Supplemental File

Circulating cytokines and small molecules follow distinct expression patterns in acute myeloid leukemia

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Contents:

Materials and Methods

Study population

Acute myeloid leukemia (AML) patients were selected based on newly diagnosed cases, no previous cancer history, and chemotherapeutic treatments have not been received yet. Control subjects were ensured to be free from any type of fever for at least 1 week before testing, have not taken any medications, were not pregnant, and presented no known chronic or acute diseases. Additional information of the patients is shown in Supplemental Table S1. A total of 2-ml peripheral blood samples were collected in plasma separating (purple top) tubes from 38 individuals (19 AML cases and 19 healthy controls) and fresh plasma was separated by centrifuging at 2000 rpm for 10 min. Plasma was aliquoted and frozen at -80^0 C until use. Cytogenetic studies were performed as a routine diagnostic test for all AML patients in Cytogenetics Laboratory, Hematology Unit, Institute for Medical Research, Kuala Lumpur. All the patients provided written informed consent. All experimental protocols and medical ethics were approved by the Medical Ethics Committee of Institute for Medical Research, Malaysia (NMRR-16-1384-31900 S1 R0).

Circulating cytokines and small molecules (analytes) selection

We searched available peer-reviewed literature that reported human circulating cancer biomarkers and curated a list of biomarkers in different cancers, including AML. We selected 22 analytes that been reported in AML patients by different research groups. Then, we selected another 10 analytes that have been previously reported in some other cancers but not in AML. A total of 32 analytes were selected for this study to profile from plasma samples (Table S2).

Analytes profiling

To measure the concentration of the analyte in plasma, the multiplex analysis was performed using the Luminex 200™ on a Bioplex (Bio-Rad) cytometer which enabled simultaneous detection and quantification of multiple analytes per sample. One antibody specific for a single plasma analyte is bound to magnetic beads with distinctive fluorescence. All the antibodies were mixed together and used for each sample. The magnetic beads used for this study were supplied by EMD Millipore. Technical details and procedures are given at MILIPLEX MAP Human Circulating Cancer Biomarker Magnetic Bead Panel section (www.emdmillipore.com).

Patient plasma was diluted and mixed with magnetic beads. Two 96-well Plates were incubated overnight at 4° C with shaking (500 rpm), washed 3 times with wash buffer and incubated for 1 hour with detection antibodies. Streptavidin-phycoerythrin was then added and incubated for 30 min, washed 3 times and sheath fluid was added for 5 min then read using calibrated Luminex 200™ system on a Bioplex (Bio-Rad) cytometer. After measurement of fluorescence intensity by Bioplex cytometer, a digital processor captured the raw data and Bioplex Manager (Version 6.1) was used to analyze the data. Each 96-well plate contained negative controls (duplicate) that were used as background intensity. All the samples, quality controls, and standards were run in duplicates. In order to draw a standard curve, 7-wells (duplicate) of consecutive 3-fold dilution of known standards were used to fit a 6-parameter logistic curve for each analytes. The intensity of the magnetic beads was compared to the corresponding fitted curve to measure the concentration of the proteins in each duplicate sample and mean concentration was calculated from the duplicate. If the intensities of the samples were beyond the standard curve range, the values were considered as out-of-range above (OOR>) or out-of-range below (OOR<) and the values were excluded. Some analytes (IL-6, IL-8, bHCG, TGFa, and VEGF) were OOR< in some of the healthy controls.

Data analysis and visualization

We used R platform (http://www.r-project.org/, version 3.1.3) for data analysis and visualization. In order to measure differential expression between AML and control, Mann-Whitney U-test was performed and a significant threshold was set up ($p<0.005$) for this study. Power analysis was performed for the sample size and the probability of getting significant deregulation (Table S5). Median fold changes were calculated by dividing healthy controls median expression. Distribution of the analytes across the AML patients was visualized using the R package OncoPrint. Pearson correlation test was performed for determining correlation coefficients and the ggplot2 package was used for visualization of the correlation coefficients. For multivariate analysis and low dimensional representation of the dataset, we applied principal component analysis (PCA). The BimodalIndex package [1] was used for determining bimodal distribution patterns of the analytes and 18 of them were selected as significantly informative (BI>1.4) for clustering. The packages gplots and beeswarm were used for the generation of heatmaps and beeswarm plots, respectively.

To validate the deregulation of analytes, and additional exploratory analysis was performed using The Cancer Genome Atlas (TCGA) data[2]. Promoter methylation and gene expression of the corresponding genes were analyzed using RnBeads package (http://rnbeads.mpi-inf.mpg.de/) and custom R scripts[3].

Supplemental Tables

Table S1: Demographics of the samples.

