Harmonisation Efforts in AML MRD

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Sylvie Freeman DISCLOSURES OF COMMERCIAL SUPPORT

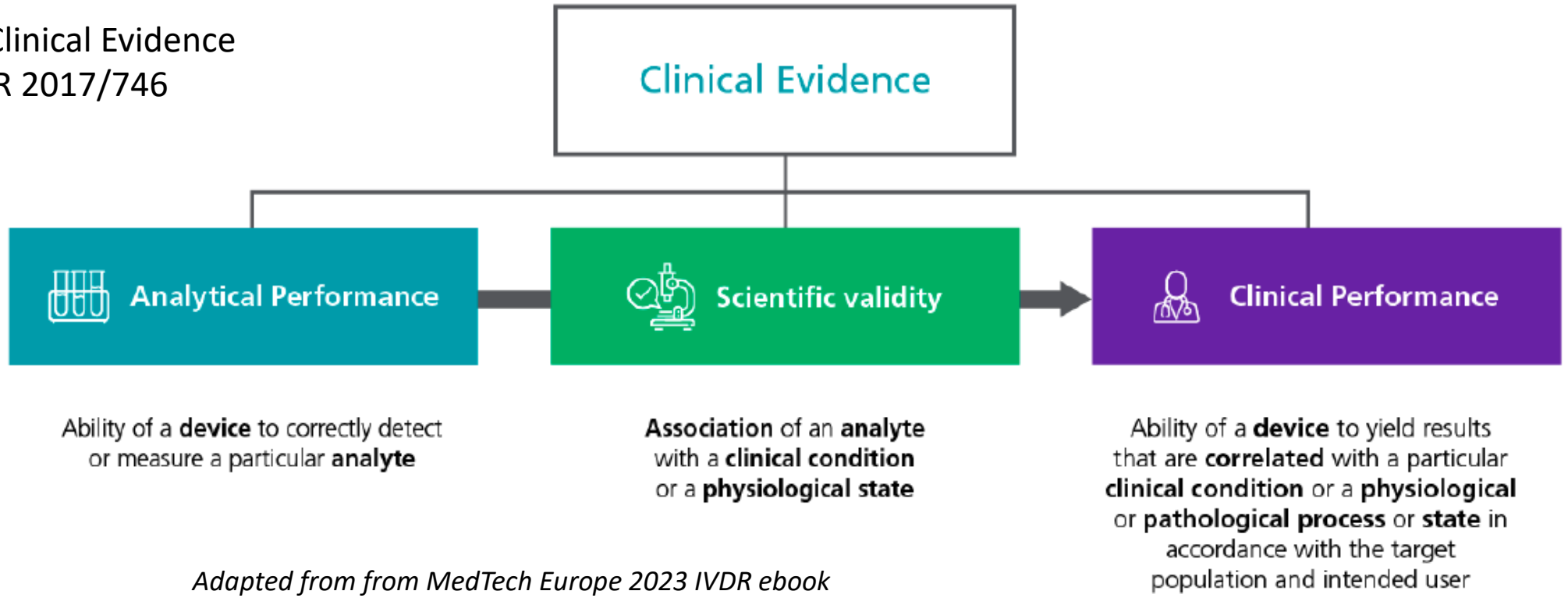
Name of Company	Research support	Employee	Consultant	Stockholder	Speaker's Bureau	Scientific Advisory Board	Other
Novartis	No	No	No	No	Yes	Yes	
Jazz	Yes	No	No	No	Yes	No	
BMS	Yes	No	No	No	No	No	

Flow cytometric MRD in AML is a high risk test (IVDR C)

Intended Purpose

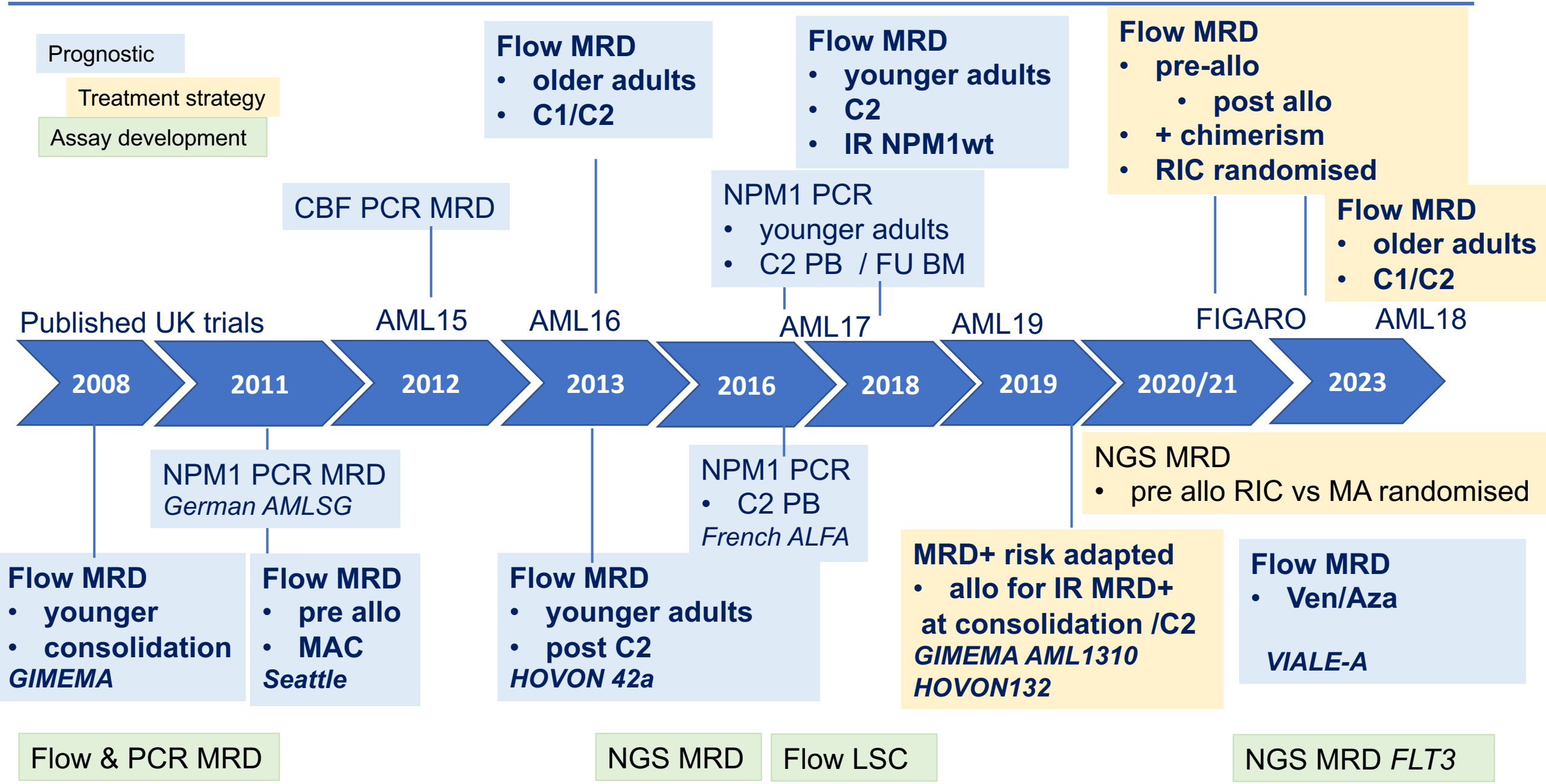
- To monitor response to treatment
- As an aid to treatment choices
  - intensification and transplant related decisions
    time-points post induction, pre-transplant, post-transplant
  - more recently enrollment criterion for some trials

