Advances in Multiparameter Flow Cytometry for the Diagnostic Work-Up of Myelodysplastic Syndromes

A collaboration between Amsterdam UMC & Erasmus MC Rotterdam

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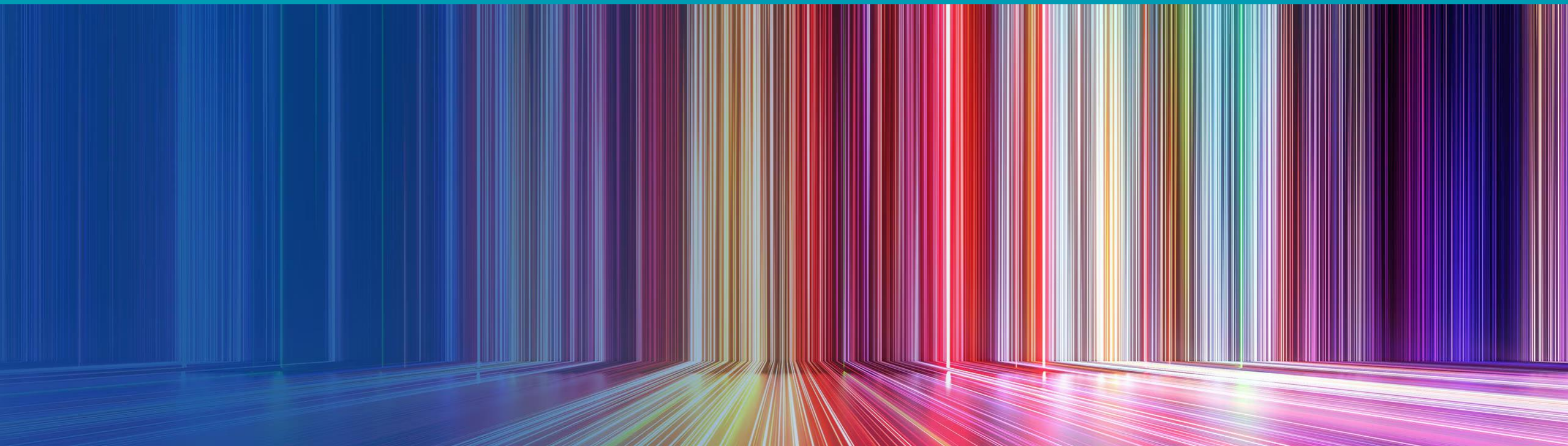


Disclosures

- Nothing to disclose

Content

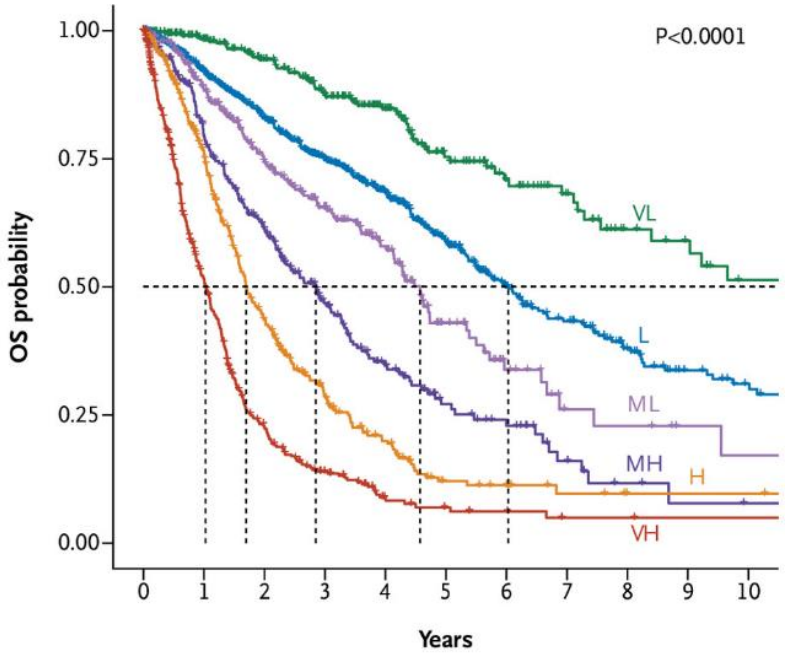
- Flow Cytometry in the Diagnostic Work-Up of MDS
- ELN iMDS-Defined Immunophenotypic Markers: Validation and Application
- Future Perspectives

Myelodysplastic Syndromes (MDS)

- Group of heterogeneous clonal hematopoietic stem cell diseases
  - *Peripheral blood cytopenia*
  - *Morphologic dysplasia of hematopoietic cells*
  - *Potential for rapid progression to acute myeloid leukemia (AML)*

