Amsterdam UMC
University Medical Centers

Relevance of Flow Cytometry for Myelodysplastic Syndromes' diagnosis

2022 recommendations of the ELN iMDS Flow working group

Arjan van de Loosdrecht

Department of Hematology, Cancer Center Amsterdam,
Amsterdam University Medical Centers
Location Vrije Universiteit, VU University Medical Center
Amsterdam, The Netherlands

Lund 2022, Sweden
European Association for Haematopathology
XV EBMWG
May 28th - 31st, 2022

VU Hematologie

Amsterdam UMC
University Medical Centers

Disclosures:
A.A. van de Loosdrecht

None relevant to this presentation

Integrated diagnostics in Myelodysplastic Syndromes

A. Prerequisite Criteria

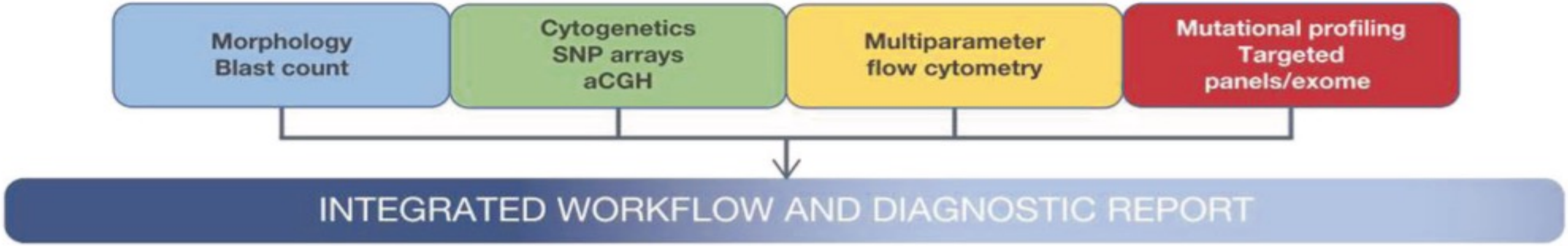
- persistent cytopenia in one or more cell lineages
- exclusion of all other (non)hematopoietic disorders

B. MDS-related (decisive) Criteria

- dysplasia in >10% of all cells in one of the lineages
- or > 15% ring sideroblasts (iron stain) in BM smears or 5–14% RS and SF3B1 mutation
- 5–19% blast cells in bone marrow smears
- typical chromosomal abnormality (karyotyping or FISH)

C. Co-criteria

- abnormal findings in histology/immunohistochemistry of bone marrow biopsies
- abnormal immunophenotype of bone marrow cells
- evidence of a clonal population of myeloid cells determined by molecular (sequencing) studies (NGS) revealing MDS-related mutations



Morphology
Blast count

Cytogenetics
SNP arrays
aCGH

Multiparameter
flow cytometry

Mutational profiling
Targeted
panels/exome

INTEGRATED WORKFLOW AND DIAGNOSTIC REPORT

Arber AD, et al., Blood 2016, 127(20): 2391-2405; Valent P, et al., Oncotarget 2017, 8(43): 73483-73500; Mufti G, Van de Loosdrecht AA et al., Leukemia 2018, 32(8):1679-1696; Van de Loosdrecht AA, et al., Cytometry B Clin Cytom 2021; Dec 13. doi:10.1002

WHO-2016 guidelines on FC in MDS

- Percentages myeloid progenitors are informative
  - cannot replace differential blast counts on smears
  (fibrosis, hemodilution)

- Abnormal phenotypes of CD34$^+$ may be additional evidence of dysplasia

- Aberrant differentiation patterns (myeloid and erythroid) can indicate dysplasia

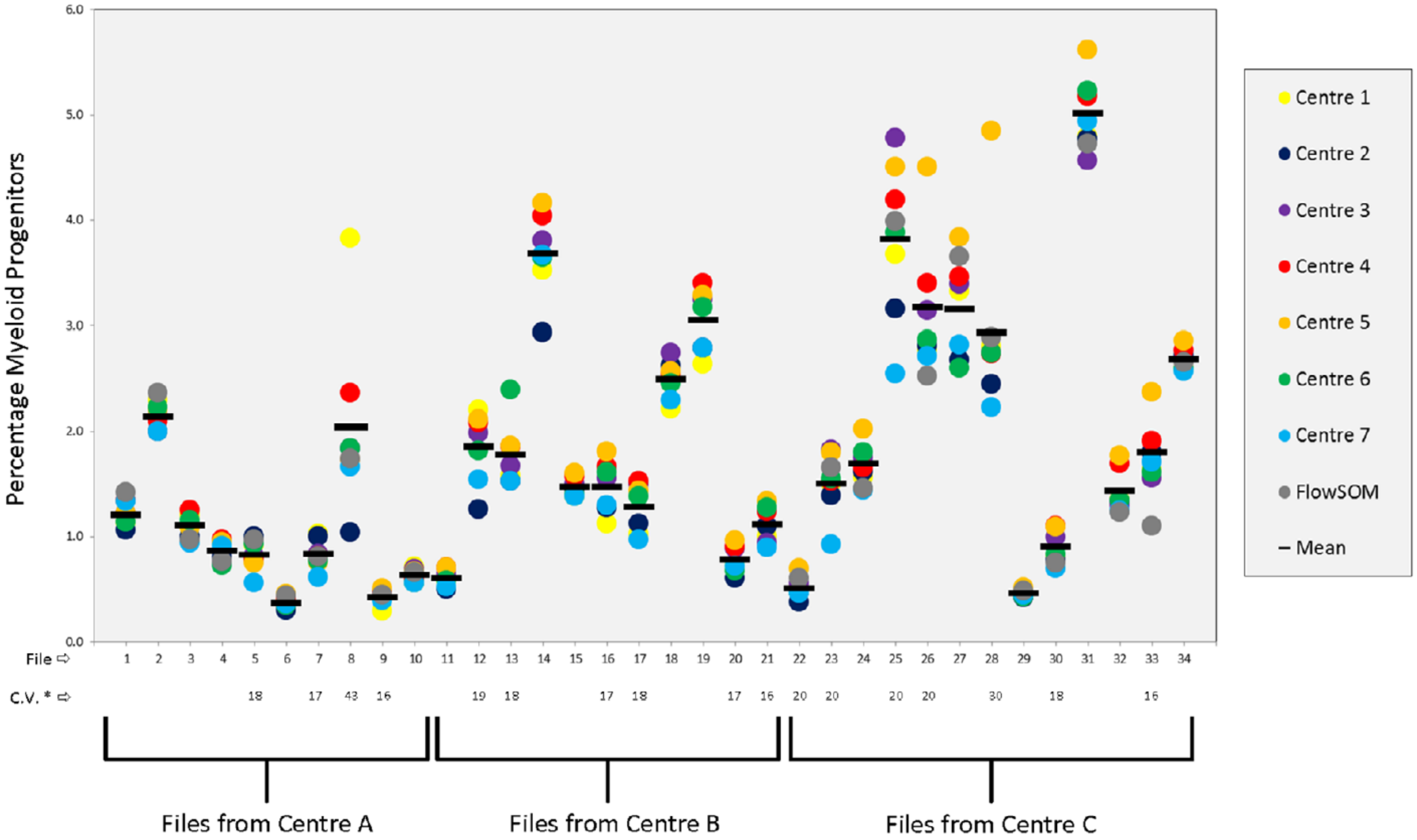
- Aberrant findings in at least three tested features (not specified) and at least two cell compartments are highly associated with MDS or MDS/MPN

- FC in suspected MDS if performed acc. to recommended panels (ELN)

- FC as part of an integrated report

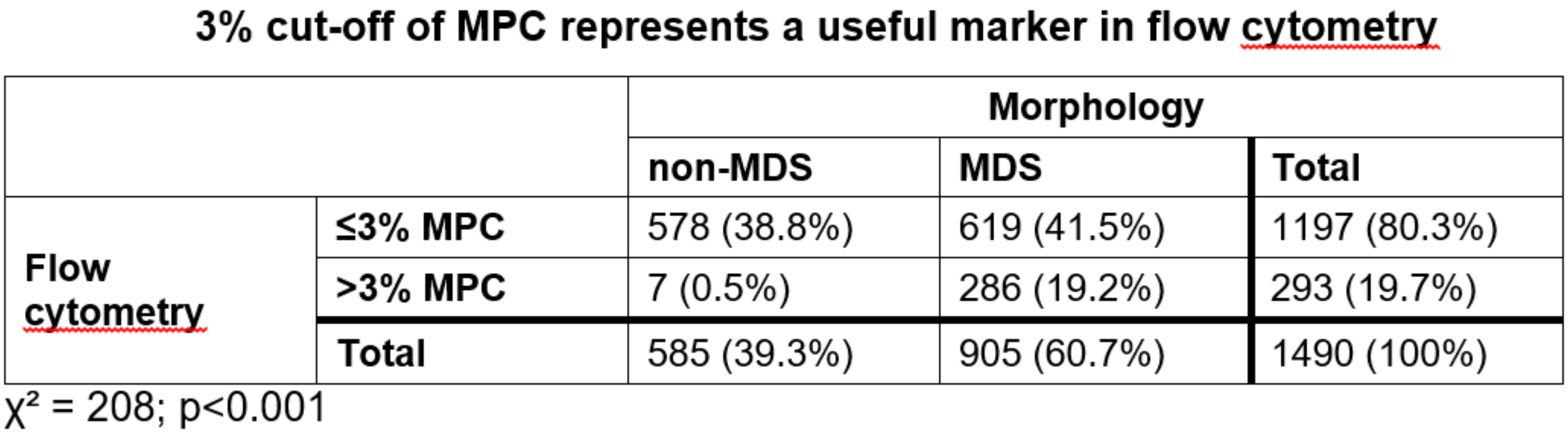
Arber DA and Hasserjian RP. Hematology 2015;294-298
Porwit A, et al., Leukemia 2014:28:1793-98
Arber DA, et al., Blood 2016;127:2391-2405

Reproducibility of myeloid progenitor cell count in MDS by FC



Johansson U, et al. Cytometry B Clin Cytom 2021: Dec 21. doi: 10.1002

ELN Multicenter study on MDS and FC: myeloid progenitors (MPC)



3% cut-off of MPC represents a useful marker in flow cytometry

		Morphology		
		non-MDS	**MDS**	**Total**
Flow cytometry	**≤3% MPC**	578 (38.8%)	619 (41.5%)	1197 (80.3%)
	>3% MPC	7 (0.5%)	286 (19.2%)	293 (19.7%)
	Total	585 (39.3%)	905 (60.7%)	1490 (100%)

$\chi^2 = 208; p<0.001$

Kern W, et al., Cytometry B Clin Cytom 2022; (provisionally accepted)

Recommendations 2022 of the ELN iMDS Flow WG on pre-analytical and technical issues

Recommended antibodies

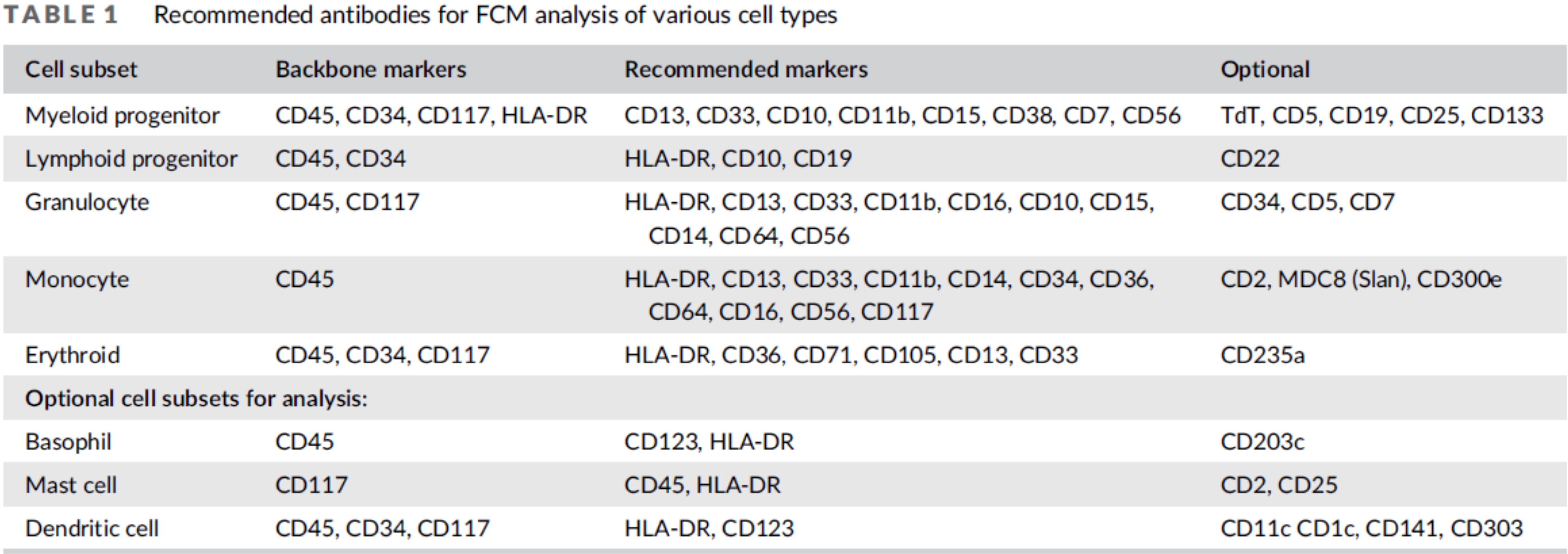


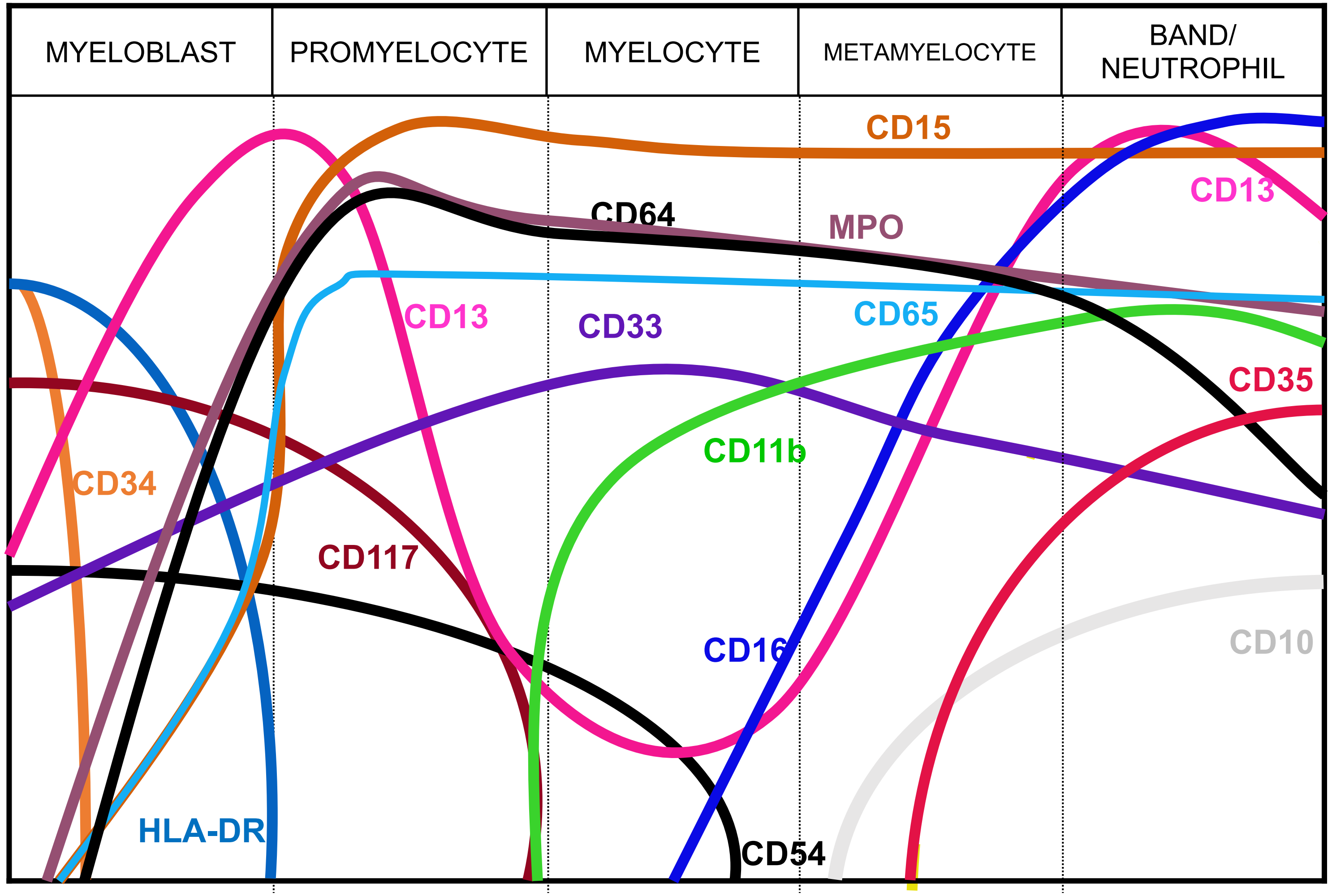
TABLE 1 Recommended antibodies for FCM analysis of various cell types

