

Flow cytometric MRD in AML: Emerging Approaches

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Disclosures

Name of Company	Research support	Consultant	Speaker's Bureau	Scientific Advisory Board
Novartis	No	No	Yes	Yes
Jazz	Yes	No	Yes	No
BMS	Yes	No	No	No
MPAACT	No	No	No	Yes
Cytek	Yes	No	No	No
AstraZeneca	Yes	No	No	No

Clinical Use of Flow AML MRD is established and increasing

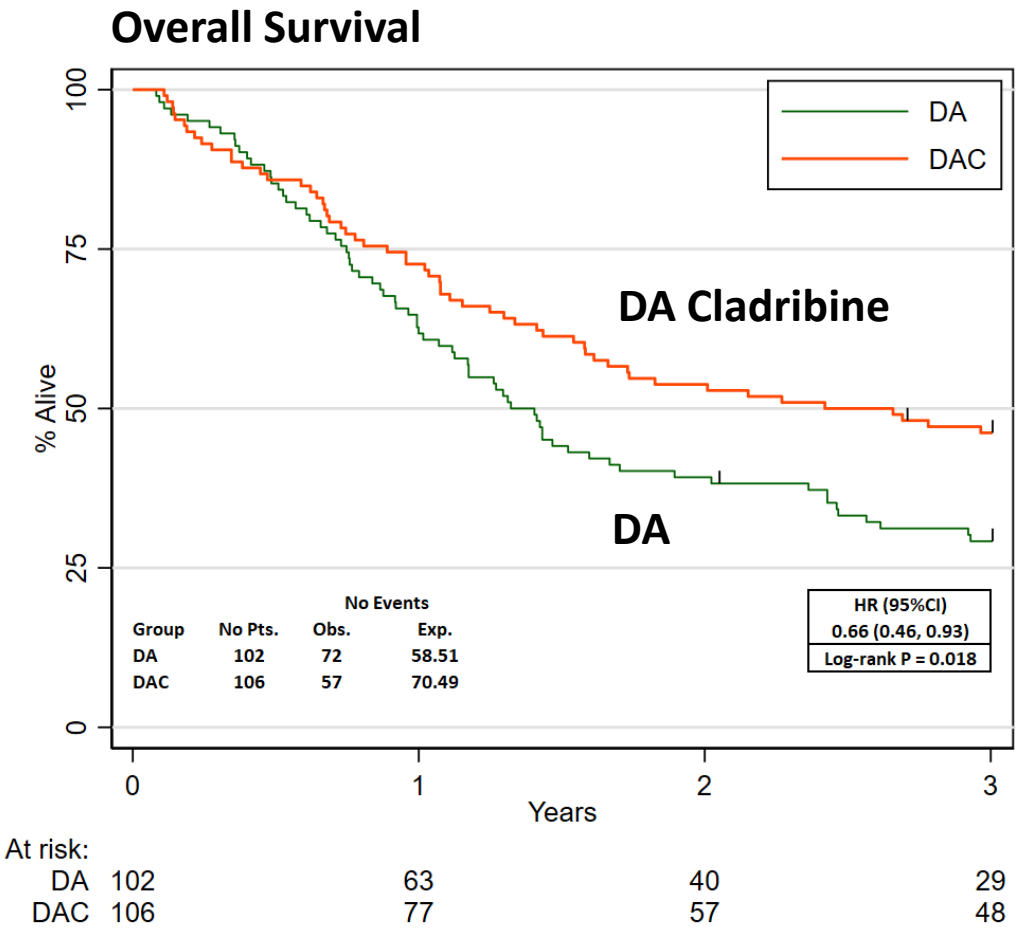
*2026 based on Jan –Sep numbers

Flow MRD guided Intensification can improve Outcomes

AML 18 trial: older adults 60+yrs

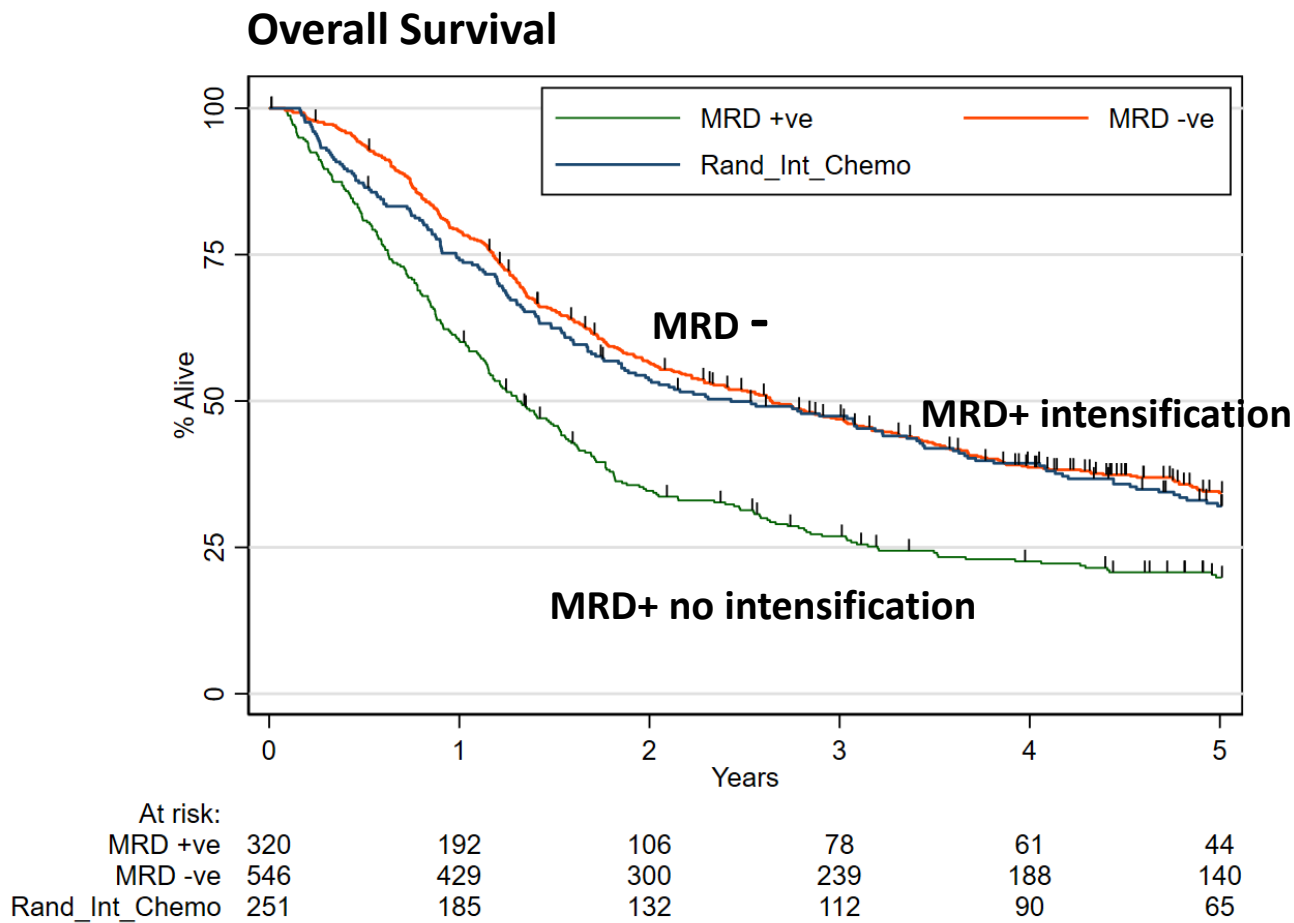
Residual disease by Flow MRD
post course 1