- Diagnostics (ICC 2022[1] & WHO 2022[2])
  - *Hematological parameters, morphology, Cytogenetics, Next Generation Sequencing & Flow Cytometry*

- Risk classification
  - *IPSS-Molecular[3]*
  - *94% of MDS carry genetic lesion*



No. at risk

VL	344	267	224	180	126	82	57	42	28	24	18
L	852	640	496	382	270	176	112	83	57	40	31
ML	295	214	152	111	72	35	18	8	7	4	3
MH	278	191	134	80	48	27	20	9	4	2	1
H	367	235	121	65	37	15	12	6	3	3	3
VH	460	200	77	37	14	9	6	3	3	2	2

1. Arber et al, Blood, 2022
2. Khoury et al, Leukemia, 2022
3. Bernard et al, NEJM Evidence, 2022

Figure adapted from: Bernard et al. NEJM Evidence. 2022

The role of flow cytometry in MDS

Received: 29 September 2021 | Revised: 20 November 2022 | Accepted: 29 November 2022
DOI: 10.1002/cyto.b.22108
REVIEW ARTICLE
CLINICAL CYTOMETRY WILEY

Multiparameter flow cytometry in the evaluation of myelodysplasia: Analytical issues

Recommendations from the European LeukemiaNet/International Myelodysplastic Syndrome Flow Cytometry Working Group

Anna Porwit[1] | Marie C. Béné[2] | Carolien Duetz[3] | Sergio Matarraz[4] | Uta Oelschlaegel[5] | Theresia M. Westers[3] | Orianne Wagner-Ballon[6,7] | Shahram Kordasti[8] | Peter Valent[9] | Frank Preijers[10] | Canan Alhan[3] | Frauke Bellos[11] | Peter Bettelheim[12] | Kate Burbury[13] | Nicolas Chapuis[14,15] | Eline Cremers[16] | Matteo G. Della Porta[17,18] | Alan Dunlop[19] | Lisa Eidenschink-Brodersen[20] | Patricia Font[21] | Michaela Fontenay[14,15] | Willemijn Hobo[9] | Robin Ireland[22] | Ulrika Johansson[23] | Michael R. Loken[20] | Kiyoyuki Ogata[24] | Alberto Orfao[4] | Katherina Psarra[25] | Leonie Saft[26] | Dolores Subira[27] | Jeroen te Marvelde[28] | Denise A. Wells[20] | Vincent H. J. van der Velden[28] | Wolfgang Kern[11] | Arjan A. van de Loosdrecht[3]

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DOI: 10.1002/cyto.b.22044
ORIGINAL ARTICLE
CLINICAL CYTOMETRY WILEY

Clinical application of flow cytometry in patients with unexplained cytopenia and suspected myelodysplastic syndrome: A report of the European LeukemiaNet International MDS-Flow Cytometry Working Group

Arjan A. van de Loosdrecht[1] | Wolfgang Kern[2] | Anna Porwit[3] | Peter Valent[4] | Sharham Kordasti[5] | Eline Cremers[6] | Canan Alhan[1] | Carolien Duetz[1] | Alan Dunlop[7] | Willemijn Hobo[8] | Frank Preijers[8] | Orianne Wagner-Ballon[9,10] | Nicolas Chapuis[11,12] | Michaela Fontenay[11,12] | Peter Bettelheim[13] | Lisa Eidenschink-Brodersen[14] | Patricia Font[15] | Ulrika Johansson[16] | Michael R. Loken[14] | Jeroen G. te Marvelde[17] | Sergio Matarraz[18] | Kiyoyuki Ogata[19] | Uta Oelschlaegel[20] | Alberto Orfao[18] | Katherina Psarra[21] | Dolores Subirá[22] | Denise A. Wells[14] | Marie C. Béné[23] | Matteo G. Della Porta[24,25] | Kate Burbury[26] | Frauke Bellos[2] | Vincent H. J. van der Velden[17] | Theresia M. Westers[1] | Leonie Saft[27] | Robin Ireland[28]

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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]

- Recognized by the WHO as a valuable additional diagnostic tool[1]
- Constant development; International/European LeukemiaNet Working Group for Flow Cytometry in MDS

1. Wang W, Khoury JD. Where diagnosis for myelodysplastic neoplasms (MDS) stands today and where it will go: The role of flow cytometry in evaluation of MDS. Cytometry B Clin Cytom. 2023 Jan;104(1):12-14. doi: 10.1002/cyto.b.22110. Epub 2022 Dec 27. PMID: 36573832.

