

# Minimal residual disease in CLL

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Clinic III – Hematology, Oncology, Palliative Medicine***

# Conflicts of interest

**Research funding:** Roche Basel, Genentech San Francisco, AbbVie North Chicago, Celgene Summit, Becton Dickinson Heidelberg, Janssen-Cilag Neuss

**Honoraria:** Roche, AbbVie, Novartis, Becton Dickinson, Janssen, Astra-Zeneca, Sanofi

**Advisory Boards:** Roche Basel

**Patents:** Flow cytometric MRD, Immunphenotyping using flow (EuroFlow)

Founding member of EuroFlow

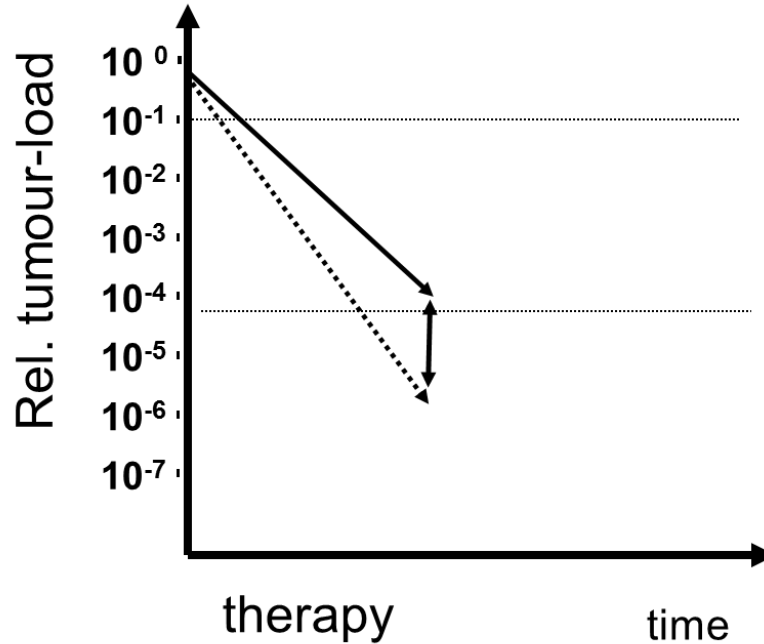
# MRD in CLL

- Why measure MRD in CLL
  - MRD quantification: a prognostic factor
  - MRD & iwCLL response
  - MRD as surrogate for PFS
  - MRD tailored treatment
- Improve current MRD assessments
  - MRD beyond  $10^{-4}$
  - Work in progress: EuroFlow CLL MRD panel design and validation



# **MRD QUANTIFICATION: A PROGNOSTIC FACTOR**

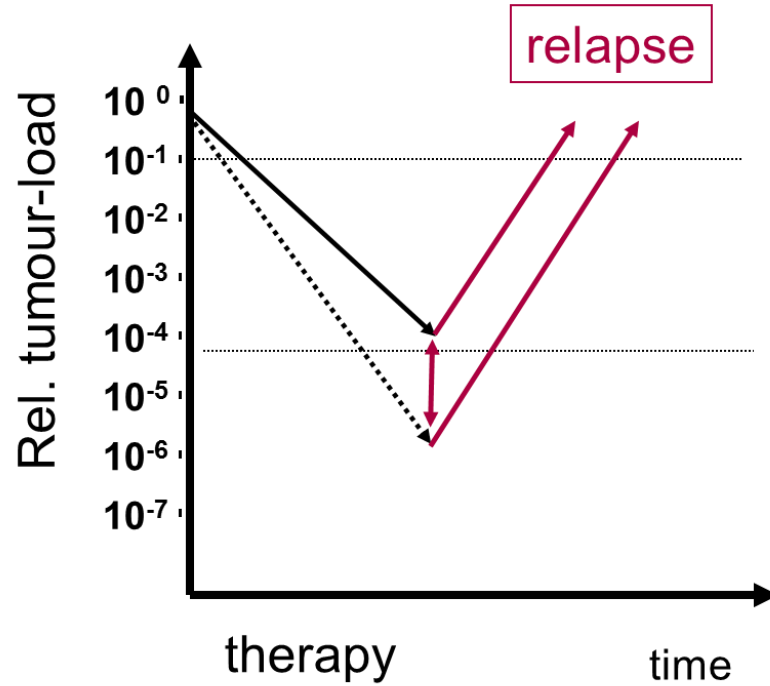
# Model



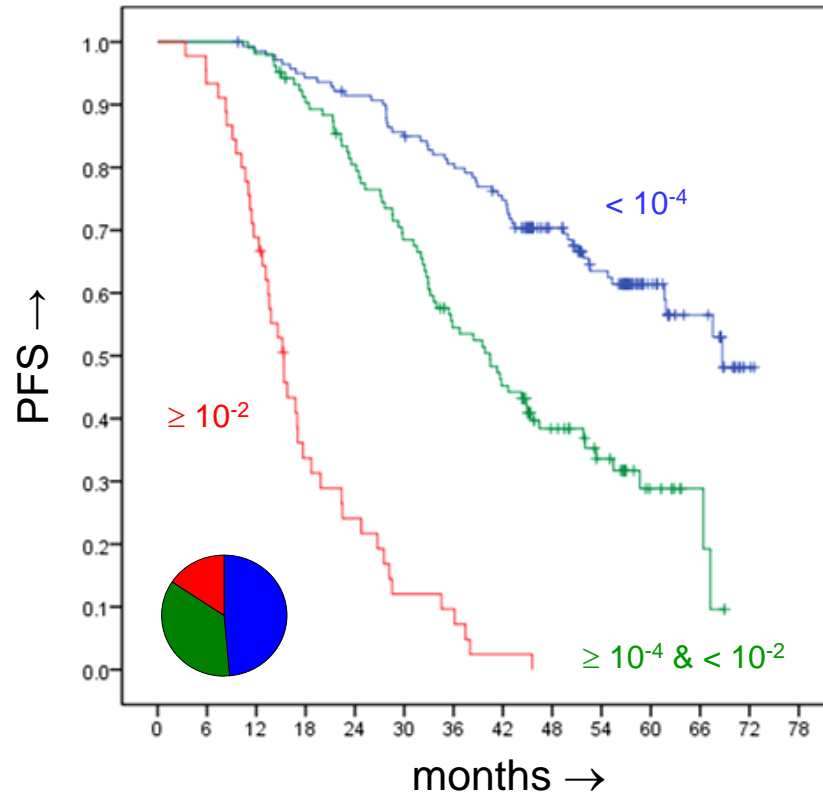
$$\Delta = \Sigma$$

- treatment regimen
- effective dosage
- pharmacogenetics
- apoptosis resistance
- microenvironment

# Model



# CLL8 trial: PFS prediction from Final Restaging (PB)



# PFS vs. MRD @ 2018 iwCLL standard ( $10^{-4}$ ) - no maintenance -

	Reference	N	Therapy	Technique	MRD threshold	PFS by MRD group	p value
1	Moreno 2006	22	auto SCT	3/4-color MRD flow	$10^{-4}$	16 mos vs 75 mos	< 0.001
2	Bosch 2008	44	FCM	4-color MRD flow	$10^{-4}$	MRD+ CR < MRD- CR (RD)	0.2
3	Böttcher 2012	290	FC/ FCR	4-color MRD flow	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 15 mos $\geq 10^{-4}$ to < $10^{-2}$ 41 mos < $10^{-4}$ 69 mos	< 0.001
4	Fischer 2012	45	BR	4-color MRD flow	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 12 mos $\geq 10^{-4}$ to < $10^{-2}$ 32 mos < $10^{-4}$ 47 mos	< 0.001
5	Pettitt 2012	25	Cam-HDMP	4-color MRD flow	$10^{-4}$	10 mos vs. 24 mos	0.009
6	Bouvet 2013	106	FCR	4-color MRD flow	$10^{-4}$	30 mos vs. NR	< 0.001
7	Santacruz 2014	96	various	3/4-color MRD flow	$10^{-4}$	40 mos vs. 76 mos (TFS, CR only)	< 0.001
8	Strati 2014	161	FCR	4-color MRD flow	$10^{-4}$	NR	< 0.001
9	Kwok 2016	133	various	4-color MRD flow	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 24 mos $\geq 10^{-4}$ to < $10^{-2}$ 40 mos < $10^{-4}$ 91 mos	< 0.001
10	Munir 2017	184	FCR /FCMR	4-color MRD flow	$10^{-4}$	42 mos vs. 70 mos	Not reported
11	Howard 2017	149	FCR /FCM-miniR	4-color MRD flow	$10^{-4}$	42 mos vs. NR	Not reported
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# PFS vs. MRD @ 2018 iwCLL standard ( $10^{-4}$ ): no maintenance

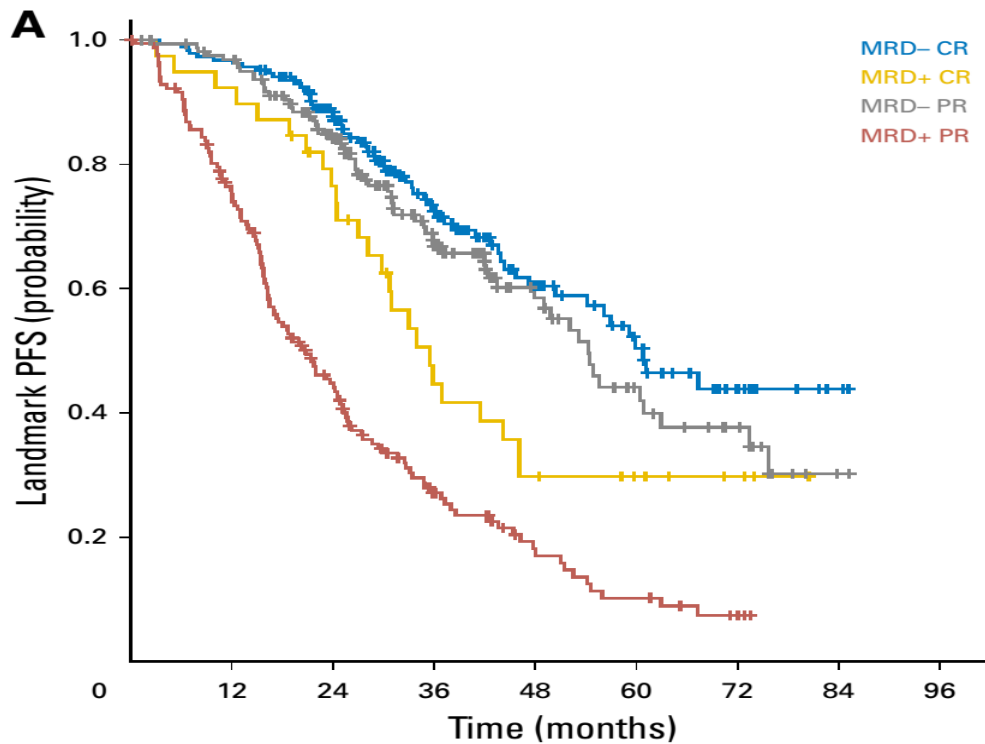
## - continued -

	Reference	N	Therapy	Technique	MRD threshold	PFS by MRD group	p value
	.....						
12	Stilgenbauer 2018	105	O-B	4-color MRD flow	$10^{-4}$	24 mos vs. NR	Not reported
13	Langerak 2019	474	C1b-G/ C1b-R	ASO RQ-PCR	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 14 mos $\geq 10^{-4}$ to $< 10^{-2}$ 24 mos $< 10^{-4}$ 56 mos	< 0.001
14	Al-Sawaf 2020 & 2021	362	C1b-G/ Ven-G	ASO RQ-PCR	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 12 mos $\geq 10^{-4}$ to $< 10^{-2}$ 30 mos $< 10^{-4}$ NR	< 0.001
15	Herling 2020	53	FCR	4-color MRD flow	$10^{-4}$	41 mos vs NR	< 0.001
16	Lestestu 2020	350	FCR	6-color MRD flow / 8-color MRD flow	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 13 mos $\geq 10^{-4}$ to $< 10^{-2}$ 52 mos $< 10^{-4}$ 67 mos	< 0.001
17	Kater 2020 & 2022	119	Ven-R	ASO RQ-PCR / 4-color MRD flow	$10^{-4}$ and $10^{-2}$	$\geq 10^{-2}$ 3 mos $\geq 10^{-4}$ to $< 10^{-2}$ 24 mos $< 10^{-4}$ NR	< 0.001
$\Sigma$		<b>2718</b>					

