How we diagnose and treat vitreoretinal lymphoma

Falko Fend,1 Andrés J. M. Ferreri2 and Sarah E. Coupland3

1Institute of Pathology and Neuropathology and Comprehensive Cancer Centre, Tübingen University Hospital, Tübingen, Germany, 2Department of Onco-Hematology, Unit of Lymphoid Malignancies, Division of Onco-Hematological Medicine, IRCCS San Raffaele Scientific Institute, Milano, Italy and 3Department of Cellular and Molecular Pathology, University of Liverpool, Liverpool, England

Summary

The eye is a rare site for the development of malignant lymphoma. Based on cell type and involved intraocular structures, which as a whole represent an immune-privileged site, several subtypes of primary intraocular lymphoma need to be discerned. Primary vitreoretinal lymphoma (PVRL), the most common form, is an aggressive B-cell malignancy and considered a subtype of primary central nervous system (CNS) lymphoma. Ocular symptoms are non-specific and often mimic uveitis, frequently resulting in delayed diagnosis. Bilateral ocular involvement and dissemination/relapse in the CNS are common. Diagnosis of PVRL is usually based on the analysis of vitreous biopsy material. In addition to cytological and immunocytochemical examination, measurements of cytokine levels and molecular determination of B-cell clonality and recurrent mutations increase the diagnostic yield. Both systemic chemotherapy and exclusively local treatment, including ocular radiotherapy and intravitreal chemotherapy, are successful approaches for the management of PVRL, although it is currently not predictable which patients require systemic treatment in order to avoid cerebral dissemination, a complication associated with a considerably worse prognosis.

Keywords: primary CNS lymphoma, intraocular lymphoma, vitreoretinal lymphoma.

Malignant lymphomas involving the eye and surrounding structures are rare, representing less than 10% of extranodal lymphoma. Due to the unique anatomical situation, the limitations for invasive procedures and the desire to preserve vision, both diagnosis and treatment of these lymphomas pose specific challenges and require close collaboration between ophthalmologists, pathologists and oncologists.

Based on clinical and pathological features and topography, two major groups of ocular lymphomas need to be discerned, namely: (i) lymphomas involving the ocular adnexae and the orbit, which mainly include extranodal marginal zone B-cell lymphoma of ‘Mucosa-Associated Lymphoid Tissue’ (‘MALT’) type and involvement by different types of systemic non-Hodgkin lymphoma (NHL); and (ii) those affecting intraocular structures, which, for the most part, are aggressive lymphoma entities (Coupland et al, 2004; Coupland & Damato, 2008). The latter group will be the subject of this review.

Based on anatomical compartments within the eye, a number of types of primary intraocular lymphoma with distinct distribution patterns, histology and behaviour can be discerned (Coupland & Damato, 2008; Coupland et al, 2009). Their salient clinical and pathological features are summarized in Table I. Secondary lymphomatous involvement of intraocular structures is less common and tends to represent a secondary manifestation of a systemic NHL, most commonly affecting the uvea (i.e. iris, ciliary body or choroid).

The term ‘intraocular lymphoma’ was first used more than 60 years ago (Cooper & Riker, 1951; Qualman et al, 1983) but later replaced with the now obsolete term ‘ocular reticulum cell sarcoma’. Given that there are great differences in the biology, clinical behaviour and prognosis depending on the affected anatomical compartment of the eye and neoplastic cell type, it is recommended that the vague term ‘intraocular lymphoma’ be replaced by a more specific terminology, reflecting both the precise location and histological subtype of lymphoma, as outlined in Table I (Coupland et al, 2009). The most common primary intraocular lymphoma by far is primary vitreoretinal lymphoma (PVRL), an aggressive disease classified as diffuse large B-cell lymphoma (DLBCL) and regarded as a manifestation of primary central nervous system lymphoma (PCNSL), reflecting the common embryological origin of the two organs (Kluin et al, 2008). Given the more common occurrence of PCNSL, much of the current state of knowledge about biology and clinical behaviour of VRL is actually inferred from the investigation of PCNSL. This review is a critical analysis of the current state-of-the-art knowledge and understanding of the biology of VRL, and the recommendations for its diagnosis and treatment. This
Biological and pathological features of PVRL