Current Panel in use Amsterdam UMC

	FL-1	FL-2	FL-3	FL-4	FL-5	FL-6	FL-7	FL-8
	FITC	PE	PerCP-Cy5.5	PC7	APC	APC-H7	V450	KO
1			CD34	CD117			HLA-DR	CD45
2	CD16	CD13	CD34	CD117	CD11b	CD10	HLA-DR	CD45
3	CD2	CD64	CD34	CD117	IREM2	CD14	HLA-DR	CD45
4	CD36	CD105	CD34	CD117	CD33	CD71	HLA-DR	CD45
5	CD5	CD56	CD34	CD117	CD7	CD19	HLA-DR	CD45
6	CD15	CD25	CD34	CD117	CD123	CD38	HLA-DR	CD45
7	CD7	CD235a	CD34	CD117	CD13	CD71	HLA-DR	CD45
			BB	BB			BB	BB

NB! BB: backbone labeling

Euroflow-like;
Slightly different from published MDS/AML panel by van Dongen et al. Leukemia, 2012

Porwit et al, Cytometry B Clin Cytom, 2023
van Dongen et al. Leukemia, 2012; doi:10.1038/leu.2012.120

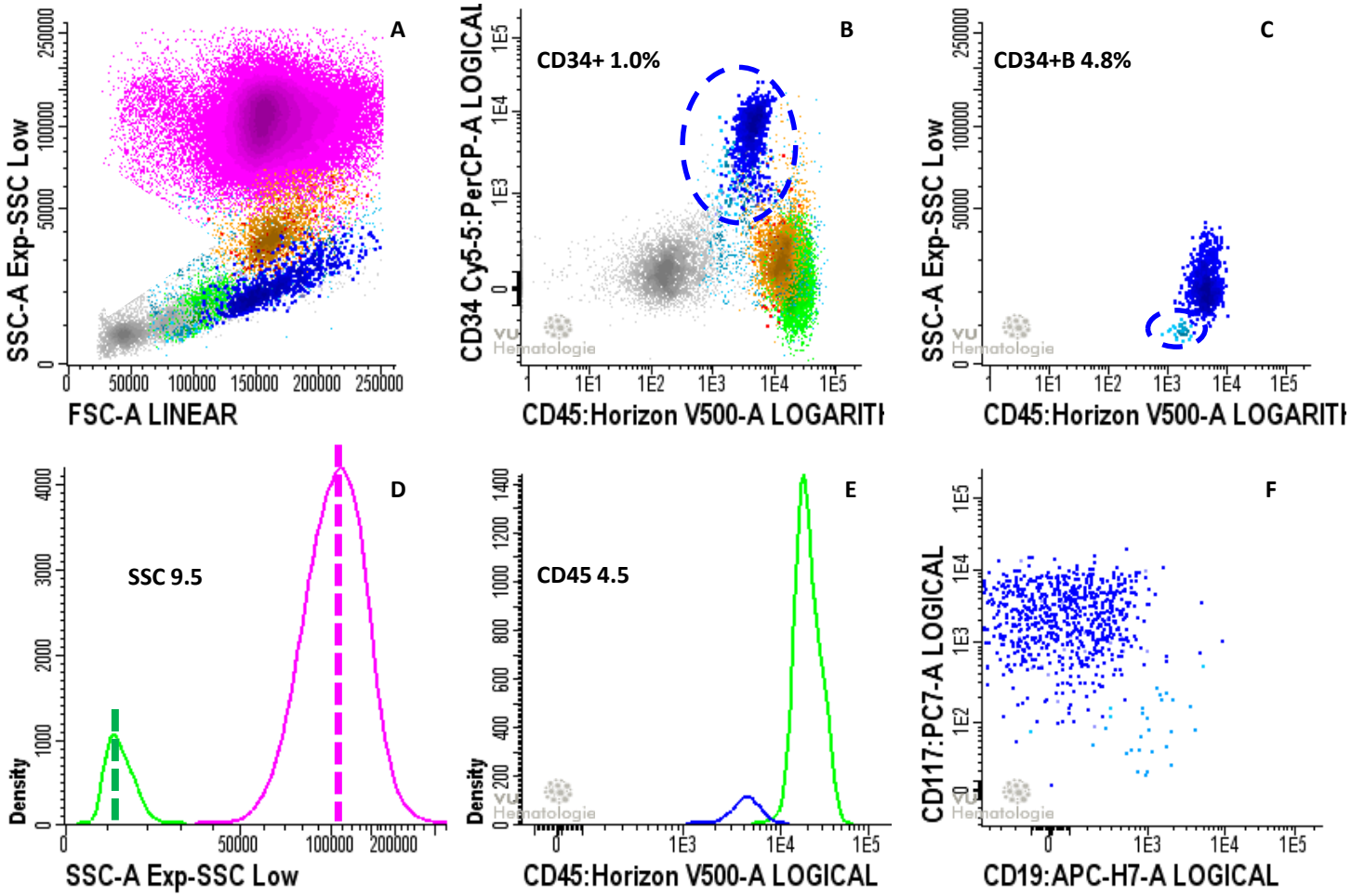
Current approach in Amsterdam UMC

	Ogata Score	Integrated Flow Score (iFS)
# Parameters	4	40+
Lineages assessed	Mainly progenitors	Multi-lineage
Erythroid evaluation	No	Yes
Output format	Low (0–1) / High (≥2)	A / B / C classification
Specificity	94%	86%
Sensitivity	57%	79%
Accuracy	65%	80%