# **MRD & IWCLL RESPONSE**

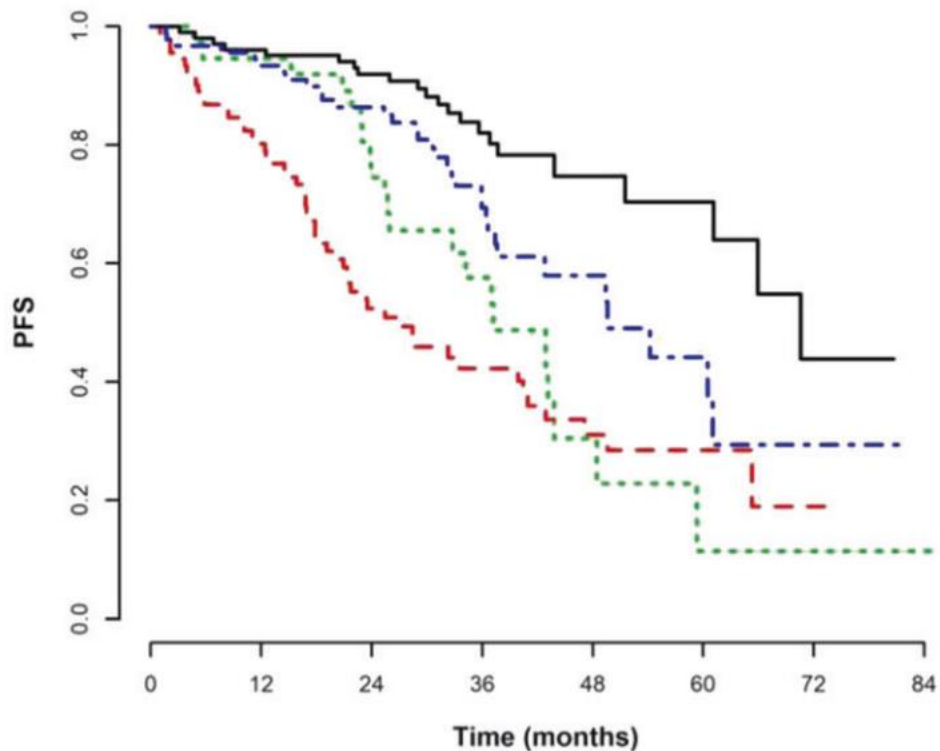
# PFS by PB MRD and clinical response

- combined landmark-analyses of GCLLSG CLL8 and CLL10 trials (n = 554) -



# PFS by PB MRD and clinical response

- combined landmark-analyses of 3 FILO trials (n = 321) -

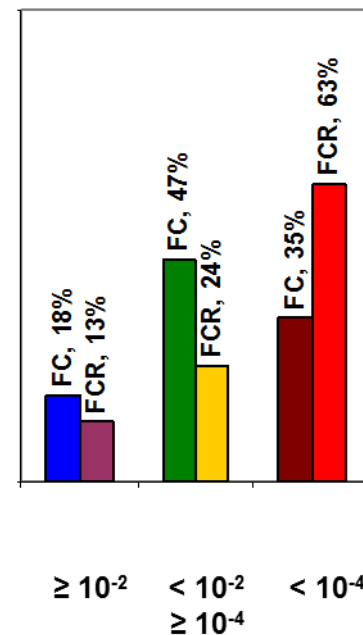
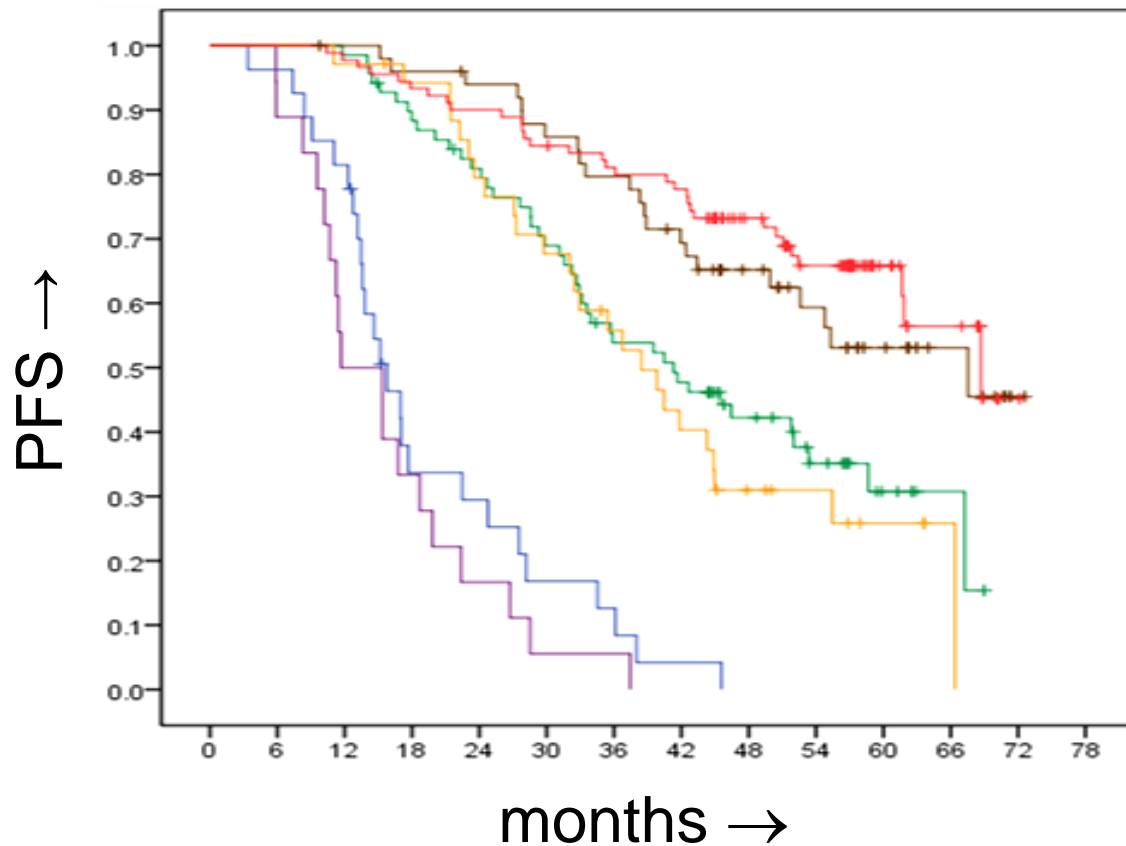


Blood MRD	Median (months)	Comparison with Und-MRD CR HR [95% CI]
Und-MRD CR	70.6	---
Und-MRD PR	49.6	p=0.019 1.91 [1.11-3.28]
Any MRD+ CR	37.2	p=0.0001 3.17 [1.74-5.78]
Any MRD+ PR	26.6	p<0.0001 4.26 [2.59-7.02]

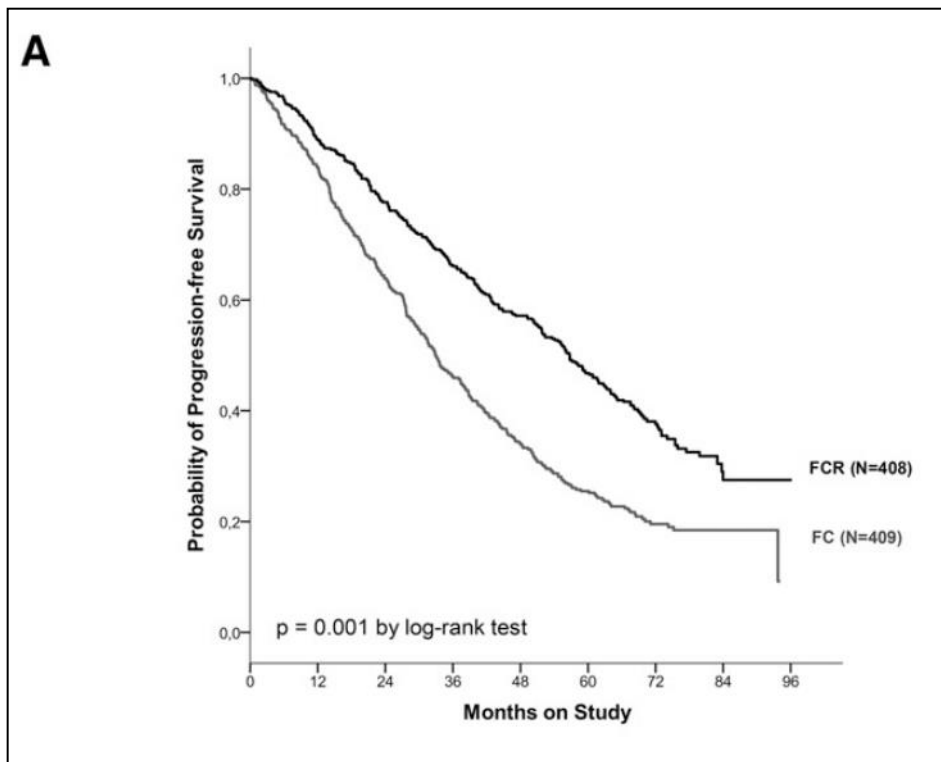
**SURROGATE MARKER FOR PFS**

# CLL8 trial: PFS prediction from Final Restaging (PB)

-Treatment arm -

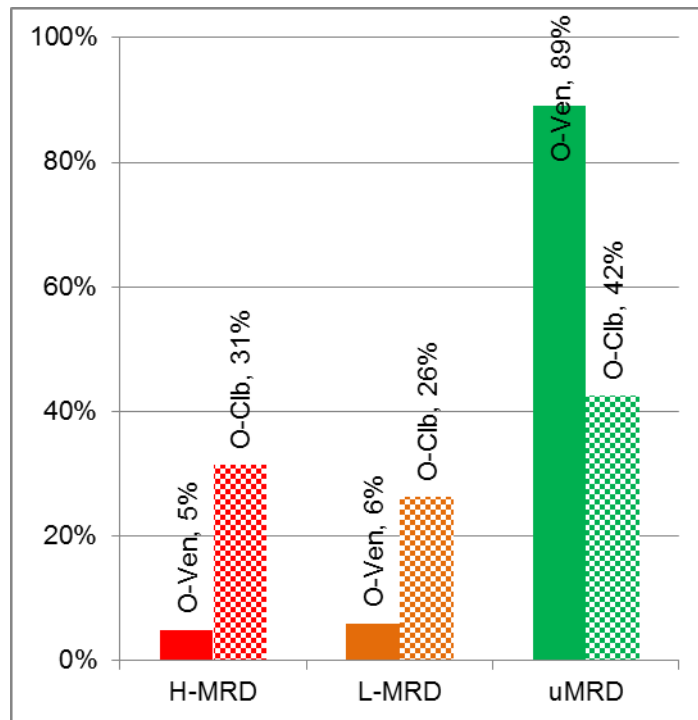
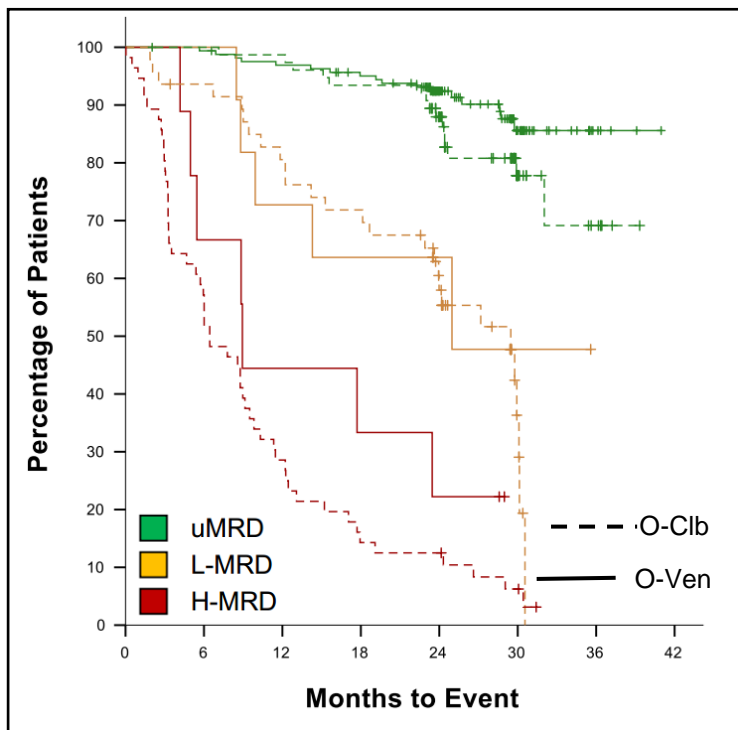