PVRL involving the retina, the vitreous or both structures is by far the most common form of primary intraocular lymphoma. Involvement of the central nervous system (CNS) is common, occurs in 16–34% of cases at presentation and develops in 35–90% during the course of the disease. Conversely, approximately 15–25% of patients with PCNSL develop VRL (Grimm et al., 2007, 2008). In contrast to systemic DLBCL and similar to PCNSL, extracerebral dissemination is very rare. The reasons for this are unclear: some
immunophenotypical studies have shown distinct patterns of chemokine and chemokine receptor expression on the malignant cells of PCNSL, which might explain their dissemination characteristics. Interaction of B-cell chemokines CXCL12 and CXCL13 with their receptors CXCR4 and CXCR5 mediates chemotaxis and may promote B-cell survival (Chan et al., 2003; Smith et al., 2003, 2007; Montesinos-Rongen et al., 2008; Deckert et al., 2014). Elevated levels of CXCL13 in cerebrospinal fluid (CSF) have been associated with poor prognosis and may be used for diagnostic purposes (Rubenstein et al., 2013a). However, studies confirming these site-specific expression profiles and their potential impact on clinical behaviour of VRL are lacking.

Furthermore, the eye, similar to the brain and the testis, represents an immune-privileged site in which normal mechanisms of immune recognition of foreign antigen and immune-mediated inflammation are inactive (Coupand et al., 2009). The absence of local immune surveillance might result in acquisition of distinct biological features by the neoplastic cells, which in turn could prevent their efficient dissemination to extracerebral sites. However, manifest VRL often contains an abundance of reactive T-cells and macrophages, indicating that the development of clinically manifest lymphoma is associated with a breakdown of the immune-privileged state. Of note, PCNSL as well as DLBCL arising in the testis, another immune-privileged site, frequently show lack of human leucocyte antigen (HLA) class I and II expression, due to deletions of chromosome 6p21-32 harbouring the HLA locus, which may allow escape from immune attack (Riemersma et al., 2000).

Cell of origin and immunophenotype of PVRL

The vast majority (~95%) of PVRL can be classified as DLBCL. (Fig 1); however, due to unique clinical and biological features, PCNSL including PVRL, is recognized as a specific subtype of lymphoma in the World Health Organization classification (Kluin et al., 2008). Primary DLBCL of the CNS (including VRL) belong to the activated B-cell (ABC) type of DLBCL, according to gene expression profile and mutational status, in 80–90% of cases (Montesinos-Rongen et al., 2008). In addition to pan-B-cell markers, such as CD20, PAX5 and CD79a, they therefore express MUM1/IRF4, commonly BCL6 and BCL2, and usually lack CD10 and plasma cell markers, showing that, biologically, they are arrested at a late germinal centre B-cell differentiation stage (Coupand et al., 2005a; Camilleri-Broet et al., 2006). VRL usually expresses monoclonal immunoglobulin light chains and IgM or IgM and IgD heavy chains (Coupand et al., 2009). Their proliferation and apoptotic rate is high, also reflected in commonly high numbers of necrotic cells. As B-cells that have passed through the germinal centre reaction, VRL commonly shows a high rate of somatic mutation of the rearranged immunoglobulin genes, in most cases without evidence for on-going hypermutation and, similar to PCNSL frequently exhibit immunoglobulin rearrangements using IGHV4-34 (Montesinos-Rongen et al., 1999; Coupland et al., 2005b; Malumbres et al., 2007).

Genetics of PVRL

Genetic studies of VRL are sparse, and most published data stem from studies of PCNSL. Earlier studies of VRL using polymerase chain reaction (PCR) have demonstrated a high frequency of IGH/BCL2 rearrangements as a result of the t(14;18) translocation, which occur in 85-90% of follicular lymphoma and in about 30% of DLBCL of germinal centre B-cell type (Chan, 2003; Wallace et al., 2006). These results, however, are somehow at odds with the notion that most PVRL are of ABC type, which lacks BCL2 rearrangements, and with the fact that BCL2 translocations are rare in PCNSL (Montesinos-Rongen et al., 2002). Translocations involving the BCL6 oncogene occur in 17-47% of PCNSL, and activation of this master regulator of the germinal centre reaction may be partly responsible for the arrest in the terminal B-cell differentiation stage (Montesinos-Rongen et al., 2002; Cady et al., 2008). Their presence in VRL has not been studied to date. Using a high-resolution single nucleotide polymorphism (SNP) array for the identification of copy number changes, large numbers of alterations with common gains on chromosomes 1q, 18q and 19q and frequent losses on 6q, alterations, which are also frequently identified in PCNSL, have been demonstrated in VRL (Wang et al., 2014).

