

# Unsupervised Flow Cytometry Analysis Allows for an Accurate Identification of Minimal Residual Disease Assessment in Acute Myeloid Leukemia

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**Table S1.** Complement on patient characteristics

Patient (#)	WHO classification	Molecular markers used for MRD	FLT3 status	Karyotype	Number of FU
1	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	ITD	normal	2
2	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 A</i>	WT	normal	4
3	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	WT	normal	3
4	AML with BCR-ABL	<i>NPM1 A</i>	WT	t(9;22)	1
5	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	WT	normal	2
6	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	WT	normal	1
7	AML with mutated NPM1 (with maturation)	<i>NPM1 A</i>	WT	normal	2
8	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	ITD	normal	2
9	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 A</i>	WT	complex	2
10	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	ITD	normal	1
11	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	ITD	normal	1
12	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	ITD	normal	1
13	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 A</i>	WT	normal	1
14	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	WT	normal	1
15	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	ITD	normal	3
16	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	ITD	normal	2
17	AML with mutated NPM1 (with maturation)	<i>NPM1 A</i>	WT	normal	2
18	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 A</i>	TKD	normal	4
19	AML with mutated NPM1 (with maturation)	<i>NPM1 A</i>	ITD	trisomy 8	4
20	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	ITD	normal	2
21	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	WT	normal	4
22	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	WT	normal	4
23	AML with mutated NPM1 (without maturation)	<i>NPM1 A</i>	ITD	normal	3
24	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 A</i>	ITD	normal	1
25	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 B</i>	WT	normal	2
26	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 B</i>	ITD	normal	2
27	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 B</i>	TKD	normal	1
28	AML with mutated NPM1 (monoblastic/monocytic)	<i>NPM1 D</i>	ITD	normal	1
29	AML with mutated NPM1 (myelomonocytic)	<i>NPM1 D</i>	WT	normal	1
30	AML with recurrent cytogenetic abnormalities	<i>RUNX1-RUNXT1</i>	NA	t(8;21)	2
31	AML with recurrent cytogenetic abnormalities	<i>RUNX1-RUNXT1</i>	NA	t(8;21)	5
32	AML with recurrent cytogenetic abnormalities	<i>RUNX1-RUNXT1</i>	NA	t(8;21)	1
33	AML with recurrent cytogenetic abnormalities	<i>RUNX1-RUNXT1</i>	NA	t(8;21)	3

34	AML with recurrent cytogenetic abnormalities	<i>RUNX1-RUNXT1</i>	NA	t(8;21)	5
35	AML with recurrent cytogenetic abnormalities	<i>RUNX1-RUNXT1</i>	NA	t(8;21)	4
36	AML with recurrent cytogenetic abnormalities	<i>CBFB-MYH11</i>	NA	inv(16)	6
37	AML with recurrent cytogenetic abnormalities	<i>CBFB-MYH11</i>	NA	inv(16)	2
38	AML with recurrent cytogenetic abnormalities	<i>CBFB-MYH11</i>	NA	inv(16)	3
39	AML with recurrent cytogenetic abnormalities	<i>CBFB-MYH11</i>	NA	inv(16)	3
40	AML with BCR-ABL	<i>BCR-ABL</i>	NA	t(9;22)	2

**Table S2.** Detailed point by point patient status and results of MRD Assessment.

FU: Follow-up; d: days after diagnosis; FS-SORN: FlowSOM sum of retained nodes; CR: complete remission.

