The young side of
LYMPHOMA

gli under 40 a confronto

Milano, 14-15 aprile 2023

MARCATORI BIOLOGICI E TARGET MOLECOLARI NEI LINFOMI DEL SISTEMA NERVOSO CENTRALE

Dott.ssa Lucia Bongiovanni
IRCCS Ospedale San Raffaele
Università Vita-Salute San Raffaele

Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
			NO DISCLOSURES				

CLINICAL CASE #1

Male 63 yo → left hemisoma paresthesias + left leg hyposthenia + dizziness and persistent vomitus → Contrast brain MRI

↓

Right thalamic mass with a moderate amount of perilesional oedema + encephalic trunk

↓

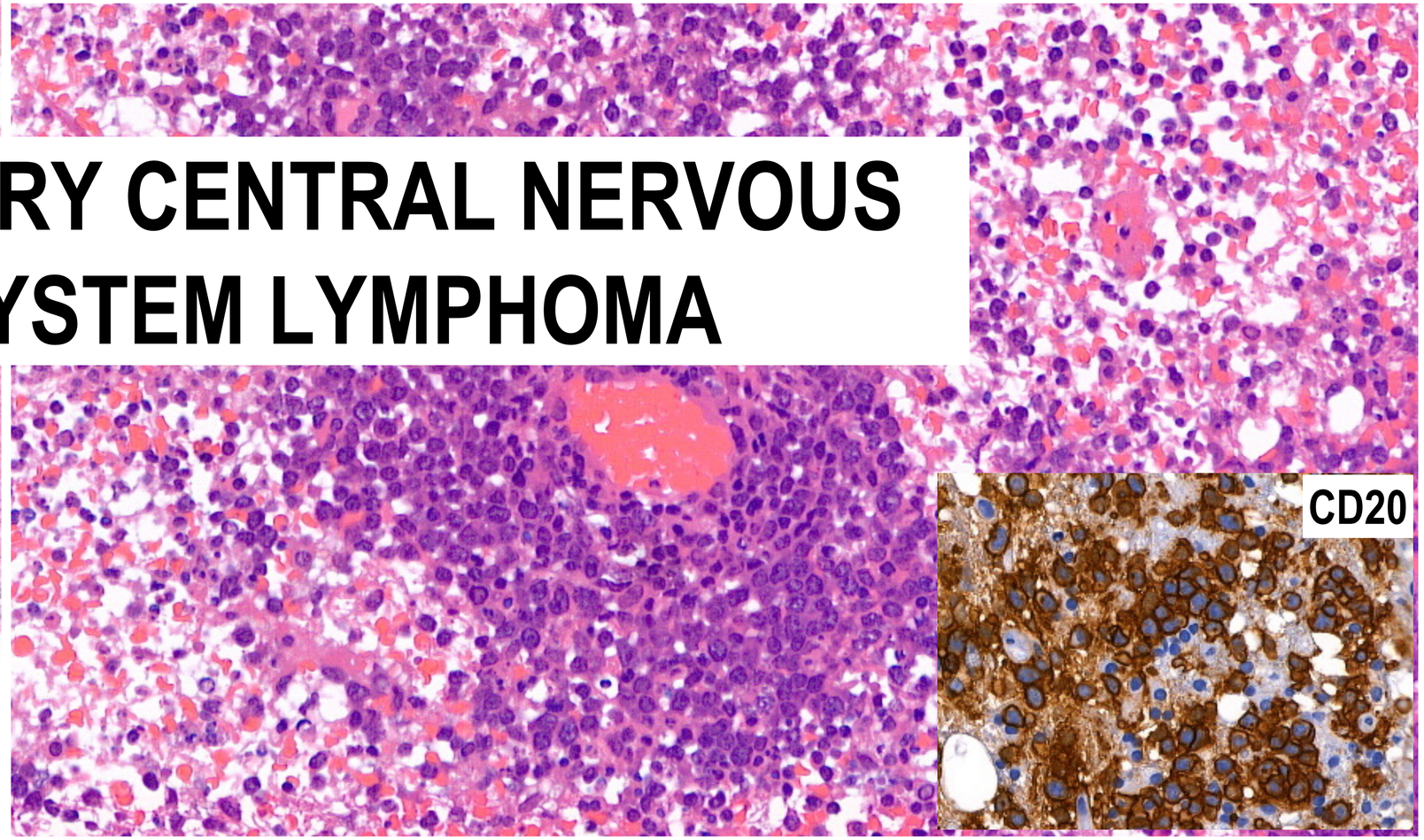
HD steroids for five days with improvement of the sintomatology

↓

Stereotactic biopsy

STEREOTACTIC BIOPSY

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA



CLINICAL CASE #1

Male 63 yo → left hemisoma paresthesias + left leg hyposthenia + dizziness and persistent vomitus → Contrast brain MRI

↓

Right thalamic mass with a moderate amount of perilesional oedema + encephalic trunk

↓

HD steroids for five days with improvement of the sintomatology

↓

Stereotactic biopsy → **PCNSL** → **MATRix polychemiotherapy**

HD-MTX/cytarabine/thiotepa/rituximab

↑

Consolidative whole-brain irradiation

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

PRIMARY LARGE B CELL LYMPHOMA OF THE IMMUNOPRIVILEGED SITES WHO 5ed.

Non invasive options

Cerebrospinal fluid vs **Peripheral blood**

Low sensitivity

original reports

Circulating Tumor DNA Profiling for Detection, Risk Stratification, and Classification of Brain Lymphomas JCO, 2022

Jurik A. Mutter, BSc[1,2]; Stefan K. Alig, MD[3]; Mohammad S. Esfahani, PhD[3]; Eliza M. Lauer, MD[1]; Jan Mitschke, PhD[1]; David M. Kurtz, MD, PhD[3]; Julia Kühn, MD[1]; Sabine Bleul[1]; Mari Olsen, PhD[3]; Chih Long Liu, PhD[3]; Michael C. Jin, BS[3]; Charles W. Macaulay, MSc[3]; Nicolas Neidert, MD[4,5]; Timo Volk, MD[6]; Michel Eisenblaetter, MD[7]; Sebastian Rauer, MD[6]; Dieter H. Heiland, MD[4]; Jürgen Finke, MD[1]; Justus Duyster, MD[1]; Julius Wehrle, MD[1]; Marco Prinz, MD[8,9,10]; Gerald Illerhaus, MD[11]; Peter C. Reinacher, MD[12,13]; Elisabeth Schorb, MD[1]; Maximilian Diehn, MD, PhD[14]; Ash A. Alizadeh, MD, PhD[3]; and Florian Scherer, MD[1,15]

Cytology

Low diagnostic accuracy

Sensitivity 13,3%

Flow cytometry

Sensitivity 23,3%

(antigens and free light chains)