Recently, several studies using conventional techniques or next generation sequencing have analysed the mutational landscape of PCNSL and have identified high frequencies of mutations in MYD88, the gene encoding Myeloid Differentiation Factor 88 (MYD88), a member of the toll-like receptor pathway, and members of the B-cell receptor signalling pathway, including CD79B, as well as other genes resulting in a constitutive activation of NF-kB signalling (Gonzalez-Aguilar et al., 2012; Kraan et al., 2013; Bruno et al., 2014; Bonzheim et al., 2015; Braggio et al., 2015; Nakamura et al., 2015). Furthermore, data generated by whole exome sequencing suggest a major impact of aberrant somatic hypermutation (SHM) on the mutational profile of PCNSL. SHM is the process by which mutations are introduced into the rearranged immunoglobulin genes of germinal centre B-cells in order to increase the binding affinity of the B-cell receptor. In addition to well-known targets, such as MYC, PAX5 and PIM1, aberrant SHM seems to target additional genes in PCNSL, some of which are involved in CNS development (Vater et al., 2015).

Mutations affecting MYD88, most commonly the canonical L265P mutation, also found in the vast majority of lymphoplasmacytic lymphomas/Waldenström macroglobulinaemia, and CD79B, although also present in DLBCL of ABC type of other locations, seem to be enriched specifically in DLBCL of immune-privileged sites, namely the testis and the CNS. MYD88 mutations have been found in 35–79% of
PCNSL (Montesinos-Rongen et al, 2011; Gonzalez-Aguilar et al, 2012; Kraan et al, 2013; Braggio et al, 2015; Nakamura et al, 2015) and were recently identified in 69% of VRL with or without concomitant cerebral involvement (Bonzheim et al, 2015).

Very little is known so far about epigenetic alterations and micro-RNA (miRNA) expression profiles in PVRL, and only few studies have addressed these topics in PCNSL (Deckert et al, 2014). Of interest, a recent study has compared miRNA expression profiles in vitreal aspirates of PVRL and uveitis, and identified miR155 as consistently differentially expressed and a potential novel biomarker (Tuo et al, 2014); however, further validation studies are needed to confirm the value of miRNA analysis for PVRL diagnosis.

VRL in immunosuppressed patients

Epstein-Barr virus (EBV) is identified at a very high frequency in immunosuppressed patients with PCNSL, especially in the setting of acquired immunodeficiency syndrome (AIDS) (MacMahon et al, 1991), but absent from lymphomas in immunocompetent patients. The same seems true for patients with VRL, although the number of cases studied for the presence of EBV is low (Chan, 2003).

Other types of primary and secondary intraocular lymphoma

As outlined in Table I, primary choroidal and ciliary body lymphoma is usually a low-grade B-cell lymphoma, and can be subtyped as an extranodal marginal zone lymphoma; occasionally, primary choroidal lymphomas may extend extraocularly into the ocular adnexae, e.g. into the subconjunctival space (Coupland & Damato, 2008). Distinction of primary choroidal lymphoma from PVRL is extremely important, because the former run an indolent course and lack any involvement of the CNS. Consequently, patients with primary choroidal lymphoma tend to have a very good prognosis following treatment with low-dose external beam radiotherapy (Fig 2).

Other NHL subtypes including T-cell lymphoma with primary retinal manifestation have been mostly reported as sin-
gle case reports (Ponzoni et al, 2002; Coupland et al, 2005c). The few cases of T-VRL reported to date have shown a less aggressive course compared to B-VRL (Coupland & Damato, 2008).

Secondary intraocular involvement by systemic lymphoma is relatively rare, and the disease is usually confined to the choroid. The most common subtype is systemic DLBCL (Coupland & Damato, 2008) but secondary infiltration of the eye by other B-NHL subtypes include chronic lymphocytic leukaemia (Coupland et al, 2001), plasma cell neoplasms (Fung et al, 2005) and, rarely, Burkitt lymphoma (Payne et al, 1971; Wysenbeek et al, 1987) as well as intravascular large B-cell lymphoma (Mudhar et al, 2007). A peculiar phenomenon is the increased risk for secondary vitreoretinal or CNS involvement by DLBCL of the testis, another immune-privileged site, which shares many features with PVRL and PCNSL, including predominance of the ABC type, common loss of HLA class I and II expression and common MYD88 mutations (Riemersma et al, 2000; Kraan et al, 2013). Of interest, joint MYD88 L265P mutations, indicating a common clonal origin, were identified in two cases of VRL with a history of testicular lymphoma in our recent VRL series (Bonzheim et al, 2015).

**Epidemiology**

The true incidence of PVRL is unknown because no central database exists yet for this rare disease: hopefully, this major deficiency will be overcome soon through the efforts of ‘The American Joint Committee on Cancer Ophthalmic Oncology Task Force’, who are presently establishing an international multicentre VRL registry. It can be confidently stated that PVRL is one of the rarest primary ocular tumours: a 20-year retrospective study at a large Canadian hospital estimated the incidence of VRL in British Columbia to be between 0.017-0.048/100,000 people between the years 1990 and 2010 (Levasseur et al, 2013). Better data exist for PCNSL: the Central Brain Tumor Registry of the United States published the incidence of PCNSL in the U.S. as 0.46 per 100,000 person-years between 2004-2007 and 0.45 per 100,000 person-years between 2005-2009. The incidence was higher in males (0.54) than in females (0.39), with a male:female ratio of 1:3.8. Similar incidence rates have been published in Europe (Phillips et al, 2014; Hoang-Xuan et al, 2015).

