MB&C26 course
February 2026

Understanding ocular lymphoma: a challenging diagnosis

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PhD Pharm Clin Biol


SPECIFICITY
TECHNICALLY
INCIDENCE
STAGING
CD79B
CYTOLOGY
OCULAR LYMPHOMA
ADNEXAL
SECUNDARY
SENSITIVITY
SURVIVAL
RISKS TO PATIENTS' VISION
MULTIDISCIPLINARY TEAM
MYD88
LARGE B-CELL LYMPHOMA
LABORATORY
MATURE B-CELLS
NGS
UVEAL
CNS
ICC
BIOPSY
DIAGNOSIS
FOLLOW UP
IMMUNE PRIVILEGED SITES
PRIMARY
VITRORETINAL
SMALL B-CELL LYMPHOMA
INTERLEUKINES
EXTRA-NODAL
VITREOUS HUMOR
CHALLENGING
DELICATE
DISEASE
QOL
UVEITIS
HISTOLOY

Ocular lymphoma

1 What is ocular lymphoma?

Clinical presentation
Classification WHO
Incidence/survival

2 Diagnosis PVR-LBCL

Sampling
Laboratory diagnosis

3 PVR-LBCL @UZA

Workflow
2023 – 2025 experience
Immunophenotyping ⇔ MD
ddPCR ⇔ NGS
Case study

4 What's next @UZA for PVR-LBCL?

5 Take home messages

6 Teamwork

UZA

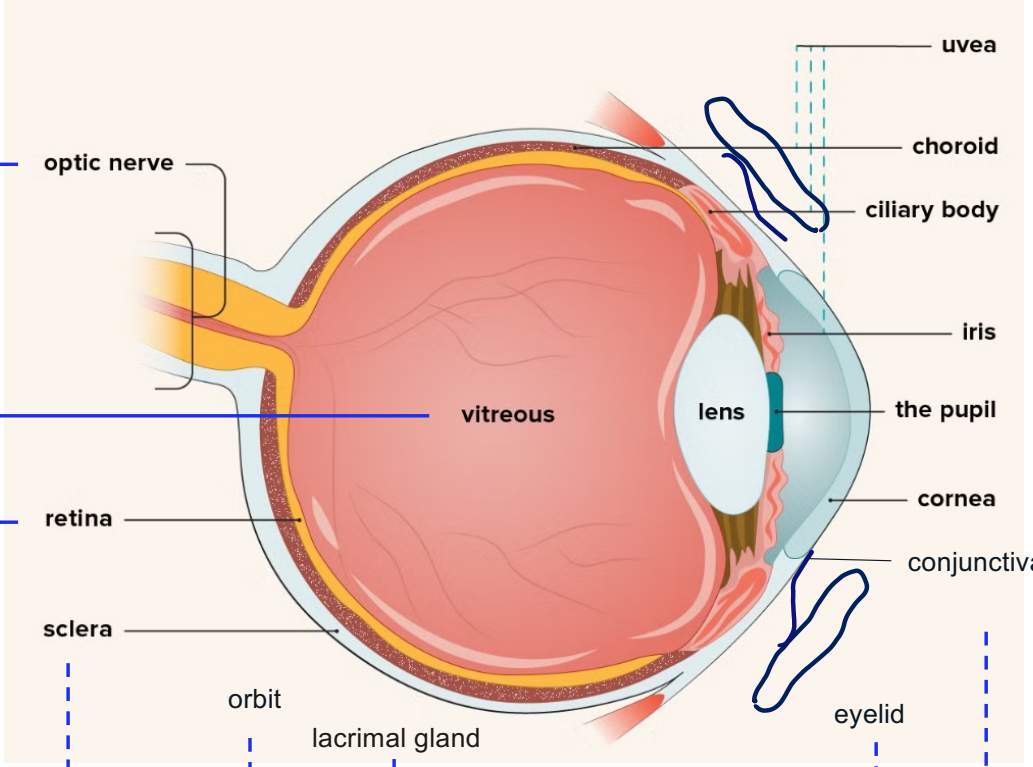
What is ocular lymphoma?

>75% primary
<25% secondary

<5-10% intraocular lymphoma

Vitreoretinal lymphoma

- PVR-LBCL
High-grade large B-cell lymphoma
~ poor prognosis



Uveal lymphoma (rare)

- **Primary**: low-grade small B-cell lymphoma
~ good prognosis

>90-95% adnexal lymphoma

- **Primary**: 85-90% Low-grade small B-cell lymphoma
MALT association with infection (H. pylori, EBV, HIV)
~ good prognosis

What is ocular lymphoma?

How to classify?

WHO 2012/2017 Mature B-cell neoplasms → Large B-cell lymphoma → Primary DLBCL of the CNS (involving brain, spinal cord, leptomeninges or eye) → ~~= primary CNS/intraocular lymphoma~~

WHO 2022 Mature B-cell neoplasms → Large B-cell lymphoma (LBCL) → Primary LBCL of immune-privileged sites* → - CNS (PCNS-LBCL)** **- Vitreoretinal (PVR-LBCL)** - Testes (PT-LBCL)

ICC Mature B-cell neoplasms → Primary DLBCL of the CNS (including PVRL)

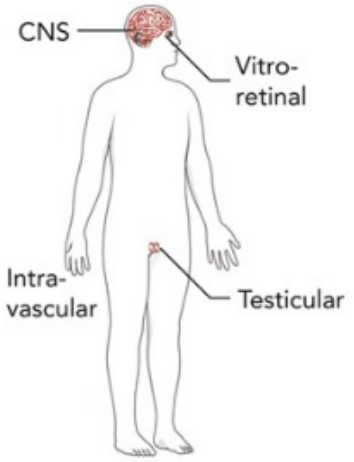
WHO/ICC → **Other B-cell neoplasm according to subtype**

* In immunocompetent hosts, exclusion of secondary involvement
** 15-25% involvement of CSF or eyes

PVR-LBCL with CNS invasion
<=> PCNS-LBCL with ocular invasion

Immune-privileged site
= a location in the body that can tolerate foreign antigens or transplanted tissue without triggering a destructive inflammatory immune response

Immune-Privileged Lymphomas



➔ Tumor cells within these immune-privileged sites are less susceptible to antitumor immune responses from T/NK-cells.

Roschewski et al 2024

What is ocular lymphoma?

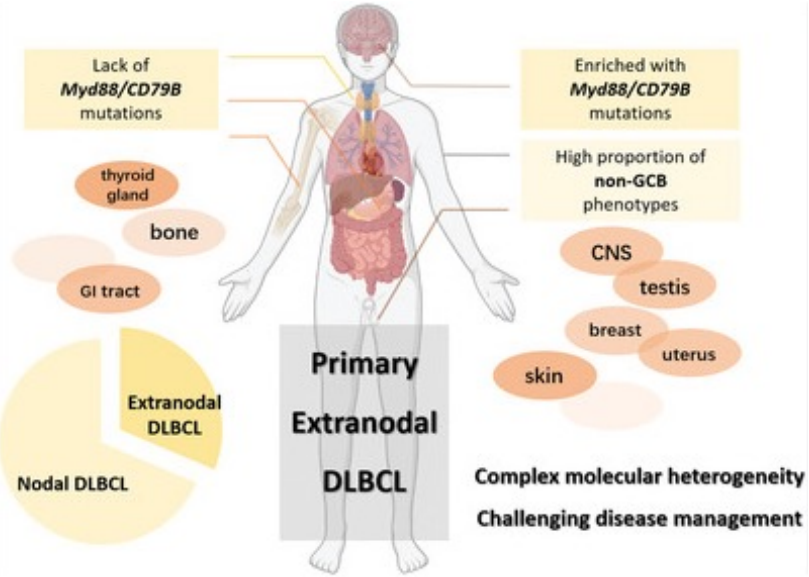
How to classify PVR-LBCL?

Primary LBCL of immune-privileged sites share common features:

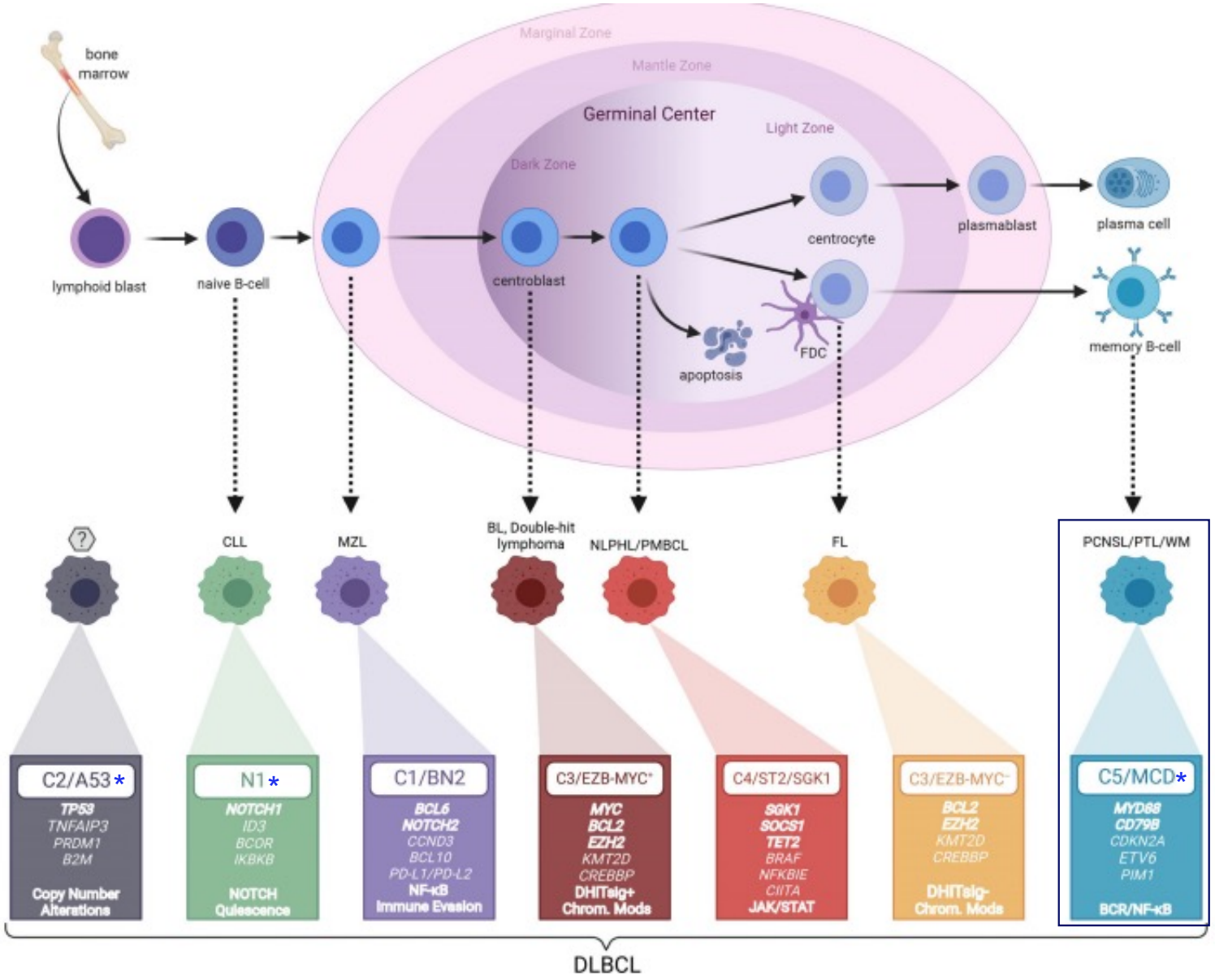
- Clinical
  - Confined to immune-privileged sites (≠ other extranodal LBCL)
  - Strong CNS tropism
- Immunotypic
  - Non-GCB (or ABC) origin LBCL
- Molecular
  - MYD88 L265P and/or CD79B mutations
  - Other genetic alterations promoting immune evasion (gains/losses, rearrangements)

➔ Justifying to group these diseases under the umbrella term PLBCL-IPS

Other extranodal LBCL ⇔ PLBCL-IPS



Roschewski et al 2024


bone marrow
lymphoid blast
naive B-cell
Marginal Zone
Mantle Zone
Germinal Center
Light Zone
Dark Zone
centroblast
centrocyte
plasmablast
plasma cell
memory B-cell
FDC
apoptosis
CLL
MZL
BL, Double-hit lymphoma
NLPHL/PMBCL
FL
PCNSL/PTL/WM
C2/A53*
TP53
TNFAIP3
PRDM1
B2M
Copy Number Alterations
N1*
NOTCH1
ID3
BCOR
IKBKB
NOTCH Quiescence
C1/BN2
BCL6
NOTCH2
CCND3
BCL10
PD-L1/PD-L2
NF-κB Immune Evasion
C3/EZB-MYC+
MYC
BCL2
EZH2
KMT2D
CREBBP
DHITsig+ Chrom. Mods
C4/ST2/SGK1
SGK1
SOCS1
TET2
BRAF
NFKBIE
CIITA
JAK/STAT
C3/EZB-MYC-
BCL2
EZH2
KMT2D
CREBBP
DHITsig- Chrom. Mods
C5/MCD*
MYD88
CD79B
CDKN2A
ETV6
PIM1
BCR/NF-κB
DLBCL

* ABC-type

What is ocular lymphoma?