Components of Clinical Evidence
according to IVDR 2017/746



Adapted from from MedTech Europe 2023 IVDR ebook

Published Evidence Source



Why

ELN-DAVID MRD ASSESSMENT and VALIDATION in AML

Expert Consensus Recommendations

2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party

blood

Michael Heuser,[1] Sylvie D. Freeman,[2] Gert J. Ossenkoppele,[3] Francesco Buccisano,[4] Christopher S. Hourigan,[5] Lok Lam Ngai,[3] Jesse M. Tettero,[3] Costa Bachas,[3] Constance Baer,[6] Marie-Christine Béné,[7] Veit Bücklein,[8] Anna Czyz,[9] Barbara Denys,[10] Richard Dillon,[11] Michaela Feuring-Buske,[12] Monica L. Guzman,[13] Torsten Haferlach,[6] Lina Han,[14] Julia K. Herzig,[12] Jeffrey L. Jorgensen,[15] Wolfgang Kern,[6] Marina Y. Konopleva,[14] Francis Lacombe,[16] Marta Libura,[17] Agata Majchrzak,[18] Luca Maurillo,[4] Yishai Ofran,[19] Jan Philippe,[10] Adriana Plesa,[20] Claude Preudhomme,[21] Farhad Ravandi,[14] Christophe Roumier,[21] Marion Subklewe,[8] Felicitas Thol,[1] Arjan A. van de Loosdrecht,[3] Bert A. van der Reijden,[22] Adriano Venditti,[4] Agnieszka Wierzbowska,[23] Peter J. M. Valk,[24] Brent L. Wood,[25] Roland B. Walter,[26] Christian Thiede,[27,28] Konstanze Döhner,[12] Gail J. Roboz,[13] and Jacqueline Cloos[3]

Technical Aspects of Flow Cytometry-based Measurable Residual Disease Quantification in Acute Myeloid Leukemia: Experience of the European LeukemiaNet MRD Working Party

HemaSphere

Jesse M. Tettero[1], Sylvie Freeman[2], Veit Buecklein[3], Adriano Venditti[4], Luca Maurillo[4], Wolfgang Kern[5], Roland B. Walter[6], Brent L. Wood[7], Christophe Roumier[8], Jan Philippé[9], Barbara Denys[9], Jeffrey L. Jorgensen[10], Marie C. Bene[11], Francis Lacombe[12], Adriana Plesa[13], Monica L. Guzman[14], Agnieszka Wierzbowska[15], Anna Czyz[16], Lok Lam Ngai[1], Adrian Schwarzer[17], Costa Bachas[1], Jacqueline Cloos[1], Marion Subklewe[3], Michaela Fuering-Buske[18], Francesco Buccisano[4]

Harmonisation as an aid to evidence of Analytical and Clinical Performance

Equivalent MRD results between laboratories

Cytometer and settings

Wet protocols

Antibody panels

Analysis strategy

Sample quality assessment

Control BMs

Denominator

Antibody Panels

2021 ELN Recommended core combination

CD34 CD117 CD33 CD13 HLADR CD45 CD7 CD56

ELN Tube

Fluorochorme	FITC	PE	PerCP-Cy5.5	PECy7	APC	Alexa Fluor750	PB	V500	KO
Antigen	CD34	CD13	CD7	CD33	CD56	CD117	HLA-DR	CD45	
Clone	8G12	L138	M-T701	D3HL60.251	NCAM16.2	104D2D1	Immu357	HI30	J33
Manufacturer	BD	BD	BD	BC	BD	BC	BC	BD	BC
Order No.	345801	347406	561602	B92408	341027	B92450	B36291	560777	B36294
Amount	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl

Hovon P1 Tube

Fluorochorme	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7	BV421	KO
Antigen	CD7	CD56	CD34	CD117	CD33	HLA-DR	CD13	CD45
Clone	M-T701	MY31	8G12	104D2D1	P67.6	L243 (G46-6)	WM15	J33
Manufacturer	BD	BD	BD	BC	BD	BD	BD	BC
Order No.	555360	345810	347222	B49221	345800	641411	562596	B36294
Amount	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl	5 µl

Also highly relevant

- Myeloid maturation / monocytic markers
- CD38 with CD34 and ‘LSC’ markers (markers absent on normal CD34+CD38low/neg cells)

Antibody Panels

Progress in technology

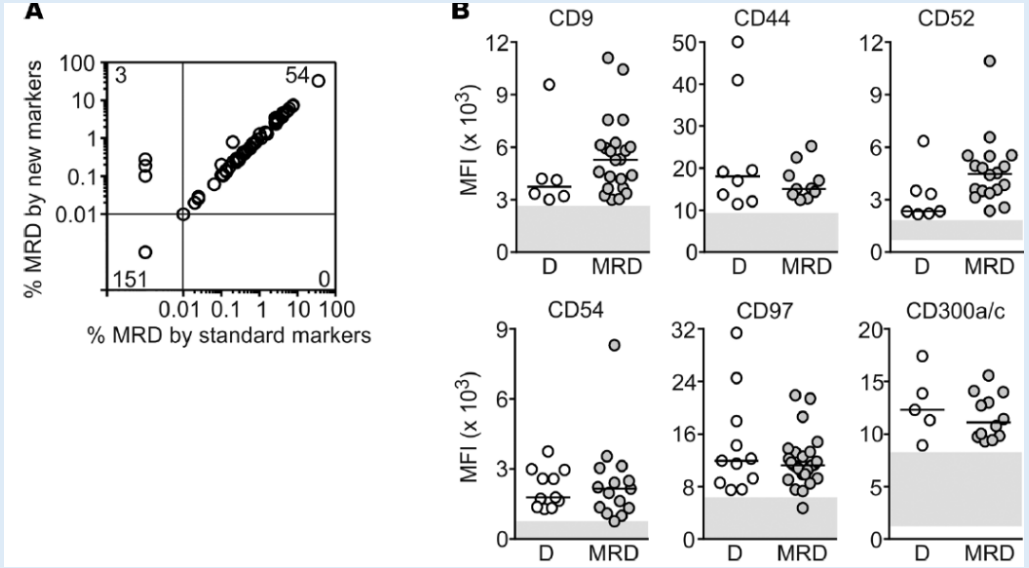
- More colours with new cytometers
- More / new markers

