in multivariate analysis [58, 64].

Mutational analysis of genes encoding the components of the B-cell receptor signaling pathway demonstrated a high frequency of aberrations in the coding region of CD79B, (20%) suggesting dysregulation of the B-cell receptor pathway in PCNSL as a potential therapeutic target [65].

Given the overall small size of these clinical series, it is difficult to ascertain whether the mutational frequency of the components of the B-cell receptor and toll-like receptor pathways are different from the frequency reported for systemic DLBCL. Nevertheless, taken together, these data suggest that pharmacologic agents that attenuate proximal signals that promote NFκB signaling in PCNSL may hold promise in the treatment of this variant form of DLBCL. Candidate agents which merit evaluation are of course pharmacologic antagonists of the B-cell receptor and downstream mediators including SYK, Protein kinase C-δ, PI-3 kinase-δ and MALT1 [66–68].

Transcriptional profile analyses comparing PCNSL with both nodal DLBCL as well as extranodal DLBCL have also provided useful insights into disease mechanisms, which underlie its pathogenesis. For example, relative upregulation of the MYC oncogene in PCNSL was demonstrated using a platform that compared miRNA transcript expression levels between PCNSL and nodal DLBCL [38]. An independent study comparing microRNA’s (miRNA’s) between PCNSL and nodal DLBCL also demonstrated the upregulation of several miRNA’s associated with the MYC pathway (miR-17-5p, miR-20a, miR-9) [69]. Finally, immunohistochemical analysis of diagnostic specimens of PCNSL patients enrolled in CALGB (Alliance) 50202 also identified a high relative degree of significant MYC protein expression in PCNSL, although MYC did not correlate with prognosis [27, 70].
Another potential pharmacologic target in PCNSL is the PIM family of serine/threonine kinases, which may synergize with MYC in pro-survival signaling and in drug resistance [71]. PIM expression appears to be upregulated in CNS lymphomas, both at diagnosis and potentially at relapse [22, 38]. In addition, somatic mutations in PIM1 have been identified in PCNSL, consistent with aberrant activation in this disease [36, 58]. These results may suggest a role for pharmacologic antagonists of the PIM pathway in CNS lymphoma, perhaps as a means to sensitize tumors to chemotherapy [72, 73].

Several lines of evidence suggest that the JAK/STAT pathway may also contribute to survival signaling in PCNSL. CNS lymphoma tumor cells express IL-10 transcript and protein, and elevated levels of this B-cell survival factor which signals via the JAK/STAT pathway, have been reported in CSF and correlate with adverse prognosis [74, 75]. Notably, IL-10 concentration has reproducibly been demonstrated to be elevated in the vitreous fluid in intraocular lymphoma, consistent with the overlapping biological properties of CNS and intraocular lymphomas as closely related entities [76, 77]. Expression of another B-cell growth factor, IL-4, has also been shown to be upregulated in CNS lymphoma and localized to the tumor vasculature; IL-4 may also mediate survival signaling via the JAK/STAT pathway. In addition, JAK1 transcripts have been reproducibly demonstrated to be upregulated in PCNSL [38, 78] and there is evidence for selective JAK1 activation in PCNSL cases [75]. Finally, there is evidence for both expression and/or activation of STAT3 and STAT6 in PCNSL tumors, [38, 79, 80] as well as genomic data demonstrating amplification of the locus encoding STAT6 on chromosome 12, detected in greater than 50% of PCNSL specimens [57]. Data implicating upregulated expression of IL-10 and the JAK/STAT pathway are consistent with underlying activation of the MyD88 pathway in this disease [81]. Taken together, these findings support the evaluation of pharmacologic antagonists of the immune suppressive cytokines IL-4 and IL-10, their receptors, as well as the downstream mediator the Janus kinases in PCNSL.

One of the IL-4 target genes that has been shown to be upregulated in PCNSL and which has been reproducibly associated with adverse prognosis is XBP-1, a marker of plasma cell differentiation and a transcriptional regulator of the unfolded protein response (UPR) pathway [38, 82, 83]. Given the mounting evidence that the UPR may be a druggable target in cancer and in other diseases [84, 85], the implementation of novel agents which target this pathway may be useful in high-risk cases of PCNSL as well.

Finally, there is also significant interest in the development of novel therapies in cancer via agents that target protein synthesis. A variety of evidence demonstrates upregulation of translational regulatory proteins EIF4A, EIF4E and EIF5 in PCNSL. Notably there is recent evidence that pharmacologic antagonism of translational initiation targeting EIF4E may be effective in a preclinical model, supporting further analysis of this potential strategy in PCNSL [38, 86]. Pharmacologic inhibition of the mTOR pathway may also effectively target translational inhibition and suppress PCNSL progression.