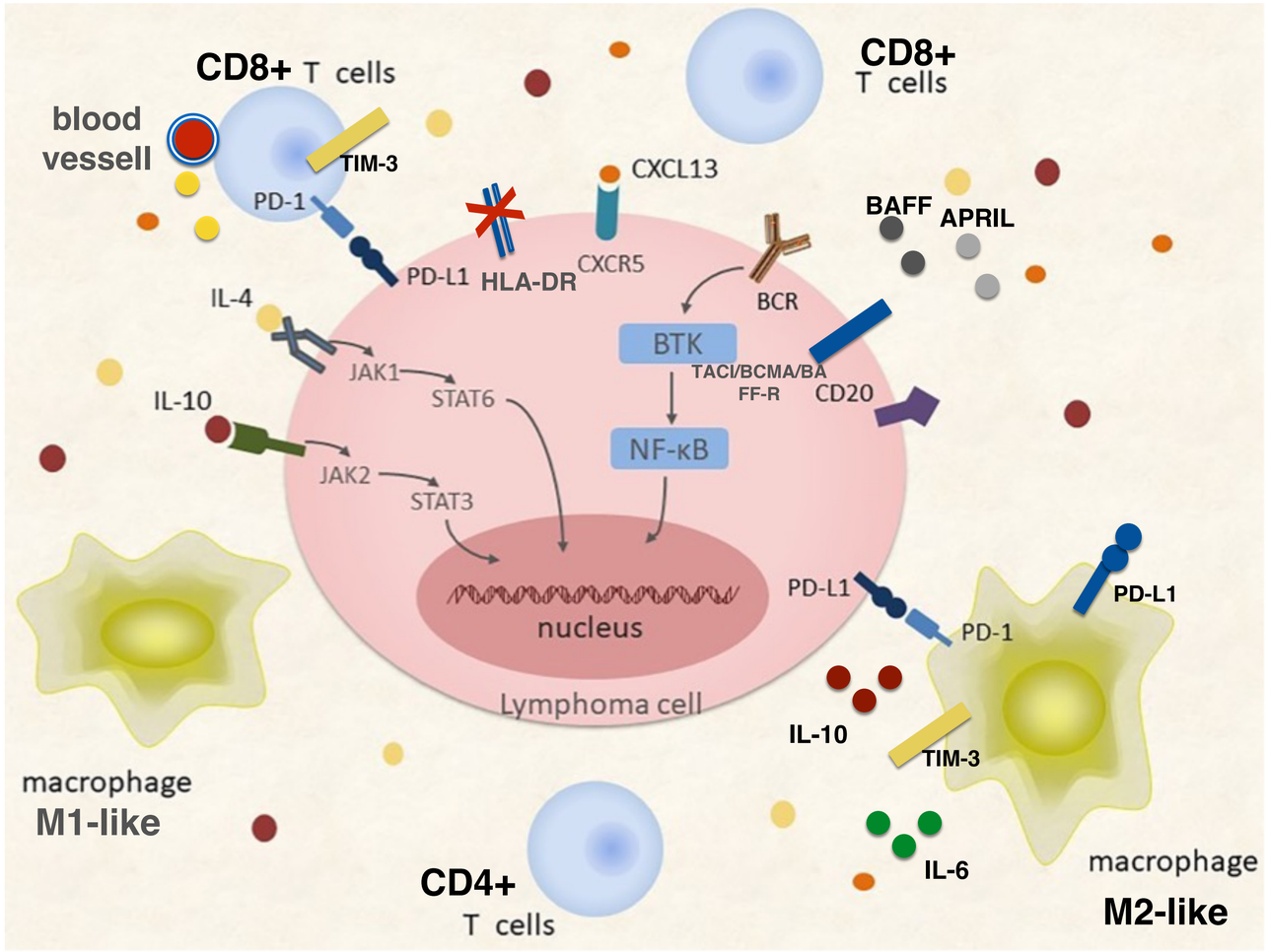
Biochemical analysis (Interleukines, Chemokines, APRIL, BAFF, TACI, BCMA, PD-L1, metabolic markers…)

Molecular analysis (clonality studies, ctDNA)

Schroers et al. Eur Journal of Haematology, 2010

Canovi et al. Diagnostic pathology, 2016

TUMOR MICROENVIRONMENT IN PCNSL



Within tumor, **CD8+ tumor infiltrating lymphocytes (TILs)** and tumor associated macrophages (TAMs) are the most represented components of TME in PCNSL.

Around tumors, CD4-positive cells predominate

- **Anti-tumoral M1-like CD68+ CD163^{low}**
- **Pro-tumoral M2-like CD68+ CD163^{high}**

M2-like macrophages are positioned in the **central portion of the tumor**, where they form palisades around the necrotic areas.

