

Supplementary Table 1: Clinical characteristics of AML patients tested in this study. Blue boxes indicate patients for whom extended mutational testing was performed. Pink boxes denote patients with relapsed or refractory disease. Purple boxes indicate patients who achieved CR after biopsy. Orange boxes indicate patients who were not treated or died during treatment. Green boxes denote patients alive at last follow-up. Yellow boxes indicate patients who were receiving hydroxyurea at the time of biopsy.

Patient	Sex	Age	Cytogenetics	Mutations	Status at Biopsy	CR	Treatment	Survival (d)	Blast %	HU
Core Binding Factor AML										
AML5	F	44	t(8;21)	None	New Dx.	Yes	4+3	619	75%	No
AML26	F	45	t(8;21)	NRAS ⁺	New Dx.	Yes	Vorinostat+3+4	Alive	15%	No
AML35	M	58	t(8;21)	Kit N822K ⁺	New Dx.	Yes	7+3 (90)	Alive	57%	No
AML10	M	37	Inv(16)	None	New Dx.	Yes	7+3 (90)	Alive	76%	Yes
AML32	F	60	t(16;16),+22	FLT3-TKD ⁺	Relapsed	unknown	Aza + GO	unknown	82%	No
Acute Promyelocytic Leukemia										
APL1	M	64	t(15;17)	FLT3-ITD ⁺	New Dx.	Yes	Ida + ATRA	unknown	96%	Yes
APL3	M	18	t(15;17)	FLT3-ITD ⁺	New Dx.	Yes	Arsenic + ATRA	Alive	21%	No
APL4	M	55	t(15;17)	PML-RAR ⁺ , FLT3-ITD ⁺ ,	New Dx.	Yes	Arsenic + ATRA	Alive	93%	No
APL5	M	48	t(15;17)	PML-RAR ⁺ , FLT3-ITD ⁺	New Dx.	N/A	ATRA + Dauno	2	94%	Yes
Normal Karyotype AML, FLT3-TKD+										
AML6	M	66	Failed; Later Normal	FLT3 TKD ⁺	New Dx.	Refractory	7+3 x2	142	76%	Yes
AML8	M	66	Normal	FLT3 TKD ⁺	Refractory	Refractory	Decitabine	107	34%	No
AML31	F	37	Normal	NPM1 ⁺ , FLT3-TKD ⁺ , IDH1 ⁺	Relapse	No	MEC	227	92%	Yes
Normal Karyotype AML, FLT3-ITD+										
AML7	F	72	Normal	FLT3-ITD ⁺	New Dx.	N/A	No Tx.	32	78%	No
AML9	F	26	Normal	FLT3-ITD ⁺ , NPM1 ⁺	New Dx.	Yes	7+3 x2	533	21%	No
AML20	M	29	Normal	FLT3-ITD ⁺ , BiCEBPa ⁺	New Dx.	Yes	G-CLAC	229	90%	Yes
AML21	F	27	Normal	FLT3-ITD ⁺ , NPM1 ⁺	Relapsed	No	G-CLAC x 2	173	76%	No
AML23	F	62	Normal	DNMT3a ⁺ , FLT3-ITD ⁺ ,	New Dx.	N/A	No Tx.	11	92%	No