ORIGINAL ARTICLE

CLINICAL CYTOMETRY

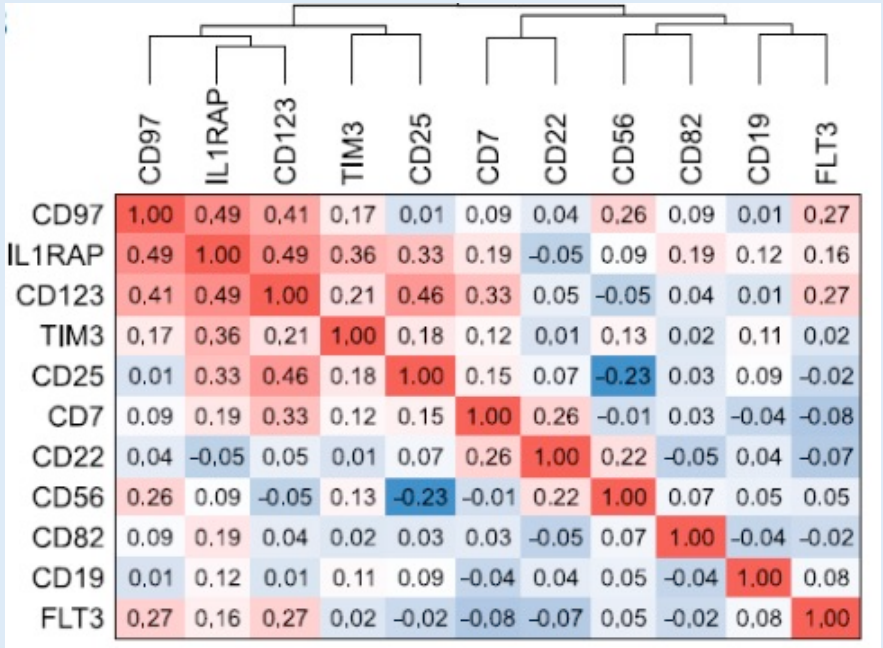
Validation of a 12-color flow cytometry assay for acute myeloid leukemia minimal/measurable residual disease detection Wang et al August 2023

gene expression array
28 markers but identified <1% further MRD+



Adapted from Coustan-Smith et al. *JCI Insight* 2018

plasma membrane proteomics
overlap in identified aberrant markers



Adapted from de Boer, et al. *Cancer Cell* 2018

Antibody Panels

- AML MRD panel composition survey: 11 ELN Laboratories
  Belgium (1), France (2), Germany (2), Israel (1), Netherlands (1), Poland (1), UK (1), USA (2)

Excluding 8c ELN core combination - 29 markers in use.

Asynchronous	Lymphoid	LSC-specific	Monocytic	Misc
CD38	CD19	CD38	CD64	CD123
CD133	CD22	CD45RA	CD14	CD36
	CD2	CLL-1/CLEC12A	CD300e	CD54
CD11b	CD5	TIM3		CD25
CD15	CD9	CD90		CD71
CD65		CD97		
		GPR56		
		CD81		
		CD97		
		CD200		
		CD44		

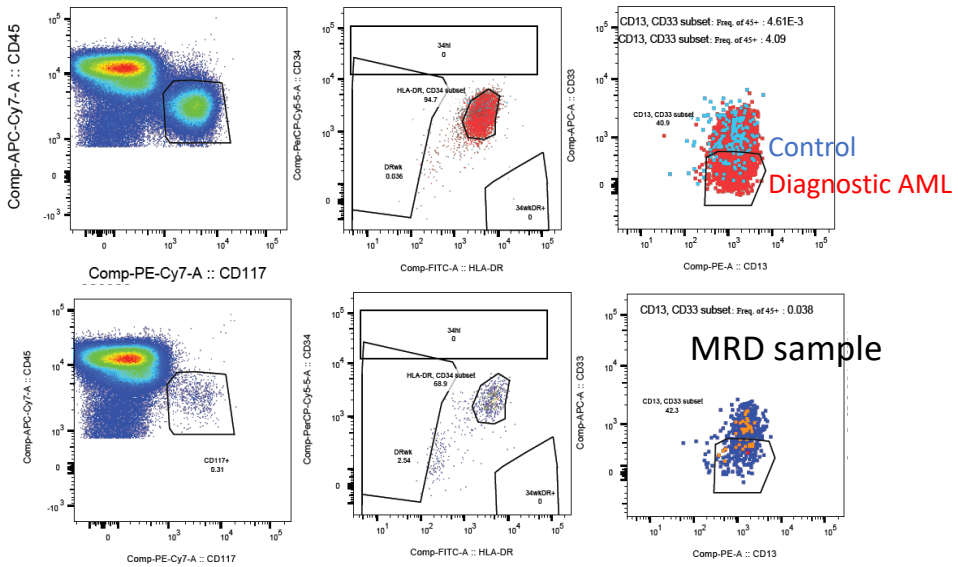
Work in progress:

- Updating Consensus markers for:
  - 10c tube
  - 12c tube
  - 13-16c tube
  - LSC specific
  - Monocytic specific

Analytical performance

Use of proficiency testing material to harmonize results

1. Sample exchange schemes
2. External quality assurance schemes
   - UK NEQAS Leukocyte immunophenotyping



UK NEQAS
Leucocyte Immunophenotyping

Results and Performance

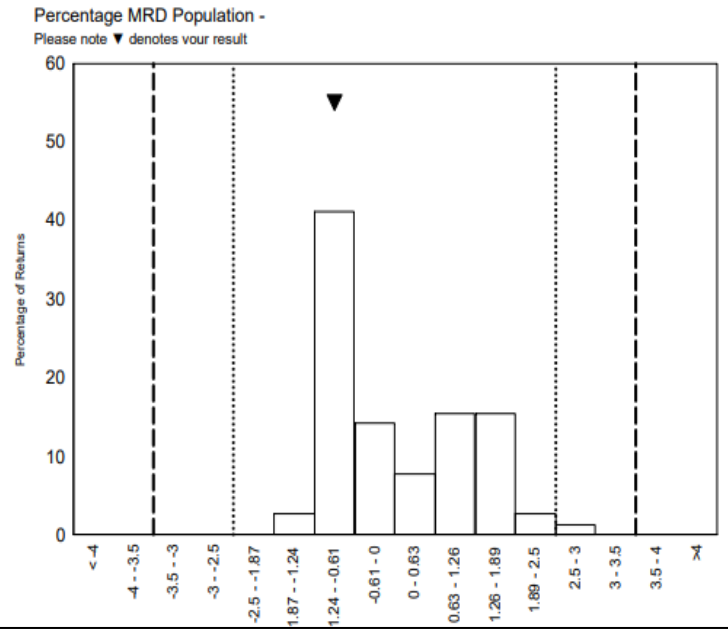
Percentage MRD Population	Your Results (%)	Robust Mean (%)	Robust SD (%)
	0.0490	0.2000	0.1582

Percentage MRD Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
			12	0	0

***z Score Limits Definitions**

Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.

