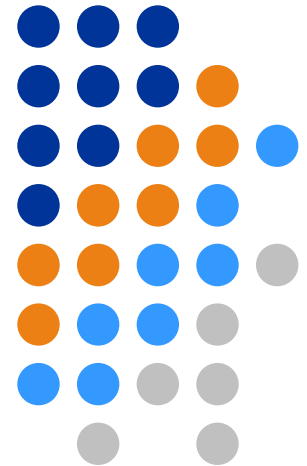


Flowcytometrische detectie van afwijkende erytroïde en myelomonocyttaire cellen in MDS



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Frank Preijers, Hans Veenstra; UMCN St Radboud
Christa Homburg; Sanquin Diagnostiek
Canan Alhan, Marisa Westers; VUmc



Workshop Masterclass flowcytometrie in MDS

5 september 2012

Antistof panel



tube					lineage
1	PBS	PBS	CD45	PBS	
2	CD235a	CD117	CD45	CD71	erythroid
3	CD16	CD13	CD45	CD11b	myelomonocytic
4	CD36	CD33	CD45	CD14	myelomonocytic
5	HLA-DR	CD15	CD45	CD11b	myelomonocytic
6	CD13+33	CD117	CD45	CD34	myelomonocytic
7	HLA-DR	CD123	CD45	CD34	immature/basophilic/DC
8	CD7	CD56	CD45	CD34	immature/lymphocytes
9	CD5	CD19	CD45	CD34	immature/lymphocytes
10*	CD15	CD11b	CD45	CD34	myelomonocytic

NB! Geen beperking in de te gebruiken antistofklonen, fluorochromen en xx-kleurenfluorescentie

Definiëren van subpopulaties in NBM



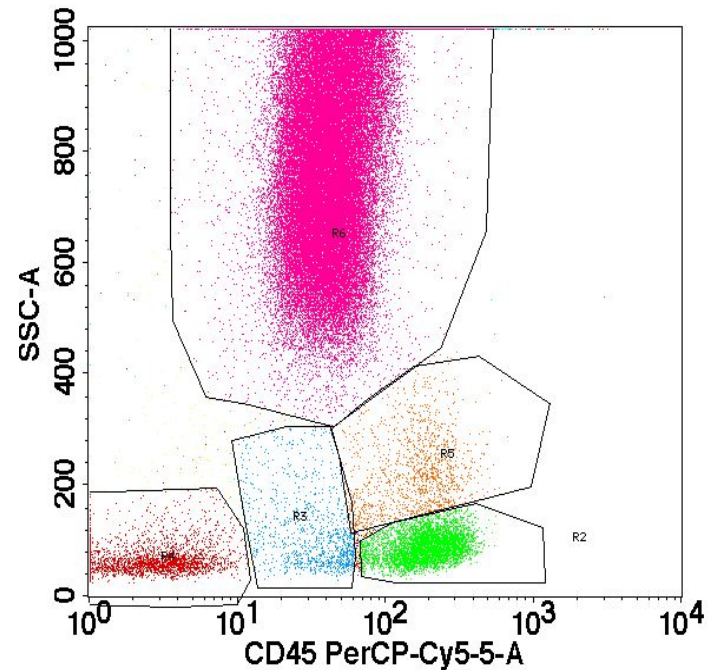
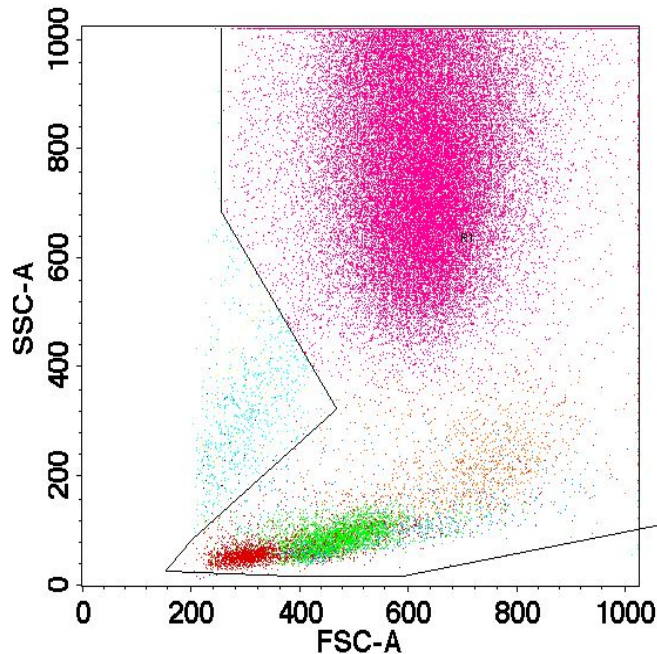
- Bulk lysis met NH₄Cl, acquisitie van minimaal 100,000 events
- Analyse van populaties door middel van de CD45, FSC en SSC karakteristieken in combinatie met de specifieke merkers

Erytroïde voorlopers	CD45 ⁻ /dim/SSC ^{low} /CD235a ⁺ /CD71 ⁺
Granulocyten	CD45 ⁺ /SSC ^{high} /CD33 ^{dim} /CD14 ⁻
Monocyten	CD45 ⁺ /SSC ^{int} /CD33 ^{bright} /CD14 ⁺
Lymfocyten	CD45 ^{bright} /SSC ^{low}

Definiëren van supopulaties in NBM gating strategie



Exclusie van debris en niet-kernhoudende rood & vervolgens analyse door middel van de CD45, FSC en SSC karakteristieken



Pitfalls



- Debris/niet-kernhoudende rood is niet altijd eenvoudig te excluderen.
- Apoptose die patronen kan verstoren
- Neutrofielen: hypogranulair, gating contaminatie van monocyten gate
- Monocyten: hypogranulair, weinig mono's, CD14- (onrijp)
- Lymfo plak en aspecifieke expressie van lymfocyttaire merkers

Interpretatie van de populaties/patronen



- Detectie van afwijkingen in:
 - Erytroïde uitrijping (71/235a, 117/71)
 - Myeloïde uitrijping (11b/13, 16/13, 16/11b, DR/11b, onder- of over-expressie en aberranties)
 - Monocytaire uitrijping (36/14, 14/64, DR/11b, 16/13, onder- of over-expressie en aberranties)
- Flow score om de verschillende afwijkingen te scoren en uiteindelijk een conclusie te kunnen trekken

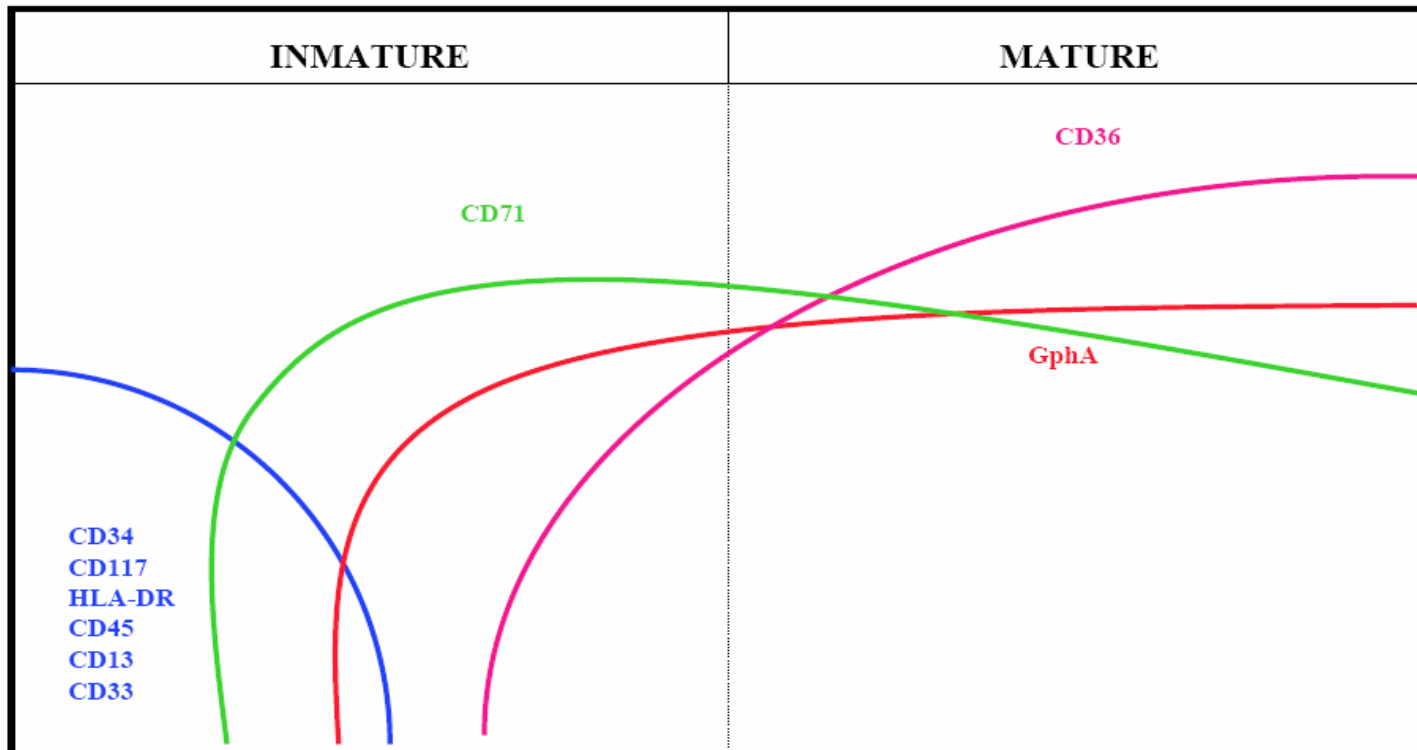
1-Erytroïd



Uitrijping erytroïd



Normale expressie tijdens de uitrijping



Uitrijping erytroïd



I



Pro-erythroblast

CD45_{dim}
CD117
CD71

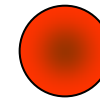
II



Erythroblast

CD45_{dim/neg}
CD71
CD235a

III



Erythrocyt

CD45_{neg}
CD235a

Te scoren afwijkingen in erythroïde reeks



- Percentage kernhoudende rode cellen
- Afwijkende relatie CD71/CD235a
- Afwijkende expressie CD117

Antistof panel

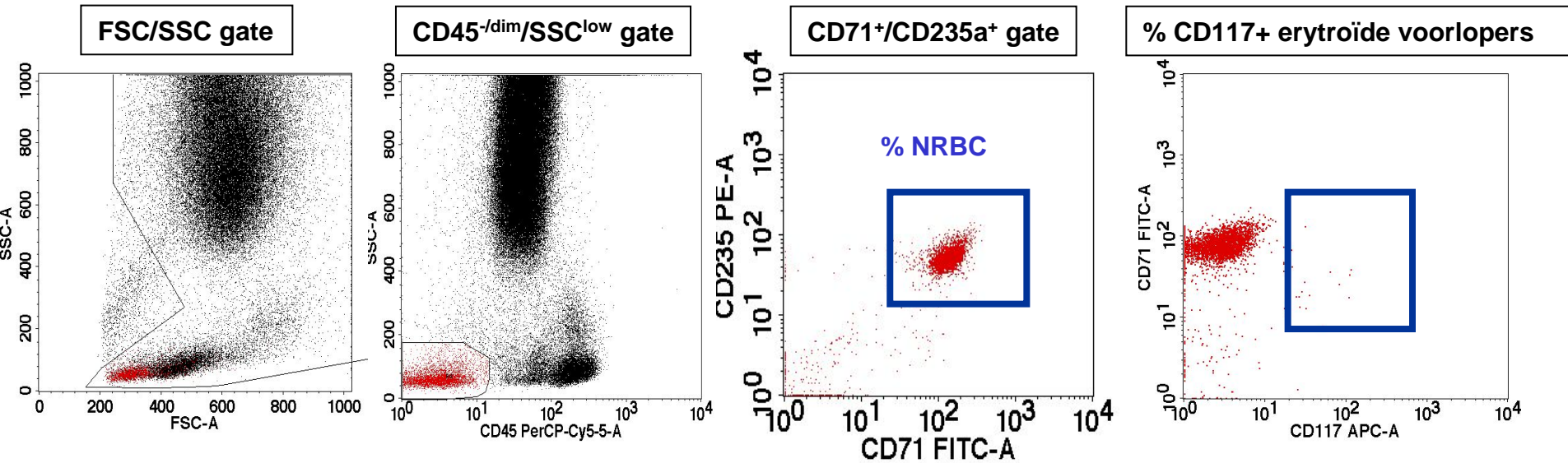


tube					lineage
1	PBS	PBS	CD45	PBS	
2	CD235a	CD117	CD45	CD71	erythroid
3	CD16	CD13	CD45	CD11b	myelomonocytic
4	CD36	CD33	CD45	CD14	myelomonocytic
5	HLA-DR	CD15	CD45	CD11b	myelomonocytic
6	CD13+33	CD117	CD45	CD34	myelomonocytic
7	HLA-DR	CD123	CD45	CD34	immature/basophilic/DC
8	CD7	CD56	CD45	CD34	immature/lymphocytes
9	CD5	CD19	CD45	CD34	immature/lymphocytes
10*	CD15	CD11b	CD45	CD34	myelomonocytic

NB! Geen beperking in de te gebruiken antistofklonen, fluorochromen en xx-kleurenfluorescentie

Gating erythroïde voorlopers (NBM)

CD71/CD235a/CD45/CD117

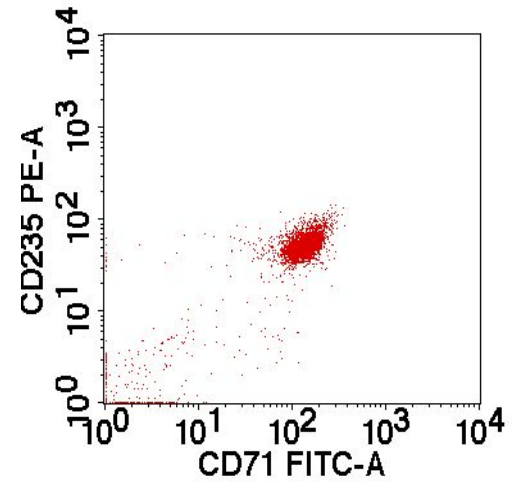
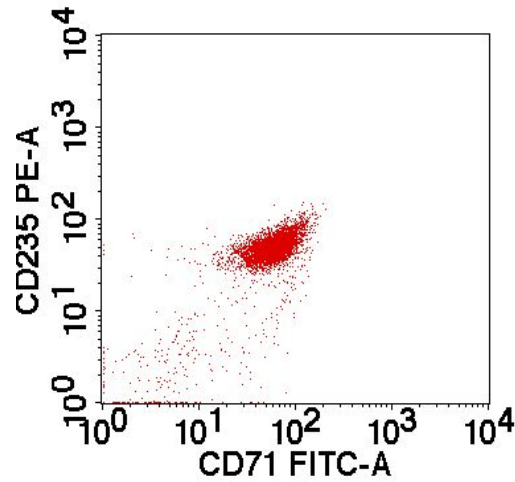
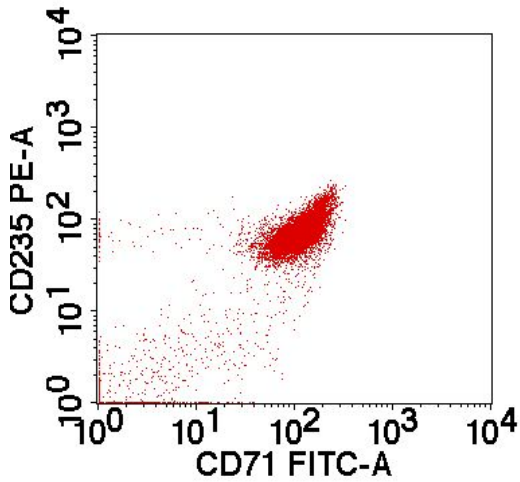


Voorbeeld normaal vs. afwijkend

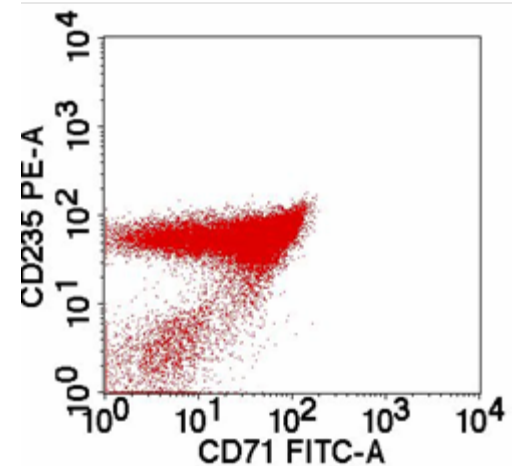
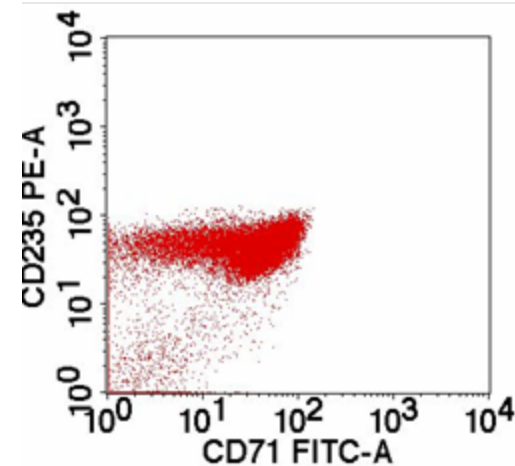
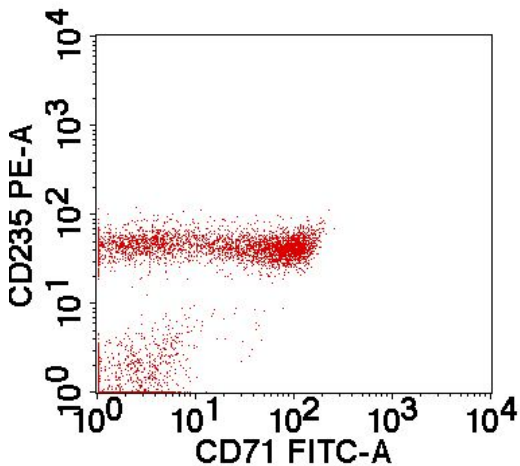
CD71/CD235a



NBM



Afw.

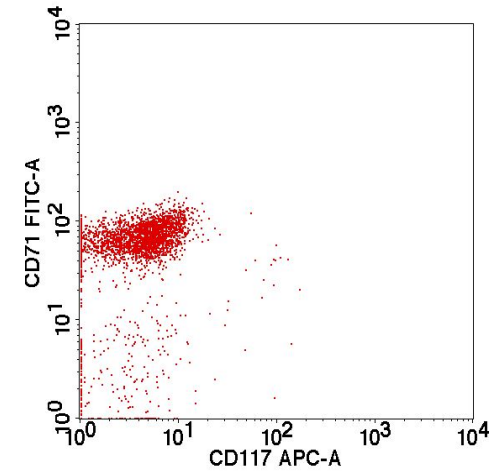
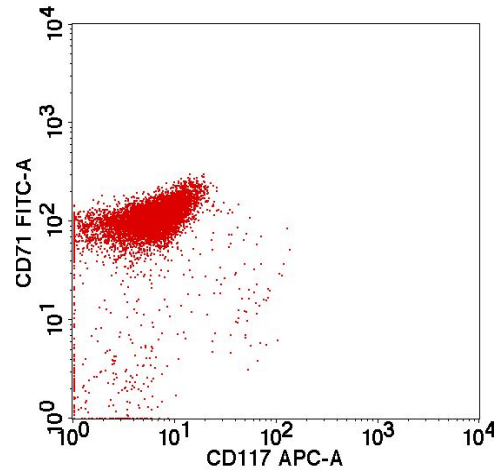
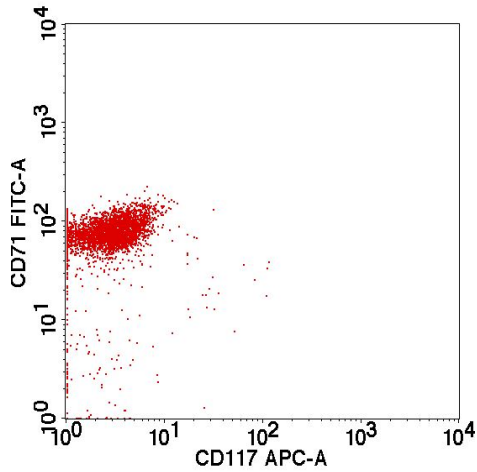


Voorbeeld normaal vs. afwijkend

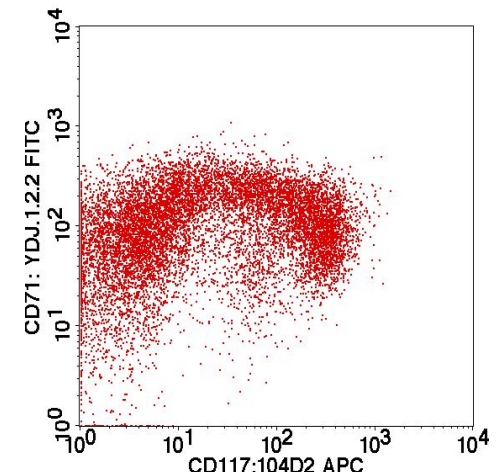
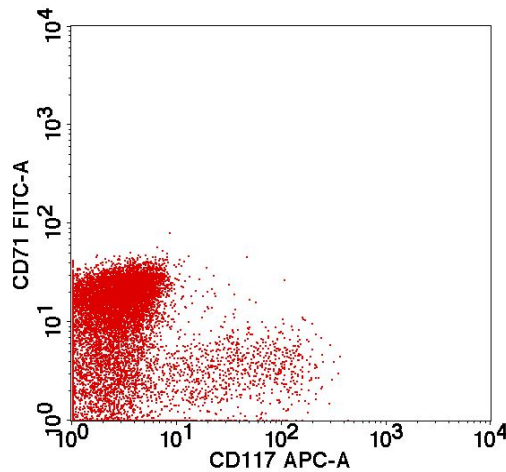
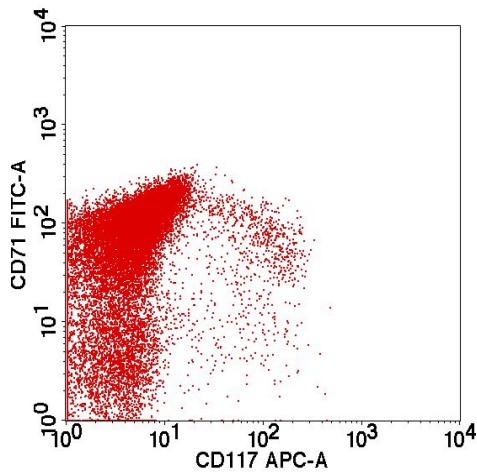
CD117/CD71



NBM



Afw.