				sCEBPa ⁺						
AML27	M	53	Normal	NPM1 ⁺ , FLT3-ITD ⁺	New Dx.	Yes	7+3 (90)	Alive (BMT)	96%	Yes
AML30	F	67	Normal	NPM1 ⁺ , FLT3-ITD ⁺ , DNMT3a ⁺	New Dx.	Yes	Decitabine + midostaurin	357	89%	Yes
AML37	M	71	Normal	NPM1 ⁺ , FLT3-ITD ⁺ , DNMT3a ⁺	New Dx.	N/A	No Tx.	2	84%	Yes
AML38	M	33	Normal	FLT3-ITD ⁺ , NRAS ⁺	New Dx.	PR	4+3	230	39%	No
AML 39	M	59	Normal	DNMT3a ⁺ , FLT3-ITD ⁺	New Dx.	PR	7+3, 5+2 (90)	Alive (BMT)	69%	Yes
AML 40	M	58	Normal	FLT3-ITD ⁺ , NPM1 ⁺ , sCEBPa ⁺	Residual	PR	GCLAC	267	49% / 68%	No
Normal Karyotype AML, wt FLT3										
AML13	M	50	Normal	None	Relapsed	Refractory	Aza + GO	74	27%	No
AML14	F	55	Normal	None	Relapsed	Refractory	Aza + GO	65	43%	No
AML15	M	72	Normal	BiCEBPa ⁺	Refractory	Refractory	NEDD-8 inhib.	181	24%	No
AML42 (RAEB-2)	M	80	Normal	None	Progression	PR	Decitabine	390	16% / 11%	No
AML36 (RAEB-2)	M	68	Normal	None	Residual	N/A	No Tx.	207	9%/13 %	No
AML33	M	32	normal	BiCEBPa ⁺	Relapse	Yes	MEC	Alive	28%	No
Adverse-Risk Karyotype										
AML4	M	66	Monosomal	None	New Dx.	No	Aza-Rev	6	43%	No
AML18	F	30	Inv(1), t(11;15)	FLT3-ITD ⁺ , sCEBPa ⁺	Relapsed	PR	MEC x 2	unknown	81%	No
AML19	F	76	Monosomy 7	None	Relapsed	No	Temozolomide	22	82%	Yes
AML22	F	42	t(10;11) [MLL]	Kit(D816V) ⁺ , KRAS(G12V) ⁺	New Dx.	No	7+3 x 2	257	95%	No
AML25	F	67	Monosomy 7	JAK2(V617F) ⁺	New Dx.	N/A	No Tx.	98	28%	No
AML29	M	57	Hyperdiploid, Complex, 5q-	DNMT3a ⁺	New Dx.	Yes	Vorinostat +3+4	412	90%	No
AML41	F	62	t(1;3)	NRAS ⁺ , SF3b1 ⁺	Residual	PR	7+3 + Panobinostat	unknown	13% / 8%	No

Complete Response (CR) / Complete Response with in complete count recovery (CRi)										
CR2	F	48	Normal	IDH2 ⁺	CR s/p 7+3	N/A	N/A	Alive	1%	No
CR3	F	53	Normal	NPM1 ⁺ , FLT3 ⁺ , IDH1 ⁺	CR s/p 4+3	N/A	N/A	269	2%/1%	No
CR5	M	49	Normal	None	CRi s/p 7+3	N/A	N/A	185	2%	No
CR6	F	81	Normal	None	CRi	N/A	N/A	327	4%	No
CR7	F	80	del 13q	None	CRi	N/A	N/A	112	4%	No

Chemotherapy regimens as follows: 4+3, high dose cytarabine x 4 days plus idarubicin x 3 days; 7+3, standard dose cytarabine x 7 days plus idarubicin x 3 days; 7+3 (90), standard dose cytarabine x 7 days plus high-dose daunorubicin x 3days; 5+2, standard dose cytarabine x 5days plus idarubicin x 2 days; Aza + GO, azacitidine plus gemtuzumab; Arsenic + ATRA, arsenic trioxide plus all-trans retinoic acid; ATRA + Dauno, all-trans retinoic acid plus daunorubicin; MEC, mitoxantrone, etoposide and intermediate-dose cytarabine; G-CLAC, fligrastrim, clofarabine and high-dose cytarabine; Ida + ATRA, idarubicin plus all-trans retinoic acid. Note: Samples AML6 and AML8 came from the same patient, all others samples were from separate patients. Samples in italics were not analyzed for in all experiments (APL1 was collected 3 hours after biopsy and could not be analyzed for cell cycle or signaling; AML33 was collected fresh, but IdU was not added so this sample could not be analyzed for cell cycle).

Supplementary Table 2: Antibodies used in this study. The staining panel (A, B, or both) is indicated for each. The “Analyses” column indicates whether the marker was used for each dimensionality reduction analysis (S, SPADE analysis; V, viSNE analysis; B, multidimensional binning). Coefficients of variation (CV) were calculated based on the average of gated cell populations with expression of the marker. CVs could not be calculated for two of the markers (“N/A”); cleaved PARP-positive cells were too rare to allow accurate estimation of the CV, and pSMAD1/5 did not have sufficiently bright staining to perform any analyses.