Case ID	Age	Gender	Race	Karyotype	Control ID	Age	Gender	Race
S1	53	M	Malay	Tri-11	C1	60	M	Malay
S2	46	F	Malay	Normal	C2	36	M	Chinese
S3	61	M	Malay	Normal	C3	54	M	Chinese
S4	65	M	Chinese	t(8;21)	C4	35	M	Chinese
S5	40	F	Malay	t(8;21)	C5	56	F	Chinese
S6	21	F	Malay	Tri-21	C6	33	M	Chinese
S7	22	F	Chinese	Normal	C7	55	F	Chinese
S8	25	F	Malay	inv(16)	C8	49	M	Chinese
S9	52	M	Malay	Normal	C19	44	M	Chinese
S10	22	M	Chinese	Normal	C10	30	M	Chinese
S11	21	M	Chinese	inv(16)	C11	50	F	Chinese
S12	58	M	Chinese	Normal	C12	45	F	Chinese
S13	42	M	Malay	Normal	C13	50	F	Chinese
S14	24	F	Chinese	Normal	C14	47	F	Chinese
S15	34	M	Malay	t(8;21)	C15	32	M	Malay
S16	47	F	Malay	Normal	C16	27	M	Chinese
S17	38	M	Malay	Normal	C17	58	M	Malay
S18	20	M	Malay	t(15;17)	C18	62	F	Chinese
S19	26	M	Malay	Normal	C19	42	F	Chinese

Blue header= AML cases (n=19, S1-S19); Green header= Healthy Controls (n=19, C1-C19).

Table S2: Information of the analytes.

Proteins	Gene Name	Ensembl	Category	Description
AFP	AFP	ENSG00000081051	Serum protein	Alpha Fetoprotein
Total PSA	KLK3	ENSG00000142515	Protease	Kallikrein Related Peptidase 3
CA 15.3	MUC1	ENSG00000185499	Cell surface molecule	Carbohydrate antigen 15-3 (Mucin 1)
CA 19.9	FUT3	ENSG00000171124	Cell surface molecule	Carbohydrate antigen 19-9
MIF	MIF	ENSG00000240972	Signaling molecules	Macrophage Migration Inhibitory Factor
TRAIL	TNFSF10	ENSG00000121858	Signaling molecules	Tumor Necrosis Factor-related apoptosis-inducing ligand
Leptin	LEP	ENSG00000174697	Hormone	Satiety hormone
IL6	IL6	ENSG00000136244	Cytokine	Interleukin 6
sFasL	FASLG	ENSG00000117560	Signaling molecules	Fas Ligand

CEA	CEACAM5	ENSG00000105388	Cell surface molecule	Carcinoembryonic Antigen-Related Cell Adhesion Molecule 5
CA125	MUC16	ENSG00000181143	Cell surface molecule	Mucin 16
IL8	IL8	ENSG00000169429	Chemokine	Interleukin 8
HGF	HGF	ENSG00000019991	Hormone	Hepatocyte Growth Factor
sFas	FAS	ENSG00000026103	Signaling molecules	Soluble Fas, Cell Surface Death Receptor
TNFa	TNF	ENSG00000232810	Cytokine	Tumor Necrosis Factor
Prolactin	PRL	ENSG00000172179	Hormone	Pituitary hormone
SCF	KITLG	ENSG00000049130	Growth factor	Stem Cell Factor
CYFRA21.1	KRT19	ENSG00000171345	Cytokeratin	Keratin 19
OPN	SPP1	ENSG00000118785	Integrin	Secreted Phosphoprotein 1
FGF2	FGF2	ENSG00000138685	Cytokine	Fibroblast Growth Factor 2
bHCG	CGB	ENSG00000104827	Hormone	Chorionic Gonadotropin hormone
HE4	WFDC2	ENSG00000101443	Protease inhibitor	WAP Four-Disulfide Core Domain 2
TGFa	TGFA	ENSG00000163235	Cytokine	Transforming Growth Factor Alpha
VEGF	VEGFA	ENSG00000112715	Growth factor	Vascular Endothelial Growth Factor A
Galectin	LGALS3	ENSG00000131981	Integrin	Galactose-Specific Lectin 3
Cathepsin D	CTSD	ENSG00000117984	Protease	Lysosomal Aspartyl Protease
FAPa	FAP	ENSG00000078098	Integrin	Fibroblast Activation Protein Alpha
MIA	MIA	ENSG00000261857	Growth factor	Melanoma Inhibitory Activity
MPO	MPO	ENSG00000005381	Serum protein	Myeloperoxidase
SHBG	SHBG	ENSG00000129214	Serum protein	Sex Hormone Binding Globulin
IGFBP3	IGFBP3	ENSG00000146674	Serum protein	Insulin-Like Growth Factor Binding Protein 3
Ferritin	FTH1	ENSG00000167996	Serum protein	Ferritin Heavy Chain 1

Red= Reported-Analytes. Olive-Green= Novel-Analytes.

Table S3: Expression levels in healthy controls and comparison with the literature.

Analytes	N	Gender	Age	Ethnicity	Median	Range	Method	Reference	Bioplex-Median	Bioplex-SD	Biolex-Range	Unit
AFP	270		40.7	American	3040	500-11600	RIA	4	1276	791.07	848.1-3380	pg/ml
	284	M=173, F=111	20-64	Danish	2600	600-12600	ELISA	5				
	49	M=25, F=24	18-60	Turkish	2138	736-4164	ELISA	6				
Total PSA	3222	M=3222, F=0	40-79	Canadian	1000	700-1900	Hybritech	7	972.1	447.71	327.3-2022	pg/ml
	8422	M=8422, F=0	40-79	Chinese	3705	2150-5370	Hybritech	8				
	5805	M=5805, F=0	30-79	Korean	3650	2000-6300	Hybritech	9				
	513	M=513, F=0	20-79	Singaporean	3716	1400-6600	ELISA	10				
	583	M=583, F=0	20-89	Indian	1625	800-2200	ELISA	11				
	286	M=286, F=0	40-79	Japanis	3500	2000-5000	ELISA	12				
CA 15-3	323	M=0, F=323	20-62	Norwegian	10.9	5-35	IRMA	13	7.72	4.6	4.29-17.67	U/ml
CA 19-9	49	M=25, F=24	18-60	Turkish	5.098	1.90–12.253	ELISA	6	12.13	5.32	7.19-20.57	U/ml
MIF	53	M=9, F=44	20-40	Turkish	4971.2	1917-8025	ELISA	14	1706	664.44	533.9-2861	pg/ml