# CLL8: PFS



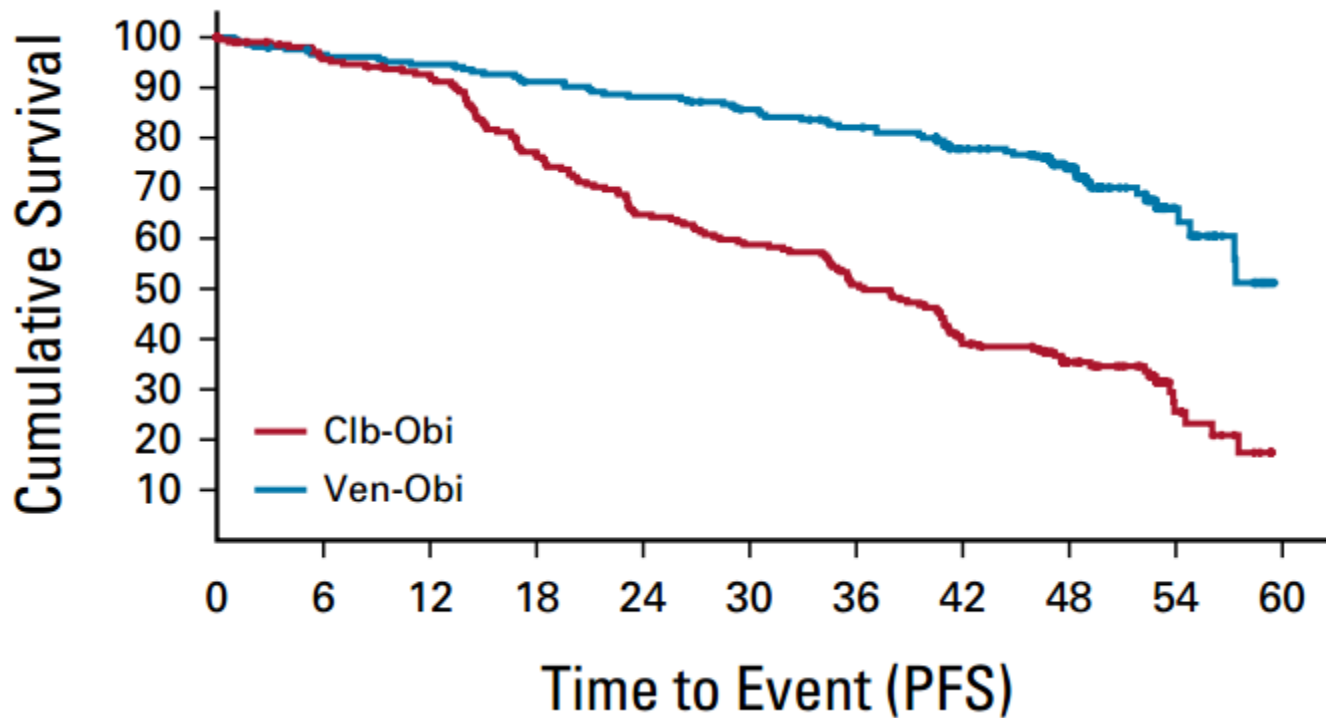
# CLL14 trial: PFS prediction from Final Restaging (PB)

-Treatment arm -





# CLL14 trial: PFS





EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

17 December 2015  
EMA/CHMP/703715/2012 Rev. 2  
Committee for Medicinal Products for Human Use (CHMP)

## **MRD as an endpoint for licensure**

A difference in MRD response rates can be used as primary evidence of clinical benefit to obtain early licensure in randomised CLL trials designed to show superiority in terms of PFS but where mature PFS data will only become available at a later stage. Regulatory considerations (e.g. legal basis of the marketing authorisation application or other considerations, for example conditional approval) should be decided on a case by case basis.

# FDA 2020



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## **Hematologic Malignancies: Regulatory Considerations for Use of Minimal Residual Disease in Development of Drug and Biological Products for Treatment Guidance for Industry**

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- MRD status should be measured by a standardized method with a quantitative lower limit of detection sufficient to evaluate the prospective cutoff in the trial and at least less than  $10^{-4}$  (0.01%). Currently, MRD is most commonly assessed using RT-qPCR and flow cytometric methods, but NGS can also reliably assess MRD in CLL.
  - A challenge in MRD testing is that CLL is a multicompartmental disease involving the BM, blood, lymph nodes, liver, and spleen; after treatment, one or more of these sites may serve as a reservoir for residual disease. Sponsors should carefully consider for assessment the sample source, which should be the same throughout the trial. This is especially important as therapeutic intervention differentially affects MRD measurement in peripheral blood and BM, as has been demonstrated with certain therapeutics (e.g., anti-CD20 monoclonal antibodies, alemtuzumab).

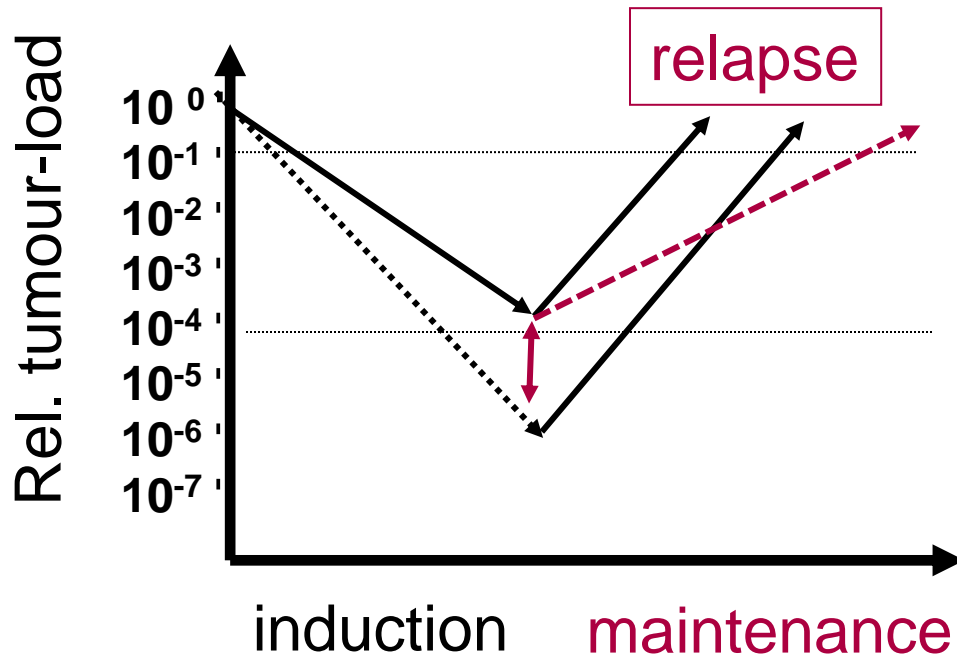
U.S. Department of Health and Human Services  
Food and Drug Administration  
Oncology Center of Excellence (OCE)  
Center for Drug Evaluation and Research (CDER)  
Center for Biologics Evaluation and Research (CBER)

January 2020  
Clinical/Medical

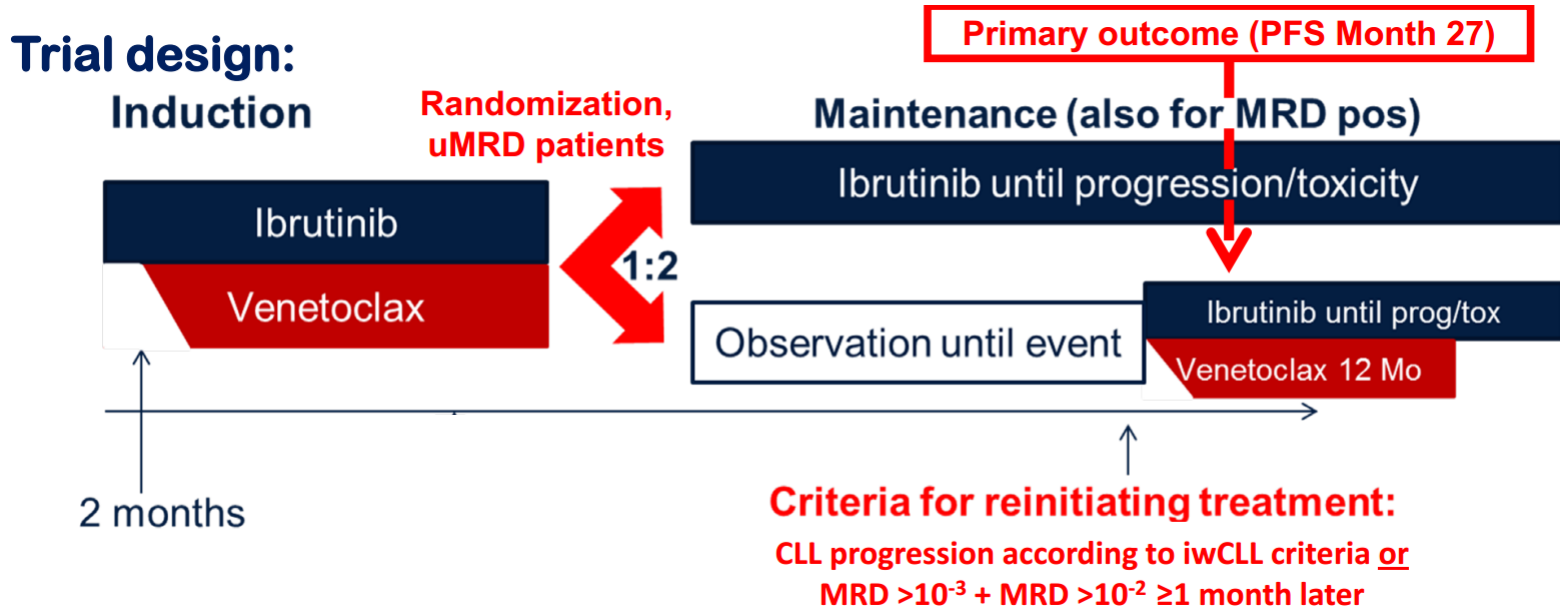
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# **MRD TAILORED TREATMENT**