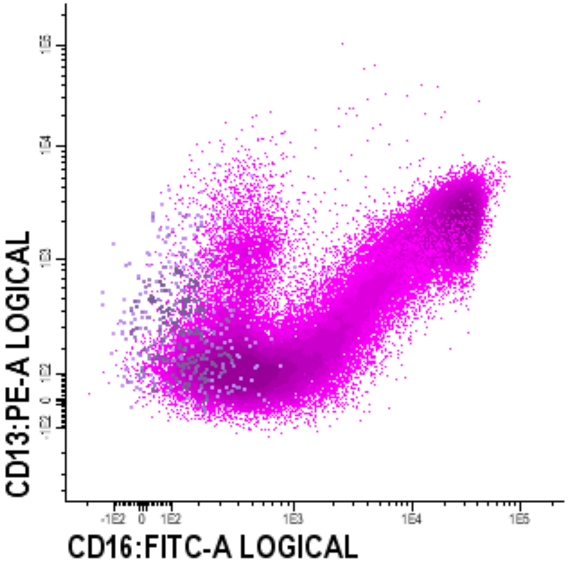
Cell subset	Backbone markers	Recommended markers	Optional
Myeloid progenitor	CD45, CD34, CD117, HLA-DR	CD13, CD33, CD10, CD11b, CD15, CD38, CD7, CD56	TdT, CD5, CD19, CD25, CD133
Lymphoid progenitor	CD45, CD34	HLA-DR, CD10, CD19	CD22
Granulocyte	CD45, CD117	HLA-DR, CD13, CD33, CD11b, CD16, CD10, CD15, CD14, CD64, CD56	CD34, CD5, CD7
Monocyte	CD45	HLA-DR, CD13, CD33, CD11b, CD14, CD34, CD36, CD64, CD16, CD56, CD117	CD2, MDC8 (Slan), CD300e
Erythroid	CD45, CD34, CD117	HLA-DR, CD36, CD71, CD105, CD13, CD33	CD235a
Optional cell subsets for analysis:			
Basophil	CD45	CD123, HLA-DR	CD203c
Mast cell	CD117	CD45, HLA-DR	CD2, CD25
Dendritic cell	CD45, CD34, CD117	HLA-DR, CD123	CD11c CD1c, CD141, CD303

van der Velden VHJ, et al., Cytometry B Clin Cytom 2021: Dec 11. doi:10.1002

Antigen expression during neutrophil differentiation:

the concept





Adapted from: A Orfao, ELNet Flow MDS 2008-2022, Amsterdam

Standardization of FC in MDS:

ELNet 2012-2021 recommendations

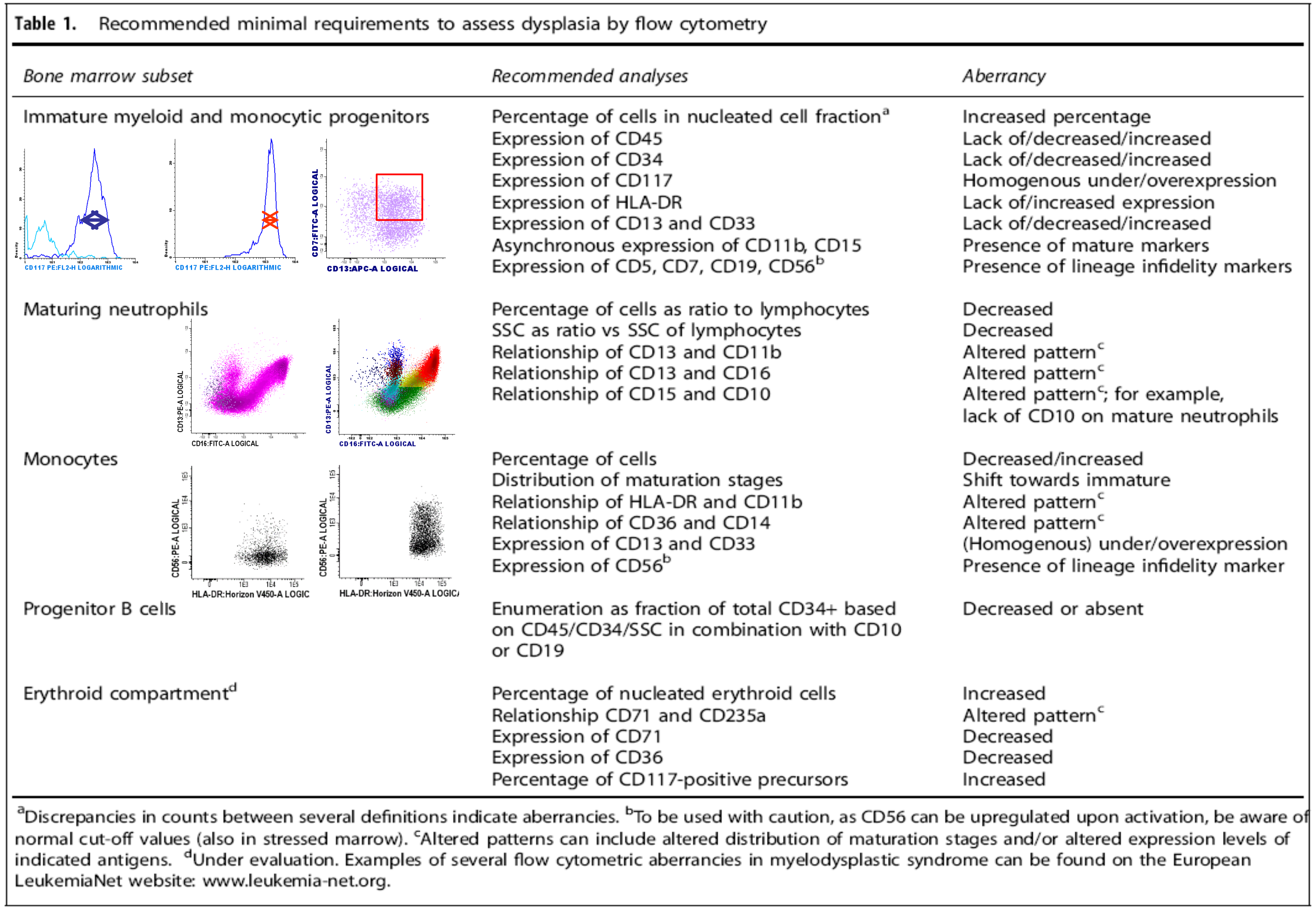


Table 1. Recommended minimal requirements to assess dysplasia by flow cytometry

Bone marrow subset	*Recommended analyses*	*Aberrancy*
Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction[a]	Increased percentage
	Expression of CD45	Lack of/decreased/increased
	Expression of CD34	Lack of/decreased/increased
	Expression of CD117	Homogenous under/overexpression
	Expression of HLA-DR	Lack of/increased expression
	Expression of CD13 and CD33	Lack of/decreased/increased
	Asynchronous expression of CD11b, CD15	Presence of mature markers
	Expression of CD5, CD7, CD19, CD56[b]	Presence of lineage infidelity markers
Maturing neutrophils	Percentage of cells as ratio to lymphocytes	Decreased
	SSC as ratio vs SSC of lymphocytes	Decreased
	Relationship of CD13 and CD11b	Altered pattern[c]
	Relationship of CD13 and CD16	Altered pattern[c]
	Relationship of CD15 and CD10	Altered pattern[c]; for example, lack of CD10 on mature neutrophils
Monocytes	Percentage of cells	Decreased/increased
	Distribution of maturation stages	Shift towards immature
	Relationship of HLA-DR and CD11b	Altered pattern[c]
	Relationship of CD36 and CD14	Altered pattern[c]
	Expression of CD13 and CD33	(Homogenous) under/overexpression
	Expression of CD56[b]	Presence of lineage infidelity marker
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment[d]	Percentage of nucleated erythroid cells	Increased
	Relationship CD71 and CD235a	Altered pattern[c]
	Expression of CD71	Decreased
	Expression of CD36	Decreased
	Percentage of CD117-positive precursors	Increased

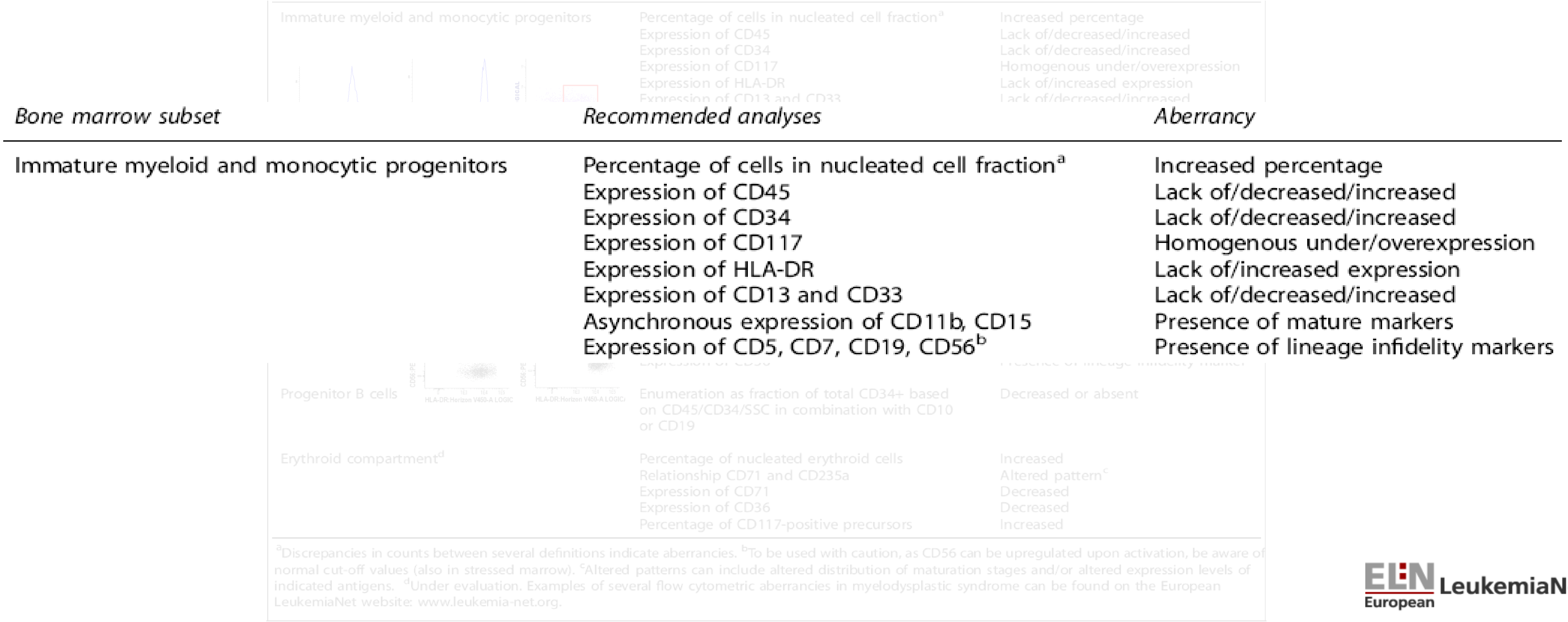
[a] Discrepancies in counts between several definitions indicate aberrancies. [b] To be used with caution, as CD56 can be upregulated upon activation, be aware of normal cut-off values (also in stressed marrow). [c] Altered patterns can include altered distribution of maturation stages and/or altered expression levels of indicated antigens. [d] Under evaluation. Examples of several flow cytometric aberrancies in myelodysplastic syndrome can be found on the European LeukemiaNet website: www.leukemia-net.org.

European LeukemiaNet

Van de Loosdrecht AA, et al., Haematologica 2009; 94:1124-34

Westers TM, et al., Leukemia 2012;36:422-30; Porwit A, et al., Leukemia 2014:28:1793-98

Standardization of FC in MDS:
ELNet 2012-2021 recommendations



Bone marrow subset	*Recommended analyses*	*Aberrancy*
Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction[a] Expression of CD45 Expression of CD34 Expression of CD117 Expression of HLA-DR Expression of CD13 and CD33 Asynchronous expression of CD11b, CD15 Expression of CD5, CD7, CD19, CD56[b]	Increased percentage Lack of/decreased/increased Lack of/decreased/increased Homogenous under/overexpression Lack of/increased expression Lack of/decreased/increased Presence of mature markers Presence of lineage infidelity markers

Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction[a] Expression of CD45 Expression of CD34 Expression of CD117 Expression of HLA-DR	Increased percentage Lack of/decreased/increased Lack of/decreased/increased Homogenous under/overexpression Lack of/increased expression

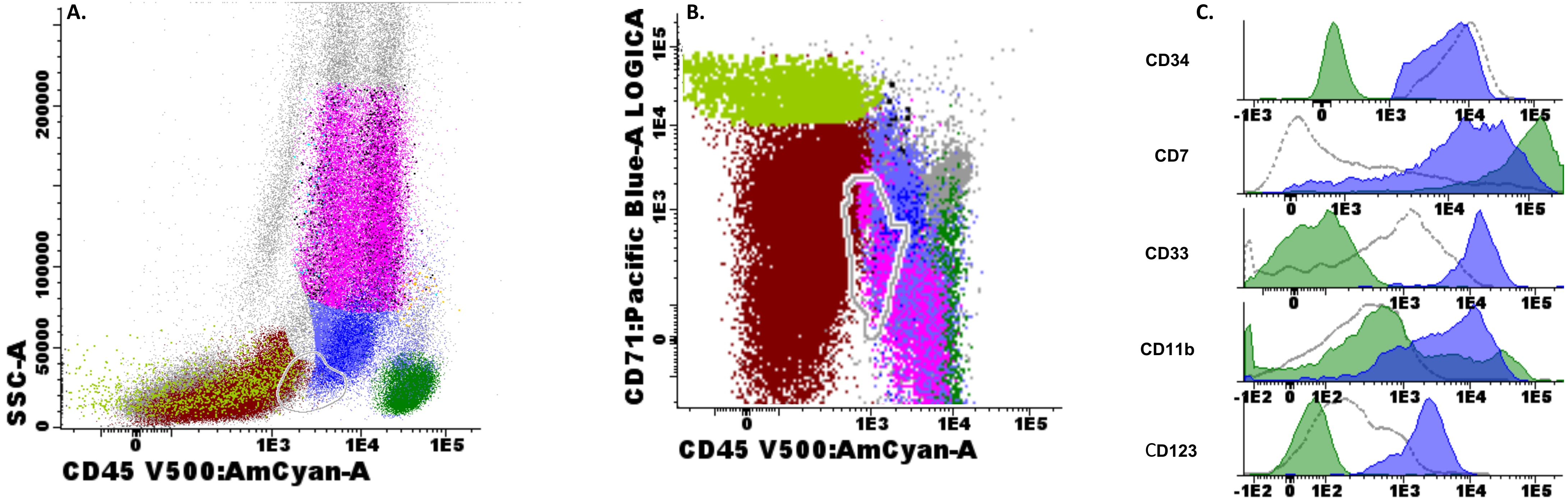
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment[d]	Percentage of nucleated erythroid cells Relationship CD71 and CD235a Expression of CD71 Expression of CD36 Percentage of CD117-positive precursors	Increased Altered pattern[c] Decreased Decreased Increased

[a] Discrepancies in counts between several definitions indicate aberrancies. [b] To be used with caution, as CD56 can be upregulated upon activation, be aware of normal cut-off values (also in stressed marrow). [c] Altered patterns can include altered distribution of maturation stages and/or altered expression levels of indicated antigens. [d] Under evaluation. Examples of several flow cytometric aberrancies in myelodysplastic syndrome can be found on the European LeukemiaNet website: www.leukemia-net.org.

