SUPPLEMENTARY INFORMATION

Systemic Metabolomic Profiling of Acute Myeloid Leukemia Patients before and During Disease-Stabilizing Treatment Based on All-Trans Retinoic Acid, Valproic Acid, and Low-Dose Chemotherapy

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Table S1. Clinical and biological characteristics of the included patients[1]. Responders to treatment are listed at the upper part of the table; the non-responders are listed at the lower part.

ID[1]	Gender	Age	Previous disease	FAB	Membrane molecule expression[2]					Karyotype	FLT3	NPM1	Additional mutations	WBC counts (x10^8)	Survival (days)[3]
					CD13	CD14	CD15	CD33	CD34						
RESPONDERS															
1*	M	73	MDS	M1	+	-	-	+	+	multiple	ITD, TKD	wt	PHF6, RUNX1	<0.5	392
2*	F	61	1st relapse	M1	+	-	+	+	+	multiple	wt	wt	NRS, SF3B1	55.8	644
3*	M	62	2nd relapse	M2	+	-	+	+	+	-7	wt	wt	nt	4.9	350
4*	M	80	*de novo*	M1	+	-	-	+	+	multiple	wt	wt	nt	8	58
5*	M	78	MDS	M1	+	-	-	+	+	nt	nt	nt	nt	142	69
6*	F	68	1st relapse	M1	+	-	+	+	+	normal	wt	wt	TET2, ASXL1, BCOR	15.6	105
7*	F	81	MDS	M1	+	-	-	+	+	normal	wt	wt	TET2, ASXL1, GATA2, CEBPA, SRSF2	6	147
8*	M	86	*de novo*	M4	+	+	+	+	-	nt	nt	nt	nt	18.7	59
9	M	81	Polycytemia-vera	M2	-	-	-	-	+	-7	wt	wt	ASXL1, SRSF2, RAD21	22.3	610
10	M	77	MDS	M1	+	-	-	+	+	normal	wt	wt	NRAS, RUNX1, CEBPA, SRSF1, STAG2	2.1	419
11	F	85	MDS	M1	-	-	-	-	+	multiple	nt	nt	nt	3.9	171
12	F	83	MDS	M2	+	-	+	-	+	normal	nt	nt	nt	0.3	196
13	M	66	MDS	M2	-	-	+	-	-	14q	nt	nt	nt	2,6	239
14	M	83	MDS	M0	-	-	-	-	+	normal	wt	wt	nt	1.1	102
15	M	73	*de novo*	M1						nt	wt	Ins	TKD, IDH2, SRSF1	12.1	383
16	M	74	*de novo*	M0	+	-	-	+	+	multiple	wt	wt	TP53	18.7	151
17	F	72	MDS	M2	+	-	-	+	-	t(1;5), t(2;3)	ITD	wt	KMT3, RUNX1	42.6	132
18	F	77	MDS	M2	+	-	-	+	+	normal	wt	wt	NRAS, TET2, ASXL1, RUNX1, SRSF1, STAG2, BCDR	142	132
NON-RESPONDERS															
19*	F	81	*de novo*	M1	+	-	-	+	+	multiple	nt	nt	nt	1.7	192
20*	M	74	*de novo*	M0	+	-	-	-	+	multiple	wt	wt	IDH2	13.3	112
21*	F	70	MDS	M1	+	-	-	+	+	multiple	wt	wt	TP53	3.6	142
22*	M	67	1st relapse	Nt	nt	nt	nt	nt	nt	normal	TKD	wt	nt	15.6	73