#	FU (d)	Disease status	Gene	Gene/ABL (%)	Molecular MRD	FS-SORN (%)	MFC-MRD
1	diag	AML4	<i>NPM1 A</i>	892		63.6	
	39	CR		0.0001	-	0	-
	530	CR		0.0001	-	0	-
2	diag	AML5	<i>NPM1 A</i>	1120		56.2	
	74	CR		0.0072	+	1.00	+
	162	CR		0.0001	-	0	-
	428	relapse		1897	+	2.14	+
	470	CR		0.0010	-	0	-
3	diag	AML4	<i>NPM1 A</i>	1309		52.68	
	38	CR		0.7174	+	1.55	+
	407	relapse		444	+	6.85	+
	837	CR		0.006	+	5.00	+
4	diag	AML2	<i>NPM1 A</i>	710		40.16	
	28	CR		0.1029	+	0.23	+
5	diag	AML MRC	<i>NPM1 A</i>	6000		58.59	
	53	CR		0.0142	+	2.7	+
	217	CR		0.0051	+	0.23	+
6	diag	AML4	<i>NPM1 A</i>	1317		73.37	
	39	CR		0.1233	+	7.37	+
	38	CR		8.4	+	0.48	+
7	diag	AML2	<i>NPM1 A</i>	1337		45.44	
	38	CR		7.57	+	2.36	+
	148	CR		1623		65.9	
8	diag	AML4	<i>NPM1 A</i>	555	+	8.50	+
	36	refractory		3337	+	32.53	+
	198	relapse		1374		40.19	
9	diag	AML5	<i>NPM1 A</i>	0.0001	-	3.87	+
	38	refractory		0.0001	-	2.33	+
	48	refractory		40	+	0.32	+
10	diag	AML4	<i>NPM1 A</i>	4539	+	0.13	+
	44	refractory		3647		63.05	
11	diag	AML1	<i>NPM1 A</i>	2457		81.71	
	47	CR		1770		31.47	
12	diag	AML5	<i>NPM1 A</i>	40	+	1.47	+
	38	CR		0.0289	+	0	-
13	diag	AML5	<i>NPM1 A</i>	0.8026	+	0	-
	72	CR		2533		41.06	
	49	CR		0.0001	-	0	-
14	diag	AML1	<i>NPM1 A</i>	2718		74.69	
	35	CR		44	+	0	-
	68	CR		0.001	-	0	-
	209	CR		0.001	-	0	-
15	diag	AML4	<i>NPM1 A</i>	2253		48.33	
	70	CR		0.0317	+	0	-

	149	CR		0.0251	+	0	-
17	diag	AML2	<i>NPM1 A</i>	335	-	53.01	-
	52	CR		0.001	-	0	-
	91	CR		0.001	-	0.77	+
18	diag	AML5	<i>NPM1 A</i>	846	-	81.13	-
	39	CR		0.0685	+	1.85	+
	74	CR		0.040	+	4.76	+
	111	CR		0.001	-	9.71	+
	256	CR		0.001	-	5.30	+
19	diag	AML2	<i>NPM1 A</i>	1700	-	65.72	-
	43	CR		0.770	+	1.20	+
	77	CR		0.000	-	0	-
	112	CR		0.000	-	2.16	+
	146	CR		0.000	-	0	-
20	diag	AML5	<i>NPM1 A</i>	970	-	81.14	-
	36	CR		0.5742	+	0.09	+
	76	CR		0.001	-	0	-
21	diag	AML1	<i>NPM1 A</i>	6220	-	45.83	-
	39	CR		0.088	+	1.43	+
	77	CR		0.001	-	0	-
	112	CR		0.001	-	0	-
	145	CR		0.001	-	0	-
22	diag	AML1	<i>NPM1 A</i>	690	-	69.56	-
	36	CR		2.5	+	1.86	+
	71	CR		0.04	+	3.29	+
	106	CR		0.000	-	3.53	+
	140	CR		0.02	+	1.11	+
23	diag	AML1	<i>NPM1 A</i>	2176	-	76.27	-
	61	CR		0.038	+	0.96	+
	95	CR		0.001	-	0	-
	138	CR		0.001	-	2.06	+
24	diag	AML MRC	<i>NPM1 A</i>	2665	-	50.37	-
	49	CR		0.0061	+	0.23	+
25	diag	AML5	<i>NPM1 B</i>	776	-	54.7	-
	40	CR		0.0117	+	1.52	+
	503	CR		0.0001	-	0	-
26	diag	AML5	<i>NPM1 B</i>	3156	-	78.44	-
	35	CR		0.9153	+	4.76	+
	242	CR		0.0001	-	3.01	+
27	diag	AML5	<i>NPM1 B</i>	2356	-	41.44	-
	35	CR		0.238	+	1.65	+
28	diag	AML5	<i>NPM1 D</i>	2015	-	78.21	-
	39	CR		0.1841	+	0	-
29	diag	AML4	<i>NPM1 D</i>	1705	-	20.18	-
	35	CR	<i>RUNX1-</i>	3.832	+	3.53	+
	39	CR	<i>RUNX1T1</i>	209	-	30.26	-
30	diag	AML2	<i>RUNX1T1</i>	2.938	+	2.07	+
	190	relapse		488	+	9.17	+