# Consolidation in MRD<sup>+</sup>

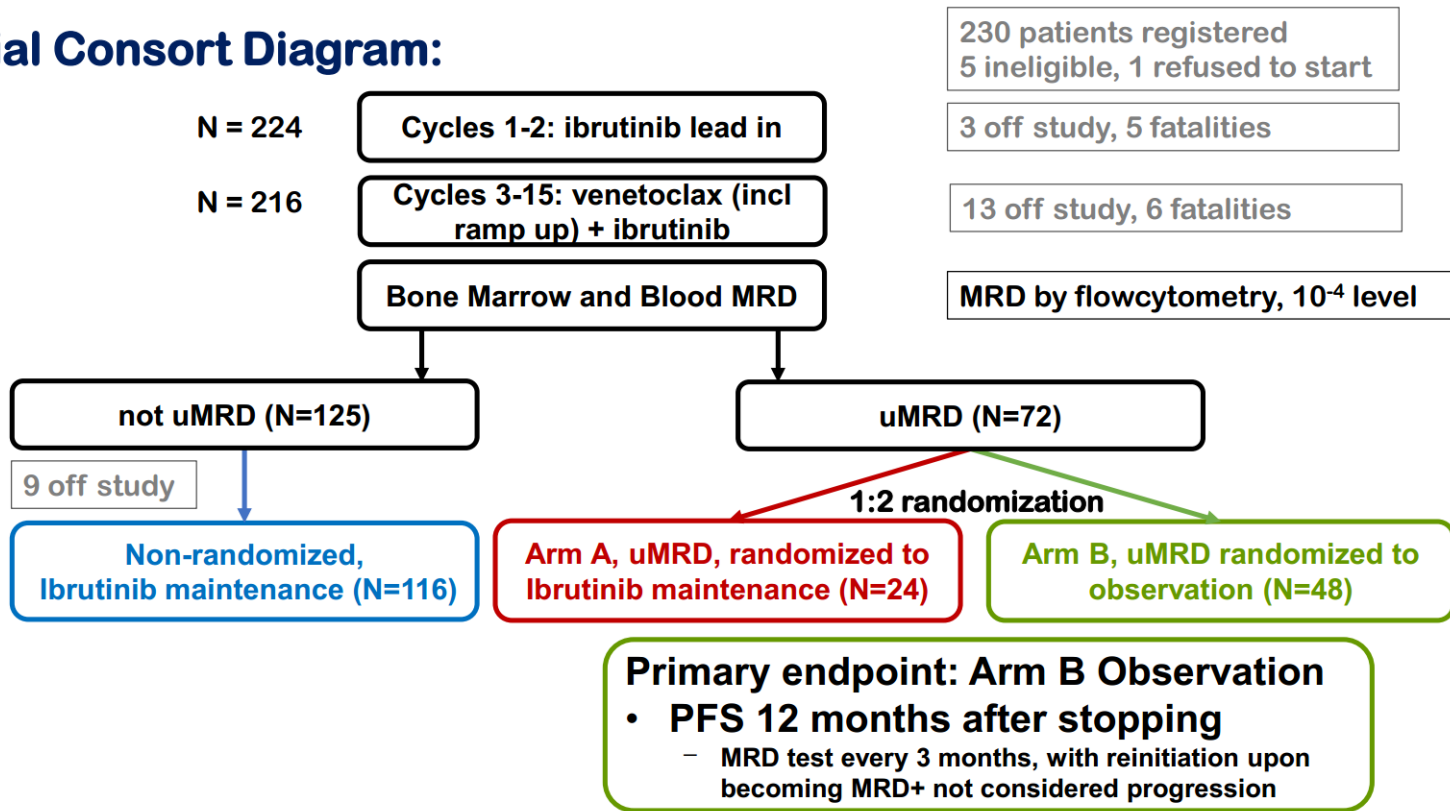


# VISION



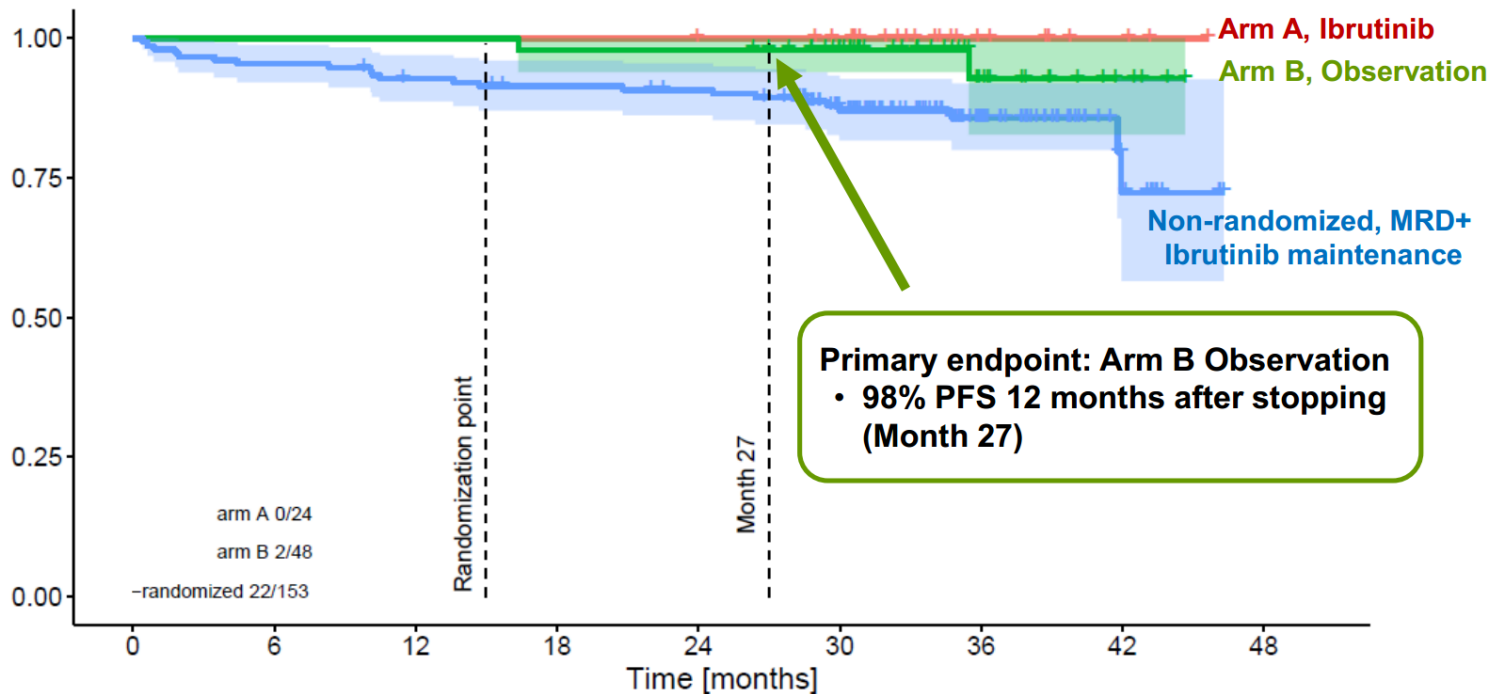
# VISION

## Trial Consort Diagram:



# VISION

## Progression Free Survival (PFS)

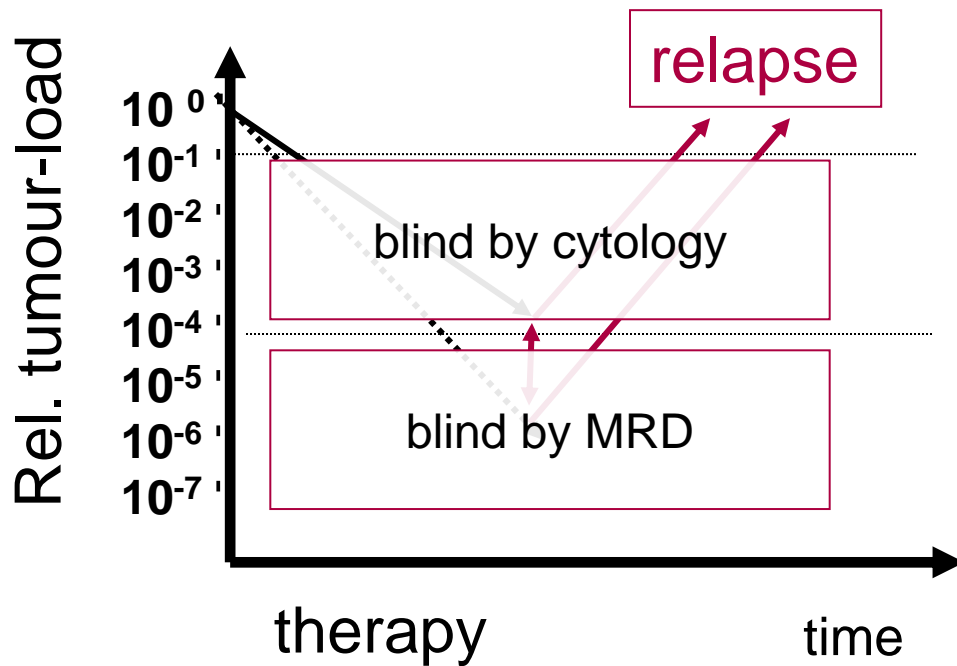


All patients with events prior to cycle 15 included in non-randomized group

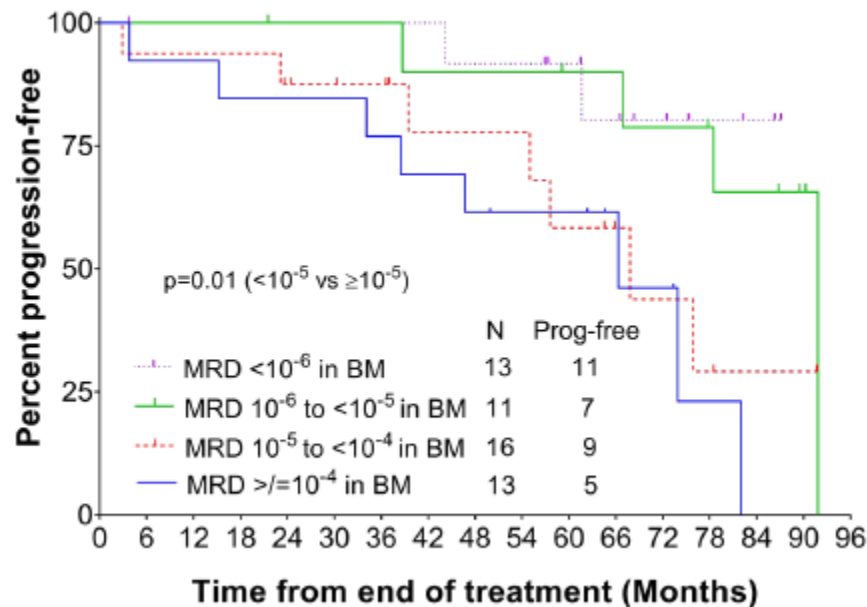


**MRD BEYOND  $10^{-4}$**

# MRD eradication is a misnomer



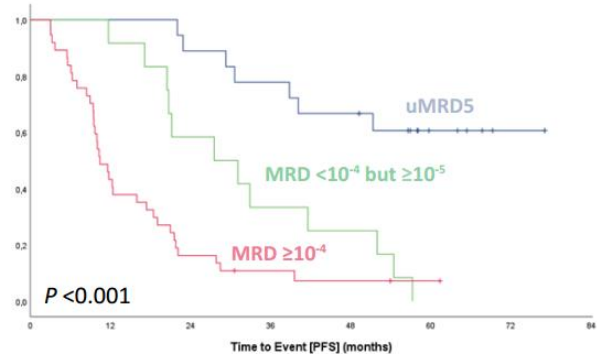
# MRD neg patients: PFS by MRD level using NGS - MDACC FCR series -



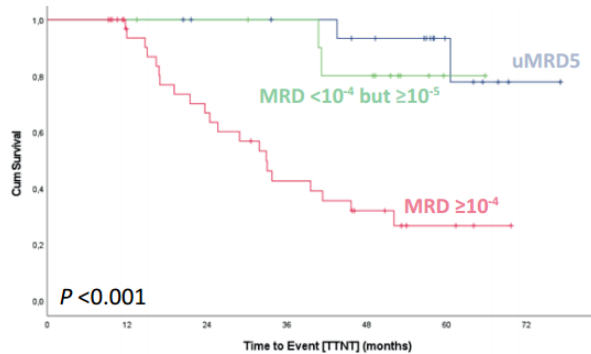
# PFS, TTNT, OS by PB MRD5

- IGHV leader based NGS MRD from CLL11, n=67 -

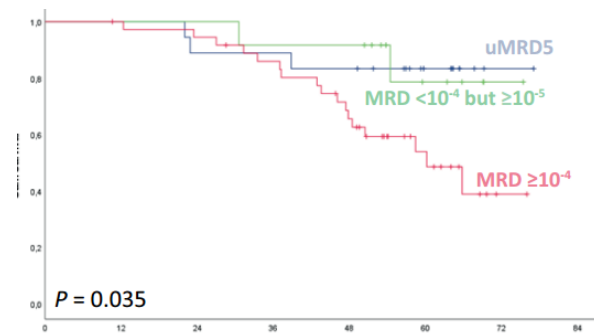
## PFS



## TTNT

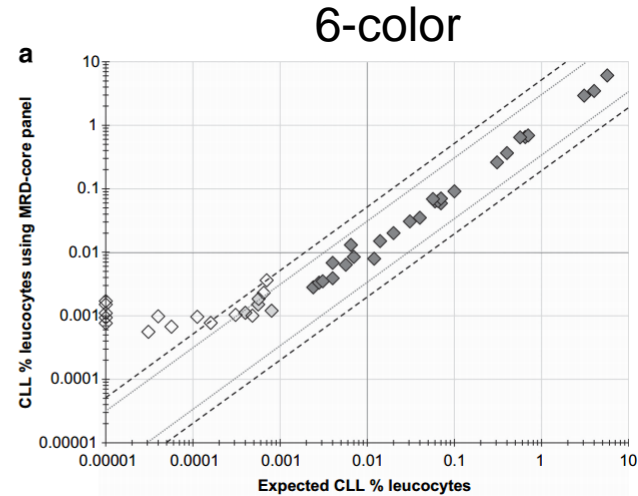
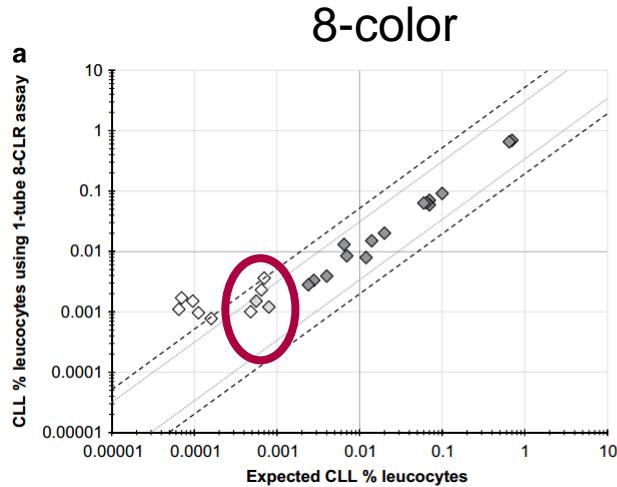