Clinical presentation PVR-LBCL

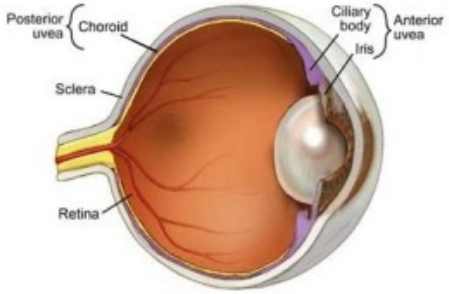
- Patients present with non-specific clinical manifestations
- Can mimic other inflammatory or benign conditions
- Develops insidiously
- Bilateral ocular involvement possible

➔ Delay in diagnosis

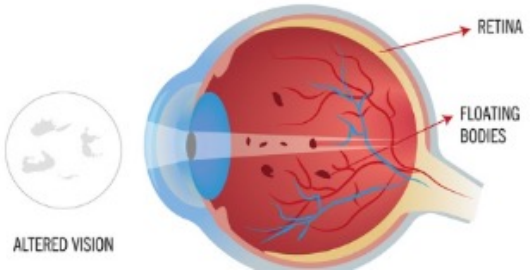
➔ CNS involvement and poor prognosis

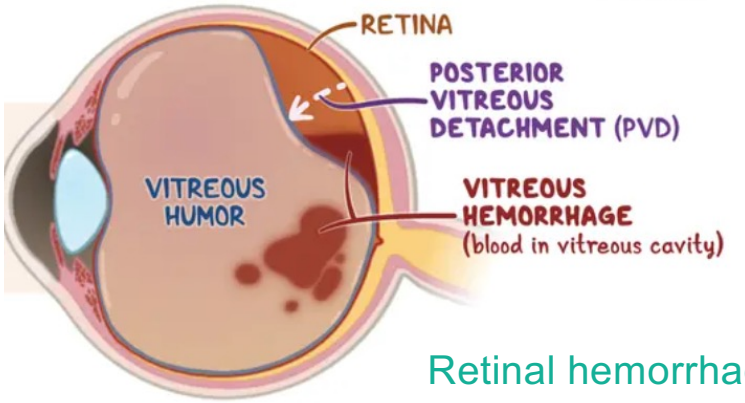
Blurred vision

Posterior uveitis (inflammation involving the choroid)



Floaters (spots in your vision)



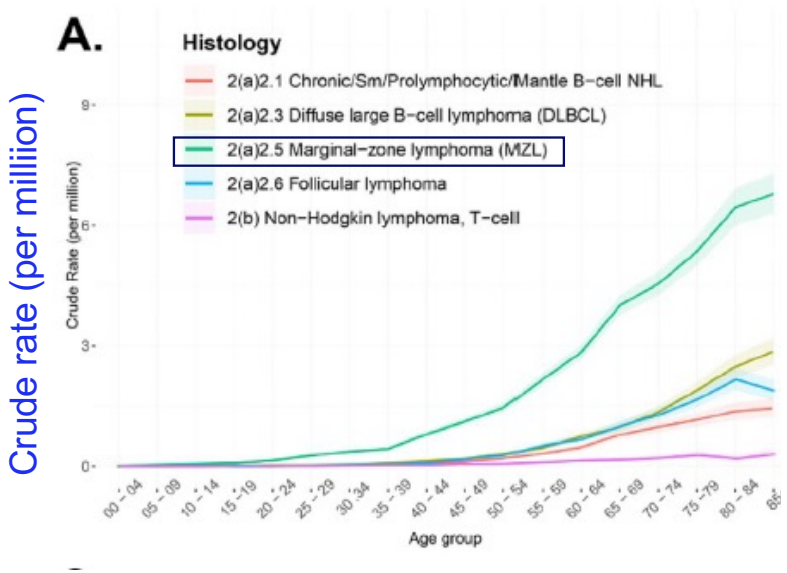


Retinal hemorrhage and/or detachment

What is ocular lymphoma?

Incidence

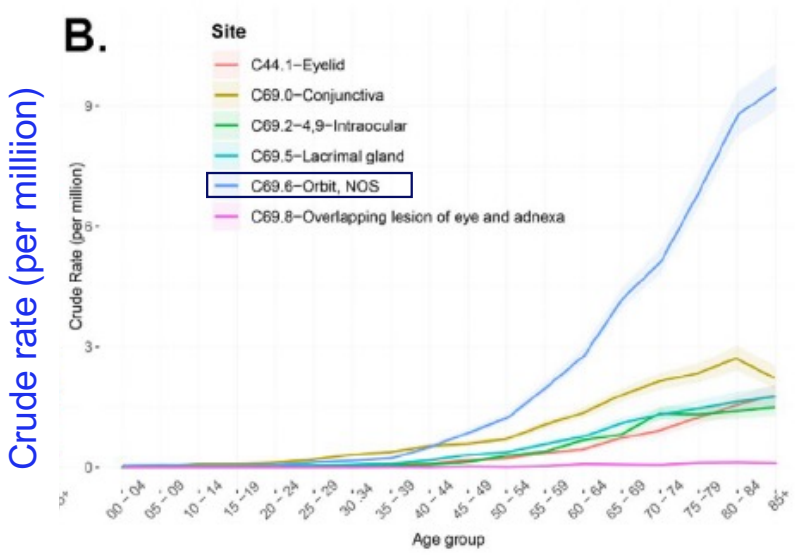
US (1995 – 2018)



Incidence rate per age group in the major histological groups

➔ ↑ incidence = ↑ age

➔ Mainly MZL



Incidence rate per age group of ocular sites

➔ Mainly orbit lymphoma

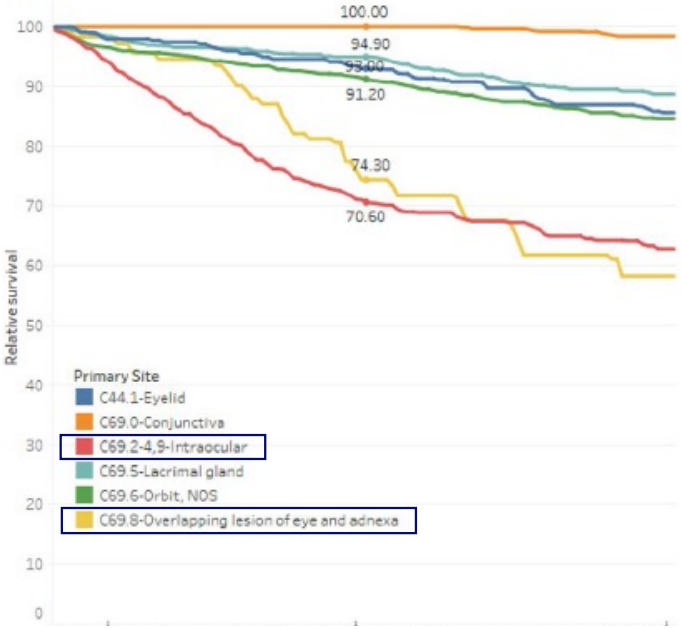
Ahmad Samir Alfaar et al (Nature Scientific Reports)

What is ocular lymphoma?

Survival

US (1995 – 2018)

By site



12 mo 60 mo 120 mo

By histology

12 mo 60 mo 120 mo

- Shortest survival with
  - Intraocular lymphoma and overlapping lesions of eye/adnexa
  - Burkitt lymphoma/DLBCL
- Longest survival
  - MZL, FL,CLL,…

Cumulative relative survival of all patients between 1995 and 2018

Ahmad Samir Alfaar et al (Nature Scientific Reports)

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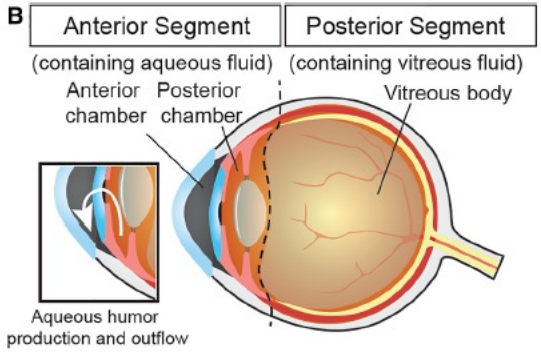
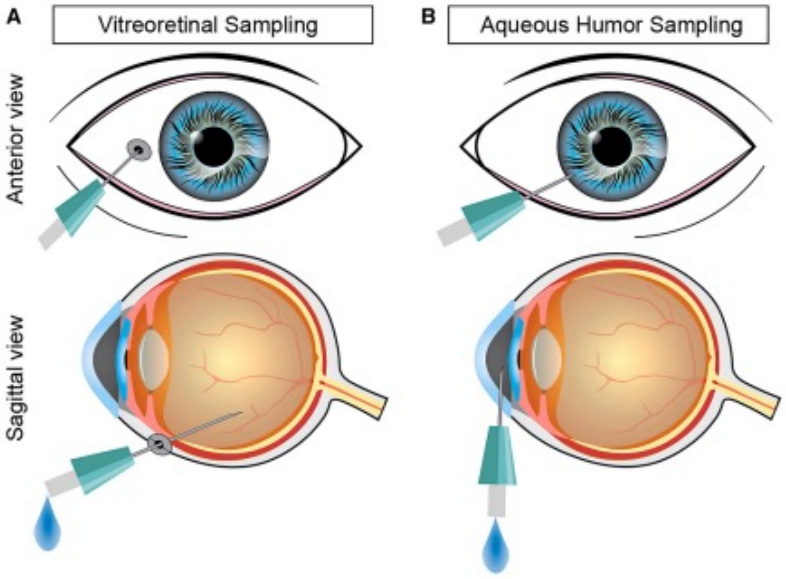
Diagnosis PVR-LBCL

Sampling

- Direct tumor biopsies
  - Too risky, not useful for FU
- Vitreum biopsy (= vitreous humor (VH) sampling)
  - Less risky but not totally save, performed under anesthesia in an operating room
  - PRL sens: 30-82% (spec: 100%)
    - Sens = dependent on laboratory technique and circumstances
      - Steroid treatment (lymphoma cell lysis)
      - Sparse tumor cells in a substantial volume
      - Rapid degeneration of malignant cells
- Aqueous humor (AH)
  - Minimally invasive, ↓ risk of adverse events, easy to perform
  - PRL sensitivity: 90,6%, specificity 99,3%
    - Small volume (0,2 – 0,3 mL): relatively concentrated reservoir of biomarkers
    - Cell-free and cell-based MYD88 L265P mutation
    - FU after treatment with no MYD88 L265P mutation = no clinical evidence of recurrence of lymphoma (9 months FU)





Cheslow et al 2025
Demirci et al 2022

Diagnosis PVR-LBCL

Performance of laboratory tests

Test	Sensitivity	Specificity	Notes
Cytology	31-87%	98-100%	+ Gold standard - Lowest sensitivity (fragile lymphoma cells)
Flow cytometry	82-100%	95-100%	+ Higher sensitivity - False neg when few cells or fragile/large cells
IL-10/IL-6 ratio >1.0	75-90%	75-100%	+ Serial monitoring disease - Not applicable to patients with extensive retinal infiltration?
Aqueous IL-10 level	90%	90%	+ More easy access
IgH rearrangement	40-100%	79-99%	- Low sensitivity, false positivity (few cells?) (pseudoclonality)
MYD88 L265P mutation*	67-91%	92-100%	+ Serial monitoring of disease, aqueous humor, other fluids (CSF) - Higer cost/mutation detection infrastructure

*PCR, ddPCR, NGS?