2-Rijp myeloïd



Rijp myeloïd

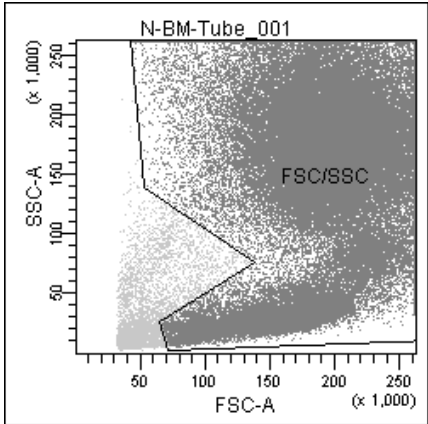


- Gating procedure
- MDS parameters:
 - Percentage granulocyten
 - Mate van granulariteit (SSC)
 - Uitrijpingspatronen & expressie niveaus

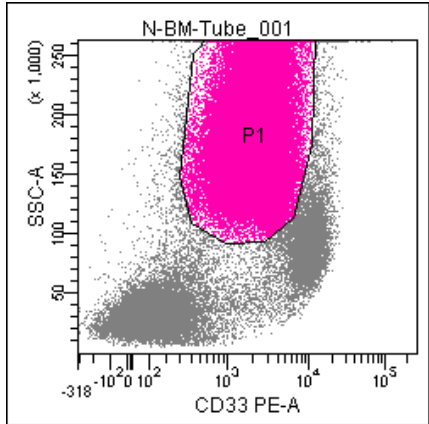
Gating procedure



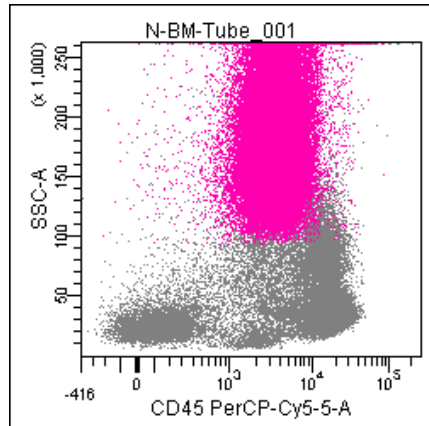
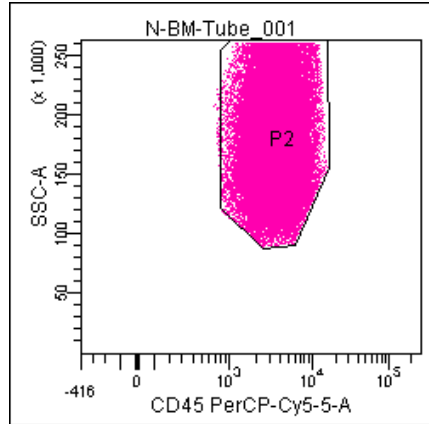
FSC /SSC



CD33+ /SSC^{high}

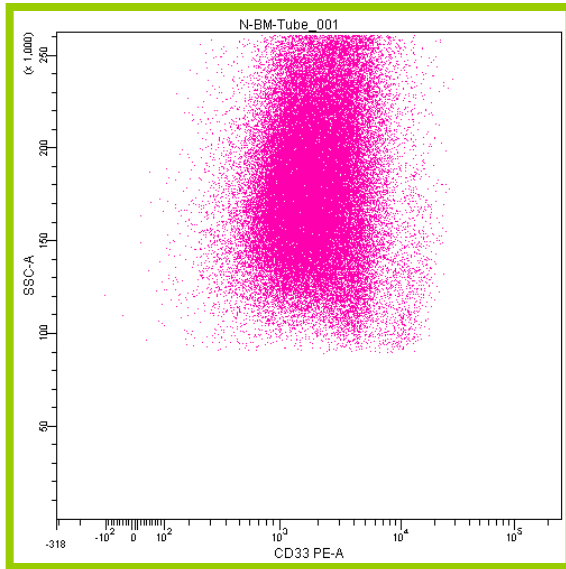


CD45^{dim} / SSC^{high}

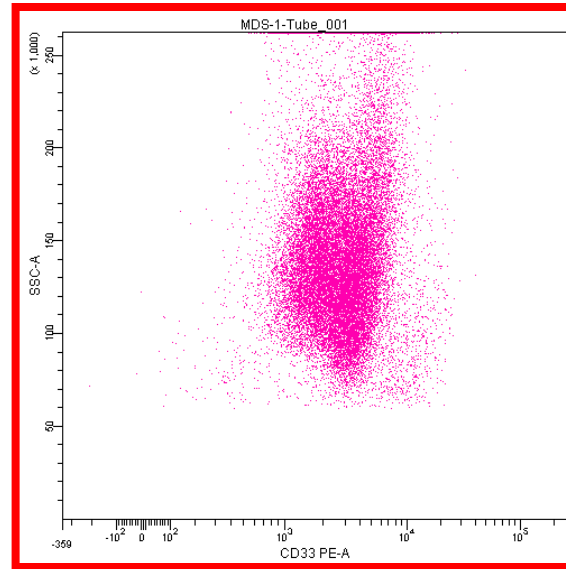


SSC rijpe myeloïde cellen

Ratio ten opzichte van de lymfocyt



Ratio: 8.04

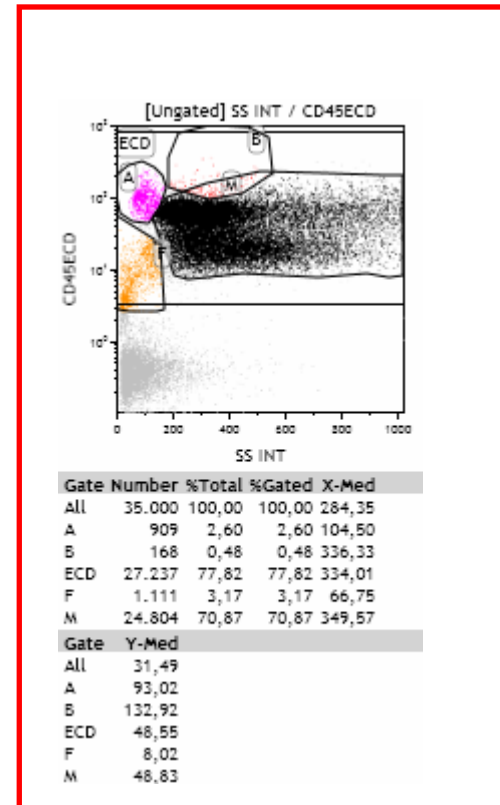
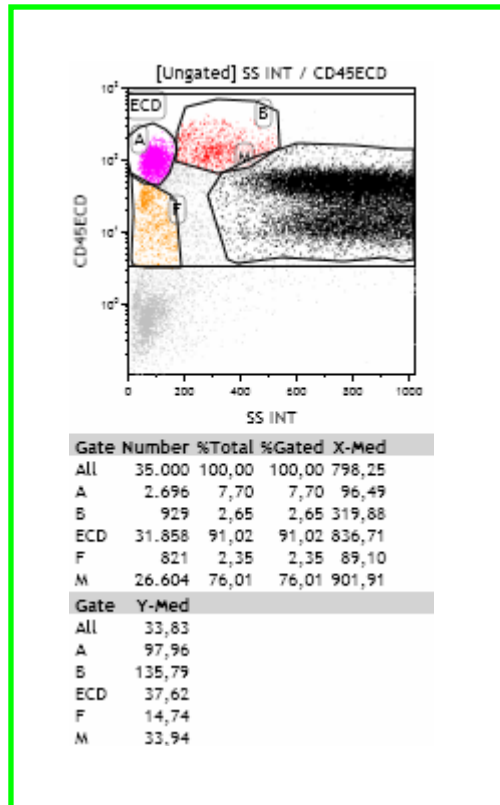


Ratio: 4.71

Referentiewaarden: > 6 (*Ogata et al.*)
SSC waarden zijn instrument setup afhankelijk.

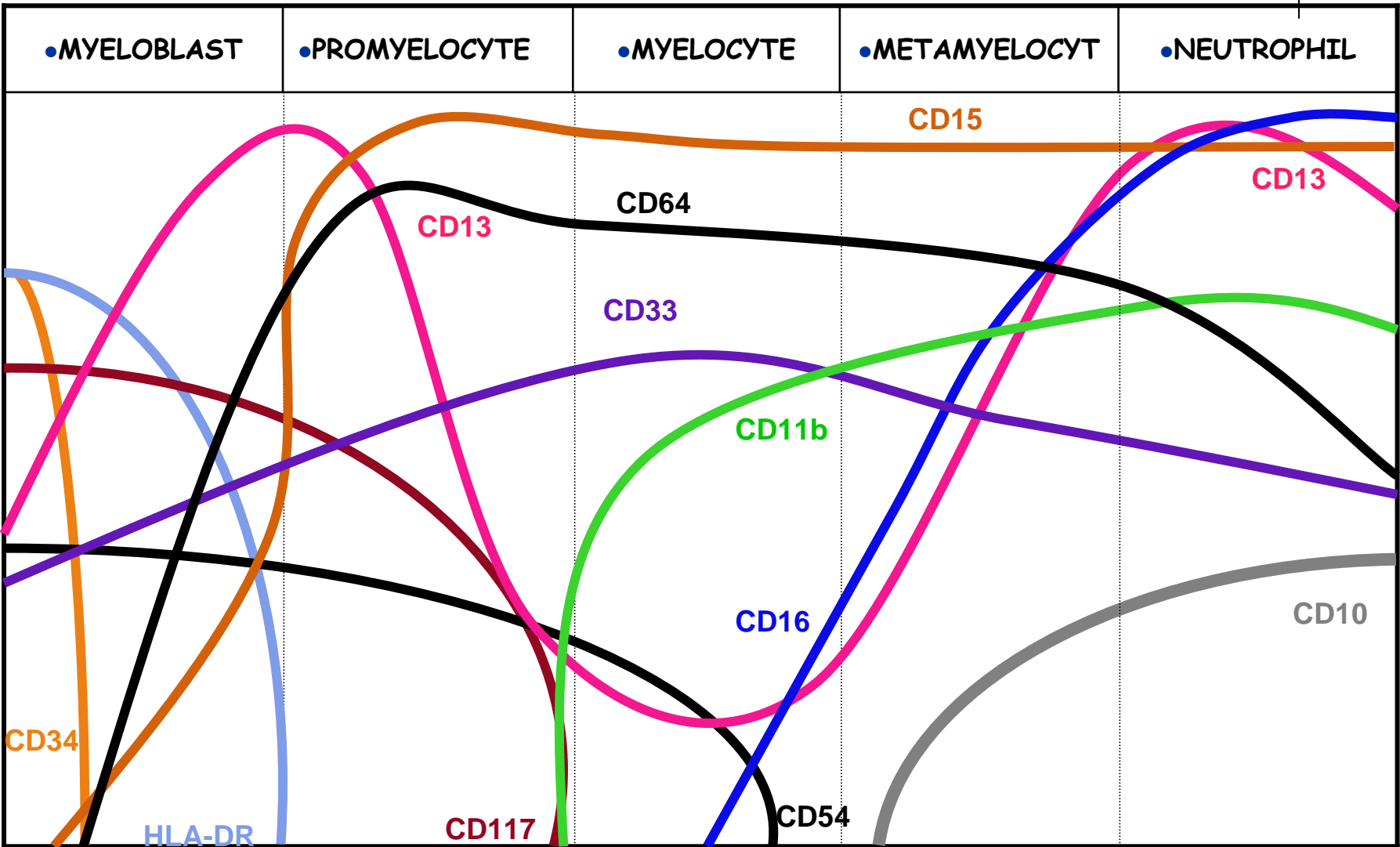
SSC rijpe myeloïde cellen

Ratio ten opzichte van de lymfocyten

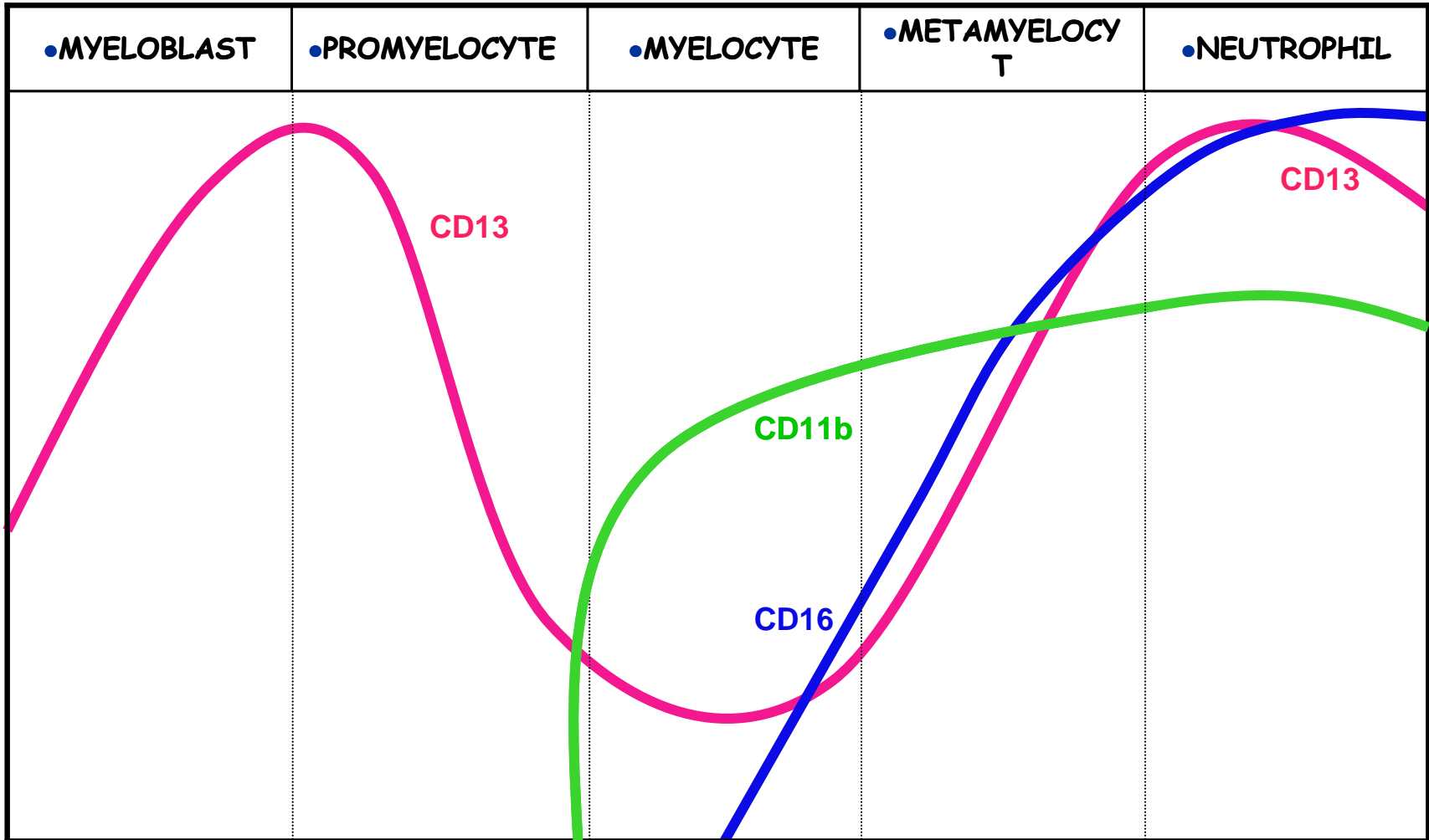


Expressiepatronen

Normale expressie tijdens de uitrijping

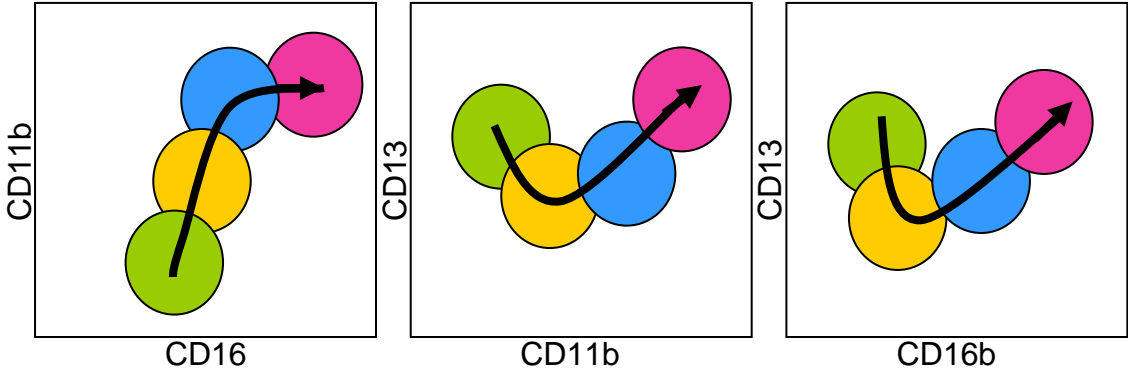


Expressiepatronen CD13, CD16, CD11b



Expressiepatronen

CD13, CD16, CD11b



Promvelocyt



Mvelocyt



Metamvelocyt



Neutrofiel



CD117

CD13^{high}
CD33^{high}
CD15

CD13^{dim}
CD33^{dim}
CD15
CD11b

CD13
CD33^{dim}
CD15
CD11b
CD16

CD13^{high}
CD33
CD15
CD11b^{high}
CD16^{high}

Antistof panel



tube					lineage
1	PBS	PBS	CD45	PBS	
2	CD235a	CD117	CD45	CD71	erythroid
3	CD16	CD13	CD45	CD11b	myelomonocytic
4	CD36	CD33	CD45	CD14	myelomonocytic
5	HLA-DR	CD15	CD45	CD11b	myelomonocytic
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8	CD7	CD56	CD45	CD34	immature/lymphocytes
9	CD5	CD19	CD45	CD34	immature/lymphocytes
10*	CD15	CD11b	CD45	CD34	myelomonocytic

NB! Geen beperking in de te gebruiken antistofklonen, fluorochromen en xx-kleurenfluorescentie

Voorbeeld van flurochromoomkeuze



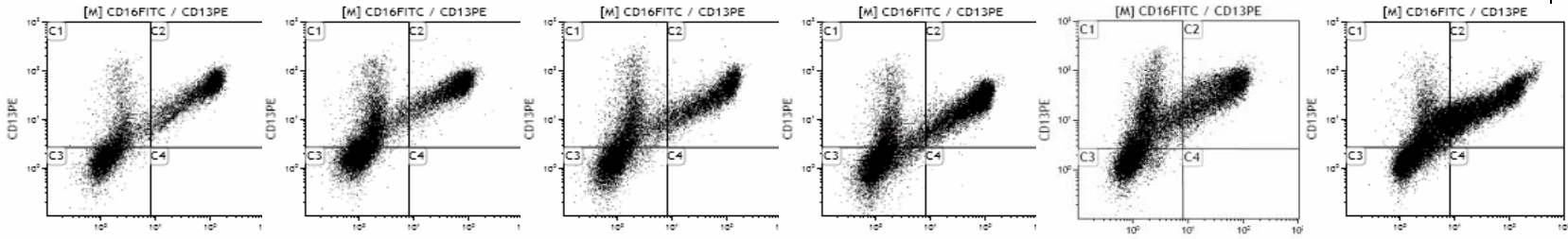
- BC:

CD16^F CD13^{PE} CD45^{ECD} CD11b^{PE-Cy5}

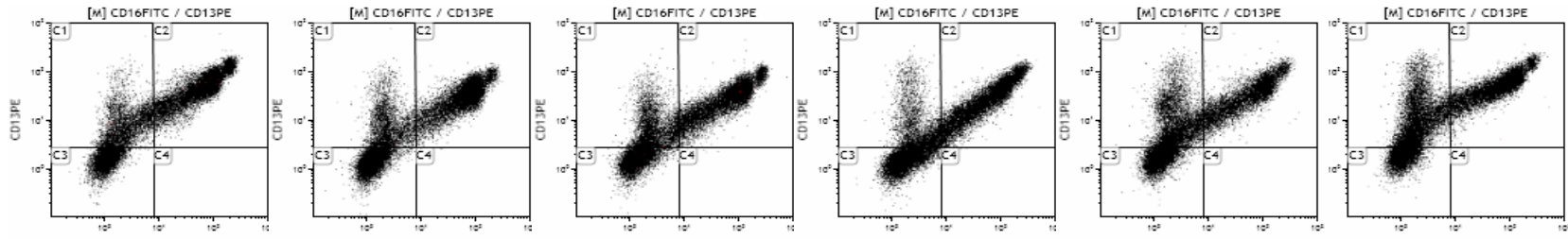
- BD:

CD16^F CD13^{PE} CD45^{PerCP} CD11b^{APC}

Patronen CD16/CD13

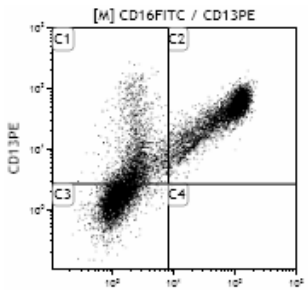


CD16FITC				CD16FITC				CD16FITC				CD16FITC				CD16FITC				CD16FITC									
Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean
All	13.011	37,58	100,00	35,92	All	20.254	53,93	100,00	19,23	All	18.941	42,74	100,00	23,81	All	24.384	61,82	100,00	28,79	All	20.115	62,52	100,00	17,10	All	29.415	84,04	100,00	23,86
C1	2.608	7,53	20,04	2,73	C1	6.298	16,77	31,10	2,11	C1	5.184	11,70	27,37	2,25	C1	3.950	10,01	16,20	2,53	C1	7.233	22,48	35,96	2,45	C1	7.984	22,81	27,14	4,27
C2	5.303	15,32	40,76	85,51	C2	6.124	16,31	30,24	60,11	C2	5.894	13,30	31,12	73,25	C2	10.484	26,58	43,00	64,68	C2	6.626	20,59	32,94	48,26	C2	11.855	33,87	40,30	54,92
C3	5.097	14,72	39,17	1,33	C3	7.829	20,84	38,65	1,04	C3	7.861	17,74	41,50	0,96	C3	9.904	25,11	40,62	1,35	C3	6.255	19,44	31,10	1,02	C3	9.562	27,32	32,51	1,73
C4	3	0,01	0,02	10,48	C4	3	0,01	0,01	10,99	C4	2	0,00	0,01	9,24	C4	46	0,12	0,19	12,38	C4	1	0,00	0,00	10,57	C4	14	0,04	0,05	9,51
Gate Y-Mean				Gate Y-Mean				Gate Y-Mean				Gate Y-Mean				Gate Y-Mean				Gate Y-Mean									
All	21,74	All	19,29	All	17,17	All	12,43	All	21,29	All	12,97	All	19,75	All	18,45	All	10,97	All	23,52	All	1,59	All	2,35						
C1	15,20	C1	14,47	C1	17,93	C1	13,23	C1	18,45	C1	10,97	C1	15,38	C1	11,67	C1	12,03	C1	37,02	C1	1,47	C1	2,38						
C2	44,40	C2	46,68	C2	37,54	C2	22,67	C2	43,02	C2	59,57	C2	35,35	C2	57,99	C2	37,02	C2	3,47	C2	1,84	C2	2,40						
C3	1,53	C3	1,74	C3	1,41	C3	1,32	C3	1,56	C3	1,57	C3	1,64	C3	1,70	C3	1,47	C3	1,47	C3	1,84	C3	1,84						
C4	2,17	C4	2,61	C4	2,63	C4	2,21	C4	2,69	C4	2,16	C4	2,28	C4	2,20	C4	2,16	C4	2,38	C4	2,38	C4	2,38						

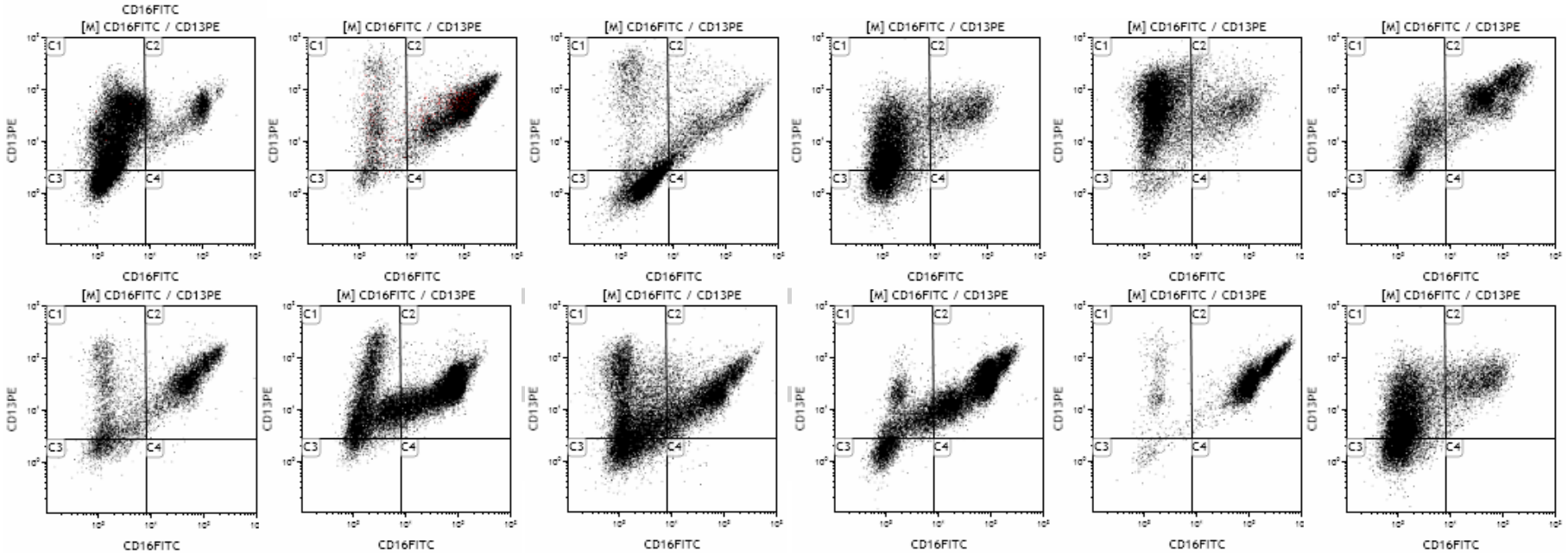


CD16FITC				CD16FITC				CD16FITC				CD16FITC				CD16FITC				CD16FITC									
Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean
All	24.749	70,71	100,00	48,13	All	26.473	75,64	100,00	40,52	All	27.582	78,81	100,00	44,93	All	26.792	76,55	100,00	36,69	All	23.826	68,07	100,00	28,52	All	26.525	75,79	100,00	33,07
C1	4.587	13,11	18,53	3,02	C1	4.103	11,72	15,50	2,89	C1	5.046	14,42	18,29	3,16	C1	4.148	11,85	15,48	3,72	C1	7.364	21,04	30,91	2,98	C1	9.760	27,89	36,79	2,41
C2	13.999	40,00	56,56	83,58	C2	13.535	38,67	51,13	77,45	C2	13.005	37,16	47,15	92,96	C2	11.968	34,19	44,67	79,11	C2	8.358	23,88	35,08	77,29	C2	10.250	29,29	38,64	82,53
C3	6.162	17,61	24,90	1,16	C3	8.825	25,21	33,34	1,41	C3	9.513	27,18	34,49	1,49	C3	10.621	30,35	39,64	1,89	C3	8.102	23,15	34,00	1,42	C3	6.515	18,61	24,56	1,19
C4	1	0,00	0,00	8,62	C4	10	0,03	0,04	12,63	C4	18	0,05	0,07	13,56	C4	55	0,16	0,21	10,05	C4	2	0,01	0,01	10,43	C4	1	0,00	0,00	13,53
Gate Y-Mean				Gate Y-Mean				Gate Y-Mean				Gate Y-Mean				Gate Y-Mean				Gate Y-Mean									
All	36,25	All	21,28	All	19,04	All	18,46	All	19,75	All	29,35	All	19,75	All	17,64	All	19,75	All	17,64	All	1,84	All	2,40						
C1	11,67	C1	12,03	C1	9,88	C1	13,26	C1	15,38	C1	11,67	C1	12,03	C1	12,03	C1	12,03	C1	12,03	C1	1,47	C1	2,38						
C2	59,57	C2	37,02	C2	35,35	C2	35,37	C2	41,13	C2	57,99	C2	35,35	C2	35,35	C2	35,35	C2	35,35	C2	3,47	C2	2,40						
C3	1,57	C3	1,47	C3	1,64	C3	1,51	C3	1,70	C3	1,57	C3	1,64	C3	1,70	C3	1,64	C3	1,64	C3	1,84	C3	1,84						
C4	2,16	C4	2,38	C4	2,28	C4	2,31	C4	2,20	C4	2,16	C4	2,28	C4	2,20	C4	2,16	C4	2,16	C4	2,38	C4	2,38						