## OS



# HOW TO ACHIEVE $10^{-5}$ BY MRD FLOW

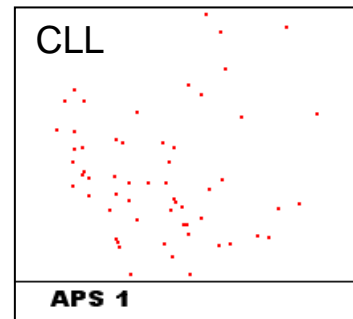
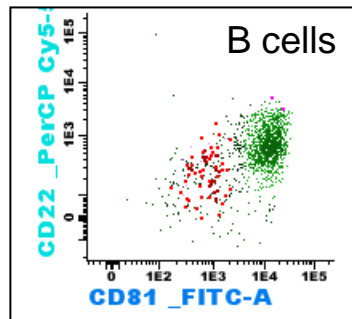
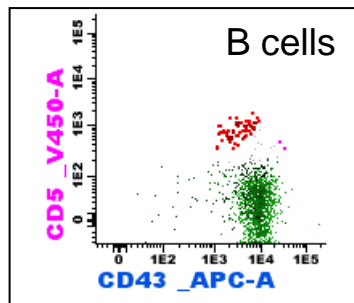
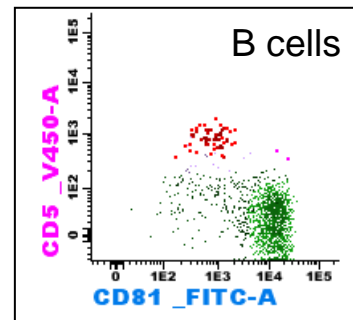
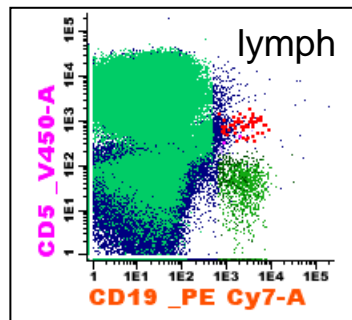
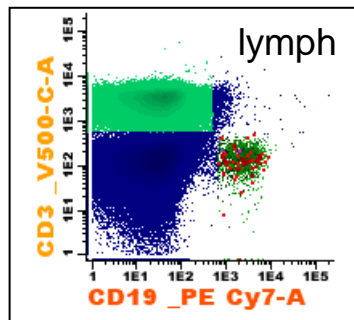
# ERIC 8-color and 6-color core panel MRD



V450	V500-C	FITC	PE	PerCP Cy5.5	PE Cy7	APC	APC H7
CD5	CD3	CD81	CD79b	CD22	CD19	CD43	CD20

# 8 color MRD flow according to ERIC : BM example

- CLL cells shown in red -



MRD negative by iwCLL: 58 CLL / 4606278 leukocytes = 0.0013 % =  $1.3 \times 10^{-5}$

# FDA 2020

## 1. *Cellular Technology Platforms*

Sponsors should consider the following when using cellular technology platforms for MRD assessments in clinical trials:

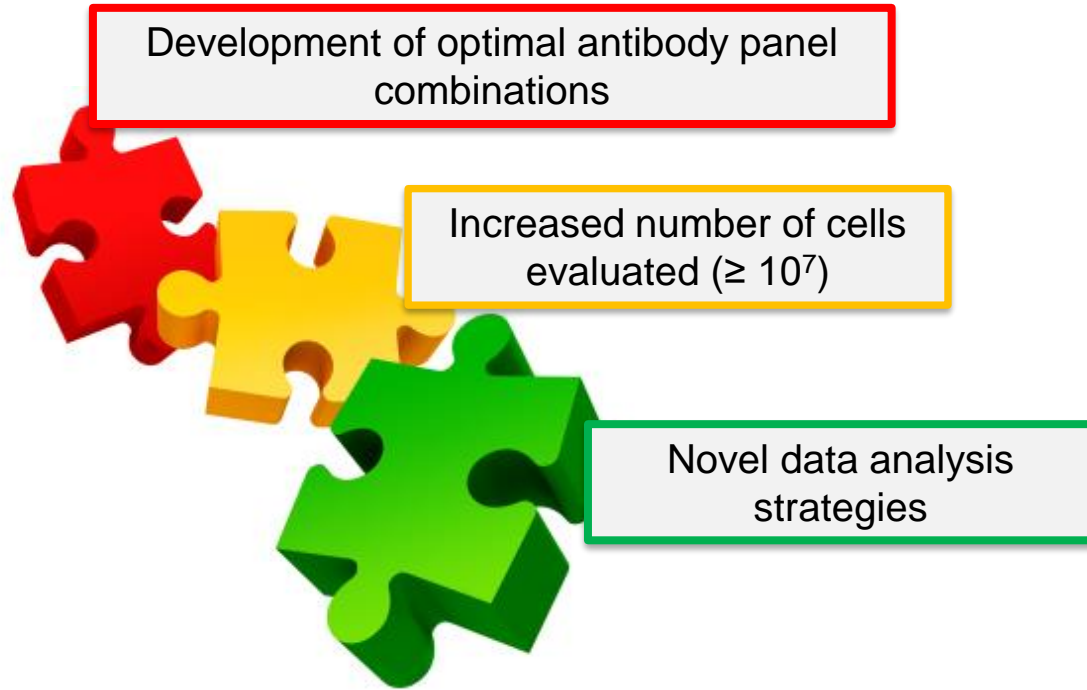
- Prespecify the total number of events to be collected
- Use a consistent panel of antibodies and fluorochromes, as no single antigen is specific for any neoplasm
- Consider sample stability, which may limit the utility of flow cytometry
- Use a consistent analysis template (e.g., gating strategy)
- Determine whether the therapy affects the detectability of the specific antigens targeted by the antibody panels of the flow cytometry assay
- Evaluate the potential for the flow assay to detect normal BM cells that are regenerating after chemotherapy to reduce the likelihood that those cells are misinterpreted as abnormal cells

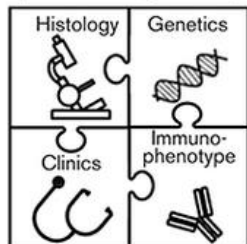


# Aim for EuroFlow MRD Panel

- LOD  $10^{-5}$
- Operator-independent
- Insensitive to treatment

# Standardized EuroFlow-IMF NGF-MRD MM Approach

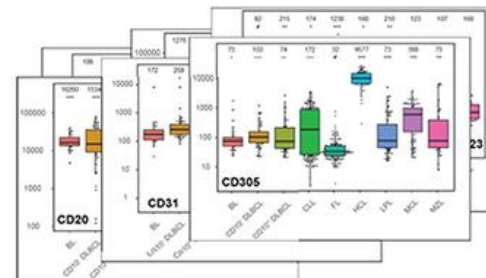


**1****662 patients  
(WHO diagnosis)**

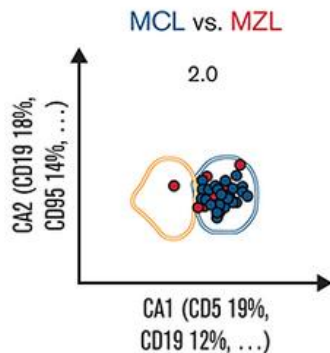
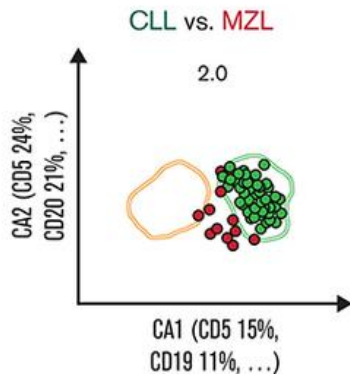
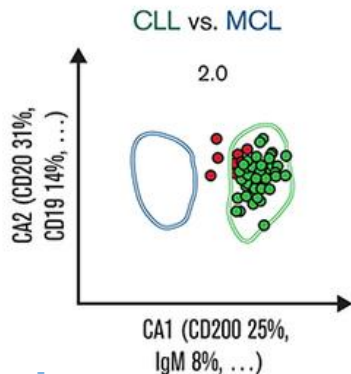
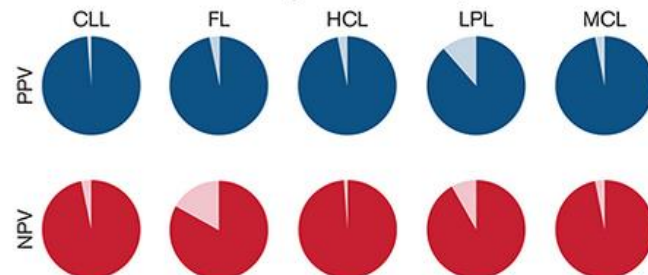
Representative of the  
9 most frequent mature  
B-cell neoplasms

**2**

**Standardized flow cytometry  
@ 9 different centers**

**3****662 patients, 662 samples**

**26 flow cytometric parameters per patient**

**4****176 patients (training set)****5****486 patients (validation set using  
1 as gold standard)**

Lesser unequivocal PPV/NPV in BL, DLBCL, MZL,  
but limited differential diagnoses



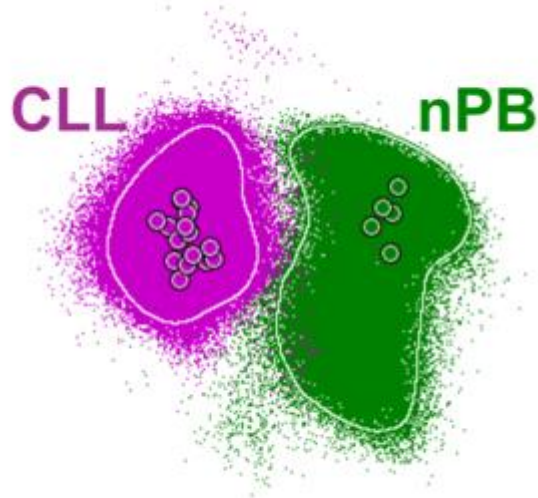
# EuroFlow B-CLPD panel: tool for rational MRD marker identification

	Pac Blue	Pac Orange	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7
<b>1=LST</b>	CD20 / CD4	CD45	λ/CD8	κ/CD56	CD5	CD19 / TCRγ/δ	CD3	CD38
<b>2</b>	CD20	CD45	CD23	CD10	CD79b	CD19	CD200	CD43
<b>3</b>	CD20	CD45	CD31	LAIR	CD11c	CD19	IgM	CD81
<b>4</b>	CD20	CD45	CD103	CD95	CD22	CD19	CXCR5	CD49d
<b>5</b>	CD20	CD45	CD62L	CD39	HLA-DR	CD19	CD27	