Table adapted from: Porwit et al, Cytometry B Clin Cytom, 2023 & Oelschlaegel et al, Cytometry B Clin Cytom, 2023

Example of the Ogata score (normal)

- progenitors
- granulocytes
- monocytes
- lymphocytes (internal reference)



Score based on reference ranges:

SSC gran/Ly	>6	0
% CD34+ myeloid	<2%	0
% CD34 B cell progenitors	>5%	1
CD45 Ly/My	4-7.5	0
		1

Figure by courtesy of dr. Westers, Amsterdam UMC

Westers and Van de Loosdrecht, 2025 in book review
(Multiparameter Flow Cytometry in the Diagnosis of Hematologic Malignancies; ed. Porwit and Bené)

Example of the Ogata score: indicative of MDS

- progenitors
- granulocytes
- monocytes
- lymphocytes (internal reference)

Score based on reference ranges:

SSC gran/Ly	>6	1
% CD34+ myeloid	<2%	1
% CD34 B cell progenitors	>5%	1
CD45 Ly/My	4-7.5	1
		4

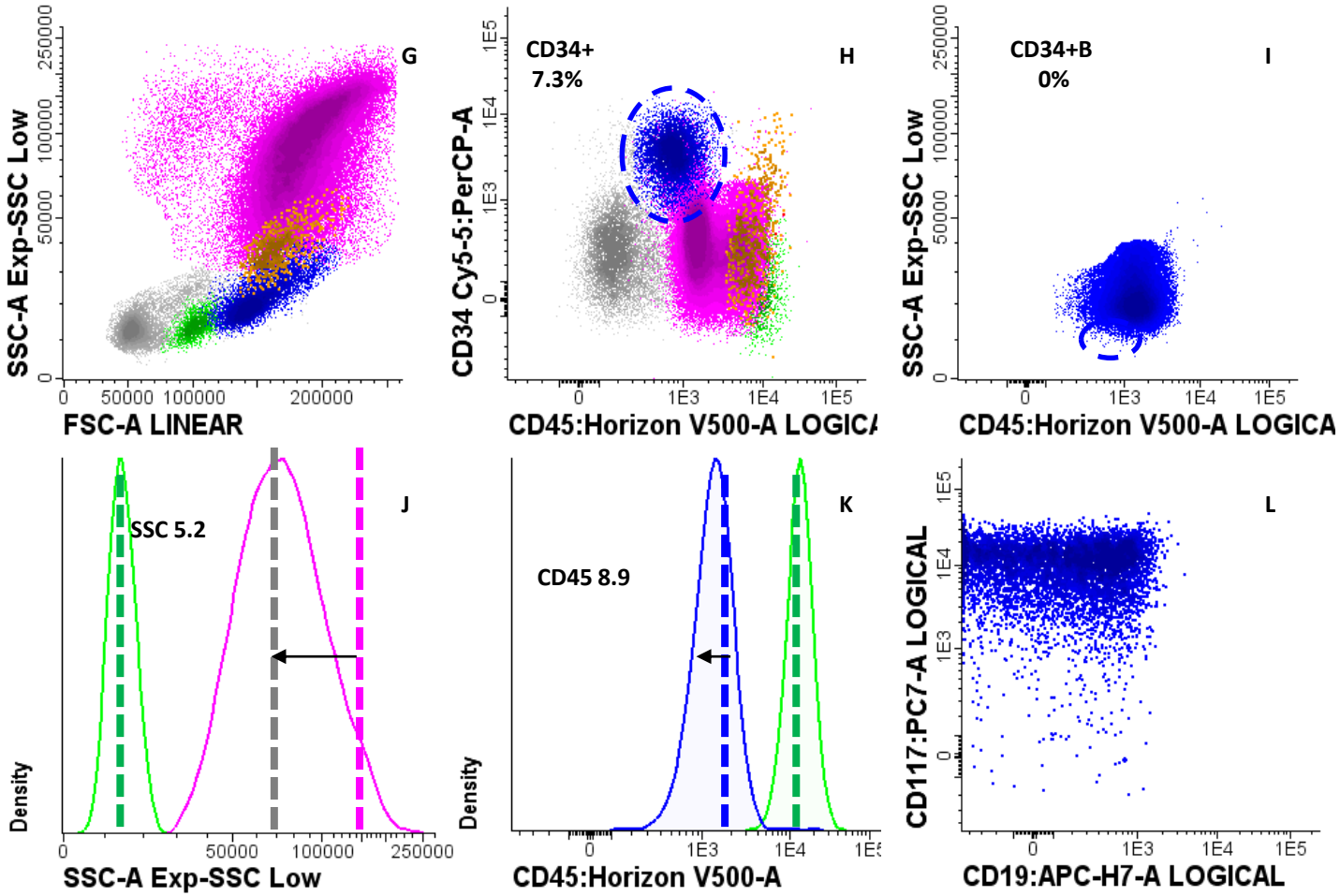
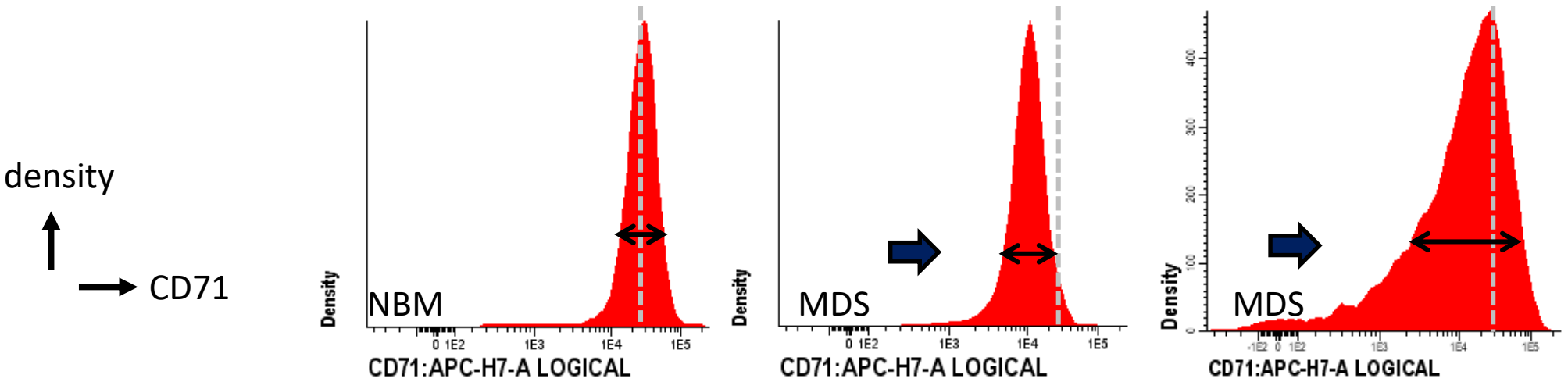