PVRL usually occurs in adults from the third to the eighth decades of life, with the median age at diagnosis in these patients being 63 years, with no gender prevalence (Grimm et al, 2007). Whilst the most important risk factors for
PCNSL are human immunodeficiency virus (HIV) status and EBV infection status (Phillips et al., 2014), there are no other known risk factors for PVRL.

**Clinical features of VRL**

Clinical presentation of VRL varies largely according to the involved ocular structures and to the presence or absence of concomitant brain and/or meningeal disease. Ocular symptoms are usually the only expression of disease in patients with PVRL. Most of these patients have good performance status, with a history of a prior malignancy in 10–15% of cases (Hoang-Xuan et al., 2015).

The mean duration of PVRL symptoms prior to diagnosis is 6 months, but, in some cases, symptoms precede diagnosis by 2–3 years. The most common presenting symptoms are non-specific, and include blurred vision in 40–50% of patients, decreased visual acuity in 25–30% and floaters in 20–25%. Signs and symptoms of PRVL may mimic other intraocular conditions, such as uveitis, thus making PVRL a ‘masquerade’ syndrome (Al-Qahtani et al., 2014). Bilateral ocular involvement is common, occurring in 60–90% of patients, but may appear clinically as unilateral involvement due to uneven distribution of disease. CSF dissemination is detected during staging workup in only 10–15% of cases (Grimm et al., 2007).

Patients’ characteristics and clinical presentation do not differ greatly between patients with PVRL and patients with concomitant brain and ocular lymphoma. Median age and gender distribution are the same, whereas performance status is usually poorer in the latter group (Hoang-Xuan et al., 2015). Ocular symptoms are similar to those above reported for PVRL patients, and can be concomitant to other neurological symptoms or precede the onset of brain lesions by weeks or months, and sometimes years. The most commonly associated neurological symptoms, which often span weeks to months, are focal deficits, personality changes and increased intracranial pressure: behavioural/cognitive changes in 25–30% of cases, hemiparesis in 10–15%, headache in 10–15%, aphasia in 10–15%, seizure in 5%, ataxia in 4% (Ferreri et al., 2002; Hoang-Xuan et al., 2015). Patients with ocular symptoms and asymptomatic brain disease are uncommon (3% of cases) (Grimm et al., 2008). The average duration of symptoms prior to diagnosis is usually shorter than that reported for PVRL (3 months). CSF infiltration is detected in 20–25% during staging in patients with concomitant ocular and cerebral disease.

**Diagnosis of VRL**

Ophthalmological examination in VRL frequently demonstrates the presence of vitritis, usually in association with infiltrates of the retina and the retinal pigment epithelium, sometimes giving the characteristic ‘leopard skin’ pigmentation (Fig 1) on fundoscopy and fluorescein angiography (Fardeau et al., 2009), whereas alterations in the anterior segment of the eye are usually absent (Coupland & Damato, 2008; Chan & Sen, 2013; Sagoo et al., 2014). Recently, there has been an interest in the autofluorescence findings in VRL patients, with a granular pattern in autofluorescence being suggestive of VRL (Egawa et al., 2014). Optical Coherence Tomography has also been described as being informative in VRL, with the visualization of nodular hyper-reflective lesions in the retinal pigment epithelium corresponding to lymphomatous cell deposition (Fardeau et al., 2009; Egawa et al., 2014).

In patients with suspected concomitant ocular and cerebral lymphoma, Contrast-enhanced cranial magnetic resonance imaging (MRI) is the best imaging modality for assessing the cerebral disease (Kuker et al., 2005; Hoang-Xuan et al., 2015). Lesions are often isointense to hypointense on T2-weighted MRI, with variable surrounding oedema and a homogeneous and strong pattern of enhancement. In patients where MRI is a contraindication, contrast-enhanced cranial computerized tomography (CT) scans are recommended.

Although clinical examination and imaging procedures often lead to a high suspicion of lymphoma, VRL frequently mimics chronic posterior uveitis, including an initial response to steroids. As a classical ‘masquerade syndrome’, VRL hence requires diagnostic confirmation through invasive procedures providing morphological, phenotypical and/or molecular evidence for malignancy. Important differential diagnoses, including fungal or bacterial endophthalmitis, sarcoidosis as well as ocular syphilis and tuberculosis, need to be excluded with special stains, cultures or molecular techniques.

