Multicentric study underlining the interest of adding CD5, CD7 and CD56 expression assessment to the flow cytometric Ogata score in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms

Valérie Bardet,[1] Orianne Wagner-Ballon,[2] Julien Guy,[3] Céline Morvan,[4] Camille Debord,[1] Franck Trimoreau,[4] Emmanuel Benayoun,[2] Nicolas Chapuis,[1] Nicolas Freynet,[2] Cédric Rossi,[3] Stéphanie Mathis,[1] Marie-Pierre Gourin,[5] Andréa Toma,[6] Marie C. Béné,[7] Jean Feuillard,[4] and Estelle Guérin;[4] on behalf of the Groupe Francophone des Myélodysplasies (GFM) and the Groupe d'Etude Immunologique des Leucémies (GEIL)

[1]Service d'Hématologie Biologique, Hôpitaux Universitaires Paris Centre-Cochin, Faculté de Médecine Paris Descartes, INSERM U1016, UMR 8104, Paris; [2]Département d'Hématologie et d'Immunologie Biologiques, Hôpitaux Universitaires Henri Mondor, APHP, Faculté de Médecine UPEC, Créteil; [3]Service d'Hématologie Biologique, CHU de Dijon; [4]Service d'Hématologie Biologique, Hôpital Dupuytren, CHU de Limoges, Faculté de Médecine et UMR CNRS 7276, Limoges; [5]Service d'Hématologie Clinique, Hôpital Dupuytren, CHU de Limoges; [6]Service d'Hématologie Clinique, Hôpitaux Universitaires Henri Mondor, APHP, Créteil; and [7]Service d'Hématologie Biologique, CHU de Nantes, France

		Centre 1 n=178	Centre 2 n=56	Centre 3 n=179	Centre 4 n=239
Low risk (N=218)	RA n=50	12	6	14	18
	RCMD n=126	65	9	23	29
	RARS n=31	6	7	10	8
	5q- n=11	7	0	3	1
	Low risk proven	52	12	15	22
	Low risk not proven	38	10	25	34
High risk (n=128)	RAEB 1 n=90	10	4	26	50
	RAEB 2 n =38	10	4	9	15
MDS/MPN (n=53)	CMML n=43	6	3	14	20
	Other n=10	2	1	5	2
Controls (n=253)		60	22	75	96

Supplementary table 1 : distribution of patients between the 4 participating centers

Panel	Antibodies
4 colors panel	FITC conjugated CD45 monoclonal antibody (mAb) (CD45-FITC, clone ALB12), ref IM0647 PE-cyanine 5 (Cy5) conjugated CD19 mAb (CD19-PC5, clone J3-119), ref A07771 PE-cyanine 7 (Cy7) conjugated CD34 mAb (CD34-PC7, clone 581), ref A21691 PE conjugated CD10 mAb (CD10-PE,clone ALB1), ref A07760
7 colors panel	FITC conjugated CD45 monoclonal antibody (mAb) (CD45-FITC, clone ALB12), ref IM0647 PE-cyanine 7 (Cy7) conjugated CD34 mAb (CD34-PC7, clone 581), ref A21691 PE conjugated CD10 mAb (CD10-PE,clone ALB1), ref A07760 Pacific blue (PB) conjugated CD19 mAb (CD19-PB, clone J3-119), ref A86355 PE-cyanine 5.5 (Cy5.5) conjugated CD19 mAb (CD19-PC5,5 clone J3-119), refA66328 Allophycocyanin (APC) conjugated CD56 mAb (CD56-APC, clone N901(NKH-1), ref IM2474 APC Alexa Fluor (A) 700 conjugated CD7 mAb (CD7-APC-A700, clone 8H8.1), ref A70201 APC-A750 conjugated CD5 mAb (CD5-APC-A750, clone BL1A), ref A78836

Supplementary table 2: Antibodies used in this study. All were purchased by Beckman Coulter (Miami, FL)