Immunological targets —> PD-L1 TIM-3

The absolute count of CD163+ M2 macrophages is not prognostically relevant

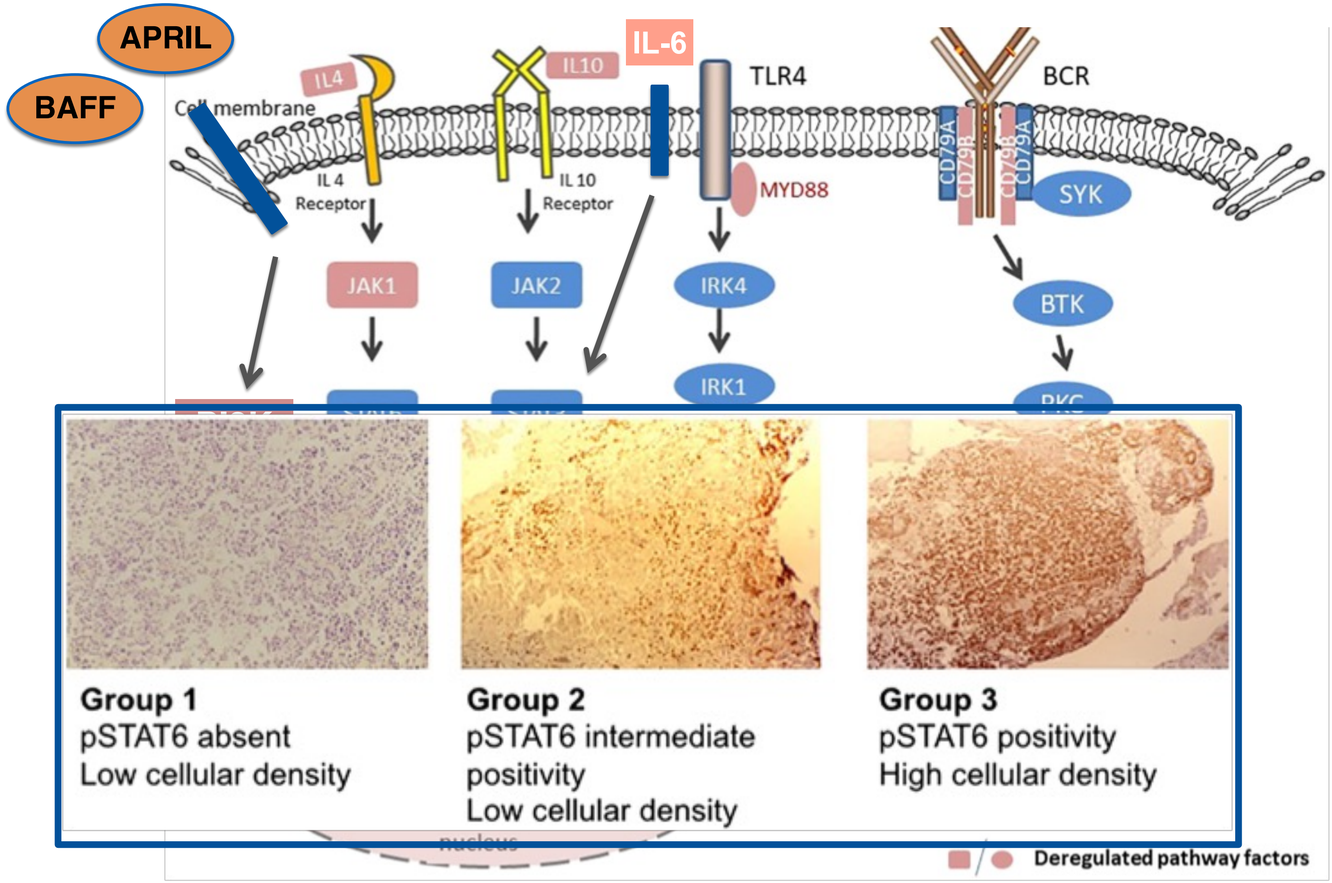
—> low M1/M2 ratio a worse outcome

Modified from Cai et al. 2019
Marcelis et al. 2020
Sasayama et al. 2016

PATHWAYS IN PCNSL

- **BAFF and APRIL** can active mTOR/AKT pathway
- **IL-10 and IL-6** can activate STAT3 and can be produced by both cancer cells and TAMs
- **MYD88 mutations** are detectable in 58% to 86% of PCNSL cases

Elevated expression of **pSTAT6** in PCNSL, in association with the expression of **IL-4 in neoplastic cells and in the endothelium**, correlated with **higher levels of IL-10 and IL-4 in the CSF and a worse prognosis**.



Zhou et al. Front Imm, 2020

From, Chapuy et al. Blood, 2016

From Cai et al. 2019

Mondello P et al. 2019

IL10 and CXCL13

CSF IL-10 in patients with PCNSL or systemic DLBCL is significantly higher than in other brain tumors, lymphomas and non-neoplastic disorders

Biomarker/Method.	Number of Patients	Body Fluid	Sensitivity (%)	Specificity (%)	References
IL-10 (cut-off 9.5 pg/mL)	66	CSF	71	100	Sasayama et al.
IL-10 (cut-off 4 pg/mL)	119	CSF	88.6	88.9	Nguyen-Them et al.
IL-10 (cut-off 8.2 pg/mL)	102	CSF	95.5	96.1	Song et al.
IL-10 (cut-off 8.3 pg/mL)	108	CSF	59	98	Shao et al.
IL-10/IL-6 ratio (cut-off 1.6 pg/mL)	108	CSF	66	91	Shao et al.
IL-10/IL-6 ratio (cut-off 0,72 pg/mL)	102	CSF	95.5	100	Song et al.
CXCL13	220	CSF	69.9	92.7	Rubenstein et al.
Combination of CXCL13 and IL-10	77	CSF	76.7	90.9	Mabray et al.
Combination of CXCL13, IL10, sIL2R, β2-microglobulin	248	CSF	97	97	Maeyama et al 2020

IL-10

- CSF IL-10 levels display **high specificity and sensitivity** and is a useful non-invasive tool for diagnosis of CNS lymphomas. The optimal cut-off level is still debated.

CXCL-13

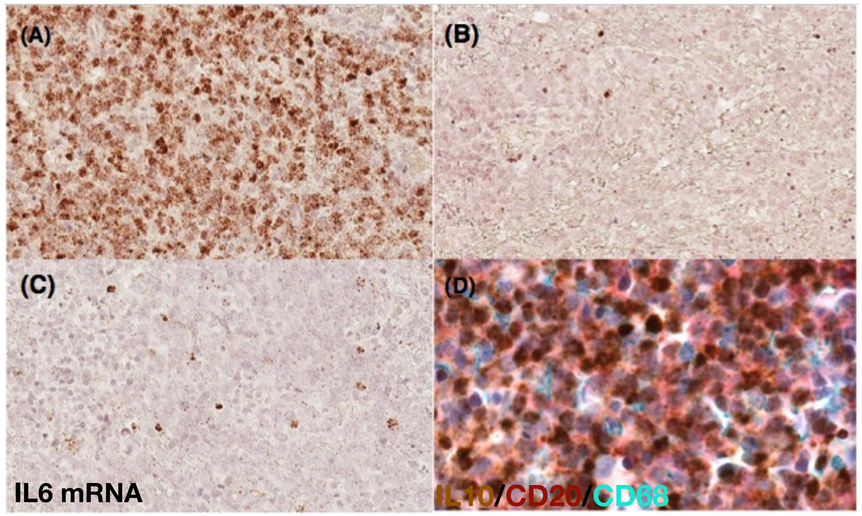
- Elevated levels detected in PCNSL and secondary DLBCL have been proposed as **negative prognosticators**.
- The CXCL-13/CXCR-5 axis has also been **targeted by drugs**

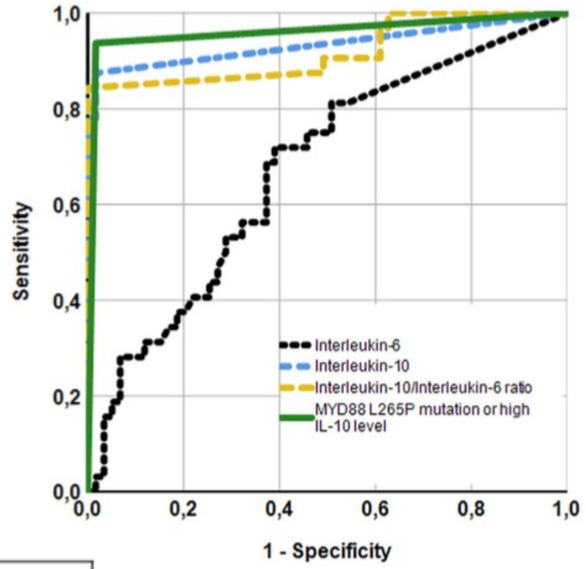
Modified from Baraniskin et al, Cancers 2021

MYD88 L265P mutation and interleukin-10 in PCNSL

Neoplastic B lymphocytes are the main source of IL10

IL10 mRNA expression in PCNSL





	Sensitivity	Specificity	AUC
Interleukin-6	72%	52%	0·66 (0·55 - 0·78)
Interleukin-10 >2pg/mL	88%	99%	0·94 (0·86 - 1·00)
IL-10/IL-6 ratio	85%	99%	0·92 (0·84 - 0·99)
MYD88 mutational status & IL-10	94%	98%	0·96 (0·91 - 1·00)