In cases with concomitant CNS involvement, a positive CSF examination or stereotactic brain biopsy may obviate the need for intraocular biopsy. The standard approach to diagnosis of PVRL is vitrectomy or vitreous aspirate biopsy. In case this approach does not render a diagnosis, subretinal aspirate biopsy or chorioretinal biopsy or of other intraocular structures may be used (Coupland, 2012). For a detailed review of the surgical procedures, the reader is referred to the ophthalmological literature. The aspirated material can be used for cytological examination, immunocytochemistry, flow cytometry, molecular examinations and determination of cytokine levels. Therefore, an adequate triage system to maximize the use of the limited material for the different techniques should be in place in the pathology laboratory, which ideally should be experienced in handling these samples. Pre-analytical conditions are of major importance. Cytological material should either be worked within an hour after aspiration, or alternatively put either into culture medium or a mild fixative, such as HOPE (Hepes-glutamic acid buffer-mediated organic solvent protection effect) solution, which preserves cytological detail, as well as immunoreactivity and nucleic acids (Coupland, 2012). Cytological specimens are usually prepared with the cytospin technique; alternatively, the cell-block technique can be employed for cell-rich specimens.
Cytology and immunocytochemistry

Cytological examination reveals the presence of large, atypical lymphoid cells with increased nuclear/cytoplasmic ratio, basophilic cytoplasm and irregular nuclei with one to several nucleoli in cases of VRL. However, large numbers of reactive lymphocytes, poor preservation of cytological detail due to degenerative changes and necrosis, and paucicellular aspirates due to limited involvement of the vitreous or an antecedent steroid therapy often preclude a diagnosis of malignancy based on morphology. Furthermore, atypical large cells may also occur in reactive conditions, such as acute viral infection. The reported rates of sensitivity and specificity of cytology for the diagnosis of VRL vary widely, but cytology alone is able to confirm VRL in 45–60% of cases, and false positive results are considered rare (Davis et al., 2005; Wittenberg et al., 2008; Kimura et al., 2012).

Immunocytochemistry is a valuable tool for confirming a diagnosis of VRL, with a predominance of large cells expressing pan B-cell markers such as CD20, PAX5 and CD79a. Alternatively, multicolour flow cytometry has been successfully employed for phenotyping of vitreal aspirates, with a reported sensitivity of 82% and 100% specificity (Missotten et al., 2013). Again, however, poor cellular preservation and abundant reactive T-cells may limit the diagnostic yield (Davis et al., 2012).

Molecular diagnosis of VRL

Molecular examination of vitreous specimens is a valuable tool to confirm a diagnosis of lymphoma, and the application of modern PCR techniques has reduced the necessary amount of material considerably. Identification of clonal immunoglobulin gene rearrangements using consensus primer sets such as those developed by the BIOMED-2 consortium is a mainstay in VRL diagnosis. The sensitivity of clonality studies ranges between 65% and 95%, depending on the choice of primer sets and quality of material (Coupcland et al., 2003, 2005b; Merle-Beral et al., 2004; Baehring et al., 2005; Wang et al., 2011; Kimura et al., 2012). Depending on the number of primer sets used – which can be limited by the available material – VRL of DLBCL type may also yield false negative results due to somatic hypermutation abrogating primer binding. On the other hand, due to the unique situation of the eye as immune-privileged site, inflammatory conditions can also result in oligoclonal or even clonal expansions of lymphocytes and may lead to false positive results, especially in cases with low cellularity (Sugita et al., 2009; Bonzheim et al., 2015). In order to avoid misdiagnosis of minor clonal expansions as evidence for lymphoma, all tests should be run in duplicate to confirm the presence of a dominant clone, and results should be interpreted with caution and only in the context of clinical and morphological findings. Determination of clonality is especially valuable for cases in which suspected intraocular dissemination or relapse of PCNSL or systemic lymphoma can be confirmed by proving or disproving clonal relationship.

In order to increase the diagnostic yield of vitreous specimens, we recently made use of the common occurrence of MYD88 mutations in PCNSL, identified in 50–70% of cases. Arguing that VRL, as a subtype of PCNSL, should show a similar high frequency, we used a sensitive allele-specific PCR for the most common mutation MYD88 L265P, and conventional sequencing for exons 3 and 4 in a large series of archival vitreous specimens from two institutions (Bonzheim et al., 2015). We detected MYD88 mutations, in 20/28 samples of confirmed VRL, with the canonical L265P in all but a single case (Fig 1). None of the cases classified as reactive was positive for MYD88 mutations. Importantly, this approach confirmed a diagnosis of VRL in 6 cases initially diagnosed as either only suspicious for lymphoma or reactive based on cytology, immunocytochemistry and clonality analysis, thus increasing the sensitivity of vitreous biopsy for VRL diagnosis from 62% to 90%. Although these findings need to be confirmed in a prospective manner, mutational analysis using sensitive techniques, such as allele-specific PCR or next generation sequencing using mutation-specific panels, will probably provide a valuable additional tool for the diagnosis and, perhaps, the follow-up of VRL.