			<i>RUNX1-</i>			
31	diag	AML2	<i>RUNX1T1</i>	329		60.54
	44	CR		2.525	+	0
	73	CR		0.1989	+	0
	167	CR		36	+	0.17
	199	relapse		147	+	20.62
	341	CR		0.0001	-	0
			<i>RUNX1-</i>			
32	diag	AML2	<i>RUNX1T1</i>	348		43.62
	39	CR		5.567	+	0.63
			<i>RUNX1-</i>			
33	diag	AML2	<i>RUNX1T1</i>	1262		82.9
	37	CR		29	+	0.82
	104	CR		1.861	+	0.96
	125	CR		1.412	+	1.34
			<i>RUNX1-</i>			
34	diag	AML2	<i>RUNX1T1</i>	1372		58.89
	38	CR		2.952	+	2.08
	75	CR		0.725	+	1.92
	115	CR		0.073	+	3.29
	144	CR		0.115	+	0
	322	CR		9.6	+	0.65
			<i>RUNX1-</i>			
35	diag	AML2	<i>RUNX1T1</i>	584		31.13
	33	CR		0.96	+	1.98
	68	CR		0.145	+	1.26
	103	CR		0.0135	+	0.23
	139	CR		0.0001	-	0
			<i>CBFB-MYH11</i>			
36	diag	AML4	<i>CBFB-MYH11</i>	243		55.93
	42	CR		0.0137	+	3.60
	78	CR		0.022	+	0
	463	CR		0.061	+	0
	492	relapse		0.012	+	0.38
	533	CR		0.012	+	0.67
	654	relapse		0.0001	-	0
			<i>CBFB-MYH11</i>			
37	diag	AML4	<i>CBFB-MYH11</i>	107		62.94
	35	CR		0.204	+	1.43
	97	CR		0.21	+	2.11
			<i>CBFB-MYH11</i>			
38	diag	AML4	<i>CBFB-MYH11</i>	ND		43.40
	39	CR		0.008	+	1.93
	181	CR		0.01	+	0
	277	CR		0.0001	-	0
			<i>CBFB-MYH11</i>			
39	diag	AML4	<i>CBFB-MYH11</i>	84		20.60
	40	CR		0.11	+	2.03
	74	CR		0.055	+	1.16
	111	CR		0.017	+	0.89
			<i>BCR-ABL</i>			
40	diag	A-CML	<i>BCR-ABL</i>	127		22.41
	63	refractory		84	+	6.51
	91	CR		82	+	3.22

