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How we diagnose and treat vitreoretinal lymphoma

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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.

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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

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Table I. Summary of the morphological, immunophenotypic, genotypic and clinical features known to date for the various intraocular lymphoma subtypes.

Intraocular Lymphoma	Vitreoretinal	Uveal	
		Primary	Secondary
Most common WHO subtype	DLBCL	EMZL	Dependent on systemic NHL (majority DLBCL)
Immunoprofile	CD79a+ CD20 + PAX5 + BCL2 + BCL6 + /- MUM1/IRF4 + CD10-/+ CD43- IgM+ Ki67 rate: high (>80%)	CD79a+ CD20 + PAX5 + /- BCL2 + BCL6- MUM1/IRF4 + /- CD10- CD43 + /- IgM+ Ki67 rate: low (5-15%)	Dependent on systemic NHL
Genotype	High somatic *IGH* mutation load Few on-going somatic mutations Chromosomal translocations: currently not known *MYD88* mutations in ~70%	Low-to-moderate somatic *IGH* mutation load Few on-going somatic mutations Chromosomal abnormalities: t(11;18)(q21;q21) *MYD88* mutations – not known	Dependent on systemic NHL
Putative Cell of Origin	Possibly 2 different types: (i) Early post-germinal centre B cell = DLBCL of ABC type (majority) (ii) Germinal centre cell = DLBCL of GCB type (minority)	Post-germinal centre (memory) B cell	Dependent on systemic NHL
Clinical Features	Typically present in 6th and 7th decade Symptoms: 'Floaters' Painless decrease in visual acuity Signs: Vitreous infiltrates, possibly with subretinal involvement. Rare involvement of choroid Often bilateral RPE changes on FA with 'leopard skin'-like alterations caused by RPE hyper- and hypo-pigmentation. CNS involvement (70–80% of patients) Treatment: chemotherapy+/-radiotherapy+/- Poor prognosis	Typically present in 4th and 5th decade Symptoms: Blurring of vision Metamorphopsia Signs: Clear vitreous Diffuse thickening of iris and/or choroid Usually unilateral Extraocular extension frequent No CNS involvement Treatment: low dose radiotherapy. Good prognosis	Typically >60 years Symptoms: Previous history of systemic NHL Decrease in VA Signs: Clear vitreous Diffuse thickening of iris or and/or choroid Uni- or bilateral involvement Concurrent secondary CNS involvement possible Treatment: dependent on NHL subtype Poor prognosis

DLBCL, diffuse large B-cell lymphoma; EMZL, extranodal marginal zone B-cell lymphoma; NHL, Non Hodgkin lymphoma; CD, cluster of differentiation; ABC, activated B-cell type; GCB, germinal centre B-cell type; VA, visual acuity; RPE, retinal pigment epithelium; FA, fluorescein angiography; WHO, World Health Organization; CNS, central nervous system.

review also briefly discusses available literature on other lymphoma entities that may infiltrate the eye.

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 (Coupland *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 (Coupland *et al*, 2005a; Camilleri-Broet *et al*, 2006). VRL usually expresses monotypic immunoglobulin light chains and IgM or IgM and IgD heavy chains (Coupland *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