➤ Standard vs intensified chemo



CR MRD+ patients post course 1

- equivalent survival to CR MRD- patients if they had the course 2 intensification



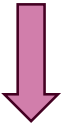
Case 66y M AML with MDS -related mutations – Allografted 7/11/2025 – Flow MRD Monitoring

Total Blast %

DLI



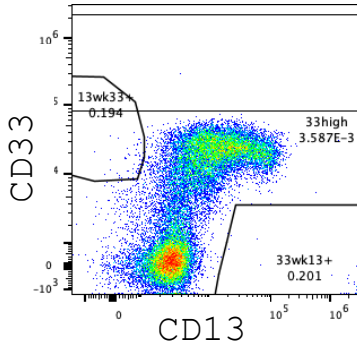
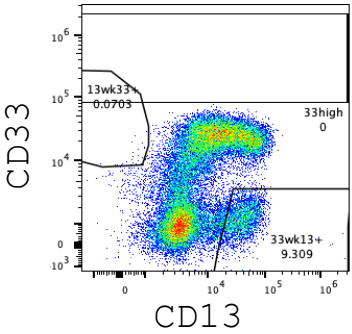
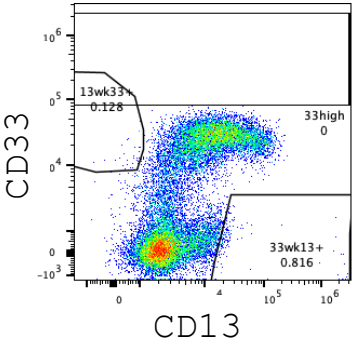
MRD relapse