Figure by courtesy of dr. Westers, Amsterdam UMC

Westers and Van de Loosdrecht, 2018 in book review
(Multiparameter Flow Cytometry in the Diagnosis of Hematologic Malignancies; ed. Porwit and Bené)

Examples of diagnostic FC aberrancies in suspected MDS - iFS



data from ammoniumchloride-based lysed BM aspirates

CD71: decreased expression, increased CV

ELNet iMDSFlow-WG previously also defined:

- increase in CV of CD36 expression
- de/increase of % of CD117^{+} erythroid progenitors

2 or more aberrancies associated with erythroid dysplasia (2017)

erythroid

Figure by courtesy of dr. Westers, Amsterdam UMC

Westers et al., Haematologica, 2017, 102(2):308-319
Porwit et al. Cytometry B Clin Cytom 2023; 104:27-50

VU Hematologie

Schematic representation of flow 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 FCM myeloid progenitors	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Dysplasia by FCM - Neutrophils - Monocytes	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Dysplasia by FCM - 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

Figure by courtesy of dr. Westers, Amsterdam UMC

Cremers EMP, et al., Haematologica 2017, 102(2)
based on Van de Loosdrecht and Westers. J Nat Compr Cancer Netw 2013;11:892-902)

ELN iMDS FC WG - Problem

- 229 laboratories surveyed[1]
  - Large variability in MDS flow protocols
  - Median 20 ± 4.5 markers per laboratory
  - Low adherence to iMDS recommendations
  - Limited use of standardized scoring systems