- 102 CLL, 26 PB, 9 BM samples -

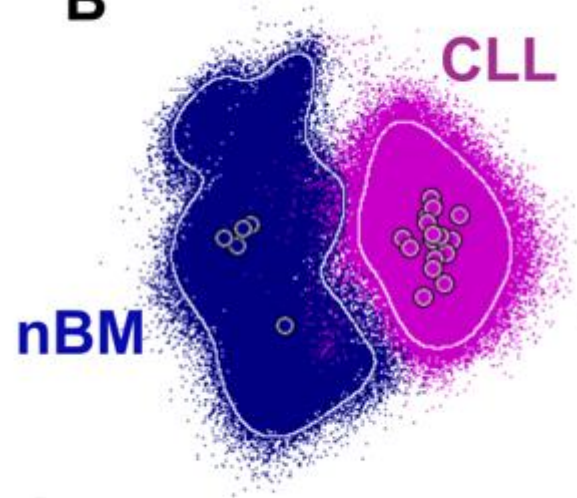
# Rational panel design aiming @ lower FP rates

**A**



**APS 1**

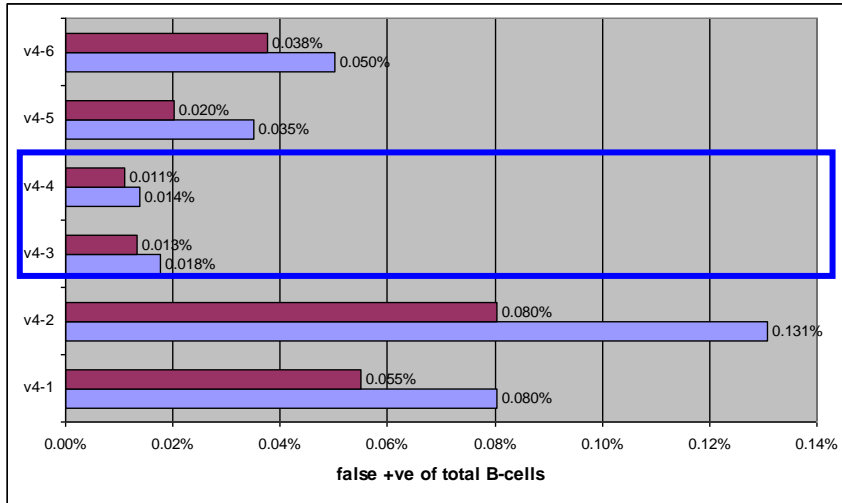
**B**



**APS 1**

# Rate of false positive events

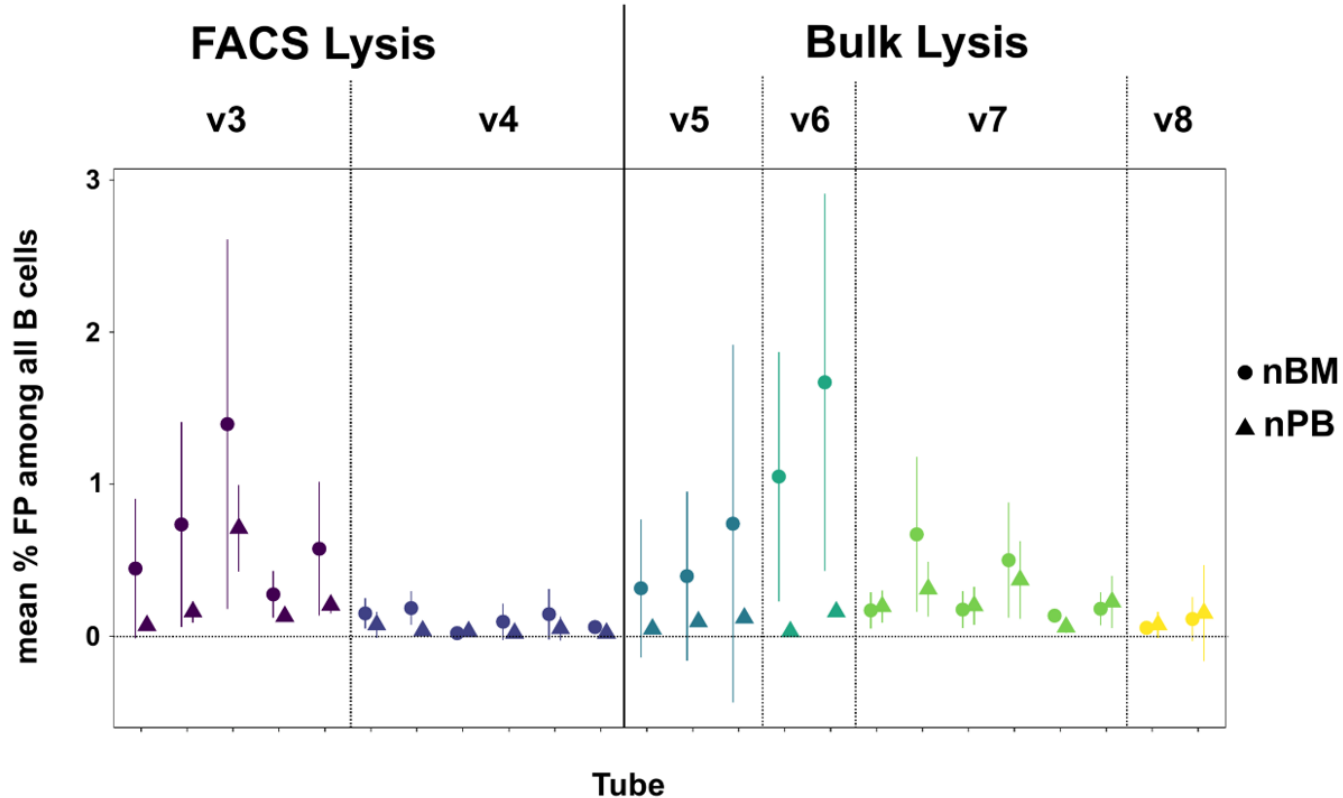
- uncalculated files from panel version 4 -



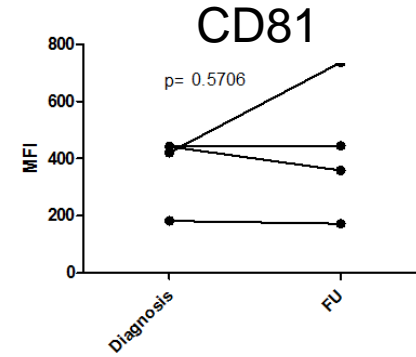
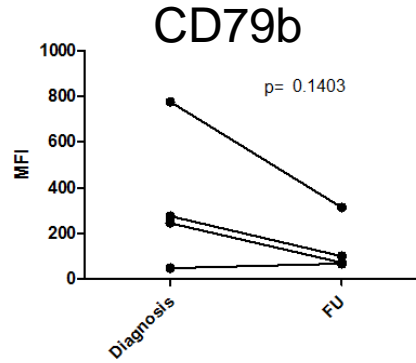
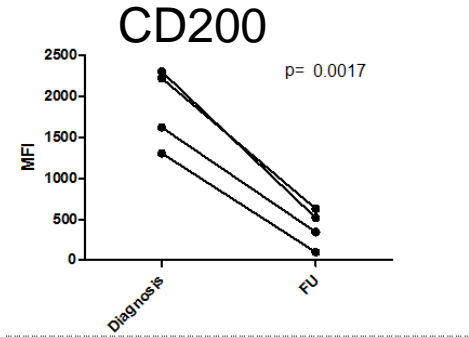
v4 - 6	CD20	CD200	ROR1
v4 - 5	CD20	CD22	ROR1
v4 - 4	CD20	CD22	CD200
v4 - 3	CD3	CD22	CD200
v4 - 2	CD20	CD200	CD22
v4 - 1	CD3	CD200	CD22