Histograms of Participant z Scores



EQA shows harmonising Flow AML MRD results continues to be a challenge

98 laboratories participating in current UK NEQAS programme

- Uses stabilised EQA material with 4 distributions per year
- Test for panel, processing and analysis
- MRD samples now also contain ‘normal’ progenitors (PB stem cells)

UK NEQAS
Leucocyte Immunophenotyping

Matthew Fletcher,
Liam Whitby,
Stuart Scott

August 2023 Exercise

Robust Mean (%)	Robust SD (%)
0.0914	0.0300

ELN MRD threshold 0.1%

Robust SD - 0.07% to 0.12%

Understanding Obstacles for harmonisation

EQAs are a tool to inform issues and guide changes in practice

ELN-NEQAS Interlaboratory study for molecular MRD

- *NPM1*^{mut} PCR MRD -High proportion of false positive results in EQA negative sample
  - Proportion attributed to use of a specific highly active reverse transcriptase
    – more likely to introduce errors (less of an issue for rearranged genes).

Scott et al Blood Advances 2023

Issues for Flow Cytometric MRD Panels vs Processing vs Analysis & Interpretation

Analytical performance – development of an electronic EQA NEQAS /ELN

EQA pilot - Flow files (FCS) of diagnostic, control and MRD samples stained with ELN tube

Items for harmonization

- Denominator used to calculate the MRD population size
- ELN Marker Staining Intensity of Diagnostic Blasts
- Presence and Ranking of Diagnostic LAIP(s)
- What is the Aberrant Immunophenoype quantified in the MRD sample? Diagnostic or emerging Different from normal
- MRD value (% and events) in MRD sample & Normal Control (ie background)
- LOQ by events criteria (to assess sample quality)

Lessons learnt from initial pilots:

Confounder for harmonized values

CD34+ vs CD117+ for reporting MRD % particularly if dominant aberrant IP changes 34 or 117 expression between diagnosis and MRD

UK NEQAS Leucocyte Immunophenotyping

Matthew Fletcher
Liam Whitby
Stuart Scott

ELN-DAVID

Analytical performance – development of an electronic EQA

MRD Population (%)	z score	Performance Status by z Score
CD34+	-0.405	Satisfactory
CD117+	-0.199	Satisfactory

z-score for MRD %, but also performance by reference value

Control BM

Diagnostic LAIP blasts

In silico dilution

→ MRD sample with n LAIP cells

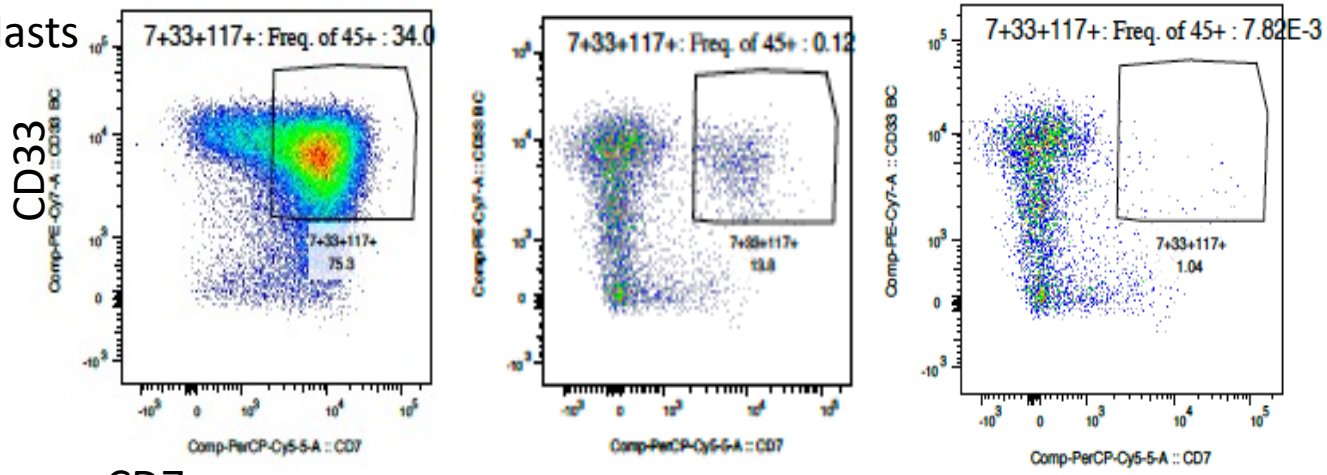
n = reference / maximum MRD events

Diagnostic

MRD BM

Control BM

117+ blasts



Participant-specific Report

UK NEQAS Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS Foundation Trust

ELN Electronic Exercise - All Participant Report

Date Issued: 03 April 2023 **Date Closed:** 21 April 2023 **Participant ID Number:** 1

Trial Comments: This electronic exercise was issued to 20 participants.

Results and Performance

Diagnostic Blast Staining

Antigen	CD7	CD13	CD33	CD34	CD56	CD117	HLADR
Your Result	Strong	Weak	Strong	Strong	Absent	Strong	Strong
Consensus	Strong	Weak	Strong	Strong	Absent	Strong	Strong

Diagnostic LAIP on CD117+ Blasts

	CD7+ (+/- CD33)	CD56+ (+/- CD33)	CD33wk/- CD13+	CD13wk/- CD33+	HLADRwk/-	HLADRwk/- CD33+
Your Result	Present*	Absent	Absent	Present	Absent	Absent
Consensus	Present	Absent	Absent	Absent	Absent	Absent

*Ranked 1st for Diagnostic LAIP

Diagnostic LAIP on CD34+ Blasts

	CD7+ (+/- CD33)	CD56+ (+/- CD33)	CD33wk/- CD13+	CD13wk/- CD33+	HLADRwk/-	HLADRwk/- CD33+
Your Result	Present*	Absent	Absent	Present	Absent	Absent
Consensus	Present	Absent	Absent	Absent	Absent	Absent

*Ranked 1st for Diagnostic LAIP

Follow Up Sample

	Your Result	Consensus	Maximum	Minimum	Reference Value
Total CD45+ Events	768081	729910	771572	400000	
Calculated %LOQ**	0.007	0.007	0.013	0.006	0.007
Total CD34+ Blast Events	8130	8130	10283	3019	
Total CD34+ MRD Events	781	841	5120	164	
%CD34+ MRD Events	0.10	0.115	0.708	0.020	
Total CD117+ Blast Events	6646	7150	35030	4193	
Total CD117+ MRD Events	917	953	13290	156	917
%CD117+ MRD Events	0.120	0.126	1.760	0.020	0.12
Interpretation (0.1% cut off)	MRD Present	MRD Present			MRD Present