- IL6 expression was negligible in brain biopsies—> low discriminating sensitivity
- 82% concordance in MYD88mut in brain biopsies and CSF (taqPCR)
- Combined MYD88mut and IL10 in CSF have elevated accuracy in both **newly diagnosed and relapsed PCNSL**

Use of inhibitors of IL-10 and IL-10R deserve to be investigated

Ferreri A.J.M., BJH, 2021

BAFF, APRIL and PD-L1

- **BAFF and APRIL** and its receptor are expressed by neoplastic B cells in PCNSL promoting neoplastic cell growth by activation of **PI3K and AKT/mTOR pathway**
- CSF levels of BAFF and APRIL in PCNSL correlated with a **more aggressive disease and worse outcome**
  - **TACI and BCMA** levels are significantly more elevated in patients with PCNSL compared to other neurological diseases

- **PD-L1 levels** are elevated in newly diagnosed and relapsed PCNSL
- Higher CSF PD-L1 levels correlated with high sLDH levels, leptomeningeal and deep-brain involvement.
- CSF PD-L1 could predict **poor survival** in PCNSL

Biomarker	Number of patients	Sample	Methods	Sensitivity	Specificity	Reference
APRIL	53 CNSL (30 PCNSL) and 63 controls	CSF	ELISA	62.3%	93.7%	Mulazzani *et al.*[43]
BAFF		CSF	ELISA	47.1%	93.7%	
Elevation of APRIL and/or BAFF				77.3%	96.1%	
TACI	33 PCNSL and 143 controls	CSF	ELISA	87.9%	88.3%	Thaler *et al.*[44]
BCMA			ELISA	72.7%	71.8%	
Combination of TACI and BCMA				63.9%	96.7%	
Combination of TACI and BAFF	9 PCNSL and 73 controls	CSF	ELISA	100%	100%	Mizutani *et al.*[45]

Diagnosis	Number	Age (median)	Sex (M/F)	sPD-L1 (ng/ml)
Newly diagnosed PCNSL	35	64	23/12	0.498 (0–4.736)
Relapsed PCNSL	11	68	5/6	0.565 (0.015–1.568)
Other primary brain tumours	10	65	7/3	0.039 (0.007–0.446)
Metastatic brain tumours	31	46	23/8	0.04 (0–1.904)
Neuro-inflammation/infection	70	50	37/33	0.02 (0–1.659)
Other tumours without CNS involvement	42	45	19/23	0.002 (0–0.137)

Modified from Zhai et al, Ther Adv Med Onc 2022
Modified from Cheng et al, BJH 2022

miRNA espression

Individual miRNAs and miRNA signatures potent non-invasive diagnostic and prognostic tools

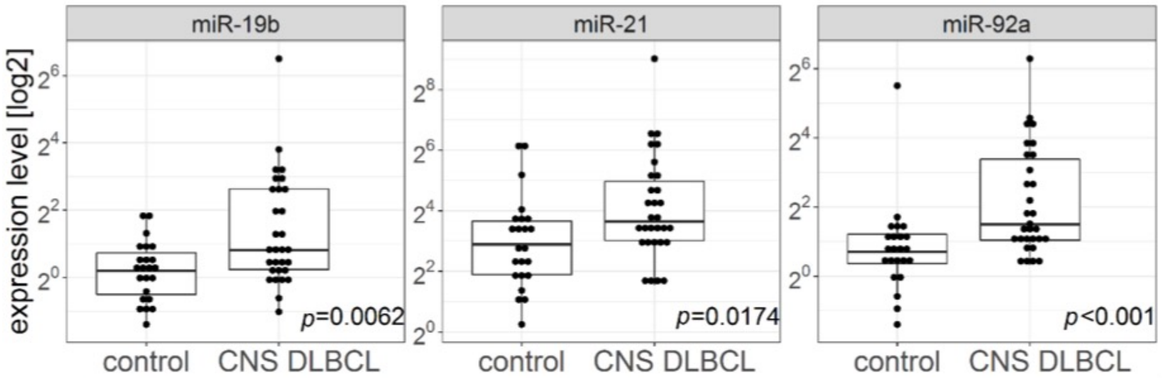
Nevertheless, the available data are scarce and further validation is necessary

Controversial results

CSF **miR21, miR19b and miR92a** showed **95%sensitivity and 97% specificity** for CNSL compared with other neurological disorders (Baraniskin et al.)

A set of CSF **miR-21, miR-19b, and miR-92a** differentiated CNS DLBCL from n-ML, with a **specificity of 80.77% and a sensitivity of 63.33%** (Zajdel et al.)

MicroRNA expression in CSF samples of cerebral lesions from patients with CNS DLBCL and non-malignant brain lesions