TRAIL	25	M=0,F=25	21-48	Egypt	468.93	383-554	ELISA	15	173.7	35.09	93.14-238.9	pg/ml
	60	M=47, F=13	40-75	Italian	85	70-100	ELISA	16				
Leptin	713	M=312, F=401	5.8-19.9	German	2855	710-5000	RIA	17	10100	12584.28	1150-36360	pg/ml
	20		20-50	Omanis	10600	6400-14800	ELISA	18				
IL-6	22	M=6, F=16	23-71	American	0.56	0-2.67	Bioplex	19	24.05	50.66	23.35-111.4	pg/ml
	20	M=16, F=6	30-82	German	1	0.5-1.5	Multiplex	20				
	14	M=8, F=6	5.0-7.0	Hispanic	4.1	2.4-5.8	Bioplex	21				
	26				4.3	0-72	LabMAP	22				
	30	M=14, F=16	20-40	Turkish	4.6	4.3-4.9	ELISA	23				
	23	M=0, F=23	20-40		5		Sandwich	24				
	50	M=0, F=50	59		6.6	0.3-32.3	LINCOplex	25				
	45	M=0, F=45	36-76		8.8	0-64.1	LabMAP	26				
	23			Turkish	4.2	0-12.7	ELISA	27				
	28	M=20, F=8	50-70		20	1.3-339.3	Luminex	28				
	378				22.8	0-2394.2	Multiplex	29				
	9	M=5, F=4	19-55		31	20-40	Multiplex	30				
	44	M=18, F=26	20-50	Chinese	38.76	23.47-54.05	ELISA	31				
	19	M=11, F=8	24-57	American	32.63	19.28-118.63	Multiplex	32				

sFasL	10			Polish	90	15-165	ELISA	33	21.1	6.59	11.41-36.9	pg/ml
	15	M=12, F=3	61	Polish	326.7	151-870	ELISA	34				
CEA	98	M=98, F=0	62	Hungary	1900	1400-2500	CMIA	35	1382	577.76	735.6-2829	pg/ml
	49	M=25, F=24	18-60	Turkey	1014	540-1693	ELISA	6				
CA 125	323	M=0, F=323	20-62	Norway	10.9	5-35	IRMA	13	5.98	1.24	5.08- 9.01	U/ml
	98	M=98, F=0	62	Hungary	9.8	6.8-12.9	CMIA	35				
	50	M=0, F=50		Polish	9.94	5.06-36.60	ELISA	36				
IL-8	22	M=6, F=16	23-71	American	0.13	0.05-0.18	Bioplex	19	4.69	1.97	3.62-8.77	pg/ml
	20	M=16, F=6	30-82	German	0.5	0-5.6	Multiplex	20				
	30	M=14, F=16	20-40	Turkish	5.3		ELISA	23				
	23	M=0, F=23	20-40		5.7		Sandwich	24				
	50	M=0, F=50	59		7.1	3.1-18.8	LINCOplex	25				
	14	M=8, F=6	5.0-7.0	Hispanic	8.1		Bioplex	21				
	28	M=20, F=8	50-70		8.1	3.9-15.3	Luminex	28				
	26				9	3.3-18.8	LabMAP	22				
	378				9.56	0-87.6	Multiplex	29				
	45	M=0, F=45	36-76		10.2	2.3-51.4	LabMAP	26				
	44	M=18,	20-50	Chinese	30.55		ELISA	31				

		F=26										
	23			Turkish	12.9	0-50.4	ELISA	27				
	9	M=5, F=4	19-55		139		Multiplex	30				
	19	M=11, F=8	24-57	American	10.94	3.94-52.10	Multiplex	32				
	35	M=9,F=26	21-86	Italy	29.3	24.4-35.9	Multiplex	37				
HGF	35	M=9,F=26	21-86	Italy	319.7	196.6-477.9	Multiplex	37	176.4	47.19	156-319.6	pg/ml
	11		19-60	American	364.2	164-522.3	Sandwich	38				
sFas	15	M=12, F=3	61	Polish	227.3	135.3-319.3	ELISA	34	1474	417.26	729.1-2439	pg/ml
	30	M=15, F=15	40-60	German	6270		ELISA	39				
TNFa	14	M=8, F=6	5.0-7.0	Hispanic	0.6		Bioplex	21	5.55	2.04	3.45-9.61	pg/ml
	23	M=0, F=23	20-40		4		Sandwich	24				
	20	M=16, F=6	30-82	German	5.8	0-115.8	Multiplex	20				
	30	M=14, F=16	20-40	Turkish	6.4		ELISA	23				
	28	M=20, F=8	50-70		8.4	1.7-62.6	Luminex	28				
	44	M=18, F=26	20-50	Chinese	21.08		ELISA	31				
	278				34.32	0-3847.6	Multiplex	30				
	9	M=5, F=4	19-55		73		Multiplex	21				
Prolactin	133	M=133, F=0	46-55		16900	15300-18700	Multiplex	40	23020	8288.79	10320-40600	pg/ml