**Using 50 CD45+ Event Threshold Criteria

Percentage MRD Population	Your Result (%)	Robust Mean (%)	Robust SD (%)
CD34+	0.100	0.115	0.038
CD117+	0.120	0.126	0.032

MRD Population (%)	z score	Performance Status by z Score	Performance Status by Reference Value
CD34+	-0.405	Satisfactory	
CD117+	-0.199	Satisfactory	
MRD Events			(Reference Range 733.6 to 1100.4)
CD34+			Satisfactory
CD117+			Satisfactory

Use of absolute Limit of Quantification in AML MRD

Myeloma / CLL / ALL MRD LOD 20-30 cells LOQ 50 cells

Absolute LOQ - allows harmonization of sample quality assessment for AML MRD
Critical to qualify a negative result

Sample LOQ of 0.01% if 500,000 leukocytes acquired

Next steps to consider

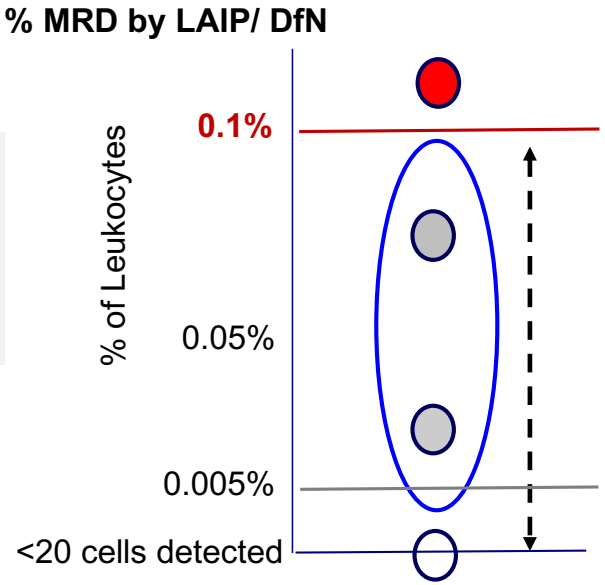
- blast “LOQ” - need 500 blast events to detect MRD target present in 1 in 10 blasts

- assessment of hemodilution - particularly if MRD <0.1%

Use of absolute Limit of Quantification in AML MRD

Flow MRD target

Target background may be higher than absolute LOQ



0.1% = MRD positivity by ELN criteria

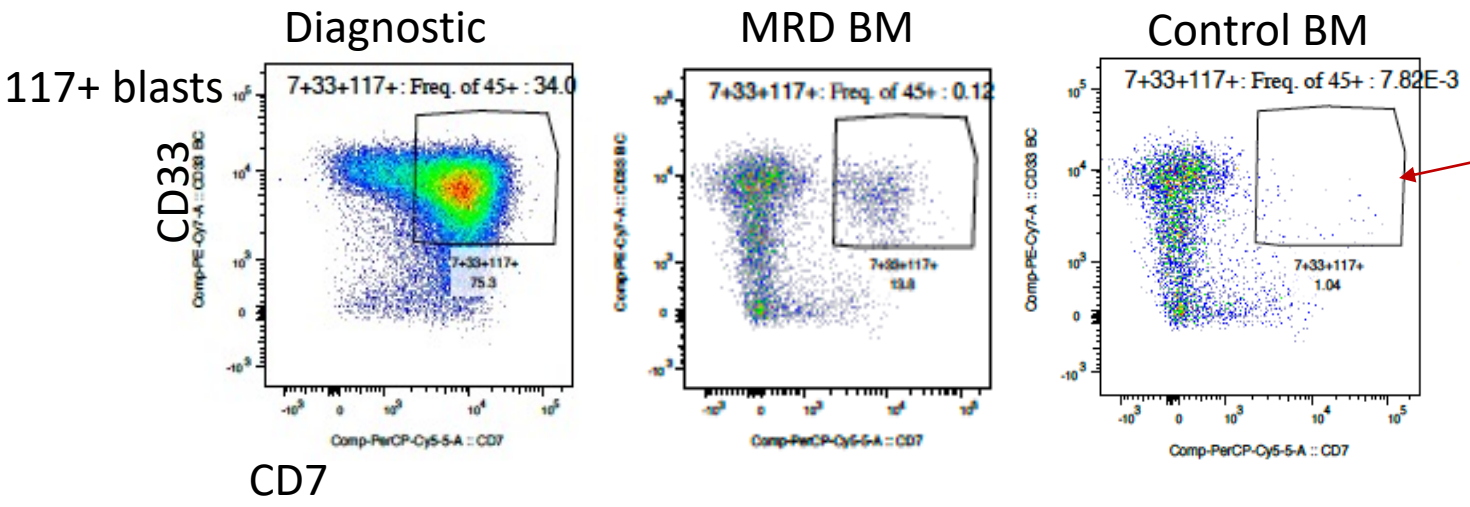
significance of <0.1% events in LAIP/DfN gate depends on:

sample quality / target background

biological background from non-AML cells

Absolute LOQ (50 cells) if 1 million leukocytes acquired from good quality BM

Absolute LOD

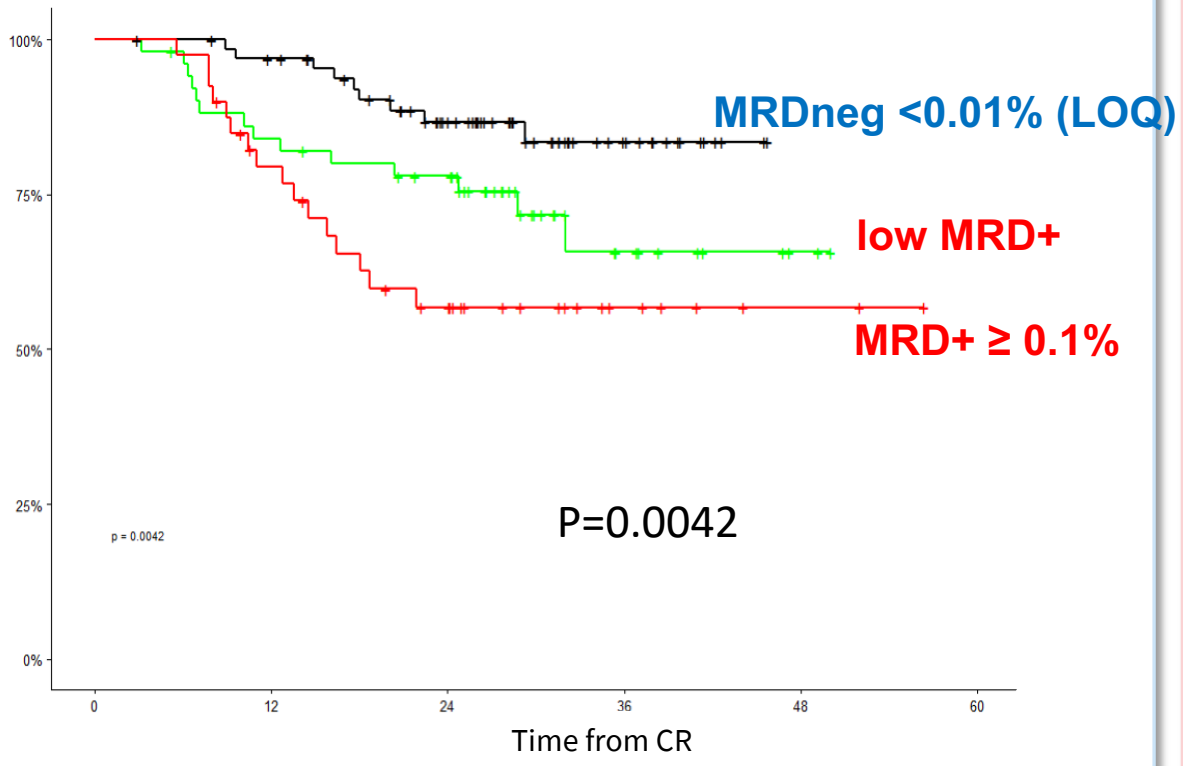


60 cells in 760,000 cells
>50 cells but <0.01%

Improving Flow cytometric MRD prognostic accuracy - Clinical Performance

GIMEMA 1310 Trial

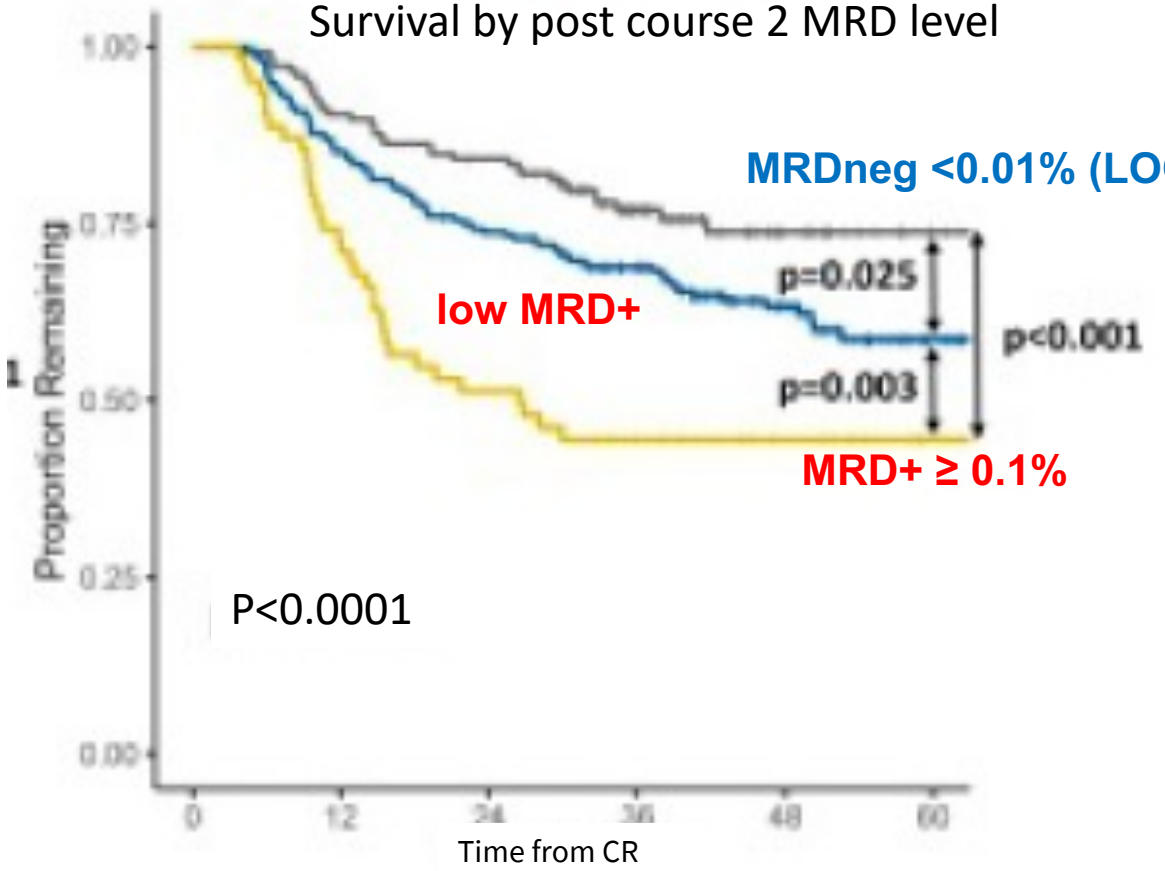
Survival by post consolidation MRD level



F. Buccisano, personal communication
& *Buccisano et al Haematologica. 2022*

HOVON-SAK 132 Trial

Survival by post course 2 MRD level



*J Tettero et al EHA 2023 Hemasphere. 2023 Aug; 7(*Suppl)

Analysis strategies: considerations for performance and harmonization

LAIP - leukemia immunophenotypic footprint

- number of Boolean gates and exact gate positions varies between SOPs, operators and samples
- can achieve $MRD^{negative}$ < absolute LOQ with multiple Boolean gates + empty space footprint

Different from Normal

- applicable to: immunophenotypic shifts, when no diagnostic sample & longer-term monitoring
- screens several plots of 2 marker combinations, usually focused on blast compartments
- can be standardized by fixed gates for the most useful empty spaces
  - allows rapid analysis
- may have higher background from normal controls than multiple Boolean gates

Flow cytometric MRD target assessment needs control bone marrows

Leukemic **A**berrant **I**mmuno**P**henotype **LAIP**

= abnormal immunophenotype of diagnostic leukaemic blasts

Sensitive / specific LAIPs for tracking MRD amongst normal regenerating blasts are in immunophenotypic 'empty spaces' of normal blasts