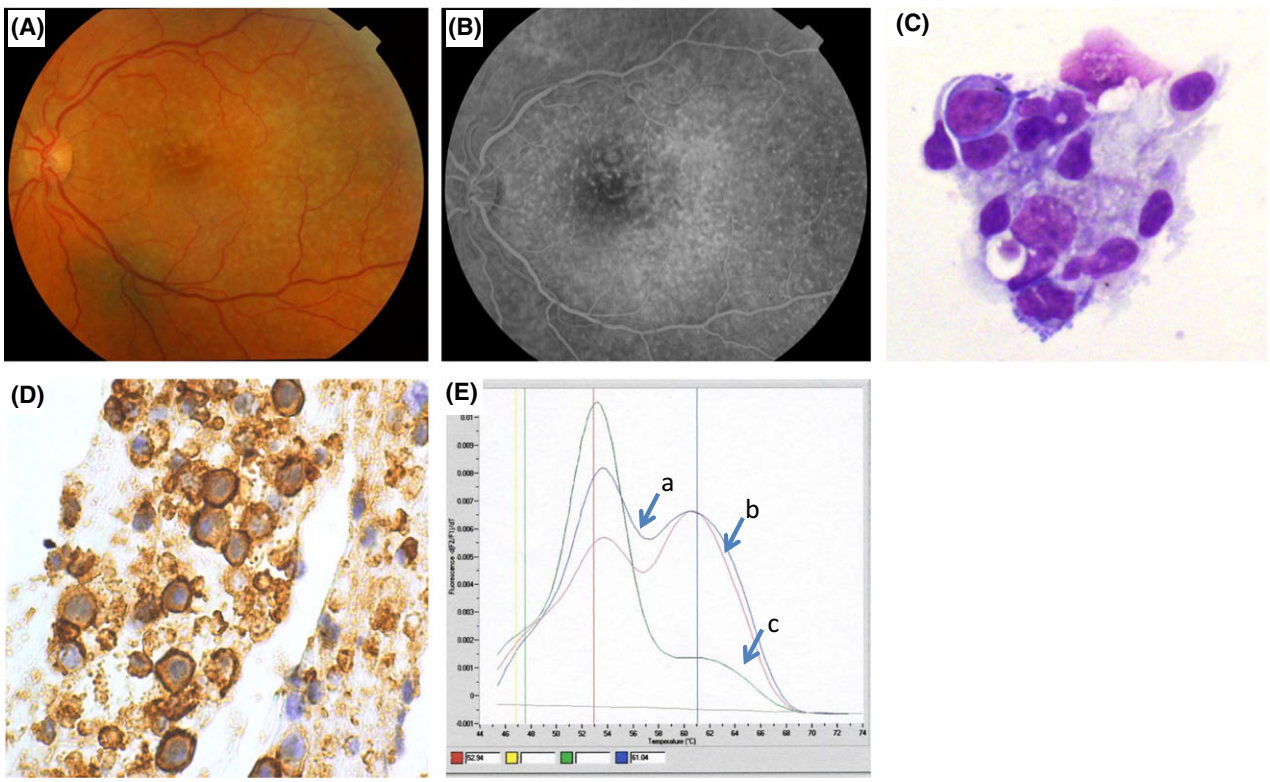

Fig 1. A 72-year-old female with a 17-month history of recurrent posterior uveitis of both eyes. No response to steroids. (A) Fundoscopy of the left eye – diffuse orange-yellow retinal infiltrates. (B) Fluorescein angiography of the retina shows the typical variegated hyper- and hypopigmentation ('leopard skin' appearance). (C) Intraocular vitrectomy sample stained with MGG shows an aggregate of large atypical lymphocytes with some background macrophages (objective 60x); (D) CD79a+ B-cells are the dominant infiltrating cell population (objective 60x); (E) Allele-specific polymerase chain reaction with melting curve analysis for *MYD88* mutation. Wild-type T 53°C, *MYD88* L265P 61°C; Positive control (a), patient DNA with (b) and without (c) WT-suppressing LNA. Diagnosis: vitreoretinal B-cell Lymphoma (VRL). Staging: concurrent central nervous system disease. Treatment: Intrathecal and systemic R-CHOP with methotrexate. The patient succumbed to CNSL within one year of VRL diagnosis.

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-

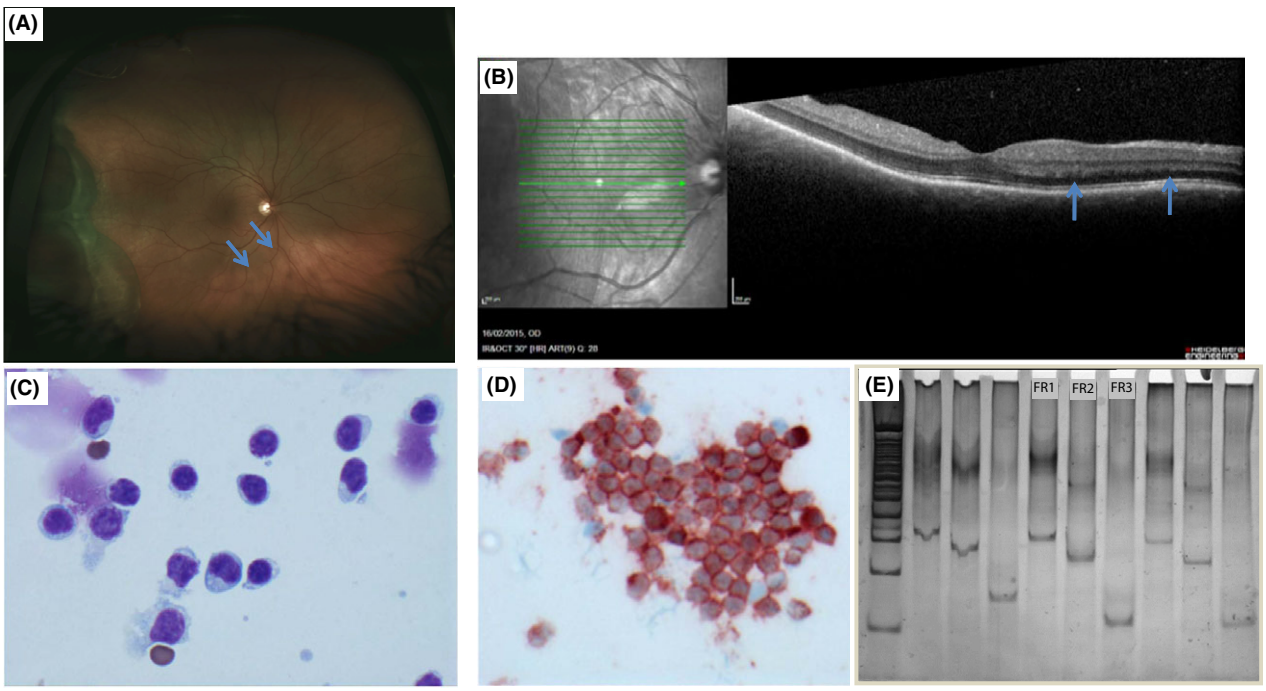

Fig 2. A 45-year-old male, with a 4-month history of blurred vision of the right eye. No previous medical history. Non-smoker. (A) Fundoscopy – creamy choroidal infiltrates also close to the macula (arrow). (B) Optical Coherence Tomography (OCT) demonstrates a thickening of the choroid with partial detachment of the retina (arrow). (C) Intraocular biopsy stained with May-Grünwald-Giemsa shows small atypical lymphocytes with some scattered background erythrocytes (objective 60x); (D) CD79a+ B-cells are the dominant cell population (objective 40x); (E) *IGH*-polymerase chain reaction electrophoresis gel with bands at 310, 260 and 120 base pairs (FR1, FR2 and FR3, respectively). Diagnosis: primary choroidal 'Extranodal Marginal Zone B-cell Lymphoma' (EMZL). Staging investigations: no systemic or CNS disease. Treatment: low-dose radiotherapy with good response.