	195	M=0, F=195	25-30	Italian	14000		Immunoassay	41				
SCF	62	M=22, F=40	41	Japanis	939		ELISA	42	61.96	17.81	40.75-97.74	pg/ml
	45	M=19, F=26	68		997.7		ELISA	43				
CYFRA 21-1	124	M=71, F=53	31-61	Chinese	380	160-480	RIA	44	1546	466.48	1189-2233	pg/ml
	98	M=98, F=0	62	Hungary	1400	1200-2100	CMIA	35				
	84	M=74, F=10	28-75	Korean	1400	200-5700	Immunoassay	45				
OPN	20	M=10, F=10	58		156700		ELISA	46	32690	11050.84	12540-49080	pg/ml
	30	M=16, F=14	46-70		525500	131000-1009000	ELISA	47				
FGF2	26				43	0-521	LabMAP	22	138.5	49.56	103.9-255.5	pg/ml
	9	M=5, F=4	19-55		224		Multiplex	30				
	19	M=11, F=8	24-57	American	37.91	0.08-155.19	Multiplex	32				
	35	M=9,F=26	21-86	Italy	41.7	33.2-49.5	Multiplex	37				
bHCG	193	M=193, F=0	23-50	Denish	Not detected		RIA	48	1	0.75	0.37-2.31	mU/ml
HE4	50	M=0, F=50		Polish	44.15 pmol/L	6.20-122.30	ELISA	36	6720	5322.93	3525-17950	pg/ml
	98	M=98, F=0	62	Hungary	62.2 pmol/L	47.2-76.1	CMIA	35				
TGFa	74		21-58	American	147		RIA	49	7.47	4.96	5.54-16.54	pg/ml
	44			Spanish	6.2	0-14.5	ELISA	50				

VEGF	50	M=0, F=50		Polish	39.39	2.30-467.10	ELISA	36	298.6	187.9	89.01-592.1	pg/ml
	378				76.6	0-1151	Multiplex	29				
	45	M=0, F=45	36-76		90.7	18-306	LabMAP	26				
	23	M=0, F=23	20-40		99.7		Sandwich	24				
	21	M=5, F=16	29-96		101	0-377	ELISA	51				
	9	M=5, F=4	19-55		623		Multiplex	37				
	35	M=9,F=26	21-86	Italy	61.6	32-118.9	Multiplex	32				
	19	M=11, F=8	24-57	American	35.5	0.46-389.5	Multiplex	36				
Galectin-3	46	M=44, F=2	46.1	Chinese	3.63		Miliplex	52	5.95	1.53	4.3-9.51	ng/ml
	20		34.5	German	3.7		ELISA	53				
	20	M=15, F=5	58	Netherlands	9.7		ELISA	54				
Cathepsin D	56			German	0.73		ELISA	55	99.55	29.48	68.99-182.6	ng/ml
	409	M=245, F=164	60.2	Swedish	163 AU	128-210 AU	Multiplex	56				
	11	M=7, F=4	55-70	Spanish	0.73	0.53-1.41	ELISA	57				
FAPa	501	M=183, F=318	18-57	Netherlands	90.3	72.2-106.6	ELISA	58	208.6	71.81	145.2-442	ng/ml
	30	M=15, F=15	18-72	Netherlands	107	67-157	ELISA	59				
MIA	5	M=2, F=3	24-66	Brazil	5.64	4-8.6	ELISA	60	27.31	8.45	18.41-49.53	ng/ml

	20				6.91		ELISA	61				
MPO	46	M=30, F=16	24-69	Turkish	20		ELISA	62	21.8	15.37	8.26-69.07	ng/ml
	146		42-83	New Zealand	39	29-49	ELISA	63				
SHBG	193	M=193, F=0	23-50	Denish	37	12-108	RIA	48	92.12	50.78	41.24-203	nM
	250	M=250, F=0	25-100		45.5	35.5-65.9	RIA	64				
IGFBP 3	30			Turkish	4235	2600-5950	Immulite 2000	65	1740	467.33	750.9-2569	ng/ml
	15	M=0, F=15	37.1	Poland	4400	4100-4700	RIA	66				
Ferritin	167	M=0,F=167		American	100	7-1182	RIA	67	499.5	249.81	131.1-1050	ng/ml
	331	M=331, F=0		American	168	1-1479	RIA	67				
	20			Thailand	37	26-48	Immunoturbidimetric assay	68				

Orange header is the baseline analytes levels for healthy controls reported in peer-reviewed literature. Green header (right side shaded part) is the baseline analytes levels for healthy controls in this study.

Table S4: Expression levels in AML cases and comparison with the literature.