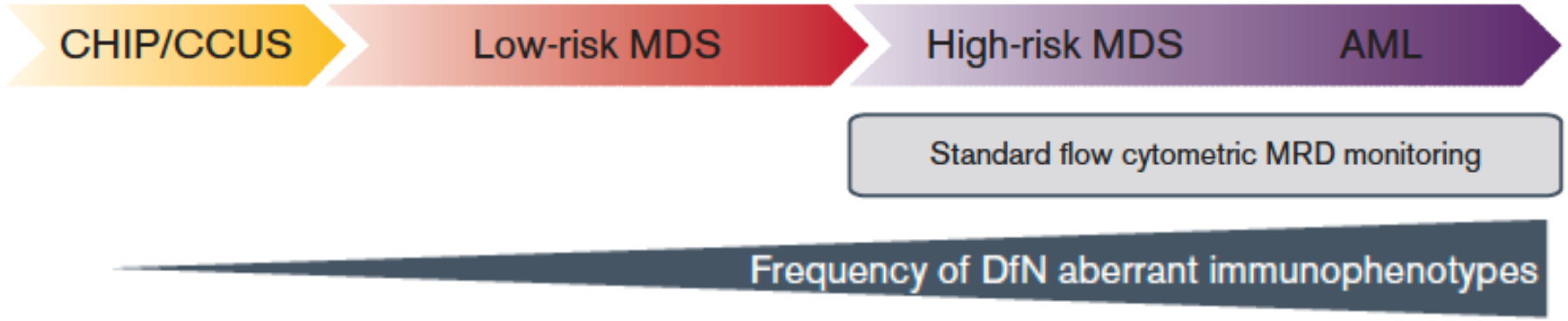
- Control BMs required to interpret positive result vs background

Different **f**rom **N**ormal **DfN** analysis relies on these empty spaces defined by set panel

- Control BMs required to define empty spaces and their background

Empty spaces? - Consider "aberrant immunophenotypes with indeterminate leukemic potential"

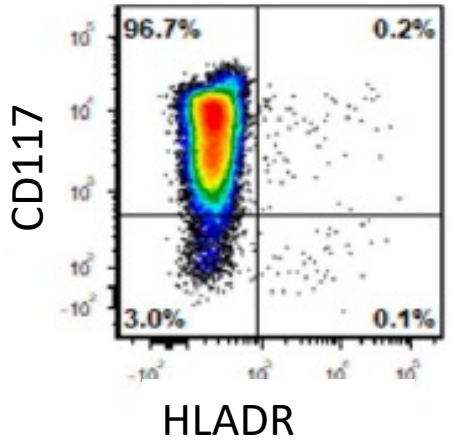
Aberrant immunophenotypes with indeterminate leukemic potential



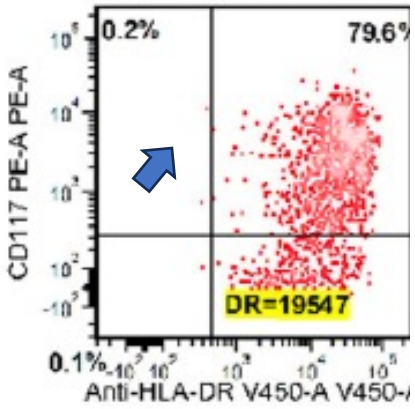
Impacts: Reporting, scientific validity and clinical performance

- Library of reference 'control' bone marrows - need sufficient number and subtypes