Patronen CD16/CD13

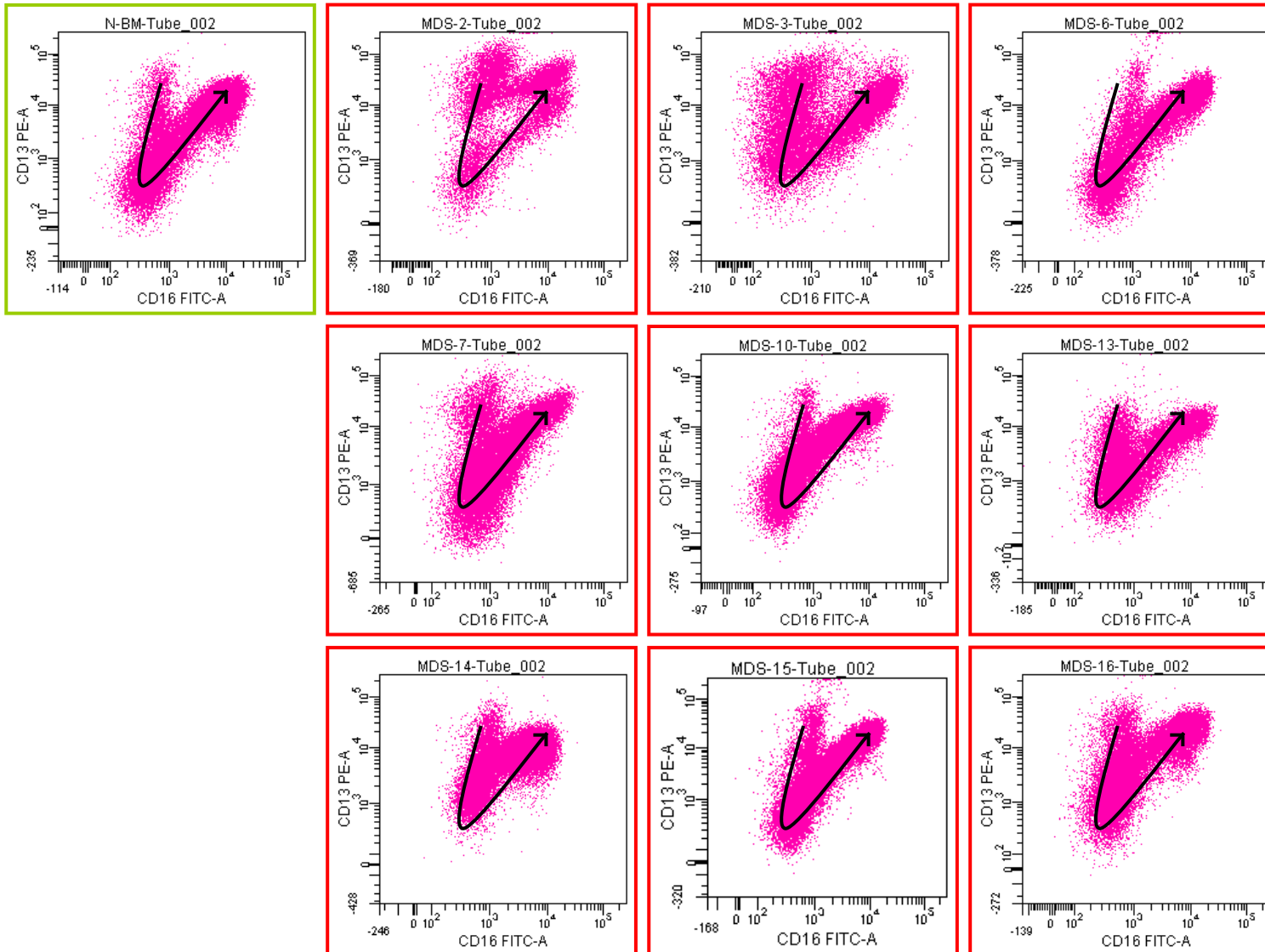


normaal

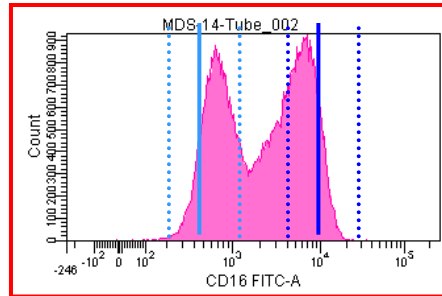
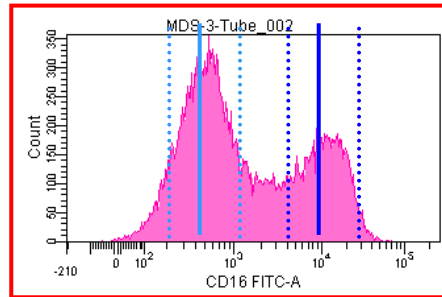
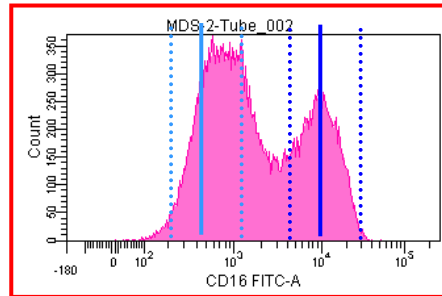
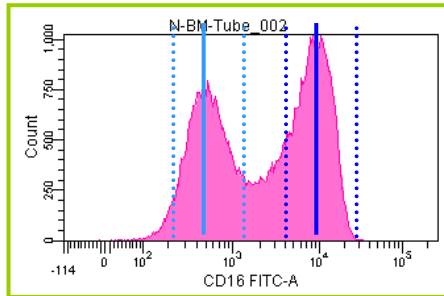


Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean					
All	8.145	23,27	100,00	40,52	All	29.055	83,01	100,00	48,23	All	23.585	67,39	100,00	25,07	All	27.360	78,17	100,00	61,43	All	8.640	30,47	100,00	141,53	All	21.592	61,69	100,00	6,36
C1	2.412	6,89	29,61	2,31	C1	9.495	27,13	32,68	2,76	C1	10.121	28,92	42,91	2,42	C1	3.853	11,01	14,08	3,38	C1	521	1,84	6,03	2,16	C1	13.274	37,93	61,48	1,71
C2	4.709	13,45	57,81	68,55	C2	18.641	53,26	64,16	73,70	C2	8.376	23,93	35,51	66,38	C2	20.964	59,90	76,62	79,42	C2	7.948	28,03	91,99	153,68	C2	2.814	8,04	13,03	38,34
C3	1.017	2,91	12,49	1,51	C3	915	2,61	3,15	1,31	C3	4.952	14,15	21,00	1,84	C3	2.540	7,26	9,28	1,08	C3	170	0,60	1,97	1,42	C3	5.502	15,72	25,48	1,20
C4	7	0,02	0,09	25,64	C4	4	0,01	0,01	10,22	C4	136	0,39	0,58	13,27	C4	3	0,01	0,01	25,49	C4	1	0,00	0,01	9,20	C4	2	0,01	0,01	10,69
Gate Y-A-Mean					Gate Y-A-Mean					Gate Y-A-Mean					Gate Y-A-Mean					Gate Y-A-Mean					Gate Y-A-Mean				
All	39,46				All	29,69				All	20,82				All	49,23				All	14,06				All	14,06			
C1	33,10				C1	30,26				C1	26,38				C1	10,82				C1	52,74				C1	13,58			
C2	50,87				C2	30,76				C2	25,69				C2	39,19				C2	50,02				C2	40,25			
C3	1,96				C3	2,14				C3	1,77				C3	1,66				C3	1,52				C3	1,81			
C4	1,76				C4	2,31				C4	2,02				C4	2,73				C4	2,72				C4	2,41			

Patronen CD16/CD13



Expressie CD16

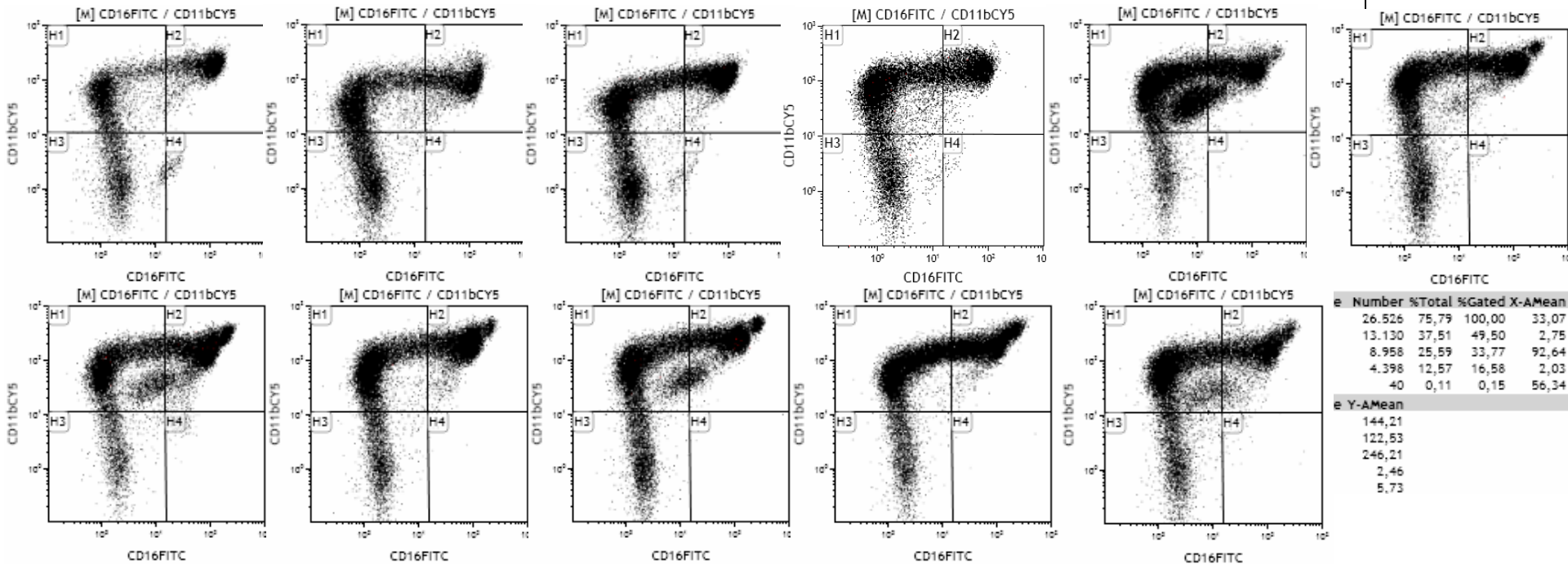


Expressie CD16 afwijkend op de verschillende subpopulaties.

Patronen CD16/CD11b



Eosinofilie



e	Number	%Total	%Gated	X-A-Mean
	26.526	75,79	100,00	33,07
	13.130	37,51	49,50	2,75
	8.958	25,59	33,77	92,64
	4.398	12,57	16,58	2,03
	40	0,11	0,15	56,34

e	Y-A-Mean
	144,21
	122,53
	246,21
	2,46
	5,73

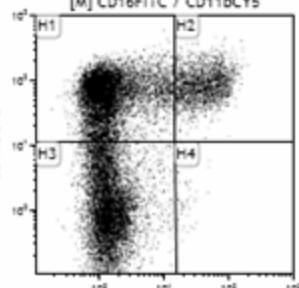
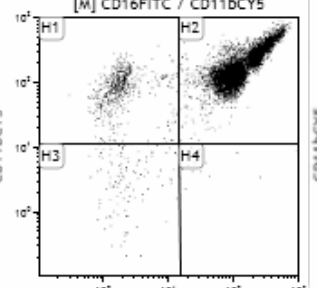
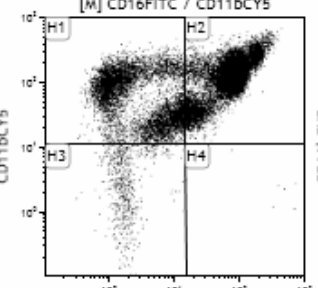
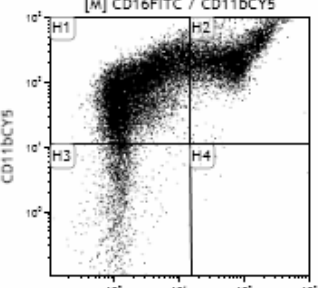
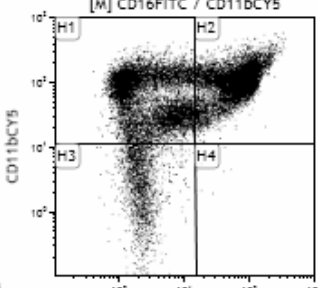
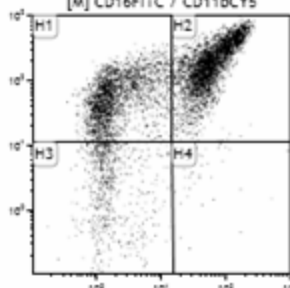
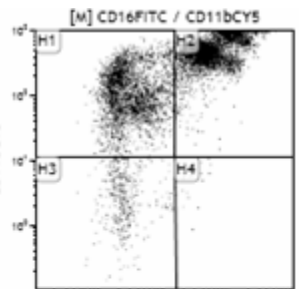
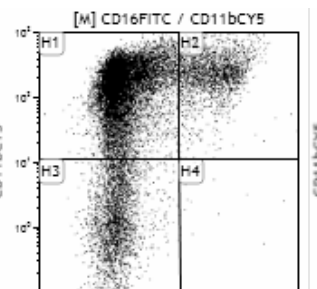
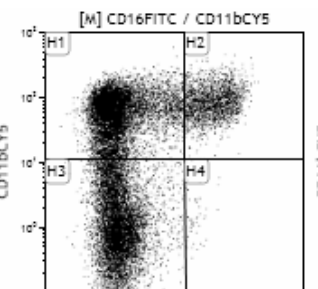
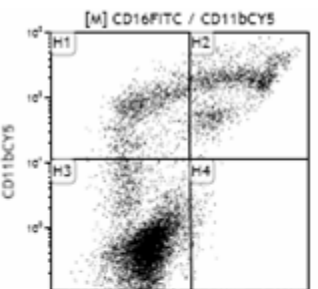
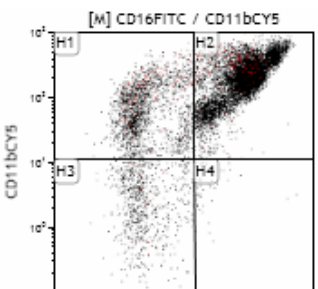
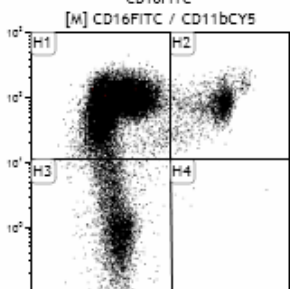
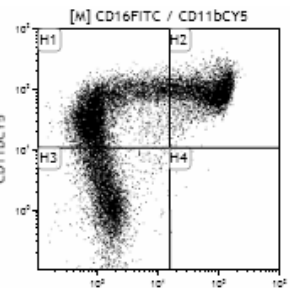
Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean	Gate	Number	%Total	%Gated	X-Mean
All	24.749	70,71	100,00	48,13	All	26.473	75,64	100,00	40,52	All	27.582	78,81	100,00	44,93	All	26.792	76,55	100,00	36,69
H1	10.198	29,14	41,21	3,24	H1	10.555	30,16	39,87	2,52	H1	12.334	35,24	44,72	3,20	H1	13.838	39,54	51,65	3,64
H2	12.384	35,38	50,04	92,81	H2	12.715	36,33	48,03	81,72	H2	11.469	32,77	41,58	103,75	H2	10.127	28,93	37,80	91,33
H3	2.015	5,76	8,14	2,15	H3	3.184	9,10	12,03	2,09	H3	3.771	10,77	13,67	2,13	H3	2.817	8,05	10,51	2,10
H4	152	0,43	0,61	28,39	H4	19	0,05	0,07	22,72	H4	8	0,02	0,03	243,93	H4	10	0,03	0,04	169,43

Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean
All	122,98	All	136,31	All	149,17	All	124,15	All	92,33
H1	84,61	H1	89,01	H1	95,67	H1	86,98	H1	78,09
H2	175,47	H2	209,23	H2	255,00	H2	208,81	H2	167,90
H3	3,33	H3	2,71	H3	2,60	H3	2,82	H3	2,59
H4	6,94	H4	7,58	H4	6,82	H4	6,09	H4	6,18

Patronen CD16/CD11b



normaal



Gate	Number	%Total	%Gated	X-A-Mean
All	8.145	23,27	100,00	40,52
H1	2.698	7,71	33,12	3,07
H2	4.378	12,51	53,75	72,03
H3	1.028	2,94	12,62	2,05
H4	41	0,12	0,50	105,17

Gate	Number	%Total	%Gated	X-A-Mean
All	29.055	83,01	100,00	48,23
H1	9.785	27,96	33,68	4,26
H2	16.787	47,96	57,78	80,61
H3	2.471	7,06	8,50	2,44
H4	12	0,03	0,04	26,76

Gate	Number	%Total	%Gated	X-A-Mean
All	23.585	67,39	100,00	25,07
H1	14.494	41,41	61,45	3,42
H2	6.768	19,34	28,70	79,28
H3	2.310	6,60	9,79	1,28
H4	13	0,04	0,06	176,55

Gate	Number	%Total	%Gated	X-A-Mean
All	27.360	78,17	100,00	61,43
H1	7.605	21,73	27,80	4,71
H2	19.051	54,43	69,63	86,06
H3	694	1,98	2,54	2,23
H4	10	0,03	0,04	380,07

Gate	Number	%Total	%Gated	X-A-Mean
All	8.640	30,47	100,00	141,53
H1	604	2,13	6,99	2,60
H2	7.873	27,77	91,12	154,84
H3	144	0,51	1,67	3,00
H4	19	0,07	0,22	90,21

Gate	Number	%Total	%Gated	X-A-Mean
All	21.592	61,69	100,00	6,36
H1	9.250	26,43	42,84	2,14
H2	2.096	5,99	9,71	47,38
H3	10.189	29,11	47,19	1,66
H4	57	0,16	0,26	22,28

Gate	Y-A-Mean
All	144,80
H1	71,12
H2	224,87
H3	2,76
H4	110

Gate	Y-A-Mean
All	91,29
H1	79,15
H2	111,40
H3	3,16

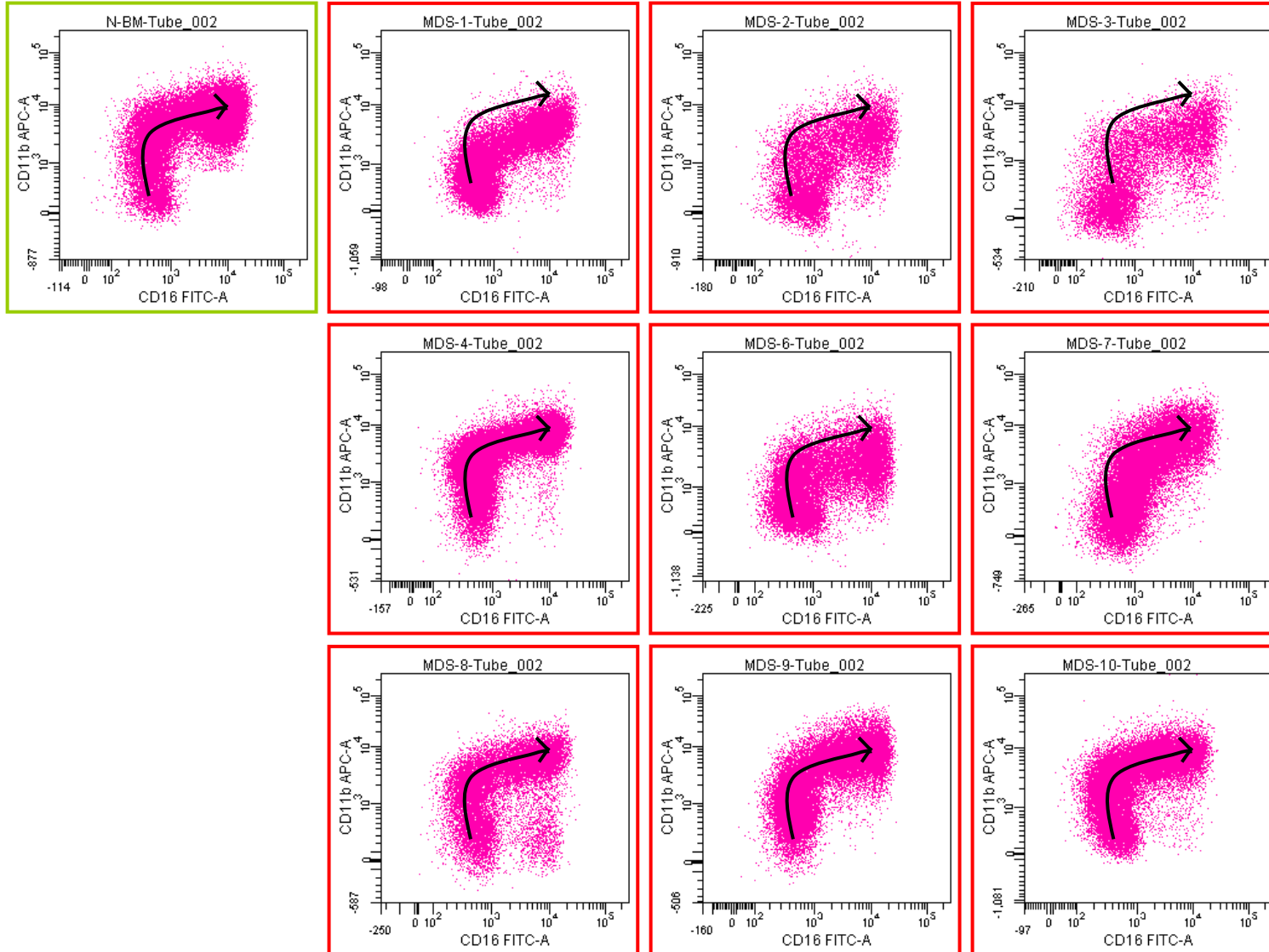
Gate	Y-A-Mean
All	143,87
H1	105,39
H2	274,66
H3	2,89

Gate	Y-A-Mean
All	122,37
H1	80,77
H2	143,35
H3	4,04

Gate	Y-A-Mean
All	178,35
H1	92,69
H2	188,54
H3	3,50

Gate	Y-A-Mean
All	39,34
H1	68,02
H2	96,93
H3	1,67
H4	2,64

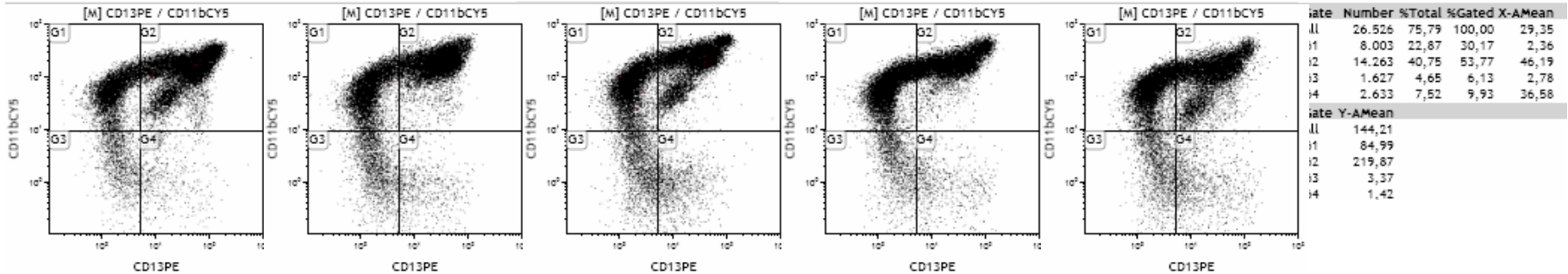
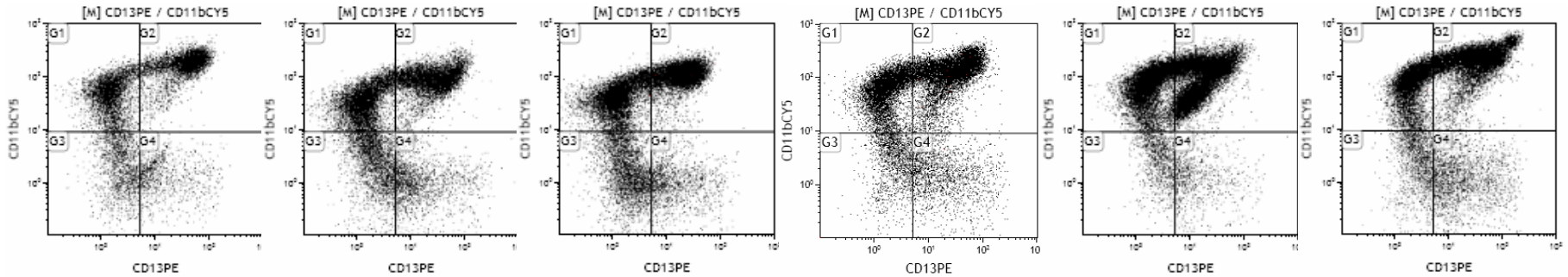
Patronen CD16/CD11b