Van de Loosdrecht AA, et al., Haematologica 2009; 94:1124-34
Westers TM, et al., Leukemia 2012;36:422-30; Porwit A, et al., Leukemia 2014:28:1793-98

Aberrant antigen expression on the CD34+ myeloid progenitor population

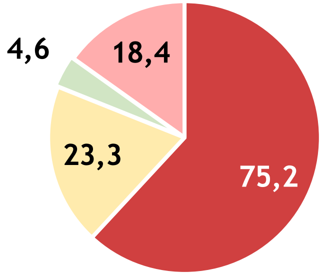
a 30y pregnant female presenting with rapidly progressive anemia and no clear evidence of dysplasia by cytomorphology



Westers TM, et al., Cytometry B Clin Cytom 2022: Feb 18. doi:10.1002

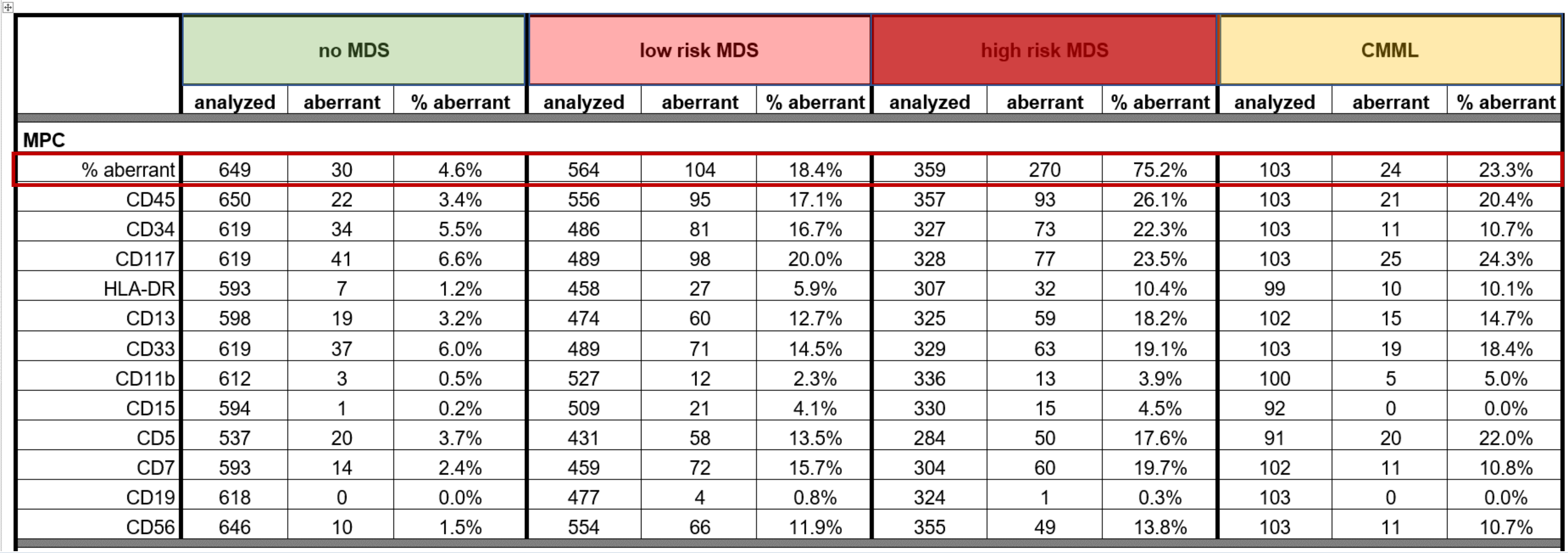
ELN Multicenter study on MDS and FC: myeloid progenitors (MPC)

Univariate analysis



European LeukemiaNet

Table 10: Aberrancies in MDS-Subtypes

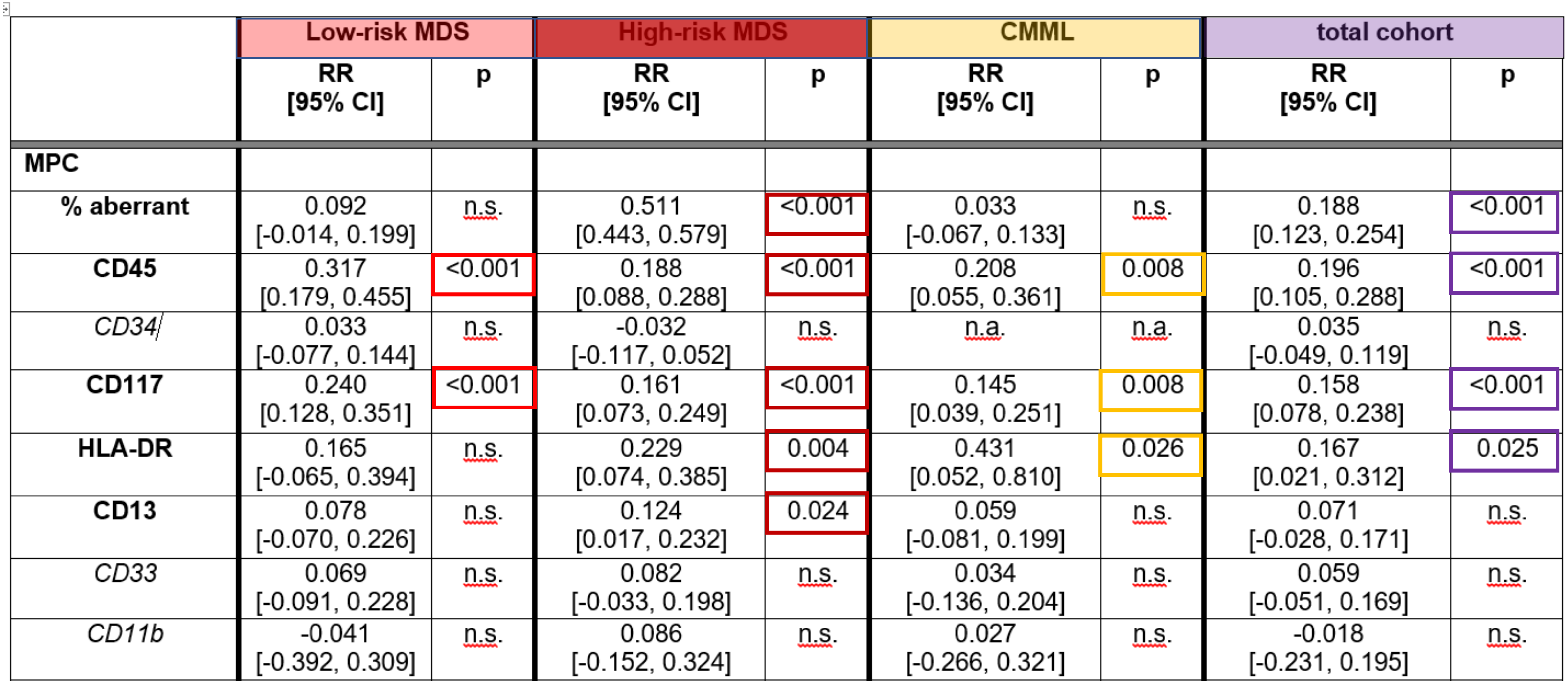


	no MDS			low risk MDS			high risk MDS			CMML		
	analyzed	aberrant	% aberrant	analyzed	aberrant	% aberrant	analyzed	aberrant	% aberrant	analyzed	aberrant	% aberrant
MPC												
% aberrant	649	30	4.6%	564	104	18.4%	359	270	75.2%	103	24	23.3%
CD45	650	22	3.4%	556	95	17.1%	357	93	26.1%	103	21	20.4%
CD34	619	34	5.5%	486	81	16.7%	327	73	22.3%	103	11	10.7%
CD117	619	41	6.6%	489	98	20.0%	328	77	23.5%	103	25	24.3%
HLA-DR	593	7	1.2%	458	27	5.9%	307	32	10.4%	99	10	10.1%
CD13	598	19	3.2%	474	60	12.7%	325	59	18.2%	102	15	14.7%
CD33	619	37	6.0%	489	71	14.5%	329	63	19.1%	103	19	18.4%
CD11b	612	3	0.5%	527	12	2.3%	336	13	3.9%	100	5	5.0%
CD15	594	1	0.2%	509	21	4.1%	330	15	4.5%	92	0	0.0%
CD5	537	20	3.7%	431	58	13.5%	284	50	17.6%	91	20	22.0%
CD7	593	14	2.4%	459	72	15.7%	304	60	19.7%	102	11	10.8%
CD19	618	0	0.0%	477	4	0.8%	324	1	0.3%	103	0	0.0%
CD56	646	10	1.5%	554	66	11.9%	355	49	13.8%	103	11	10.7%

Kern W, et al., Cytometry B Clin Cytom 2022; (provisionally accepted)

Multicenter study: myeloid progenitors

Table 11: Multivariate analysis to identify aberrancies independently related to MDS/CMML



	Low-risk MDS		High-risk MDS		CMML		total cohort	
	RR [95% CI]	p	RR [95% CI]	p	RR [95% CI]	p	RR [95% CI]	p
MPC								
% aberrant	0.092 [-0.014, 0.199]	n.s.	0.511 [0.443, 0.579]	<0.001	0.033 [-0.067, 0.133]	n.s.	0.188 [0.123, 0.254]	<0.001
CD45	0.317 [0.179, 0.455]	<0.001	0.188 [0.088, 0.288]	<0.001	0.208 [0.055, 0.361]	0.008	0.196 [0.105, 0.288]	<0.001
CD34	0.033 [-0.077, 0.144]	n.s.	-0.032 [-0.117, 0.052]	n.s.	n.a.	n.a.	0.035 [-0.049, 0.119]	n.s.
CD117	0.240 [0.128, 0.351]	<0.001	0.161 [0.073, 0.249]	<0.001	0.145 [0.039, 0.251]	0.008	0.158 [0.078, 0.238]	<0.001
HLA-DR	0.165 [-0.065, 0.394]	n.s.	0.229 [0.074, 0.385]	0.004	0.431 [0.052, 0.810]	0.026	0.167 [0.021, 0.312]	0.025
CD13	0.078 [-0.070, 0.226]	n.s.	0.124 [0.017, 0.232]	0.024	0.059 [-0.081, 0.199]	n.s.	0.071 [-0.028, 0.171]	n.s.
CD33	0.069 [-0.091, 0.228]	n.s.	0.082 [-0.033, 0.198]	n.s.	0.034 [-0.136, 0.204]	n.s.	0.059 [-0.051, 0.169]	n.s.
CD11b	-0.041 [-0.392, 0.309]	n.s.	0.086 [-0.152, 0.324]	n.s.	0.027 [-0.266, 0.321]	n.s.	-0.018 [-0.231, 0.195]	n.s.

Kern W, et al., Cytometry B Clin Cytom 2022; (provisional accepted)

Schematic representation of FC results: Integrated Flow Score (iFS)

Ogata score	<2	<2	<2	<2	<2	<2	<2	<2	≥2	≥2	≥2	≥2	≥2	≥2	≥2	≥2
Dysplasia by FC myeloid prog.	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Dysplasia by FC - Neutrophils - Monocytes	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Dysplasia by FC - Nucl. erythroid	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Conclusion	A	B	B	C	B	C	C	C	B	C	C	C	C	C	C	C

A = 'results show no MDS-related features'
B = 'results show limited number of changes associated with MDS'
} not enough to consider MDS
C = 'results are consistent with MDS'

VU Hematologie

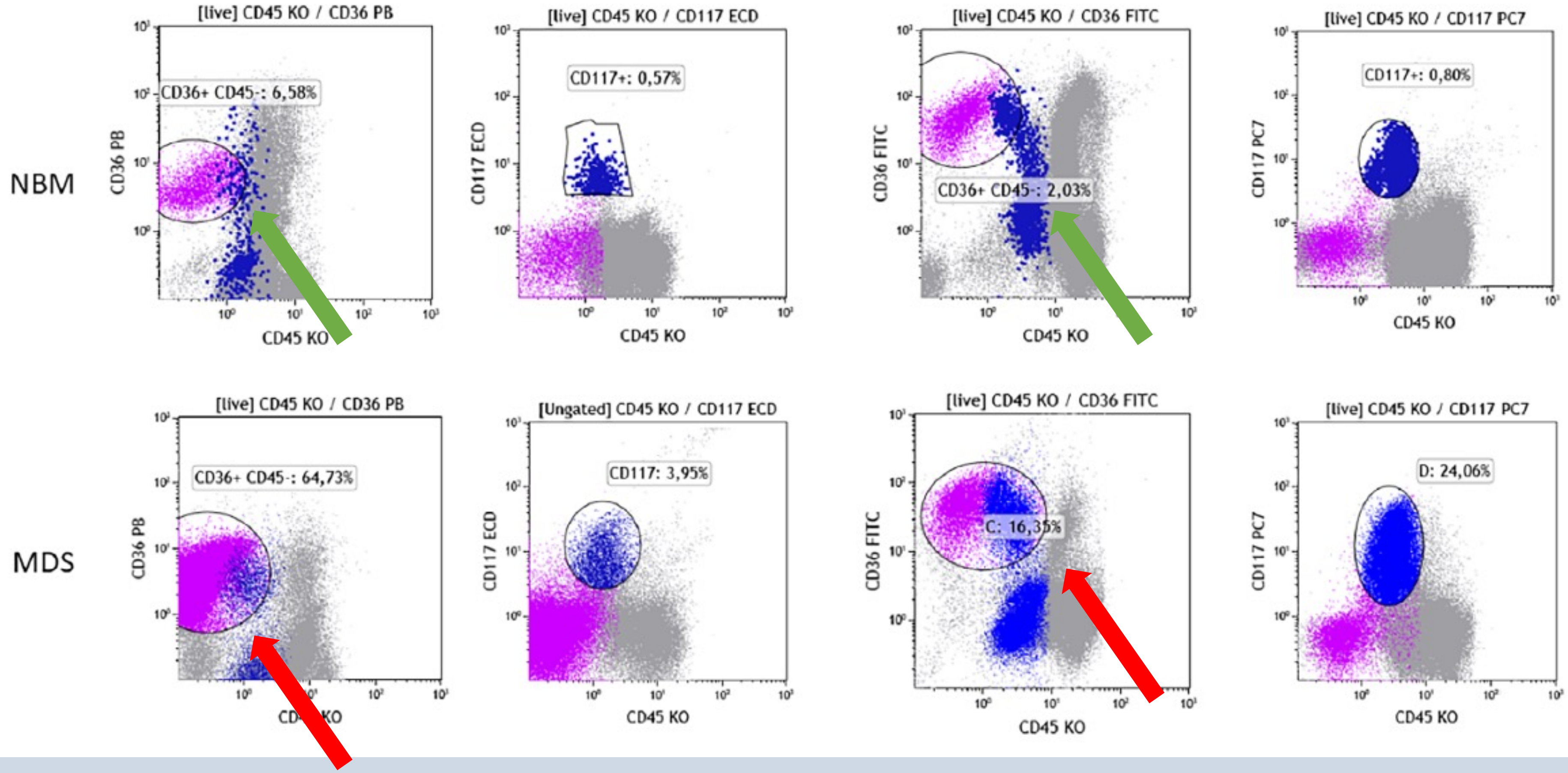
Cremers EMP, et al., Haematologica 2017, 102(2)
Van de Loosdrecht AA and Westers TM. J Nat Compr Cancer Netw 2013;11:892-902)
Porwit A, et al. Cytometry B Clin Cytom 2022 (provisionally accepted)

Recommendations 2022 on pre-analytical and technical issues

Impact of lysis

No lysis gate on DRAQ5+ cells under acquisition

Bulk Lyse with Pharmlyse, stain and wash



European LeukemiaNet

van der Velden VHJ, at al., Cytometry B Clin Cytom 2021: Dec 11. doi:10.1002

Prospective multicenter trial on FC in MDS by the ELN iMDS Flow working group 2022