Different subset of control cases

Baraniskin et al, Cancers 2021

Zaidel Cancers, 2019

CLINICAL CASE #2

Female 51 yo

July, 2020 → Phosphenes, myodesopsia, decline in vision

Oct, 2021 → Worsening of symptoms

Treatment with Luxazone for hemovitreus

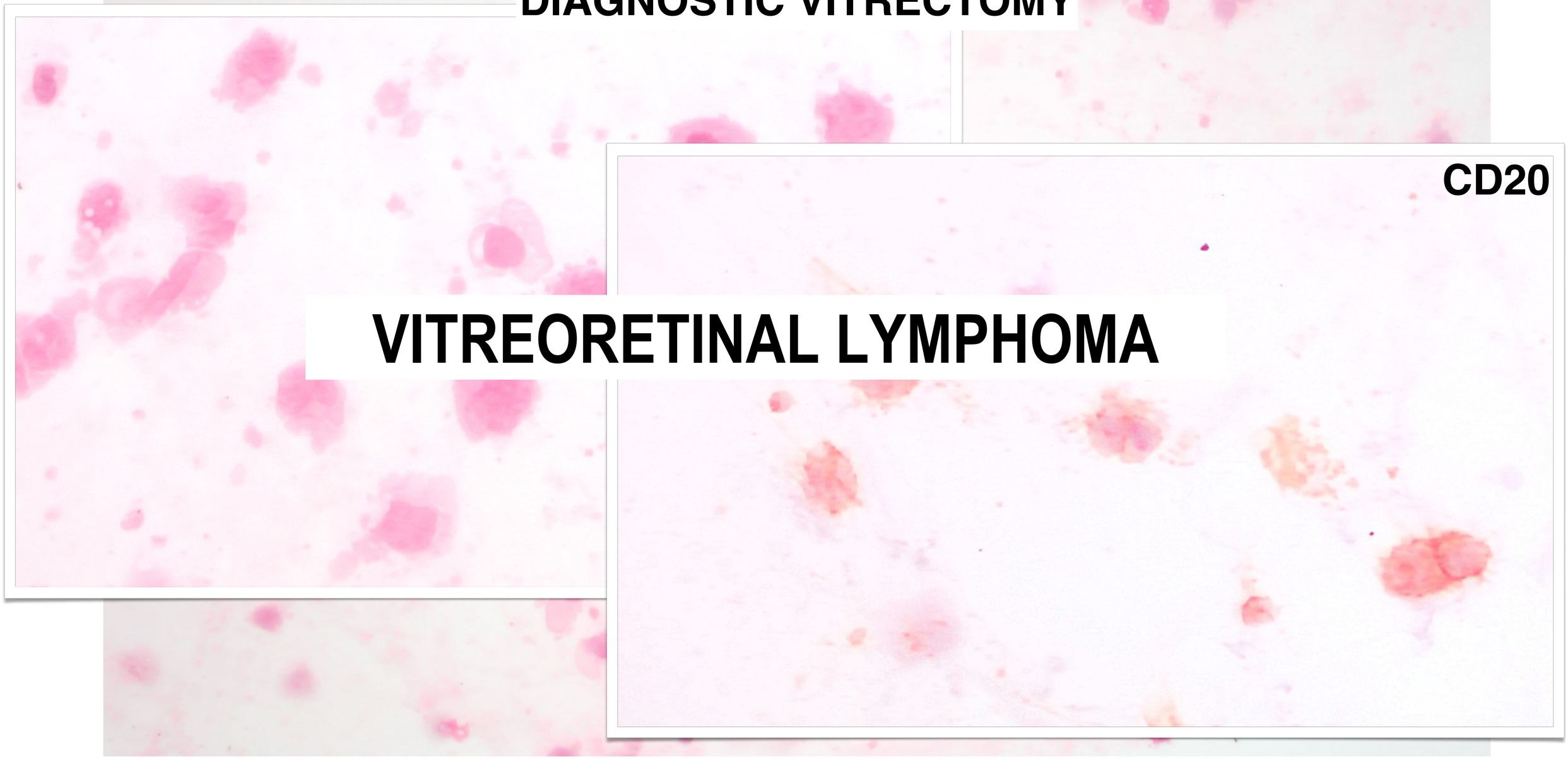
Nov, 2021 → Bilateral severe vitreitis OD>OS with sheets and clumps of retinic cells, small detachment of the RPE

Dec, 2021

Diagnostic vitrectomy

DIAGNOSTIC VITRECTOMY

VITREORETINAL LYMPHOMA



CLINICAL CASE #2

Female 51 yo

July, 2020

Phosphenes, myodesopsia, decline in vision

Oct, 2021

Worsening of symptoms

Treatment with Luxazone for hemovitreus

Nov, 2021

Bilateral severe vitreitis OD>OS with sheets and clumps of retinic cells, small detachment of the RPE

Dec, 2021

Diagnostic vitrectomy

VRL

No SNC involvement at MRI

MATRix polychemiotherapy

HD-MTX/cytarabine/thiotepa/rituximab

ASCT+ RT

VIREORETINAL LYMPHOMA

PRIMARY LARGE B CELL LYMPHOMA OF THE IMMUNOPRIVILEGED SITES WHO 5ed.

Subset of PCNSL affecting the **retina** with/out vitr
no evidence of brain or CSF i

56%–90% of patients with VRL develop CNS c
CNS lymphoma spreads via the optic nerve to

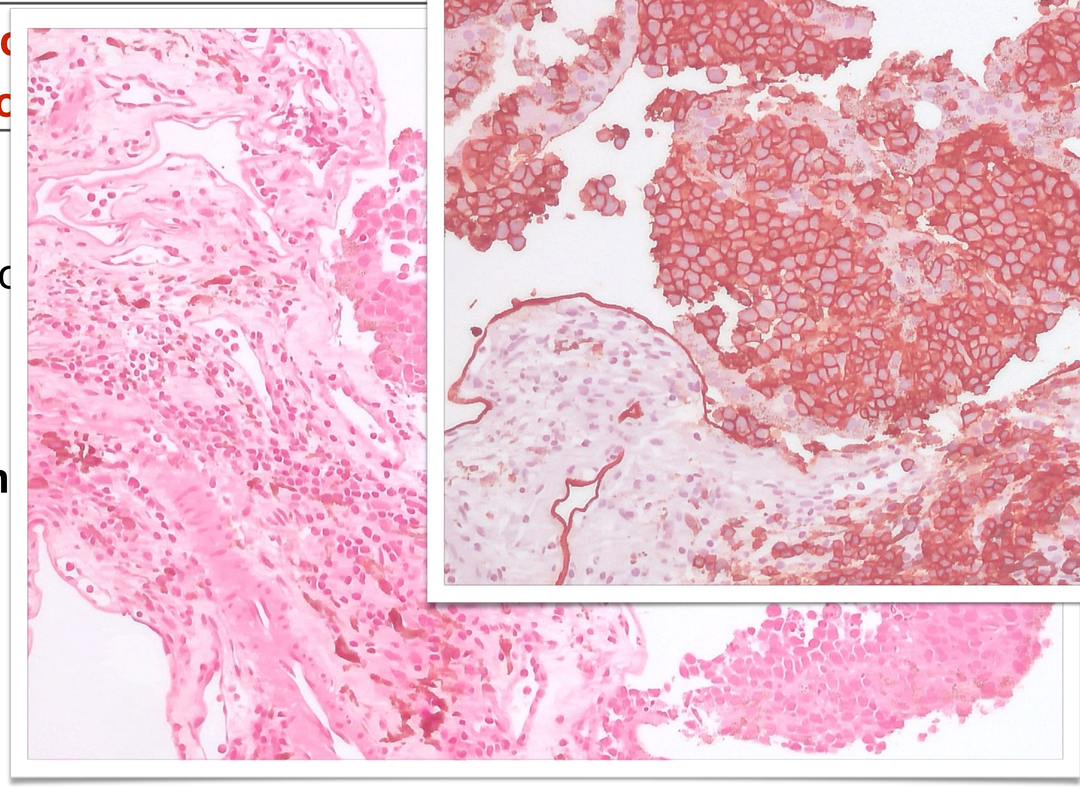
High index of suspicion (mimic uveitis)