Patronen CD11b/CD13



Eosinofilie



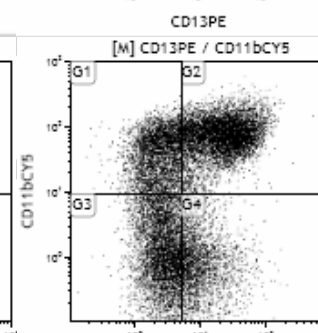
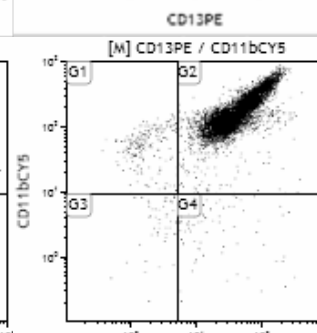
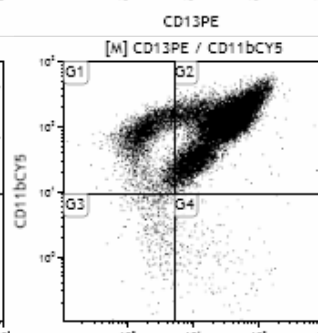
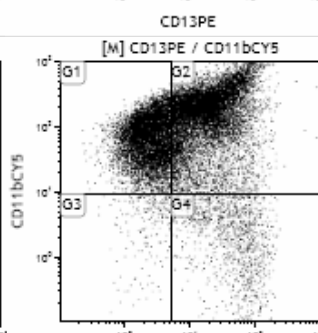
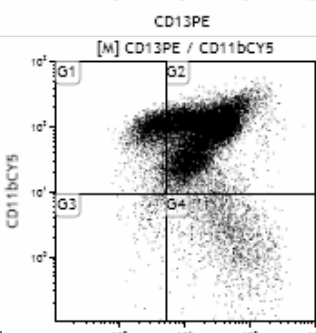
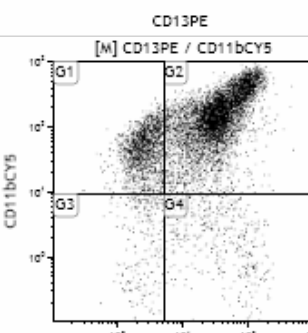
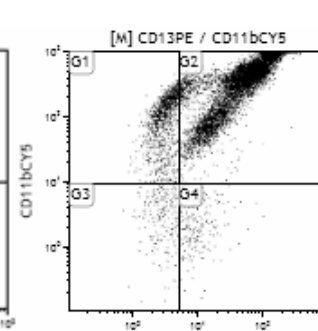
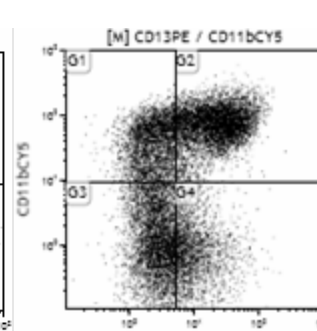
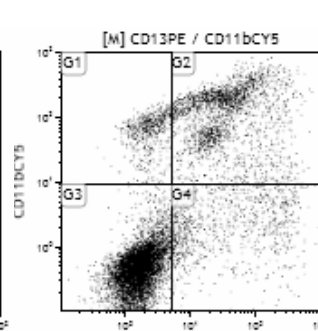
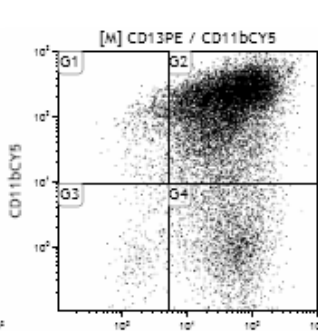
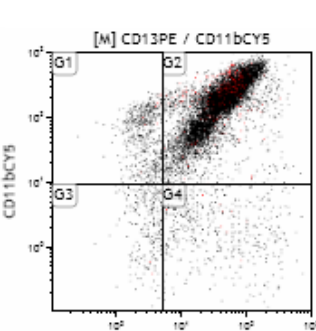
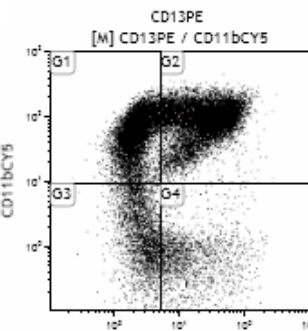
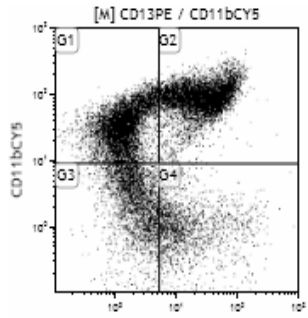
Gate	Number	%Total	%Gated	X-A-Mean
Gate				
i1	26,526	75,79	100,00	29,35
i2	8,003	22,87	30,17	2,36
i3	14,263	40,75	53,77	46,19
i4	1,627	4,65	6,13	2,78
i4	2,633	7,52	9,93	36,58
Gate Y-A-Mean				
i1	144,21			
i2	84,99			
i3	219,87			
i4	3,37			
i4	1,42			

Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean					
All	24,749	70,71	100,00	36,25	All	26,473	75,64	100,00	21,28	All	27,582	78,81	100,00	19,04	All	26,792	76,55	100,00	18,46	All	23,826	68,07	100,00	19,75
G1	6,288	17,97	25,41	1,91	G1	8,376	23,93	31,64	1,76	G1	9,686	27,67	35,12	1,93	G1	11,689	33,40	43,63	1,92	G1	8,978	25,65	37,68	2,14
G2	16,454	47,01	66,48	52,46	G2	15,056	43,02	56,87	34,43	G2	14,306	40,87	51,87	33,13	G2	12,408	35,45	46,31	34,82	G2	11,289	32,25	47,38	34,43
G3	1,304	3,73	5,27	2,29	G3	2,115	6,04	7,99	2,09	G3	2,219	6,34	8,05	2,58	G3	1,672	4,78	6,24	2,25	G3	1,887	5,39	7,92	2,56
G4	703	2,01	2,84	26,96	G4	926	2,65	3,50	27,88	G4	1,371	3,92	4,97	19,57	G4	1,023	2,92	3,82	35,39	G4	1,672	4,78	7,02	34,60
Gate Y-A-Mean					Gate Y-A-Mean					Gate Y-A-Mean					Gate Y-A-Mean									
All	122,98				All	136,31				All	149,17				All	124,15				All	92,33			
G1	63,61				G1	70,22				G1	82,51				G1	75,55				G1	66,82			
G2	160,29				G2	200,14				G2	231,19				G2	196,37				G2	141,03			
G3	3,37				G3	2,98				G3	2,91				G3	3,45				G3	3,05			
G4	2,50				G4	0,87				G4	1,07				G4	0,87				G4	1,25			

Patronen CD11b/CD13



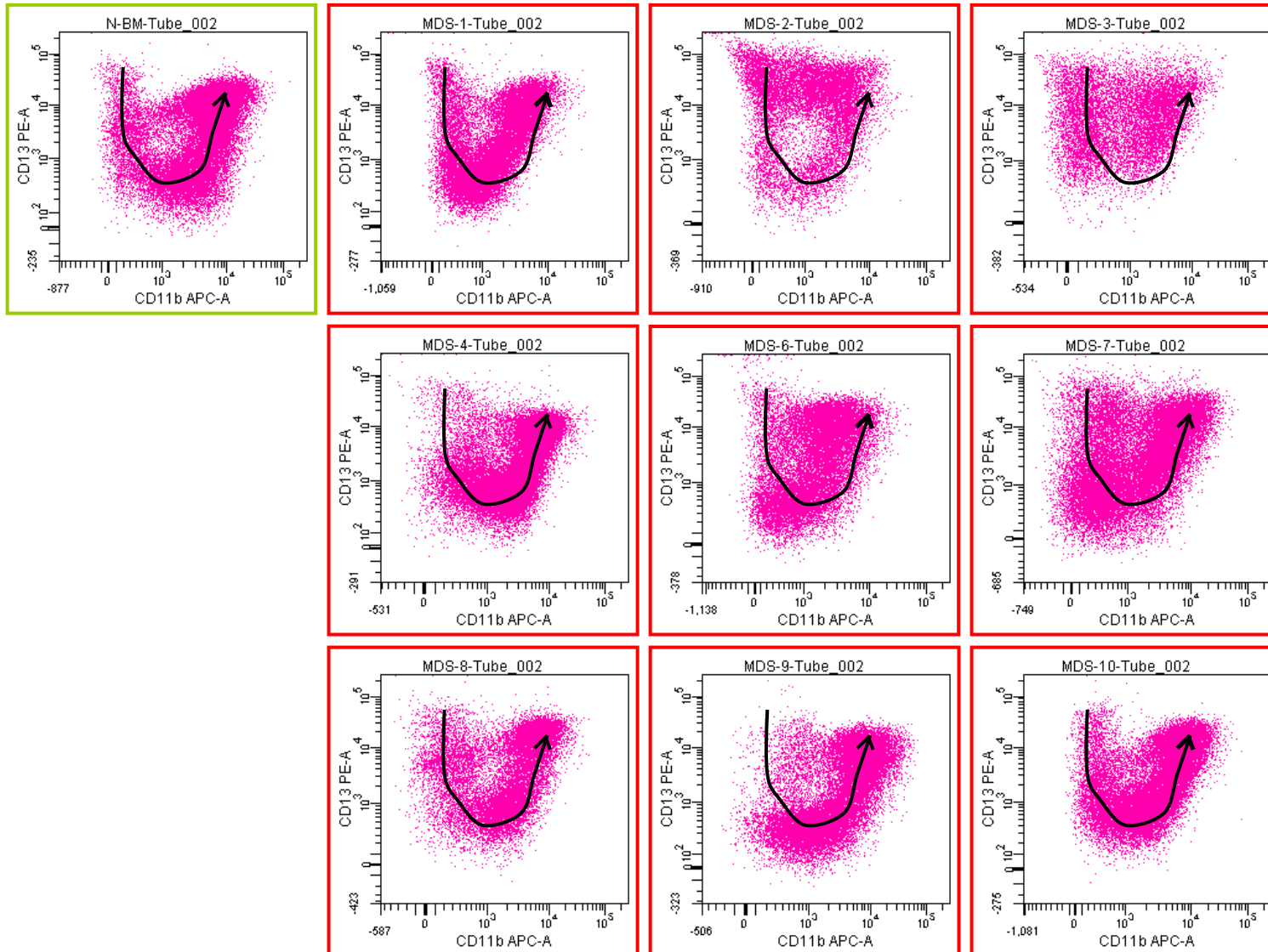
normaal



Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean	Gate	Number	%Total	%Gated	X-A-Mean					
All	8.145	23,27	100,00	39,46	All	29.055	83,01	100,00	29,69	All	23.585	67,39	100,00	20,82	All	27.360	78,17	100,00	31,71	All	8.640	30,47	100,00	49,23	All	21.592	61,69	100,00	14,06
G1	1.551	4,43	19,04	2,77	G1	2.705	7,73	9,31	3,36	G1	8.548	24,42	36,24	2,63	G1	3.452	9,86	12,62	2,37	G1	192	0,68	2,22	2,05	G1	3.928	11,22	18,19	2,55
G2	5.612	16,03	68,90	46,75	G2	24.026	68,65	82,69	27,69	G2	12.911	36,89	54,74	25,62	G2	23.281	66,52	85,09	36,55	G2	8.301	29,28	96,08	50,40	G2	7.651	21,86	35,43	28,02
G3	290	0,83	3,56	2,61	G3	203	0,58	0,70	3,14	G3	264	0,75	1,12	2,55	G3	383	1,09	1,40	2,80	G3	52	0,18	0,60	2,80	G3	5.992	17,12	27,75	2,78
G4	692	1,98	8,50	77,99	G4	2.121	6,06	7,30	88,44	G4	1.862	5,32	7,89	73,67	G4	244	0,70	0,89	29,57	G4	95	0,34	1,10	67,17	G4	4.021	11,49	18,62	15,52

Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean	Gate	Y-A-Mean
All	144,80	All	91,29	All	143,87	All	122,37	All	178,35	All	39,34	All	39,34
G1	56,21	G1	87,93	G1	85,76	G1	79,70	G1	59,53	G1	50,63	G1	50,63
G2	194,24	G2	100,24	G2	205,67	G2	131,91	G2	184,20	G2	83,11	G2	83,11
G3	2,87	G3	4,87	G3	4,07	G3	4,22	G3	3,67	G3	1,85	G3	1,85
G4	1,88	G4	2,48	G4	1,96	G4	1,77	G4	2,81	G4	0,92	G4	0,92

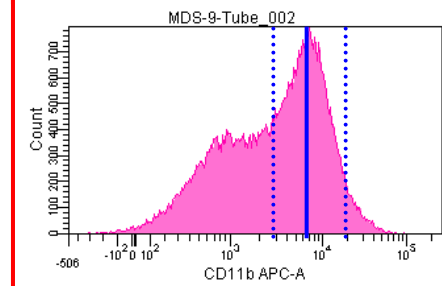
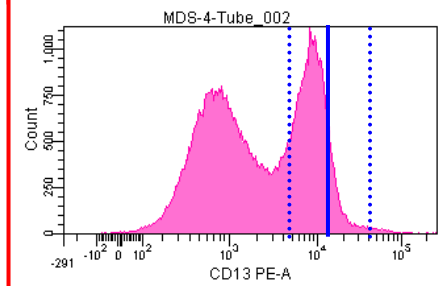
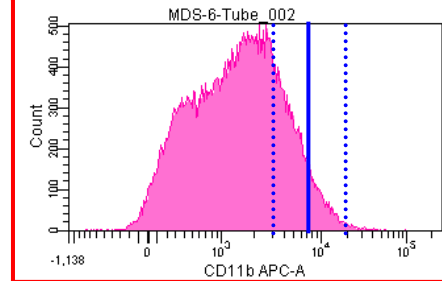
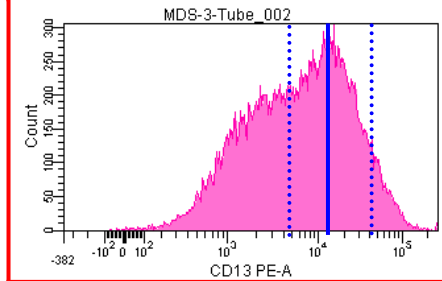
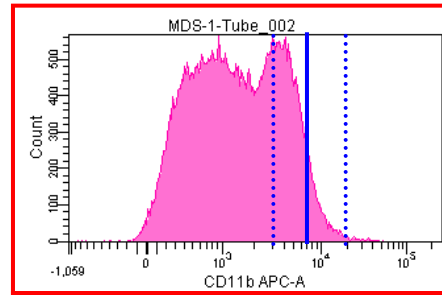
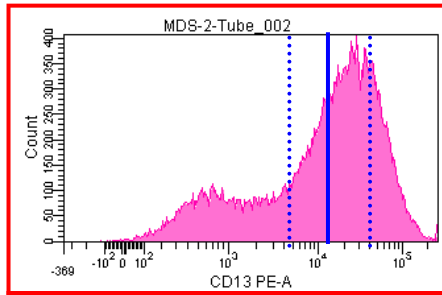
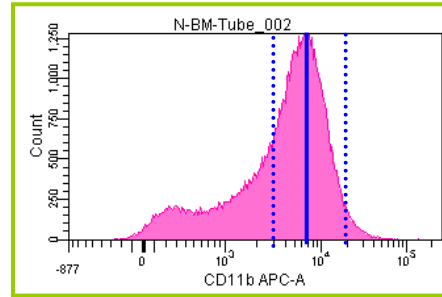
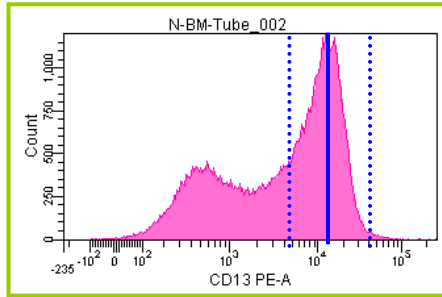
Patronen CD11b/CD13



Verdeling
binnen een
patroon kan
afwijkend zijn,
terwijl de
expressie
normaal is.

Hoe dient dit
beoordeeld te
worden?

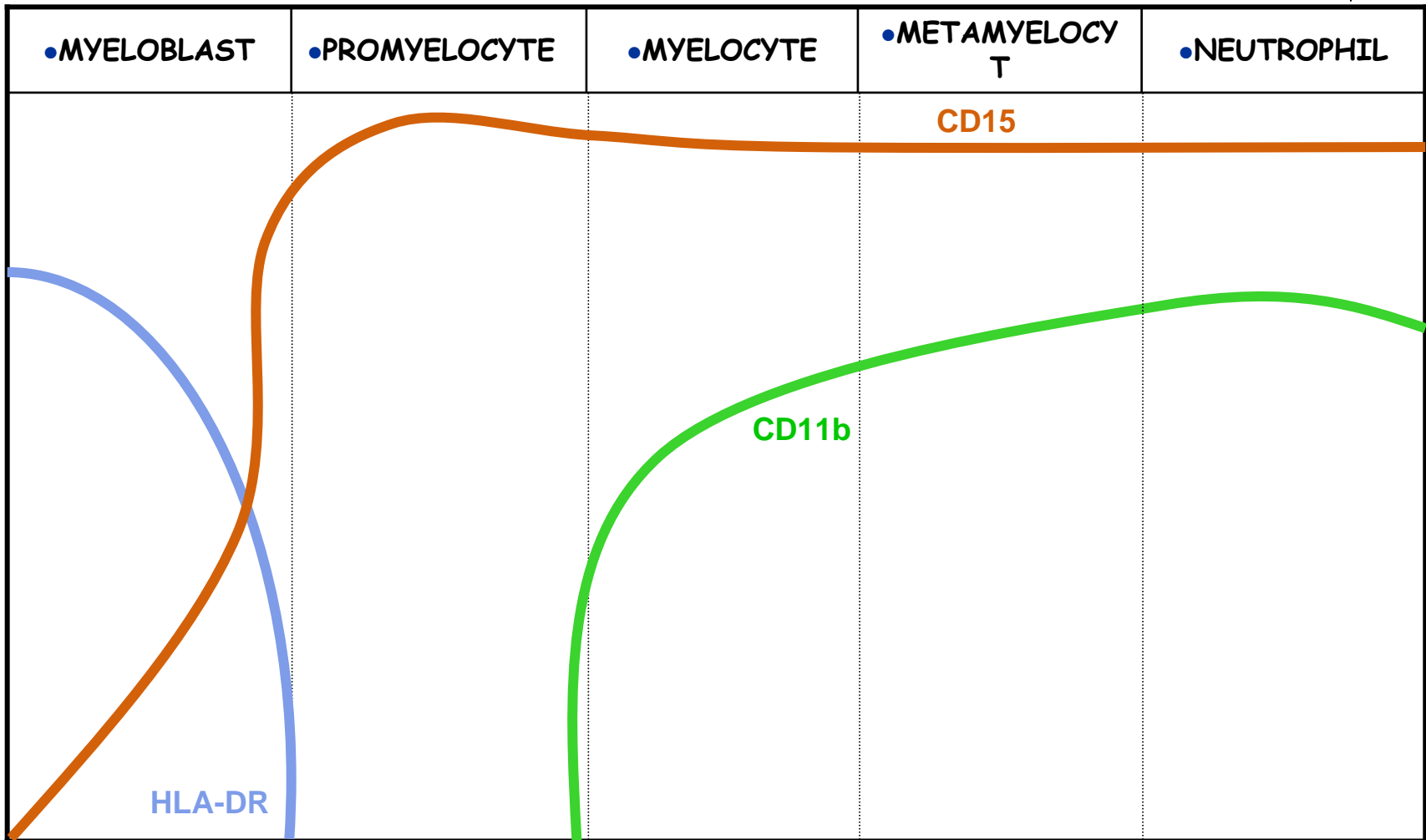
Expressie CD13 en CD11b



Onder- of overexpressie:
Minimaal een halve log (factor 3.3)
verschil met het normale BM.

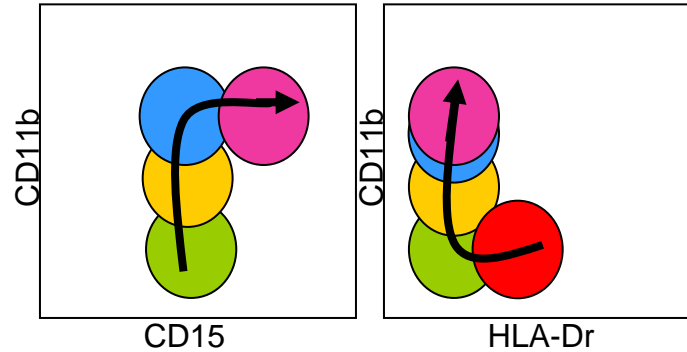
Expressiepatronen

CD15, CD11b, HLA-Dr



Patronen

CD15 – HLA-Dr – CD11b



Myeloblast



CD34
CD117
HLA-DR
CD13^{high}
CD33^{high}

Promyelocyt



CD117
CD13^{high}
CD33^{high}
CD15

Myelocyt



CD13^{dim}
CD33^{dim}
CD15
CD11b

Metamyelocyt



CD13
CD33^{dim}
CD15
CD11b
CD16

Neutrofiel



CD13^{high}
CD33
CD15
CD11b^{high}
CD16^{high}

Antistof panel



tube					lineage
1	PBS	PBS	CD45	PBS	
2	CD235a	CD117	CD45	CD71	erythroid
3	CD16	CD13	CD45	CD11b	myelomonocytic
4	CD36	CD33	CD45	CD14	myelomonocytic
5	HLA-DR	CD15	CD45	CD11b	myelomonocytic
6	CD13+33	CD117	CD45	CD34	myelomonocytic
7	HLA-DR	CD123	CD45	CD34	immature/basophilic/DC
8	CD7	CD56	CD45	CD34	immature/lymphocytes
9	CD5	CD19	CD45	CD34	immature/lymphocytes
10*	CD15	CD11b	CD45	CD34	myelomonocytic

NB! Geen beperking in de te gebruiken antistofklonen, fluorochromen en xx-kleurenfluorescentie