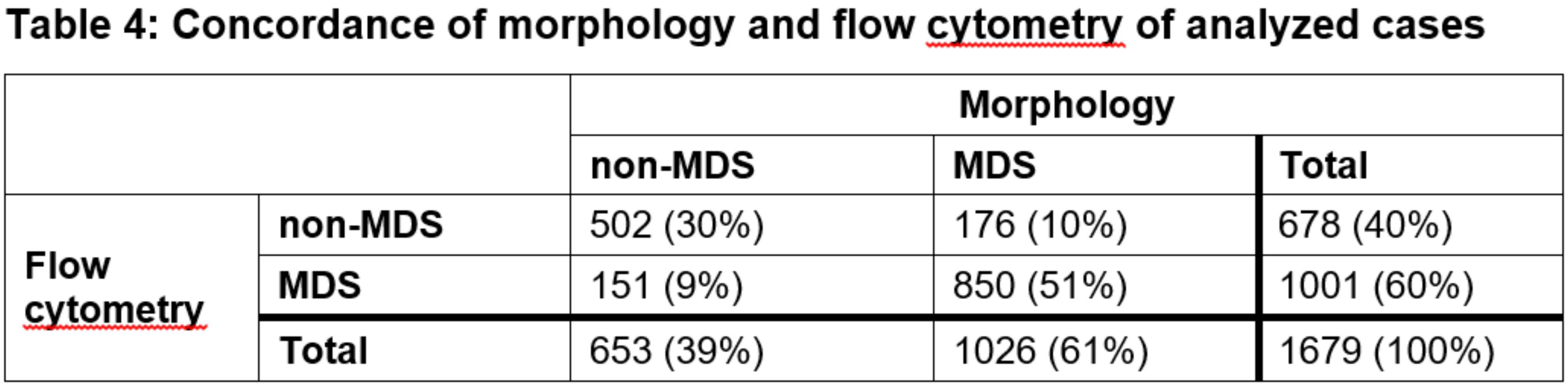


Table 4: Concordance of morphology and flow cytometry of analyzed cases

		Morphology		
		non-MDS	**MDS**	**Total**
Flow cytometry	**non-MDS**	502 (30%)	176 (10%)	678 (40%)
	MDS	151 (9%)	850 (51%)	1001 (60%)
	Total	653 (39%)	1026 (61%)	1679 (100%)

Kern W, et al., Cytometry B Clin Cytom 2022; (provisionally accepted)

Prognostic scoring systems in MDS: integrated flow score (iFS) vs. IPSS-R

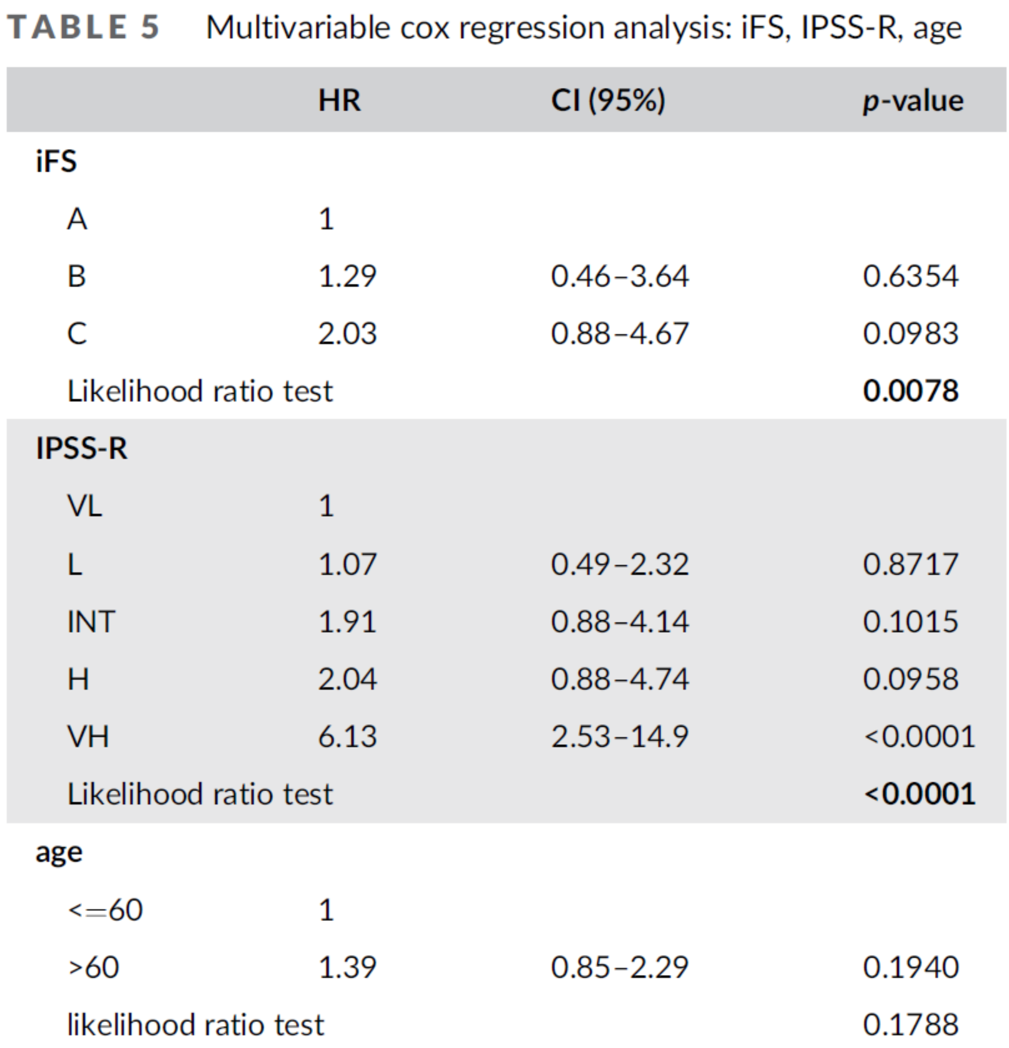
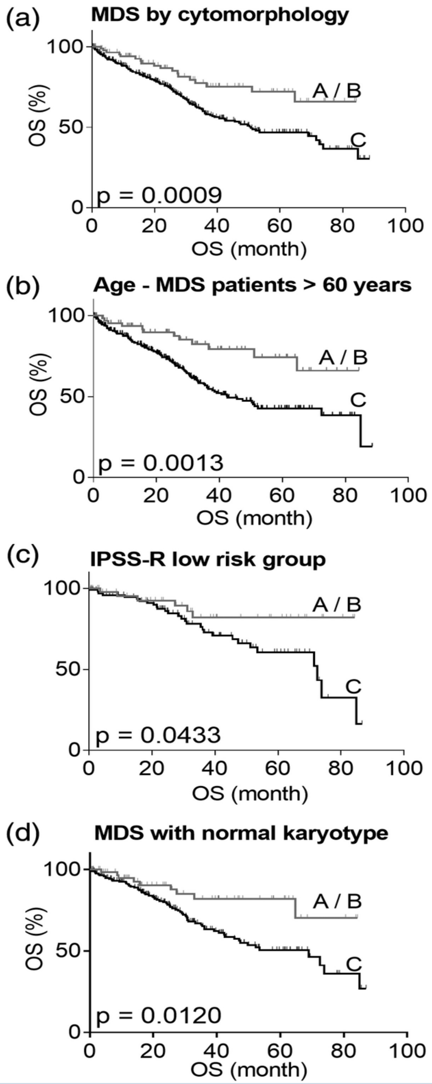


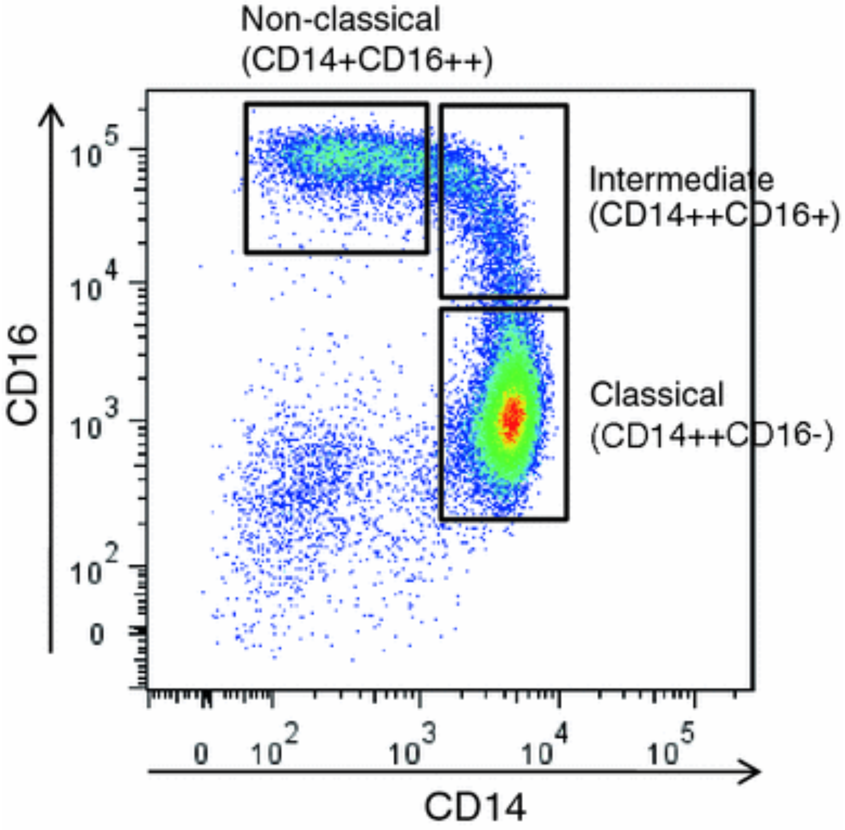
TABLE 5 Multivariable cox regression analysis: iFS, IPSS-R, age

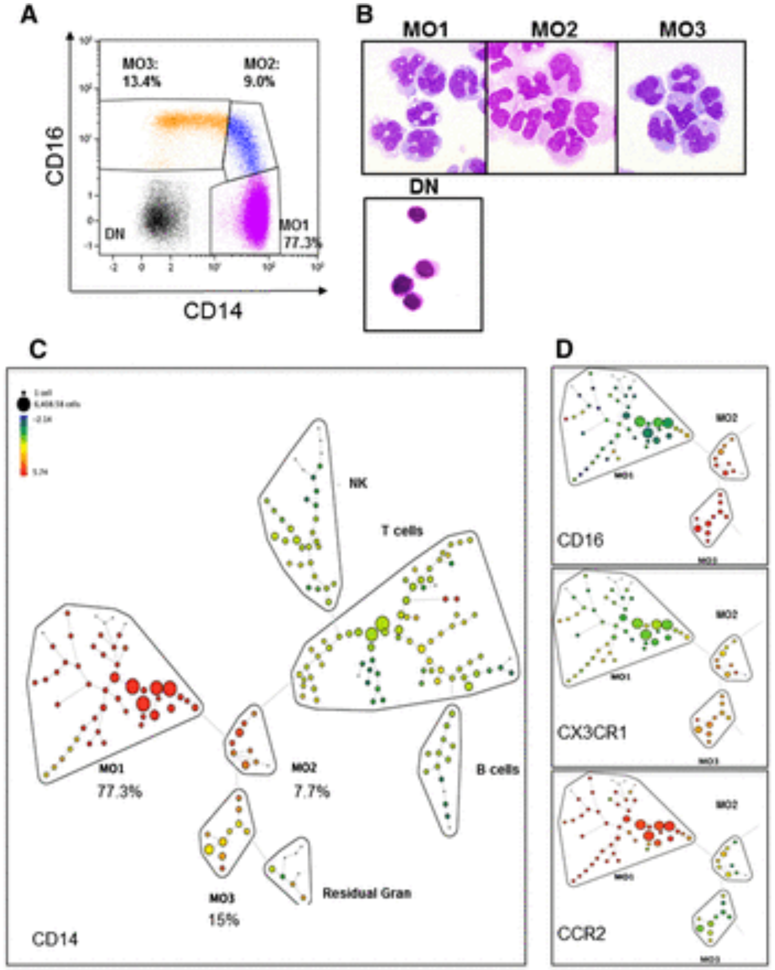
	HR	CI (95%)	*p*-value
iFS			
A	1		
B	1.29	0.46–3.64	0.6354
C	2.03	0.88–4.67	0.0983
Likelihood ratio test			**0.0078**
IPSS-R			
VL	1		
L	1.07	0.49–2.32	0.8717
INT	1.91	0.88–4.14	0.1015
H	2.04	0.88–4.74	0.0958
VH	6.13	2.53–14.9	<0.0001
Likelihood ratio test			**<0.0001**
age			
<=60	1		
>60	1.39	0.85–2.29	0.1940
likelihood ratio test			0.1788



Oelschlaegel U, et al., Cytometry B Clin Cytom 2021: aug 14. doi:10.1002

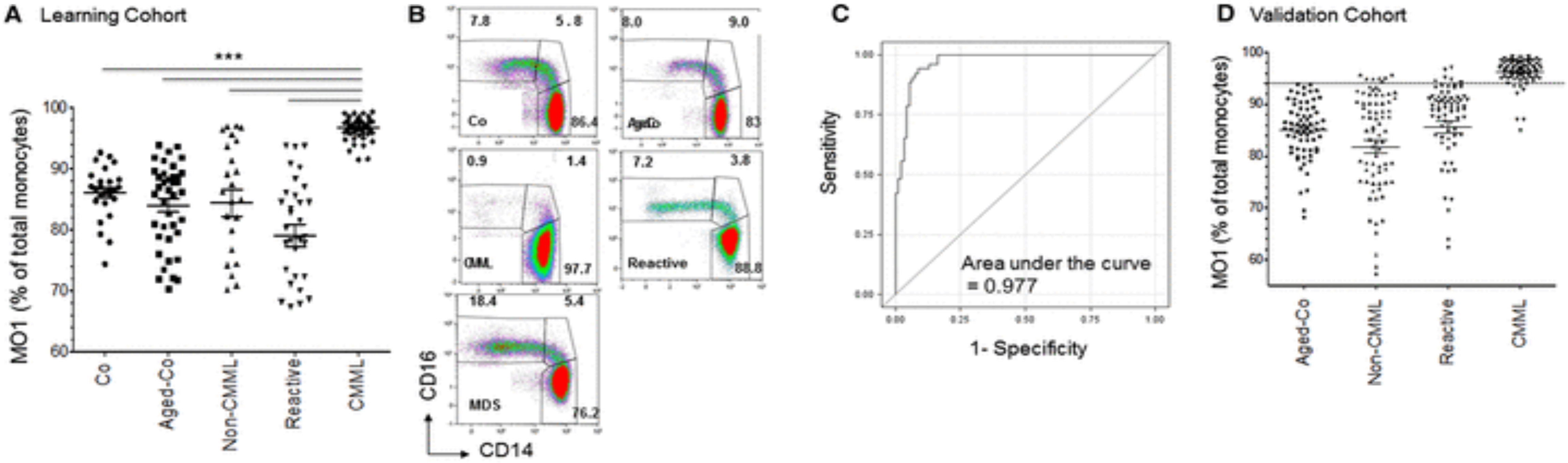
FC in CMML: Normal monocyte subset definitions





Zawada A et al., Immunobiol 2017:831-840; Ziegler-Heitbrock-L. Front Immunol 2015:6;423; Wong KL et al., Blood 2011;118:e16-31
Wong KL et al., Immunol Res 2012;53:41-57; Selimoglu-Buet, L, et al., Blood 2015;125:3618-26