Supplementary legends

**Supplementary figure 1 : gating strategy**

Events were first gated on SSC/FCS (A) and secondly on CD45/SSC (B) biparametric histograms. $CD34^+$ events were gated on a CD45/CD34 histogram (C). $CD34^+$ cells were backgated on a CD45/SSC histogram (D). $CD19^+$ $CD34^+$ $CD10^+$ hematogones were counted on a CD34/CD19 histogram (E). $CD19^-$ $CD10^-$ $CD34^+$ myeloid blasts were counted on a CD45/CD10 histogram (F). SSC peak intensity for lymphocytes and granulocytes was estimated on an SSC monoparametric histogram (G). CD45 mean intensity of lymphocytes and myeloid progenitors was measured on a CD45 monoparametric histogram. From A to G, lymphocytes are stained in black. Lymphocytes and granulocytes were gated on CD45/SSC biparametric histograms (not shown)
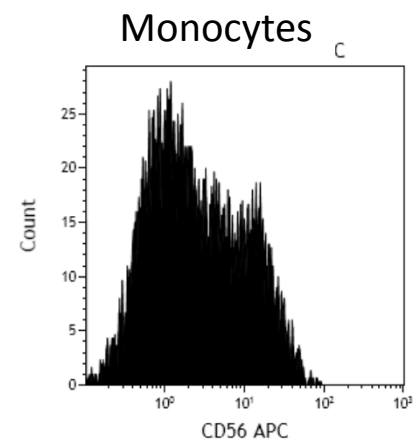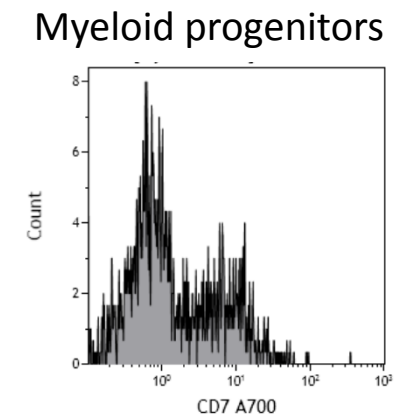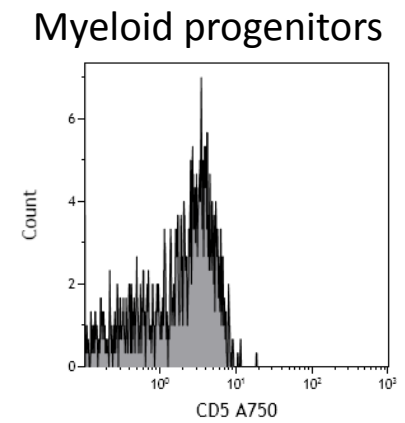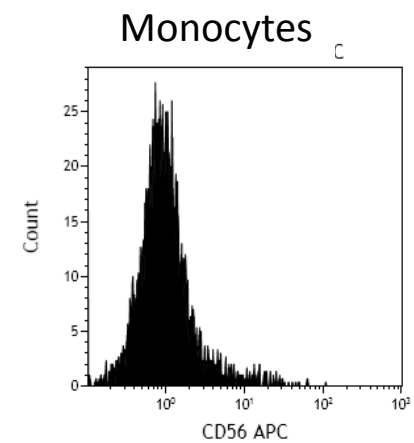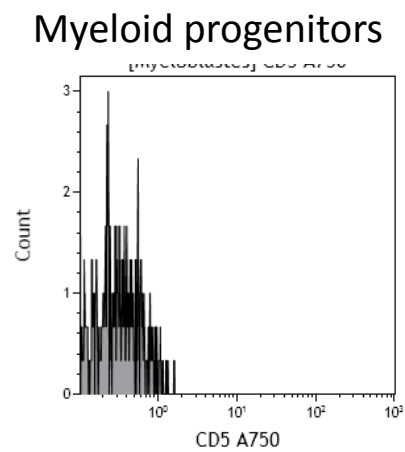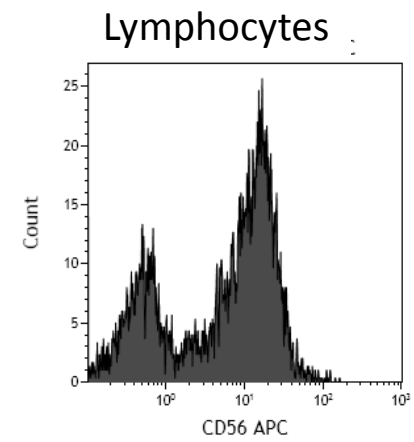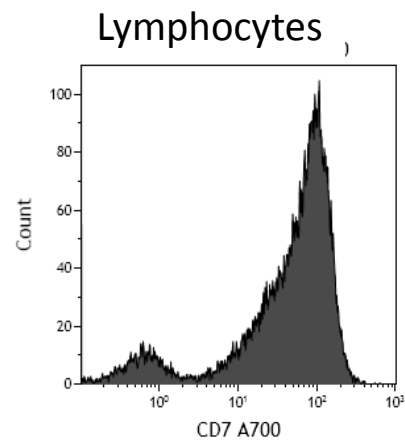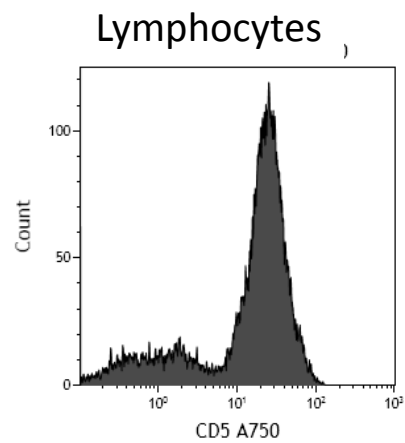
Supplementary figure 2