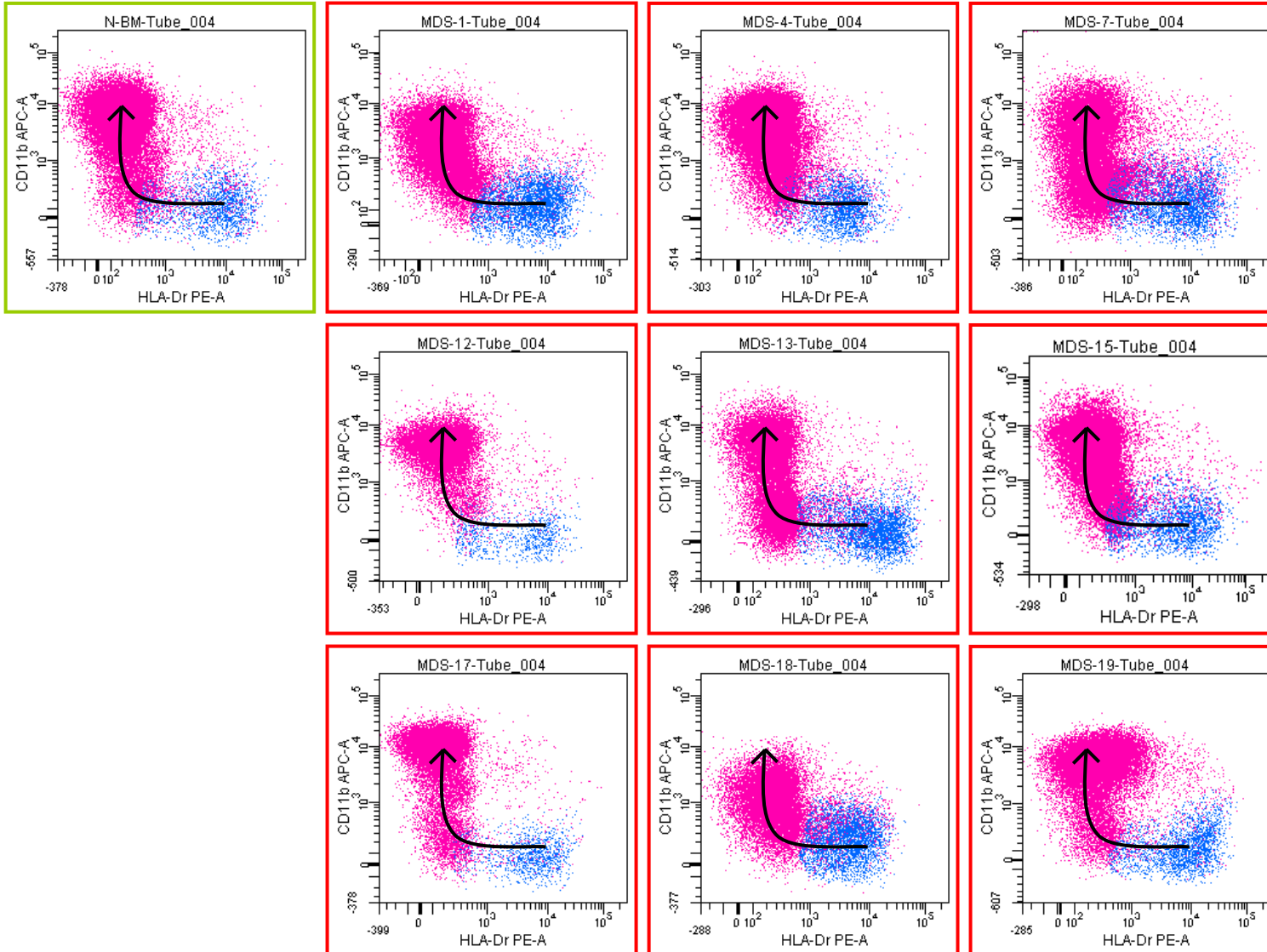
Voorbeeld van flurochromoomkeuze



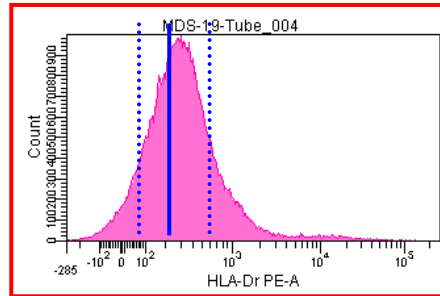
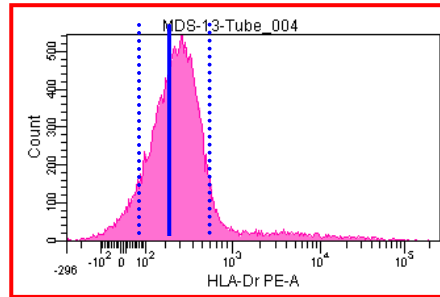
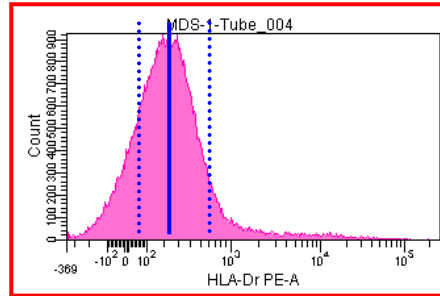
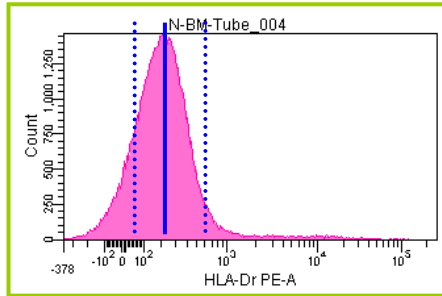
- BC:
CD15^F HLA-Dr^{PE} CD45^{ECD} CD11b^{PE-Cy5}
- BD:
CD15^F HLA-Dr^{PE} CD45^{PerCP} CD11b^{APC}

Patronen

HLA-Dr/CD11b



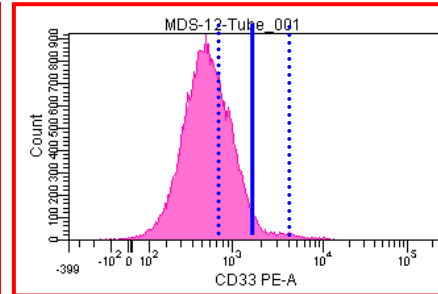
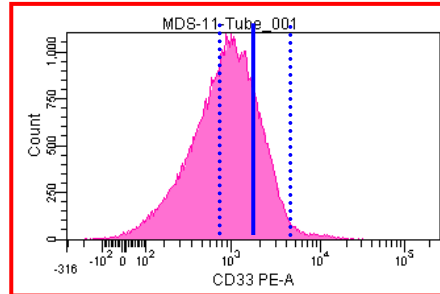
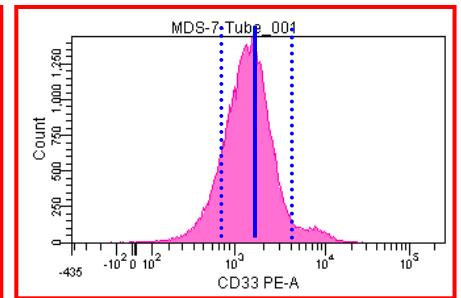
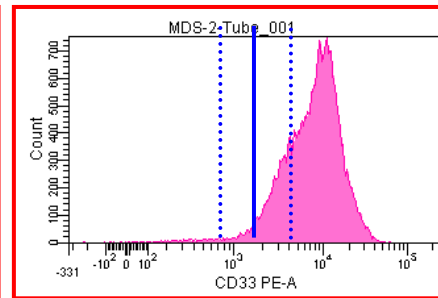
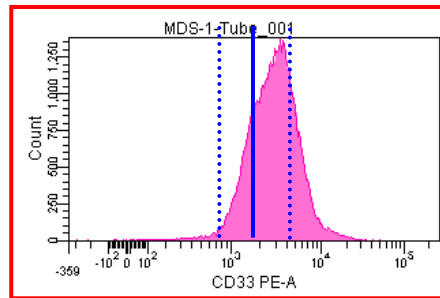
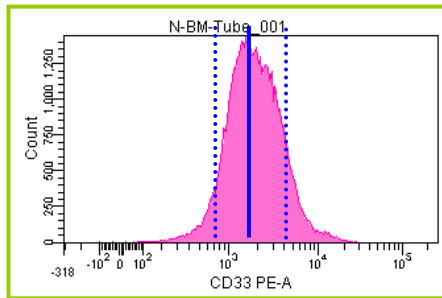
Expressie HLA-Dr



Ondanks de afwijkende patronen (HLA-Dr/
CD11b) geen verhoogde of verlaagde
expressie niveau's van HLA-Dr

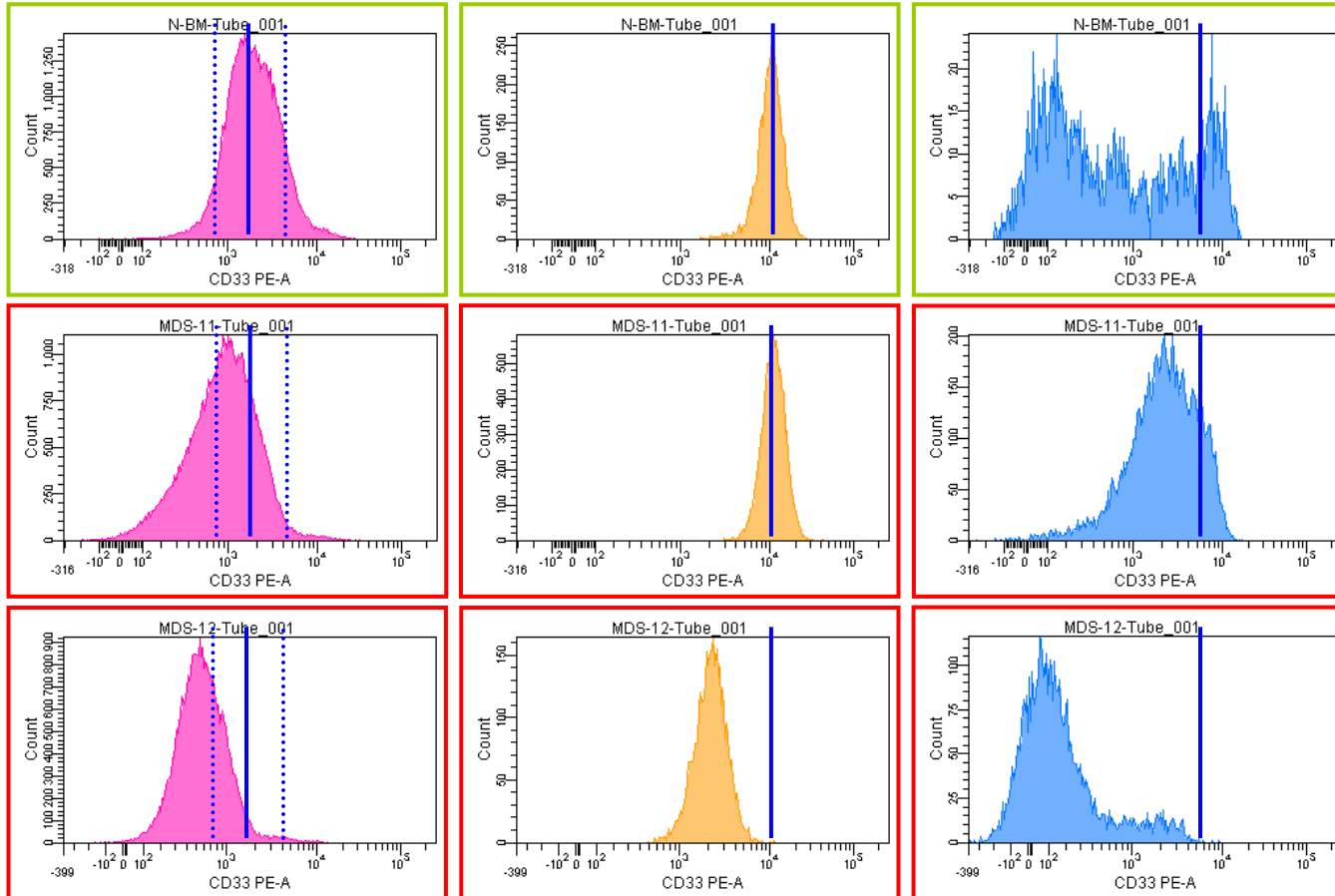
Mogelijk breed verhoogde expressie

Expressie CD33



Expressie CD33 beoordelen in relatie tot de monocyt en onrijpe cellen.

Expressie CD33 - Polymorfisme



Zwakke CD33 expressie op de rijp en onrijp myeloïde cellen. CD33 expressie op monocyten is normaal.

Zwakke CD33 expressie op de rijp en onrijp myeloïde cellen. CD33 expressie op monocyten is ook zwak.
→ Wrs. polymorfisme

Aberrante markers op granulocyten



- CD5
- CD7
- CD14
- CD19
- CD34
- CD36
- CD56

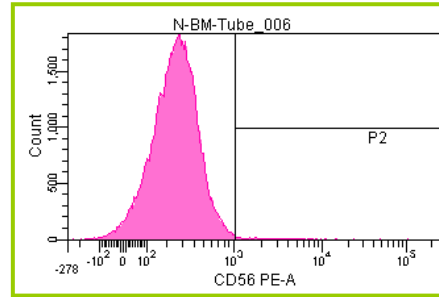
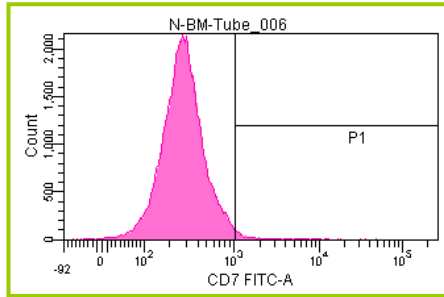
Antistof panel



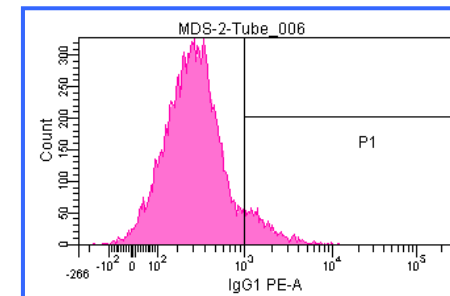
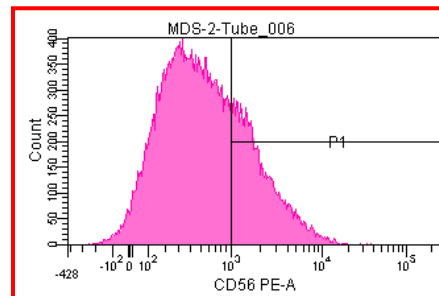
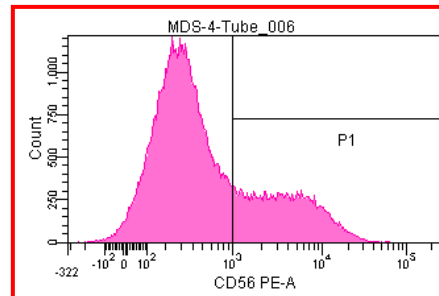
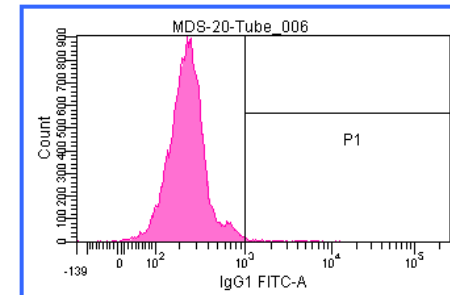
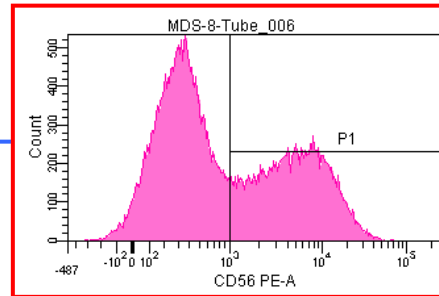
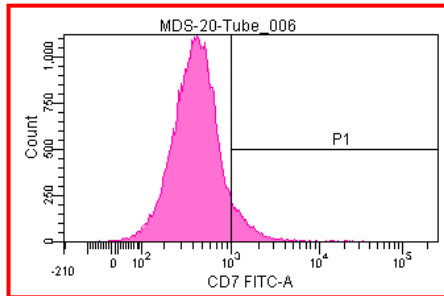
tube					lineage
1	PBS	PBS	CD45	PBS	
2	CD235a	CD117	CD45	CD71	erythroid
3	CD16	CD13	CD45	CD11b	myelomonocytic
4	CD36	CD33	CD45	CD14	myelomonocytic
5	HLA-DR	CD15	CD45	CD11b	myelomonocytic
6	CD13+33	CD117	CD45	CD34	myelomonocytic
7	HLA-DR	CD123	CD45	CD34	immature/basophilic/DC
8	CD7	CD56	CD45	CD34	immature/lymphocytes
9	CD5	CD19	CD45	CD34	immature/lymphocytes
10*	CD15	CD11b	CD45	CD34	myelomonocytic

NB! Geen beperking in de te gebruiken antistofklonen, fluorochromen en xx-kleurenfluorescentie

Expressie CD7 en CD56



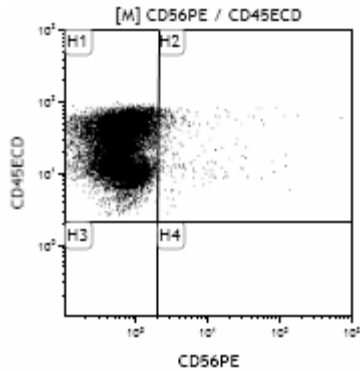
Expressie beoordelen icm
achtergrond aankleuring



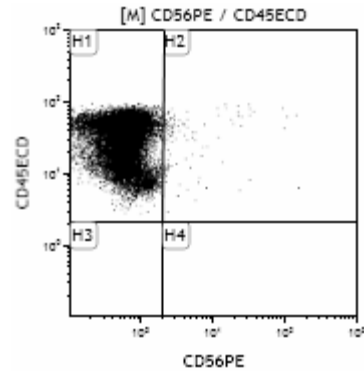
Expressie CD56



NBM

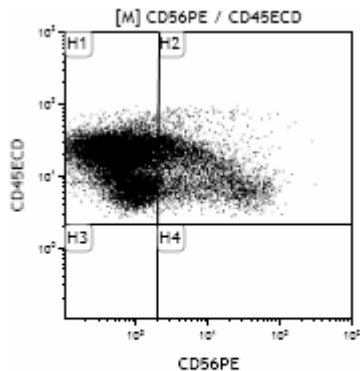


Gate	X-A-Mean	Y-A-Mean
All	0,85	32,74
H1	0,72	32,62
H2	9,55	40,16
H3	N/A	N/A
H4	N/A	N/A

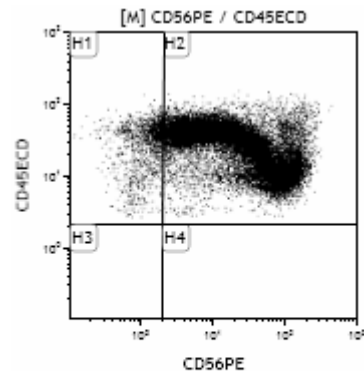


Gate	X-A-Mean	Y-A-Mean
All	0,70	36,16
H1	0,64	36,15
H2	9,46	38,35
H3	N/A	N/A
H4	N/A	N/A

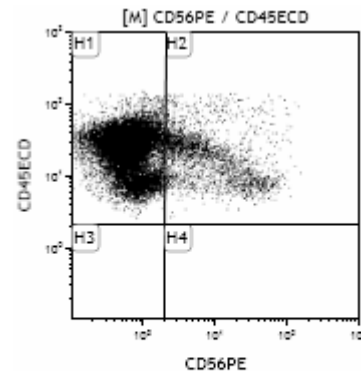
MDS



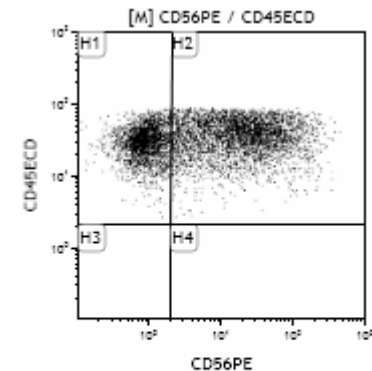
Gate	X-A-Mean	Y-A-Mean
All	2,79	19,01
H1	0,73	19,40
H2	11,83	17,25
H3	N/A	N/A
H4	N/A	N/A



Gate	X-A-Mean	Y-A-Mean
All	41,04	30,00
H1	1,28	33,14
H2	43,33	29,82
H3	N/A	N/A
H4	N/A	N/A

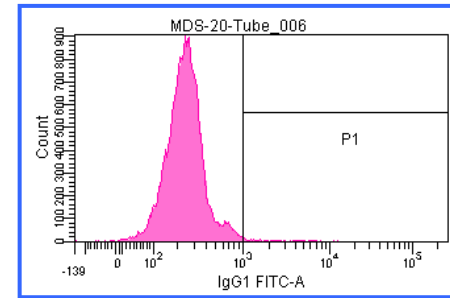
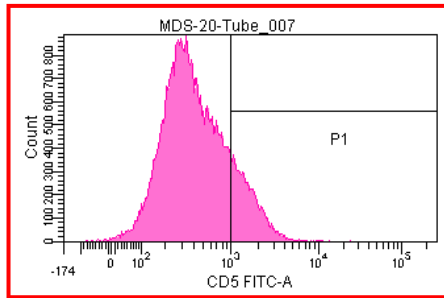
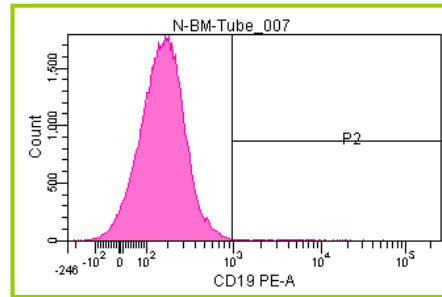
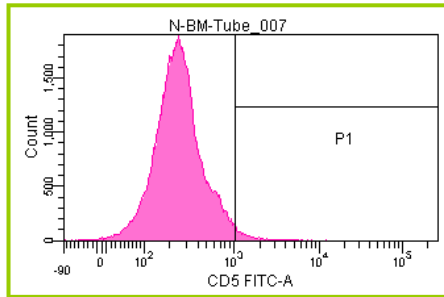


Gate	X-A-Mean	Y-A-Mean
All	1,97	27,71
H1	0,72	27,98
H2	13,63	25,16
H3	N/A	N/A
H4	N/A	N/A



Gate	X-A-Mean	Y-A-Mean
All	21,05	36,31
H1	0,98	31,72
H2	33,50	39,12
H3	N/A	N/A
H4	N/A	N/A

Expressie CD5 en CD19

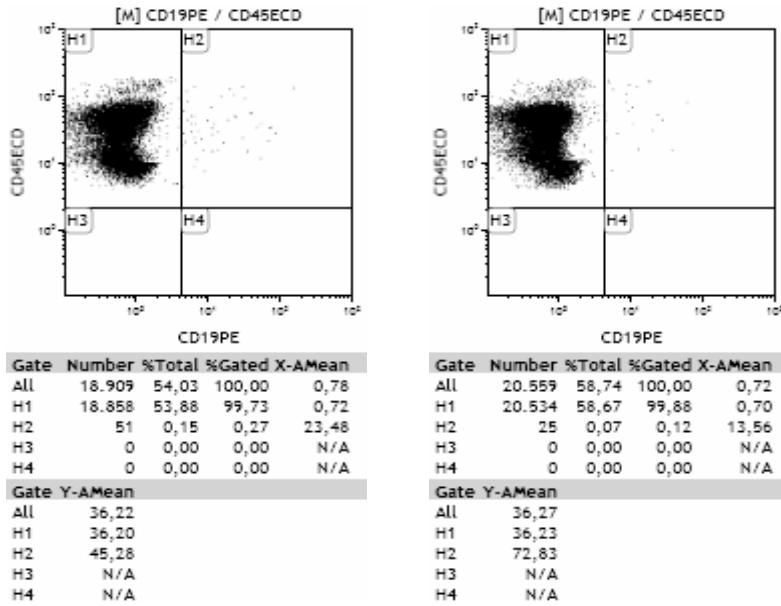


Expressie beoordelen in
achtergrond aankleuring

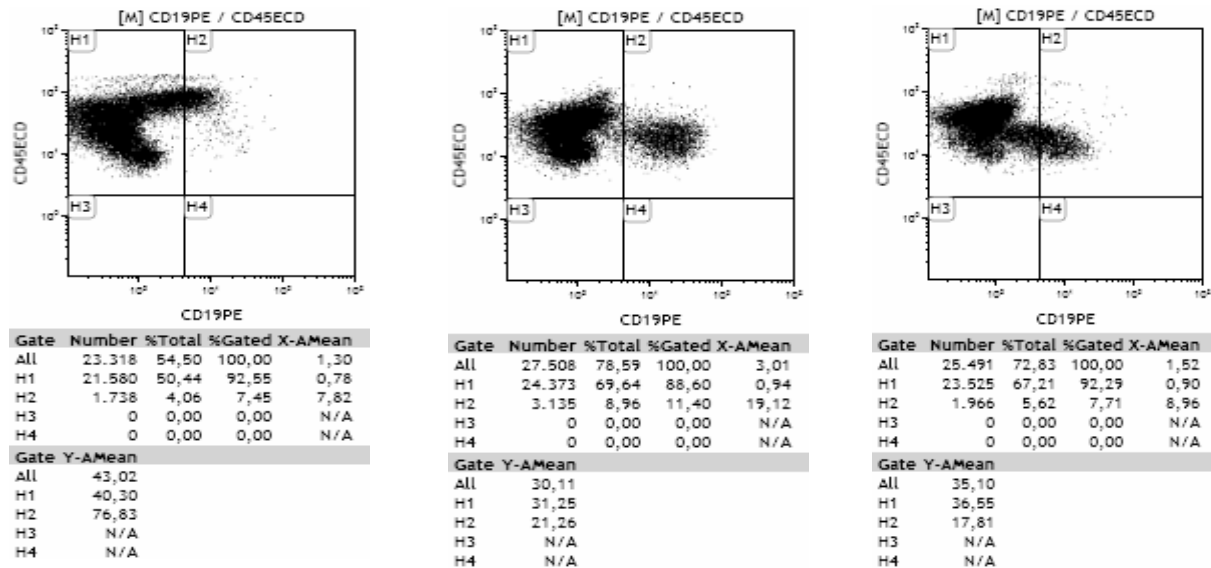
Expressie CD19



NBM



MDS

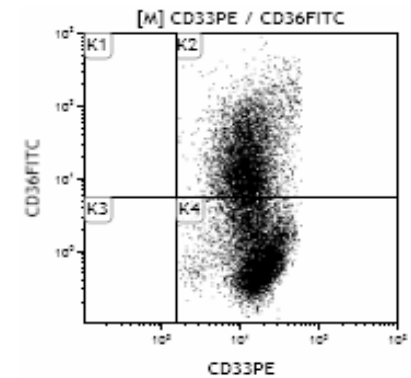
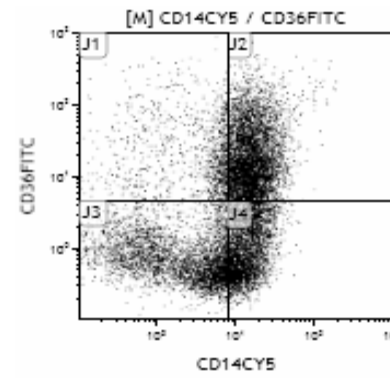
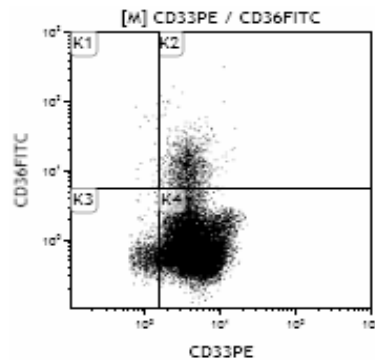
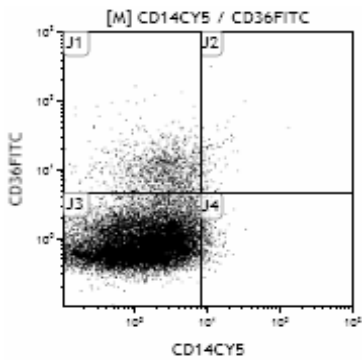