- **<u>Need for harmonization and optimization</u>**

- **Focus on lower-risk MDS → greatest diagnostic challenge**

1. Grille Montauban et al. 2019; Jensen et al. 2019 in Porwit et al, Cytometry B Clin Cytom, 2023; 104:27-50:

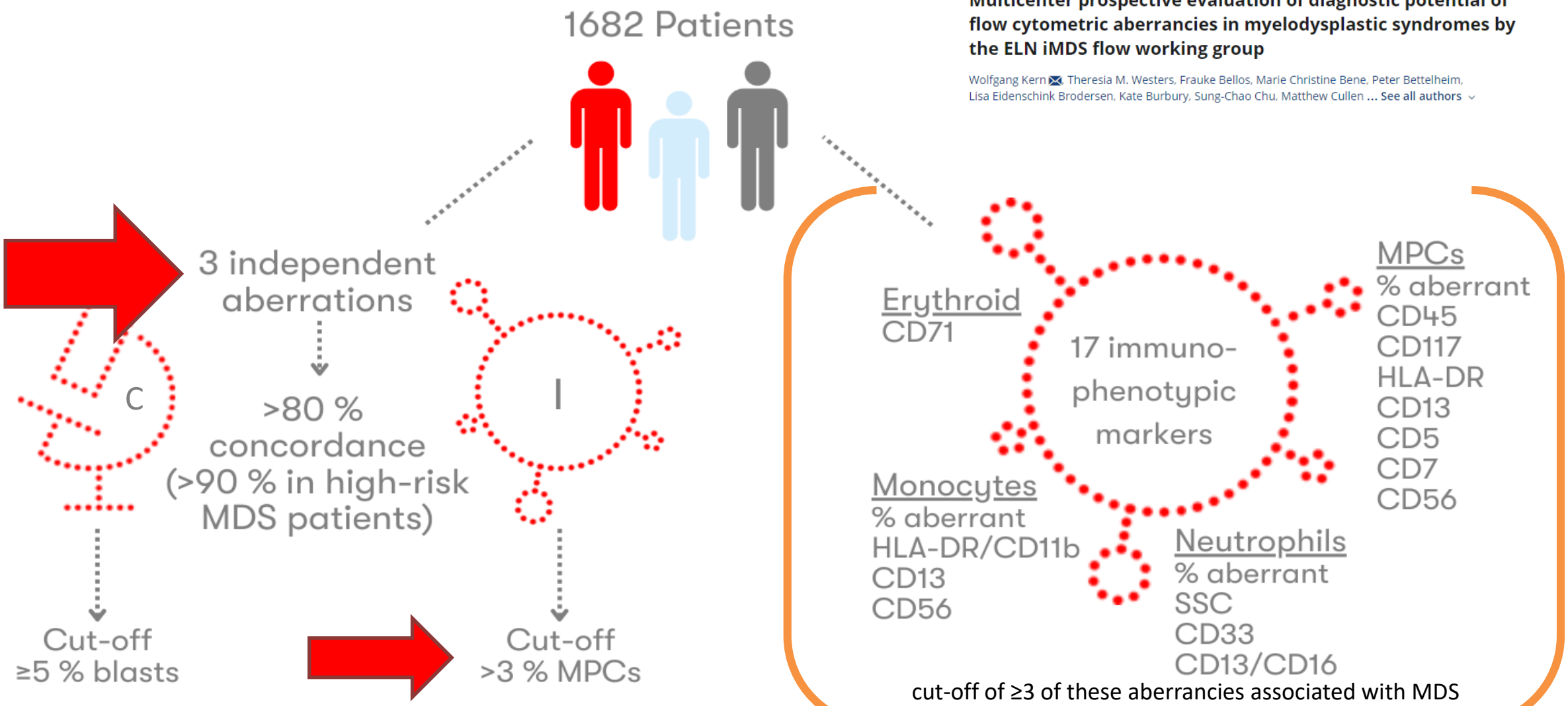
ELN Scoring System

THE OFFICIAL JOURNAL OF THE INTERNATIONAL CLINICAL CYTOMETRY SOCIETY
Cytometry PART B CLINICAL CYTOMETRY

ORIGINAL ARTICLE | Free Access

Multicenter prospective evaluation of diagnostic potential of flow cytometric aberrancies in myelodysplastic syndromes by the ELN iMDS flow working group

Wolfgang Kern, Theresia M. Westers, Frauke Bellos, Marie Christine Bene, Peter Bettelheim, Lisa Eidenschink Brodersen, Kate Burbury, Sung-Chao Chu, Matthew Cullen ... See all authors



cut-off of ≥3 of these aberrancies associated with MDS (not necessarily in two lineages!)

Figure by courtesy of Prof. dr. med. Wolfgang Kern, MLL, Munich, Germany

Kern et al., Cytometry B Clin Cytom. 2023; 104:51-65;
Porwit et al. Cytometry B Clin Cytom 2023; 104:27-50