Examples of CD5 and CD7 monoparametric histograms gated on lymphocytes and myeloid progenitors and of CD56 monoparametic histograms gated on lymphocytes and monocytes. Monocytes on a CD45/FSC histogram were gated in a Boolean fashion after exclusion of $CD19^+$, $CD10^+$, $CD5^+$ and/or $CD7^+$ events.

Supplementary Figure 3 :

Contingency table showing the partition of Ogata FCM scores in MDS/MPN patients (A), controls (B) and in the whole cohort (C) according to the degree of BM hemodilution. There is no significant difference in the percentages of classified cases.
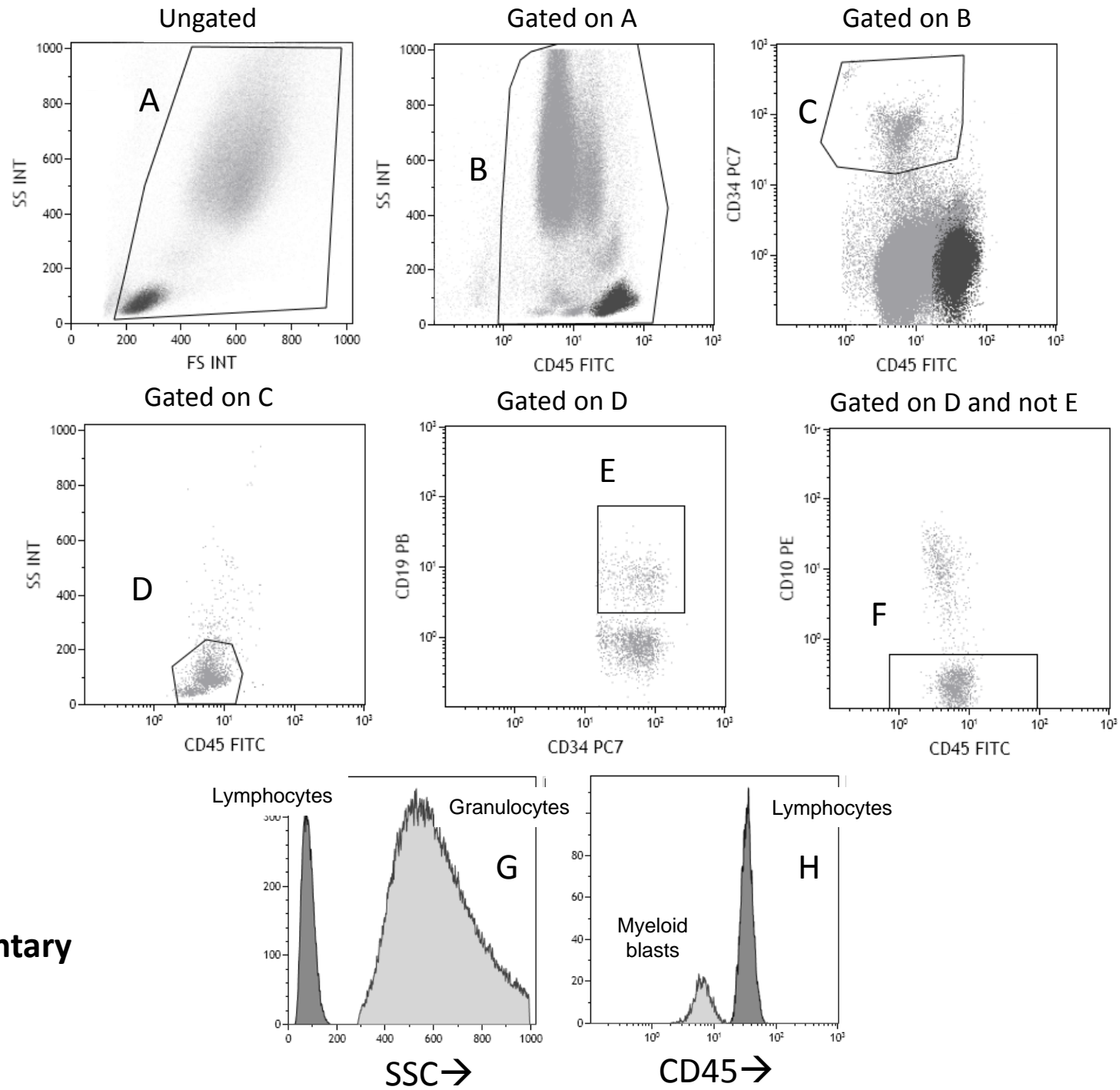
Supplementary figure 1
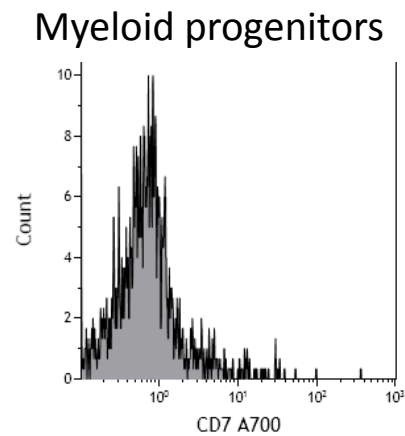
Supplementary figure 2

A

MDS/MPN	BM purity <80%	BM purity ≥80%	Total
Ogata FCM score ≥ 2	37 (59%)	102 (59%)	139
Ogata FCM score <2	26 (41%)	72 (41)	98
	63	174	237

P=0.9879

B

Controls	BM purity <80%	BM purity ≥80%	Total
Ogata FCM score ≥ 2	2 (5%)	9 (9%)	11
Ogata FCM score <2	38 (95%)	86 (91%)	124
	40	95	135

P=0.6009

C

All patients	BM purity <80%	BM purity ≥80%	Total
Ogata FCM score ≥ 2	39 (38%)	111 (41%)	150
Ogata FCM score <2	64 (62%)	158 (59%)	222
	103	269	372

P=0.5497

Supplementary figure 3