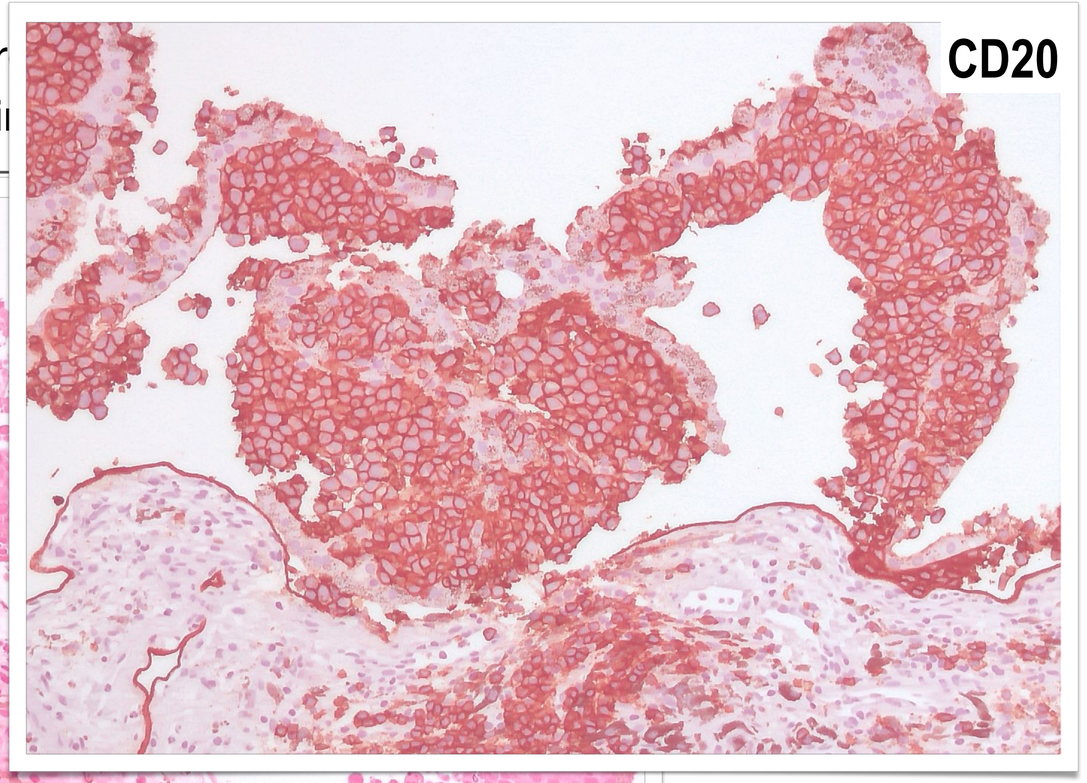
Frequent false negative at diagnostic cytopatholo

Multiple diagnostic interventions are needed

Cytology (vitrectomy) + flow cytometry **gold stan**

Chorioretinal biopsies or subretinal aspirates





Carbonell et al Ocul Imm and infllamm 2021

VIREORETINAL LYMPHOMA

Diagnosis

OCULAR IMMUNOLOGY AND INFLAMMATION
2021, VOL. 29, NO. 3, 507–520
https://doi.org/10.1080/09273948.2021.1878233

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Check for updates

Consensus Recommendations for the Diagnosis of Vitreoretinal Lymphoma

Denise Carbonell, MD[a], Sarakshi Mahajan, MD[b*], Soon-Phaik Chee, MBBS, M Med(Ophth), FRCS(Glasg), FRCS(Ed), FRCOphth[a,c,d,e], Bianka Sobolewska, MD[f], Rupesh Agrawal, FRCS, MMed, DNB, DO[g], Tanja Bülow, MSc[h], Vishali Gupta, MD[i], Nicholas P. Jones, BSc MBChB DO FRCSEd FRCOphth[j], Massimo Accorinti, MD, PhD[k], Mamta Agarwal, MD, Tracy Batchelor, MD, MPH[m], Jyotirmay Biswas, MS, FAMS, FIC Path, FAICO[l], Luca Cimino, MD[n], Ninette H. tenDam-van Loon, MD[o], Alejandra de-la-Torre, MD, PhD[p], Shahar Frenkel, MD, PhD[q], Jacob Pe'er, MD[q], Michal Kramer, MD, MPH[r], Elisabetta Miserocchi, MD, PhD[s], Manabu Mochizuki, MD, PhD[t], Thomas Ness, MD, PhD[u], James T. Rosenbaum, MD[v], H. Nida Sen, MD, MHS[w], Mr. Michael Simion (VRL patient)[x], Helmut Sitter, MD[y], Daniel V. Vasconcelos-Santos, MD, PhD[z], Zohar Habot-Wilner, MD[aa], Sarah E. Coupland, MBBS, PhD[bb], José S. Pulido, MD, MBA, MPH[cc], Justine Smith, MD, FRANZCO, PhD, FARVO[dd], Jennifer E. Thorne, MD, PhD[ee,ff], Manfred Zierhut, MD, PhD[f], and the Study Group for Vitreoretinal Lymphoma Diagnostics[**f]

IL10 levels intraocular fluids (both aqueous and vitreous)

IL10/IL 6 ratio >1 intraocular fluids seen

MYD88 L265P mutation by RT-PCR (70

—> NEGATIVE RESULT DOES

JAMA Ophthalmology | Original Investigation

Potential Diagnosis of Vitreoretinal Lymphoma by Detection of *MYD88* Mutation in Aqueous Humor With Ultrasensitive Droplet Digital Polymerase Chain Reaction

Laura S. Hiemcke-Jiwa, MD; Ninette H. ten Dam-van Loon, MD; Roos
Jeannette Ossewaarde-van Norel, PhD; Joke H. de Boer, PhD; Floor F
Manon M. H. Huibers, PhD; Jolanda D. F. de Groot-Mijnes, PhD; Jonas

—> Vitreous testing sensitivity 75%

—> aqueous testing sensitivity 67%

Clonality IGH and IGL by PCR or flow cytometry

—> sensitivity between 46%-95%, depending on the choice of primer sets

—> false positive

Carbonell et al Ocul Imm and infllamm 2021

BAFF AND APRIL

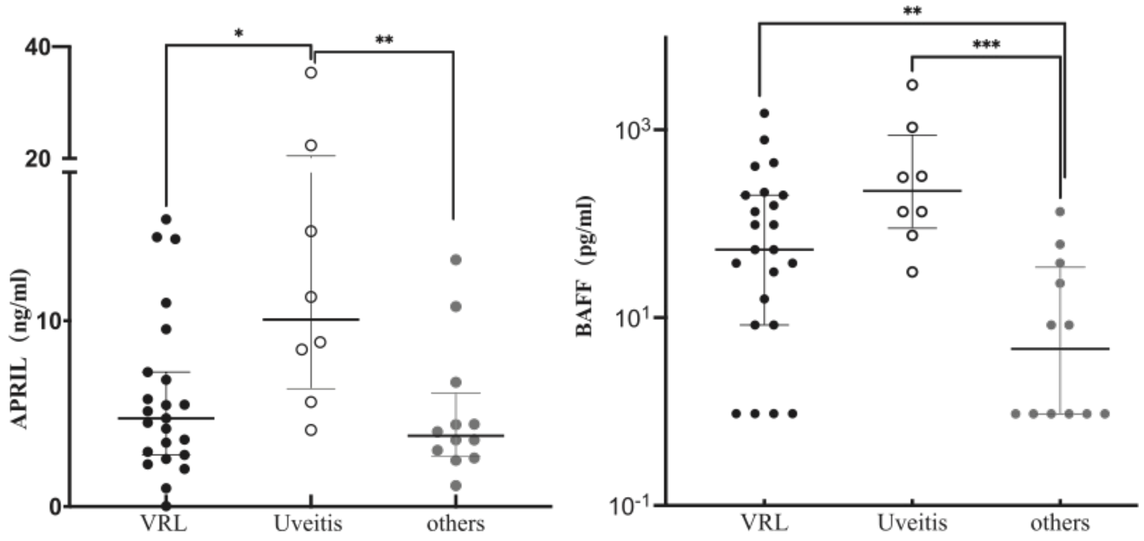
APRIL and BAFF appeared to distinguish between uveitis and VRL