Abnormal repartition of monocyte subsets in CMML



ROC: cut-off: 94% MO1
sensitivity 90%
specificity 95%

Selimoglu-Buet, L, et al., Blood 2015;125:3618-26

Validation of monocyte subsets in CMML by ELN iMDS Flow WG

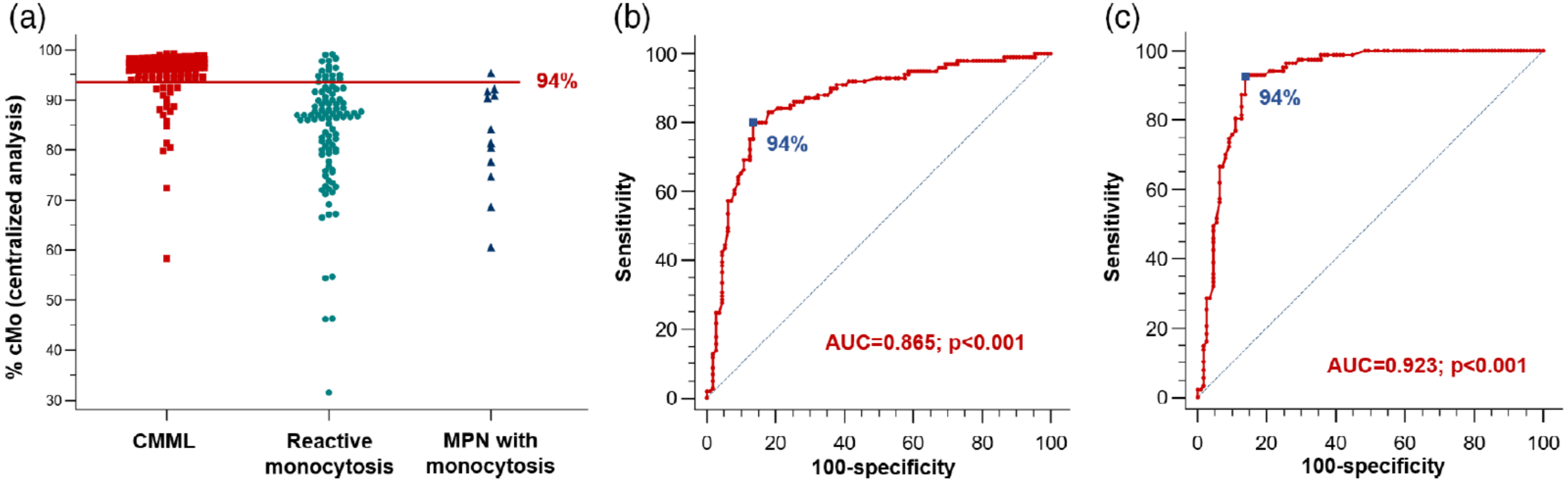
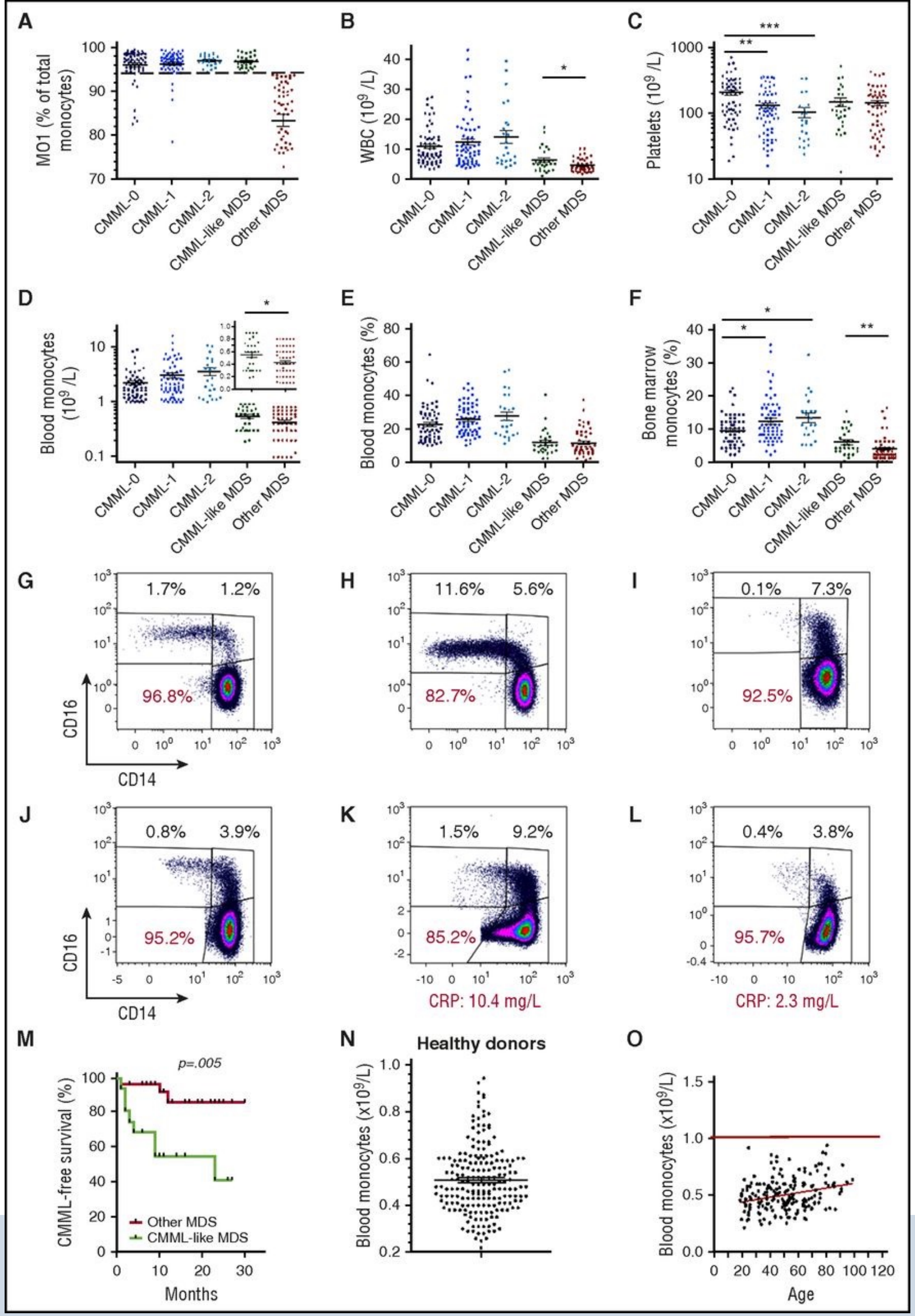


FIGURE 3 Sensitivity and specificity of the "monocyte assay". (a) cMo percentages determined by centralized analysis for 101 CMML patients (median: 96.2% [range: 58.2–99.3]), 99 patients with reactive monocytosis (median: 87.0% [range: 31.5–99.1]) and 12 patients suffering from MPN with monocytosis (median: 82.8% [range: 60.5–95.3]). (b) Receiver operator curve (ROC) establishing from the 212 flow profiles a 94% cMo cut-off value. (c) Receiver operator curve (ROC) establishing from the 196 flow profiles without a bulbous aspect a 94% cMo cut-off value

Wagner-Ballon O, et al., Cytometry B Clin Cytom 2021: Dec 30. doi:10.1002

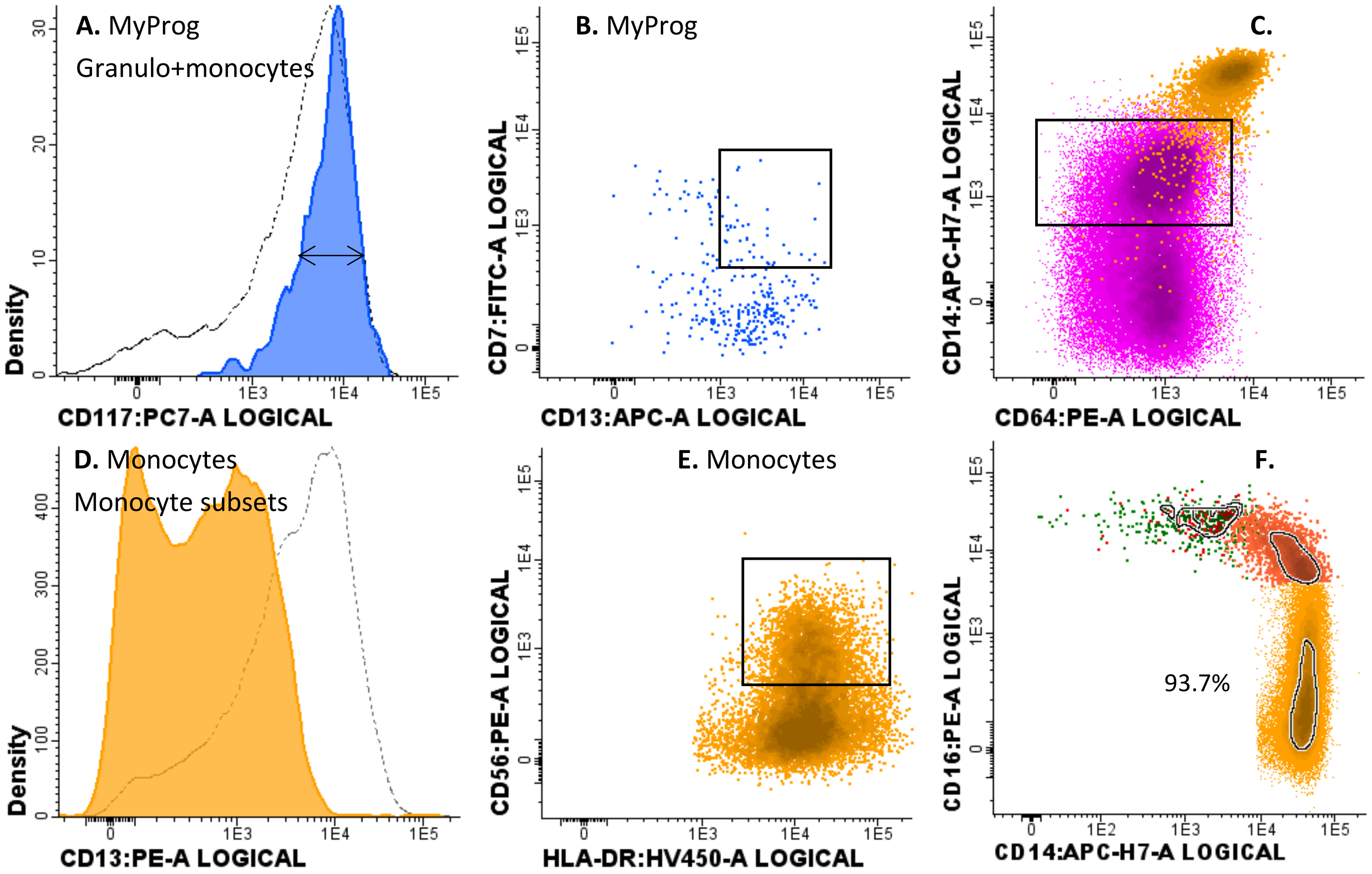


CMML-like MDS (not fulfilling CMML criteria) with >94% MO1 evolve to overt CMML (A-F/M)

(K-L): Associated inflammatory conditions give rise to false-neg FC CMML diagnosis

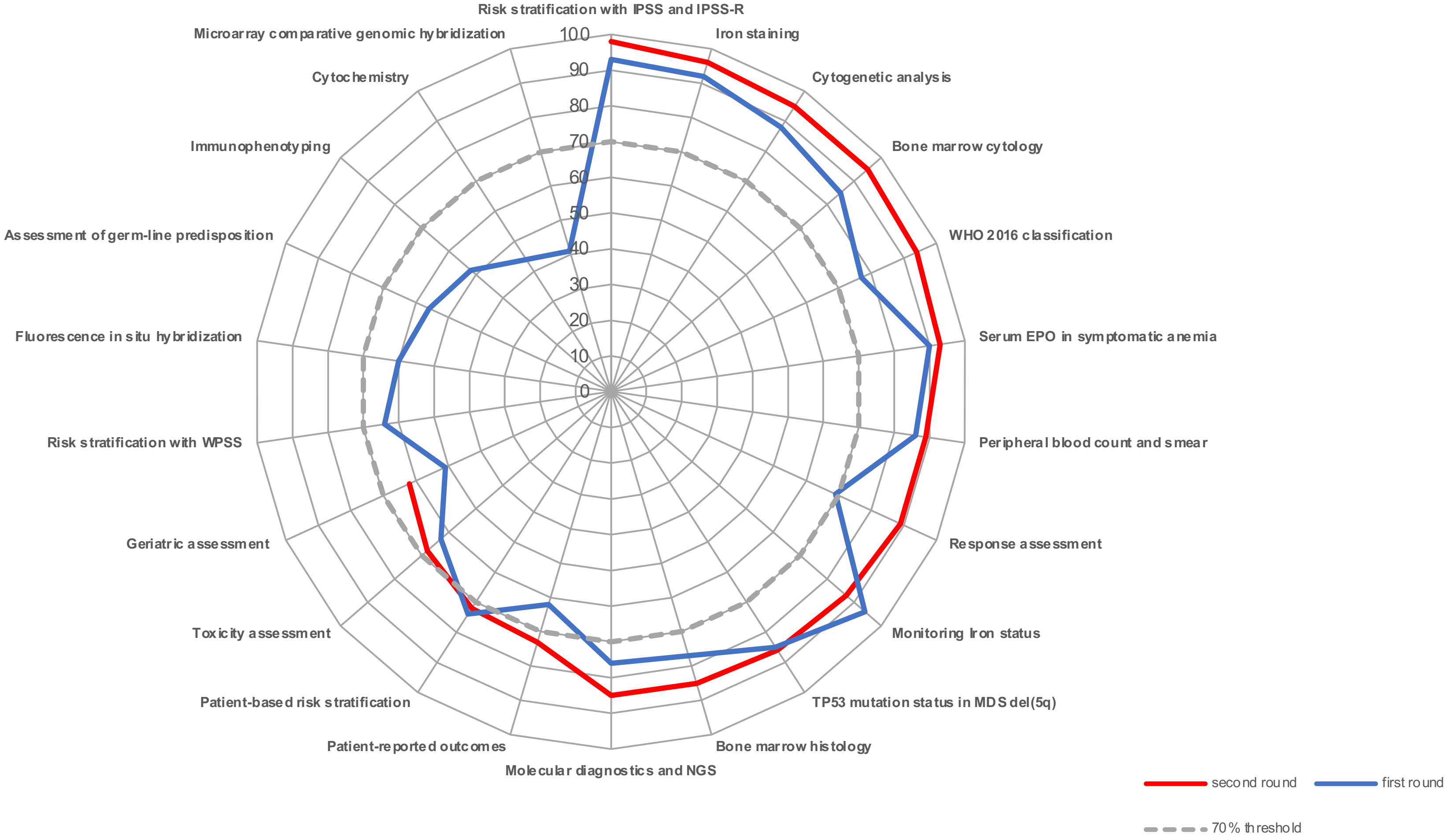
Selimoglu-Buet D, et al, Blood 2017

Immunophenotypic aberrancies in a case of MDS/CMML



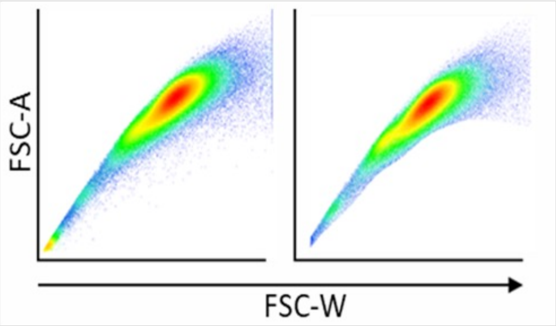
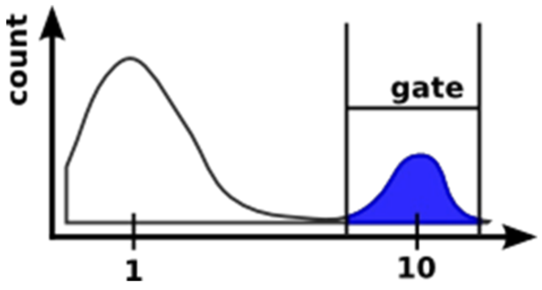
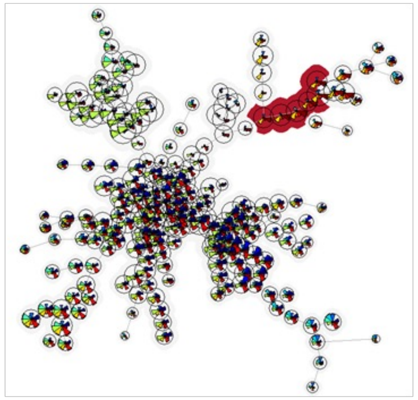
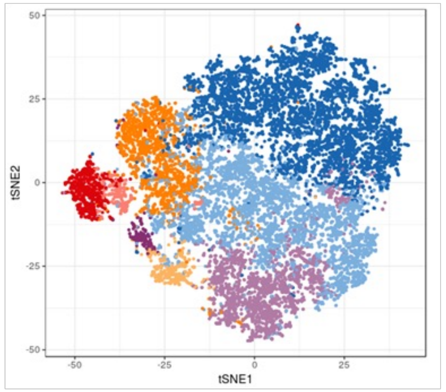
Westers TM, et al., Cytometry B Clin Cytom 2022: Feb 18

Guideline Based Indicators: *MDS Diagnosis* (DELPHI rating rounds)



Stojkov K, Bonadies N., et al., Blood Adv. 2020;4:4029-44

Several types of analysis

Manual/expert gating	Computer-assisted gating	Clustering	Dimensionality reduction
			

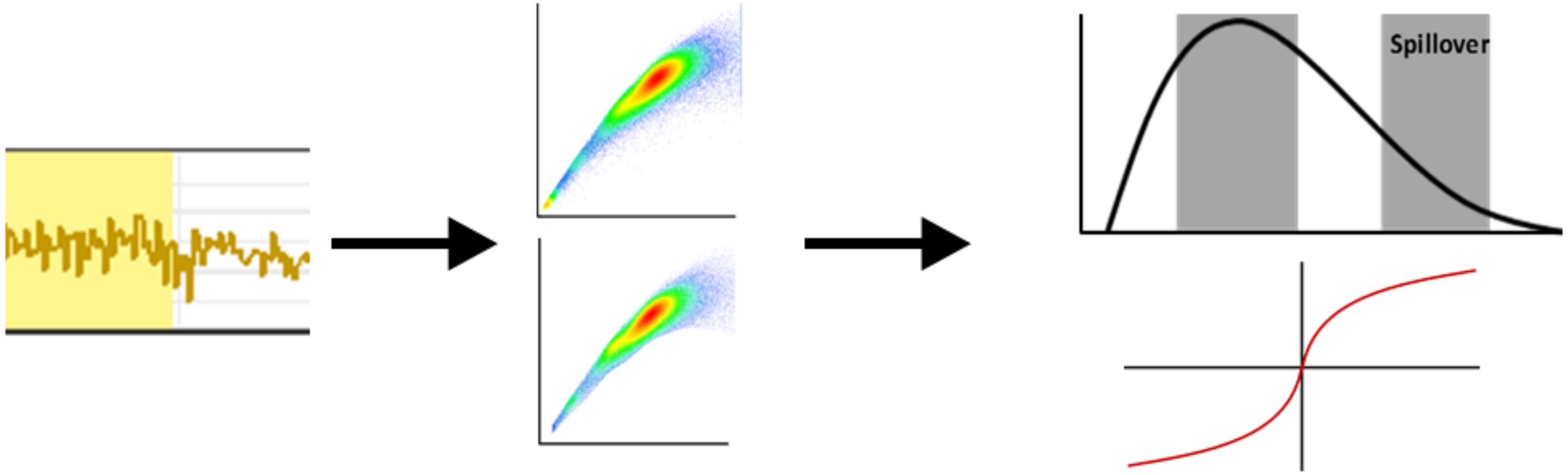
Saeys, Y., Van Gassen, S. & Lambrecht, B. *Nat Rev Immunol* 16, 449-462 (2016).