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 1·38. 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 (AlQahtani *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 patents 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 only 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 (Coupland *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*,

2004; Cassoux *et al*, 2007; Saleh *et al*, 2012; Fisson *et al*, 2013; Raja *et al*, 2013; Mehta *et al*, 2015).

Our approach

In our institutions, we immediately work up the freshly received vitrectomy specimens, preparing cytospins 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 testicular sonography, given the propensity of testicular 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 radiation – see also below) (Smith *et al*, 2002; Helbig *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; Helbig *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 irradiation 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 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-mentioned international PVRL registry.

Conclusion

Though no standardized recommendations exist for the diagnosis and treatment of PVRL, significant advances have been made in the field in the last decades regarding earlier detection of the disease, both clinically and using novel pathological 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 targeted and efficacious therapy. Multicentre collaborative international 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.

References

Abrey, L.E., Ben-Porat, L., Panageas, K.S., Yahalom, J., Berkey, B., Curran, W., Schultz, C., Leibel, S., Nelson, D., Mehta, M. & DeAngelis, L.M. (2006) Primary central nervous system lymphoma: the Memorial Sloan-Kettering Cancer Center prognostic model. *Journal of Clinical Oncology*, **24**, 5711–5715.

AlQahtani, A., Touitou, V., Cassoux, N., Aknin, C., Merle-Beral, H., Bodaghi, B. & LeHoang, P. (2014) More than a masquerade syndrome: atypical presentations of vitreoretinal lymphomas. *Ocular Immunology and Inflammation*, **22**, 189–196.

Baehring, J.M., Androudi, S., Longtine, J.J., Betensky, R.A., Sklar, J., Foster, C.S. & Hochberg, F.H. (2005) Analysis of clonal immunoglobulin heavy chain rearrangements in ocular lymphoma. *Cancer*, **104**, 591–597.

Batchelor, T.T., Kolak, G., Ciordia, R., Foster, C.S. & Henson, J.W. (2003) High-dose methotrexate for intraocular lymphoma. *Clinical Cancer Research*, **9**, 711–715.

Bonzheim, I., Giese, S., Deuter, C., Susskind, D., Zierhut, M., Waizel, M., Szurman, P., Federmann, B., Schmidt, J., Quintanilla-Martinez, L., Coupland, S.E., Bartz-Schmidt, K.U. & Fend, F. (2015) High frequency of MYD88 mutations in vitreoretinal B-cell lymphoma: a valuable tool to improve diagnostic yield of vitreous aspirates. *Blood*, **126**, 76–79.

Braggio, E., Van Wier, S., Ojha, J., McPhail, E., Asmann, Y.W., Egan, J., da Silva, J.A., Schiff, D., Lopes, M.B., Decker, P.A., Valdez, R., Tibes, R., Eckloff, B., Witzig, T.E., Stewart, A.K., Fonseca, R. & O'Neill, B.P. (2015) Genome-wide analysis uncovers novel recurrent alterations in primary central nervous system lymphomas. *Clinical Cancer Research*, **21**, 3986–3994.

Bruno, A., Boisselier, B., Labreche, K., Marie, Y., Polivka, M., Jouvet, A., Adam, C., Figarella-Branger, D., Miquel, C., Eimer, S., Houillier, C., Soussain, C., Mokhtari, K., Daveau, R. & Hoang-Xuan, K. (2014) Mutational analysis of primary central nervous system lymphoma. *Oncotarget*, **5**, 5065–5075.

Cady, F.M., O'Neill, B.P., Law, M.E., Decker, P.A., Kurtz, D.M., Giannini, C., Porter, A.B., Kurtin, P.J., Johnston, P.B., Dogan, A. & Remstein, E.D. (2008) Del(6)(q22) and BCL6 rearrangements in primary CNS lymphoma are indicators of an aggressive clinical course. *Journal of Clinical Oncology*, **26**, 4814–4819.

Camilleri-Broet, S., Criniere, E., Broet, P., Delwail, V., Mokhtari, K., Moreau, A., Kujas, M., Raphael, M., Iraqi, W., Sautes-Fridman, C., Colombat, P., Hoang-Xuan, K. & Martin, A. (2006) A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. *Blood*, **107**, 190–196.

Cassoux, N., Giron, A., Bodaghi, B., Tran, T.H., Baudet, S., Davy, F., Chan, C.C., Lehoang, P. & Merle-Beral, H. (2007) IL-10 measurement in aqueous humor for screening patients with suspicion of primary intraocular lymphoma. *Investigative Ophthalmology & Visual Science*, **48**, 3253–3259.

Chan, C.C. (2003) Molecular pathology of primary intraocular lymphoma. *Transactions of the American Ophthalmological Society*, **101**, 275–292.

Chan, C.C. & Sen, H.N. (2013) Current concepts in diagnosing and managing primary vitreoretinal (intraocular) lymphoma. *Discovery Medicine*, **15**, 93–100.