Analytes	N	Gender	Age	Ethnicity	Median	Range	Method	Reference	Bioplex-Median	Bioplex-SD	Bioplex-Range	Unit
AFP	1				2500			69	1294	1530.66	833.6-5697.0	pg/ml
Total PSA									500.3	267.55	61.21-856.70	pg/ml
CA 15.3*	17				11.1	2-200	ELISA	70	30.48	41.16	13.3-173.2	U/ml
CA 19.9									20.87	31.27	18.6-97.55	U/ml
MIF*									50220	32624.72	12460-111000	pg/ml
TRAIL*									63.08	32.41	17.25-142.6	pg/ml
Leptin	14	M=6, F=8	18-71	Turkish		1376.1-60237.4	ELISA	71	2481	4537.12	994.7-15320	pg/ml
IL6	176			American	36.11		Bioplex	32	23.35	23.26	5.05-72.93	pg/ml
					6.7	0-194		72				
	32		25-56	Egypt	24.83	0-64.23	ELISA	73				
sFasL*	16				600	200-1700	ELISA	74	105.4	14.37	70.74-109.40	pg/ml
CEA	17					0-7000		75	1235	604.6	647-2634	pg/ml
CA125	20	M=10, F=10	34.8		12.4		IMMULITE	76	8.22	9.09	5.14-34.72	U/ml
IL8*	176			American	170.83		Bioplex	32	72.51	74	10.24-261.1	pg/ml

					27.5	0-1587		72				
HGF*	59				843.5	101.9 - 12819.5		38	2968	5280.45	565.9-17560	pg/ml
	63				899.6	101.9-12819		77				
sFas*	16				11	8.5-14	ELISA	74	3454	1204.89	1436-5576	pg/ml
TNFa*	63				8.9	7.3-19		77	18.88	5.17	7.8-24.32	pg/ml
					5.7	1.3-42.8		78				
	176			American	62.88		Bioplex	32				
Prolactin	56	M=24, F=32	24-86		30000		MEIA	79	27110	13891.65	10520-57980	pg/ml
SCF	13				1750	1000-2500		80	93.11	31.85	42.51-130.8	pg/ml
CYFRA21.1									2934	1371.05	1648-5939	pg/ml
OPN*	68				745000	0-10780000	ELISA	47	90770	86609.87	43750-373700	pg/ml
FGF2*	176			American	32.19		Bioplex	32	255.5	130.6	94-532.5	pg/ml
					2.8	1.4-14.3		81				
	113				6.48	4.66-203.73		77				
bHCG	1				3			69	2.08	0.54	1.28-3.02	mU/ml
HE4									17930	7103.42	3525-24390	pg/ml
TGFa	49				0	0-199.4		77	46.83	39.34	20.1-158.2	pg/ml
VEGF	115				30.43	21.47-439.25		77	449.8	170.61	218.9-759.3	pg/ml

					54.3	2.8-455.4		78				
	176			American	53.43		Bioplex	32				
Galectin-3*	5			American	65		ELISA	82	73.3	45.97	9.7-174.5	ng/ml
Cathepsin D*									317.1	314.91	179.8-1111	ng/ml
FAPa*									92.42	69.71	41.6-306.1	ng/ml
MIA									31.38	13.47	24.12-64.79	ng/ml
MPO*	19				100		ELISA	83	1239	508	297.7-2370	ng/ml
SHBG*									27.94	19.86	7.04-58.99	nM
IGFBP3	11		28-80		3200		ELISA	84	1597	597.7	498.3-2577	ng/ml
Ferritin*	32		25-56	Egypt	1400.25	617.25-1875.35	ELISA	73	1966	496.08	1135-2647	ng/ml

Orange header is the baseline analytes levels for AML cases reported in peer-reviewed literature. Blue header (right side shaded part) is the baseline analytes levels for AML cases in this study. Olive-Green= Novel-Analytes: no published baseline found in AML. Statistically significant analytes are shown as asterisk (*) (Mann-Whitney U-test, p-value <0.005).

Table S5: Statistical tests for analytes in AML cases compared to healthy controls.

Analytes	P-value*	Power**	BI Score***
AFP	0.09033	0.8304	0.07723
Total PSA	0.7703	0.0721	0.892257
CA15-3	**1.10E-08**	1	0.854866
CA-19-9	0.8819	0.267	0.120152
MIF	**2.79E-09**	1	**3.188746**
TRAIL	**1.08E-09**	0.999	**1.516339**
Leptin	0.01538	0.7065	**1.715155**
IL-6	0.3812	0.6761	0.168804
sFasL	**5.59E-06**	1	**4.002725**
CEA	0.6441	0.9754	1.076593
CA-125	0.02848	0.9508	0.037218
IL-8	**1.33E-06**	1	**2.450504**
HGF	**2.37E-07**	1	**2.25318**
sFas	**2.98E-06**	1	**1.537448**
TNFa	**7.37E-06**	1	**2.426188**
Prolactin	0.2844	0.611	1.063814
SCF	0.03176	0.8605	**1.915231**
CYFRA-21-1	0.1594	0.234	**2.123984**
OPN	**2.08E-06**	1	1.283494
FGF-2	**0.001855**	0.9998	**1.746355**
bHCG	0.2097	0.2422	1.080848
HE4	0.01569	0.8984	**2.067391**
TGFa	0.03596	0.9999	**2.54859**
VEGF	0.2437	0.3613	0.846097
Galectin	**1.73E-07**	1	**2.679931**
Cathepsin	**1.13E-10**	1	**1.454599**
FAPa	**0.0008892**	0.8112	**1.759841**
MIA	0.7038	0.126	1.149084
MPO	**2.37E-07**	1	**4.016934**
SHBG	**2.30E-05**	0.7126	1.054942
IGFBP3	0.212	0.2379	1.084311
Ferritin	**1.13E-10**	1	**2.285821**

*P-value was calculated by Mann-Whitney U-test. Highly significant p-values (<0.005) are shown as bold.