Kwak et al 2025
Heiferman et al 2022

Diagnosis PVR-LBCL

Performance of laboratory tests: PVR-LBCL mutational profile

- MYD88 mut ≠ all PVR-LBCL
  - ddPCR ⇔ NGS panel = OK to diagnose most of PVRL

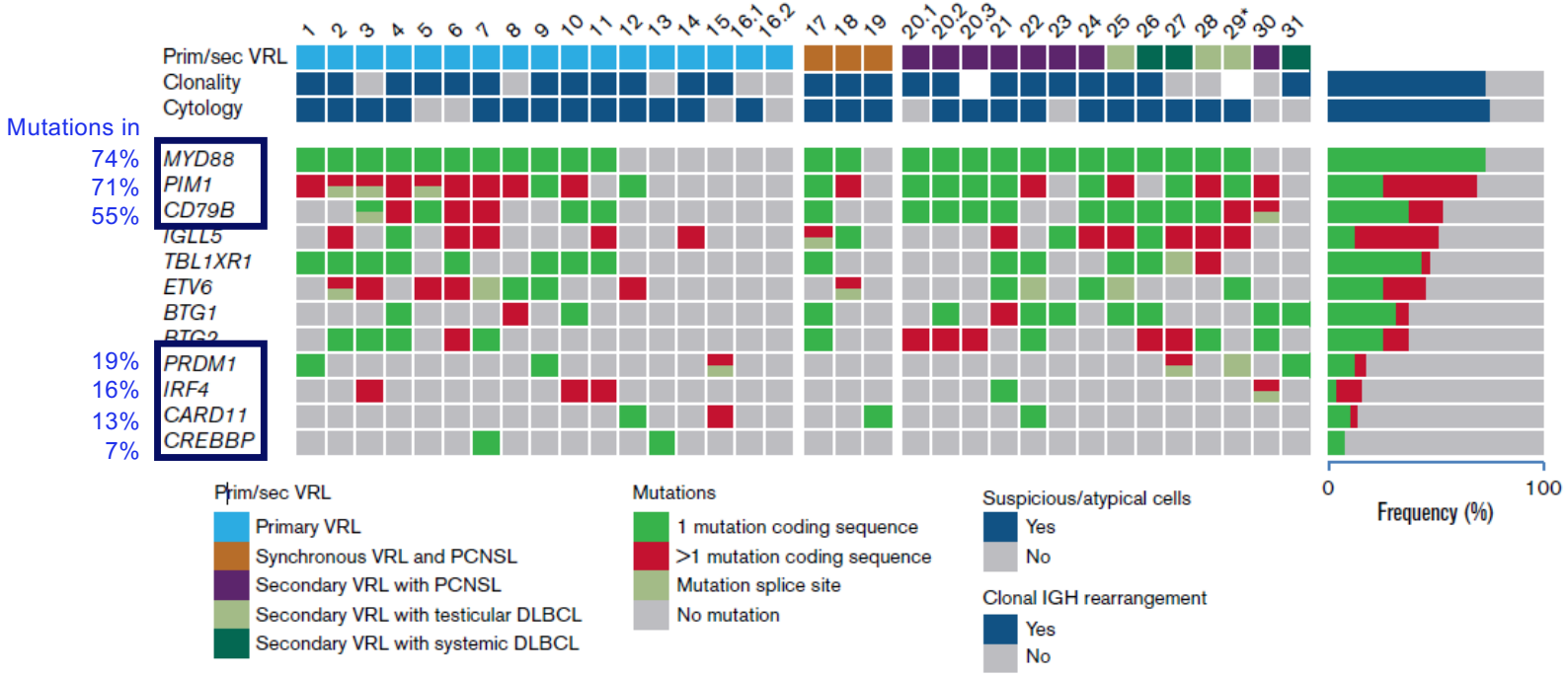


Table 2: Mutational profile in vitreoretinal lymphoma

Altered genes	Frequency (%)	Possible functions
MYD88	53.3–100	NF-kB pathway
CD79B	14–55	NF-kB pathway
IGLL5	27–88.9	B cell development
IRF4	9–60	B cell development
PIM1	29–90.9	Serine/threonine kinase
TBL1XR1	18.2–48	Transcription regulation
ETV6	23–50	Transcription regulation
CDKN2A	36–100	Tumor suppressor
BTG2	9–77.8	Tumor suppressor
BTG1	18–55.6	Tumor suppressor
PTEN	25	Tumor suppressor

In UZA NGS panel

Kwak et al 2025
Bronzheim 2022

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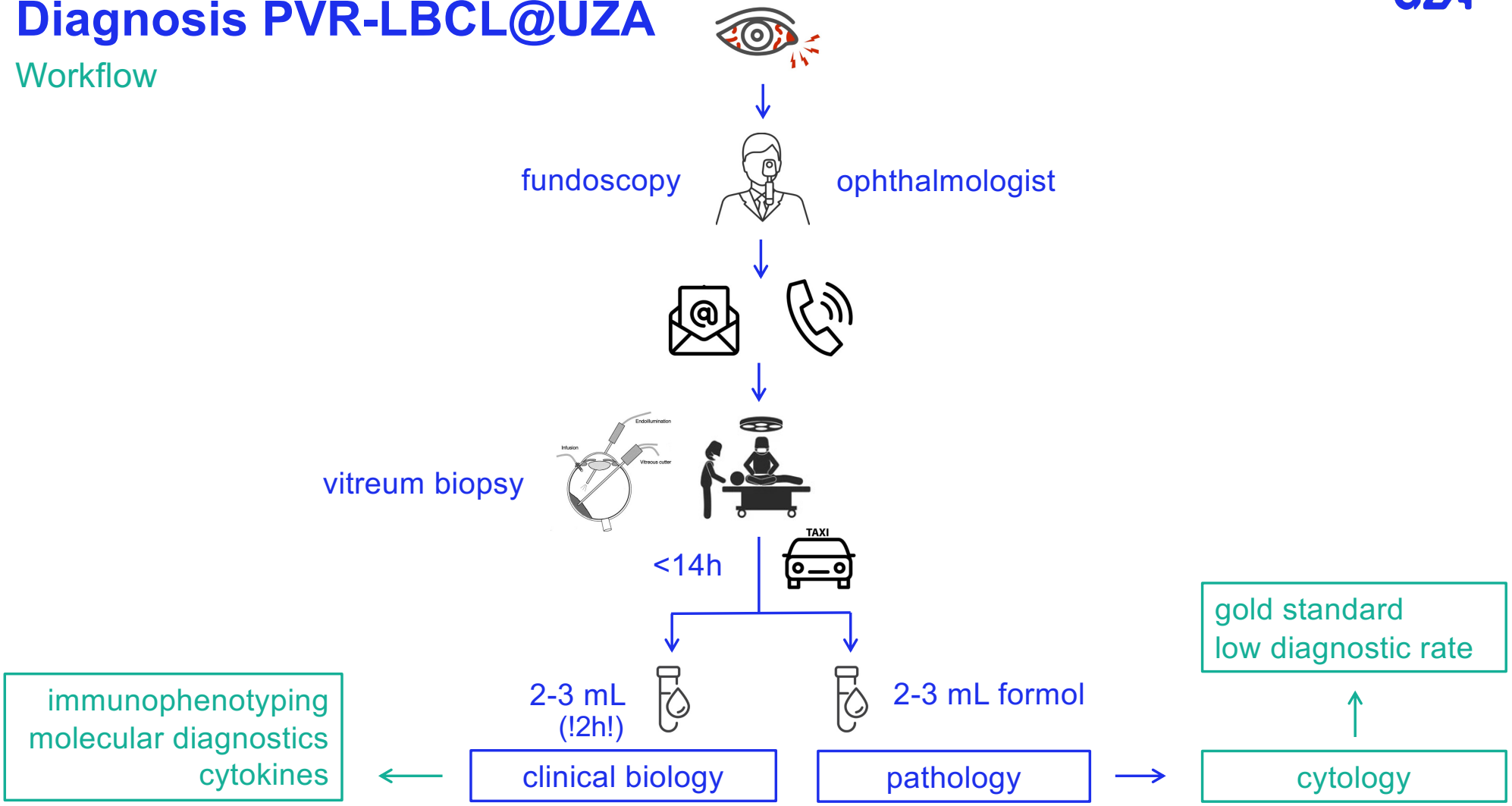
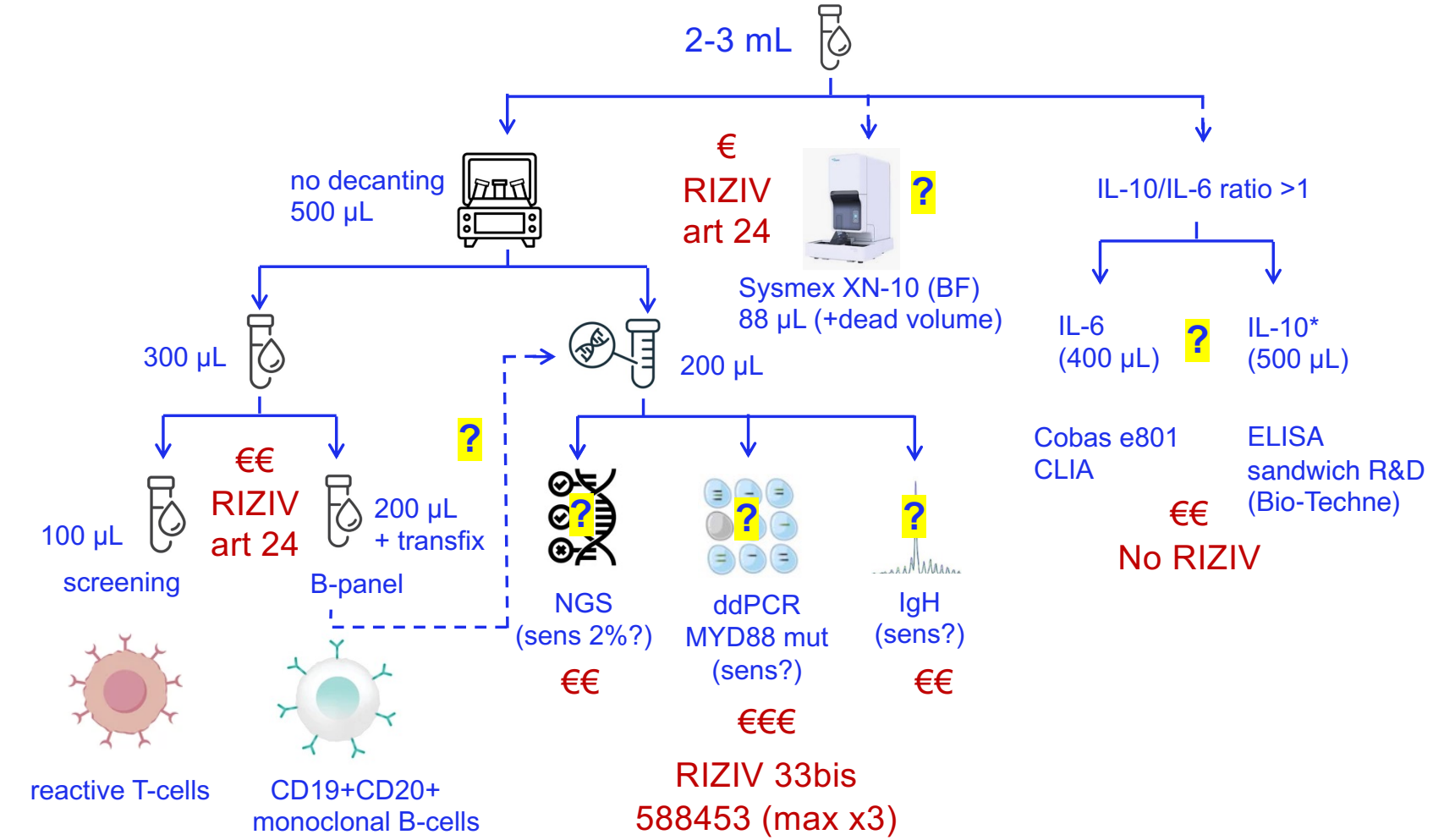
Diagnosis PVR-LBCL@UZA