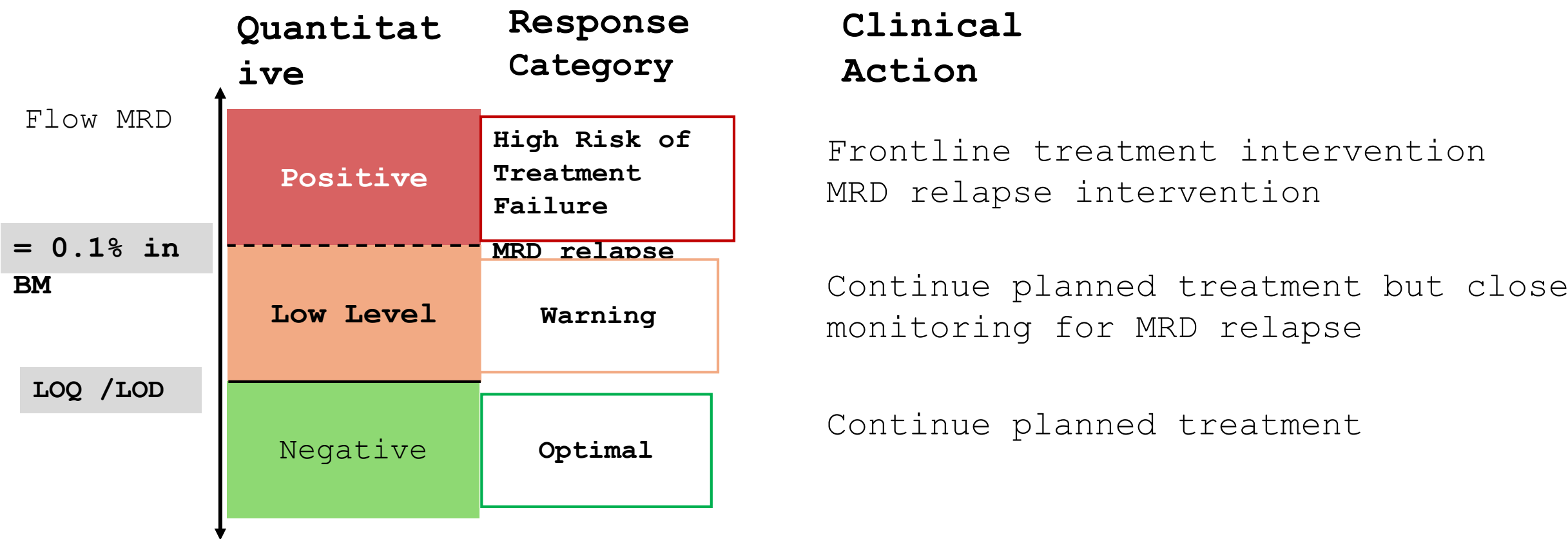
MRD negative



MRD %

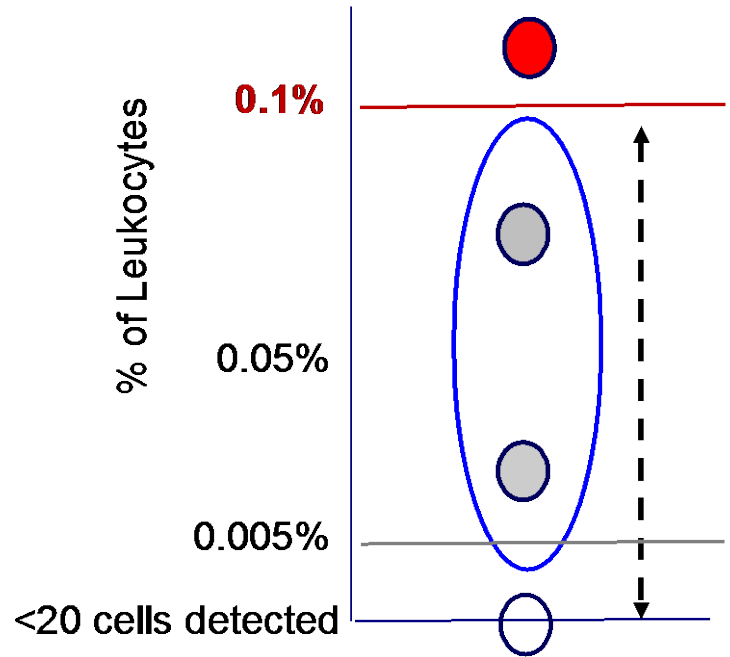


Flow MRD thresholds for clinical action in AML



Reliable Detection of Low positives in Flow AML MRD

% MRD by LAIP/ DfN

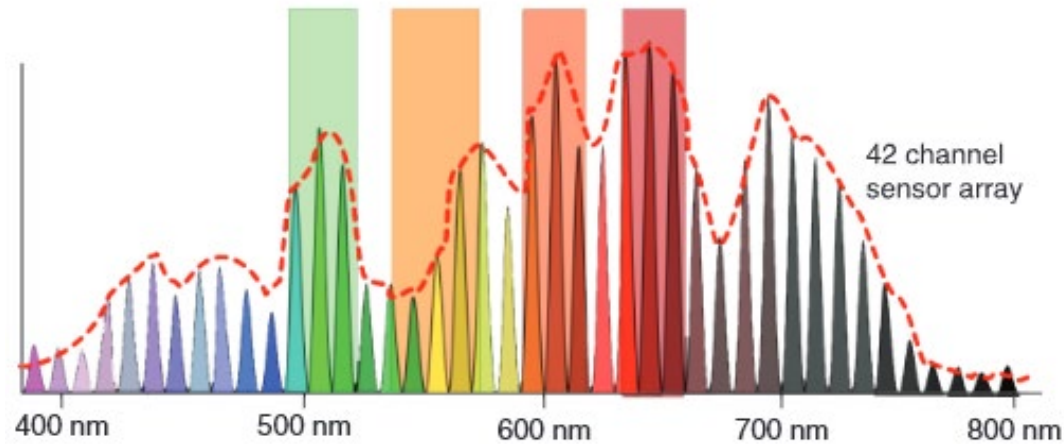


Detection of $<0.1\%$ relevant events in MRD gates depends on:

- Resolution from target background / biological “noise” of non AML cells
- sufficient sample quality

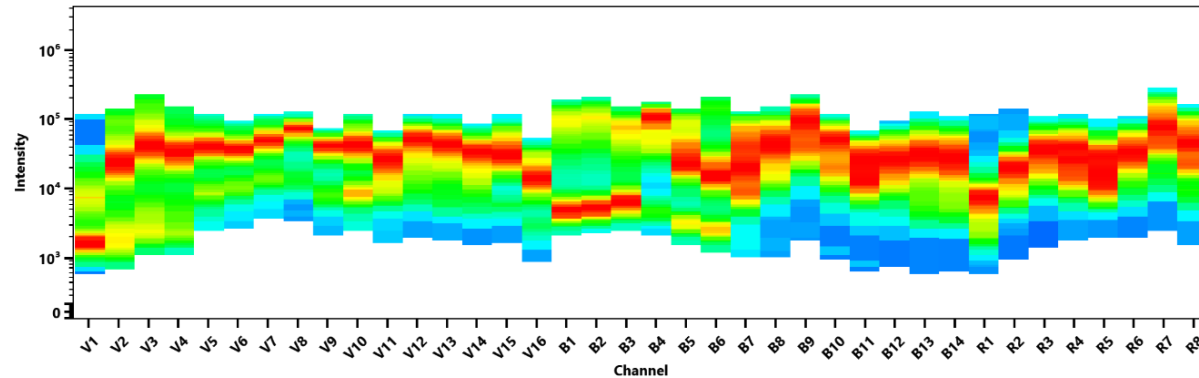
Sample sensitivity of 0.01% if 500,000 leukocytes
(based on 50 events for LOQ)

Spectral Flow Cytometry

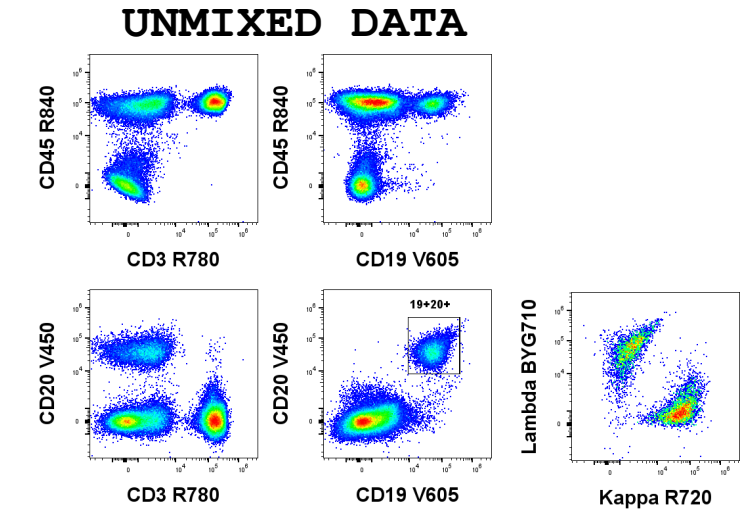


measuring the
entire emission
spectra using
30+ detectors

Multicolour stained sample; **RAW DATA**

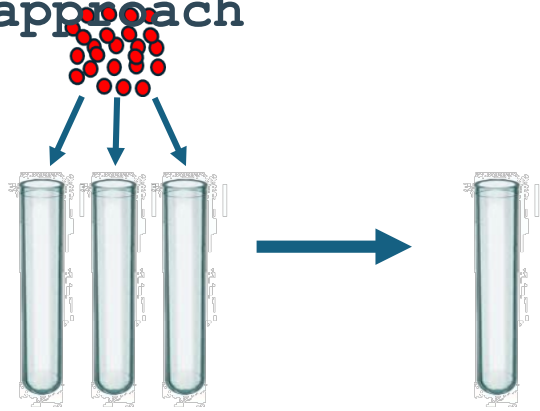


Spectral
unmixing using
single colour
stained controls
➔



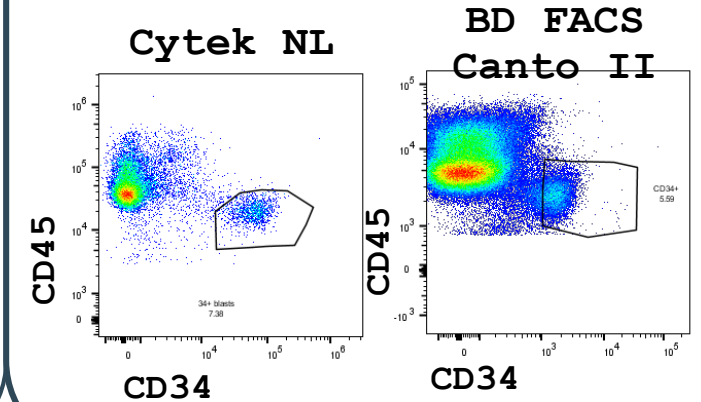
Potential advantages of spectral cytometry for MRD testing