Global		FU1									
Tube 1		Tube 2		Tubes 1 & 2		Tube 1		Tube 2		Tubes 1 & 2	
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+ 38 MFC- 29	7 22	MFC+ 46 MFC- 21	7 22	MFC+ 54 MFC- 13	9 20	MFC+ 23 MFC- 13	1 3	MFC+ 24 MFC- 12	1 3	MFC+ 30 MFC- 6	1 3
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+ 39.6% MFC- 30.2%	7.3% 22.9%	MFC+ 47.8% MFC- 21.9%	7.3% 22.9%	MFC+ 56.3% MFC- 13.5%	9.4% 20.6%	MFC+ 57.5% MFC- 32.5%	2.5% 7.5%	MFC+ 60.0% MFC- 30.0%	2.5% 7.5%	MFC+ 75.0% MFC- 15.0%	2.5% 7.5%
Concordance 62.5% Discordance 37.5%		Concordance 70.8% Discordance 29.2%		Concordance 77.1% Discordance 22.9%		Concordance 65.0% Discordance 35.0%		Concordance 67.5% Discordance 32.5%		Concordance 82.5% Discordance 17.5%	
Se 0.57 Sp 0.76 PPV 0.84 NPV 0.43 Youden 0.33 Kappa 0.27		Se 0.69 Sp 0.76 PPV 0.87 NPV 0.51 Youden 0.45 Kappa 0.39		Se 0.81 Sp 0.69 PPV 0.86 NPV 0.61 Youden 0.50 Kappa 0.48		Se 0.64 Sp 0.75 PPV 0.96 NPV 0.39 Youden 0.39 Kappa 0.17		Se 0.67 Sp 0.75 PPV 0.97 NPV 0.20 Youden 0.42 Kappa 0.19		Se 0.83 Sp 0.75 PPV 0.97 NPV 0.33 Youden 0.58 Kappa 0.38	
<b>Global</b>											
Tube 1		Tube 2		Tubes 1 & 2		Tube 1		Tube 2		Tubes 1 & 2	
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+ 40 MFC- 27	8 21	MFC+ 48 MFC- 19	7 22	MFC+ 57 MFC- 10	9 20	MFC+ 25 MFC- 13	1 3	MFC+ 25 MFC- 11	1 3	MFC+ 32 MFC- 4	1 3
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+ 41.7% MFC- 28.1%	8.3% 21.9%	MFC+ 50.0% MFC- 19.8%	7.3% 22.9%	MFC+ 59.4% MFC- 10.4%	9.4% 20.6%	MFC+ 62.5% MFC- 27.5%	2.5% 7.5%	MFC+ 62.5% MFC- 27.5%	2.5% 7.5%	MFC+ 80.0% MFC- 10.0%	2.5% 7.5%
Concordance 63.5% Discordance 36.5%		Concordance 72.9% Discordance 27.1%		Concordance 80.2% Discordance 19.8%		Concordance 70.0% Discordance 30.0%		Concordance 70.0% Discordance 30.0%		Concordance 87.5% Discordance 12.5%	
Se 0.60 Sp 0.72 PPV 0.83 NPV 0.44 Youden 0.32 Kappa 0.27		Se 0.72 Sp 0.76 PPV 0.87 NPV 0.54 Youden 0.48 Kappa 0.43		Se 0.85 Sp 0.69 PPV 0.86 NPV 0.67 Youden 0.54 Kappa 0.54		Se 0.69 Sp 0.75 PPV 0.96 NPV 0.21 Youden 0.44 Kappa 0.21		Se 0.69 Sp 0.75 PPV 0.97 NPV 0.21 Youden 0.44 Kappa 0.21		Se 0.89 Sp 0.75 PPV 0.97 NPV 0.43 Youden 0.64 Kappa 0.48	
<b>FU1</b>											
Settings: NBM max cell 1% Ratio FU/NBM ≥2											

**Figure S1:** Display of the results for all 96 time points and FU1 of the 40 patients with different threshold parameters. Top panel: settings derived from published data (15). Bottom panel: optimized setting according to Youden and kappa tests (as in Figure 2). Matrices of numbers and percentages of concordance between molecular and MFC MRD detection are shown for tube 1, tube 2 and the combination of both tubes. Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value. Youden and kappa tests were used and values displayed as well.