23*	M	71	1st relapse	M2	+	-	-	+	+	normal	TKD	Ins	TET2, GATA2, STAG2	1.5	49
24	F	82	*de novo*	M5	+	-	+	+	+	normal	ITD, TKD	wt	WT1, DNMT3	142	37
25	F	79	*de novo*	M2	+	-	+	+	+	multiple	wt	wt	TP53, BCORL1	1.7	42
26	M	73	MDS	M1	+	-	-	+	+	multiple	wt	wt	TP53, CUX1	11.1	33
27	M	58	Relapse	M1	+	-	-	+	+	-7	wt	wt	nt	0.9	65
28	F	65	Relapse	M6	+	-	-	+	+	multiple	wt	wt	TP53, IKZF1, RUNX1	1.3	65
29	M	77	Polycytemia vera		-	-	-	-	+	nt	nt	nt	nt	8.3	41
30	F	86	*de novo*							del5q	wt	wt	GATA2	249	17
31	F	65	Relapse	M6	+	-	-	+	+	multiple	wt	wt	nt	1.3	65
32	F	77	*de novo*	M1	-	-	-	-	-	normal	ITD	Ins	DNMT3A	68.5	32
33	M	62	MDS	M0	-	-	-	-	+	multiple	nt	nt	nt	8.2	57
34	M	68	MDS	M0	-	-	-	-	+	normal	wt	wt	TET2, ASXL1, BEBPA, SRSF2, STAG2	1.5	24
35	F	83	*de novo*		+	-	-	+	-	normal	ITD	Ins	nt	105	38
36*	M	78	MDS	nt	+	-	-	+	+	nt	ITD	wt	ASXL1, STAG2, ZRSR2	5.6	55
37*	M	62	3rd relapse	M1	+	nt	-	+	+	t(4;20)	wt	wt	DNMT3, IDH2	1.5	78
38*	M	68	Myelofibrosis	M1	+	-	-	+	+	Normal	wt	wt	KRAS	34.3	56
39*	F	80	*de novo*	M2	-	-	-	+	-	normal	ITD	Ins	PHF6, TET2, BCDFIL1, CSF3R	217	5
40*	F	70	Chemotherapy	M4	+	-	+	+	-	normal	wt	Ins	NRAS, DNMT3A, IDH1	73.7	7
41*	M	60	2nd relapse	M4	+	-	+	+	+	normal	ITD	wt	WT1	66	6
42	M	71	Chemotherapy	M4						nt	wt	Ins	KRAS, DNMT3A, TET2	104	2
43	M	48	Relapse	M4					-	normal	ITD, TKD	Ins	DNMT3A, IDH1	30.4	8
44	F	60	Relapse	M4	+	-	+	+	-	normal	ITD	Ins	DNMT3A, TET2	16.7	12

Abbreviations: MDS, myelodysplastic syndromes; Ins, insertion; ITD, internal tandem duplications; nt, not tested; wt, wild type.

1) Patient IDs marked with (*) indicate that these patients were included in the study by Ryningen *et al.* (PMID 19007987) (n=19) [1], while unmarked patient IDs were included in the study by Fredly *et al.* (PMID 23915396) [2].

2) Expression of a marker was defined as at least 20% positive cells compared with the corresponding negative control.

3) Survival is presented as the survival from start of treatment.

Table S2. Significantly altered serum metabolites between subsets of non-responders to antileukemic treatment based on ATRA and valproic acid; a comparison of non-responders with very aggressive (i.e. rapidly progressive) and less aggressive disease. The arrows (↑↓) indicate whether the metabolite levels were increased or decreased in patients with very aggressive disease compared to patients with less aggressive disease.

Main class / Subclassification		Metabolite	*p*-value	*q*-value	Fold change, very aggressive versus less aggressive
Amino Acid					
Glycine, Serine and Threonine Metabolism	↓	Betaine	0.0141	0.2745	0.75
Glutamate Metabolism	↑	N-acetylglutamate	0.0113	0.2642	1.51
Lysine Metabolism	↓	2-aminoadipate	0.0099	0.2639	0.56
Tyrosine Metabolism	↑	Thyroxine	0.0185	0.3107	1.33
Tryptophan Metabolism	↑	Tryptophan betaine	0.0051	0.2185	6.96
Methionine, Cysteine, SAM and Taurine	↑	Cysteine s-sulfate	0.0093	0.2587	1.85
Metabolism	↓	Hypotaurine	0.0284	0.3801	0.32
Urea cycle; Arginine and Proline Metabolism	↓	Citrulline	0.0427	0.3952	0.70
	↑	Dimethylarginine (SDMA + ADMA)	0.0066	0.2253	1.69
Polyamine Metabolism	↑	Spermidine	0.0433	0.3952	9.72
Peptide					
Dipeptide	↓	Isoleucylglycine	0.0015	0.1292	0.52
	↓	Leucylalanine	0.0000	0.0022	0.10
	↓	Threonylphenylalanine	0.0371	0.3952	0.45
Fibrinogen Cleavage Peptide	↓	Fibrinopeptide A, des-ala(1)	0.0235	0.3591	0.27
Acetylated Peptides	↓	Phenylacetylglycine	0.0070	0.2253	0.15
Carbohydrate					
Pentose Metabolism	↑	Ribonate	0.0043	0.2022	1.40
Energy					
TCA Cycle	↓	Aconitate [cis or trans]	0.0351	0.3952	0.35
	↑	Alpha-ketoglutarate	0.0369	0.3952	1.56
	↑	Succinate	0.0322	0.3904	1.45
	↑	2-methylcitrate/homocitrate	0.0128	0.2642	1.29
Lipid					
Medium Chain Fatty Acid	↓	Caprate (10:0)	0.0172	0.3107	0.57
Polyunsaturated Fatty Acid (n3 and n6)	↑	Docosatrienoate (22:3n3)	0.0274	0.3798	1.79
	↑	Docosatrienoate (22:3n6)	0.0278	0.3798	2.61
Fatty Acid, Dicarboxylate	↑	3-methylglutarate/2-methylglutarate	0.0402	0.3952	1.88