Chan, C.C., Whitcup, S.M., Solomon, D. & Nussenblatt, R.B. (1995) Interleukin-10 in the vitreous of patients with primary intraocular lymphoma. *American Journal of Ophthalmology*, **120**, 671–673.

Chan, C.C., Shen, D., Hackett, J.J., Buggage, R.R. & Tuaillon, N. (2003) Expression of chemokine receptors, CXCR4 and CXCR5, and chemokines, BLC and SDF-1, in the eyes of patients with primary intraocular lymphoma. *Ophthalmology*, **110**, 421–426.

Cooper, E.L. & Riker, J.L. (1951) Malignant lymphoma of the uveal tract. *American Journal of Ophthalmology*, **34**, 1153–1158.

Coupland, S.E. (2012) Analysis of intraocular biopsies. In: Current Concepts in Uveal Melanoma (eds M.J. Jager, L. Desjardins, T. Kivelä & B.E. Damato), pp. 96–116. **vol 49**. Dev Ophthalmol. Karger, Basel.

Coupland, S.E. & Damato, B. (2008) Understanding intraocular lymphomas. *Clinical Experiment Ophthalmology*, **36**, 564–578.

Coupland, S.E., Foss, H.D., Bechrakis, N.E., Hummel, M. & Stein, H. (2001) Secondary ocular involvement in systemic "memory" B-cell lymphocytic leukemia. *Ophthalmology*, **108**, 1289–1295.

Coupland, S.E., Bechrakis, N.E., Anastassiou, G., Foerster, A.M., Heiligenhaus, A., Pleyer, U., Hummel, M. & Stein, H. (2003) Evaluation of vitrectomy specimens and chorioretinal biopsies in the diagnosis of primary intraocular lymphoma in patients with Masquerade syndrome. *Graefes Archive for Clinical and Experimental Ophthalmology*, **241**, 860–870.

Coupland, S.E., Heimann, H. & Bechrakis, N.E. (2004) Primary intraocular lymphoma: a review of the clinical, histopathological and molecular biological features. *Graefes Archive for Clinical and Experimental Ophthalmology*, **242**, 901–913.

Coupland, S.E., Loddenkemper, C., Smith, J.R., Braziel, R.M., Charlotte, F., Anagnostopoulos, I. & Stein, H. (2005a) Expression of immunoglobulin transcription factors in primary intraocular lymphoma and primary central nervous system lymphoma. *Investigative Ophthalmology & Visual Science*, **46**, 3957–3964.

Coupland, S.E., Hummel, M., Muller, H.H. & Stein, H. (2005b) Molecular analysis of immunoglobulin genes in primary intraocular lymphoma. *Investigative Ophthalmology & Visual Science*, **46**, 3507–3514.

Coupland, S.E., Anastassiou, G., Bornfeld, N., Hummel, M. & Stein, H. (2005c) Primary intraocular lymphoma of T-cell type: report of a case and review of the literature. *Graefes Archive for Clinical and Experimental Ophthalmology*, **243**, 189–197.

Coupland, S.E., Chan, C.C. & Smith, J. (2009) Pathophysiology of retinal lymphoma. *Ocular Immunology and Inflammation*, **17**, 227–237.

Davis, J.L., Miller, D.M. & Ruiz, P. (2005) Diagnostic testing of vitrectomy specimens. *American Journal of Ophthalmology*, **140**, 822–829.

Davis, J.L., Ruiz, P. Jr, Shah, M. & Mandelcorn, E.D. (2012) Evaluation of the reactive T-cell infiltrate in uveitis and intraocular lymphoma with flow cytometry of vitreous fluid (an American Ophthalmological Society thesis). *Transactions of the American Ophthalmological Society*, **110**, 117–129.

Deckert, M., Montesinos-Rongen, M., Brunn, A. & Siebert, R. (2014) Systems biology of primary CNS lymphoma: from genetic aberrations to modeling in mice. *Acta Neuropathologica*, **127**, 175–188.

Egawa, M., Mitamura, Y., Hayashi, Y., Semba, K. & Naito, T. (2014) Changes of fundus autofluorescence and spectral-domain optical coherence tomographic findings after treatment of primary intraocular lymphoma. *Journal of Ophthalmic Inflammation and Infection*, **4**, 7.

Fardeau, C., Lee, C.P., Merle-Beral, H., Cassoux, N., Bodaghi, B., Davi, F. & Lehoang, P. (2009) Retinal fluorescein, indocyanine green angiography, and optic coherence tomography in non-Hodgkin primary intraocular lymphoma. *American Journal of Ophthalmology*, **147**, 886–894, 894 e881.

Ferreri, A.J. (2015) Who Is Who in Primary Vitreoretinal Lymphoma? *JAMA Oncology*, **1**, 977–978.

Ferreri, A.J., Blay, J.Y., Reni, M., Pasini, F., Gubkin, A., Tirelli, U., Calderoni, A., Zucca, E., Cortelazzo, S., Chassagne, C., Tinguely, M., Borisch, B., Berger, F., Ponzoni, M. & Cavalli, F. & International Extranodal Lymphoma Study Group. (2002) Relevance of intraocular involvement in the management of primary central nervous system lymphomas. *Annals of Oncology*, **13**, 531–538.