Single tube approach

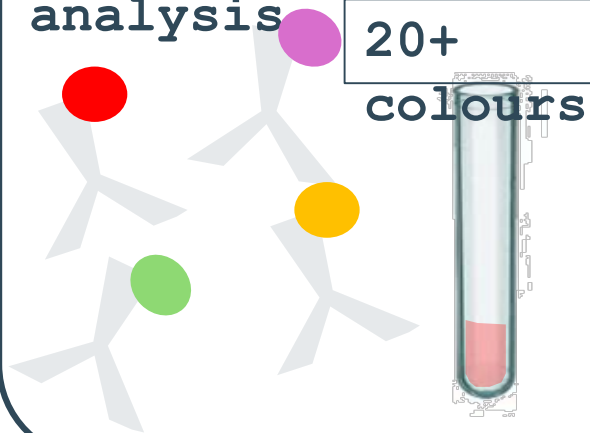


- Higher sample sensitivity
- Processing efficiency for single cell analysis

Data quality



High parameter analysis



- Improved discrimination of aberrant populations
- MRD and investigational marker single cell screen can be integrated

- Autofluorescence extraction
- Viability dye
- Fluorochrome choice

Development of a spectral cytometry tube for MRD testing

NCRI AML-MRD panel (8-colour 3 tube

	Tube 1	Tube 2	Tube 3 (LSC)
FITC	HLA-DR	CD38	CLL1/Tim-3
PE	CD13	CD56	CD123
PerCP	CD34	CD34	CD34
PE-Cy7	CD117	CD117	CD117
APC	CD33	CD33	CD19
APC-H7	CD45	CD45	CD45RA
BV421	CD11b	CD7	CD38
BV510	CD14	CD19	CD45

3 Tubes of 8 Markers = 16 unique markers

15 from NCRI /ELN MRD panel
+ 5 ELN consensus monocytic /LSC¹ + viability dye

= 21 tube



Northern Lights CLC
3 laser clinical cytometer

Spectral 1-tube assay (21-colour)

	1-tube	In 3-tube panel?	Manufacturer
BV421	CD34	Yes	BD
cFluor V450	HLA-DR	Yes	Cytek
cFluor V515	CD38	Yes	Cytek
cFluor V570	CD4	No	Cytek
cFluor V605	CD19	Yes	Cytek
cFluor V670	CD36	No	Cytek
BV711	CD56	Yes	BD
BV786	CD7	Yes	BD
cFluor B532	CD11b	Yes	Cytek
PE	CD133	No	BD
cFluor BYG610	CD14	Yes	Cytek
cFluor BYG667	CD117	Yes	Cytek
BB700	CLL1	Yes	BD
cFluor BYG710	CD123	Yes	Cytek
cFluor BYG750	CD13	Yes	Cytek
cFluor BYG781	CD64	No	Cytek
cFluor R659	CD33	Yes	Cytek
cFluor R720	CD45RA	Yes	Cytek
cFluor R685	CD15	No	Cytek
cFluor R840	CD45	Yes	Cytek
ViaDye Red	Viability	No	Cytek

1. Cloos et al Updated ELN Recommendations, Blood 2026

2. Khan, McCarthy et al ESCCA 2026

Tube design ✓

Ab titrations ✓

Single colour controls ✓

Optimised Unmixing ✓

Frozen Ab premixes ✓

Sensitivity ✓

Accuracy (21c vs 8c legacy) ✓

Precision ✓

Linearity ✓

Stability ✓

Validated for 3 weeks (12 wks by PeterMac, Australia)

3495 tests April 25 to

Periodic update of unmixing controls	Update of unmixing controls (lot number/performance change/laser replacement)
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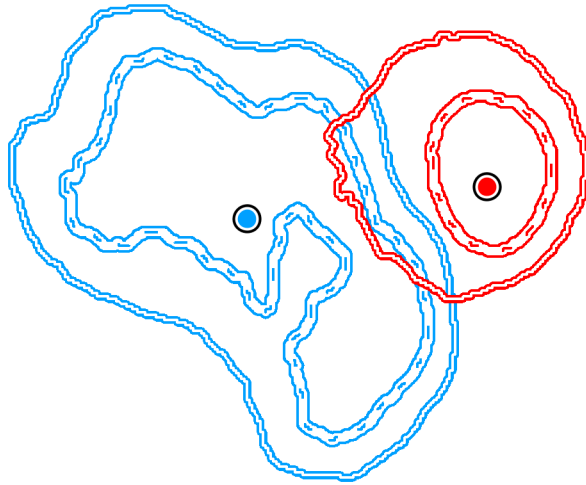
High-dimensional resolution of abnormal populations

AML case with no LAIP by ELN 8c combination

PCA -based analysis (APS) - visualization of separation between control and AML blasts

All markers

Blasts **46.0%** predicted abnormal

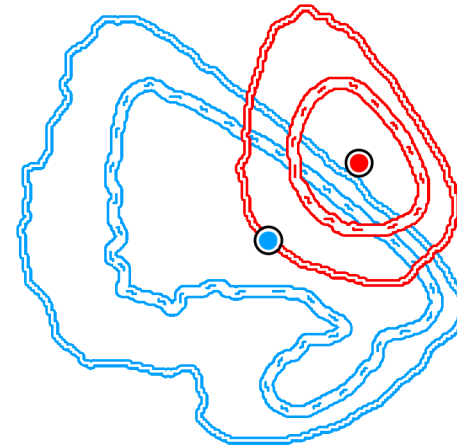


Score = 3

APS 1

ELN 8 marker combination

Blasts 4.5% predicted abnormal



Score = 1

APS 1



Spectral Subgroup

Shared Practice meetings between ELN laboratories who have validated or are validating a spectral assay

(4x meetings May to Sept 2026)

- Aim - technical recommendations with at least one spectral tube that has been validated and applied for routine AML MRD testing across ELN laboratories