Validation of the ELN Scoring System

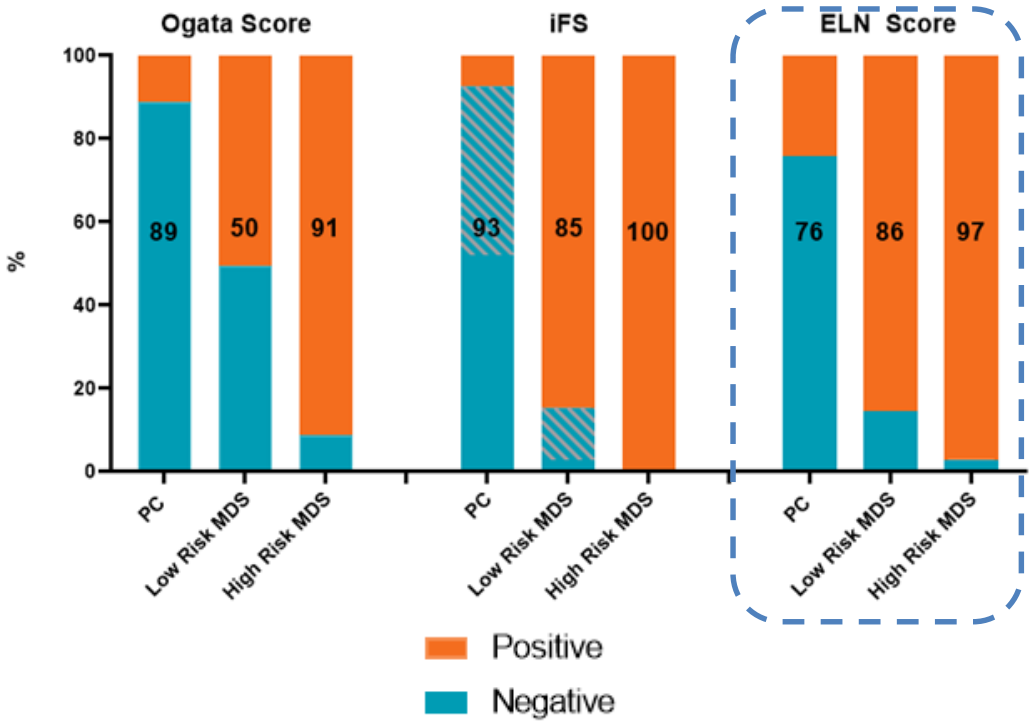
- Results validated in two independent cohorts
  - Amsterdam UMC , Amsterdam, The Netherlands[1]
  - University Hospital Carl Gustav Carus TU Dresden, Dresden, Germany[2]

1. Veenstra et al. (2025) HemaSphere; doi:10.1002/hem3.70075
2. Oelschlaegel et al. (2025) HemaSphere; doi:10.1002/hem3.70235

Validation of the ELN Scoring System

Amsterdam, The Netherlands

Risk according to IPSS-M



PC = Pathological control

Dresden, Germany

(A)	all MDS	MDS <5% blasts	MDS ≥5% blasts	MDS IPSS-M (VL, L, ML)	MDS IPSS-M (MH, H, VH)
number of patients	359	205	154	63	49
sensitivity	**89%**	**84%**	**97%**	**86%**	**94%**
accuracy (MDS/PC)	**90%**	**86%**	**97%**	**90%**	**96%**
accuracy (MDS/HBM)	**90%**	**86%**	**97%**	**89%**	**95%**

Specificity as determined by pathological controls

- ELN Orginal paper (78%), Amsterdam (76%), Dresden (98%)
- HBM cohort in Dresden specificity of 97%

- Likely influenced by differences in pathological control composition
  - Example: proportion of iron deficiency cases with dyspoietic changes[3]

1. Veenstra et al. (2025) HemaSphere; doi:10.1002/hem3.70075
2. Oelschlaegel et al. (2025) HemaSphere; doi:10.1002/hem3.70235
3. Van de Loosdrecht et al. (2023) Cytometry B Clin Cytom; doi:10.1002/cyto.b.22044

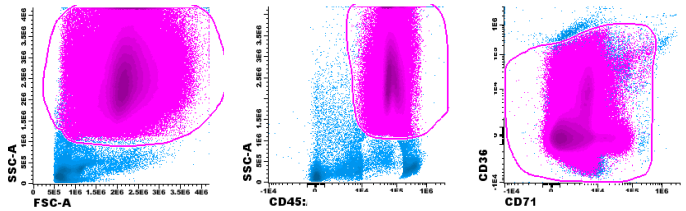
ELN Scoring System

- Independent validation confirm that “≥3 of the identified 17 aberrancies” shows high concordance with Integrated diagnostic approach, including high sensitivity in *Low Risk MDS* patients

- 12 color single tube approach

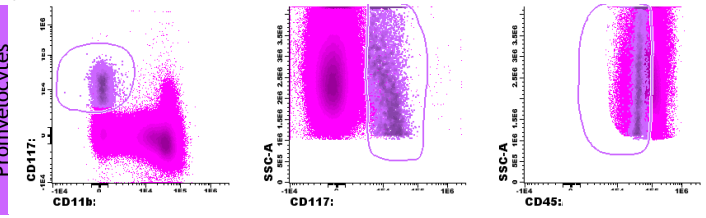
Overview granulocytes

Neutrophils



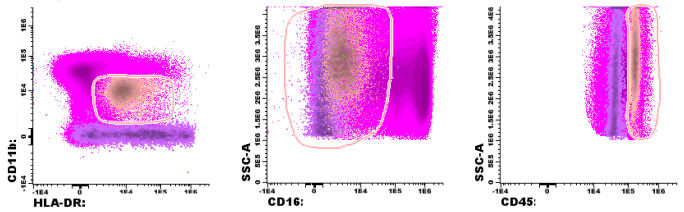
Definition: SSC-A$^{int/high}$ / FSC-A$^{int/high}$ / CD45^{+} / SSC-A^{low} / FSC-A^{int} / exclude CD71^{bright} & CD36^{bright}