Ferreri, A.J., Blay, J.Y., Reni, M., Pasini, F., Spina, M., Ambrosetti, A., Calderoni, A., Rossi, A., Vavassori, V., Conconi, A., Devizzi, L., Berger, F., Ponzoni, M., Borisch, B., Tinguely, M., Cerati, M., Milani, M., Orvieto, E., Sanchez, J., Chevreau, C., Dell'Oro, S., Zucca, E. & Cavalli, F. (2003) Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. *Journal of Clinical Oncology*, **21**, 266–272.

Ferreri, A.J., Reni, M., Foppoli, M., Martelli, M., Pangalis, G.A., Frezzato, M., Cabras, M.G., Fabbri, A., Corazzelli, G., Ilariucci, F., Rossi, G., Soffietti, R., Stelitano, C., Vallisa, D., Zaja, F., Zoppegno, L., Aondio, G.M., Avvisati, G., Balzarotti, M., Brandes, A.A., Fajardo, J., Gomez, H., Guarini, A., Pinotti, G., Rigacci, L., Uhlmann, C., Picozzi, P., Vezzulli, P., Ponzoni, M., Zucca, E., Caligaris-Cappio, F. & Cavalli, F. & International Extranodal Lymphoma Study Group. (2009) High-dose cytarabine plus high-dose methotrexate versus high-dose methotrexate alone in patients with primary CNS lymphoma: a randomised phase 2 trial. *Lancet*, **374**, 1512–1520.

Ferreri, A.J., Zucca, E., Armitage, J., Cavalli, F. & Batchelor, T.T. (2013) Ten years of international primary CNS lymphoma collaborative group studies. *Journal of Clinical Oncology*, **31**, 3444–3445.

Fisson, S., Ouakrim, H., Touitou, V., Baudet, S., Ben Abdelwahed, R., Donnou, S., Miloudi, A., Galand, C., Bodaghi, B., Lehoang, P., Brissard, M., Le Garff-Tavernier, M., Fridman, W.H., Sautes-Fridman, C., Cassoux, N. & Merle-Beral, H. (2013) Cytokine profile in human eyes: contribution of a new cytokine combination for differential diagnosis between intraocular lymphoma or uveitis. *PLoS ONE*, **8**, e52385.

Frenkel, S., Hendler, K., Siegal, T., Shalom, E. & Pe'er, J. (2008) Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. *British Journal of Ophthalmology*, **92**, 383–388.

Fung, S., Selva, D., Leibovitch, I., Hsuan, J. & Crompton, J. (2005) Ophthalmic manifestations of multiple myeloma. *Ophthalmologica*, **219**, 43–48.

Gonzalez-Aguilar, A., Idbaih, A., Boisselier, B., Habbita, N., Rossetto, M., Laurenge, A., Bruno, A., Jouvet, A., Polivka, M., Adam, C., Figarella-Branger, D., Miquel, C., Vital, A., Ghesquieres, H., Gressin, R., Delwail, V., Taillandier, L., Chinot, O., Soubeyran, P., Gyan, E., Choquet, S., Houillier, C., Soussain, C., Tanguy, M.L., Marie, Y., Mokhtari, K. & Hoang-Xuan, K. (2012) Recurrent mutations of MYD88 and TBL1XR1 in primary central nervous system lymphomas. *Clinical Cancer Research*, **18**, 5203–5211.

Grimm, S.A., Pulido, J.S., Jahnke, K., Schiff, D., Hall, A.J., Shenkier, T.N., Siegal, T., Doolittle, N.D., Batchelor, T., Herrlinger, U., Neuwelt, E.A., Laperriere, N., Chamberlain, M.C., Blay, J.Y., Ferreri, A.J., Omuro, A.M., Thiel, E. & Abrey, L.E. (2007) Primary intraocular lymphoma: an International Primary Central Nervous System Lymphoma Collaborative Group Report. *Annals of Oncology*, **18**, 1851–1855.

Grimm, S.A., McCannel, C.A., Omuro, A.M., Ferreri, A.J., Blay, J.Y., Neuwelt, E.A., Siegal, T., Batchelor, T., Jahnke, K., Shenkier, T.N., Hall, A.J., Graus, F., Herrlinger, U., Schiff, D., Raizer, J., Rubenstein, J., Laperriere, N., Thiel, E., Doolittle, N., Iwamoto, F.M. & Abrey, L.E. (2008) Primary CNS lymphoma with intraocular involvement: International PCNSL Collaborative Group Report. *Neurology*, **71**, 1355–1360.

Hashida, N., Ohguro, N. & Nishida, K. (2012) Efficacy and complications of intravitreal rituximab injection for treating primary vitreoretinal lymphoma. *Translational Vision Science and Technology*, **1**, 1.

Helbig, H., Cerny, T. & de Smet, M.D. (2003) Intravitreal chemotherapy for intraocular lymphoma. *Ophthalmologe*, **100**, 145–149.

Hoang-Xuan, K., Bessell, E., Bromberg, J., Hottinger, A.F., Preusser, M., Rudà, R., Schlegel, U., Siegal, T., Soussain, C., Abacioglu, U., Cassoux, N., Deckert, M., Dirven, C.M.F., Ferreri, A.J.M., Graus, F., Henriksson, R., Herrlinger, U., Taphoorn, M., Soffietti, R. & Weller, M. (2015) Diagnosis and treatment of primary CNS lymphoma in immunocompetent patients: guidelines from the European Association for Neuro-Oncology. *The Lancet Oncology*, **16**, e322–e332.

Itty, S. & Pulido, J.S. (2009) Rituximab for intraocular lymphoma. *Retina*, **29**, 129–132.

