Annals of Oncology 25: 316–322, 2014
doi:10.1093/annonc/mdt385
Published online 20 November 2013

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

M. Ponzoni[1], S. Issa[2], T. T. Batchelor[3] & J. L. Rubenstein[4]*

[1]*Pathology Unit and Unit of Lymphoid Malignancies, San Raffaele Scientific Institute, Milan, Italy;* [2]*Department of Haematology, Middlemore Hospital, Auckland, New Zealand;* [3]*Division of Neuro-oncology, Departments of Neurology and Radiation Oncology, Massachusetts General Hospital, Boston;* [4]*Division of Hematology/Oncology, Helen Diller Comprehensive Cancer Center University of California, San Francisco, USA*

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 penetrant 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

*Correspondence to: Prof. James L. Rubenstein, University of California, San Francisco, Division of Hematology/Oncology M1282 Box 1270 San Francisco, CA 94143, USA. Tel: +1-415-502-4430; fax: +1-415-476-0624; E-mail: jamesr@medicine.ucsf.edu

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 intraocular 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, thiotepa 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 downmodulation of MUM1/IRF-4, in a cereblon-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 I 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 oncogenically 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 DLBLCL. 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-β, PI-3 kinase- δ and MALT1̃ [66–68].

Transcriptional profile analyses comparing PCNSL with both nodal DLBCL as other 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 mRNA 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.

Table 1. Candidate investigational agents in CNS lymphoma

Candidate pathway	Investigational agent
B-cell receptor	Ibrutinib, fostamatinib, BKM120, GA101
JAK/STAT	Ruxolitinib
IRF4/MUM1	Lenalidomide, pomalidomide
BCL-6	RI-BPI
NFkB	MALT1 inhibitors
CXCL12, CXCL13	Plerixafor (AMD3100), BKM120, GA101
PIM kinases	SGI-1776
Mtor	Temsirolimus, everolimus

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 intraocular 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 R01CA139-83-01A1, 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