Workflow



Workflow clinical biology vitreum biopsy (START)



immunophenotyping	molecular diagnostics	cytokines

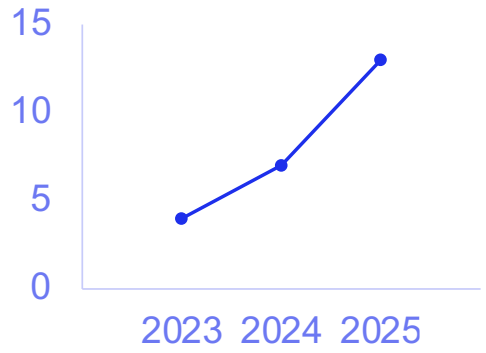
*CHU de Liège

Diagnosis PVR-LBCL@UZA

2023 – 2025 experience

26 samples

- 2x no MD and no FCM (<1 c/µL)
- 2x no FCM (<1 c/µL), MD OK
- 4x FCM but no MD (including patients with known hematological disease e.g. CLL)
- 1x IgH gen rearrangement (FCM normal)



status	FCM normal	FCM abnormal
MD no mutation	10	1 (FU sample)
MD mutation(s)	1 (ddPCR)	5

↓

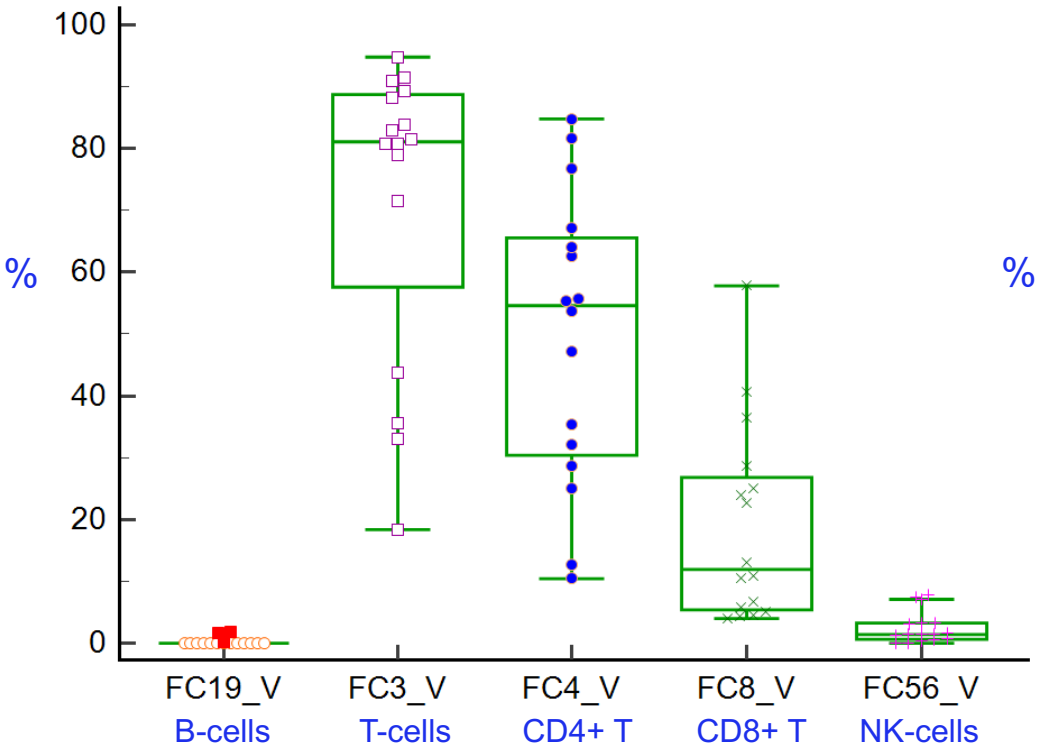
case study

FCM = flow cytometry
MD = molecular diagnostics (ddPCR of NGS)

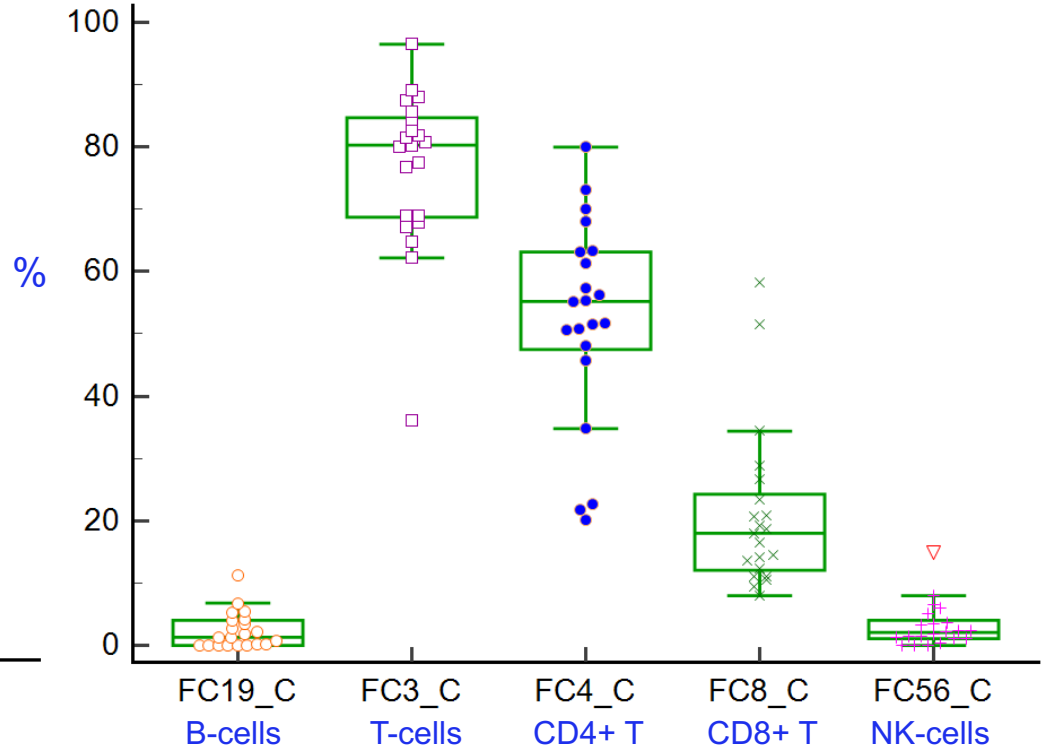
Diagnosis PVR-LBCL@UZA

2023 – 2025 experience: FCM % lymphocyte subsets

Vitreum (excluding samples with malignant cells)
N = 16



CSF (>10 WBC/µL, excluding overt bacterial infections)
N = 21



Diagnosis PVR-LBCL@UZA

2023 – 2025 experience: MYD88 mut ≠ all PVR-LBCL

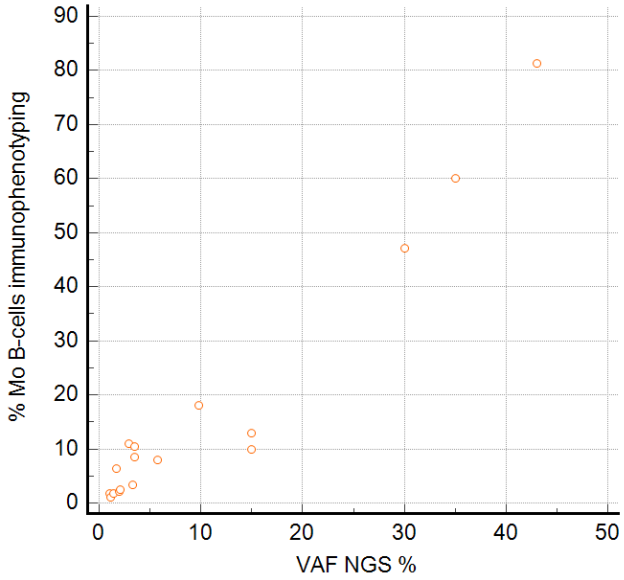
% Mo B-cells	Mo B-cell immunophenotyping	ng/µL DNA	Method MD	% VAF	Mutated genes
3	Small CD19+ cluster (3% / 20 events) without kappa/lambda expression?	17	ddPCR	1%	MYD88
25,9	Mo B-cells (CD19+ CD20+ CD5- CD10+) with high SSC	8		15,4%	
0,2	22 events clonal B-cells?	1		4%	
13,6	Mo B-cells with high FSC: LBCL? B panel: no cells: lysis of LBCL?	8	ddPCR NGS	34-52%	(ddPCR MYD88 wt) CD79B, PIM1, TNFAIP3
39,6	Mo B-cells, patient with known DLBCL	16	NGS	4-25%	MYD88, CD79B, GNA1
19,3	Mo B-cells (CD19+ CD20+ kappa+) with low FSC/SS	3	NGS/IgH	14-38%	MYD88, FOXO1, KLF2, PIM1, PRDM1 No IgH peaks
12	Mo B-cells (CD19+ CD20+ kappa+) (FU)	3	IgH FU		No IgH peaks

Diagnosis PVR-LBCL@UZA

2023 – 2025 experience: FCM ⇔ MD

- Comparison immunophenotyping (% Mo B-cells) versus NGS (VAF%) (LPL suspicion)
  - For MYD88 mutated samples
  - On peripheral blood, bone marrow (LPL indication)