Fatty Acid Metabolism(Acyl Carnitine)	↓	Docosahexaenoylcarnitine (C22:6)	0.0065	0.2253	0.31
Fatty Acid Metabolism (Acyl Choline)	↑	Arachidonoylcholine	0.0193	0.3107	1.38
Phosphatidylcholine (PC)	↑	1,2-dipalmitoyl-GPC (16:0/16:0)	0.0377	0.3952	1.36
	↑	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)	0.0404	0.3952	1.60
	↑	1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	0.0077	0.2253	1.43
	↑	1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)	0.0326	0.3904	1.18
Phosphatidylethanolamine (PE)	↑	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	0.0074	0.2253	2.09
	↑	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)	0.0205	0.3211	2.22
	↑	1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)	0.0033	0.1752	1.80
	↑	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.0010	0.1088	2.61
	↑	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)	0.0076	0.2253	1.95
	↑	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	0.0108	0.2642	1.86
	↑	1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)	0.0004	0.0568	1.60
Phosphatidylinositol (PI)	↑	1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	0.0191	0.3107	1.81
	↑	1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	0.0434	0.3952	1.50
Lysophospholipid	↑	1-oleoyl-GPC (18:1)	0.0328	0.3904	1.34
	↑	1-palmitoyl-GPE (16:0)	0.0125	0.2642	1.39
	↑	1-stearoyl-GPE (18:0)	0.0115	0.2642	1.49
	↑	2-stearoyl-GPE (18:0)	0.0385	0.3952	1.47
	↑	1-stearoyl-GPG (18:0)	0.0337	0.3941	1.71
	↑	1-arachidonoyl-GPI (20:4)	0.0463	0.3952	1.47
Plasmalogen	↑	1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P16:0/20:4)	0.0189	0.3107	1.52
Glycerolipid Metabolism	↑	Glycerophosphoglycerol	0.0003	0.0568	1.46
Monoacylglycerol	↑	2-palmitoylglycerol (16:0)	0.0422	0.3952	2.18
Diacylglycerol	↑	Palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]	0.0426	0.3952	1.63
	↑	Palmitoleoyl-linoleoyl-glycerol (16:1/18:2) [1]	0.0498	0.3952	1.61
	↑	Palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]	0.0439	0.3952	2.12
	↑	Oleoyl-oleoyl-glycerol (18:1/18:1) [1]	0.0387	0.3952	2.06
	↑	Oleoyl-oleoyl-glycerol (18:1/18:1) [2]	0.0426	0.3952	2.25
	↑	Oleoyl-linoleoyl-glycerol (18:1/18:2) [1]	0.0067	0.2253	1.83
	↑	Oleoyl-linoleoyl-glycerol (18:1/18:2) [2]	0.0193	0.3107	1.84
	↑	Linoleoyl-linoleoyl-glycerol (18:2/18:2) [1]	0.0016	0.1292	1.78
	↑	Oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]	0.0497	0.3952	2.01
Sphingolipid Metabolism	↑	Sphinganine	0.0008	0.0987	2.37