Expressie CD14 en CD36



NBM

MDS



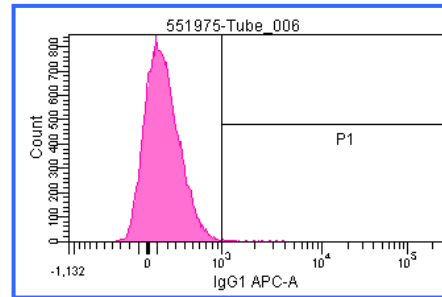
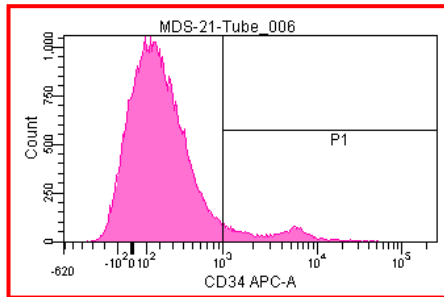
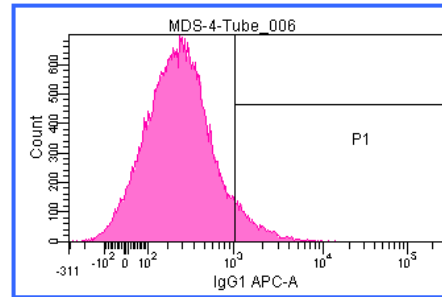
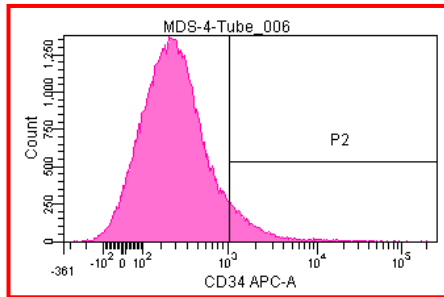
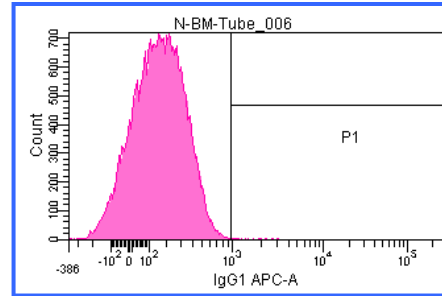
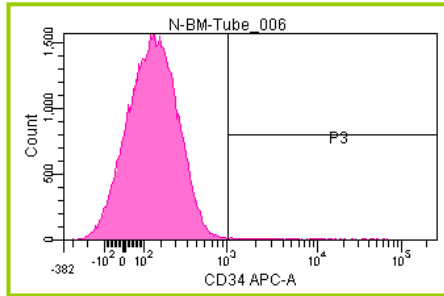
Gate	Number	%Total	%Gated	X-A-Mean
All	21.245	60,70	100,00	1,67
J1	1.258	3,59	5,92	2,66
J2	102	0,29	0,48	12,95
J3	19.581	55,95	92,17	1,41
J4	304	0,87	1,43	10,31
Gate Y-A-Mean				
All	1,75			
J1	12,81			
J2	19,87			
J3	0,95			
J4	1,37			

Gate	Number	%Total	%Gated	X-A-Mean
All	21.245	60,70	100,00	4,89
K1	22	0,06	0,10	1,21
K2	1.169	3,34	5,50	3,97
K3	836	2,39	3,94	1,15
K4	19.218	54,91	90,46	5,12
Gate Y-A-Mean				
All	1,70			
K1	31,60			
K2	14,15			
K3	0,65			
K4	0,95			

Gate	Number	%Total	%Gated	X-A-Mean
All	15.379	43,94	100,00	11,70
J1	1.296	3,70	8,43	3,53
J2	5.522	15,78	35,91	18,56
J3	4.538	12,97	29,51	2,69
J4	4.023	11,49	26,16	15,05
Gate Y-A-Mean				
All	15,26			
J1	39,68			
J2	31,34			
J3	0,98			
J4	1,43			

Gate	Number	%Total	%Gated	X-A-Mean
All	15.379	43,94	100,00	16,53
K1	0	0,00	0,00	N/A
K2	6.330	18,09	41,16	14,45
K3	0	0,00	0,00	N/A
K4	9.049	25,85	58,84	17,99
Gate Y-A-Mean				
All	15,26			
K1	N/A			
K2	35,07			
K3	N/A			
K4	1,40			

Expressie CD34



Verhoogde achtergrond

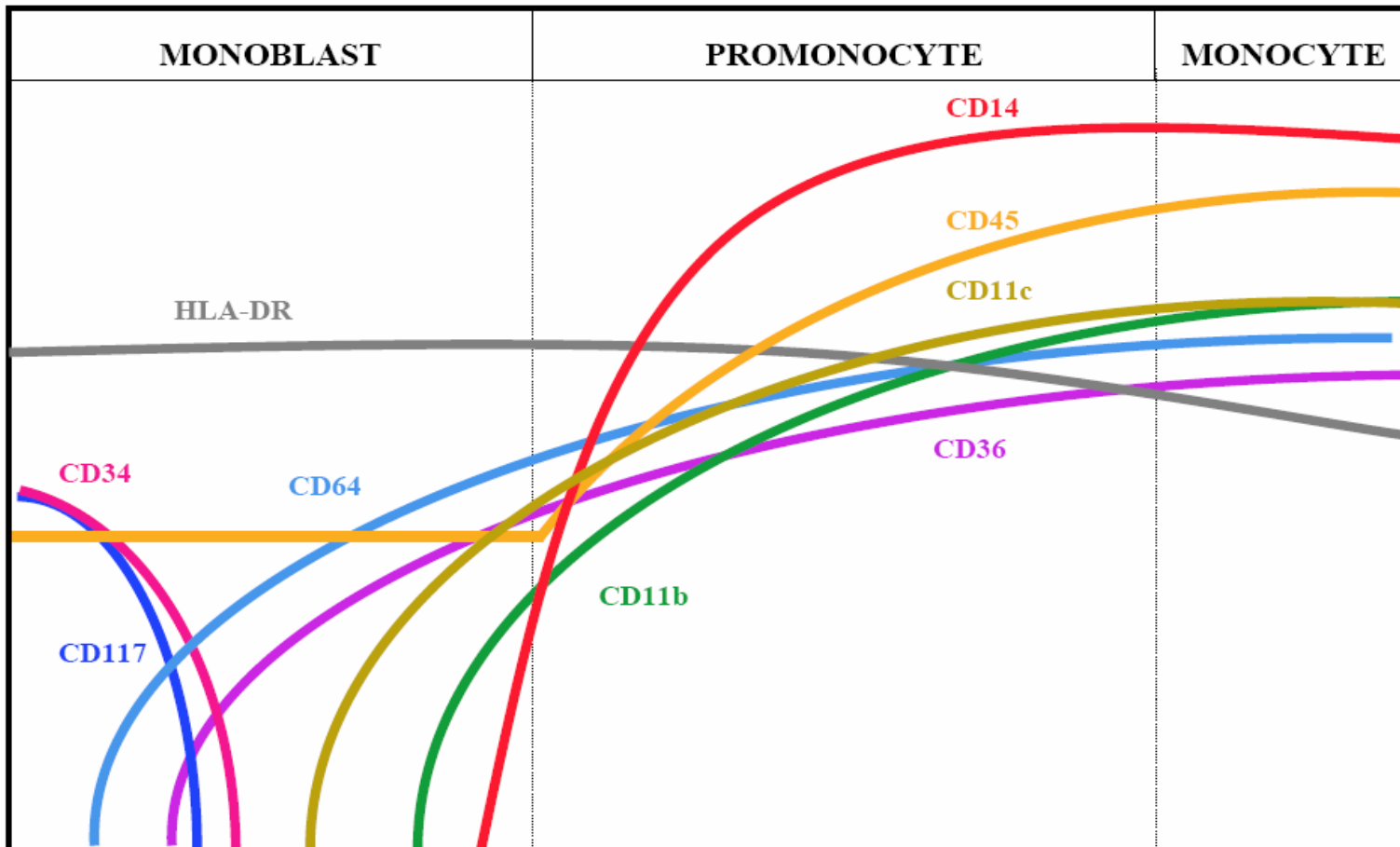
Positieve subpopulatie

3-Monocytair



Expressiepatronen

Normale expressie tijdens de uitrijping

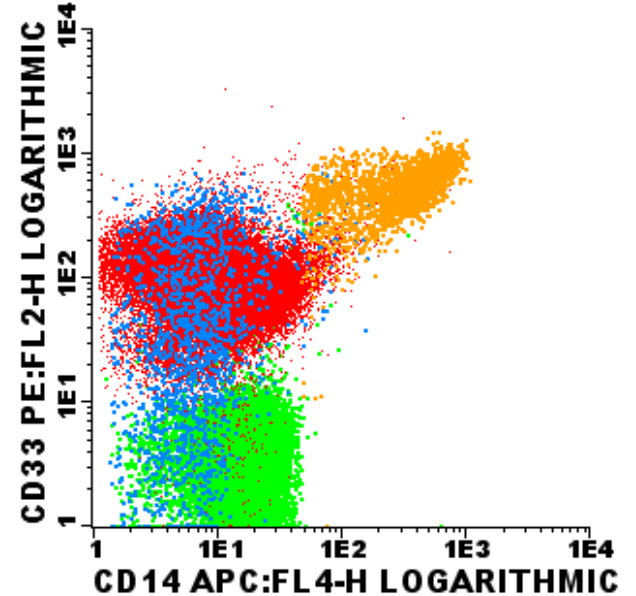
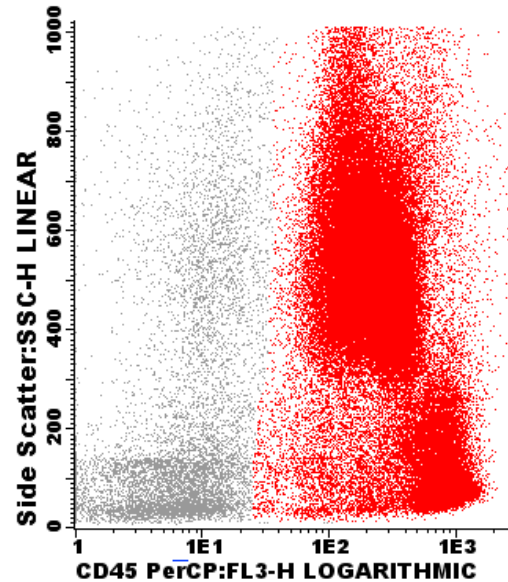
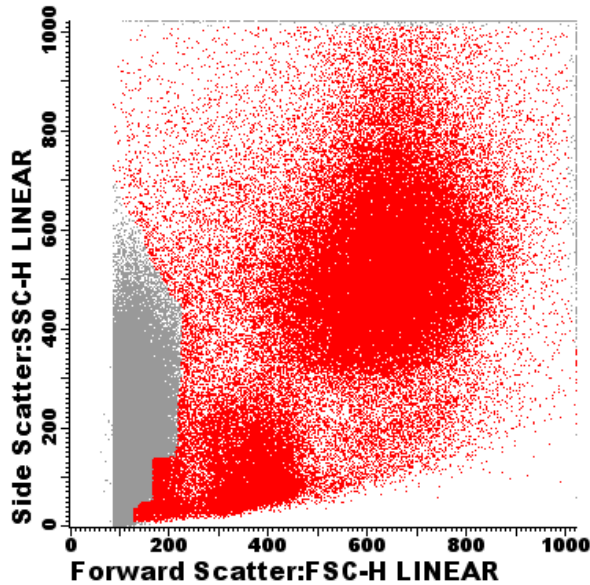


Met dank aan: A. Orfao

Gating procedure (1)



NBM



Stap 1: Débris en resterende rijpe rode bloedcellen uitgaten

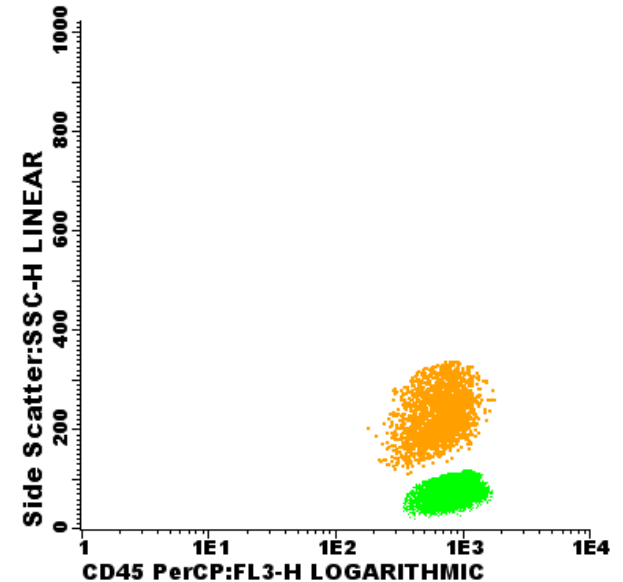
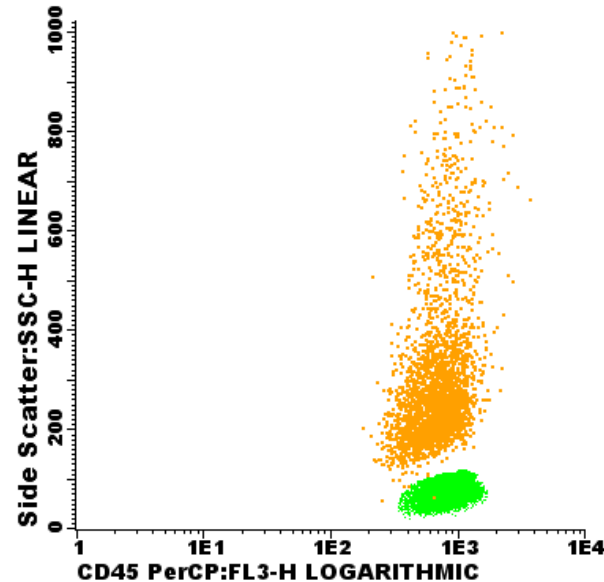
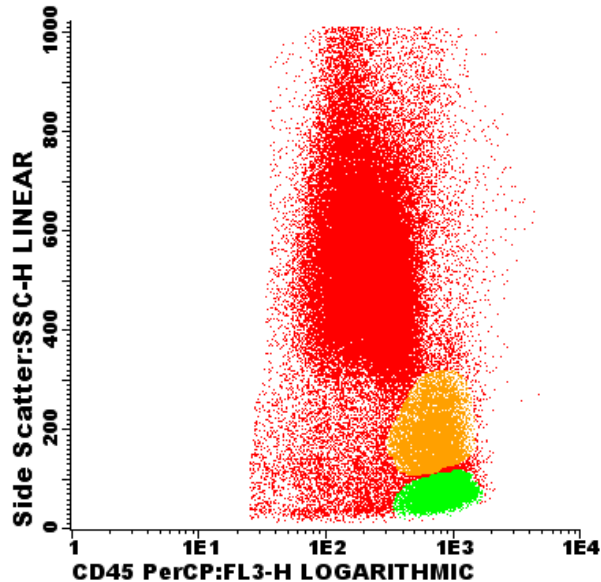
Stap 2: Op CD45pos cellen gaten

Stap 3: Op CD14pos, CD33pos cellen gaten

Gating procedure (2)



NBM



Stap 4: CD14 + cellen backgaten in SSC vs CD45 plot

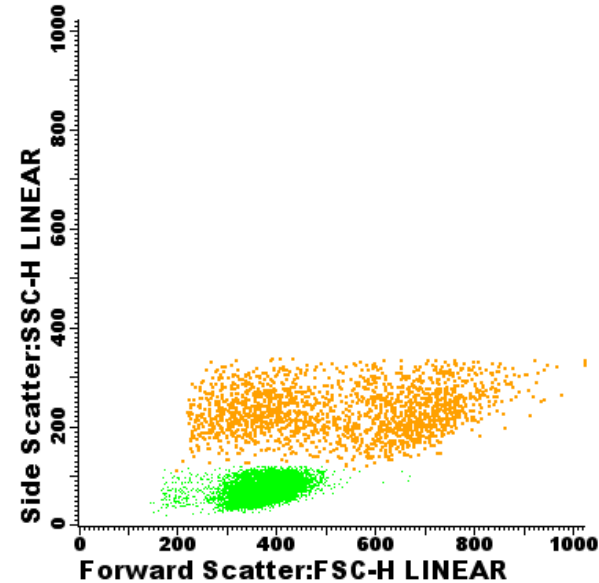
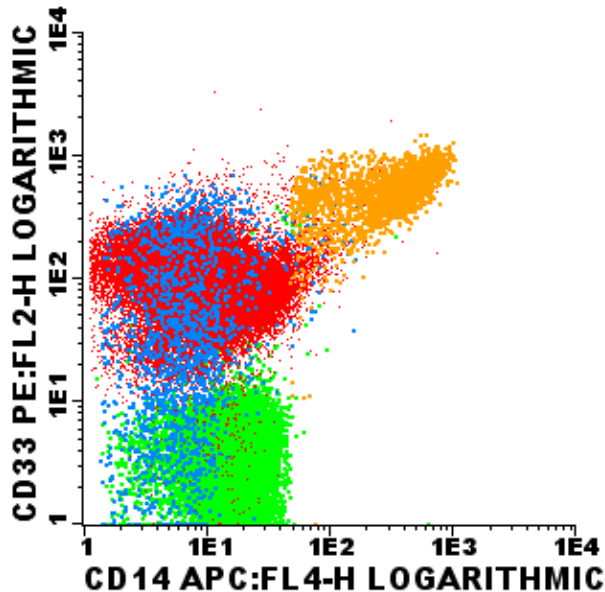
Stap 5: Verfijnen van gate, door SSChigh events eruit te gaten

Vanaf nu mono events gehighlight om het duidelijker zichtbaar te maken.

Gating procedure (3)



NBM



Stap 6: CD14+ events backgaten in FSC vs SSC plot

Kwantificeren monocyttaire cellen:

Percentage CD14+ cellen op basis van deze gating strategie ten opzichte van het percentage lymfocyten

Antistof panel



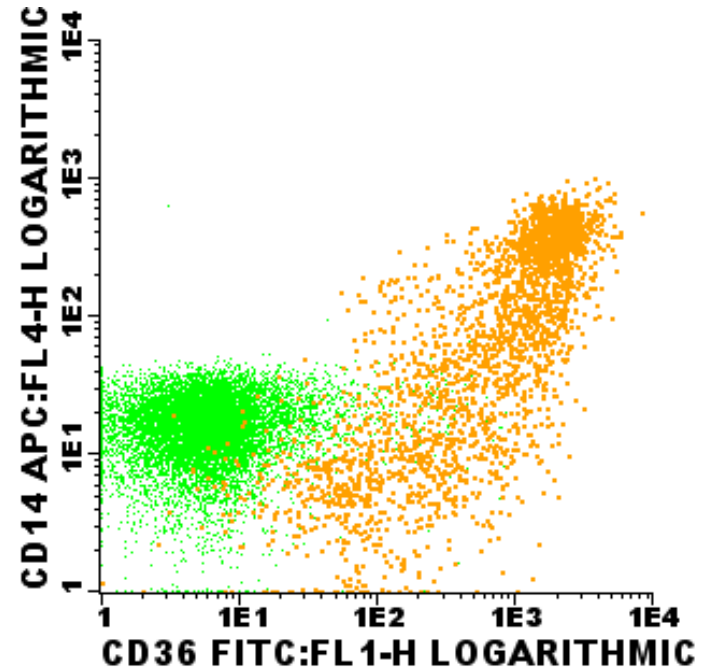
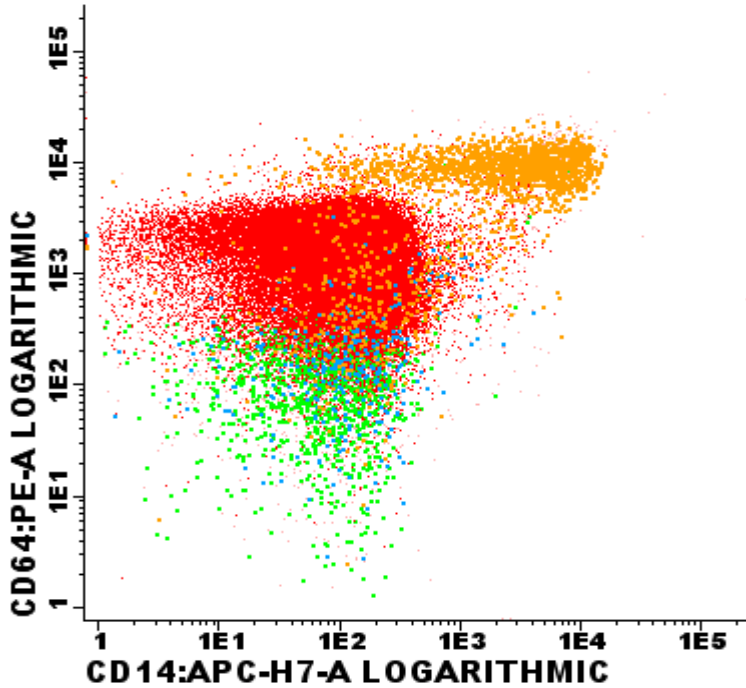
tube					lineage
1	PBS	PBS	CD45	PBS	
2	CD235a	CD117	CD45	CD71	erythroid
3	CD16	CD13	CD45	CD11b	myelomonocytic
4	CD36	CD33	CD45	CD14	myelomonocytic
5	HLA-DR	CD15	CD45	CD11b	myelomonocytic
6	CD13+33	CD117	CD45	CD34	myelomonocytic
7	HLA-DR	CD123	CD45	CD34	immature/basophilic/DC
8	CD7	CD56	CD45	CD34	immature/lymphocytes
9	CD5	CD19	CD45	CD34	immature/lymphocytes
10*	CD15	CD11b	CD45	CD34	myelomonocytic

NB! Geen beperking in de te gebruiken antistofklonen, fluorochromen en xx-kleurenfluorescentie

Differentiatiepatronen (1)



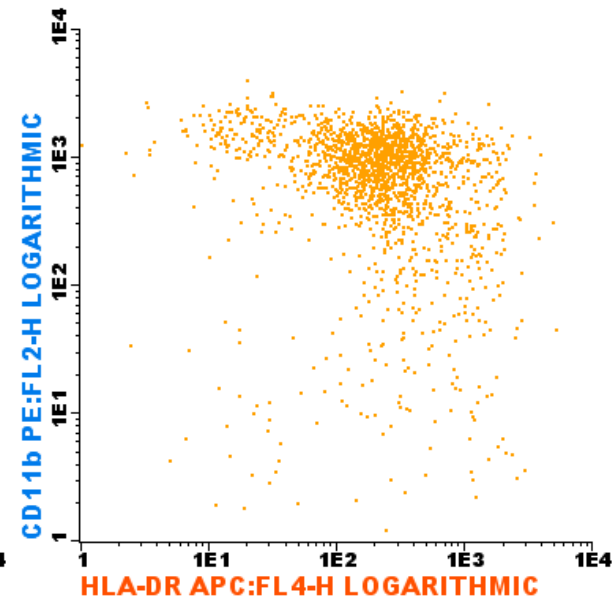
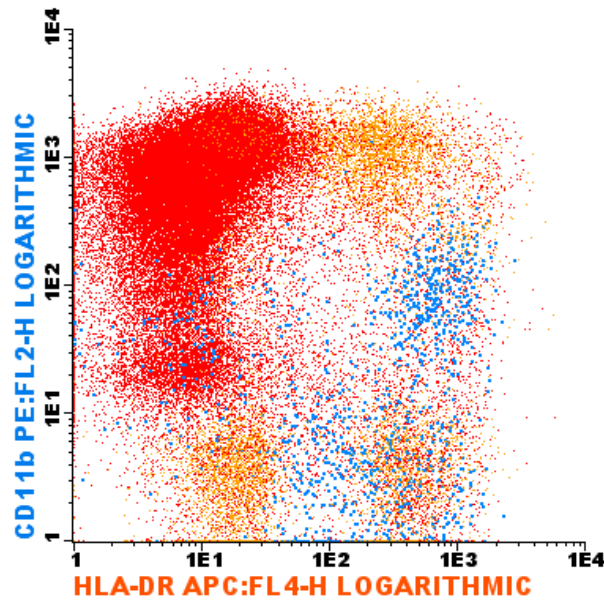
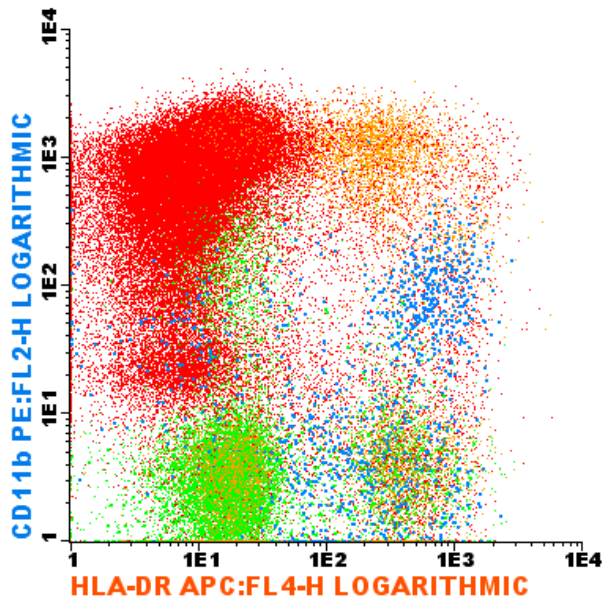
NBM