Determination of cytokine levels

Some centres advocate the use of measurement of cytokine levels within ocular fluids – i.e. aqueous humor and the vitreous – to provide diagnostic evidence in addition to cytology for the presence/absence of PVRL (Chan et al., 1995; Merle-Beral et al., 2004; Cassoux et al., 2007; Saleh et al., 2012; Fisson et al., 2013; Raja et al., 2013; Mehta et al., 2015). In particular, these centres measure the levels of interleukin (IL) 10 and IL6 and then compare their ratio: a high level of IL10 in pure vitreous or aqueous humor samples, or an IL10:IL6 ratio greater than 1 in diluted or undiluted samples, is considered indirect evidence supporting the diagnosis of PVRL. The techniques used to measure the IL10 and IL6 levels include enzyme-linked immunosorbent assay (ELISA) and multiplex-based cytometric bead array. The exact cutoff for the IL10 concentration or IL10:IL6 ratio may vary between laboratories, mainly due to differences in the methods applied, the conditions of sample harvesting and storage, techniques, and manufacturers of equipment and supplies, as well as the dilution (known or unknown) of the vitreous samples and the laboratory’s own experience. Interleukin levels within intraocular fluids have also been proposed and used with some success to monitor response of PVRL under therapy (Saleh et al., 2012; Raja et al., 2013). In larger series, the sensitivity of IL10 measurements and/or the IL10:IL6 ratio in VRL is 80–90%, with virtually no false positives reported, making cytokine determination a valuable additional diagnostic tool (Chan et al., 1995; Merle-Beral et al.,...
intravitreal Rituximab, and/or binocular external beam radiotherapy alone (e.g. using intravitreal methotrexate (MTX),
treatment of VRL patients who can be managed with ocular therapies was often combined with suboptimal systemic treatments, with consequent disappointing outcome (Riemens et al, 2015).

In summary, available studies suggest that some patients with PVRL can be safely treated with local treatment alone, while other patients should be treated with systemic chemotherapy (Smith et al, 2002; Hellbig et al, 2003; Frenkel et al, 2008; Raja et al, 2013; Larkin et al, 2014). Unfortunately, efforts to distinguish the best candidates for each strategy remain unfruitful owing to relevant selection and interpretation biases, and international efforts aimed to

Our approach
In our institutions, we immediately work up the freshly received vitrectomy specimens, preparing cytopsins for cytological and immunocytochemical examinations with a limited panel of antibodies, including at least CD20 and CD3. The remainder of the aspirate is used for molecular studies, including clonality analysis using BIOMED-2 primers and MYD88 mutational analysis with an allele-specific PCR. Of note, the results of clonality analysis are interpreted only in the context of other parameters in order to avoid overinterpretation of pseudoclonal bands.

Staging examinations
By definition, PVRL is a stage I disease, irrespective of concomitant CNS involvement. Staging in PVRL has two objectives: to determine the presence and extent of CNS involvement, and to rule out systemic lymphoma (Hoang-Xuan et al, 2015), which is rare in patients presenting with VRL. As mentioned above, neuroimaging with contrasted MRI is the method of choice for determining cerebral disease (Kuker et al, 2005; Hoang-Xuan et al, 2015). In addition, a cytological examination of CSF, potentially complemented by clonality analysis is required, but one has to keep in mind that cytology may be difficult to assess and clonality analysis can be misleading in specimens with low cellularity. Systemic studies include whole body fluorodeoxy-glucose positron emission tomography (FDG-PET) and tecticular sonography, given the propensity of tecticular lymphoma for intraocular and CNS dissemination. Complete physical and neurological examination, bone marrow biopsy, standard laboratory tests and liver, kidney and cardiac function tests and HIV serology are all included in the standard workup of PVRL patients.