1. Barnett LB, Schwartz E. Cerebral reticulum cell sarcoma after multiple renal transplants. J Neurol Neurosurg Psychiatry 1974; 37: 966–970.
2. Benjamin I, Case ME. Primary reticulum-cell sarcoma (microglioma) of the brain with massive cardiac metastasis. Case report. J Neurosurg 1980; 53: 714–716.
3. Henry JM, Heffner RR, Jr., Dillard SH et al. Primary malignant lymphomas of the central nervous system. Cancer 1974; 34: 1293–1302.
4. Ervin T, Canellos GP. Successful treatment of recurrent primary central nervous system lymphoma with high-dose methotrexate. Cancer 1980; 45: 1556–1557.
5. Skarin AT, Zuckerman KS, Pitman SW et al. High-dose methotrexate with folinic acid in the treatment of advanced non-Hodgkin lymphoma including CNS involvement. Blood 1977; 50: 1039–1047.
6. Bokstein F, Lossos A, Lossos IS et al. Central nervous system relapse of systemic non-Hodgkin's lymphoma: results of treatment based on high-dose methotrexate combination chemotherapy. Leuk Lymphoma 2002; 43: 587–593.
7. Abrey LE, DeAngelis LM, Yahalom J. Long-term survival in primary CNS lymphoma. J Clin Oncol 1998; 16: 859–863.
8. DeAngelis LM, Yahalom J, Heinemann MH et al. Primary CNS lymphoma: combined treatment with chemotherapy and radiotherapy. Neurology 1990; 40: 80–86.
9. DeAngelis LM, Yahalom J, Thaler HT et al. Combined modality therapy for primary CNS lymphoma. J Clin Oncol 1992; 10: 635–643.
10. Glass J, Gruber ML, Cher L et al. Preirradiation methotrexate chemotherapy of primary central nervous system lymphoma: long-term outcome. J Neurosurg 1994; 81: 188–195.
11. DeAngelis LM. Primary CNS lymphoma: treatment with combined chemotherapy and radiotherapy. J Neurooncol 1999; 43: 249–257.
12. Schlegel U, Pels H, Oehring R et al. Neurologic sequelae of treatment of primary CNS lymphomas. J Neurooncol 1999; 43: 277–286.
13. Gabbai AA, Hochberg FH, Linggood RM et al. High-dose methotrexate for non-AIDS primary central nervous system lymphoma. Report of 13 cases. J Neurosurg 1989; 70: 190–194.
14. Cher L, Glass J, Harsh GR et al. Therapy of primary CNS lymphoma with methotrexate-based chemotherapy and deferred radiotherapy: preliminary results. Neurology 1996; 46: 1757–1759.
15. Batchelor T, Carson K, O'Neill A et al. Treatment of primary CNS lymphoma with methotrexate and deferred radiotherapy: a report of NABTT 96–07. J Clin Oncol 2003; 21: 1044–1049.
16. Villano JL, Koshy M, Shaikh H et al. Age, gender, and racial differences in incidence and survival in primary CNS lymphoma. Br J Cancer 2011; 105: 1414–1418.
17. Plotkin SR, Betensky RA, Hochberg FH et al. Treatment of relapsed central nervous system lymphoma with high-dose methotrexate. Clin Cancer Res 2004; 10: 5643–5646.
18. Thiel E, Korfel A, Martus P et al. High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. Lancet Oncol 2010; 11: 1036–1047.
19. Shah GD, Yahalom J, Correa DD et al. Combined immunochemotherapy with reduced whole-brain radiotherapy for newly diagnosed primary CNS lymphoma. J Clin Oncol 2007; 25: 4730–4735.
20. Rubenstein JL, Treseler P, O'Brien JM. Pathology and genetics of primary central nervous system and intraocular lymphoma. Hematol Oncol Clin North Am 2005; 19: 705–17.
21. Rubenstein JL, Combs D, Rosenberg J et al. Rituximab therapy for CNS lymphomas: targeting the leptomeningeal compartment. Blood 2003; 101: 466–468.
22. Rubenstein JL, Fridlyand J, Abrey L et al. Phase I study of intraventricular administration of rituximab in patients with recurrent CNS and intraocular lymphoma. J Clin Oncol 2007; 25: 1350–1356.
23. Rubenstein JL, Li J, Chen L et al. Multicenter phase 1 trial of intraventricular immunochemotherapy in recurrent CNS lymphoma. Blood 2013; 121: 745–751.
24. Wieduwilt MJ, Valles F, Issa S et al. Immunochemotherapy with intensive consolidation for primary CNS lymphoma: a pilot study and prognostic assessment by diffusion-weighted MRI. Clin Cancer Res 2012; 18: 1146–1155.
25. Batchelor TT, Grossman SA, Mikkelsen T et al. Rituximab monotherapy for patients with recurrent primary CNS lymphoma. Neurology 2011; 76: 929–930.
26. Angelov L, Doolittle ND, Kraemer DF et al. Blood–brain barrier disruption and intra-arterial methotrexate-based therapy for newly diagnosed primary CNS lymphoma: a multi-institutional experience. J Clin Oncol 2009; 27: 3503–3509.
27. Rubenstein JL, Hsi ED, Johnson JL et al. Intensive chemotherapy and immunotherapy in patients with newly diagnosed primary CNS lymphoma: CALGB 50202 (Alliance 50202). J Clin Oncol 2013; 31: 3061–3068.
28. Doolittle ND, Jahnke K, Belanger R et al. Potential of chemo-immunotherapy and radioimmunotherapy in relapsed primary central nervous system (CNS) lymphoma. Leuk Lymphoma 2007; 48: 1712–1720.
29. Illerhaus G, Marks R, Ihorst G et al. High-dose chemotherapy with autologous stem-cell transplantation and hyperfractionated radiotherapy as first-line treatment of primary CNS lymphoma. J Clin Oncol 2006; 24: 3865–3870.
30. Illerhaus G, Muller F, Feuerhake F et al. High-dose chemotherapy and autologous stem-cell transplantation without consolidating radiotherapy as first-line treatment for primary lymphoma of the central nervous system. Haematologica 2008; 93: 147–148.
31. Abrey LE, Moskowitz CH, Mason WP et al. Intensive methotrexate and cytarabine followed by high-dose chemotherapy with autologous stem-cell rescue in patients with newly diagnosed primary CNS lymphoma: an intent-to-treat analysis. J Clin Oncol 2003; 21: 4151–4156.
32. Pels H, Schmidt-Wolf IG, Glasmacher A et al. Primary central nervous system lymphoma: results of a pilot and phase II study of systemic and intraventricular chemotherapy with deferred radiotherapy. J Clin Oncol 2003; 21: 4489–4495.