Participants

Tobias Maetzig, Adrian Schwarzer *Hannover, Germany*

Michaela Feuring *Ulm, Germany*

Prashant Tembhare *Tata Memorial Centre, Mumbai, India*

Christophe Roumier / Adriana Plesa *Lille, Lyon, France*

Nick McCarthy, Naeem Khan *Birmingham, UK*

Maura Ikoma Colturato *Hospital Amaral Carvalho - Jau - São Paulo - Brazil*

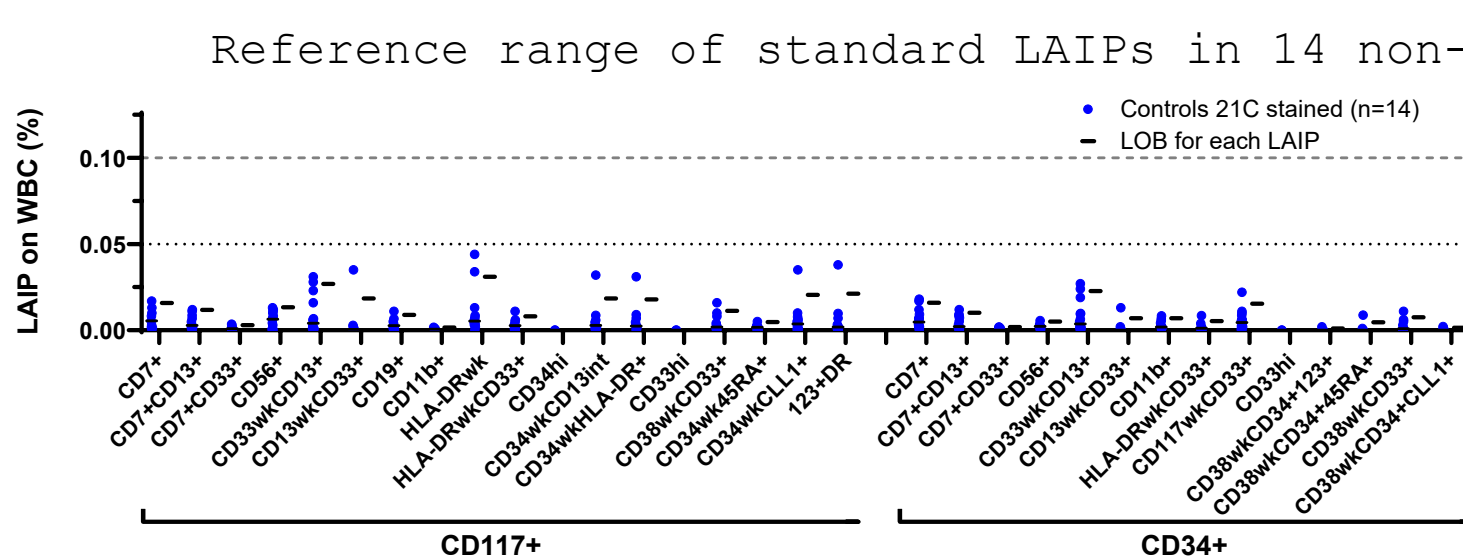
Melissa Nardi, David Westerman *PeterMac, Australia*

Lars Huygen, Costa Bachas *VUMC, Amsterdam*

Veronika Ecker, Manja Jargosch, Angelika Fischbeck, Wolfgang Kern. *MLL, Munich, Germany*

Future aims- evaluation of computational pipelines

Sensitivity in PB



All LAIPs in all controls were under 0.05%

LOB = 0.0103%

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(mean of controls +1.645*SD)
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From 8 triplicate spike-in ex

$$\text{LLOD} = 0.0143\%$$

$$\text{LLOQ} = 0.0238\%$$

Assay threshold set at 0.05

Reference range in 19 control PBs

LOBs of all 33 LAIPs (including LSC) were $<0.005\%$, -
mean **LOB** = 0.0005%

Average of 5 events per gate (range 0-15)

- 20 events as the LOD threshold for detectable MRD in PB

Flow MRD Detection in Peripheral Blood

Potential for less invasive serial monitoring for patients - routine for PCR

- More frequent tests possible - may extend treatment intervention window from diagnosis of MRD relapse
- Avoids the pitfall of heterogeneous BM sampling and extramedullary disease

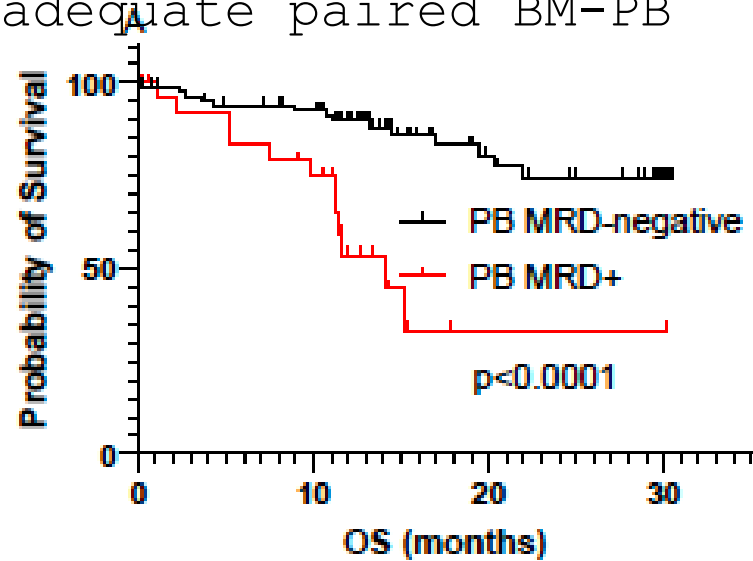
Challenges

- False negatives limiting factor for PB MRD max ~ 80% concordance with 8 colour BM MRD+ ^{1,2,3,4}
 - However a much "cleaner" assay - allowing detection below 0.01% ^{5,6}
- Most published data from testing at remission / post consolidation

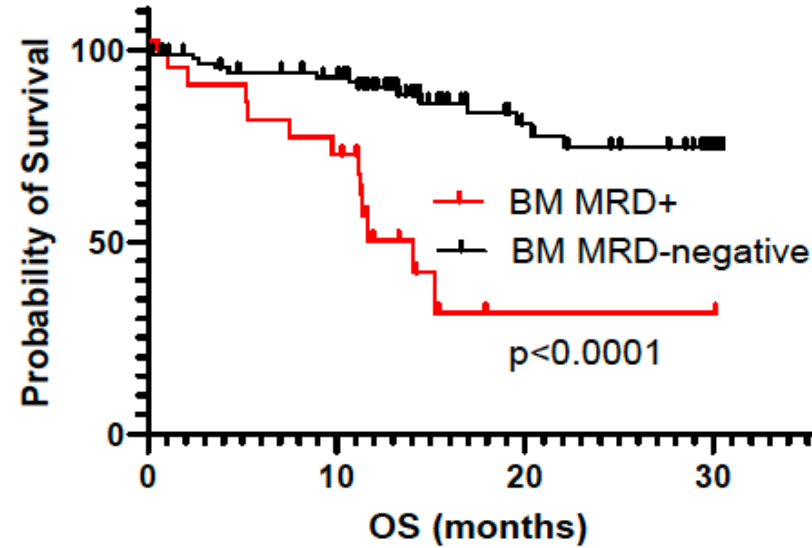
Flow MRD Detection in Peripheral Blood with 12 colour assay

Results from a 12c MRD assay - 118 paired BM -PB (post induction /consolidation =76)

109adequate paired BM-PB



98% concordance



However only 24 with BM MRD+. need to extend evaluation of false negative rate

PB AML MRD monitoring: Feasibility with spectral 21c tube

139 paired PB and BM MRD samples from 80 patients - 4-5mls PB

- recruited mainly from patients with ↑ probability of MRD+ or poorer quality samples
- 58% on lower-intensity frontline or salvage therapy (venetoclax or menin inhibitor or maintenance)

- 45% adverse genetics, median age 60 yrs

* sample LOD: BM $\geq 0.05\%$ (min 5 events)