Differentiatiepatronen (2)



NBM

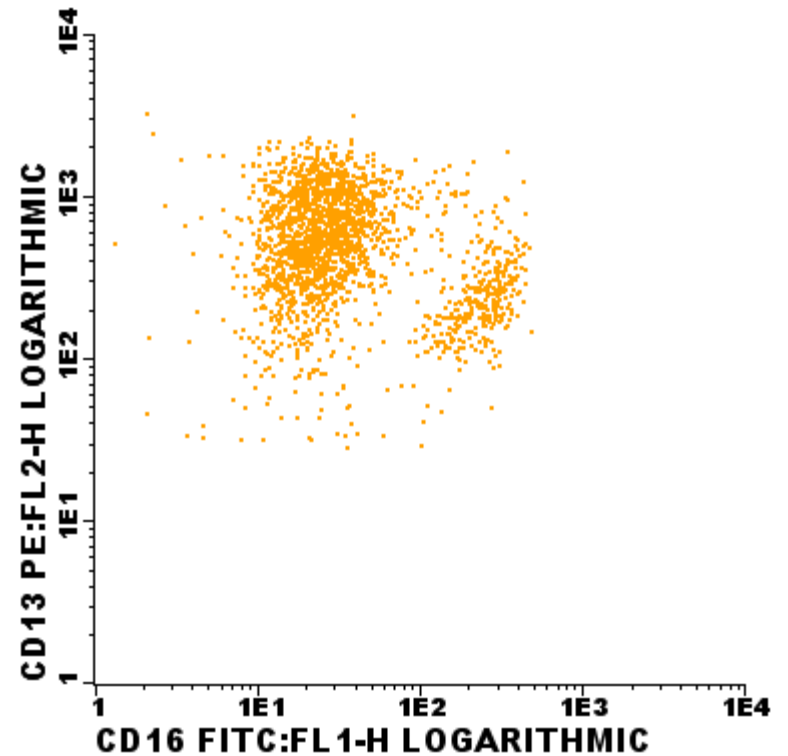
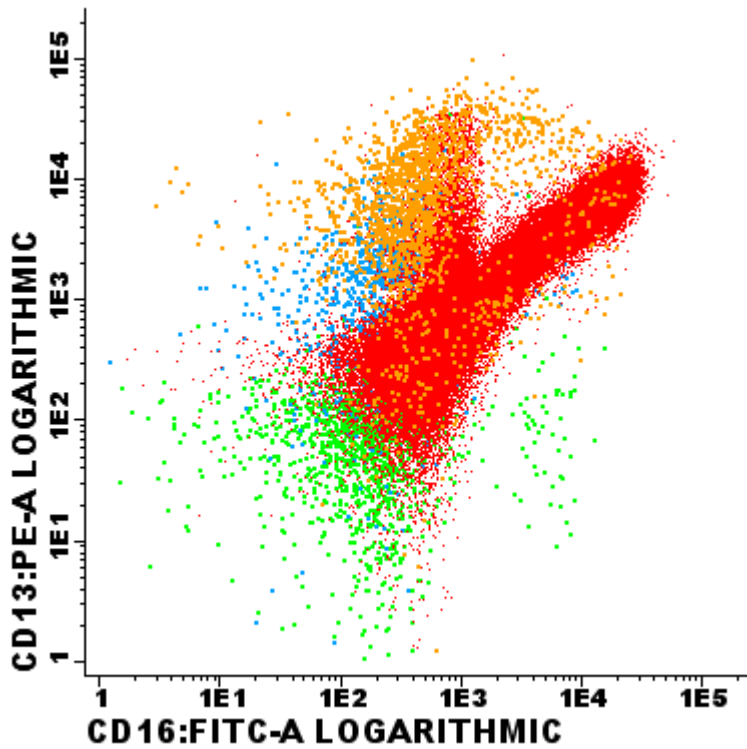


Expressiepatronen

CD13 en CD16



NBM

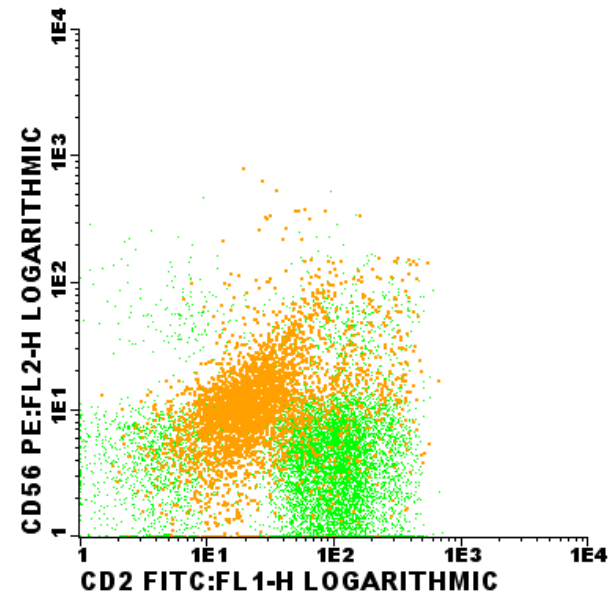
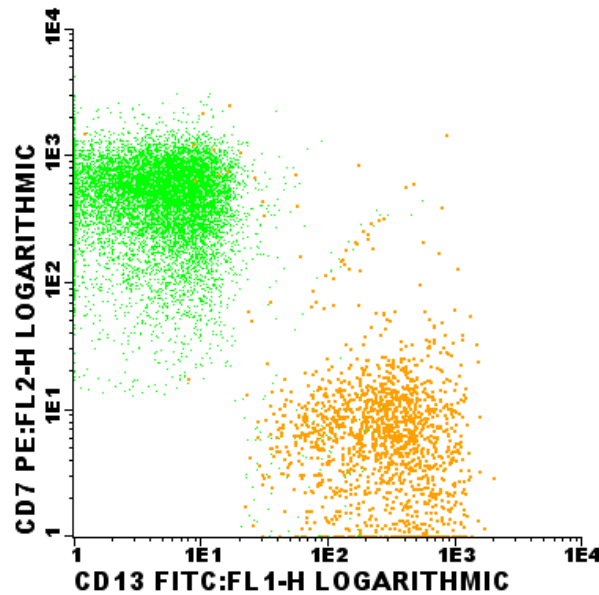
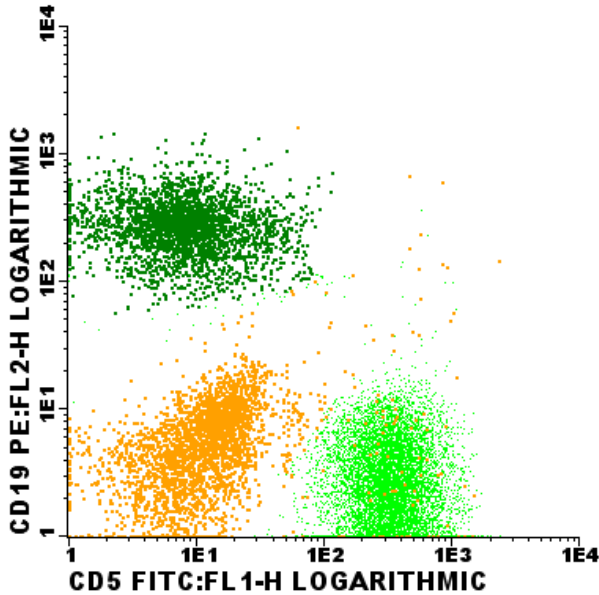


Expressiepatronen

Lineage infidelity marker en CD56



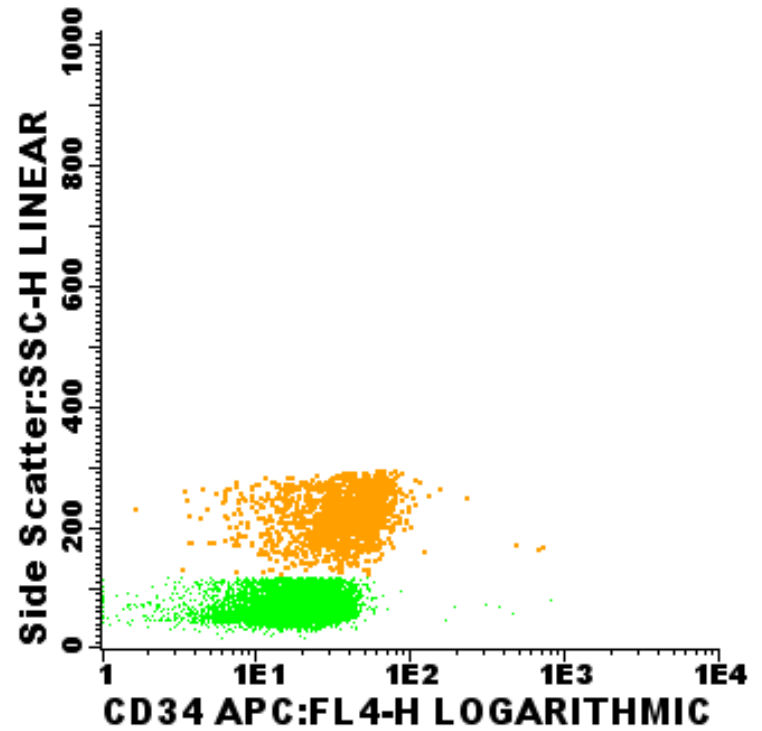
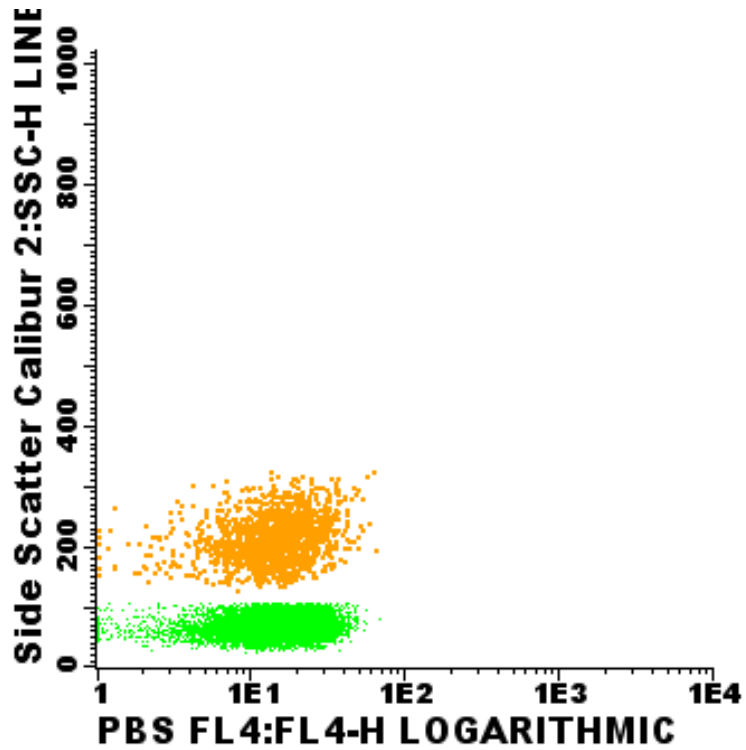
NBM



Expressiepatronen CD34



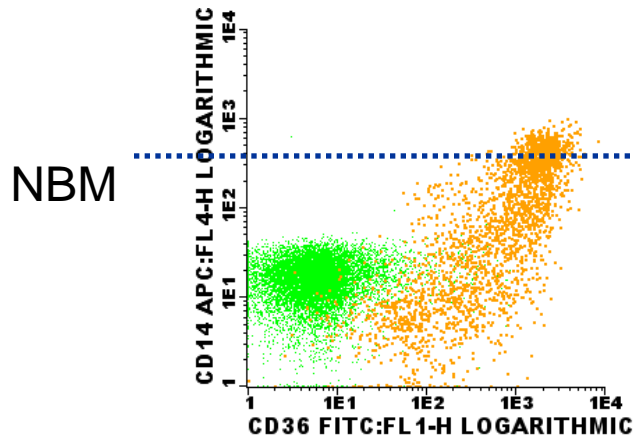
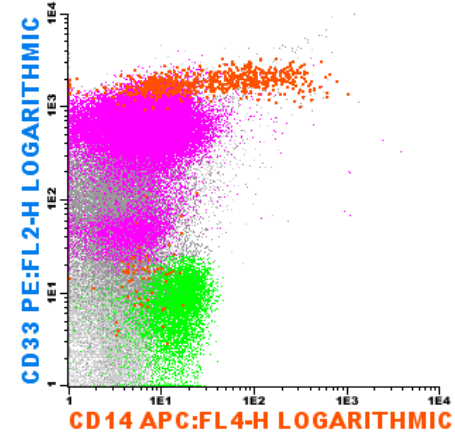
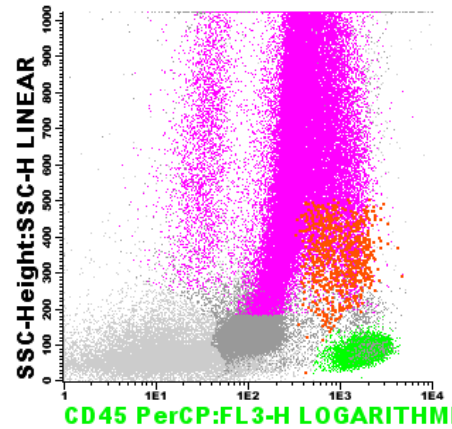
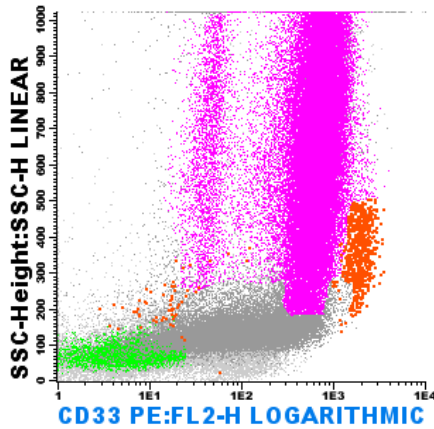
NBM



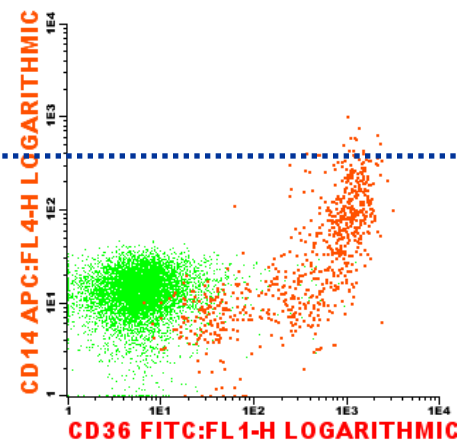
Afwijkingen in differentiatiepatronen



MDS



MDS

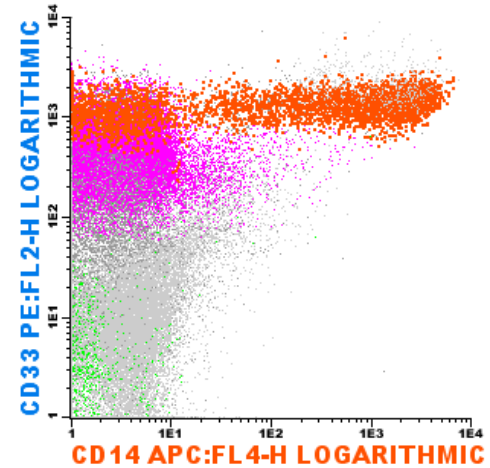
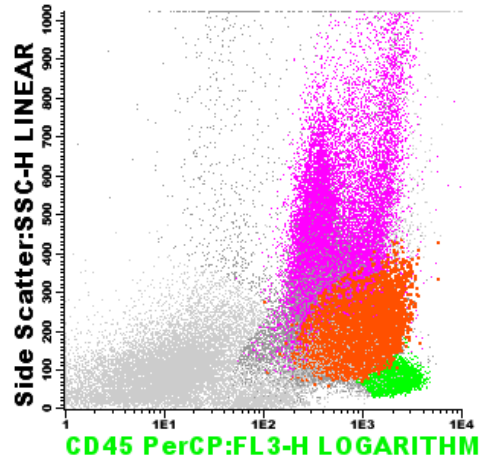
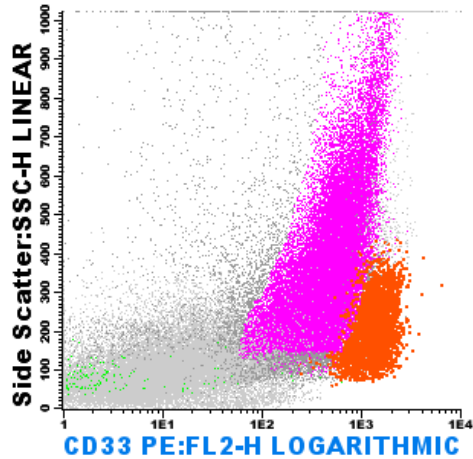


Weinig mono; CD33 hoog; lage CD14 of ontbreken rijp?

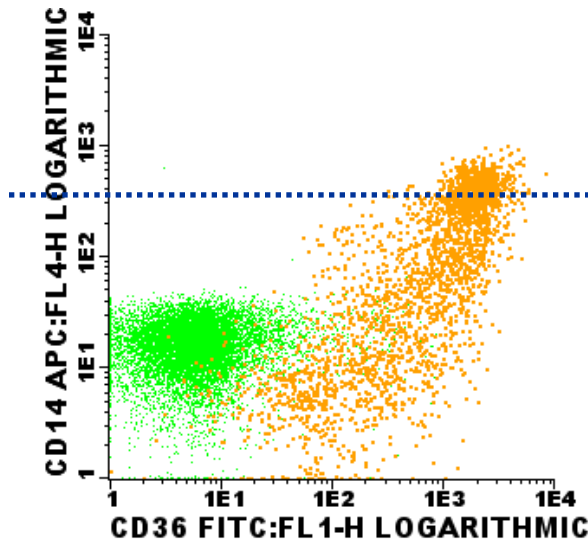
Afwijkingen in differentiatiepatronen



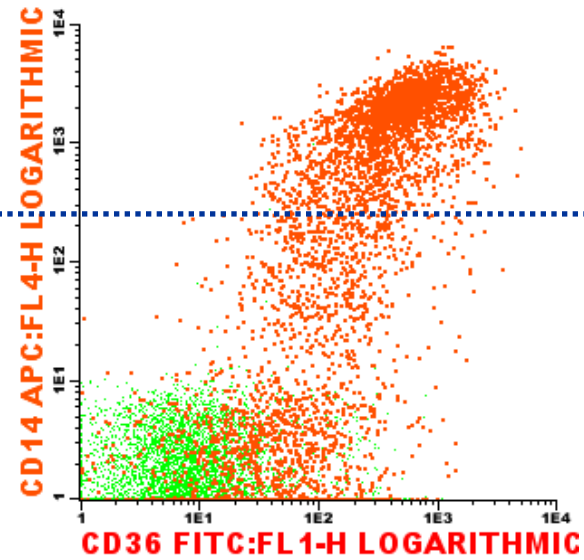
MDS



NBM



MDS

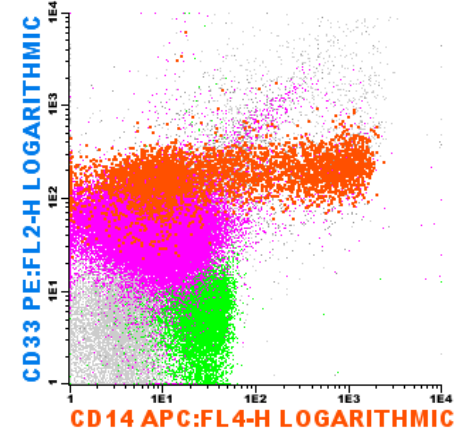
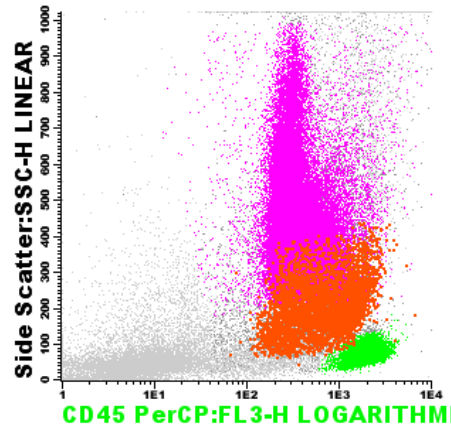
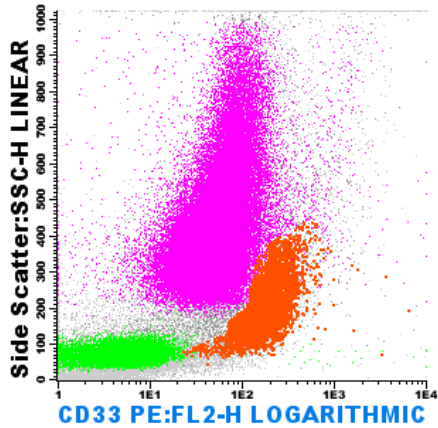