33. Juergens A, Pels H, Rogowski S et al. Long-term survival with favorable cognitive outcome after chemotherapy in primary central nervous system lymphoma. Ann Neurol 2010; 67: 182–189.
34. Braaten KM, Betensky RA, de Leval L et al. BCL-6 expression predicts improved survival in patients with primary central nervous system lymphoma. Clin Cancer Res 2003; 9: 1063–1069.
35. Camilleri-Broet S, Criniere E, Broet P et al. A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. Blood 2006; 107: 190–196.
36. Montesinos-Rongen M, Van Roost D, Schaller C et al. Primary diffuse large B-cell lymphomas of the central nervous system are targeted by aberrant somatic hypermutation. Blood 2004; 103: 1869–1875.
37. Jordanova ES, Riemersma SA, Philippo K et al. Hemizygous deletions in the HLA region account for loss of heterozygosity in the majority of diffuse large B-cell lymphomas of the testis and the central nervous system. Genes Chromosomes Cancer 2002; 35: 38–48.
38. Rubenstein JL, Fridlyand J, Shen A et al. Gene expression and angiotropism in primary CNS lymphoma. Blood 2006; 107: 3716–3723.
39. Tun HW, Personett D, Baskerville KA et al. Pathway analysis of primary central nervous system lymphoma. Blood 2008; 111: 3200–3210.
40. Booman M, Szuhai K, Rosenwald A et al. Genomic alterations and gene expression in primary diffuse large B-cell lymphomas of immune-privileged sites: the importance of apoptosis and immunomodulatory pathways. J Pathol 2008; 216: 209–217.
41. Campo E, Swerdlow SH, Harris NL et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 2011; 117: 5019–5032.
42. Hans CP, Weisenburger DD, Greiner TC et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275–282.
43. Chang CC, Kampalath B, Schultz C et al. Expression of p53, c-Myc, or Bcl-6 suggests a poor prognosis in primary central nervous system diffuse large B-cell lymphoma among immunocompetent individuals. Arch Pathol Lab Med 2003; 127: 208–212.
44. Momota H, Narita Y, Maeshima AM et al. Prognostic value of immunohistochemical profile and response to high-dose methotrexate therapy in primary CNS lymphoma. J Neurooncol 2010; 98: 341–348.
45. Levy O, Deangelis LM, Filippa DA et al. Bcl-6 predicts improved prognosis in primary central nervous system lymphoma. Cancer 2008; 112: 151–156.
46. Dalla-Favera R, Ye BH, Cattoretti G et al. BCL-6 in diffuse large-cell lymphomas. Important Adv Oncol 1996; 139–148. Review.
47. Cerchietti LC, Ghetu AF, Zhu X A small-molecule inhibitor of BCL6 kills DLBCL cells in vitro and in vivo. Cancer Cell 2010; 17: 400–411.
48. Lopez-Girona A, Heintel D, Zhang LH et al. Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response. Br J Haematol 2011; 154: 325–336.
49. Zhu YX, Braggio E, Shi CX et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood 2011; 118: 4771–4779.
50. Yang Y, Shaffer AL, 3rd, Emre NC et al. Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. Cancer Cell 2012; 21: 723–737.
51. Rubenstein JL, Treseler PA, Stewart PJ. Regression of refractory intraocular large B-cell lymphoma with lenalidomide monotherapy. J Clin Oncol 2011; 29: e595–e597.
52. Cox MC, Mannino G, Lionetto L et al. Lenalidomide for aggressive B-cell lymphoma involving the central nervous system?. Am J Hematol 2011; 86: 957.
53. Hernandez-Ilizaliturri FJ, Reddy N, Holkova B et al. Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model. Clin Cancer Res 2005; 11: 5984–5992.
54. Cady FM, O'Neill BP, Law ME et al. Del(6)(q22) and BCL6 rearrangements in primary CNS lymphoma are indicators of an aggressive clinical course. J Clin Oncol 2008; 26: 4814–4819.
55. Harada K, Nishizaki T, Kubota H et al. Distinct primary central nervous system lymphoma defined by comparative genomic hybridization and laser scanning cytometry. Cancer Genet Cytogenet 2001; 125: 147–150.
56. Boonstra R, Koning A, Mastik M et al. Analysis of chromosomal copy number changes and oncoprotein expression in primary central nervous system lymphomas: frequent loss of chromosome arm 6q. Virchows Arch 2003; 443: 164–169.