	↑	Sphingosine	0.0126	0.2642	2.08
	↓	Sphingomyelin (d18:1/22:2, d18:2/22:1, d16:1/24:2)	0.0489	0.3952	0.71
Ceramides	↑	N-palmitoyl-sphingosine (d18:1/16:0)	0.0028	0.1752	1.75
Sterol	↑	Cholesterol	0.0044	0.2022	1.46
Androgenic Steroids	↓	5alpha-androstan-3alpha,17beta-diol Monosulfate	0.0494	0.3952	0.71
Nucleotide					
Purine Metabolism, (Hypo)Xanthine/Inosine containing	↑	Xanthine	0.0264	0.3798	3.32
	↑	Xanthosine	0.0344	0.3949	3.58
Purine Metabolism, Adenine containing	↓	Adenosine 3',5'-cyclic monophosphate (cAMP)	0.0001	0.0267	0.45
	↑	N6-succinyladenosine	0.0444	0.3952	1.86
Purine Metabolism, Guanine containing	↑	7-methylguanine	0.0131	0.2642	1.74
Pyrimidine Metabolism, Orotate containing	↓	Dihydroorotate	0.0115	0.2642	0.19
	↑	Orotidine	0.0253	0.3777	2.20
Pyrimidine Metabolism, Uracil containing	↑	N-acetyl-beta-alanine	0.0481	0.3952	1.47
Pyrimidine Metabolism, Cytidine containing	↑	2'-O-methylcytidine	0.0033	0.1752	1.54
Cofactors and Vitamins					
Nicotinate and Nicotinamide Metabolism	↑	Nicotinamide	0.0020	0.1425	7.85
Tocopherol Metabolism	↓	Alpha-CEHC	0.0473	0.3952	0.97
Hemoglobin and Porphyrin Metabolism	↓	Bilirubin (E,E)	0.0275	0.3798	0.48
Xenobiotics					
Tobacco Metabolite	↓	Hydroxycotinine	0.0305	0.3904	0.21
Food Component/Plant	↑	Gluconate	0.0296	0.3883	1.27
	↓	Dihydroferulic acid	0.0163	0.3075	0.18
	↓	Umbelliferone sulfate	0.0490	0.3952	0.40
Chemical	↓	Methylnaphthyl sulfate	0.0321	0.3904	0.28

Table S3. Significantly altered metabolites after seven days of valproic acid monotherapy; a comparison of pretreatment samples versus samples collected during treatment for patients classified as responders to antileukemic therapy. The comparison is based on the results for 5 responders from the study by Fredly *et al.* (PMID 23915396) [2]. Seventy-eight metabolites were significantly altered after valproic acid therapy; 48 were significantly increased and 30 decreased. The arrows (↑↓) indicate whether the metabolite was increased or decreased by the treatment. Metabolites with $q<0.05$ are marked with yellow.

Main class / Subclassifications		Metabolite
Amino Acid (n=17)		
Glutamate Metabolism	↑	Glutamate
Lysine Metabolism	↓	N6,N6,N6-trimethyllysine
	↑	2-aminoadipate
Phenylalanine Metabolism	↑	Phenylacetate
Tyrosine Metabolism	↑	4-hydroxycinnamate sulfate
	↑	Catechol glucuronide
Tryptophan Metabolism	↓	Tryptophan
	↓	Kynurenine
	↓	Indolelactate
	↓	Indoleacetylglutamine
	↓	5-bromotryptophan
Leucine, Isoleucine and Valine Metabolism	↑	Isovalerylcarnitine (C5)
	↑	Beta-hydroxyisovalerate
	↓	3-methylglutarylcarnitine (2)
	↑	Isobutyrylcarnitine (C4)
Methionine, Cysteine, SAM and Taurine Metabolism	↑	Cysteine sulfinic acid
Urea cycle; Arginine and Proline Metabolism	↑	Homocitrulline
Peptide (n=3)		
Dipeptide	↑	Glycylvaline
Acetylated Peptides	↑	Phenylacetylcarnitine
	↓	4-hydroxyphenylacetylglutamine
Carbohydrate (n=3)		
Disaccharides and Oligosaccharides	↑	Lactose
Fructose, Mannose and Galactose Metabolism	↓	Mannose
Aminosugar Metabolism	↓	N-acetylneuraminate
Lipid (n=32)		
Medium Chain Fatty Acid	↓	10-undecenoate (11:1n1)
	↓	5-dodecenoate (12:1n7)
Polyunsaturated Fatty Acid (n3 and n6)	↓	Adrenate (22:4n6)
Fatty Acid, Dicarboxylate	↑	Adipate (C6-DC)
	↑	Suberate (C8-DC)
	↑	Sebacate (C10-DC)
	↓	3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-Cmpfp)
Fatty Acid, Amino	↓	2-aminooctanoate
Fatty Acid Metabolism (also BCAA Metabolism)	↑	Propionylcarnitine (C3)
Fatty Acid Metabolism(Acyl Glycine)	↑	Hexanoylglycine
Fatty Acid Metabolism(Acyl Carnitine)	↑	3-hydroxybutyrylcarnitine (2)
	↑	Suberoylcarnitine (C8-DC)
	↑	Adipoylcarnitine (C6-DC)
Fatty Acid, Monohydroxy	↓	2-hydroxynervonate
	↑	3-hydroxyhexanoate