Overlap CD45/SSC; CD33 hoog; hoge CD14

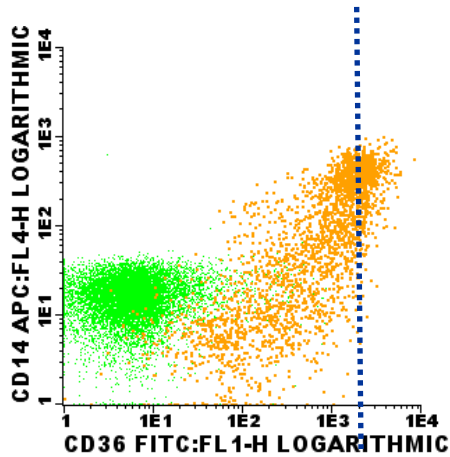
Afwijkingen in differentiatielatronen



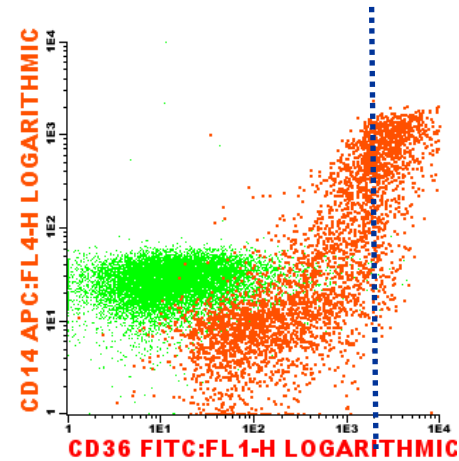
MDS



NBM



MDS

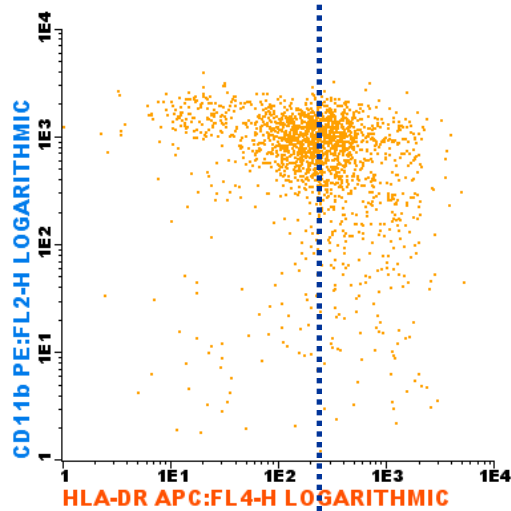


Hoge CD36; overlap gran-mono in CD45/SSC

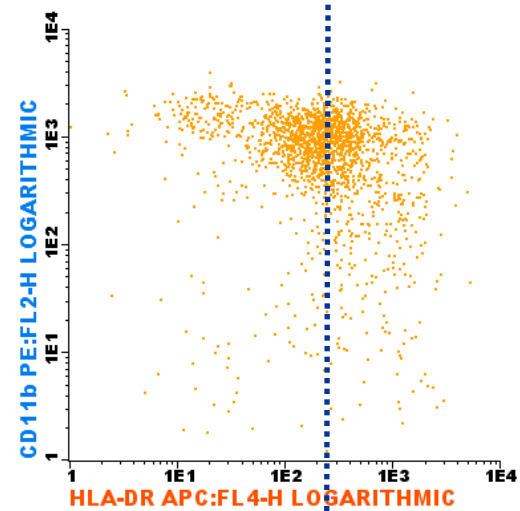
Afwijkingen in differentiatiëpatronen



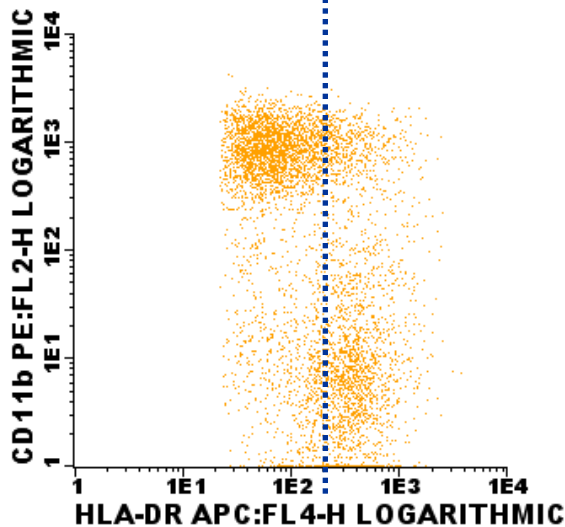
NBM



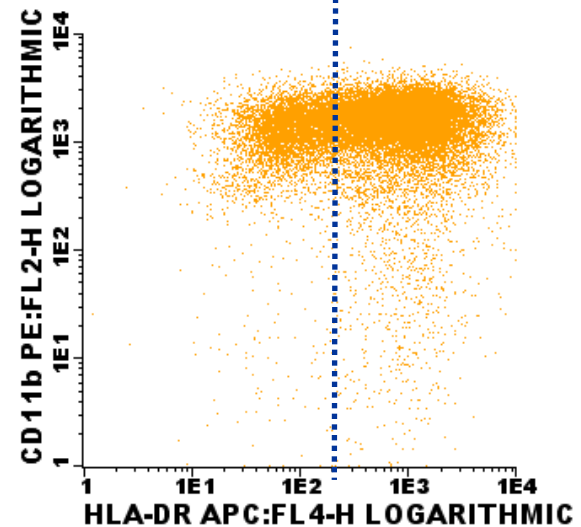
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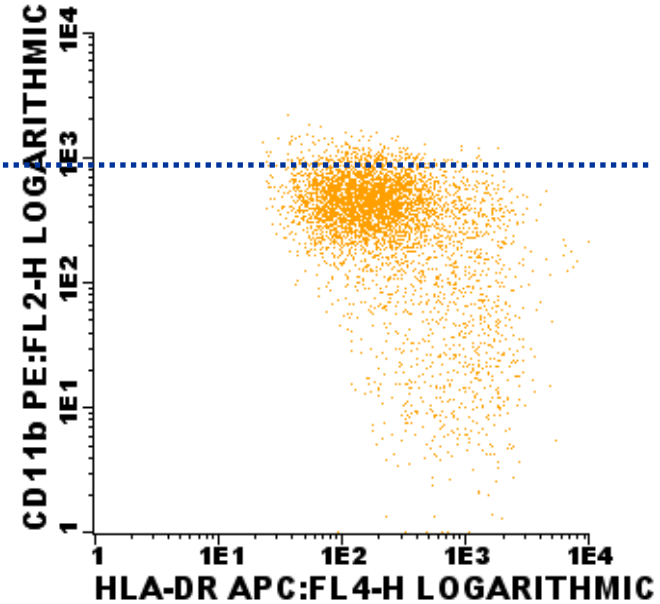
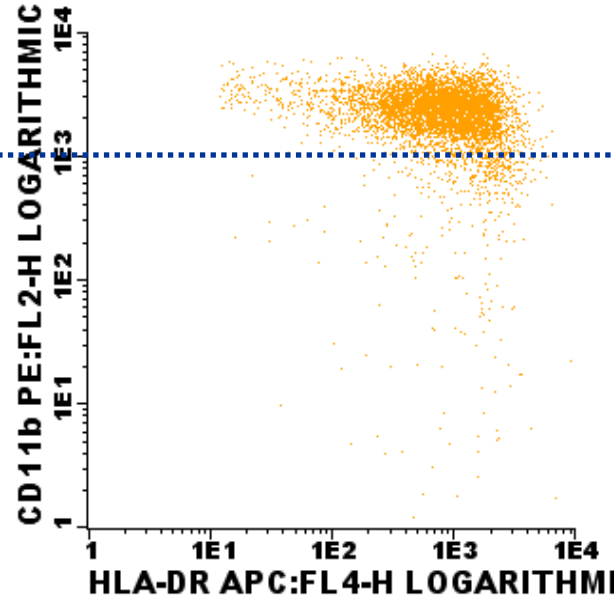
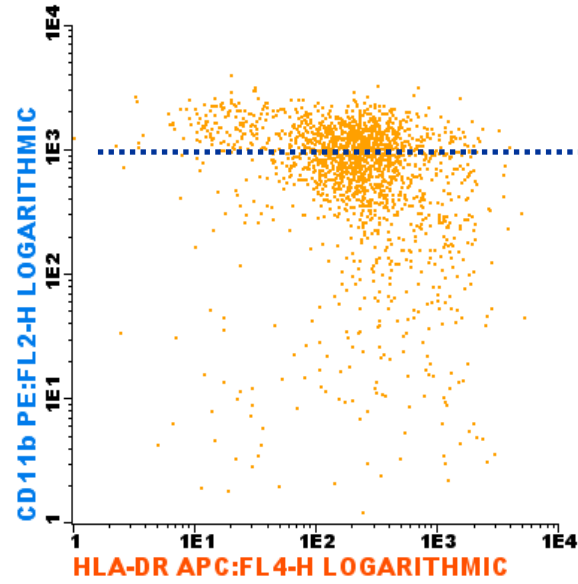
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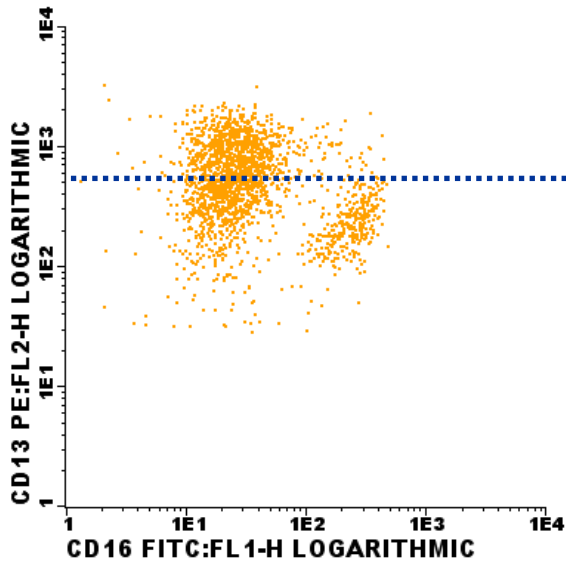
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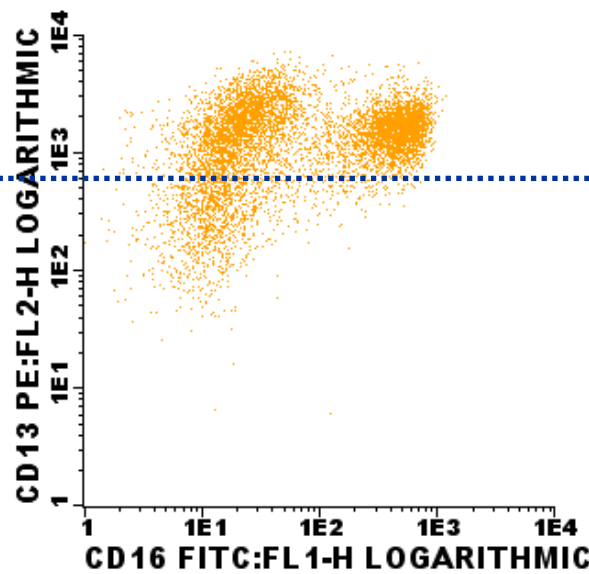
Afwijkingen in differentiatiepatronen



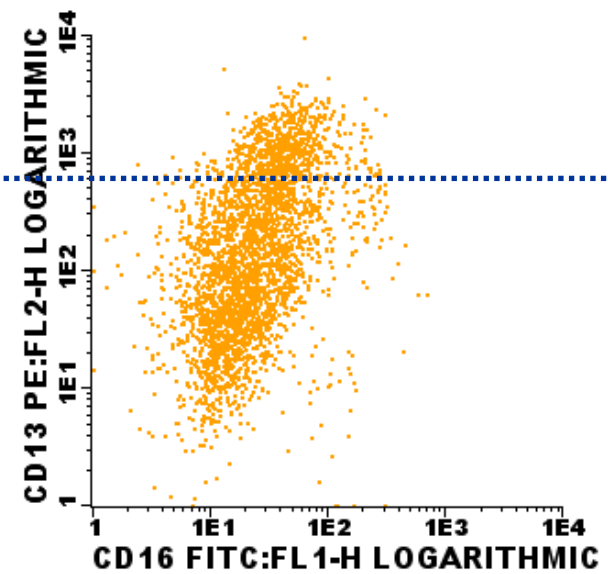
Afwijkingen in differentiatiepatronen



NBM



MDS



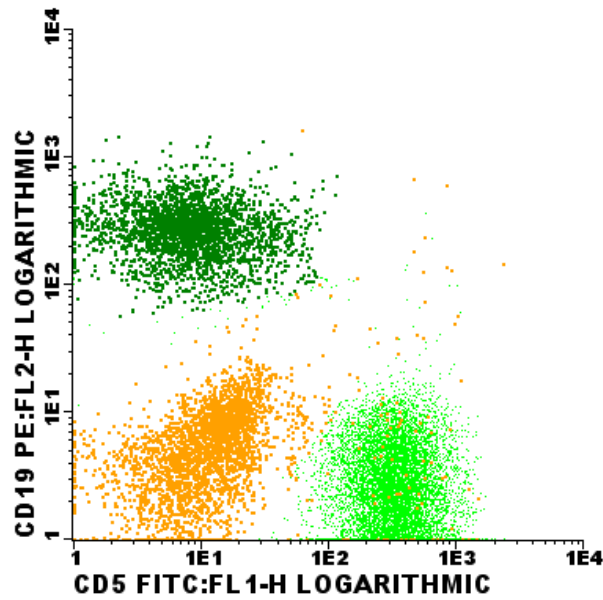
MDS

Aberrante expressie

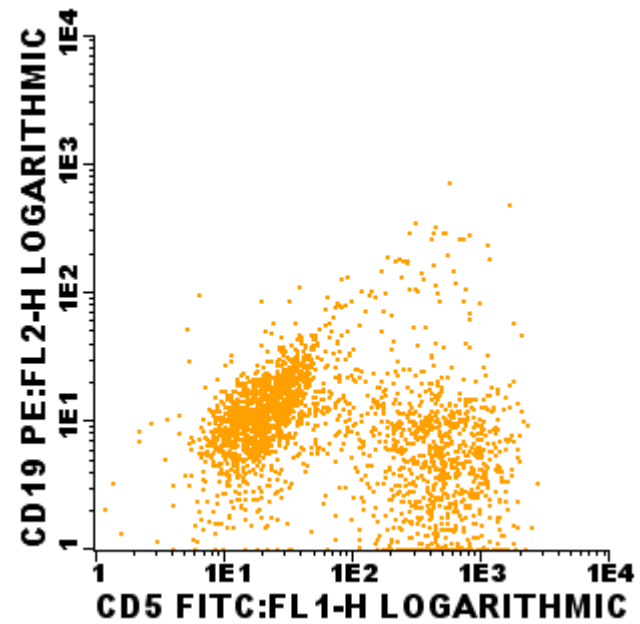
CD5 en CD19



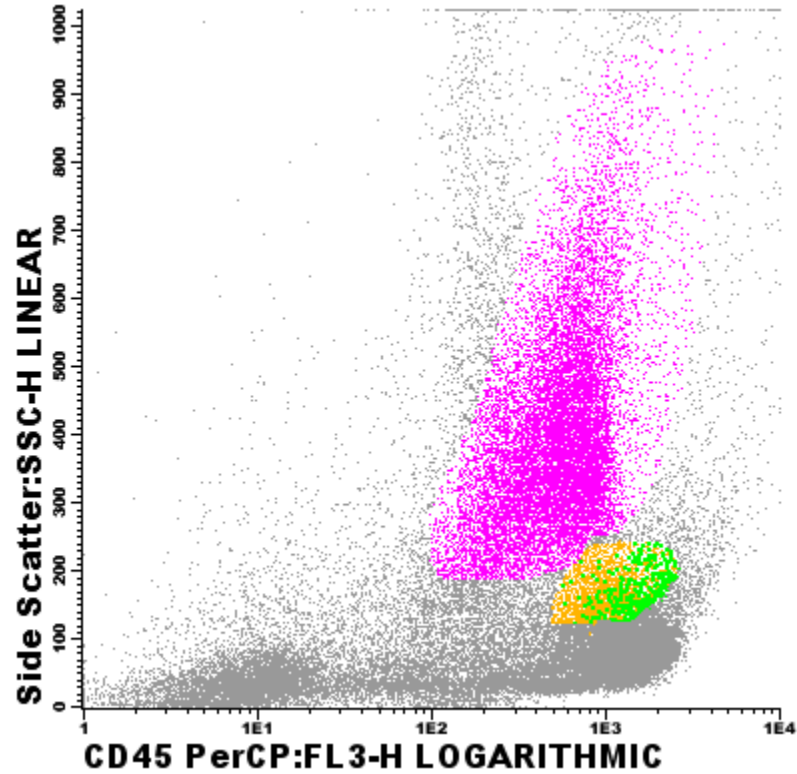
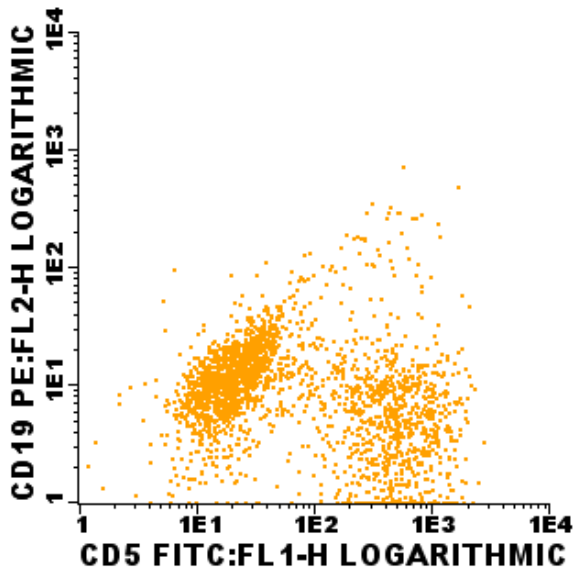
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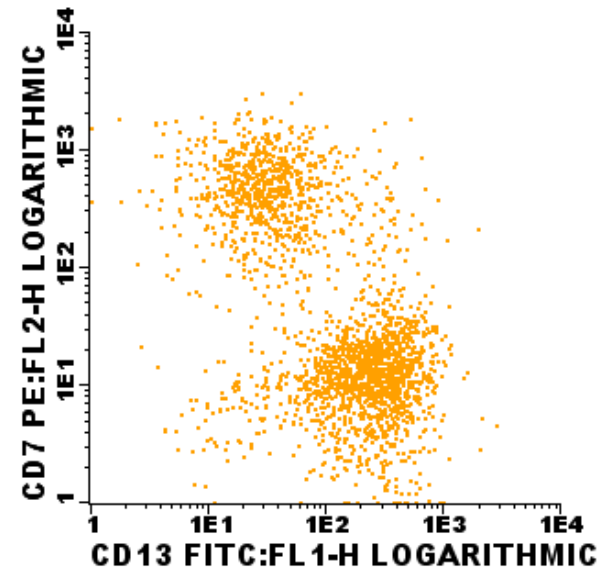
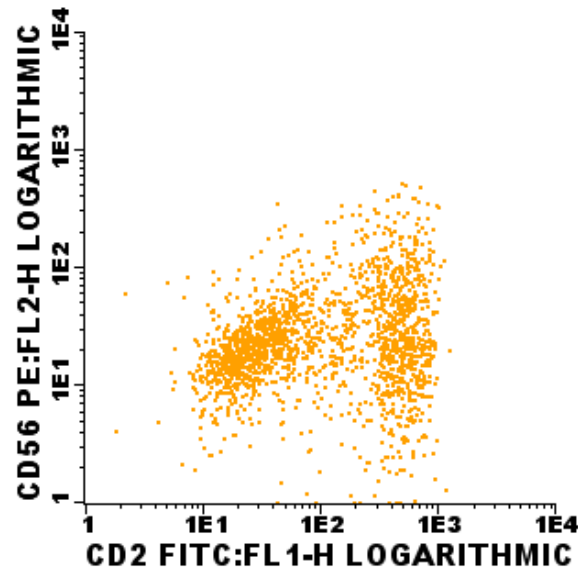
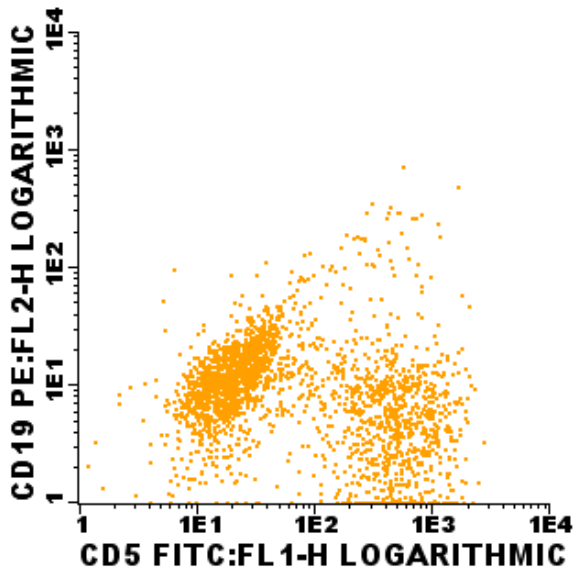
MDS



Aberrante expressie CD5 en CD19

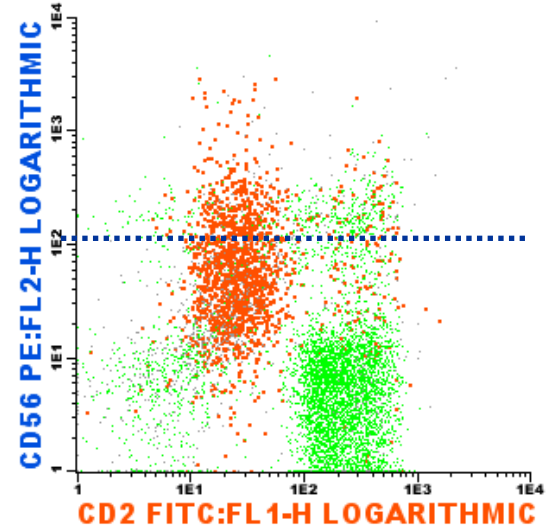
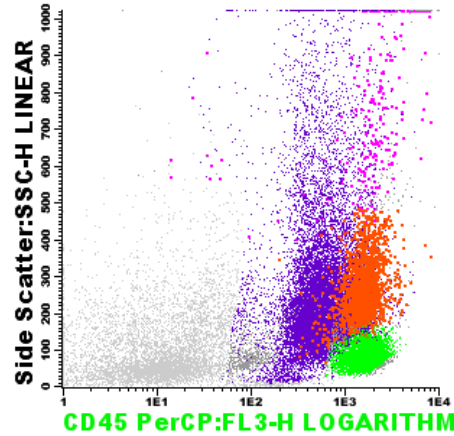
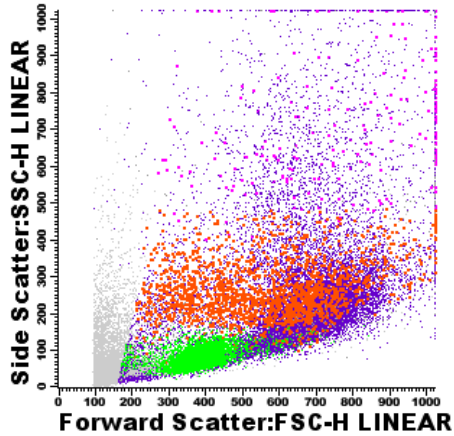


Aberrante expressie CD2, CD56 en CD7

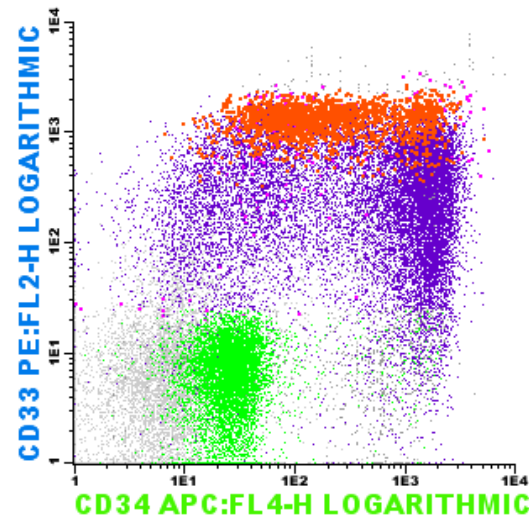
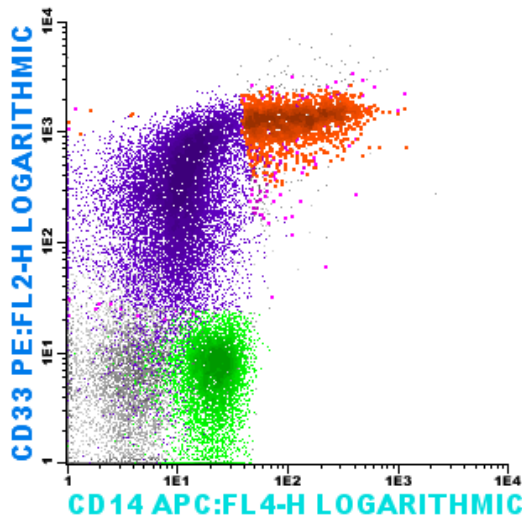


Aberrante expressie

CD34 en CD56



MDS

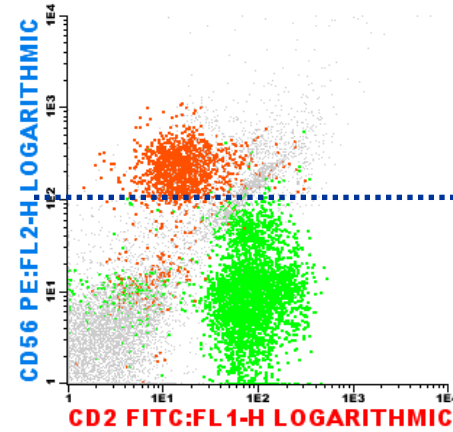
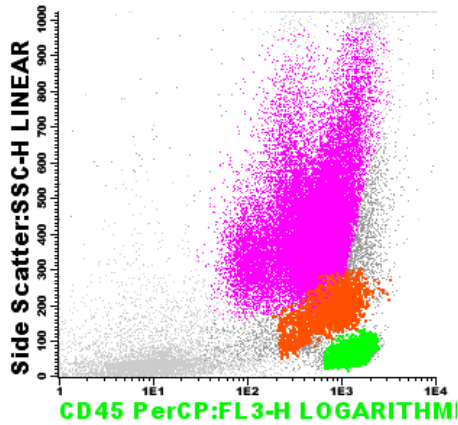


FSC/SSC, CD56+, CD34+

Voorbeelden van CD56 expressie

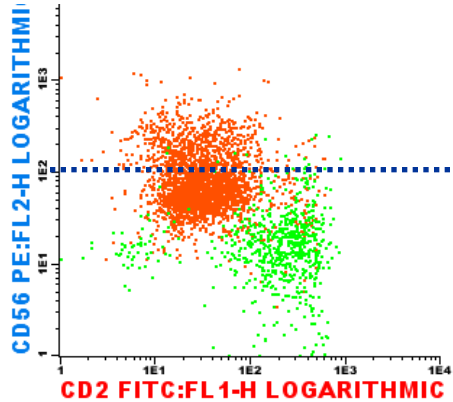
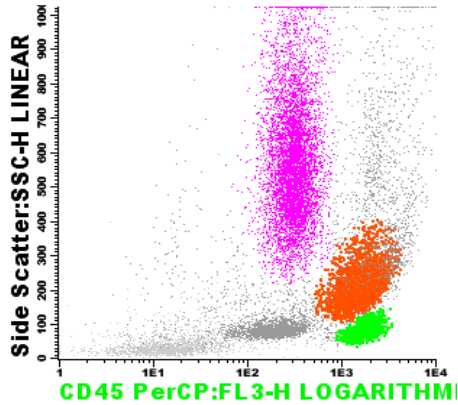


CD56+



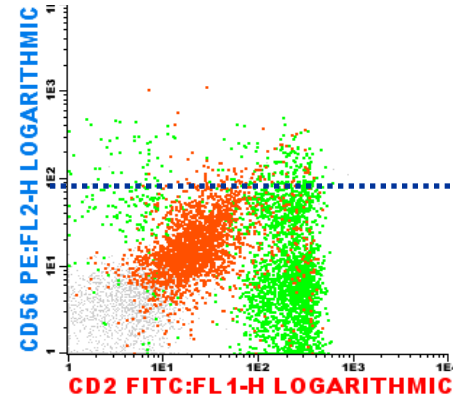
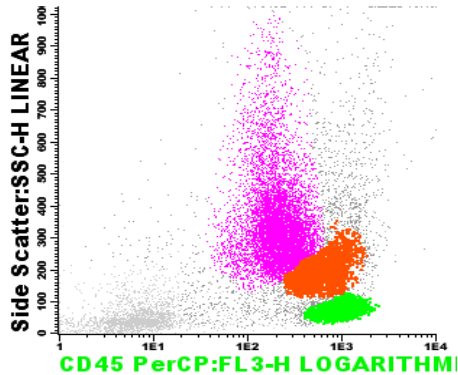
4399

CD56+



3273

CD56-



4077