57. Schwindt H, Vater I, Kreuz M et al. Chromosomal imbalances and partial uniparental disomies in primary central nervous system lymphoma. Leukemia 2009; 23: 1875–1884.
58. Gonzalez-Aguilar A, Idbaih A, Boisselier B et al. Recurrent mutations of MYD88 and TBL1XR1 in primary central nervous system lymphomas. Clin Cancer Res 2012; 18: 5203–5211.
59. Courts C, Montesinos-Rongen M, Brunn A et al. Recurrent inactivation of the PRDM1 gene in primary central nervous system lymphoma. J Neuropathol Exp Neurol 2008; 67: 720–727.
60. Nakamura M, Kishi M, Sakaki T et al. Novel tumor suppressor loci on 6q22–23 in primary central nervous system lymphomas. Cancer Res 2003; 63: 737–741.
61. Braggio E, McPhail ER, Macon W et al. Primary central nervous system lymphomas: a validation study of array-based comparative genomic hybridization in formalin-fixed paraffin-embedded tumor specimens. Clin Cancer Res 2011; 17: 4245–4253.
62. Courts C, Montesinos-Rongen M, Martin-Subero JI et al. Transcriptional profiling of the nuclear factor-kappaB pathway identifies a subgroup of primary lymphoma of the central nervous system with low BCL10 expression. J Neuropathol Exp Neurol 2007; 66: 230–237.
63. Montesinos-Rongen M, Schmitz R, Brunn A Mutations of CARD11 but not TNFAIP3 may activate the NF-kappaB pathway in primary CNS lymphoma. Acta Neuropathol 2010; 120: 529–535.
64. Montesinos-Rongen M, Godlewska E, Brunn A et al. Activating L265P mutations of the MYD88 gene are common in primary central nervous system lymphoma. Acta Neuropathol 2011; 122: 791–792.
65. Montesinos-Rongen M, Schafer E, Siebert R et al. Genes regulating the B cell receptor pathway are recurrently mutated in primary central nervous system lymphoma. Acta Neuropathol 2012; 124: 905–906.
66. Nagel D, Spranger S, Vincendeau M et al. Pharmacologic inhibition of MALT1 protease by phenothiazines as a therapeutic approach for the treatment of aggressive ABC-DLBCL. Cancer Cell 2012; 22: 825–837.
67. Fontan L, Yang C, Kabaleeswaran V et al. MALT1 small molecule inhibitors specifically suppress ABC-DLBCL in vitro and in vivo. Cancer Cell 2012;22: 812–824.
68. Young RM, Staudt LM. A new 'brew' of MALT1 inhibitors. Cancer Cell 2012; 22: 706–707.
69. Fischer L, Hummel M, Korfel A et al. Differential micro-RNA expression in primary CNS and nodal diffuse large B-cell lymphomas. Neuro Oncol 2011; 13: 1090–1098.
70. Kluk MJ, Chapuy B, Sinha P et al. Immunohistochemical detection of MYC-driven diffuse large B-cell lymphomas. PLoS One 2012; 7: e33813.
71. Allen JD, Verhoeven E, Domen J et al. Pim-2 transgene induces lymphoid tumors, exhibiting potent synergy with c-myc. Oncogene 1997; 15: 1133–1141.
72. Yang Q, Chen LS, Neelapu SS et al. Transcription and translation are primary targets of Pim kinase inhibitor SGI-1776 in mantle cell lymphoma. Blood 2012; 120: 3491–3500.
73. Lin YW, Beharry ZM, Hill EG et al. A small molecule inhibitor of Pim protein kinases blocks the growth of precursor T-cell lymphoblastic leukemia/lymphoma. Blood 2010; 115: 824–833.
74. Sasayama T, Nakamizo S, Nishihara M et al. Cerebrospinal fluid interleukin-10 is a potentially useful biomarker in immunocompetent primary central nervous system lymphoma (PCNSL). Neuro Oncol 2012; 14: 368–380.
75. Rubenstein JL, Wong VS, Kadoch C et al. CXCL13 plus interleukin-10 are highly specific for the diagnosis of CNS lymphoma. Blood. 2013; 121: 4740–4748.
76. Whitcup SM, Stark-Vancs V, Wittes RE et al. Association of interleukin 10 in the vitreous and cerebrospinal fluid and primary central nervous system lymphoma. Arch Ophthalmol 1997; 115: 1157–1160.
77. Chan CC, Whitcup SM, Solomon D et al. Interleukin-10 in the vitreous of patients with primary intraocular lymphoma. Am J Ophthalmol 1995; 120: 671–673.
78. Sung CO, Kim SC, Karnan S et al. Genomic profiling combined with gene expression profiling in primary central nervous system lymphoma. Blood 2011; 117: 1291–1300.