	PB -	PB +	inadequate*
BM -			4
BM +			8
BM inadequate*	5	2	11

PB $> 0.01\%$ (min 20 events)

Of 139 consecutive paired samples:
 8% inadequate in both BM and PB
 9% inadequate in PB only
 5% inadequate in BM only

Median patient WBC

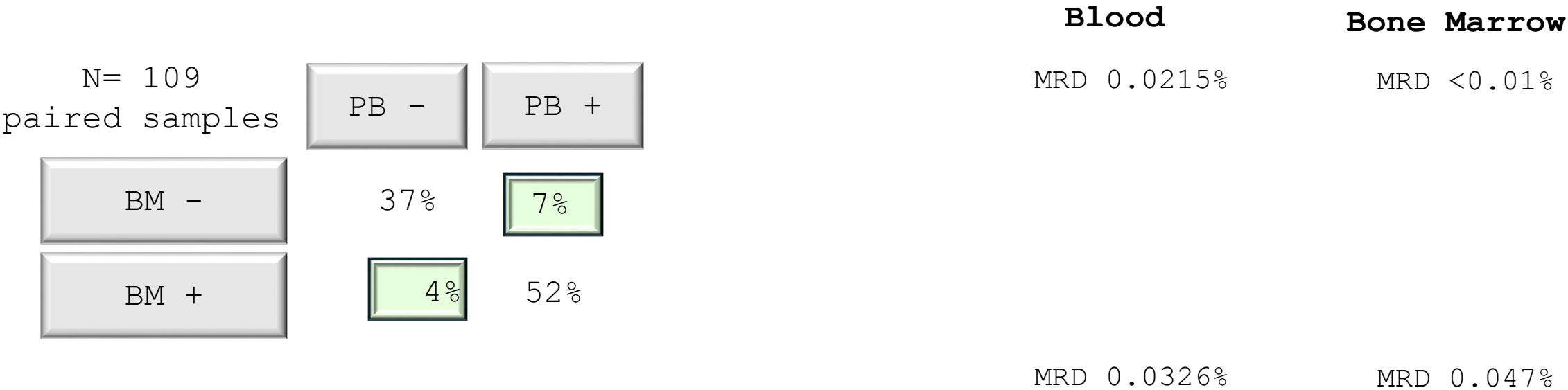
inadequate PB = $0.76 \times 10^9/L$

adequate PB = $3.10 \times 10^9/L$

PB vs BM AML MRD with spectral 21c tube

109 samples with both BM and PB adequate (includes 61 BM MRD+)

- 89% concordance
- Discrepant results: in 8 of 12 - MRD detectable in PB but below assay threshold of 0.05% in BM



PB vs BM AML MRD with spectral 21c tube

67 with detectable MRD in BM and PB (includes 17 relapses by flow)

BM and/or PB MRD+
% of leukocytes

MRD % CD45 median difference
-0.462 $\log_{10}$ (IQR, -0.12 to -
1.07)
(PCR 0.5-1 $\log_{10}$ for CBF and *NPM1m*)¹

PB vs BM AML MRD with spectral 21c tube

67 with detectable MRD in BM and PB (includes 17 relapse by flow)

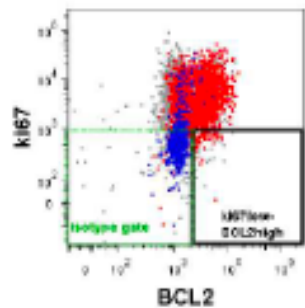
BM and/or PB MRD+BM and/or PB MRD
% of leukocytes % of blasts

MRD % of blasts median
difference
0.071 $\log_{10}$ (IQR, -0.07 to
0.22)

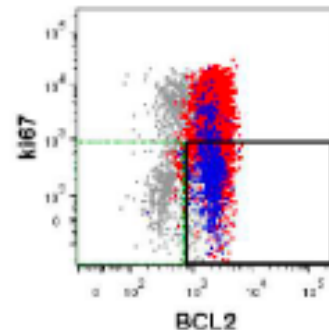
Can Flow cytometric MRD in AML be simplified

Flow MRD target identification and gating can be challenging

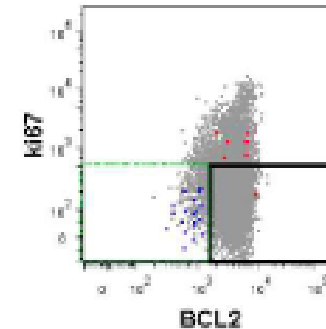
- disease heterogeneity matched by immunophenotypic heterogeneity
- diagnostic target instability due to leukemia evolution - requires surveillance of >30 DfN aberrancies
- Potentially, incorporating markers linked to functional resistance and treatment evasion into a flow cytometric assay might reveal more universal. asynchronous blast. phenotypes



Control BM:
CD34⁺CD38^{low}
CD34⁺CD38^{high}
CD34^{low}



CD34⁺ AML at diagnosis:
CD34⁺CD38^{low}
CD34⁺CD38^{high}
CD34⁻



CD34⁻ AML at diagnosis:
minor CD34⁺CD38^{low}
minor CD34⁺CD38^{high}
CD34⁻

Functional Marker MRD Assay

11 colour panel (surface then fix/ perm staining)

	Fluorochrome
Bcl-2	PE
Ki-67	Alexa Fluor 488
CD45	Brilliant Violet 510
CD34	Brilliant Violet 421
CD117	PE/Cy5
CD38	Fire 810
HLA-DR	RB780
CD36 BV650	BV650
CD123	BV480
CD64	BV 605
CD4	Spark Blue 550



Northern Lights CLC
3 laser clinical cytometer

Analysis

Blasts by CD34+ and/or CD117+ (wide mononuclear gate)

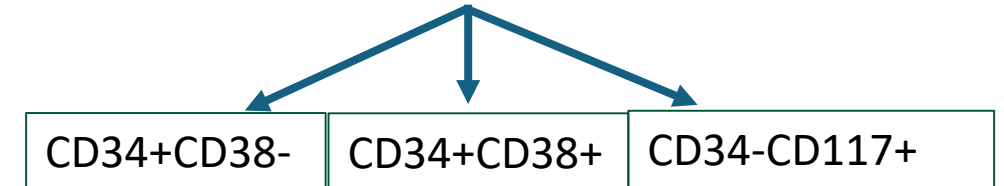


Exclude any non-blast cells

- hematogones and plasma cells (CD38vsSSC)
- basophils and PDC (CD123vsHLADR)
- erythroid and lymphocytes (CD36vsCD45)