Treatment
Primary vitreoretinal lymphoma
From a clinical standpoint, a variable proportion of patients with PVRL experience CNS dissemination, whereas the disease remains confined to the eyes for months or years in others. The goal of the treatment of PVRL is cure and vision retention, which can be achieved by controlling intraocular disease and preventing CNS dissemination. The level of evidence supporting therapeutic decisions in PVRL is very low because related literature is sparse and fragmentary, and prospective trials exclusively focused on PVRL do not exist. The main open and often debated question regards the distinction of VRL patients who can be managed with ocular treatment alone (e.g. using intravitreal methotrexate (MTX), intravitreal Rituximab, and/or binocular external beam radiotherapy – see also below) (Smith et al, 2002; Hellbig et al, 2003; Frenkel et al, 2008; Raja et al, 2013; Larkin et al, 2014) and those patients who need systemic chemotherapy. Accordingly, some investigators have reviewed multicentre retrospective series of patients with primary or secondary intraocular lymphoma with the aim to distinguish parameters predicting CNS dissemination (Grimm et al, 2007, 2008; Riemens et al, 2015). Unfortunately, reported studies display several selection and interpretation biases, and predicting parameters remain to be defined. The major criticisms of reported studies are the lack of central pathology review, which is a relevant drawback as the modest diagnostic efficacy of vitrectomy, and the confounding factor determined by the specialty of investigators performing case collection (Ferreri, 2015). In fact, the 3-year CNS relapse rate was 60% in the series collected by neuro-oncologists (Grimm et al, 2007), and 36% in the series collected by ophthalmologists (Riemens et al, 2015). Other limitations of these studies regard the inclusion of small patient subgroups receiving varied treatments being analysed together in an arbitrary way, and the long period of study, during which diagnosis and treatment of CNS lymphomas changed greatly.

Albeit with the above-mentioned biases, large retrospective studies seem to suggest that patients with newly diagnosed PVRL should be treated with local strategies, keeping the so-called ‘extensive treatments’ for brain relapses (Grimm et al, 2007; Riemens et al, 2015). This seems to be a suitable approach because local strategies are associated with negligible systemic toxicity and, in contrast to intravenous high-dose MTX, intravitreal chemotherapy results in prolonged therapeutic drug concentrations and ocular irradiation allows better local control (Ferreri et al, 2002). In fact, patients receiving ocular treatment alone show good local disease control, with an intraocular relapse rate of only 13% at a median follow-up of 4 years (Riemens et al, 2015). However, the major concern when managing PVRL with local treatment alone remains the risk of CNS dissemination, which is a devastating event that occurs in half of patients (Grimm et al, 2007), resulting in a 4-year overall survival (OS) of 32%. Conversely, patients with PVRL receiving systemic chemotherapy had a CNS relapse rate of 43%, but the reported 4-year OS is 85% (Riemens et al, 2015). These data seem to favour ‘extensive treatments’ over ‘ocular treatments’. The combination of both treatments has not been adequately assessed because ocular therapies were often combined with suboptimal systemic treatments, with consequent disappointing outcome (Riemens et al, 2015).

In summary, available studies suggest that some patients with PVRL can be safely treated with local treatment alone, while other patients should be treated with systemic chemotherapy (Smith et al, 2002; Hellbig et al, 2003; Frenkel et al, 2008; Raja et al, 2013; Larkin et al, 2014). Unfortunately, efforts to distinguish the best candidates for each strategy remain unfruitful owing to relevant selection and interpretation biases, and international efforts aimed to
distinguish predictors of CNS dissemination are needed (Ferreri, 2015). In the meantime, both high-dose MTX-based chemotherapy (with or without whole brain radiotherapy [WBRT]) and ocular therapy (intravitreal chemotherapy or ocular radiotherapy) are acceptable strategies according to European guidelines (Hoang-Xuan et al., 2015).

At our institutions, we usually treat patients with cytologically confirmed diagnosis of PVRL without brain disease with the same approach used for PCNSL in general (see next section). This is aimed to prevent the devastating event of cerebral and/or meningeal dissemination.

**PCNSL with ocular involvement**

There are no reasons to treat PCNSL patients with ocular disease differently than the other PCNSL patients (Hoang-Xuan et al., 2015). Typically, patients with PCNSL are treated with high-dose MTX-based polychemotherapy followed by consolidative WBRT or, alternatively, with either autologous stem cell transplantation (Kasenda et al., 2012) or non-myeloablative cytarabine-etoposide chemotherapy (Rubenstein et al., 2013b). Intrathecal drug delivery is less commonly used, and indeed has not been employed in recently reported (Morris et al., 2013; Rubenstein et al., 2013b; Omuro et al., 2015a) or on-going trials (Ferreri et al., 2013). It is worth noting that patients with intraocular disease represent 5–10% of considered cases in prospective trials on PCNSL: these results have not been reported separately and, consequently, it is not possible to draw definitive conclusions on the role and therapeutic efficacy of each of these strategies in the subgroup of patients with ocular disease (Ferreri et al., 2009; Morris et al., 2013; Omuro et al., 2015b).