79. Komohara Y, Horlad H, Ohnishi K et al. M2 macrophage/microglial cells induce activation of Stat3 in primary central nervous system lymphoma. J Clin Exp Hematop 2011; 51: 93–99.
80. Yang SH, Lee KS, Kim IS et al. Long-term survival in primary CNS lymphoma treated by high-dose methotrexate monochemotherapy: role of STAT6 activation as prognostic determinant. J Neurooncol 2009; 92: 65–71.
81. Ngo VN, Young RM, Schmitz R et al. Oncogenically active MYD88 mutations in human lymphoma. Nature 2011; 470: 115–119.
82. Sung KH, Lee EH, Kim YZ. Factors influencing the response to high dose methotrexate-based vincristine and procarbazine combination chemotherapy for primary central nervous system lymphoma. J Korean Med Sci 2011; 26: 551–560.
83. He M, Zuo C, Wang J et al. Prognostic significance of the aggregative perivascular growth pattern of tumor cells in primary central nervous system diffuse large B-cell lymphoma. Neuro Oncol. 2013; 15: 727–734.
84. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science 2011; 334: 1081–1086.
85. Wiseman RL, Balch WE. A new pharmacology—drugging stressed folding pathways. Trends Mol Med 2005; 11: 347–350.
86. Muta D, Makino K, Nakamura H et al. Inhibition of eIF4E phosphorylation reduces cell growth and proliferation in primary central nervous system lymphoma cells. J Neurooncol 2011; 101: 33–39.
87. Fischer L, Korfel A, Pfeiffer S et al. CXCL13 and CXCL12 in central nervous system lymphoma patients. Clin Cancer Res 2009; 15: 5968–5973.
88. Smith JR, Braziel RM, Paoletti S et al. Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. Blood 2003; 101: 815–821.
89. Smith JR, Falkenhagen KM, Coupland SE et al. Malignant B cells from patients with primary central nervous system lymphoma express stromal cell-derived factor-1. Am J Clin Pathol 2007; 127: 633–641.
90. Chunsong H, Yuling H, Li W et al. CXC chemokine ligand 13 and CC chemokine ligand 19 cooperatively render resistance to apoptosis in B cell lineage acute and chronic lymphocytic leukemia CD23+CD5+ B cells. J Immunol 2006; 177: 6713–6722.
91. Saez de Guinoa J, Barrio L, Mellado M et al. CXCL13/CXCR5 signaling enhances BCR-triggered B-cell activation by shaping cell dynamics. Blood 2011; 118: 1560–1569.
92. Hoellenriegel J, Meadows SA, Sivina M et al. The phosphoinositide 3′-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood 2011; 118: 3603–3612.
93. Kawaguchi A, Orba Y, Kimura T et al. Inhibition of the SDF-1alpha-CXCR4 axis by the CXCR4 antagonist AMD3100 suppresses the migration of cultured cells from ATL patients and murine lymphoblastoid cells from HTLV-I Tax transgenic mice. Blood 2009; 114: 2961–2968.
94. Ponzoni M, Berger F, Chassagne-Clement C et al. Reactive perivascular T-cell infiltrate predicts survival in primary central nervous system B-cell lymphomas. Br J Haematol 2007; 138: 316–323.
95. Bashir R, Chamberlain M, Ruby E et al. T-cell infiltration of primary CNS lymphoma. Neurology 1996; 46: 440–444.
96. Kadoch C, Dinca EB, Voicu R et al. Pathologic correlates of primary central nervous system lymphoma defined in an orthotopic xenograft model. Clin Cancer Res 2009; 15: 1989–1997.
97. Isobe Y, Sugimoto K, Masuda A et al. Central nervous system is a sanctuary site for chronic myelogenous leukaemia treated with imatinib mesylate. Intern Med J 2009; 39: 408–411.
98. Porkka K, Koskenvesa P, Lundan T et al. Dasatinib crosses the blood–brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. Blood 2008; 112: 1005–1012.
99. Muldoon LL, Soussain C, Jahnke K et al. Chemotherapy delivery issues in central nervous system malignancy: a reality check. J Clin Oncol 2007; 25: 2295–2305.

Annals of Oncology 25: 322–331, 2014
doi:10.1093/annonc/mdt405
Published online 26 November 2013

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

B. W. C. Tse[1], A. Collins[2], M. K. Oehler[3], A. Zippelius[4] & V. A. Heinzelmann-Schwarz[1,5*]

[1]Ovarian Cancer Group, Lowy Cancer Research Centre, Prince of Wales Clinical School; [2]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney; [3]Department of Gynaecological Oncology, Royal Adelaide Hospital, Adelaide, Australia; [4]Department of Medical Oncology, University Hospital Basel; [5]Women's University Hospital and Department of Biomedicine, University of Basel, Basel, Switzerland

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 fulfil 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

*Correspondence to: Prof V. A. Heinzelmann-Schwarz, Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, Basel CH-4031, Switzerland. Tel: +41-79-793-07-13; Fax: +41-61-265-93-99; E-mail: viola.heinzelmann@usb.ch