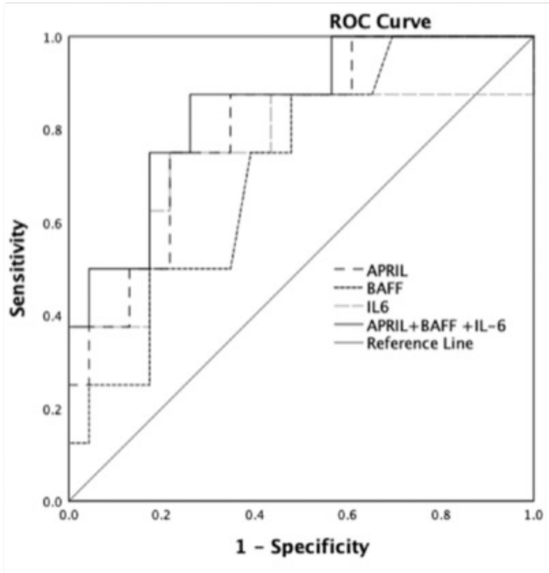
AH APRIL levels in patients with uveitis (10.07 [6.34, 20.48] ng/mL) were higher than those in patients with VRL

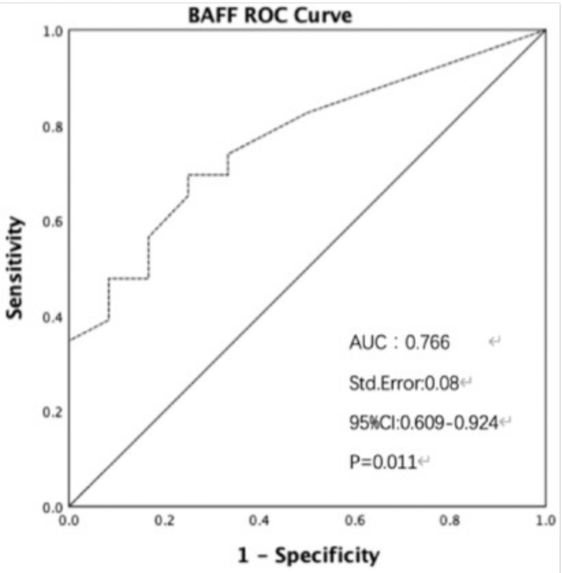
AH BAFF levels in patients with uveitis (223.50 [89.97, 875.49]) were higher than those in patients with VRL

Aqueous humor level of **APRIL** > 7.85 ng/mL with specificity of 78.3% and sensitivity of 75% for uveitis

Aqueous humor level of **BAFF** > 64 pg/mL exhibits a specificity of 52.2% and sensitivity of 87.5% for uveitis



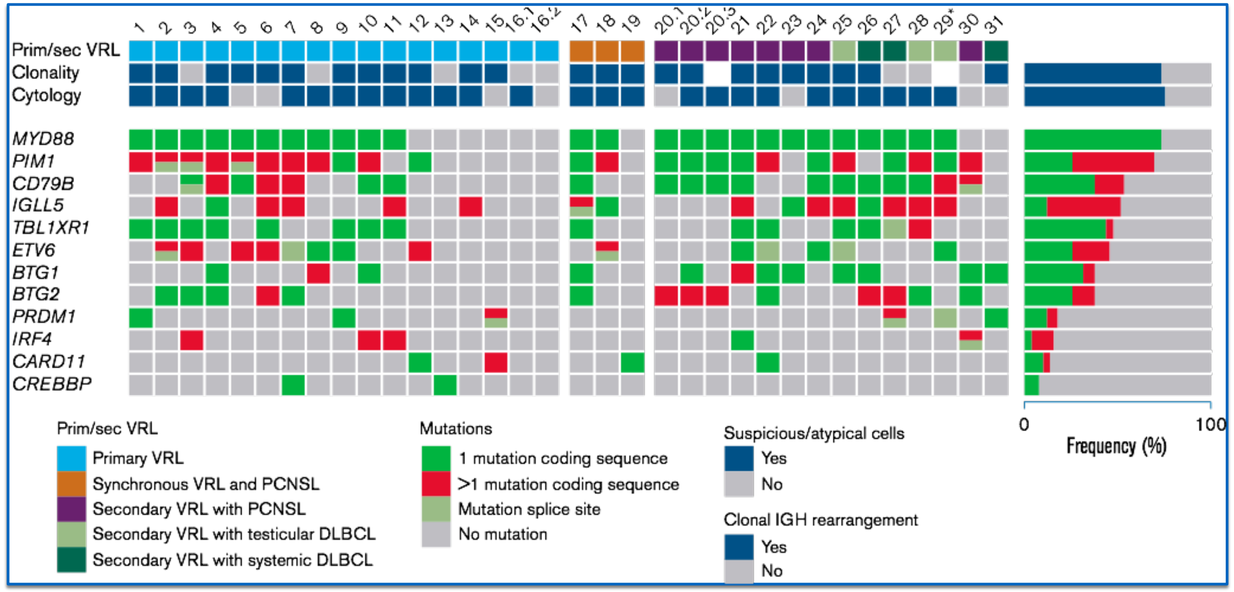




Tian el al. Clin Ch Acta 2022

VIREORETINAL LYMPHOMA

Mutational Landscape



According to Gu et al.

combined **MYD88 and ETV6** mutation in intraocular fluid high sensitivity (91,3%) and specificity (95%) for VRL

CD79B mutation has been associated with higher IL10 levels in intraocular fluids

BTG2 mutation is associated with intracranial involvement

Similar frequencies in primary, synchronous or secondary VRL

High frequency of *MYD88* (74%),*PIM1* (71%) and *CD79B* (55%) mutations

Frequently mutations *IGLL5* (52%), *TBL1XR1* (48%), and *ETV6* (45%)

Frequent homozygous deletions of *9p21/CDKN2A* (75%).

High number of CNAs (18.6 CNAs per case) reflecting genomic instability

Belhouachi et al. Blood Adv 2022

Bonzheim et al. Blood Adv 2022

Gu et al, Front Oncol, 2022

VIREORETINAL LYMPHOMA

MiRNA

MiR-92, miR-19b and miR-21 have been found to be significantly upregulated in vitreous specimens from patients with PVRL

High-throughput Vitreous and Serum miRNA analysis (Minezaki et al.)