Kasenda, B., Schorb, E., Fritsch, K., Finke, J. & Illerhaus, G. (2012) Prognosis after high-dose chemotherapy followed by autologous stem-cell transplantation as first-line treatment in primary CNS lymphoma–a long-term follow-up study. *Annals of Oncology*, **23**, 2670–2675.

Kimura, K., Usui, Y. & Goto, H. (2012) Clinical features and diagnostic significance of the intraocular fluid of 217 patients with intraocular lymphoma. *Japanese Journal of Ophthalmology*, **56**, 383–389.

Kluin, P.M., Deckert, M. & Ferry, J.A. (2008) Primary diffuse large B-cell lymphoma of the CNS. In: WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues (eds Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H., Thiele, J. & Vardiamn, J.W.), pp. 240–241. IARC Press, Lyon.

Kraan, W., Horlings, H.M., van Keimpema, M., Schilder-Tol, E.J., Oud, M.E., Scheepstra, C., Kluin, P.M., Kersten, M.J., Spaargaren, M. & Pals, S.T. (2013) High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. *Blood Cancer Journal*, **3**, e139.

Kuker, W., Nagele, T., Korfel, A., Heckl, S., Thiel, E., Bamberg, M., Weller, M. & Herrlinger, U. (2005) Primary central nervous system lymphomas (PCNSL): MRI features at presentation in 100 patients. *Journal of Neuro-Oncology*, **72**, 169–177.

Larkin, K.L., Saboo, U.S., Comer, G.M., Forooghian, F., Mackensen, F., Merrill, P., Sen, H.N., Singh, A., Essex, R.W., Lake, S., Lim, L.L., Vasconcelos-Santos, D.V., Foster, C.S., Wilson, D.J. & Smith, J.R. (2014) Use of intravitreal rituximab for treatment of vitreoretinal lymphoma. *British Journal of Ophthalmology*, **98**, 99–103.

Levasseur, S.D., Wittenberg, L.A. & White, V.A. (2013) Vitreoretinal lymphoma: a 20-year review of incidence, clinical and cytologic features, treatment, and outcomes. *JAMA Ophthalmology*, **131**, 50–55.

MacMahon, E.M., Glass, J.D., Hayward, S.D., Mann, R.B., Becker, P.S., Charache, P., McArthur, J.C. & Ambinder, R.F. (1991) Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet*, **338**, 969–973.

Malumbres, R., Davis, J., Ruiz, P. & Lossos, I.S. (2007) Somatically mutated immunoglobulin IGHV@ genes without intraclonal heterogeneity indicate a postgerminal centre origin of primary intraocular diffuse large B-cell lymphomas. *British Journal of Haematology*, **138**, 749–755.

Mehta, M., Rasheed, R.A., Duker, J., Reichel, E., Feinberg, E., Husain, D., Foster, C.S. & Laver, N.V. (2015) Vitreous evaluation: a diagnostic challenge. *Ophthalmology*, **122**, 531–537.

Merle-Beral, H., Davi, F., Cassoux, N., Baudet, S., Colin, C., Gourdet, T., Bodaghi, B. & LeHoang, P. (2004) Biological diagnosis of primary intraocular lymphoma. *British Journal of Haematology*, **124**, 469–473.

Missotten, T., Tielemans, D., Bromberg, J.E., van Hagen, P.M., van Lochem, E.G., van Dongen, J.J., Baarsma, G.S. & Langerak, A.W. (2013) Multicolor flowcytometric immunophenotyping

is a valuable tool for detection of intraocular lymphoma. *Ophthalmology*, **120**, 991–996.

Montesinos-Rongen, M., Kuppers, R., Schluter, D., Spieker, T., Van Roost, D., Schaller, C., Reifenberger, G., Wiestler, O.D. & Deckert-Schluter, M. (1999) Primary central nervous system lymphomas are derived from germinal-center B cells and show a preferential usage of the V4-34 gene segment. *American Journal of Pathology*, **155**, 2077–2086.

Montesinos-Rongen, M., Zuhlke-Jenisch, R., Gesk, S., Martin-Subero, J.I., Schaller, C., Van Roost, D., Wiestler, O.D., Deckert, M. & Siebert, R. (2002) Interphase cytogenetic analysis of lymphoma-associated chromosomal breakpoints in primary diffuse large B-cell lymphomas of the central nervous system. *Journal of Neuropathology and Experimental Neurology*, **61**, 926–933.

Montesinos-Rongen, M., Brunn, A., Bentink, S., Basso, K., Lim, W.K., Klapper, W., Schaller, C., Reifenberger, G., Rubenstein, J., Wiestler, O.D., Spang, R., Dalla-Favera, R., Siebert, R. & Deckert, M. (2008) Gene expression profiling suggests primary central nervous system lymphomas to be derived from a late germinal center B cell. *Leukemia*, **22**, 400–405.

Montesinos-Rongen, M., Godlewska, E., Brunn, A., Wiestler, O.D., Siebert, R. & Deckert, M. (2011) Activating L265P mutations of the MYD88 gene are common in primary central nervous system lymphoma. *Acta Neuropathologica*, **122**, 791–792.