Antigen	Conjugate	Clone	Concentration	Manufacturer	Panel	CV	Analyses
CD3	In-113	UCHT1	2 µg/mL	Biologend	A & B	11.7%	S, V, B
CD45	In-115	HI30	2 µg/mL	Biologend	A & B	14.1%	S, V, B
CD45RA	La-139	HI100	1.5 µg/mL	Biologend	A	14.2%	
p-RPS6 (S235/36)	La-139, Yb-175	N7-548	1 µg/mL	BD Biosciences	B (La-139), A (Yb-175)	20.2% 14.3%	
CD133	Pr-141	AC133	3 µg/mL	Milteney	A	36.5%	
pATM (S1981)	Pr-141	10H11.E12	2 µg/mL	Millipore	B	16.7%	
CD7	Nd-142	M-T701	2 µg/mL	BD Biosciences	A & B	24.1%	S, V, B
CD71	Nd-143	R17217	2 µg/mL	eBiosciences	A & B	12.4%	S
CD235	Nd-144	HIR2	6 µg/mL	Biologend	A & B	9.6%	S, V, B
CD47	Nd-145	B6H12	1.5 µg/mL	BD Biosciences	A	12.8%	
pAkt (S473)	Nd-145	D9E	2 µg/mL	CST	B	11.3%	
p21	Nd-146	SXM30	2 µg/mL	BD Biosciences	A	13.9%	
Histone 3 (K9ac)	Nd-146	C511B	0.5 µg/mL	CST	B	17.8%	
CD56	Sm-147	NCAM16.2	1.5 µg/mL	BD Biosciences	A & B	13.3%	V, B
CD34	Nd-148	8G12	3 µg/mL	BD Biosciences	A & B	16.4%	S, V, B
CD90	Sm-149	5E10	3 µg/mL	Biologend	A	16.4%	
cMyc	Sm-149	D84C12	1 µg/mL	CST	B	16.2%	
CD117	Nd-150	104D2	1 µg/mL	Biologend	A & B	27.2%	S, V, B
CD123	Eu-151	6H6	1 µL per 100 µL	DVS	A & B	15.7%	S, V, B
CD33	Sm-152	P67.6	1.5 µg/mL	BD Biosciences	A & B	15.1%	S, V, B
HLA-DR	Eu-153	L243	2 µg/mL	Biologend	A & B	12.3%	S
Cyclin A	Sm-154	BF683	2 µg/mL	BD Biosciences	A	10.4%	
pMAPKAPK2 (T334)	Sm-154	27B7	2 µg/mL	CST	B	21.5%	
Cyclin B1	Gd-156	GNS-1	3 µg/mL	BD Biosciences	A	9.8%	
pSTAT3 (Y705)	Gd-156	4	2 µg/mL	BD Biosciences	B	17.2%	
PCNA	Gd-157	PC10	0.5 µg/mL	BD Biosciences	A	15.9%	
pSTAT5 (Y694)	Gd-157	47	2 µg/mL	BD Biosciences	B	13.9%	
Ki-67	Gd-158	SolA15	1 µg/mL	eBiosciences	A & B	12.7%	
CD38	Tb-159	HIT2	1 µg/mL	Biologend	A & B	11.9%	S, V, B
CD14	Gd-160	M5E2	2 µg/mL	Biologend	A & B	15.4%	S, V, B
CD16	Dy-161	3G8	2 µg/mL	Biologend	A & B	13.1%	S, V, B
CD11b	Dy-162	ICRF44	2 µg/mL	Biologend	A & B	16.1%	S, V, B
CD15	Dy-164	W6D3	3 µg/mL	Biologend	A & B	15.3%	S, V, B

p-pRb (S807/811)	Ho-165	J112-906	0.75 µg/mL	BD Biosciences	A	20.7%	
pNF-kB (p65) (S529)	Ho-165	K10-895.12.50	2 µg/mL	BD Biosciences	B	16.1%	
CD321	Er-166	WK9	3 µg/mL	eBiosciences	A & B	13.9%	S, V, B
CD99	Er-167	HCD99	0.5 µg/mL	Biologend	A & B	24.2%	V, B
CD13	Er-168	L138	2 µg/mL	BD Biosciences	A	9.9%	
pERK 1/2 (T202/Y204)	Er-168	G2/D13	2 µg/mL	CST	B	10.4%	
cleaved-PARP(D214)	Yb-171	F21-852	1 µg/mL	BD Biosciences	A & B	N/A	
CD10	Yb-172	H110a	3 µg/mL	Biologend	A & B	12.5%	S, V, B
CD19	Yb-173	H1B19	2 µg/mL	BD Biosciences	A & B	9.9%	S, V, B
CD20	Yb-174	2H7	2 µg/mL	Biologend	A & B	13.2%	S, V, B
pSMAD1/5 (S463/S465)	Yb-175	41D10	3 µg/mL	CST	B	N/A	
pHistone H3(S28)	Yb-176	HTA28	0.5 µg/mL	Biologend	A	18.5%	
pCREB (S133)	Yb-176	87G3	1.5 µg/mL	CST	B	9.7%	

Supplementary Table 3: Comparison of clinical (fluorescent) flow cytometry (on left), to mass cytometry analysis (on right). Green boxes indicate results that were consistent between the techniques, orange boxes indicate results that were inconsistent. Not all results were reported in clinical flow cytometry pathology report and not all results could be compared (uncolored boxes). Patial expression (p) as indicated.