**The probability of getting a significant p-value in 19 AML cases was calculated by power analysis. A power of 1 means 100% probability of getting a significant p-value despite the small sample size.

*** BI= 'Bimodality Index' was calculated by R. Analytes that follow bimodal distribution patterns are called informative for samples clustering (BI>1.4, shown as bold) [1].

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Supplemental Figures:

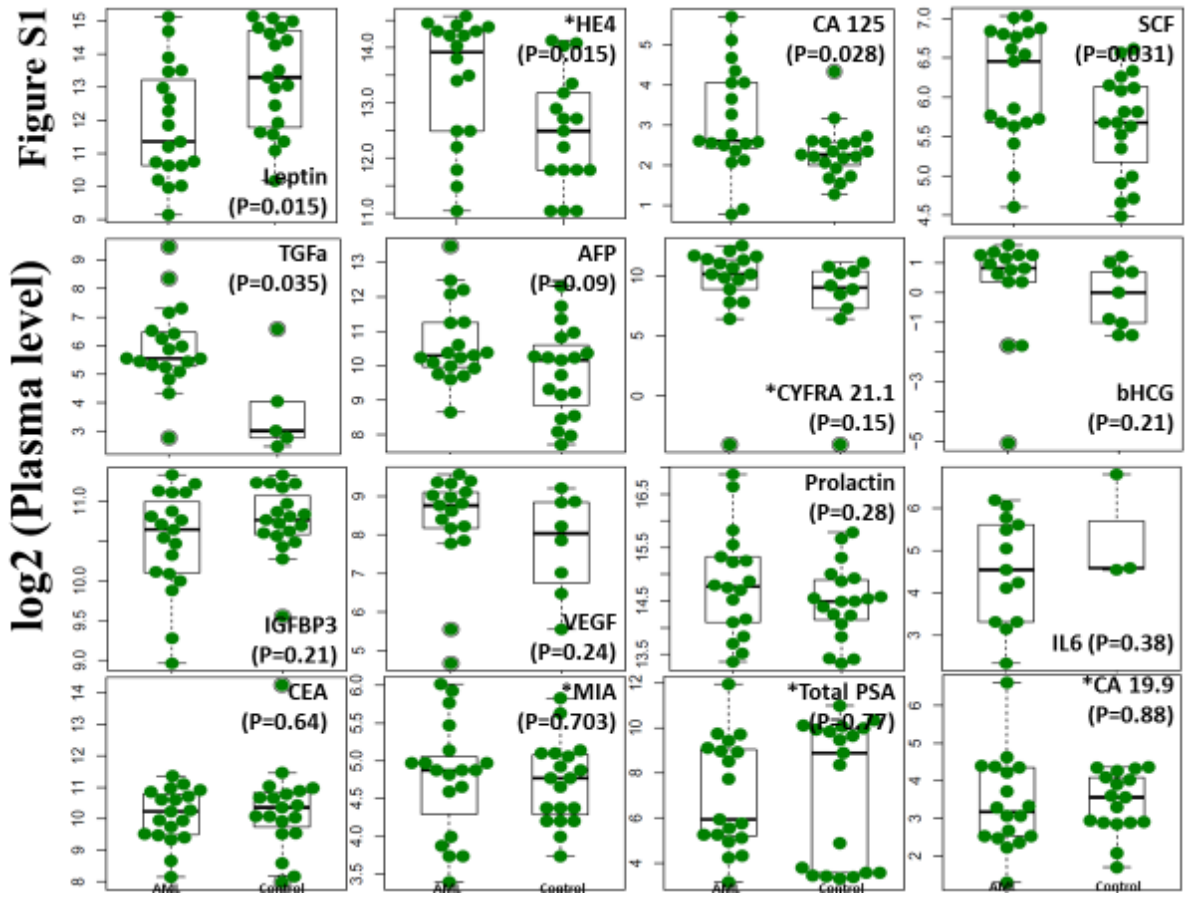

Figure S1:

Beeswarm plots for analytes not differentially expressed in AML

P-value is not significant for these 16 analytes (Mann-Whitney U test). The AML cases and healthy controls are plotted side by side in each rectangle. Novel analytes are denoted by asterisk (*). The y-axis measures plasma expression level (log_2 scale).