Morris, P.G., Correa, D.D., Yahalom, J., Raizer, J.J., Schiff, D., Grant, B., Grimm, S., Lai, R.K., Reiner, A.S., Panageas, K., Karimi, S., Curry, R., Shah, G., Abrey, L.E., DeAngelis, L.M. & Omuro, A. (2013) Rituximab, methotrexate, procarbazine, and vincristine followed by consolidation reduced-dose whole-brain radiotherapy and cytarabine in newly diagnosed primary CNS lymphoma: final results and long-term outcome. *Journal of Clinical Oncology*, **31**, 3971–3979.

Mudhar, H.S., Sethuraman, C., Khan, M.D. & Jan, S.U. (2007) Intracular, pan-uveal intravascular large B-cell lymphoma associated with choroidal infarction and choroidal tri-lineage extramedullary haemtopoiesis. *Histopathology*, **51**, 275–279.

Nakamura, T., Tateishi, K., Niwa, T., Matsushita, Y., Tamura, K., Kinoshita, M., Tanaka, K., Fukushima, S., Takami, H., Arita, H., Kubo, A., Shuto, T., Ohno, M., Miyakita, Y., Kocialkowski, S., Sasayama, T., Hashimoto, N., Maehara, T., Shibui, S., Ushijima, T., Kawahara, N., Narita, Y. & Ichimura, K.(2015) Recurrent mutations of CD79B and MYD88 are the hallmark of primary central nervous system lymphomas. *Neuropathology and Applied Neurobiology*, doi: 10.1111/nan.12259 [Epub ahead of print]

Omuro, A., Chinot, O., Taillandier, L., Ghesquieres, H., Soussain, C., Delwail, V., Lamy, T., Gressin, R., Choquet, S., Soubeyran, P., Huchet, A., Benouaich-Amiel, A., Lebouvier-Sadot, S., Gyan, E., Touitou, V., Barrie, M., Del Rio, M.S., Gonzalez-Aguilar, A., Houillier, C., Delgadillo, D., Lacomblez, L., Tanguy, M.L. & Hoang-Xuan, K. (2015a) Methotrexate and temozolomide versus methotrexate, procarbazine, vincristine, and cytarabine for primary CNS lymphoma in an elderly population: an intergroup ANOCEF-GOELAMS randomised phase 2 trial. *Lancet Haematology*, **2**, e251–e259.

Omuro, A., Correa, D.D., DeAngelis, L.M., Moskowitz, C.H., Matasar, M.J., Kaley, T.J., Gavrilovic, I.T., Nolan, C., Pentsova, E., Grommes, C.C., Panageas, K.S., Baser, R.E., Faivre, G., Abrey, L.E. & Sauter, C.S. (2015b) R-MPV followed by high-dose chemotherapy with TBC and autologous stem-cell transplant for newly diagnosed primary CNS lymphoma. *Blood*, **125**, 1403–1410.

Payne, T., Karp, L.A. & Zimmerman, L.E. (1971) Intraocular involvement in Burkitt's lymphoma. *Archives of Ophthalmology*, **85**, 295–298.

Phillips, E.H., Fox, C.P. & Cwynarski, K. (2014) Primary CNS lymphoma. *Current Hematologic Malignancy Reports*, **9**, 243–253.

Ponzoni, M., Terreni, M.R., Ciceri, F., Ferreri, A.J., Gerevini, S., Anzalone, N., Valle, M., Pizzolito, S. & Arrigoni, G. (2002) Primary brain CD30 + ALK1 + anaplastic large cell lymphoma ('ALKoma'): the first case with a combination of 'not common' variants. *Annals of Oncology*, **13**, 1827–1832.

Qualman, S.J., Mendelsohn, G., Mann, R.B. & Green, W.R. (1983) Intraocular lymphomas. Natural history based on a clinicopathologic study of eight cases and review of the literature. *Cancer*, **52**, 878–886.

Raja, H., Snyder, M.R., Johnston, P.B., O'Neill, B.P., Caraballo, J.N., Balsanek, J.G., Peters, B.E., Decker, P.A. & Pulido, J.S. (2013) Effect of intravitreal methotrexate and rituximab on interleukin-10 levels in aqueous humor of treated eyes with vitreoretinal lymphoma. *PLoS ONE*, **8**, e65627.

Riemens, A., Bromberg, J., Touitou, V., Sobolewska, B., Missotten, T., Baarsma, S., Hoyng, C., Cordero-Coma, M., Tomkins-Netzer, O., Rozalski, A., Tugal-Tutkun, I., Guex-Crosier, Y., Los, L.I., Bollemeijer, J.G., Nolan, A., Pawade, J., Willermain, F., Bodaghi, B., ten Dam-van Loon, N., Dick, A., Zierhut, M., Lightman, S., Mackensen, F., Moulin, A., Erckens, R., Wensing, B., le Hoang, P., Lokhorst, H. & Rothova, A. (2015) Treatment strategies in primary vitreoretinal lymphoma: a 17-center European collaborative study. *JAMA Ophthalmology*, **133**, 191–197.

Riemersma, S.A., Jordanova, E.S., Schop, R.F., Philippo, K., Looijenga, L.H., Schuuring, E. & Kluin, P.M. (2000) Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphomas arising in immune-privileged sites. *Blood*, **96**, 3569–3577.

Rubenstein, J.L., Wong, V.S., Kadoch, C., Gao, H.X., Barajas, R., Chen, L., Josephson, S.A., Scott, B., Douglas, V., Maiti, M., Kaplan, L.D., Treseler, P.A., Cha, S., Hwang, J.H., Cinque, P., Cyster, J.G. & Lowell, C. (2013a) CXCL13 plus interleukin 10 is highly specific for the diagnosis of CNS lymphoma. *Blood*, **121**, 4740–4748.