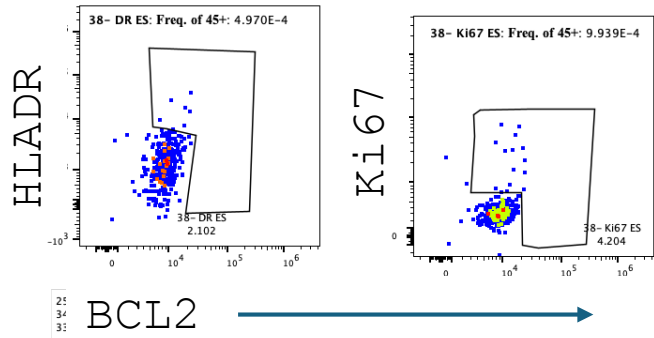
Blast compartment gating



BCL2 vs Ki67 and HLADR Phenotypes in Myeloid Blast compartments

Empty spaces (DfN) gates in Control BM

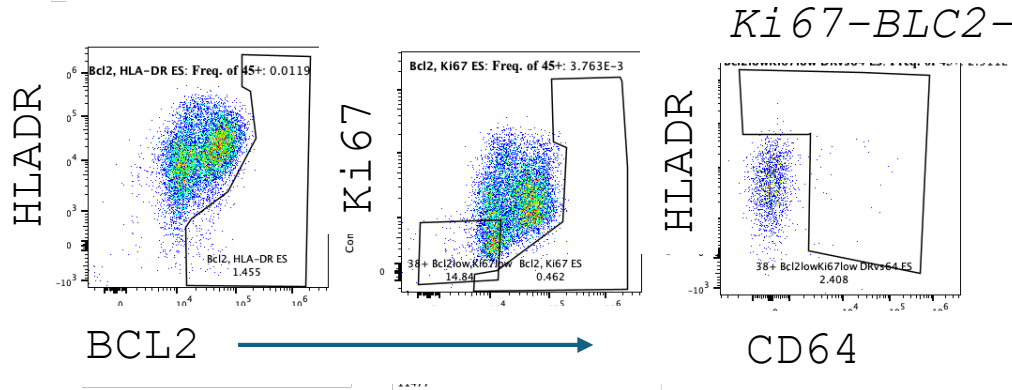
CD34+CD38-



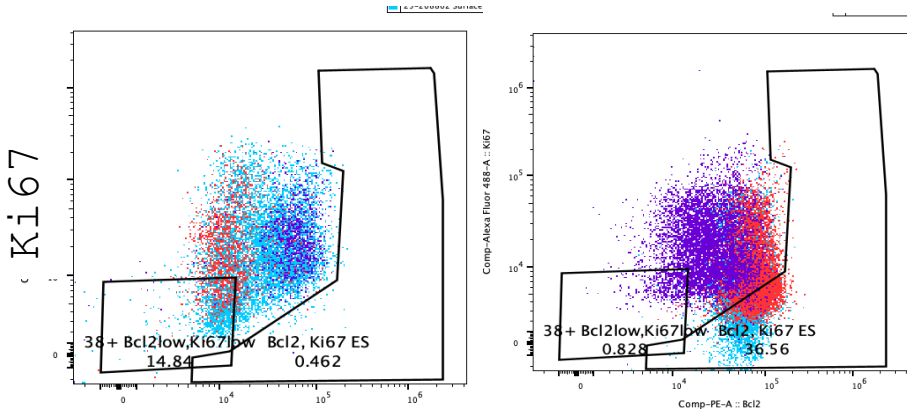
"Normal" Spaces maturation stage dysregulation