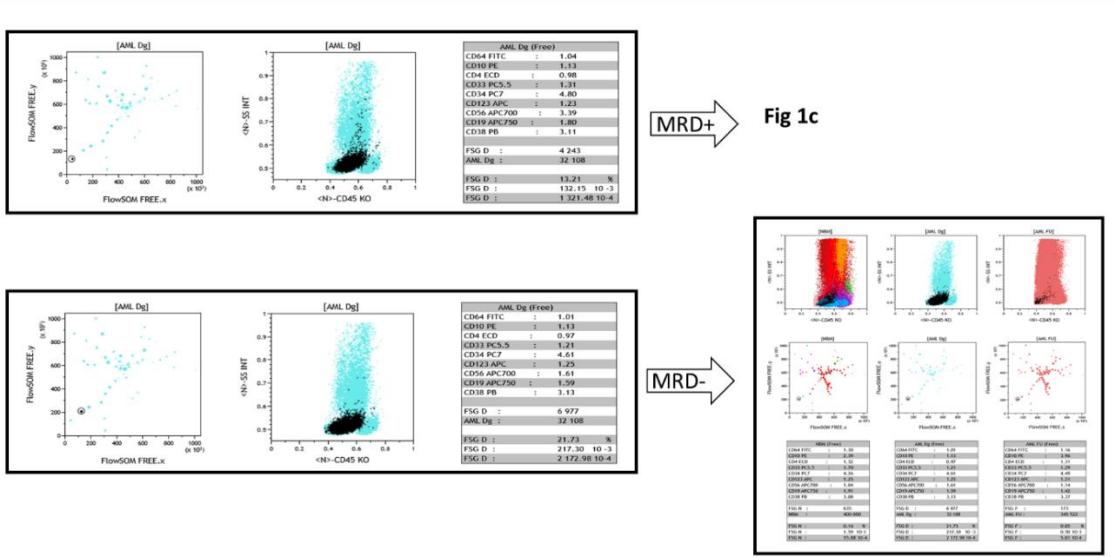
### Global NPM

Tube 1		Tube 2		Tubes 1 & 2		Tube 1		Tube 2		Tubes 1 & 2	
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+	23	8	MFC+	25	7	MFC+	31	9	MFC+	17	1
MFC-	12	17	MFC-	10	18	MFC-	4	16	MFC-	8	3
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+	38.3%	13.3%	MFC+	41.7%	11.7%	MFC+	51.7%	15.0%	MFC+	58.6%	3.4%
MFC-	20.0%	28.3%	MFC-	16.7%	30.0%	MFC-	6.7%	26.7%	MFC-	27.6%	10.3%
Concordance	66.7%		Concordance	71.7%		Concordance	78.3%		Concordance	69.0%	
Discordance	33.3%		Discordance	28.3%		Discordance	21.7%		Discordance	31.0%	
Se	0.66		Se	0.71		Se	0.89		Se	0.68	
Sp	0.68		Sp	0.72		Sp	0.64		Sp	0.75	
PPV	0.74		PPV	0.78		PPV	0.78		PPV	0.94	
NPV	0.59		NPV	0.64		NPV	0.80		NPV	0.27	
Youden	0.34		Youden	0.43		Youden	0.53		Youden	0.43	
Kappa	0.33		Kappa	0.43		Kappa	0.54		Kappa	0.25	

### Global AML 4-5

Tube 1		Tube 2		Tubes 1 & 2		Tube 1		Tube 2		Tubes 1 & 2	
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+	18	5	MFC+	25	3	MFC+	27	5	MFC+	13	1
MFC-	15	9	MFC-	8	11	MFC-	6	9	MFC-	4	2
Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-	Mol+	Mol-
MFC+	38.3%	10.6%	MFC+	53.2%	6.4%	MFC+	57.4%	10.6%	MFC+	59.1%	4.5%
MFC-	31.9%	19.1%	MFC-	17.0%	23.4%	MFC-	12.8%	19.1%	MFC-	27.3%	9.1%
Concordance	57.4%		Concordance	76.6%		Concordance	76.6%		Concordance	68.2%	
Discordance	42.6%		Discordance	23.4%		Discordance	23.4%		Discordance	31.8%	
Se	0.55		Se	0.76		Se	0.82		Se	0.68	
Sp	0.64		Sp	0.79		Sp	0.64		Sp	0.67	
PPV	0.78		PPV	0.89		PPV	0.84		PPV	0.93	
NPV	0.38		NPV	0.58		NPV	0.60		NPV	0.25	
Youden	0.19		Youden	0.54		Youden	0.46		Youden	0.35	
Kappa	0.16		Kappa	0.49		Kappa	0.45		Kappa	0.21	

**Figure S2:** Display of the results for two different subgroups of patients. Top panel: Patients with NPM1 mutation only according to all time points (N=60) or FU1 only (N=29). Bottom panel: Patients with AML4 and AML5 (myelomonocytic) only according to all time points (N=47) or FU1 only (N=22). Matrices of numbers and percentages of concordance between molecular and MFC MRD detection are shown for tube 1, tube 2 and the combination of both tubes. Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value. Youden and kappa tests were used and values displayed as well.



**Figure S3:** Unsupervised delineation of two adjacent but distinct nodes identifying subclones with different LAIP at diagnosis, respectively CD56-positive (top) and CD56-negative (bottom). The black backgating of each node on the CD45/SSC Dg histogram would not allow to distinguish these populations with classical MCF. FlowSOM identifies positive MRD for the CD56+ subclone (shown in Figure 1c) but MRD is undetectable for the CD56- subclone.