The eye is a chemotherapy sanctuary where PCNSL tumour cells can grow undisturbed, and chemotherapy efficacy depends on intraocular pharmacokinetics, which are not well understood for most cytostatics. Systemic administration of MTX and cytarabine can yield therapeutic drug levels in the intraocular fluids and clinical responses have been documented; however, drug concentrations in vitreous humor are unpredictable and intraocular relapse is common (Batchelor et al., 2003). Although the inclusion of both eyes in the radiation volume seems to improve disease control (Ferreri et al., 2002; Hoang-Xuan et al., 2015), many researchers are concerned by the risk of lymphoma cells persistence in the eyes, with the consequent increased risk of tumour relapse. Consequently, a benefit from the addition of direct intravitreal real injection of cytostatics has been hypothesized, and a large retrospective study has suggested that this strategy is associated with improved progression-free survival (Grimm et al., 2008), but its effect on OS remains to be defined. Intravitreal MTX is highly effective but does not affect OS, and is associated with important side effects in 73% of eyes and significant deterioration of visual acuity in 27% of patients (Smith et al., 2002; Frenkel et al., 2008). In fact, intravitreal chemotherapy has been associated with cataract in 73% of treated eyes, corneal epitheliopathy in 58%, maculopathy in 42% and less commonly with vitreous haemorrhage (8%), optic atrophy (4%) and sterile endophthalmitis (4%).

Another agent administered in patients with PVRL, with and without any evidence of concomitant cerebral disease, includes intravitreal rituximab, an anti-CD20 monoclonal antibody, either alone or in combination with intravitreal MTX. Most data regarding the efficacy of rituximab in treating PVRL currently comes from case reports and a few retrospective case series (Itty & Pulido, 2009; Vosganian et al., 2011; Hashida et al., 2012; Turaka et al., 2012). Although these authors suggest that rituximab is safe and efficacious in PVRL, these results do further emphasize the need for prospective clinical trials in competitive treatments for this aggressive disease.

On-going prospective trials exclusively focused on PVRL patients do not exist, while some patients with PCNSL and intraocular disease are being enrolled in a few on-going trials addressing new drugs. In particular, based on the high rates of ABC-DLBCL, CD79B and MYD88 mutations in this population, ibrutinib (NCT02542514; NCT02623010; NCT02315326), lenalidomide (NCT01542918; NCT01956695) and pomalidomide (NCT01722305), are being tested as maintenance or salvage monotherapy in PCNSL. Other drugs exploiting involved molecular pathways are being addressed in prospective trials, in particular, buparlisib (NCT02301364), a pan- phosphoinositide 3-kinase inhibitor. It is very hard to imagine the impact these trials could have on the management of PVRL, as only a minority of enrolled patients will be affected by this disorder. Nevertheless, international cooperation to perform this type of study should be strongly encouraged to offer trial participation to most PVRL patients.

**Prognosis and prognostic markers of PVRL**

To date, there is very little data concerning pathological biomarkers that predict the prognosis of PVRL. For PCNSL, the International Extranodal Lymphoma Study Group (IELSG) has identified five clinical variables that correlate with prognosis, three of which are shared with systemic NHL: elevated lactate dehydrogenase (LDH), age greater than 60 years, and an Eastern Cooperative Group performance status greater than 1; parameters specific to PCNSL include elevated CSF protein as well as tumour location within the deep regions of the brain (periventricular, basal ganglia, brainstem and/or cerebellum). The presence of 0–1, 2–3, or 4–5 adverse risk factors correlates with 2-year survival rates of 80%, 48% or 15%, respectively (Ferreri et al., 2003). While the IELSG considered age 60 years to be the cut-off point above which prognosis declines, the Memorial Sloan-Kettering prognostic index employs an age cut-off point of 50 years (Abrey et al., 2006). These prognostic models can only be used in PVRL if there is concomitant cerebral disease. A clinical prognostic index for PVRL alone has yet not
been devised, but could be an output of the above-men-
tioned international PVRL registry.

Conclusion

Though no standardized recommendations exist for the diag-
nosis and treatment of PVRL, significant advances have been
made in the field in the last decades regarding earlier detec-
tion of the disease, both clinically and using novel pathologi-
cal tests, as well as in the efficacy testing of combined
radiotherapy and chemotherapy. The advent of biologicals,
such as rituximab, creates hope in the community for a tar-
geted and efficacious therapy. Multicentre collaborative inter-
national registries and carefully designed clinical trials with
associated translational research are the only way forward to
better understand the pathogenesis of PVRL, determine the
true relative efficacy and tolerability of available therapies
and to identify abnormal pathways that could lead to the use
of new target agents.

Authorship contributions

All authors have significantly contributed to the design and
writing of the manuscript.

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