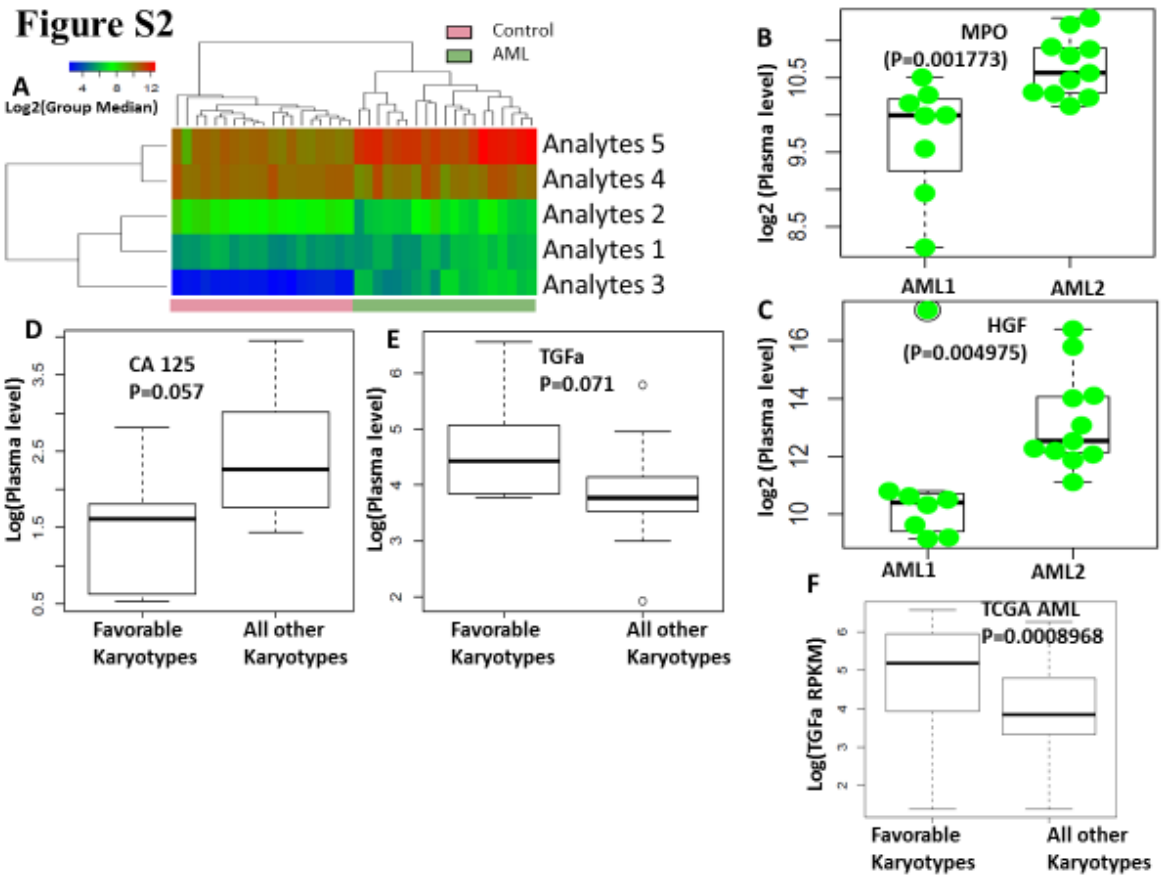

Figure S2:

Expression levels across analyte and patient groups.

(A) Aggregated median expression of the five analyte groups across all samples. Analytes4 and Analytes5, highly expressed groups, cluster together. The expression level of Analytes3 is higher in AML compared to control. (B and C) MPO and HGF expressions are significantly upregulated in the AML2 group (with 5 favorable karyotypes) compared to the AML1 group (defined in Figure 2D). (D and E) The CA-125 expression is relatively low among favorable karyotype AML patients, whereas TGFa expression is high within the favorable karyotype AML patients (although p-values are not significant based on Mann-Whitney U test). (F) TGFa mRNA expression is significantly high among favorable karyotype patients in TCGA cohort.