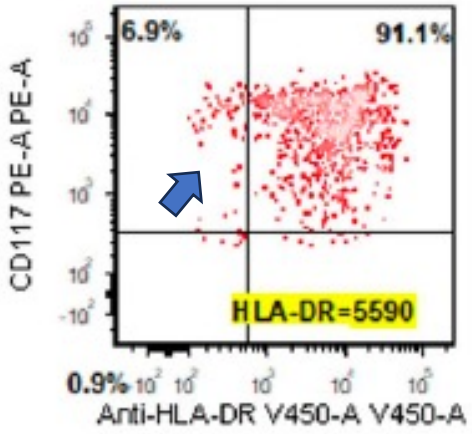
Diagnostic blasts



Normal BM CD34+

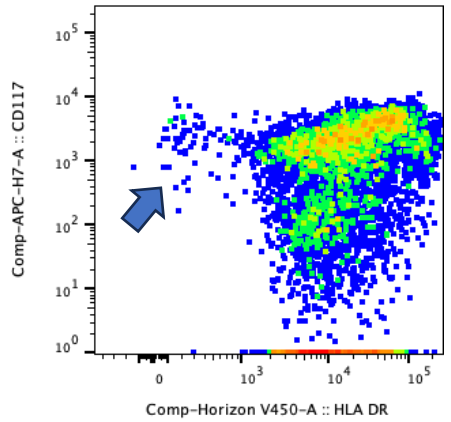


FU with CHIP only BM CD34+



Adapted from *Wang et al, Clinical Cytometry 2023*

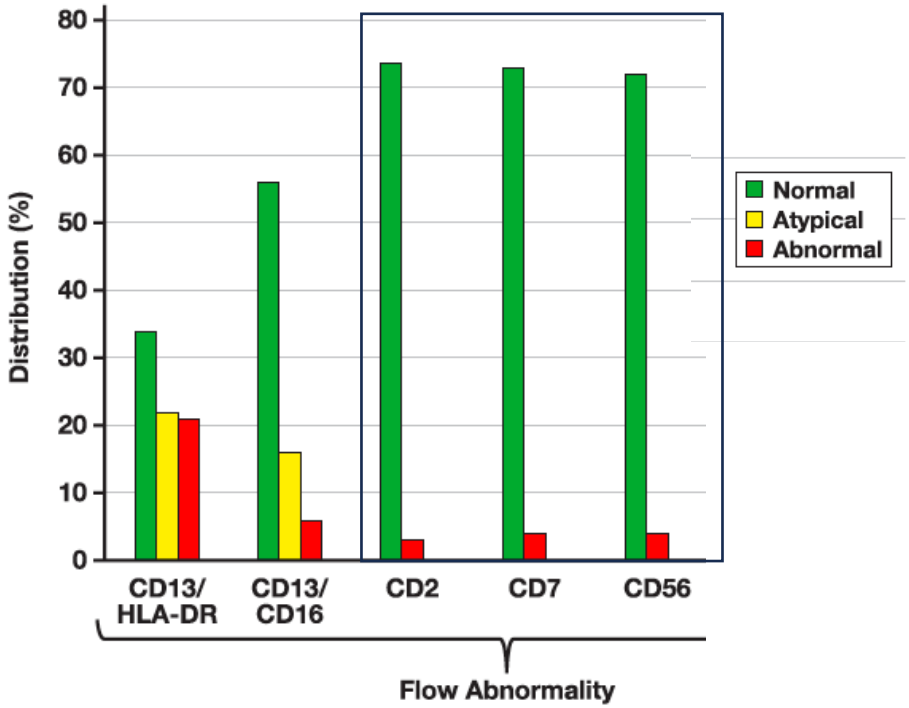
Other Lab Control BM CD34+



Aberrant immunophenotypes with indeterminate leukemic potential

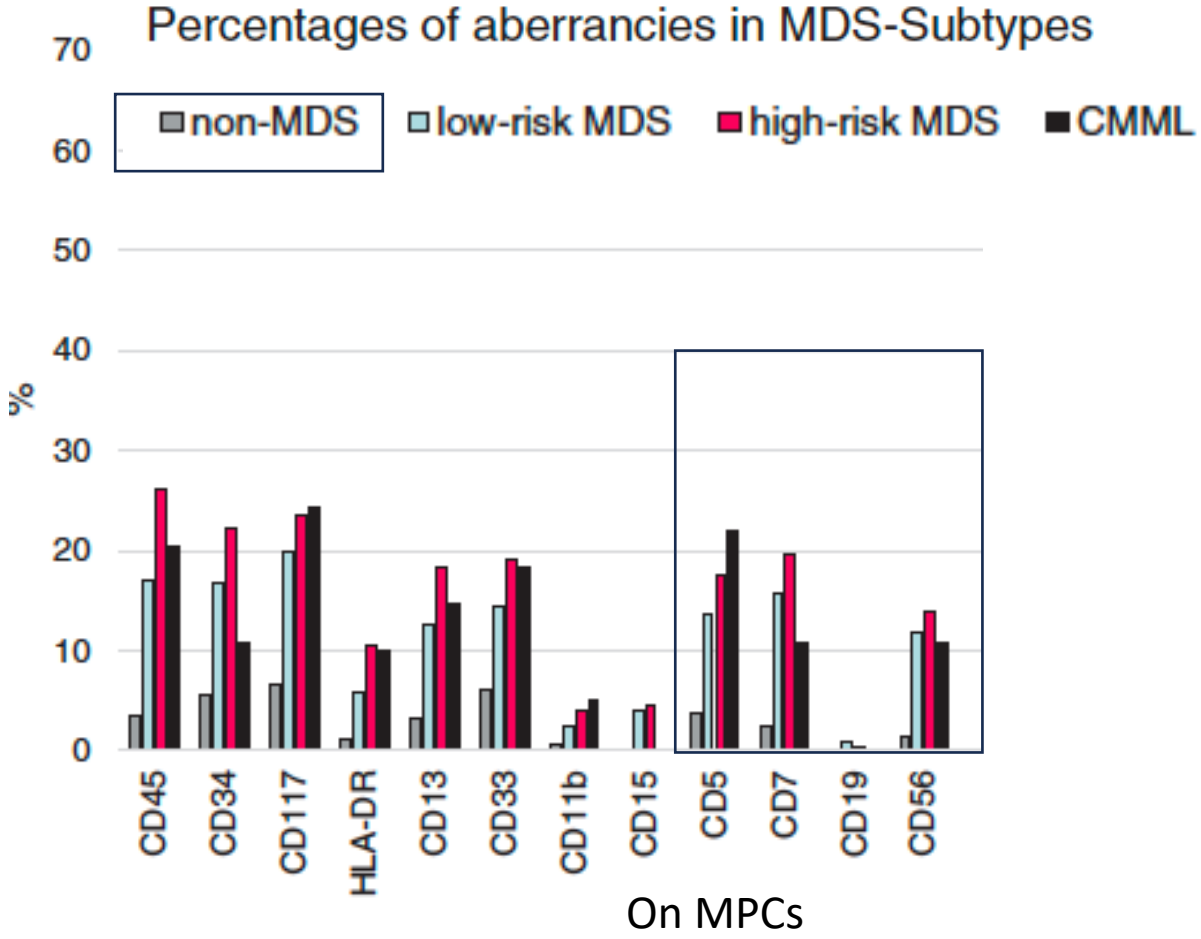
Also observed for aberrant lymphoid expression

Immunophenotypic abnormalities in CCUS
80 patients



On CD34+ blasts

Jevremovic et al, AJCP 2022



On MPCs

W Kern et al, Cytometry Part B Clinical Cytometry *2023*

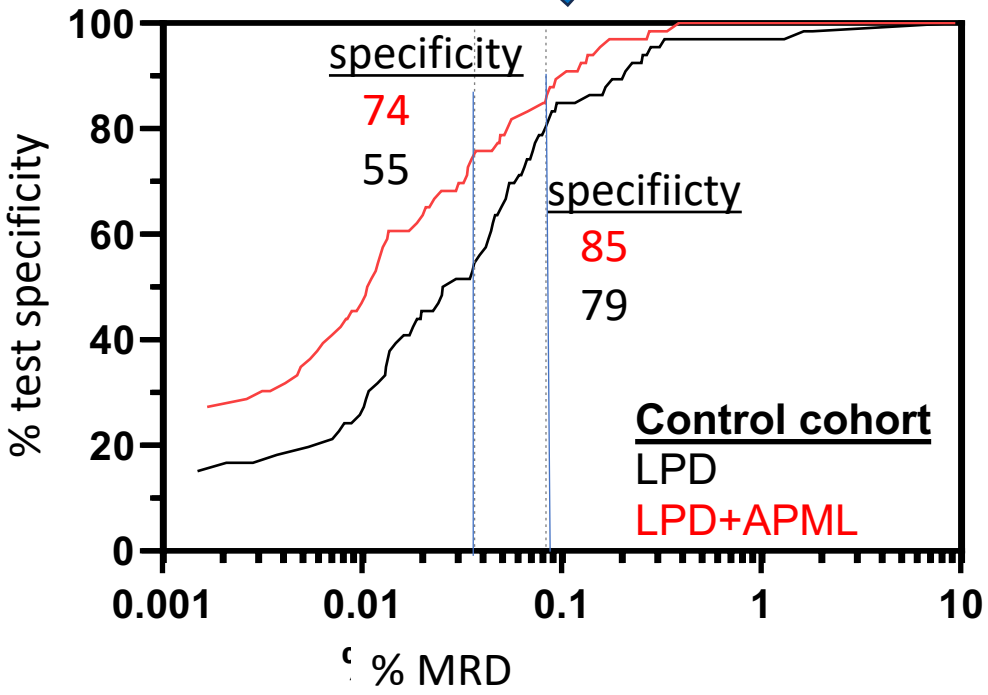
Range of control BMs impacts MRD result specificity - especially results <0.1%

Computerised Flow MRD → Identifies DfN immunophenotypes in non-relapse APML progenitors

FlowSOM clustering + Unsupervised pipeline to predict DfN immunophenotypes

Training 'normal' 'cohort (LPD staging BMs) (up to 50)

Include APML samples with DfN immunophenotypes in control BM training cohort (ML)



N McCarthy & S Freeman UK
G Gui NIH USA / F Dumezy, C Roumier, A Plesa ALFA France

Conclusions

Harmonisation leading to equivalent MRD results between laboratories - key step for Clinical Evidence

- Processing including stable cytometer settings – newer cytometers
- Using recommended antibody core markers - to be updated by ELN for ≥10 colours by 2024
- Development of an analytical EQA with reference value to monitor performance & competency

  Includes: identification and ranking of common useful LAIPs /DfN for AML MRD

  MRD % from 1) CD34+ blasts 2) CD117+ blasts

  Integration of control sample for % background by LAIP /DfN gating
- Sample quality assessment – absolute LOQ / blast events / hemodilution
- Range of control bone marrows to exclude false positives from ”aberrant immunophenotypes with indeterminate potential”
- LOQ or <0.01% may differentiate deeper responders in future