➔ % VAF NGS ~ % Mo B-cells immunophenotyping



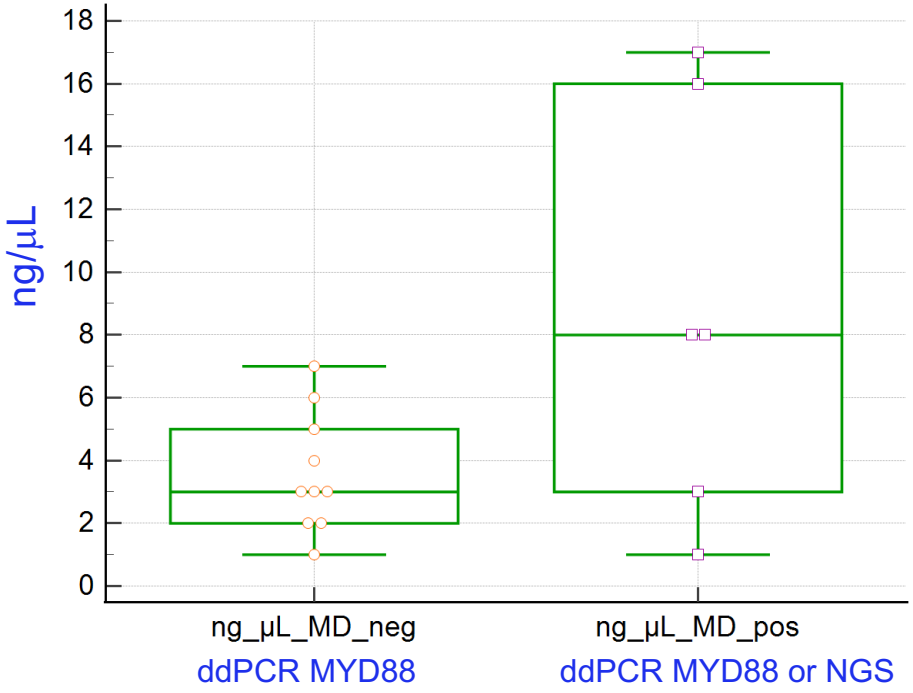
N = 17
R = 0,97
No significant deviation from linearity (P=0,95)

Diagnosis PVR-LBCL@UZA

2023 – 2025 experience: MD

- DNA concentration vitreum biopsy

➔ Usually, more DNA in malignant samples but not always



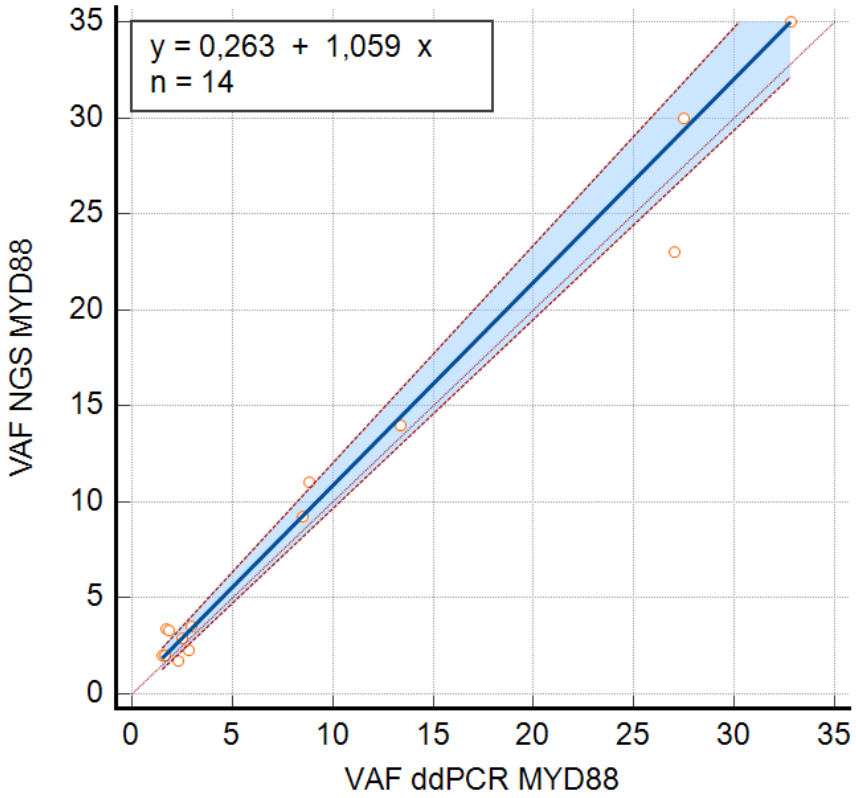
Diagnosis PVR-LBCL@UZA

2023 – 2025 experience: MD

- ddPCR MYD88
  - Sensitivity ddPCR MYD88 neg samples*
    - Criterium <1%
      - For PB, BM: **0,05%**
      - For vitreum (median): **0,27%** (n=11)
- VAF ddPCR MYD88 ⇔ NGS
  - For PB, BM
  - 1x MYD88 VAF 0,5% ddPCR (not detected by NGS)

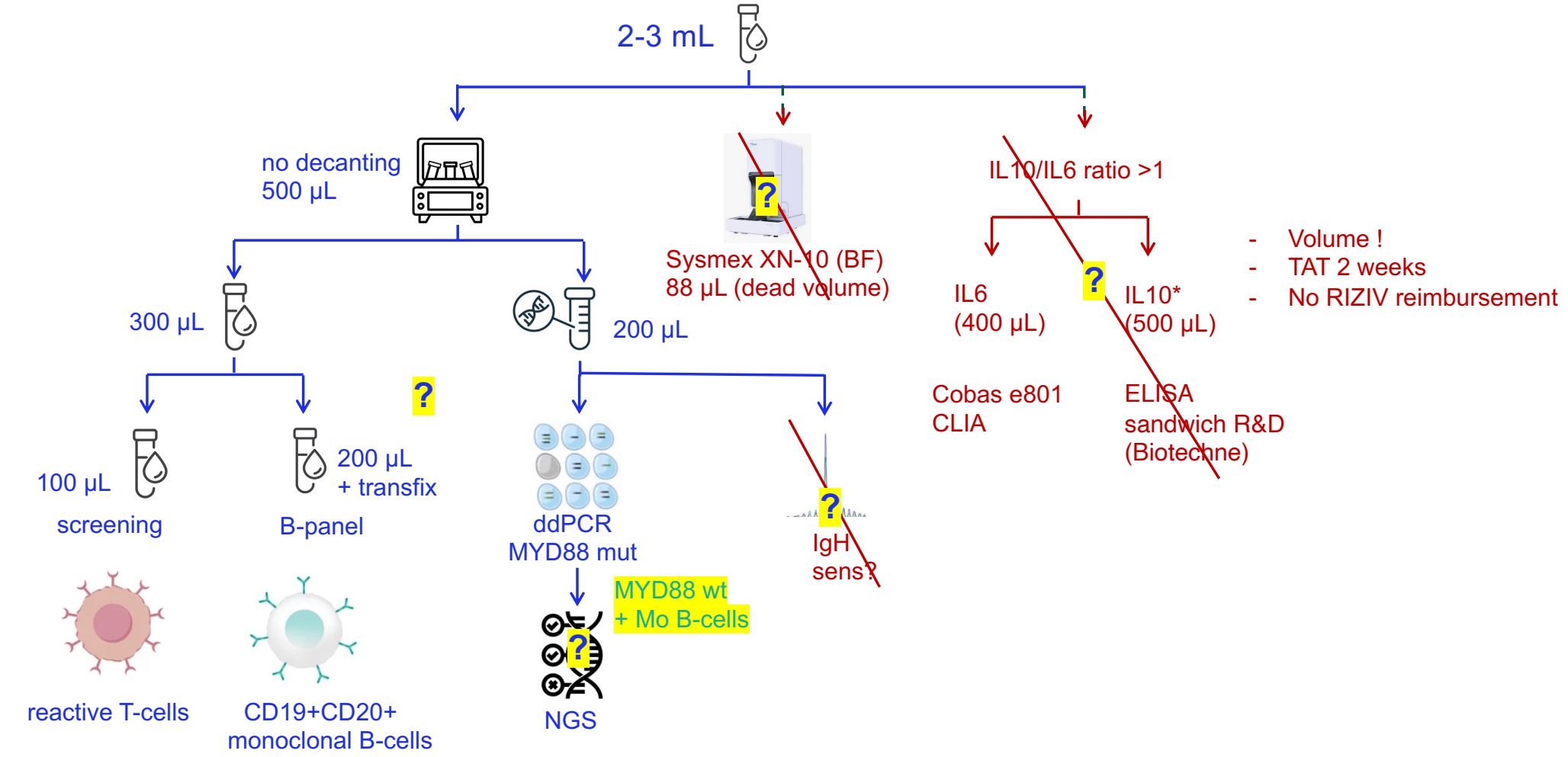
  ➔ Sensitivity NGS: **1-2%**

*sample dependent



Peripheral blood, bone marrow

Workflow clinical biology vitreum biopsy (FINAL)



*CHU de Liège

Case study PVR-LBCL (♀ °1963)

Ophthalmological history

- For 2 years before April 2023: bilateral blurred vision
- < April 2023: Pred Forte 6x/day, 1 week (stop for 2 weeks), Thealoz duo: x times a day
- April 2023: urgent referral for recurrent vitritis OD
    - Ophthalmological examination: cells in peripheral vitreous
    - Fluorescein angiography: ODS severe vitritis
- 08/2023: diagnostic vitrectomy

OD = right eye
ODS = right and left eye

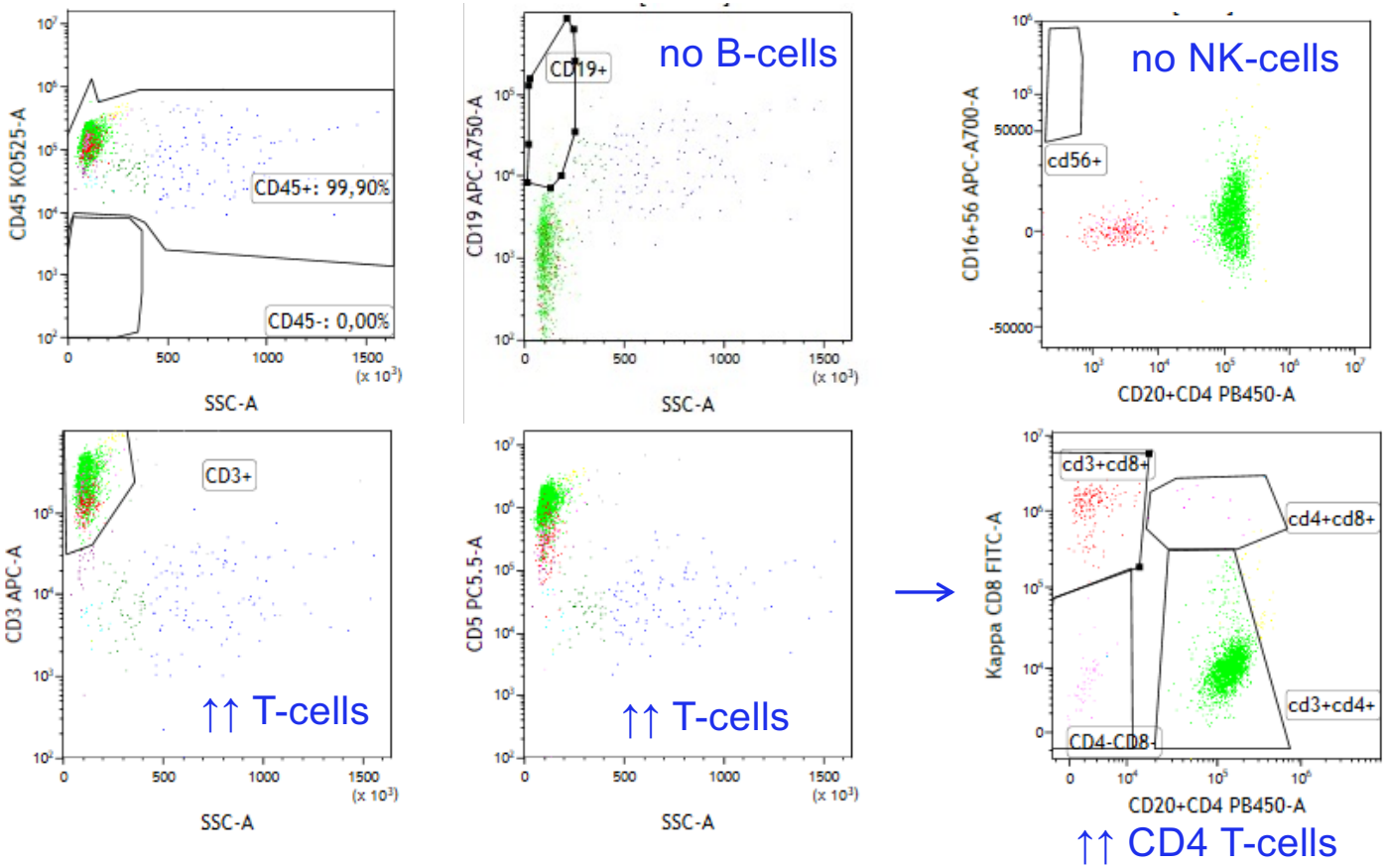
Case study PVR-LBCL

Immunophenotyping

VITREUM 08/2023

VITREUM

- Sysmex XN (BF): 114 WBC/µL (99% lymphocytes)
- FCM: T-panel = normal: reactive T-cells
- MD: not performed