Figure S3

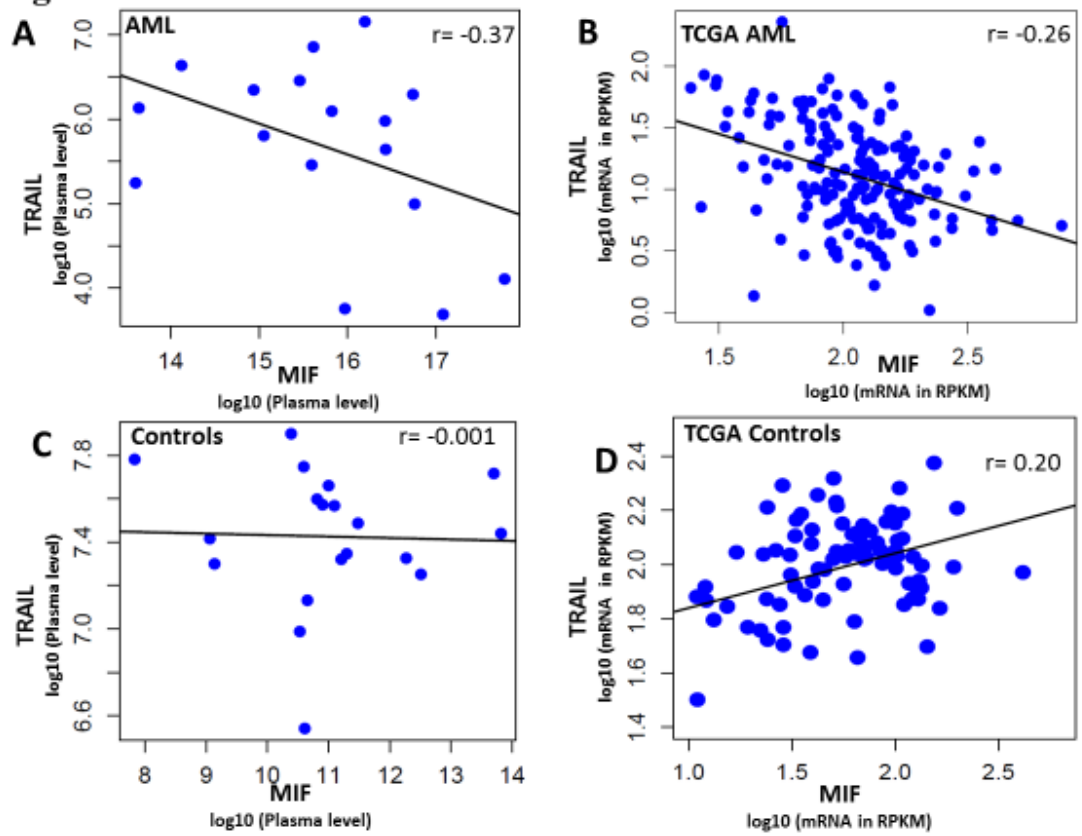

Figure S3:

Correlation between MIF and TRAIL expression levels.

Both MIF and TRAIL are found to be significantly deregulated and novel-analytes, as shown in Figure 1B. (A) MIF and TRAIL are negatively correlated in AML. (B) mRNA expression in TCGA cohort also shows a negative correlation between MIF and TRAIL, same as Figure S3A. (C) Almost no correlation is observed in controls in our study. (D) TCGA controls show opposite trends (positive correlation) compared to TCGA AML in Figure S3B. RPKM = reads per kilobase per million mapped reads.

Figure S4

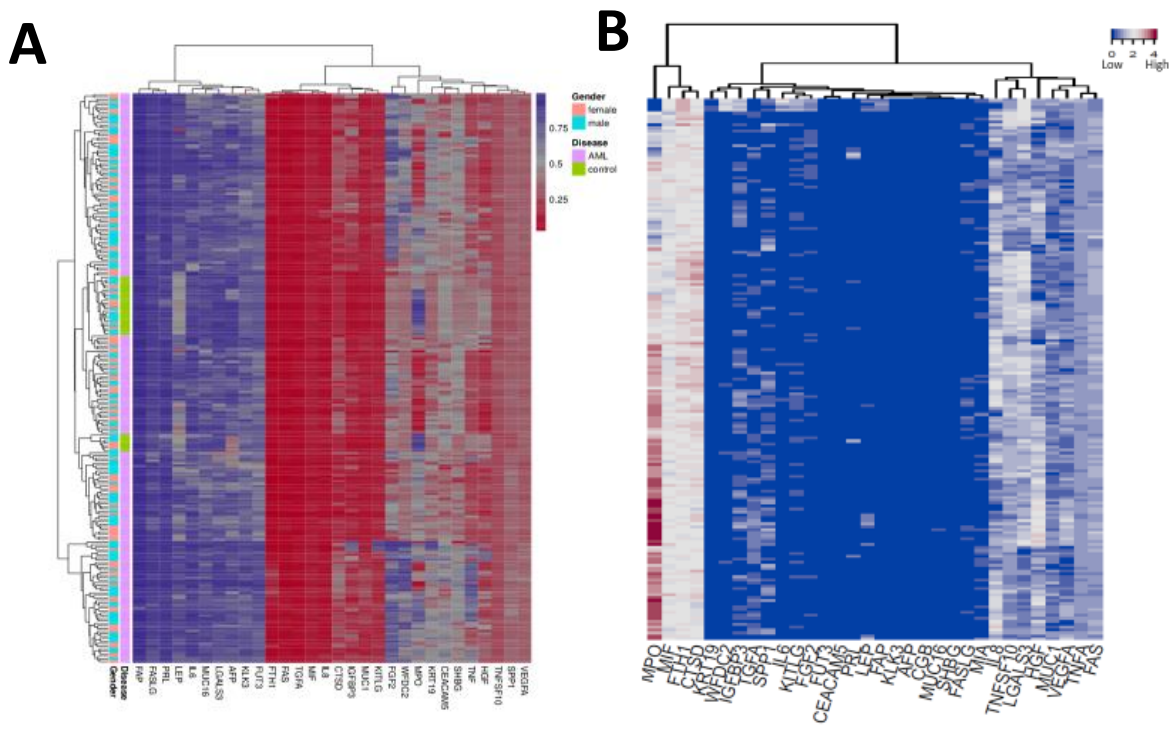

Figure S4:

Methylation and gene expression levels of the studied analytes in TCGA.

(A) Promoter methylation patterns for the 32 analytes in TCGA patients (194 AML and 30 controls). Unsupervised clustering identifies three groups of analytes: (1) the highly-methylated group contains 10 genes: FAP, FASLG, PRL, LEP, IL6, MUC16, LGALS3, AFP, KLK3, and FUT3; (2) the unmethylated group contains 9 genes: FTH1, FAS, TGFA, MIF, IL8, CTSD, IGFBP3, MUC1, and KITLG; (3) the partially methylated group consists of the remaining 13 genes. Interestingly, 7 out of the 9 unmethylated genes encoding analytes are significantly upregulated in our study (Figure 1B). (B) Gene expression patterns for 32 analytes. Unsupervised clustering identifies three groups of genes, although the number of genes in each group is not the same as promoter methylation groups.