Computational FC in MDS

1 Preprocessing

Raw fcs files were preprocessed computationally.

-Time-gating by FlowAI (1)
-Compensation for spillover
-ArcsinH tranformation
-Singlet selection (2)
-Scatter outlier removal

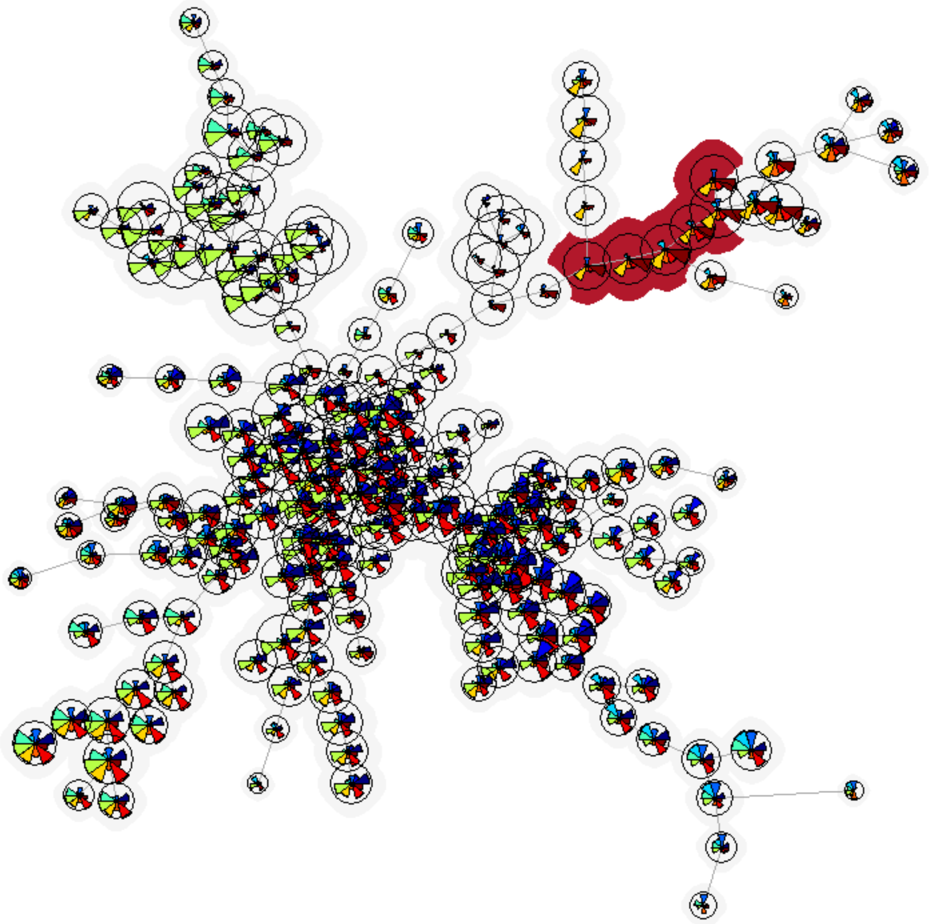


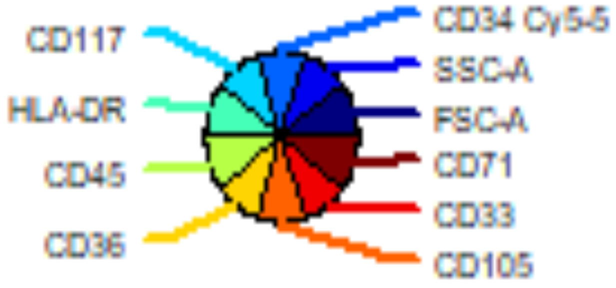
Duetz C, et al. Curr Opin Oncol. 2020;32:162-169
Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational FC in MDS

2 Feature extraction

Computational cell population detection was performed using the FlowSOM method. Relative population size, MFI and CV, were calculated per cell population (3) and patient.



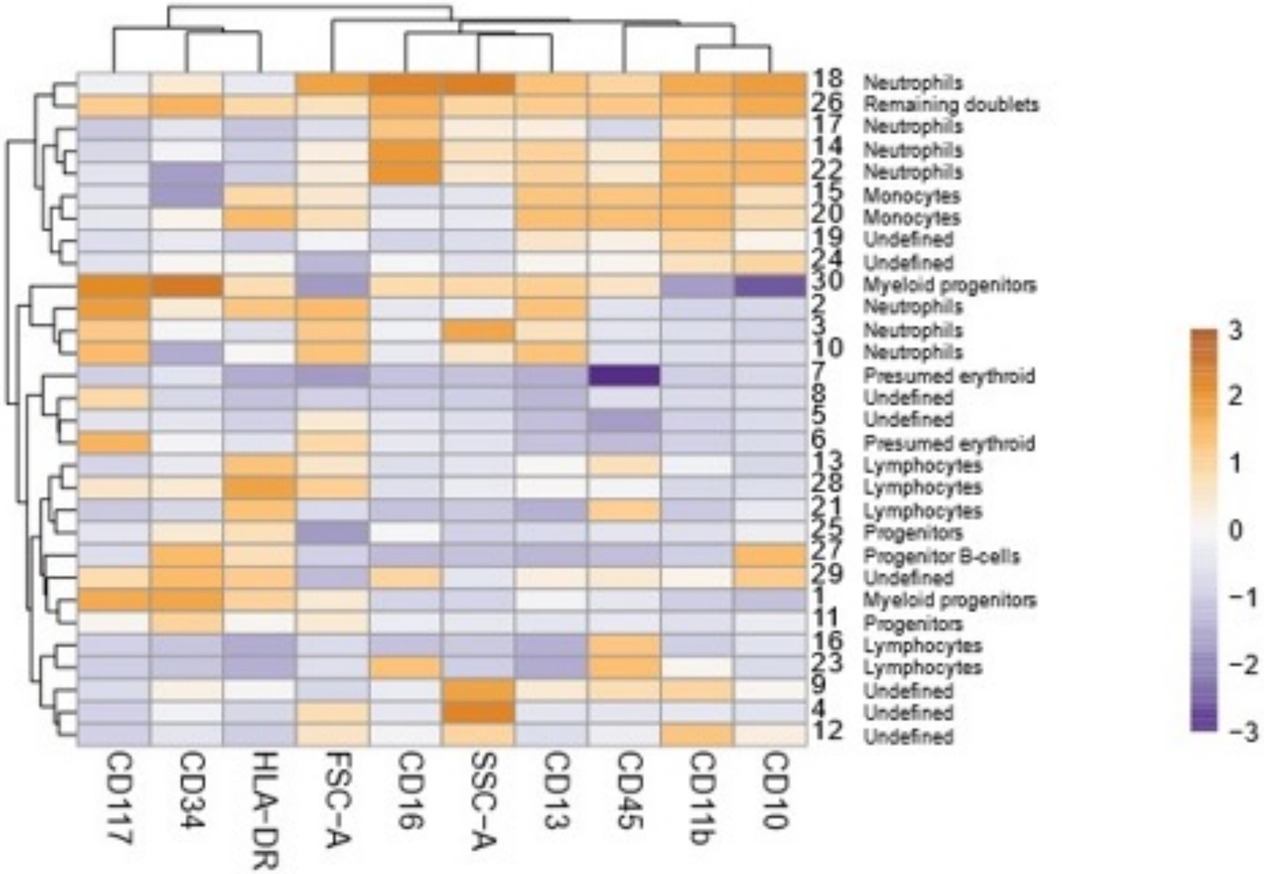


Per patient & MC:
MFI
CV
Abundance

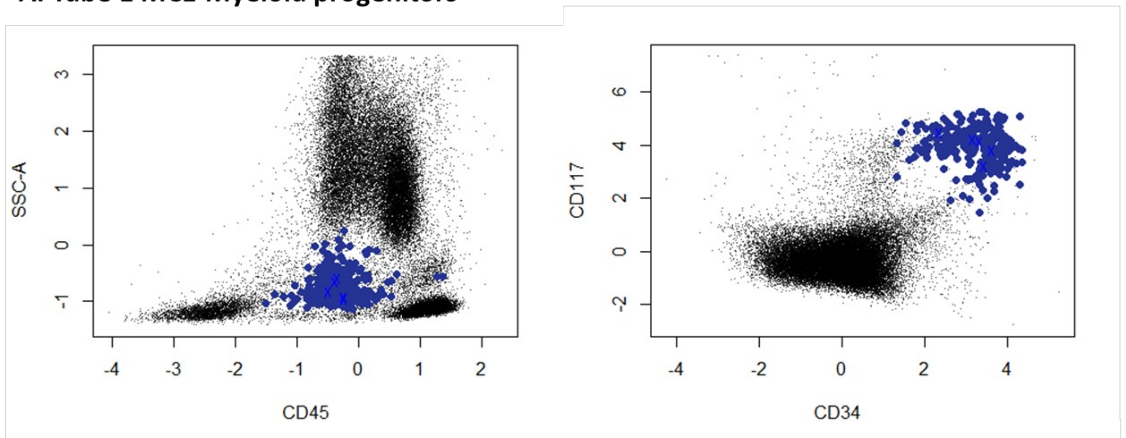
Duetz C, et al. Curr Opin Oncol. 2020;32:162-169
Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Expression heatmaps and cell subset identification

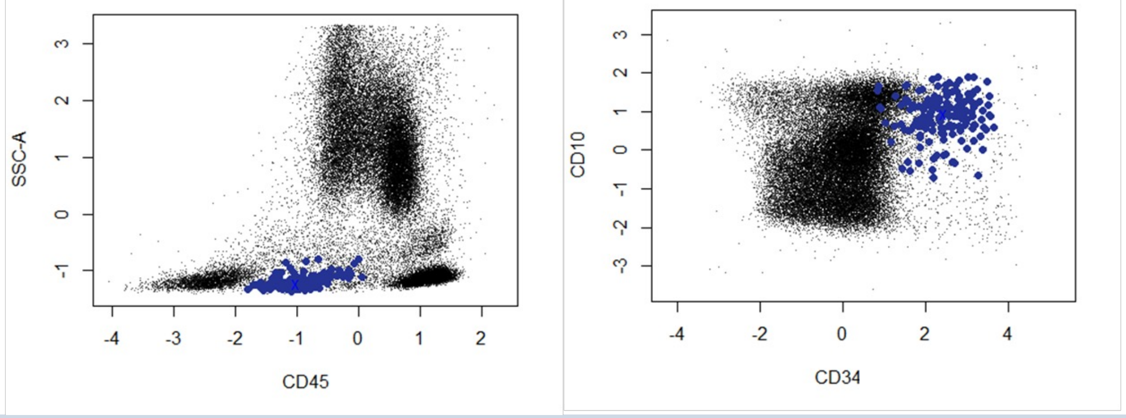
For each tube a heatmap visualizes the median marker expression levels and scatter signals (columns) over FlowSOM metacluster (rows)



A. Tube 1 MC1 Myeloid progenitors



B. Tube 1 MC27 Progenitor B-cells

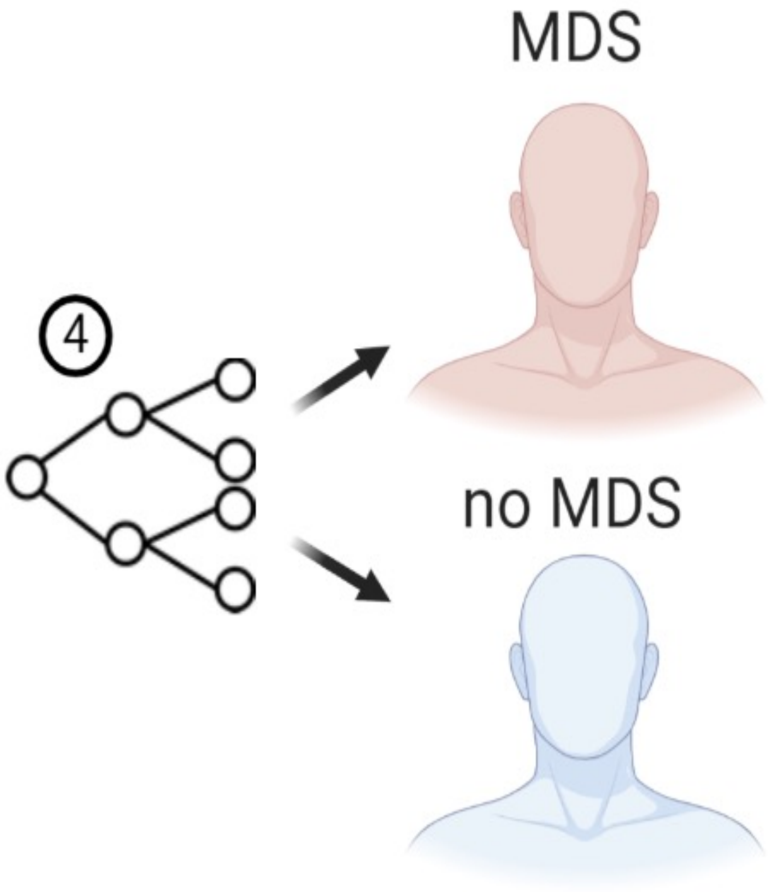


Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

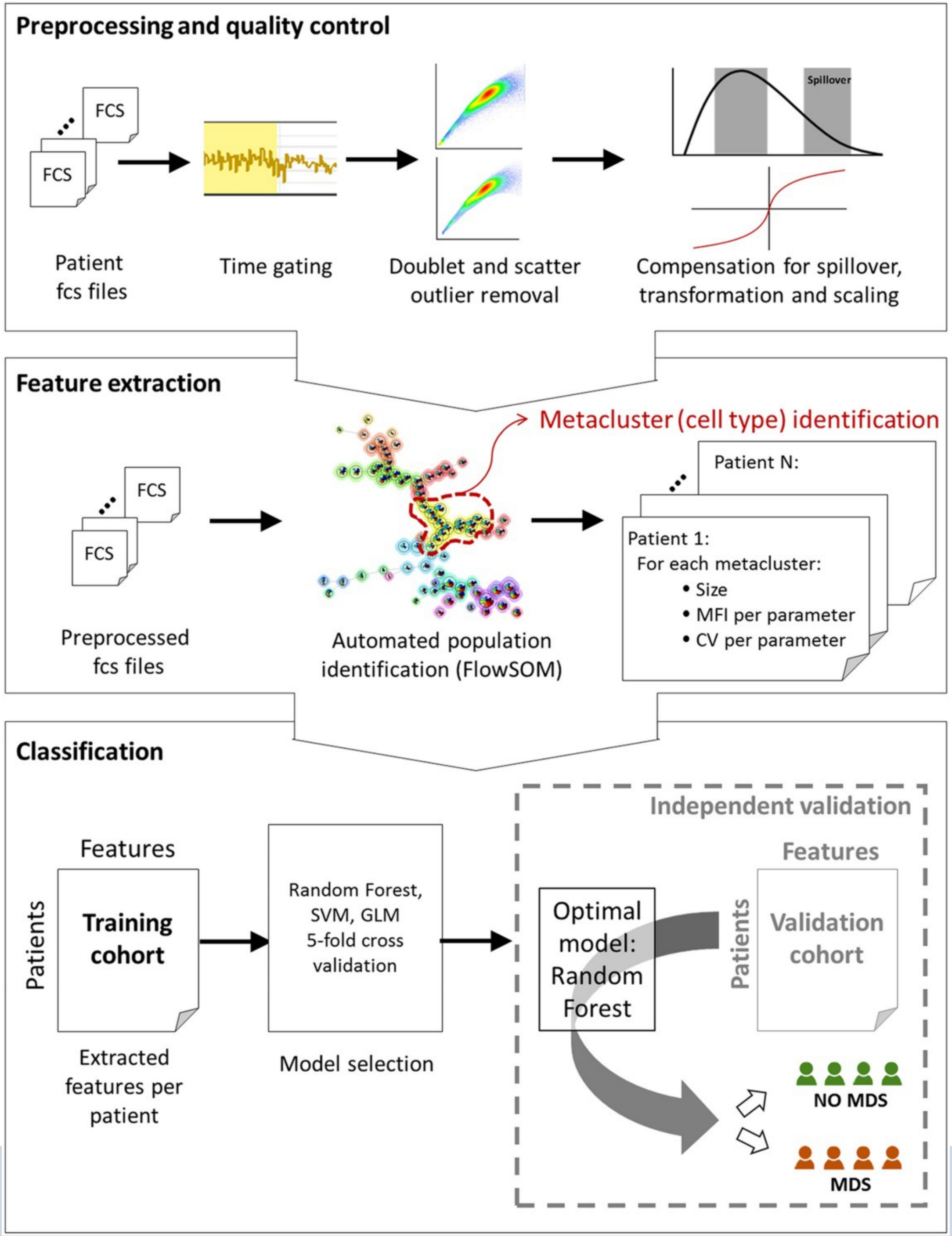
Computational FC in MDS

3 Classification

The machine learning algorithm Random Forest (4) classified patients as MDS or no MDS based on a reference cohort.