Promvelocytes



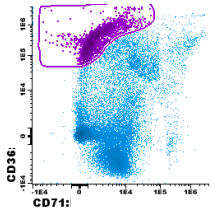
Definition: CD117^{+} / CD11b^{-} / FSC-A$^{low/high}$ / CD45^{int}

Eosinophils

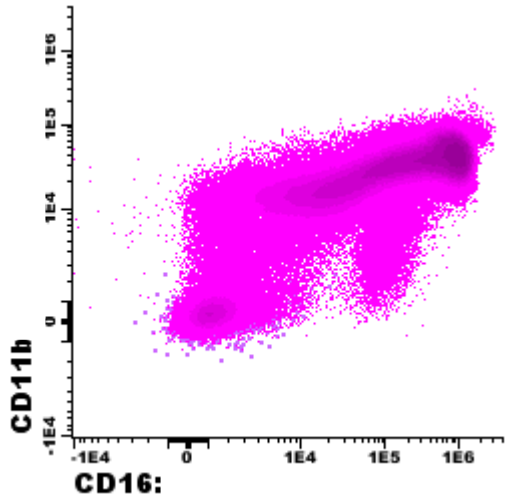


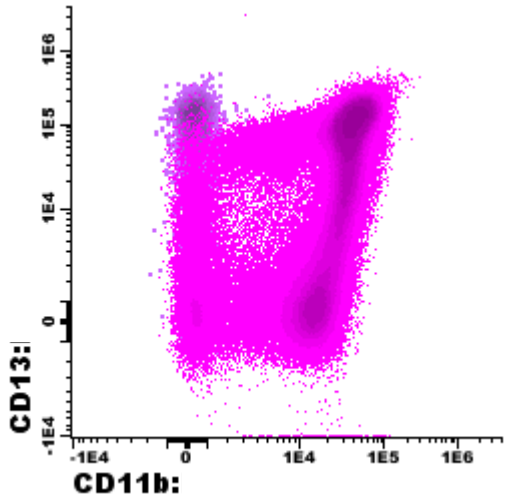
Definition: HLA-DR^{+} / CD11b^{+} / SSC-A$^{low/high}$ / CD16^{-} / CD45^{bright}

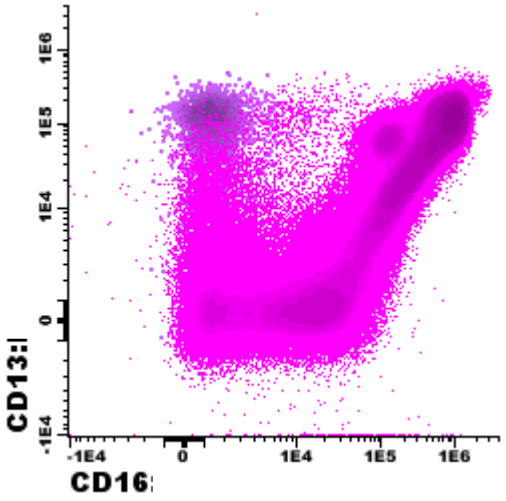
Platelets & Endothelial cells

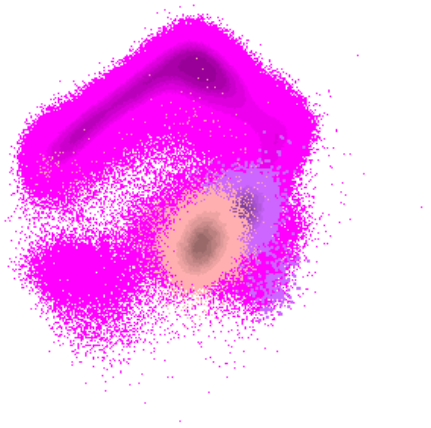


Definition: CD36^{bright}









APS 1

DO NOT SHARE

Take Home Message

- The **ELN iMDS Flow Working Group** provides extensive recommendations to harmonize MDS flow cytometry
- The **ELN Scoring System** is an accessible and sensitive tool in the diagnostic work-up of lower-risk MDS
- **Spectral flow cytometry** enables high-dimensional immunophenotyping in MDS and supports integrated single-tube diagnostic strategies

Acknowledgements

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Fleur de Bie

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ELN European LeukemiaNet