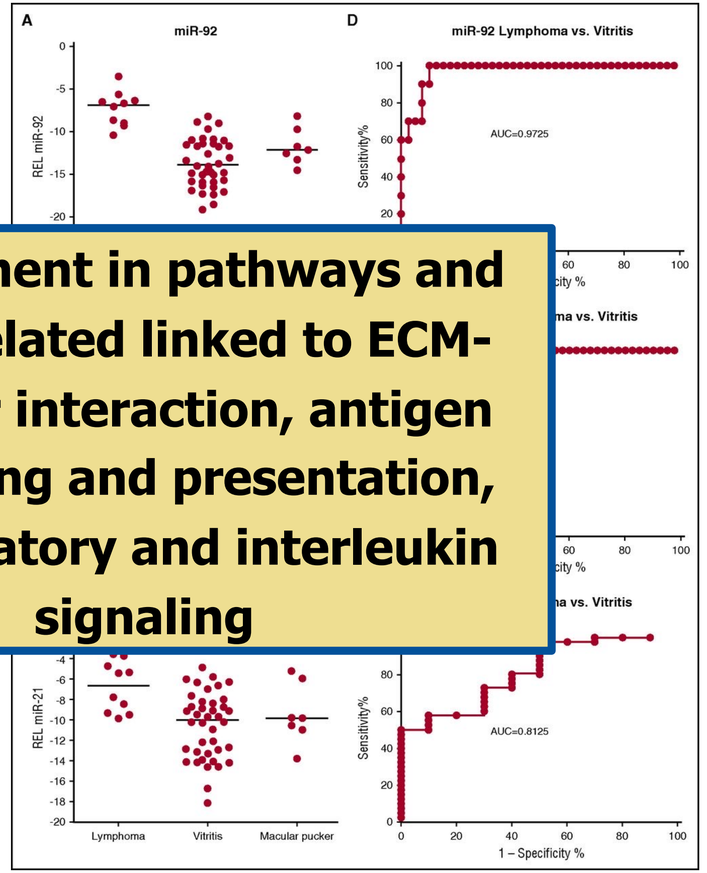
miR-6513-3p and **miR-361-3p** could discriminate VRL from uveitis with vitre

MiR-1236-3p correlated with vitreous interleukin (IL)-10 concentrations

MiR-326 appeared the most promising for differential diagnosis between PVRL and controls, such as healthy patients, or patients with uveitis, macular holes, and epiretinal membranes

Downregulation of vitreous **miR-4795-3p and miR-29b-2-5p** may be related to the reported increase of their target gene MYD88, which is strongly associated with VRL pathogenesis

Involvement in pathways and genes related linked to ECM-receptor interaction, antigen processing and presentation, inflammatory and interleukin signaling



Kakkassery et al, Blood, 2017
Minezaki et al. J. Clin. Med. 2020

TAKE HOME MESSAGES

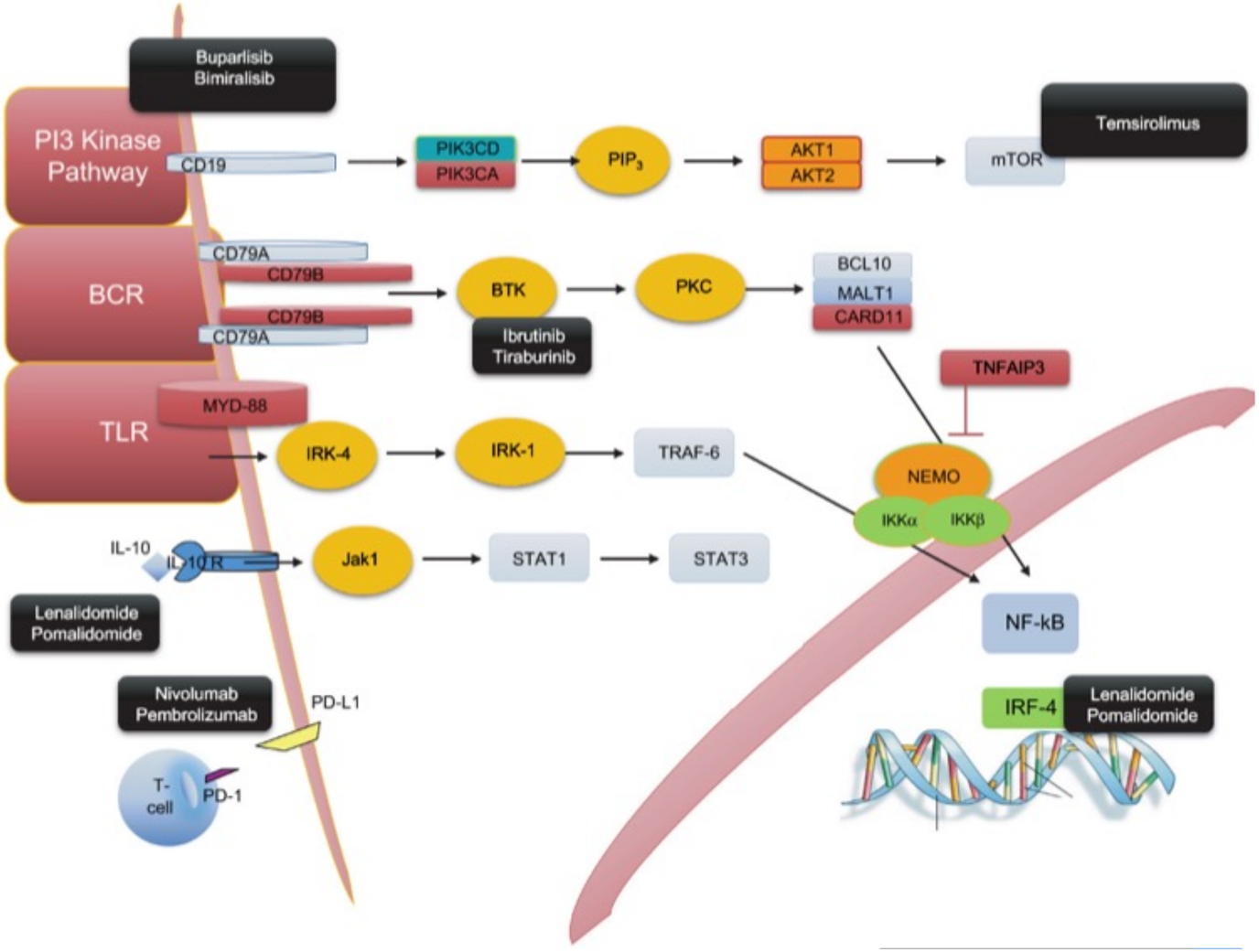
PRIMARY LARGE B CELL LYMPHOMA OF THE IMMUNOPRIVILEGED SITES WHO 5ed.

PCNSL AND VRL

- Challenging diagnosis and treatment → stereotactic biopsy/vitrectomy GOLD STANDARD
- Heterogeneous clinical outcome
- Need for biomarkers for early diagnosis and risk stratification → Molecular markers genetic alterations
- Biomarkers for treatment response
- Need for prospective and large studies for validation

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

Oncogenic Drivers and targeted therapies



Calimeri T et al, Leukemia 2021