Case study PVR-LBCL

Ophthalmological history

Ophthalmological history

- 08/2023 diagnostic vitrectomy (negative), afterwards symptoms better
- 01/2024 no cells visible, no symptoms anymore (old infection, therefore no effect of corticoids?)
- 10/2024 a few vitreous cells OS

  ➔ R/ Pred Forte 3x/d for both eyes
- 04/2025 Fluorescein angiography: enhanced coloration of the optic disc, diffuse leakage and patchy choroidal pattern in both eyes, follow-up after 4 months (CT PET whole body negative)

  Patient forgets a lot
- 07/2025 Progressive speech disorder, limping R, difficulties cycling, drops things, reduced balance, writing goes from beautiful to illegible?

  ➔ CT + MR normal

  ➔ CSF + peripheral blood??

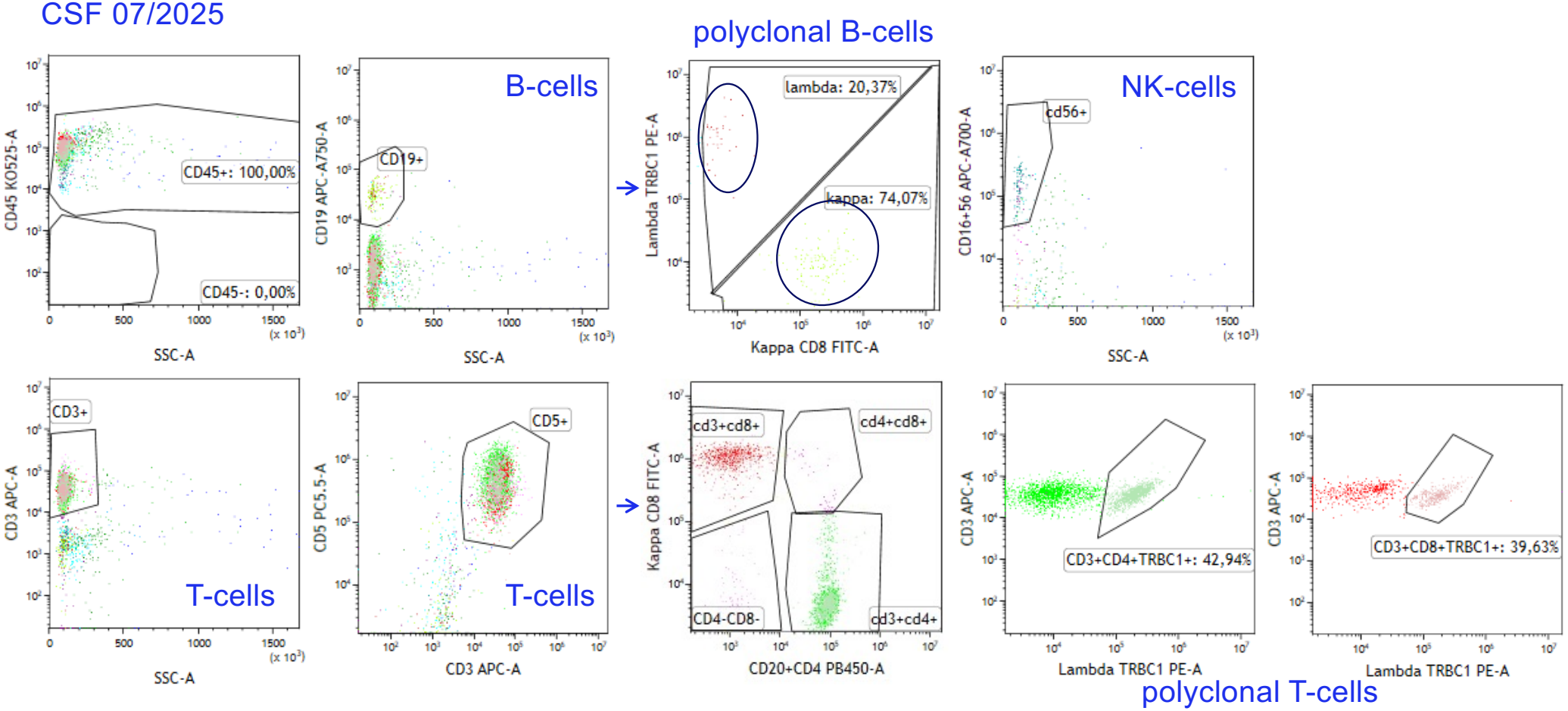
CSF

Case study PVR-LBCL

- Sysmex XN (BF): 23 WBC/µL
- FCM: normal, reactive T-cells
- MD: not determined

Immunophenotyping

CSF 07/2025



Case study PVR-LBCL

Ophthalmological history

- 07/2025 — Progressive speech disorder, limping R, difficulties cycling, drops things, reduced balance, writing goes from beautiful to illegible?
  - ➔ CT + MR normal
  - ➔ CSF + peripheral blood: negative
- 08/2025 — Fluorescein angiography: no active vitritis/retinitis, atrophic photoreceptor layer OS, vision will not restore anymore
- 09/2025 — Metamorphopsia OS sinds 2 dagen
  OCT: OS ↑ infrapapillary PED, subretinal deposits/epitheliopathy with atrophic photoreceptor layer, some vitreous cells, PELL in papillomacular bundle'(stable)
- 10/2025 — OS vitreum biopsy

VITREUM

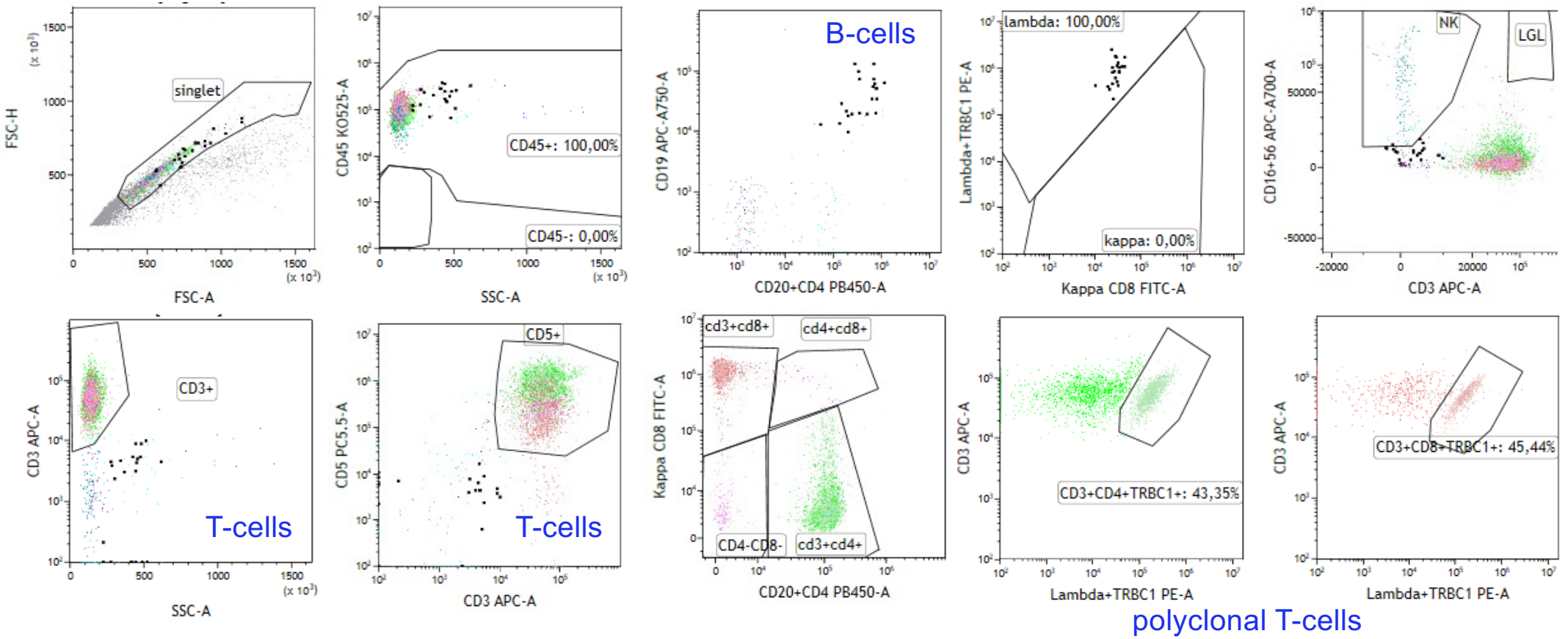
Case study PVR-LBCL

Immunophenotyping

VITREUM 10/2025

- Sysmex XN (BF): 23 WBC/µL
- FCM: 0,2 clonal B-cells?
- MD: ddPCR MYD88 mut 4%

clonal? 22 events (0,2%)