	Clinical Fluorescent Flow									Mass Cytometry								
	CD34	CD7	CD15	CD56	CD117	HLA-DR	CD33	CD38	CD13	CD34	CD7	CD15	CD56	CD117	HLA-DR	CD33	CD38	CD13
AML4	+	+		+(p)	+	+	+	+(p)	+	+	H	H	H	+	+	+	+	+
AML5	+		+	+(p)	+	+	+	+	+(p)	+	H	H	H	+	+	+	+	+
AML6	+ partial			+	+	+	+(dim)	+	+	+	H	H	H	+	+	+	+	+
AML7	negative		+		+	+	+	+(p)	+	+				+	+	+	+	+
AML9	+	+			+	+	+	+	+	+	H	H		+	+	+	+	+
AML10	+		+(p)		+	+	+	+	+	+		H		+	+	+	+	+
AML13	+ partial		+(p)		+	+	+	+	+	+		H		+	+	+	+	+
AML14	+	+			+	+	+	+	+	+		H		+	+	+	dim	+
AML15	+	+			+	+(p)	+(p)	+	+	+	H	H		+	+(p)	+	+	+
AML18	+			+(p)	+	+	+	+	+	+	H	H	H	+	+	+	+	+
AML19	+	+			+	+	+		+(dim)	+	H	H		+	+	+	+	+
AML20	+ partial	+	+(p)		+	+	+(p)	+	+(dim)	+	H	H		+	+	+	+	+
AML21	+	+			+	+	+	+(p)	+	+	H	H		+	+	+	+	+
AML22	+		+	+	negative	+(bright)	+	+	+(p;dim)	+		H	H	negative	+(bright)	+	+	negative
AML39	+ partial		+		+	+	+	+(p)	+	+	H			+	+	+	+	+
AML23	+				+	+	+	+(p;dim)	+	+		H		+	+	+	+	+
APL4	dim partial				+	negative	+	+	+	+	H			+	negative	+	dim	+
AML25	+			+(p)		+	+(dim)	+(p)	+	+				+	+	+	+	+
AML26	+		+	+	+	+	+(dim)	+(p)	+(dim)	+	H	H	H	+	+	+	+	+(dim)
AML27	+ partial	+	+		+	+	+	+(p)	+	+	H			+	+	+	+	+
AML29	+	+	+(p)	+	+	+	+	+(p)	+	+	H	H	H	+	+	+	+	+
AML30	+ partial		+		+	+	+	+	+	+				+	+	+	+	+
AML31	dim partial		+		+	+	+(dim)	+	+(dim)	+		H		+	+	+	+	+
AML32	+		+		+	+	+(dim)	+	+	+	H	H		+	+	+	+	+
AML33	+	+	+	+(p)	+	+	+	+	+	+	H	H	H	+	+	+	+	+
APL5	+ partial			+	+	negative	+	+(p;dim)	+(p;dim)	+	H	H		+	negative	+	+	+
AML35	+		+(p)	+(p)	+	+	+(dim)	+	+	+	H	H	H	+	+	+	+	+(dim)
AML36	+		+(p)		+	+	+(p)	+	+	+		H		+	+	+	dim	+
AML37	+ partial		+		+	+(p)	+(p)	+	+(p;dim)	+	H	H		+	+(p)	+	negative	+
AML38	negative	+ dim	+(p)		+	+	+	+	+	+	H			+	+	+	+	+
APL3	negative		+(p)		+	negative	+	+	+	+		H		+	negative	+	+	+
APL1	+ partial				+	+(p)	+	+(p;dim)	+	+	H	H		+	+(p)	+	+	+
AML42	+ partial			+	+	+	+	+	+	+	H			+	+	+	dim	+
AML40	rare partial	+	+		+	+	+	+	+	+	H			+	+	+	dim	+
AML41	+ partial			+	+	+	+	+	+	+	H		H	+	+	+	dim	+