CD36+CD64- CD64+

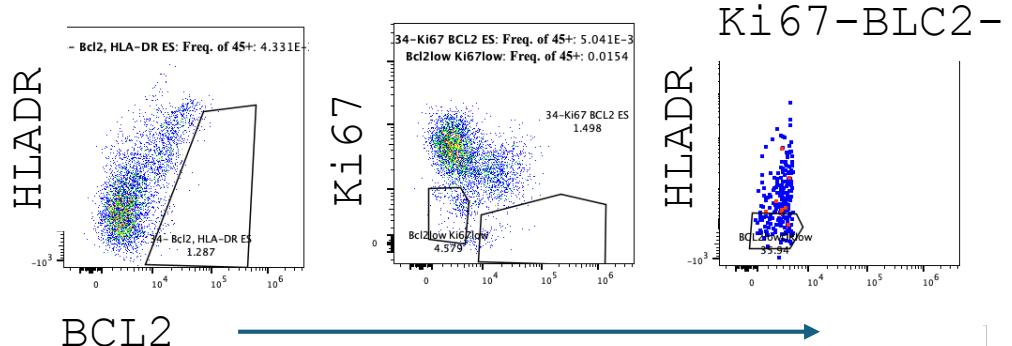
CD34+CD38+



Control AML/MDS from MDS



CD34+CD38-



CD34+CD38-

CD34+CD38+

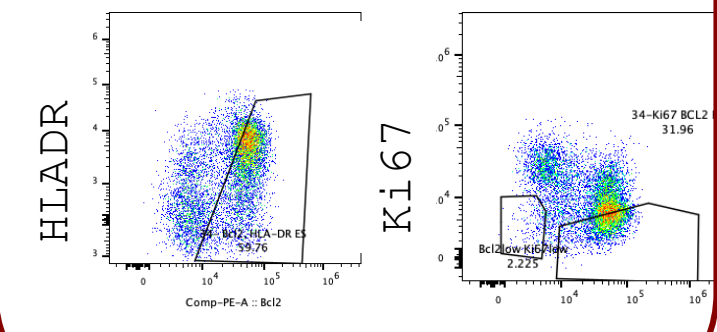
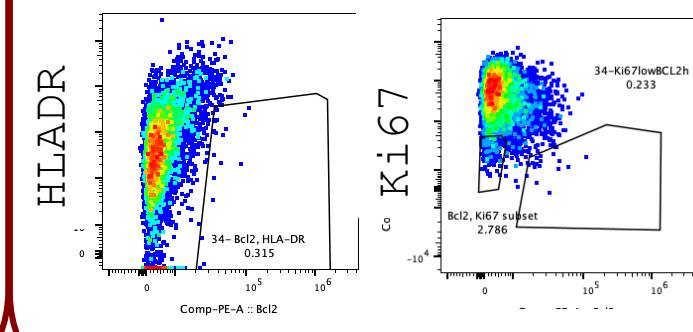
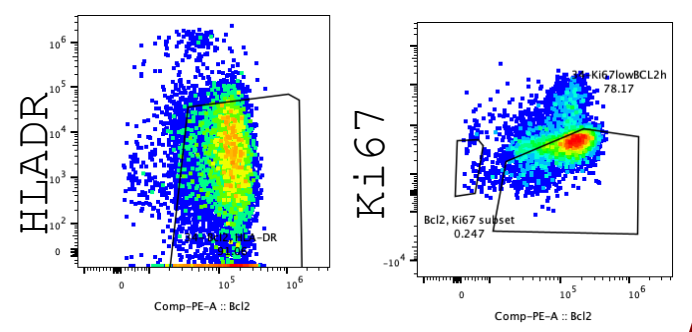
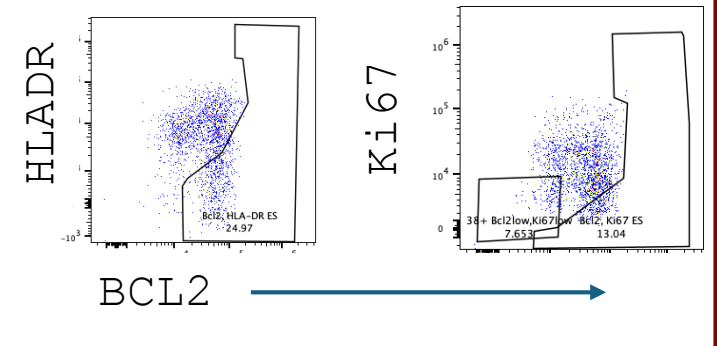
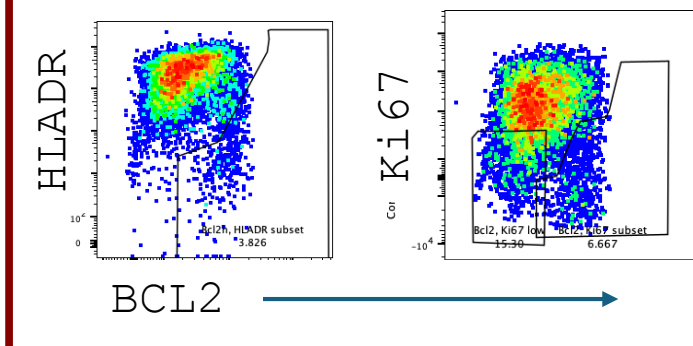
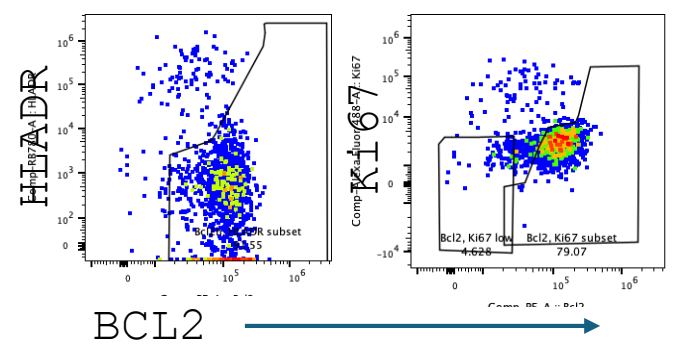
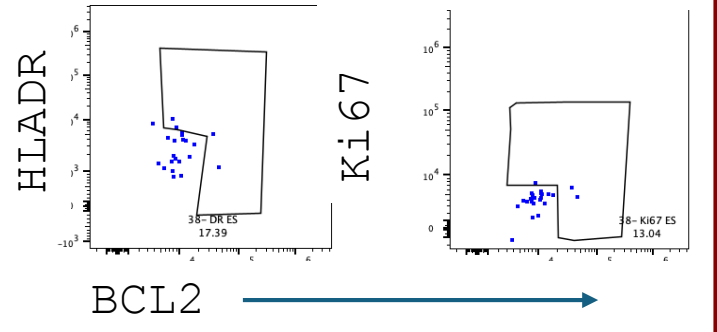
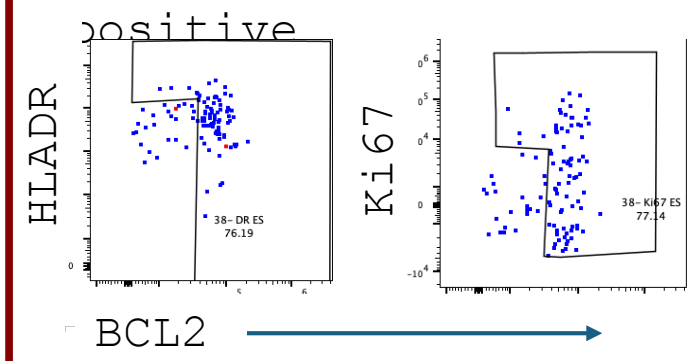
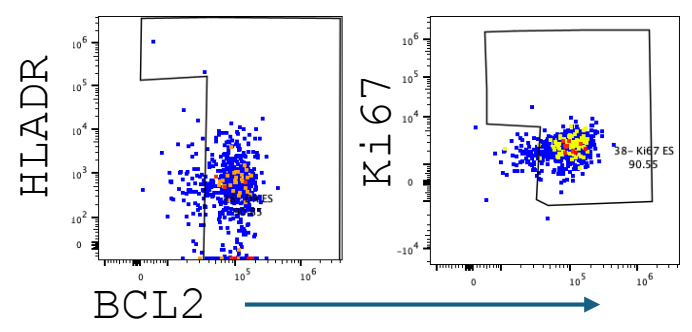
CD34+CD38-

AML FLT3-ITD, MRD relapse

AML MECOMr

MRD low

AML +8, MRD positive



Concordance of Functional marker MRD with standard Flow MRD Assay

Tested on 97 BM samples

n=8 n=4 n=5 n=15 n=4 n=45 n=6 n=10

Perspective on Emerging Approaches in Flow Cytometric AML MRD

Spectral Cytometry progresses assay resolution

- using all markers together allows phenotype of resistant cells to be better defined
 - allows combinatorial immunotherapy target screening
 - Computational tools required to extract most out of data
- Technical progress for transition to blood MRD

Flow MRD PB monitoring results very encouraging

- needs prospective study in non NPM1 / CBF AML post remission patients

Functional Marker Flow MRD has potential to simplify MRD analysis and interpretation

Note Flow, PCR/ddPCR and NGS measure different biological signals

Thank you so much for your attention

UK AML MRD network

Birmingham Flow MRD team

Nick McCarthy

Naeem Khan

Eleanor Hickman

Tatiana Raskovalova (Grenoble)

Guys Molecular MRD team

Richard Dillon

Nikki Potter

UK AML Trials

Nigel Russell

Steve Knapper

Richard Dillon

Charlie Craddock

Cardiff Trials Unit

UK NCRI /AML Research Network

Spectral Flow MRD

Collaborators for tube validation

Brazil Maura Ikoma Colturato

UK Alan Dunlop

ELN Soectral subgroup

France Christophe Roumier, Adriana Plesa,

Computational Flow MRD

Collaborators

ALFA Flow Network Christophe Roumier, Florent Dumezy, Adriana Plesa,

NIH Gege Gui / Chris Hourigan

VUMC Costa Bachas



MRD ASSESSMENT and VALIDATION in AML

Francesco Buccisano

Wolfgang Kern

Brent Wood

Sa Wang

Adriana Plesa & Chrsitophe Roumier

And other ELN-DAVID Flow members from France/ Germany/ Italy /Israel/ UK / Australia /Spain /Belgium / Austria/ Netherlands /Brazil /USA

Jacqueline Cloos

Michael Heuser