- false positivity rate  $\sim 2 \times 10^{-6}$  of total leukocytes

# Evolution of FP rates panel versions 3 to 8

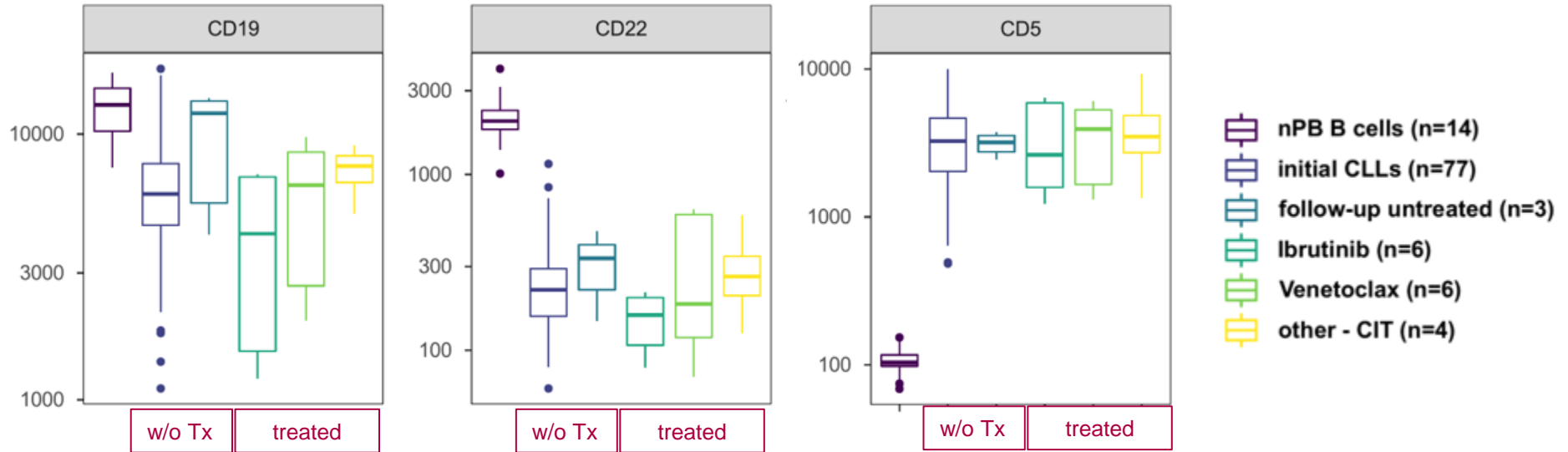


# Ibrutinib down-modulates CD200 expression



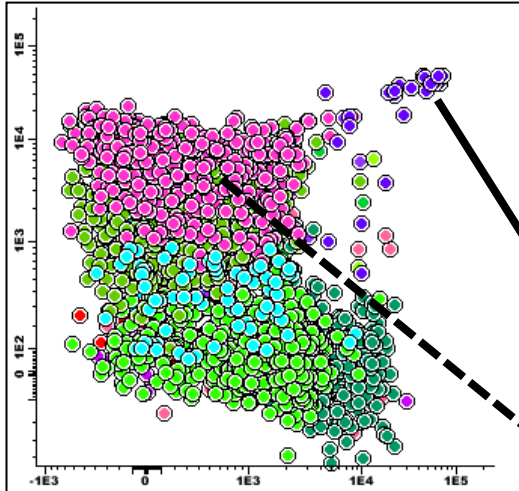


# Version 8 markers are stable regardless of tx



# Analysis strategy I

Assign B-cell clusters to NPB subpopulations using CCA



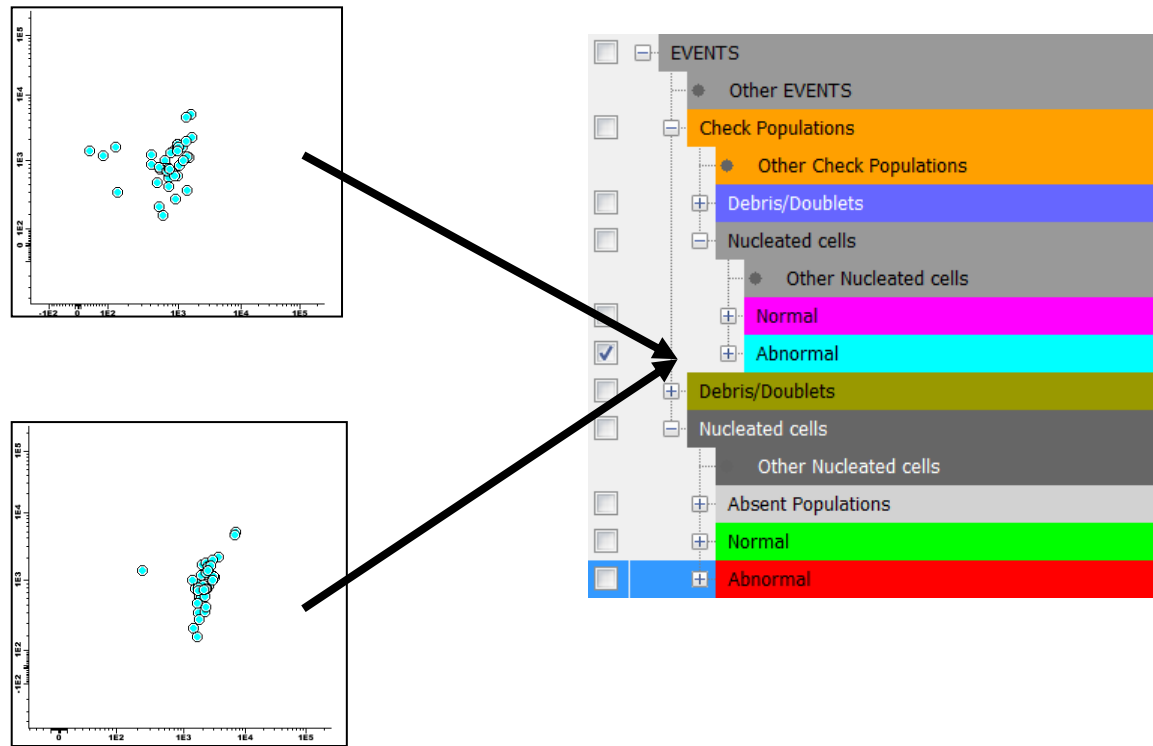
- EVENTS
  - Other EVENTS
  - Check Populations
    - Other Check Populations
    - Debris/Doublets
    - Nucleated cells
      - Other Nucleated cells
      - Normal
      - Abnormal
    - Debris/Doublets
    - Nucleated cells
      - Other Nucleated cells
  - Absent Populations
    - Normal
      - Other Normal
      - B cells
        - Other B cells
        - Immature and naive B cells
          - Other Immature and naive B cells
          - Immature/naive CD5+ B cells
          - Immature/naive CD5- B cells
          - Memory IgMD+ CD27+ B cells
            - Other Memory IgMD+ CD27+ B cells
            - clusters
            - Memory IgMD- CD27+ B cells
            - Memory IgMD-CD27- B cells
            - Plasma cells IgMD+/-



responsible: AW Langerak, S Böttcher

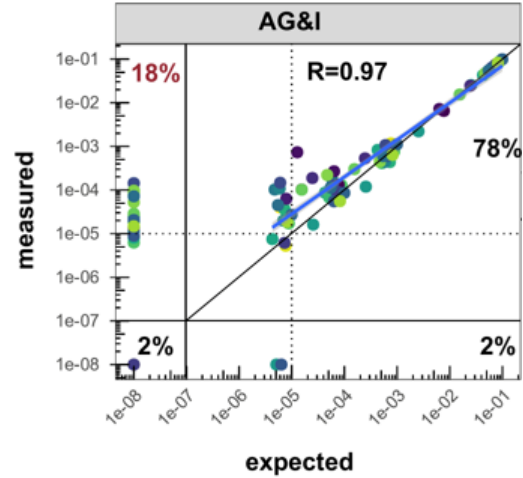
# Analysis strategy I

## Assign B-cell clusters to NPB subpopulations using CCA



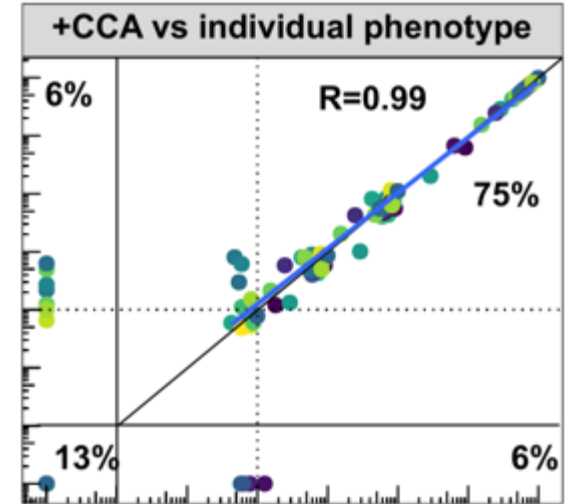
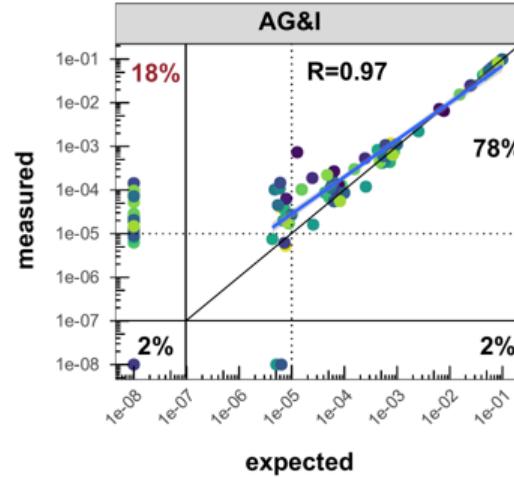
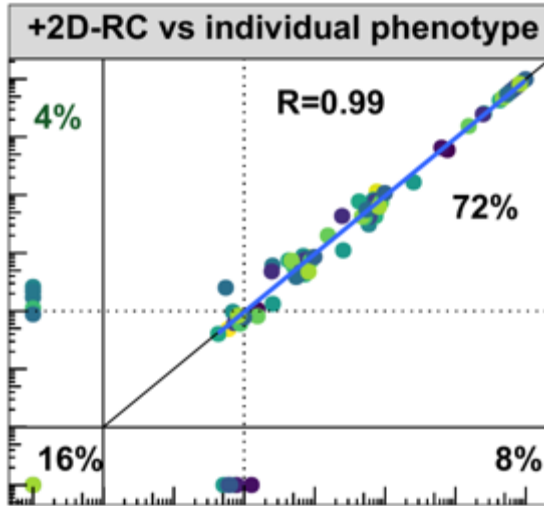
# Analysis strategy I

AG&I is sensitive, but unspecific (tube 1 only shown)



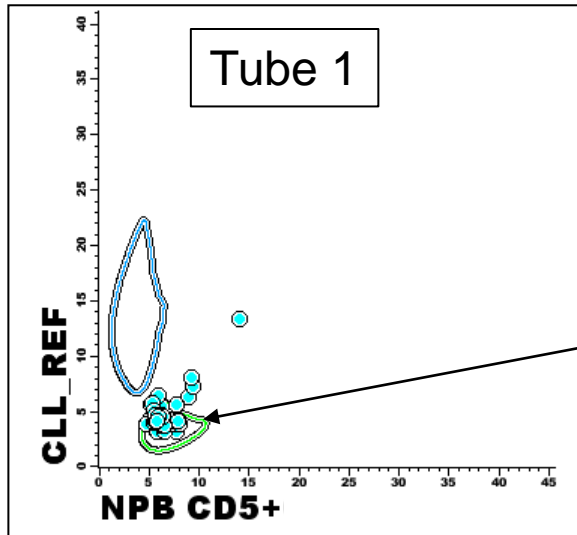
# Analysis strategy I

AG&I is sensitive, but unspecific (tube 1 only shown)