polyclonal T-cells

VITREUM

Case study PVR-LBCL

Molecular diagnostics

VITREUM 10/2025

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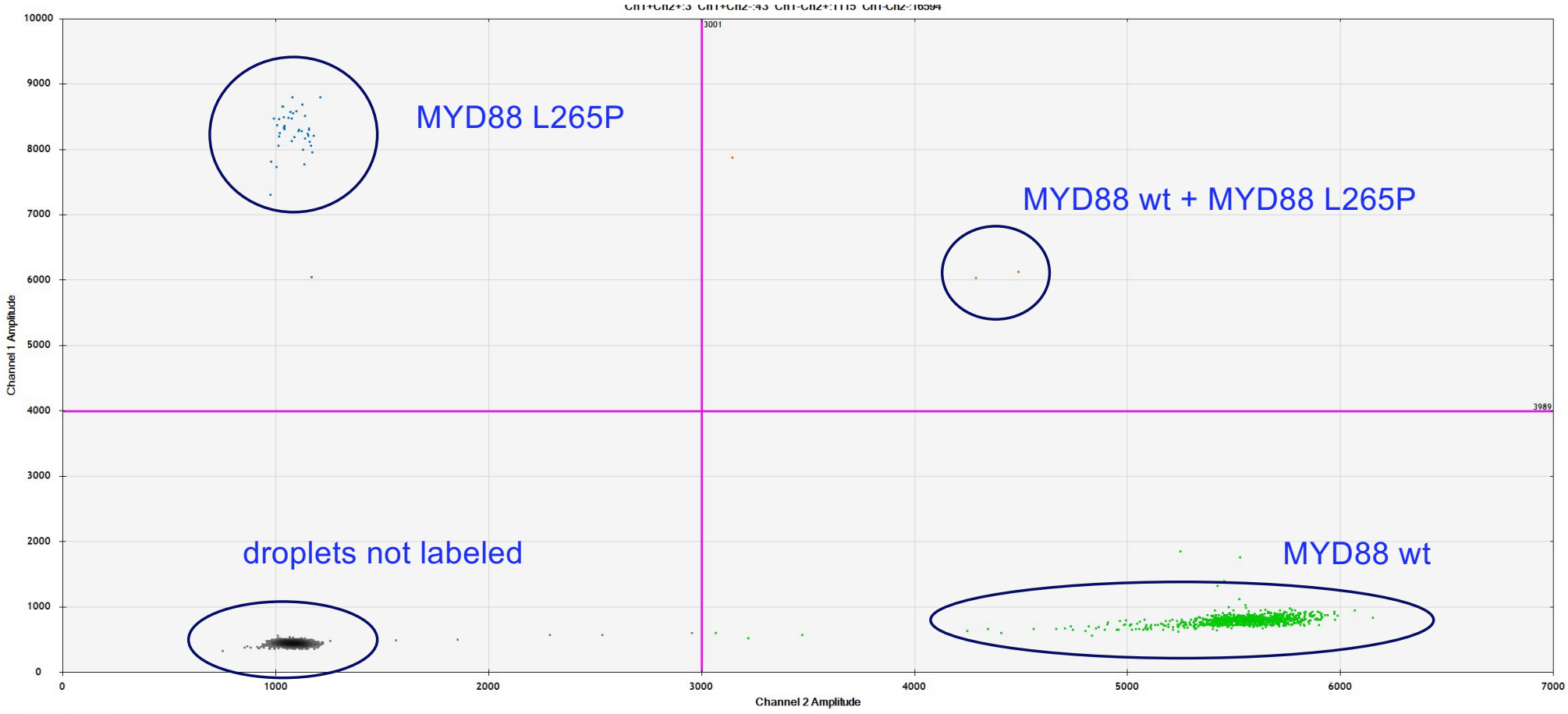

VITREUM

Case study PVR-LBCL

- Sysmex XN (BF): 23 WBC/µL
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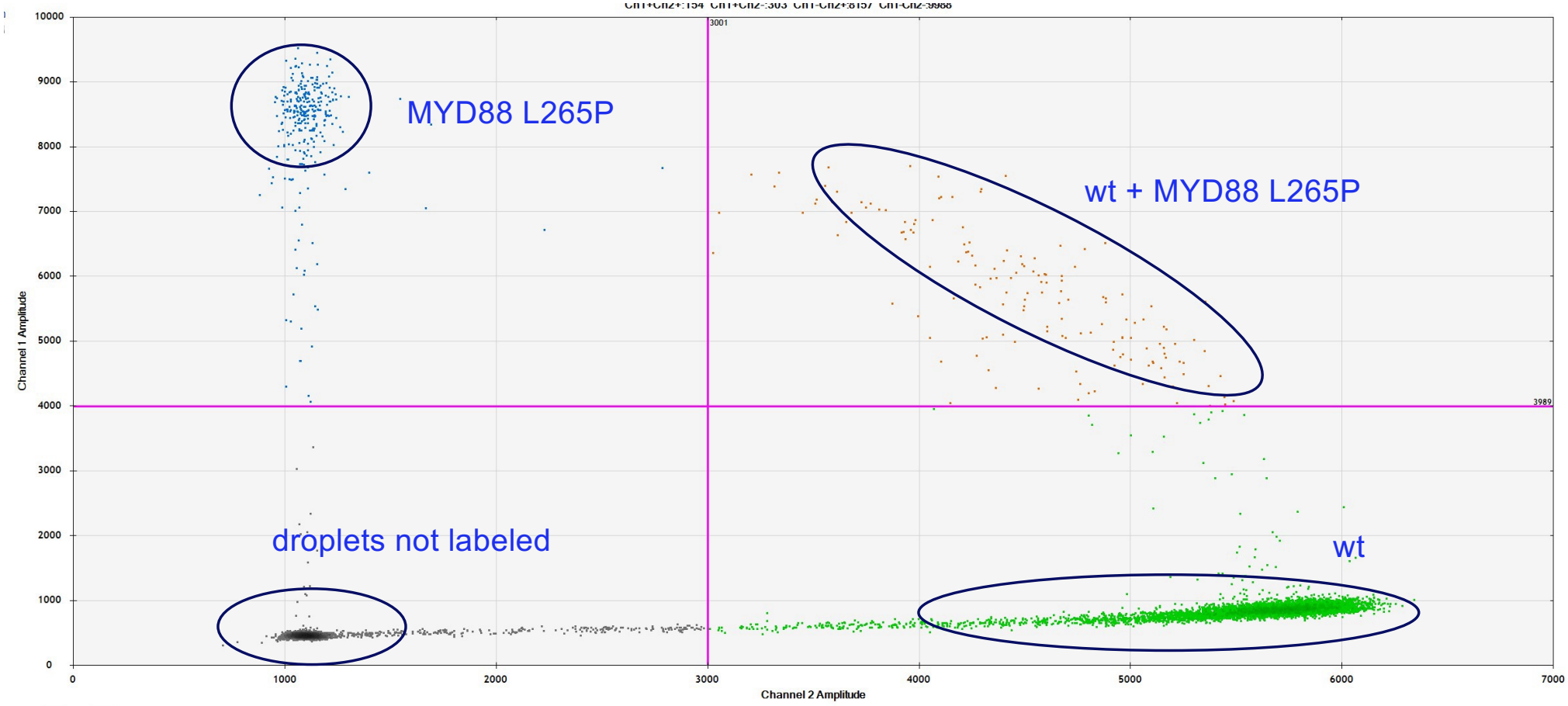
- MD: ddPCR MYD88 mut 4%

Molecular diagnostics

Positive control MYD88 mut



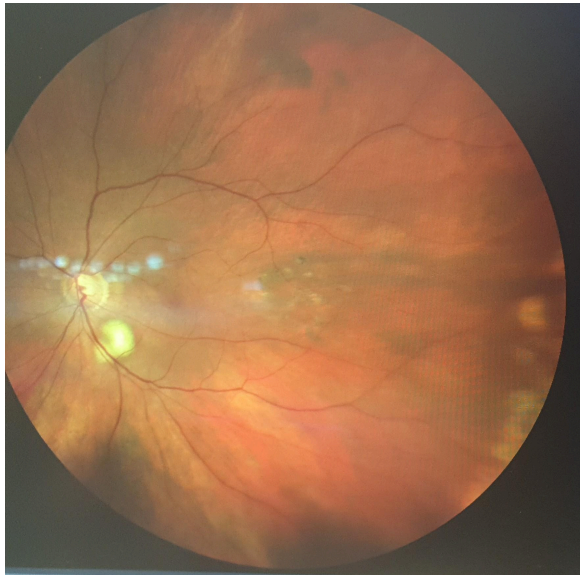
Case study PVR-LBCL

Ophthalmological history

- 10/2025 OS vitreum biopsy: FCM 0,2% Mo B-cells, MYD88 mut detected

  R/ injection **MTX** 400 µg/0.05ml + aprokam 0.1ml + luchtbel + triam subconj, AA, VVS, Pred Forte and dicloabak 3x/d for 4 weeks
  ➔ High suspicion of PVRL
  ➔ Better on MTX, repeat weekly

Choroid lesion, under the optic nerve



Before treatment

After 2 injections MTX

Case study PVR-LBCL

Ophthalmological history

- 11/2025 OS: intraocular lesion is better, OD: mild inflammation, no lesions
  New neurological events (central facial palsy left, and projectile vomiting)
  ➔ CNS invasion?
  ➔ Imaging: PET-CT: dysarthria, MR: high suspicion for lymphoma
  ➔ Sampling

CSF
- FCM: negative

Bone marrow
- Cytology/FCM: normal
- 48, XX, +3, +12[4]/46,XX[16]

Blood
- FCM: B-cells kappa>lambda? <-> lambda clone?
- MD: MYD88 L265P mut (0,15%)

Brain biopsy (pathology) ~ PCNS-LBCL
- Monotypic large B-cell infiltrate CD20+/MUM1+ (Ki-67: 90%)

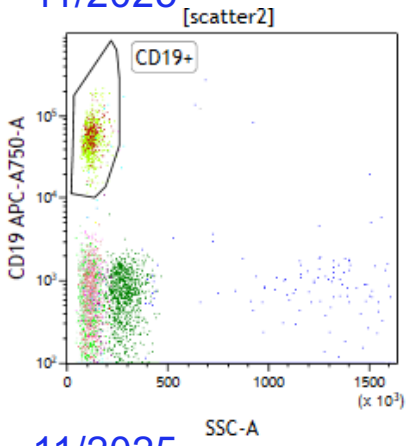
PVR-LBCL with CNS spreading <=> PCNS-LBCL with ocular spreading

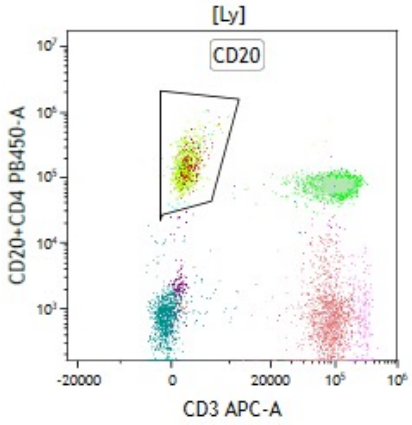
Case study PVR-LBCL

Immunophenotyping

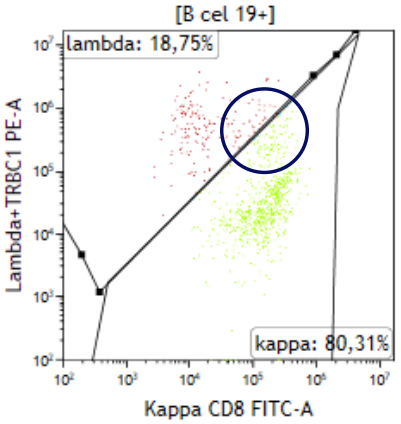
PERIPHERAL BLOOD

11/2025

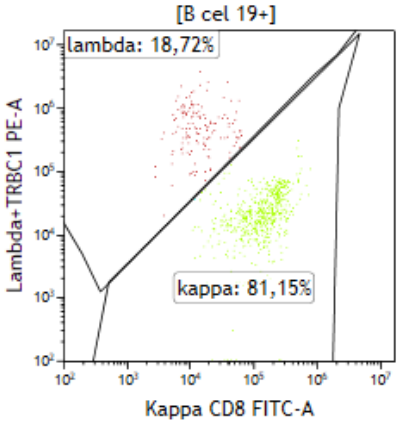




kappa > lambda?