**the tumor microenvironment in PCNSL**

The single-biological property that is most unique to PCNSL is its selective tropism to the brain microenvironment; a longstanding central question therefore is: what are the chemokines or other neurotropic signals that promote attraction and induce the retention of DLBCL cells to the brain, meninges and intraocular compartments? A variety of studies have demonstrated expression of the B-cell chemokines CXCL12 and CXCL-13 within intracranial and CNS lymphoma with brain parenchyma [87–89]. Both of these chemokines mediate chemotaxis of CNS lymphoma cells and there is evidence that elevated CXCL13 levels in CSF are associated with adverse prognosis, supporting its role as a potential survival factor [75]. Given that these chemokines potentially mediate survival signals in B-lymphoma cells via either CXCR4 as well as CXCR5 or the B-cell receptor [90, 91], there is a reason to hypothesize that targeting CXCL12 and CXCL13 may potentiate apoptotic responses and promote clinical responses in otherwise refractory tumors. Recent data also suggest that PI3 kinase inhibitors as well as pharmacologic antagonists of the CXCR4 receptor may be effective in this regard [92, 93].

While the CNS is typically assumed to be an immunologically privileged site, histopathologic evidence often demonstrates a robust inflammatory response within CNS lymphomas, including infiltrating activated macrophages and reactive T-cells. There is reproducible evidence that the presence of reactive perivascular T-cell infiltrates is associated with a favorable outcome in PCNSL [83, 94], supporting the potential of immunotherapies that potentiate T-cell-mediated immune surveillance.

Finally, given that natural killer cells are rare in both normal brain and CNS lymphomas [95], a variety of evidence suggest that infiltrating macrophages and microglia may be important mediators of immune surveillance and perhaps contribute to therapeutic efficacy of rituximab in CNS lymphomas [23, 96]. Further understanding of macrophage activation and phenotypes in these brain tumors is also likely to promote advances in patient outcomes [79].

**conclusions**

During the past 50 years, the oncology community has made remarkable progress in the treatment of PCNSL, a highly malignant type of adult brain tumor. Instead of a median survival of 12 months, it now appears that ∼40–50% of patients will exhibit long-term survival and many may be cured. Although we have made these advances with extremely limited insight into the molecular pathogenesis of this disease, and via empiric application of a relatively arcane set of chemotherapeutic agents, we are
optimistic that further progress in achieving improved rates of survival may be within reach for PCNSL patients. The systematic investigation of novel interventions based upon the biological pathways outlined in this review may facilitate this progress (Table 1). Importantly, given that not all small molecule agents are effective in achieving therapeutic concentrations across the blood–brain barrier [97, 98], an additional major challenge will be to apply strategies to safely attain effective concentrations of these agents throughout the CNS lymphoma microenvironment [99]. Advances are clearly needed in this disease, particularly given its predilection for an aging population, in which a significant proportion cannot tolerate current strategies based on high-dose chemotherapy and/or whole-brain radiotherapy.

acknowledgements
We are grateful to our patients and to our colleagues in the International Primary CNS Lymphoma Collaborative Group.

funding
The work was supported by the National Institutes of Health, University of California San Francisco-Gladstone Institute of Virology & Immunology Center for AIDS Research (P30 AI027763), NIH RO1CA139-83-01A1, UCSF Brain Tumor SPORE, the Leukemia & Lymphoma Society (JLR) and R13 CA124293-06A1 (TTB).

disclosure
JLR receives research funding from Genentech and Celgene for a phase I clinical trial. All remaining authors have declared no conflicts of interest.

references


Antibody-based immunotherapy for ovarian cancer: where are we at?

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Received 3 December 2012; revised 24 June 2013 & 6 August 2013; accepted 27 August 2013

Cytoreductive surgery and chemotherapy continue to be the mainstay of ovarian cancer treatment. However, as mortality from advanced ovarian cancer remains very high, novel therapies are required to be integrated into existing treatment regimens. Immunotherapy represents an alternative and rational therapeutic approach for ovarian cancer based on a body of evidence supporting a protective role of the immune system against these cancers, and on the clinical success of immunotherapy in other malignancies. Whether or not immunotherapy will have a role in the future management of ovarian cancer is too early to tell, but research in this field is active. This review will discuss recent clinical developments of selected immunotherapies for ovarian cancer which fulfill the following criteria: (i) they are antibody-based, (ii) target a distinct immunological pathway, and (iii) have reached the clinical trial stage. Specifically, the focus is on Catumaxomab.