Rubenstein, J.L., Hsi, E.D., Johnson, J.L., Jung, S.H., Nakashima, M.O., Grant, B., Cheson, B.D. & Kaplan, L.D. (2013b) Intensive chemotherapy and immunotherapy in patients with newly diagnosed primary CNS lymphoma: CALGB 50202 (Alliance 50202). *Journal of Clinical Oncology*, **31**, 3061–3068.

Sagoo, M.S., Mehta, H., Swampillai, A.J., Cohen, V.M., Amin, S.Z., Plowman, P.N. & Lightman, S. (2014) Primary intraocular lymphoma. *Survey of Ophthalmology*, **59**, 503–516.

Saleh, M., Nikolitch, K., Bourcier, T., Speeg, C. & Gaucher, D. (2012) Repeated IL-10 measurement in aqueous humor and OCT imaging are valuable tools to monitor intraocular lymphoma treated with intravitreal injections of methotrexate. *Graefes Archive for Clinical and Experimental Ophthalmology*, **250**, 761–764.

Smith, J.R., Rosenbaum, J.T., Wilson, D.J., Doolittle, N.D., Siegal, T., Neuwelt, E.A. & Pe'er, J. (2002) Role of intravitreal methotrexate in the management of primary central nervous system lymphoma with ocular involvement. *Ophthalmology*, **109**, 1709–1716.

Smith, J.R., Braziel, R.M., Paoletti, S., Lipp, M., Uguccioni, M. & Rosenbaum, J.T. (2003) Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. *Blood*, **101**, 815–821.

Smith, J.R., Falkenhagen, K.M., Coupland, S.E., Chipps, T.J., Rosenbaum, J.T. & Braziel, R.M. (2007) Malignant B cells from patients with primary central nervous system lymphoma express stromal cell-derived factor-1. *American Journal of Clinical Pathology*, **127**, 633–641.

Sugita, S., Takase, H., Sugamoto, Y., Arai, A., Miura, O. & Mochizuki, M. (2009) Diagnosis of intraocular lymphoma by polymerase chain reaction analysis and cytokine profiling of the vitreous fluid. *Japanese Journal of Ophthalmology*, **53**, 209–214.

Tuo, J., Shen, D., Yang, H.H. & Chan, C.C. (2014) Distinct microRNA-155 expression in the vitreous of patients with primary vitreoretinal lymphoma and uveitis. *American Journal of Ophthalmology*, **157**, 728–734.

Turaka, K., Bryan, J.S., De Souza, S., Gordon, A.J., Kwong, H.M. Jr, Ziemianski, M.C., Reddy, R. & Sell, C.H. (2012) Vitreoretinal lymphoma: changing trends in diagnosis and local treatment modalities at a single institution. *Clinical Lymphoma, Myeloma and Leukemia*, **12**, 412–417.

Vater, I., Montesinos-Rongen, M., Schlesner, M., Haake, A., Purschke, F., Sprute, R., Mettenmeyer, N., Nazzal, I., Nagel, I., Gutwein, J., Richter, J., Buchhalter, I., Russell, R.B., Wiestler, O.D., Eils, R., Deckert, M. & Siebert, R. (2015) The mutational pattern of primary lymphoma of the central nervous system determined by whole-exome sequencing. *Leukemia*, **29**, 677–685.

Vosganian, G.S., Boisot, S., Hartmann, K.I., Freeman, W.R., Sharpe, R.W., Tripuraneni, P. &

Saven, A. (2011) Primary intraocular lymphoma: a review. *Journal of Neuro-Oncology*, **105**, 127–134.

Wallace, D.J., Shen, D., Reed, G.F., Miyanaga, M., Mochizuki, M., Sen, H.N., Dahr, S.S., Buggage, R.R., Nussenblatt, R.B. & Chan, C.C. (2006) Detection of the bcl-2 t(14;18) translocation and proto-oncogene expression in primary intraocular lymphoma. *Investigative Ophthalmology & Visual Science*, **47**, 2750–2756.

Wang, Y., Shen, D., Wang, V.M., Sen, H.N. & Chan, C.C. (2011) Molecular biomarkers for the diagnosis of primary vitreoretinal lymphoma. *International Journal of Molecular Sciences*, **12**, 5684–5697.

Wang, L., Sato-Otsubo, A., Sugita, S., Takase, H., Mochizuki, M., Usui, Y., Goto, H., Koyama, T., Akiyama, H., Miura, O., Ogawa, S. & Arai, A. (2014) High-resolution genomic copy number profiling of primary intraocular lymphoma by single nucleotide polymorphism microarrays. *Cancer Science*, **105**, 592–599.

Wittenberg, L.A., Maberley, D.A., Ma, P.E., Wade, N.K., Gill, H. & White, V.A. (2008) Contribution of vitreous cytology to final clinical diagnosis fifteen-year review of vitreous cytology specimens from one institution. *Ophthalmology*, **115**, 1944–1950.

Wysenbeek, Y.S., Nissenkorn, I., Cohen, S., Ben-Sira, I., Stark, B. & Zaizov, R. (1987) Intraocular involvement of Burkitt's lymphoma in a Bedouin child. *Pediatric Hematology and Oncology*, **4**, 309–314.