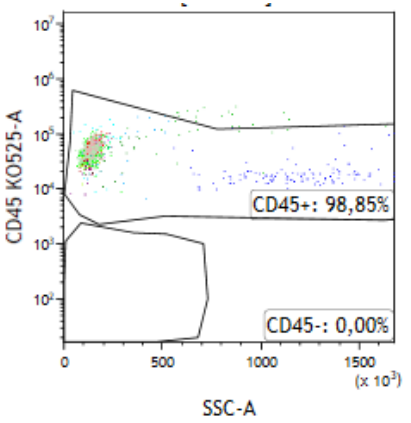


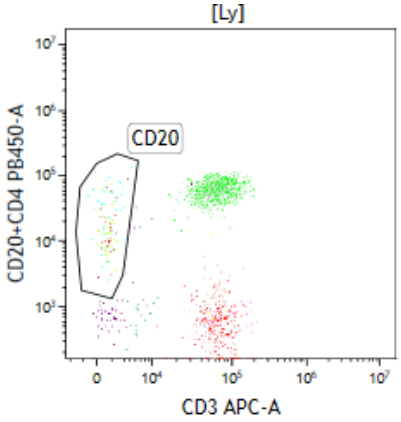
kappa > lambda?

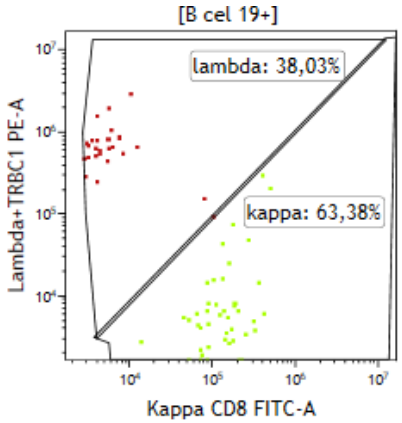


CSF (24 c/µL)

11/2025







86% T-cells
5% polyclonal B-cells
1% NK cells

Ocular lymphoma

1 What is ocular lymphoma?

Clinical presentation
Classification WHO
Incidence/survival

2 Diagnosis PVR-LBCL

Sampling
Laboratory diagnosis

3 PVR-LBCL @UZA

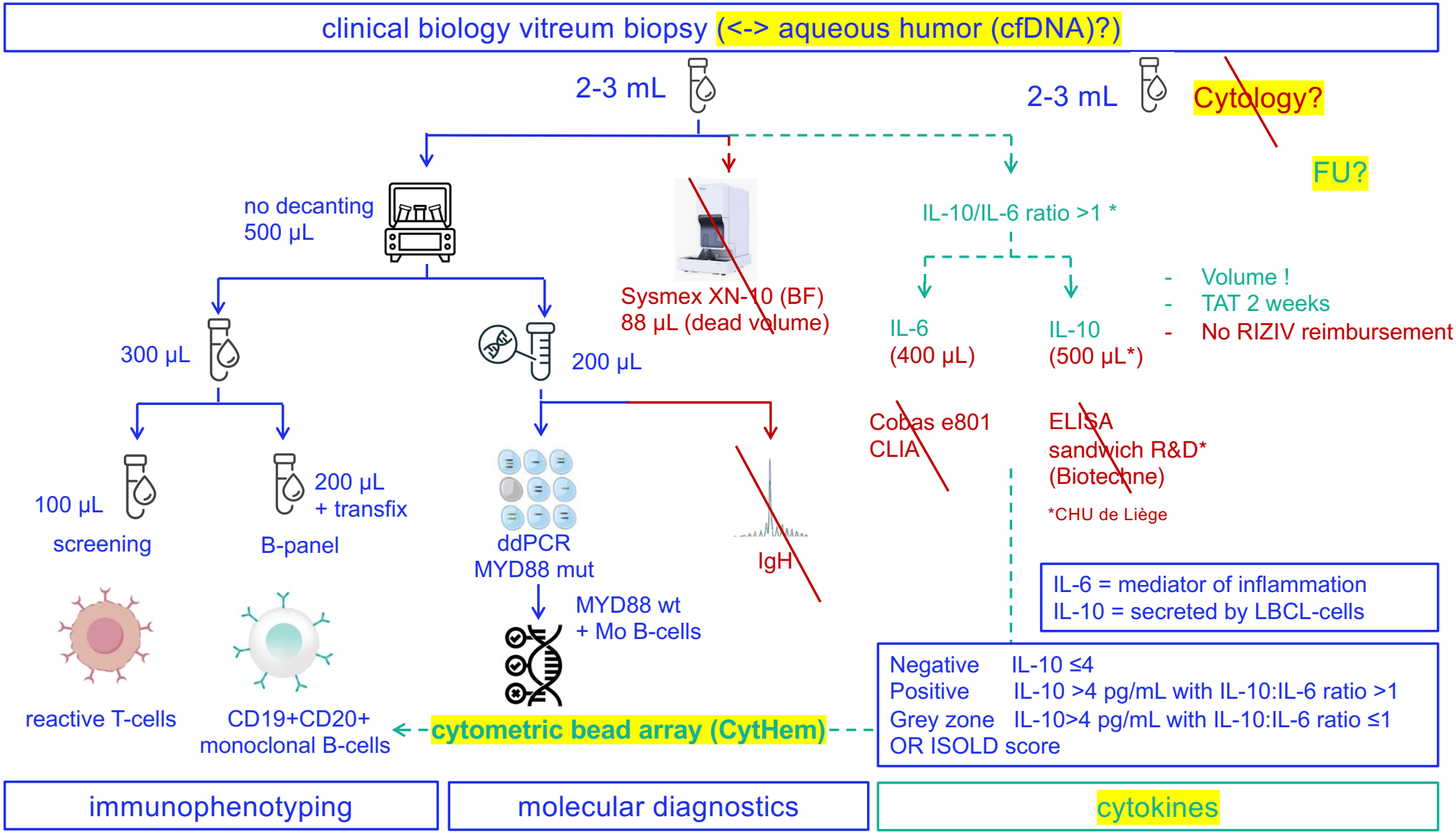
Workflow
2023 – 2025 experience
Immunophenotyping ⇔ MD
ddPCR ⇔ NGS
Case study

4 What's next @UZA for PVR-LBCL?

5 Take home messages

6 Teamwork

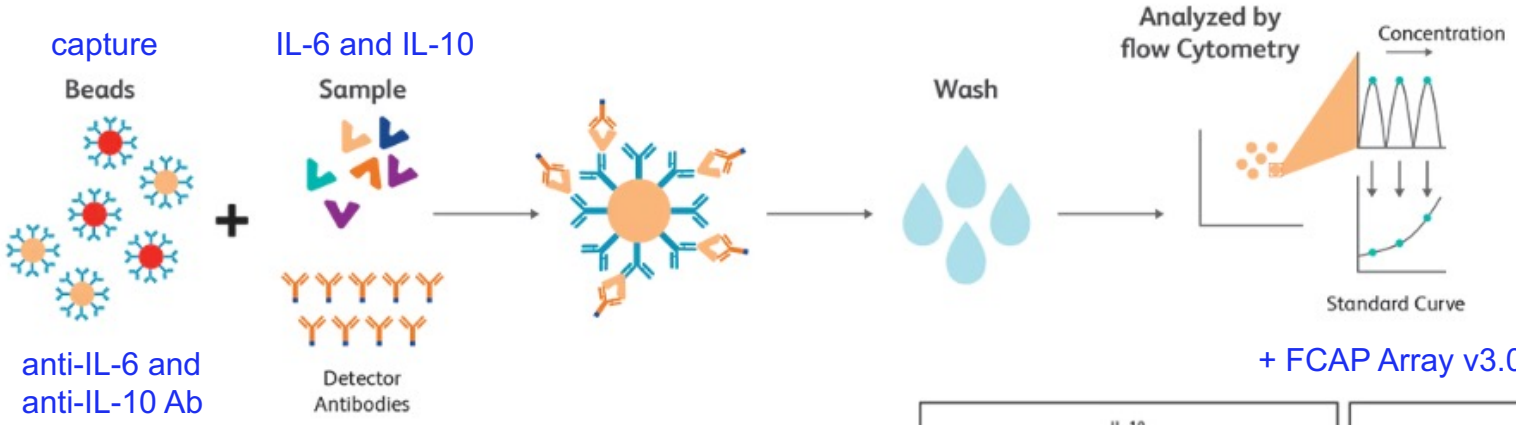
UZA


clinical biology vitreum biopsy (<-> aqueous humor (cfDNA)?)
2-3 mL
2-3 mL
Cytology?
FU?
no decanting
500 µL
Sysmex XN-10 (BF)
88 µL (dead volume)
IL-10/IL-6 ratio >1 *
- Volume !
- TAT 2 weeks
- No RIZIV reimbursement
300 µL
200 µL
IL-6
(400 µL)
IL-10
(500 µL*)
Cobas e801
CLIA
ELISA
sandwich R&D*
(Biotechne)
*CHU de Liège
100 µL
screening
200 µL
+ transfix
B-panel
ddPCR
MYD88 mut
IgH
MYD88 wt
+ Mo B-cells
IL-6 = mediator of inflammation
IL-10 = secreted by LBCL-cells
reactive T-cells
CD19+CD20+
monoclonal B-cells
cytometric bead array (CytHem)
Negative IL-10 ≤4
Positive IL-10 >4 pg/mL with IL-10:IL-6 ratio >1
Grey zone IL-10>4 pg/mL with IL-10:IL-6 ratio ≤1
OR ISOLD score
immunophenotyping
molecular diagnostics
cytokines

Ocular lymphoma

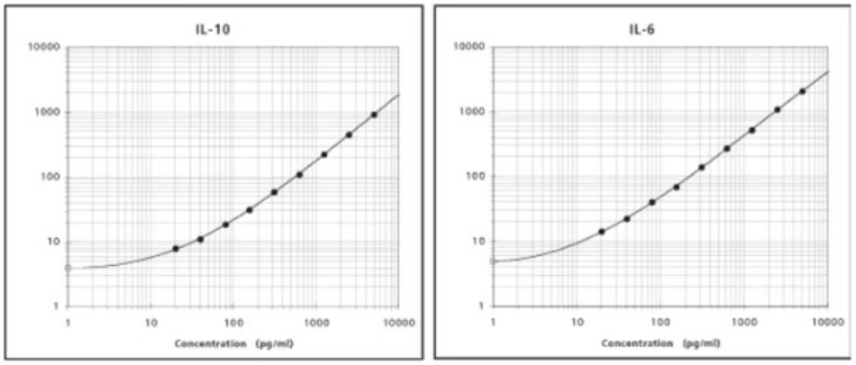
Cytokine assay

Cytometric Bead Array (BD): sample needed 25 – 50 µL



+ FCAP Array v3.0 Software

BD® CBA Flex Sets - open and configurable method of detection to build your own multiplexes for protein quantitation as low as 10 pg/mL



IL-6 and IL-10 representative standard curves

Ocular lymphoma

Take home messages

- PVR-LBCL
  - Rare but potentially deadly disease
  - Pre sampling conditions are important
  - Know your laboratory assay + sensitivity
    - Cytology can be negative
    - Immunophenotyping:
      - Mo B-cells
      - B-cells without kappa/lambda expression = suspicious!
    - Perform ddPCR MYD88 L265P (NGS when ddPCR neg and Mo B-cells)
    - (Explore cytokine analysis?)
  - Repeat sampling when no diagnosis and reoccurrence/worsening of symptoms (symptoms can fluctuate)
  - Talk to your referring ophthalmologists and pathology department

Ocular lymphoma

Teamwork

- Clinical biology UZA

Kathleen Deiteren

Marie-Berthe Maes

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Marie Le Mercier (Mol Sc)

Katrien Vermeulen (Mol Sc, FCM)

MLT's

- Ophthalmologists UZA & ZAS

Veerle Van Severen

Jordy Goemaere

Pieter-Paul Schauwvlieghe

Luc Van Os

- Pathology UZA (ddPCR)

Sasha Libbrecht

Stefan Dubois

➔ Weekly discussing between laboratories
➔ Email with opthalmologists
➔ MOC