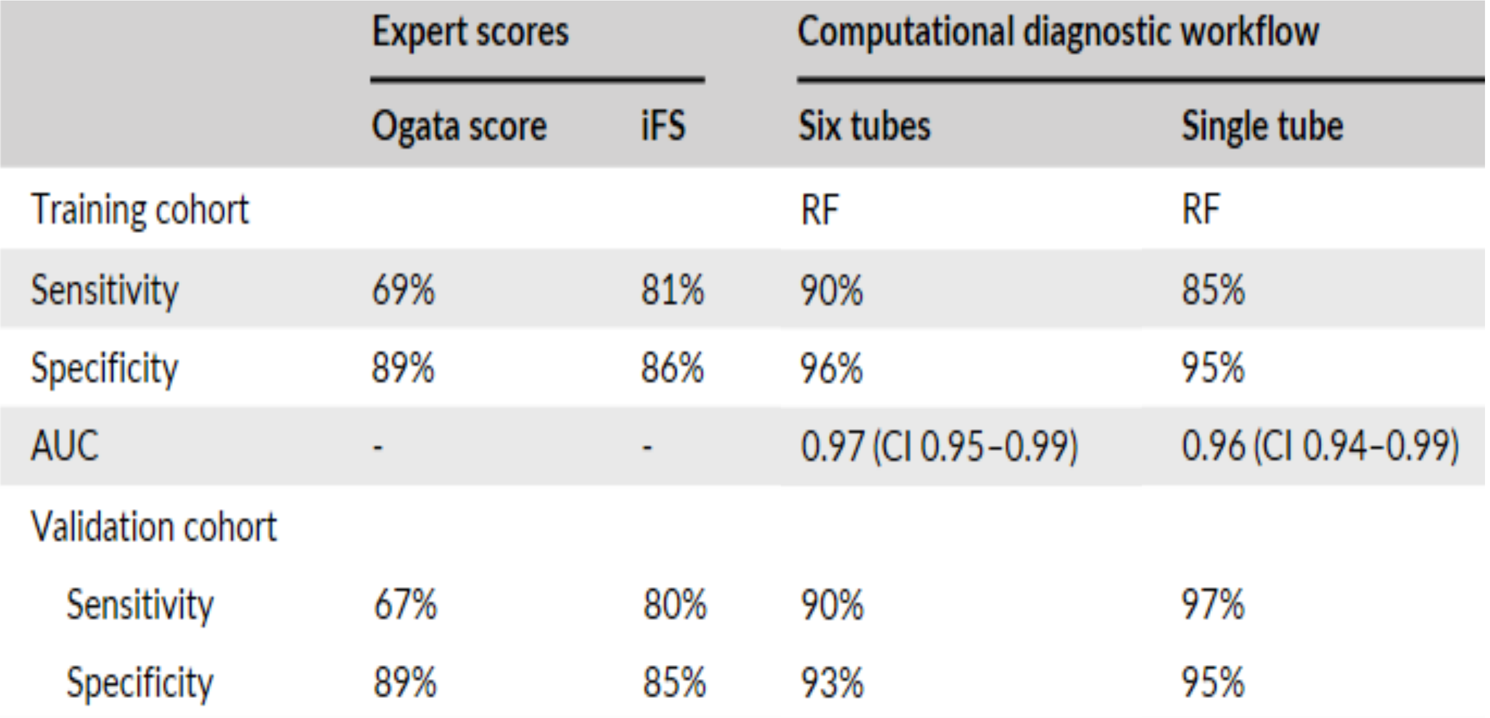
Duetz C, et al. Curr Opin Oncol. 2020;32:162-169
Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824



Computational flow cytometry in MDS

Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational FC as a diagnostic tool in suspected-MDS



	Expert scores		Computational diagnostic workflow	
	Ogata score	iFS	Six tubes	Single tube
Training cohort			RF	RF
Sensitivity	69%	81%	90%	85%
Specificity	89%	86%	96%	95%
AUC	-	-	0.97 (CI 0.95-0.99)	0.96 (CI 0.94-0.99)
Validation cohort				
Sensitivity	67%	80%	90%	97%
Specificity	89%	85%	93%	95%

Duetz C, et al. Curr Opin Oncol. 2020;32:162-169
Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational FC in MDS

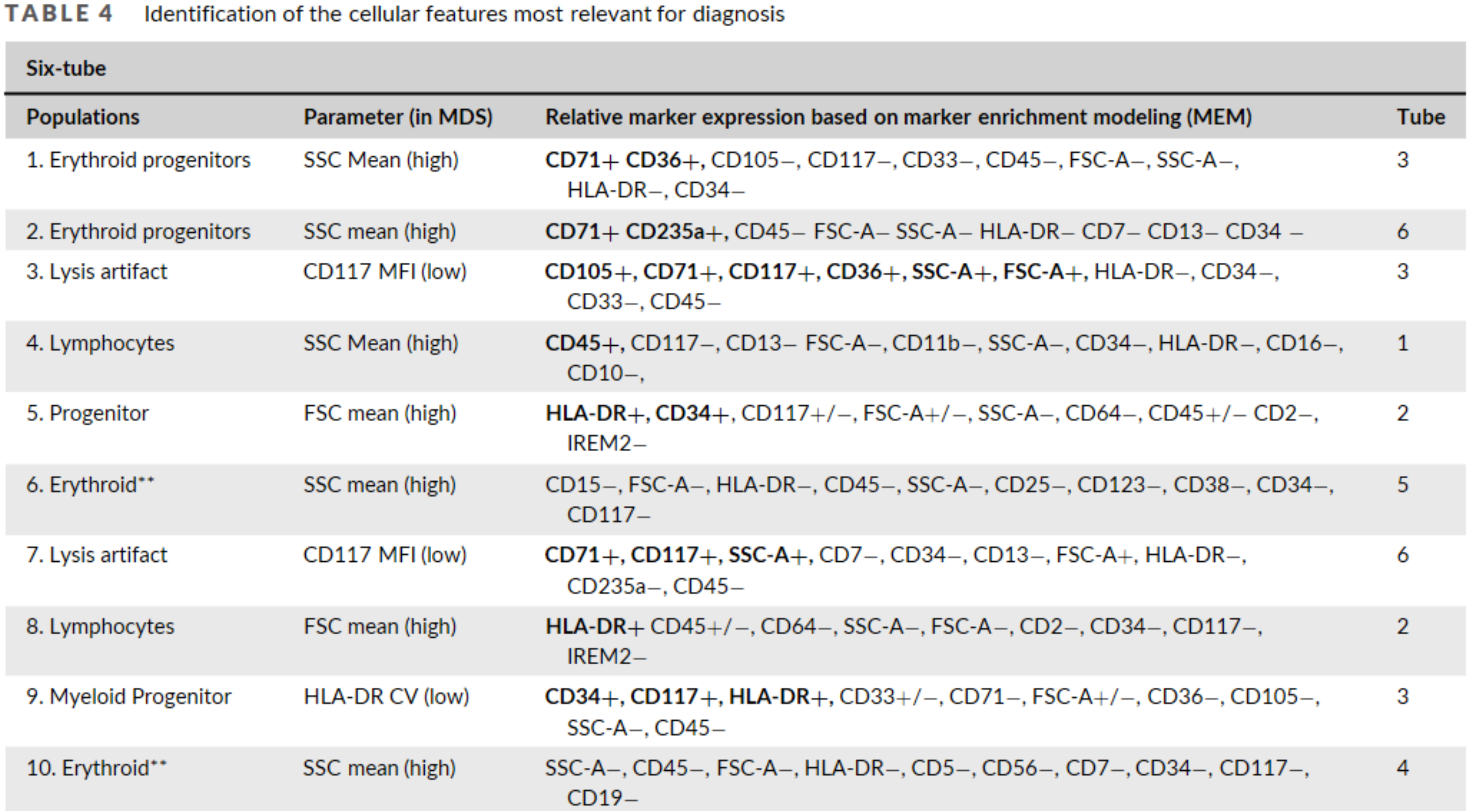
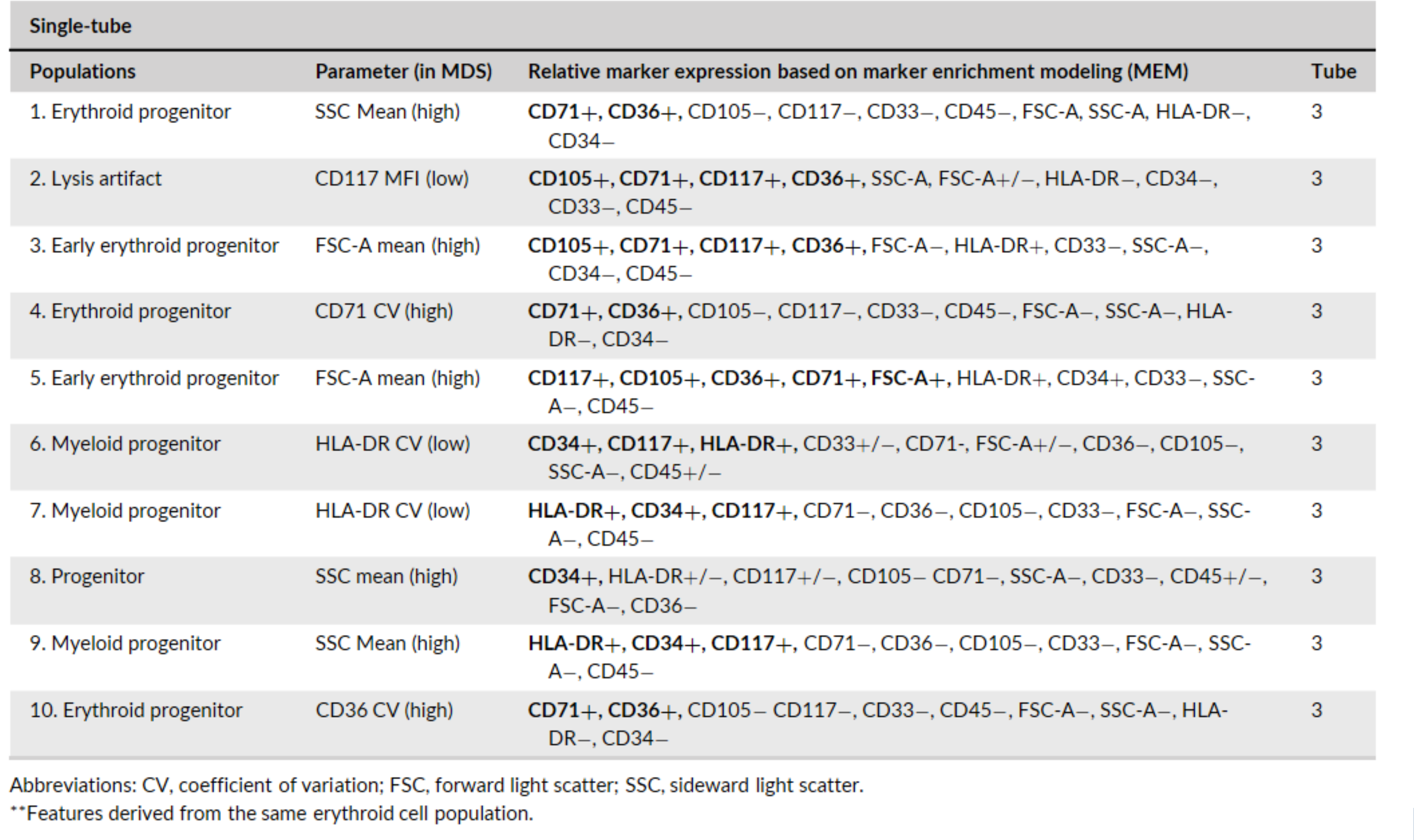


TABLE 4 Identification of the cellular features most relevant for diagnosis

Six-tube			
Populations	**Parameter (in MDS)**	**Relative marker expression based on marker enrichment modeling (MEM)**	**Tube**
1. Erythroid progenitors	SSC Mean (high)	**CD71+ CD36+,** CD105–, CD117–, CD33–, CD45–, FSC-A–, SSC-A–, HLA-DR–, CD34–	3
2. Erythroid progenitors	SSC mean (high)	**CD71+ CD235a+,** CD45– FSC-A– SSC-A– HLA-DR– CD7– CD13– CD34 –	6
3. Lysis artifact	CD117 MFI (low)	**CD105+, CD71+, CD117+, CD36+, SSC-A+, FSC-A+,** HLA-DR–, CD34–, CD33–, CD45–	3
4. Lymphocytes	SSC Mean (high)	**CD45+,** CD117–, CD13– FSC-A–, CD11b–, SSC-A–, CD34–, HLA-DR–, CD16–, CD10–,	1
5. Progenitor	FSC mean (high)	**HLA-DR+, CD34+,** CD117+/–, FSC-A+/–, SSC-A–, CD64–, CD45+/– CD2–, IREM2–	2
6. Erythroid**	SSC mean (high)	CD15–, FSC-A–, HLA-DR–, CD45–, SSC-A–, CD25–, CD123–, CD38–, CD34–, CD117–	5
7. Lysis artifact	CD117 MFI (low)	**CD71+, CD117+, SSC-A+,** CD7–, CD34–, CD13–, FSC-A+, HLA-DR–, CD235a–, CD45–	6
8. Lymphocytes	FSC mean (high)	**HLA-DR+** CD45+/–, CD64–, SSC-A–, FSC-A–, CD2–, CD34–, CD117–, IREM2–	2
9. Myeloid Progenitor	HLA-DR CV (low)	**CD34+, CD117+, HLA-DR+,** CD33+/–, CD71–, FSC-A+/–, CD36–, CD105–, SSC-A–, CD45–	3
10. Erythroid**	SSC mean (high)	SSC-A–, CD45–, FSC-A–, HLA-DR–, CD5–, CD56–, CD7–, CD34–, CD117–, CD19–	4

Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational FC in MDS



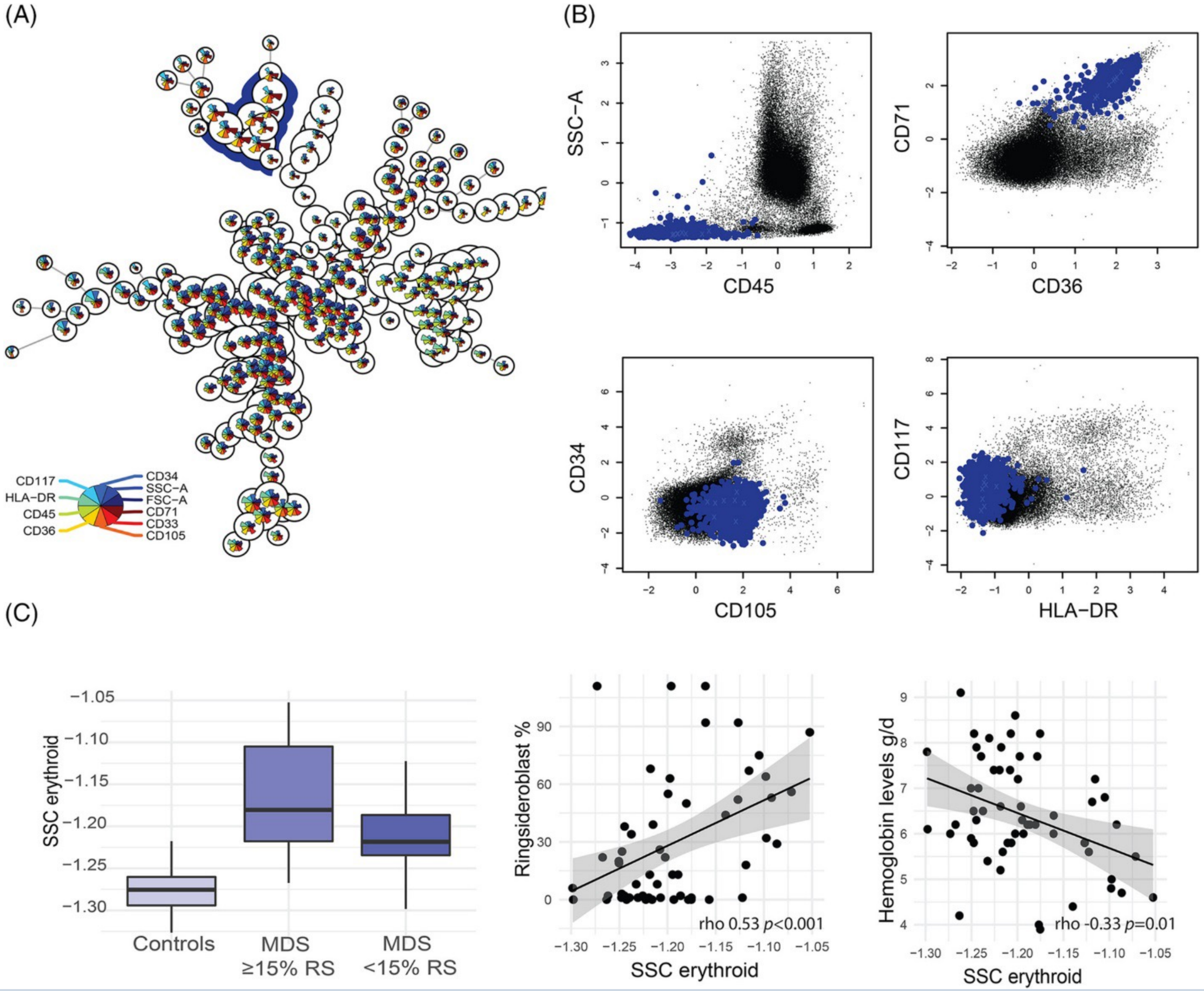
Single-tube

Populations	Parameter (in MDS)	Relative marker expression based on marker enrichment modeling (MEM)	Tube
1. Erythroid progenitor	SSC Mean (high)	**CD71+, CD36+,** CD105−, CD117−, CD33−, CD45−, FSC-A, SSC-A, HLA-DR−, CD34−	3
2. Lysis artifact	CD117 MFI (low)	**CD105+, CD71+, CD117+, CD36+,** SSC-A, FSC-A+/−, HLA-DR−, CD34−, CD33−, CD45−	3
3. Early erythroid progenitor	FSC-A mean (high)	**CD105+, CD71+, CD117+, CD36+,** FSC-A−, HLA-DR+, CD33−, SSC-A−, CD34−, CD45−	3
4. Erythroid progenitor	CD71 CV (high)	**CD71+, CD36+,** CD105−, CD117−, CD33−, CD45−, FSC-A−, SSC-A−, HLA-DR−, CD34−	3
5. Early erythroid progenitor	FSC-A mean (high)	**CD117+, CD105+, CD36+, CD71+, FSC-A+,** HLA-DR+, CD34+, CD33−, SSC-A−, CD45−	3
6. Myeloid progenitor	HLA-DR CV (low)	**CD34+, CD117+, HLA-DR+,** CD33+/−, CD71-, FSC-A+/−, CD36−, CD105−, SSC-A−, CD45+/−	3
7. Myeloid progenitor	HLA-DR CV (low)	**HLA-DR+, CD34+, CD117+,** CD71−, CD36−, CD105−, CD33−, FSC-A−, SSC-A−, CD45−	3
8. Progenitor	SSC mean (high)	**CD34+,** HLA-DR+/−, CD117+/−, CD105− CD71−, SSC-A−, CD33−, CD45+/−, FSC-A−, CD36−	3
9. Myeloid progenitor	SSC Mean (high)	**HLA-DR+, CD34+, CD117+,** CD71−, CD36−, CD105−, CD33−, FSC-A−, SSC-A−, CD45−	3
10. Erythroid progenitor	CD36 CV (high)	**CD71+, CD36+,** CD105− CD117−, CD33−, CD45−, FSC-A−, SSC-A−, HLA-DR−, CD34−	3

Abbreviations: CV, coefficient of variation; FSC, forward light scatter; SSC, sideward light scatter.
**Features derived from the same erythroid cell population.

Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational FC as a diagnostic tool in suspected-MDS



Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Conclusions and future directions

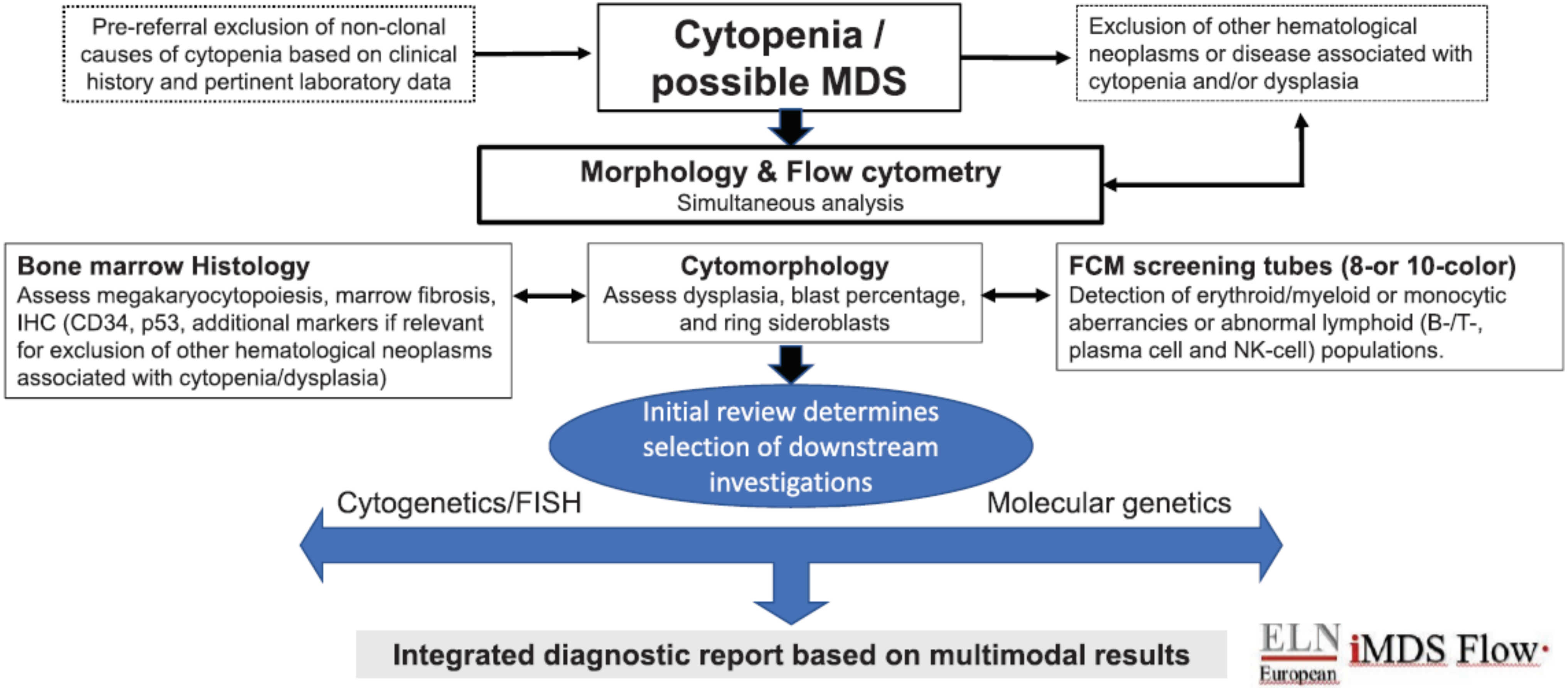
Unsupervised computational FC in MDS

- High diagnostic accuracy
- Prospectively validated
- < 30 seconds – 3 minutes
- Reduced number of cells needed including antibody use up to 86%
- Known and unknown cellular features

- (Prospective) multi-center validation ongoing
  - Challenges with scatter harmonization
  - Differences in sample preparation
  - Exploration of more MDS-specific panels
  - Optimization of the computational workflow

Duetz C, et al. Curr Opin Oncol. 2020;32:162-169
Duetz C, et al. 2021; Cytometry A. 2021;99(8):814-824

Implementation of FCM in a diagnostic algorithm for patients with cytopenia /possible MDS



Pre-referral exclusion of non-clonal causes of cytopenia based on clinical history and pertinent laboratory data

Cytopenia / possible MDS

Exclusion of other hematological neoplasms or disease associated with cytopenia and/or dysplasia

Morphology & Flow cytometry
Simultaneous analysis

Bone marrow Histology
Assess megakaryocytopoiesis, marrow fibrosis, IHC (CD34, p53, additional markers if relevant for exclusion of other hematological neoplasms associated with cytopenia/dysplasia)

Cytomorphology
Assess dysplasia, blast percentage, and ring sideroblasts

FCM screening tubes (8-or 10-color)
Detection of erythroid/myeloid or monocytic aberrancies or abnormal lymphoid (B-/T-, plasma cell and NK-cell) populations.

Initial review determines selection of downstream investigations

Cytogenetics/FISH

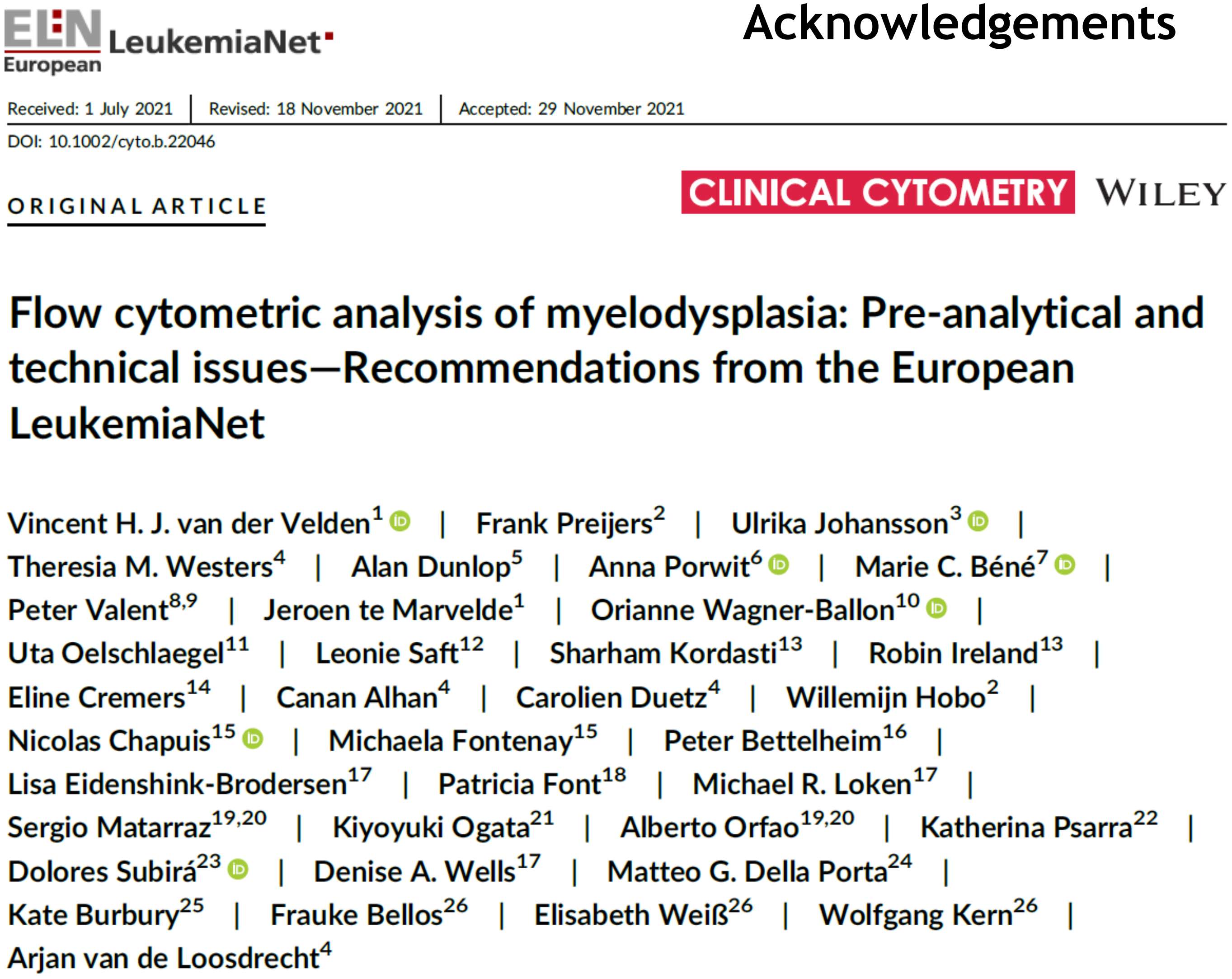
Molecular genetics

Integrated diagnostic report based on multimodal results

ELN European iMDS Flow

Loosdrecht AA van de, et al., Cytometry B Clin Cytom 2021: Dec 13. doi:10.1002/cyto.b.22044

Acknowledgements



European LeukemiaNet

Received: 1 July 2021 | Revised: 18 November 2021 | Accepted: 29 November 2021
DOI: 10.1002/cyto.b.22046

ORIGINAL ARTICLE

CLINICAL CYTOMETRY WILEY

Flow cytometric analysis of myelodysplasia: Pre-analytical and technical issues—Recommendations from the European LeukemiaNet

Vincent H. J. van der Velden[1] | Frank Preijers[2] | Ulrika Johansson[3] | Theresia M. Westers[4] | Alan Dunlop[5] | Anna Porwit[6] | Marie C. Béné[7] | Peter Valent[8,9] | Jeroen te Marvelde[1] | Orianne Wagner-Ballon[10] | Uta Oelschlaegel[11] | Leonie Saft[12] | Sharham Kordasti[13] | Robin Ireland[13] | Eline Cremers[14] | Canan Alhan[4] | Carolien Duetz[4] | Willemijn Hobo[2] | Nicolas Chapuis[15] | Michaela Fontenay[15] | Peter Bettelheim[16] | Lisa Eidenshink-Brodersen[17] | Patricia Font[18] | Michael R. Loken[17] | Sergio Matarraz[19,20] | Kiyoyuki Ogata[21] | Alberto Orfao[19,20] | Katherina Psarra[22] | Dolores Subirá[23] | Denise A. Wells[17] | Matteo G. Della Porta[24] | Kate Burbury[25] | Frauke Bellos[26] | Elisabeth Weiß[26] | Wolfgang Kern[26] | Arjan van de Loosdrecht[4]

Department of Hematology,

Amsterdam UMC, location Vumc

The Netherlands

Marisa Westers

Caroline Duetz

Margot van Spronsen

Costa Bachas

Claudia Cali,

Canan Alhan,

Eline Cremers,

Nathalie Kerkhoff,

Luca Janssen,

Adri Zevenbergen,

Geja Heeremans

VIB Inflammation Research Center and Department of Applied Mathematics, Ghent University, Belgium

Sofie Van Gassen

Yvan Saeys