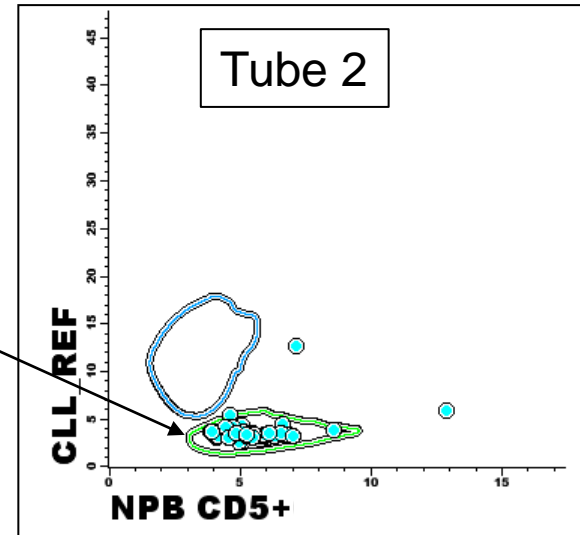


# Analysis strategy II:

RC: initial immunophenotype vs. NPB

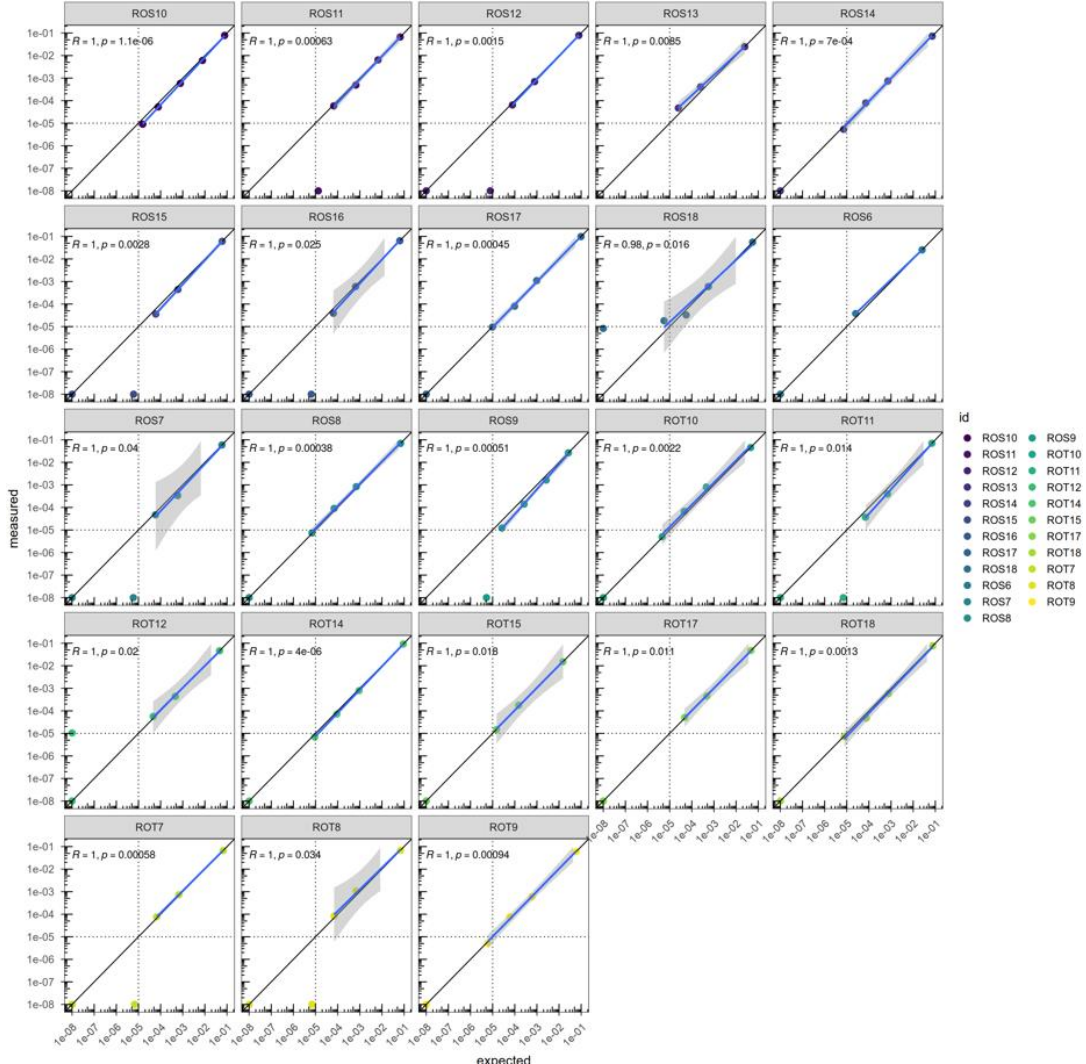


CLL



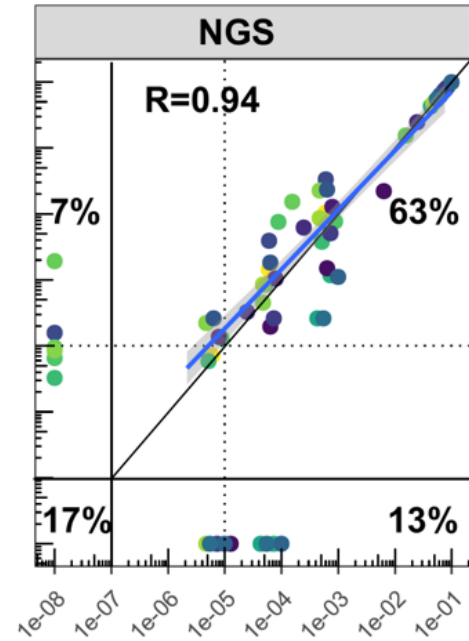
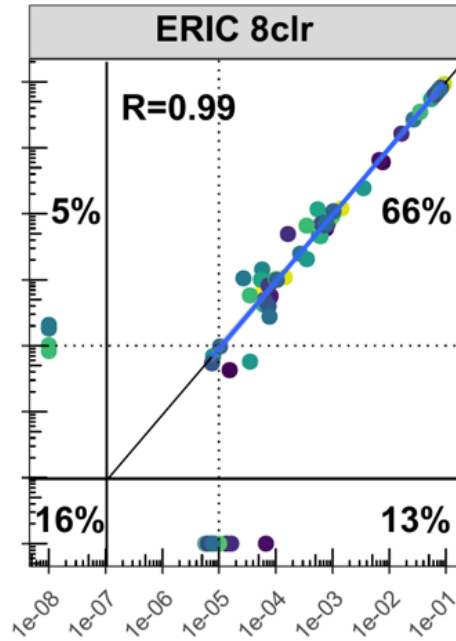
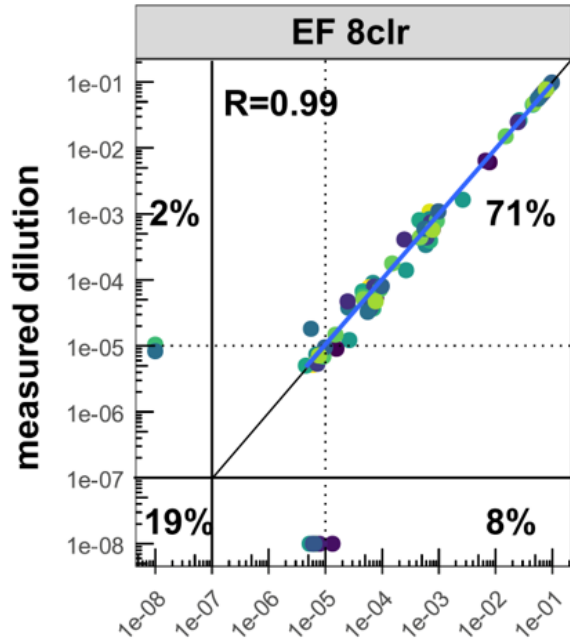
# EuroFlow

- 23 dilutional series, 115 samples -



# EuroFlow, ERIC, NGS vs expected

- 23 dilutional series, 115 samples -



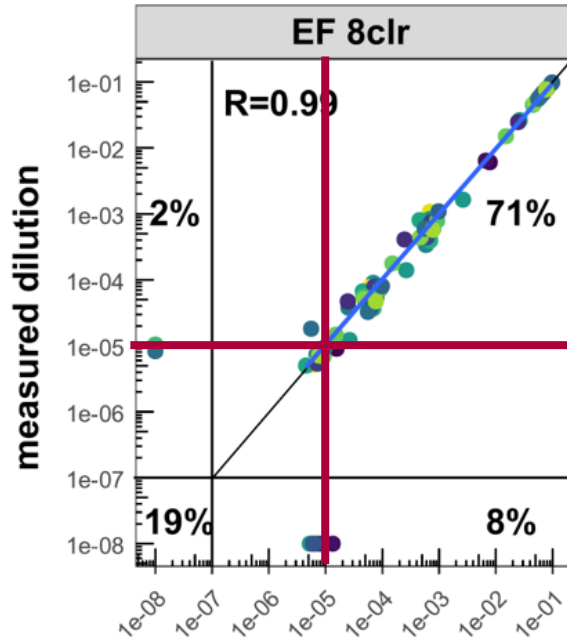
expected dilution



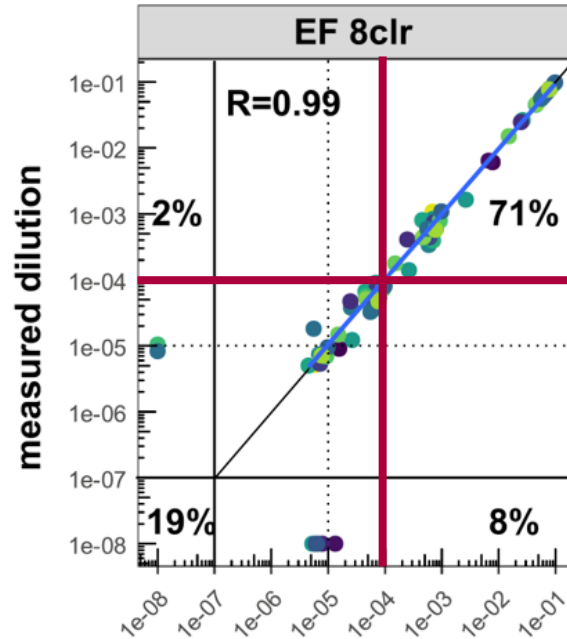


# EuroFlow vs expected – concordance rates

- 23 dilutional series, 115 samples -



98 % concordance @  $10^{-5}$

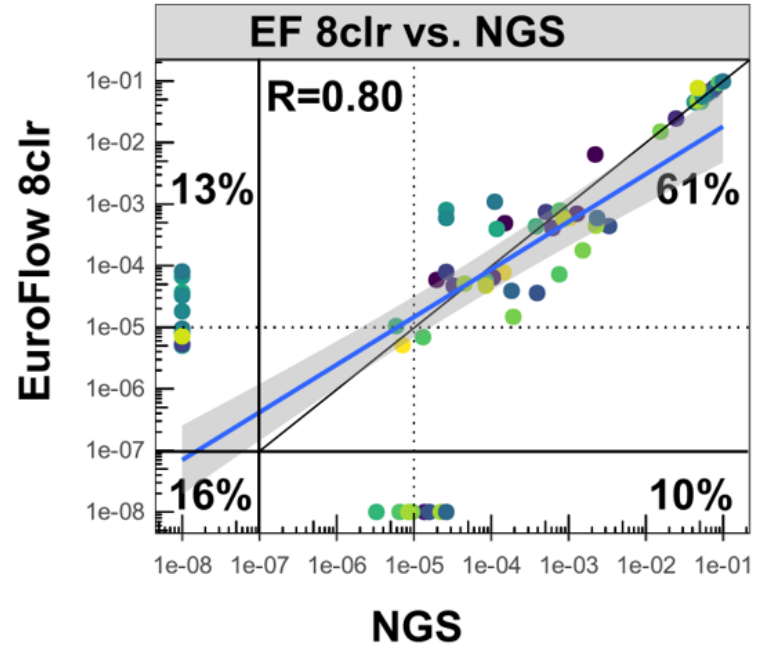
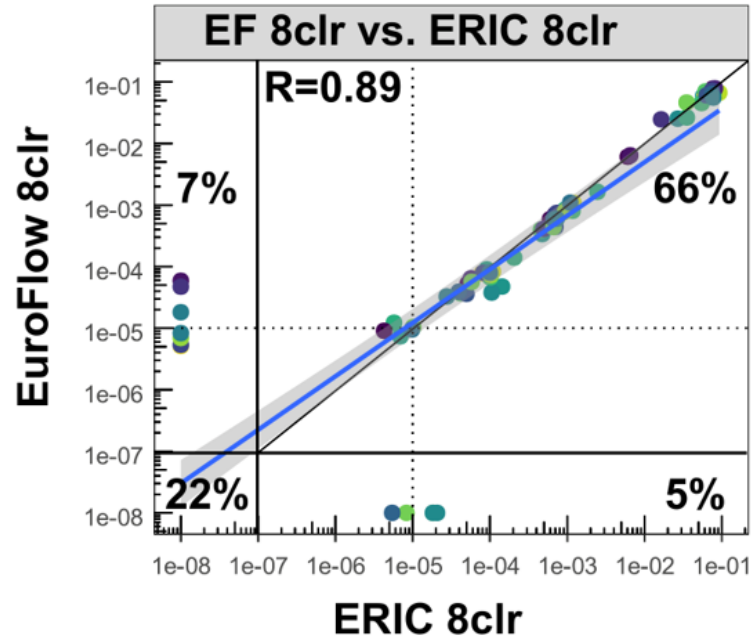


100 % concordance @  $10^{-4}$



# EuroFlow vs. ERIC, NGS

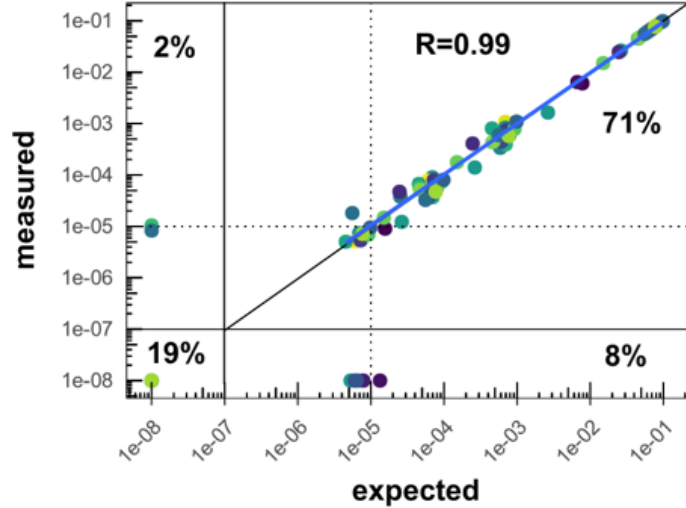
- 23 dilutional series, 115 samples -



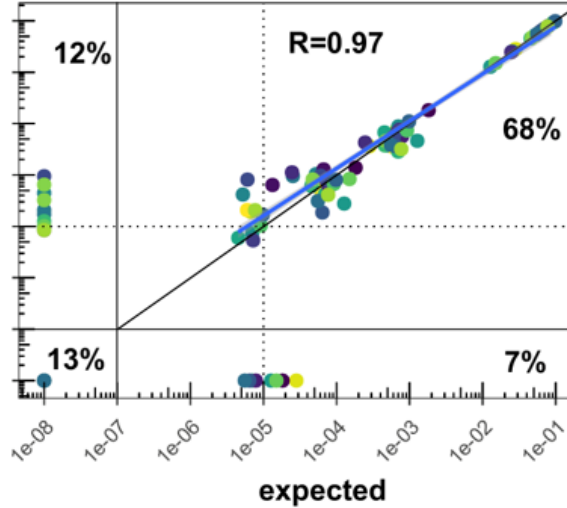
# EuroFlow generic vs. expected

- 23 dilutional series, 115 samples -

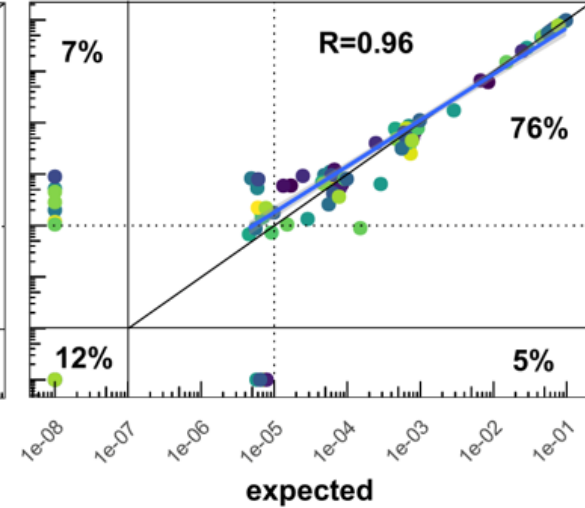
AG&I + 2D-RC  
CLL-like normal vs  
individual phenotype



AG&I + 2D-RC  
CLL-like normal vs  
75% typical CLLs



AG&I + CCA  
CLL-like normal vs  
75% typical CLLs



# Summary

- MRD: surrogate endpoint for PFS in randomized clinical trials with definite treatment duration
- MRD is increasingly being used to tailor treatment for individual patients
- MRD sensitivity beyond  $10^{-4}$  might further refine prognosis in CLL
- Fully automated, operator independent MRD flow assessments with  $10^{-5}$  sensitivity are feasible, provided the initial immunophenotype of the CLL patient is known

# Thank you for your attention.



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