	↓	3-hydroxydecanoate
	↓	3-hydroxyoleate
	↓	3-hydroxylaurate
	↑	5-hydroxyhexanoate
	↑	5-hydroxyvalproate
Phosphatidylethanolamine (PE)	↑	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)
	↑	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)
Phosphatidylinositol (PI)	↑	1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)
Diacylglycerol	↑	Diacylglycerol (14:0/18:1, 16:0/16:1) [1]
	↑	Diacylglycerol (14:0/18:1, 16:0/16:1) [2]
	↑	Palmitoyl-palmitoyl-glycerol (16:0/16:0) [2]
	↑	Oleoyl-oleoyl-glycerol (18:1/18:1) [2]
	↓	Linoleoyl-linolenoyl-glycerol (18:2/18:3) [1]
Sterol	↑	Beta-sitosterol
Androgenic Steroids	↓	Dehydroisoandrosterone sulfate (DHEA-S)
	↓	Androsterone glucuronide
Primary Bile Acid Metabolism	↑	Tauro-beta-muricholate
Nucleotide (n=2)		
Pyrimidine Metabolism, Uracil containing	↓	2'-deoxyuridine
Pyrimidine Metabolism, Thymine containing	↑	3-aminoisobutyrate
Cofactors and Vitamins (n=3)		
Ascorbate and Aldarate Metabolism	↓	Oxalate (ethanedioate)
Tocopherol Metabolism	↑	Gamma-CEHC
Hemoglobin and Porphyrin Metabolism	↓	Bilirubin (E,E)
Xenobiotics (n=16)		
Benzoate Metabolism	↑	3-(3-hydroxyphenyl)propionate sulfate
	↑	3-(3-hydroxyphenyl)propionate
Food Component/Plant	↑	Cinnamoylglycine
	↑	Dihydroferulic acid
	↑	Ferulic acid 4-sulfate
	↑	Glycyrrhetinate
	↓	Naringenin 7-glucuronide
	↓	Isoeugenol sulfate
Drug - Cardiovascular	↓	4-hydroxycoumarin
	↓	Candesartan
Drug - Neurological	↑	3-hydroxyvalproate
	↑	2-propyl-2-pentenoate (2-ene-valproate)
Chemical	↑	3-acetylphenol sulfate
	↑	HEPES
	↑	1,2,3-benzenetriol sulfate (2)
	↑	2-methoxyresorcinol sulfate
Partially Characterized Molecules (n=2)		
Partially Characterized Molecules	↑	Glucuronide of C8H16O2 (1)
	↓	Glucuronide of C14H22O4 (2)

Table S4. Significantly altered metabolites after seven days of valproic acid therapy; a comparison of pretreatment samples versus samples collected during treatment for patients classified as non-responders to antileukemic treatment. The comparison is based on the results for 5 non-responders from the study by Fredly *et al.* (23915396). A total of 105 metabolites were significantly altered after valproic acid therapy; 52 were significantly increased and 53 were decreased. The arrows (↑↓) indicate whether the metabolite was increased or decreased by the treatment. Metabolites with $q<0.05$ are marked with yellow.

Main class / Subclassifications		Metabolite
Amino Acid (n=21)		
Glycine, Serine and Threonine Metabolism	↑	Dimethylglycine
Alanine and Aspartate Metabolism	↑	N-acetylalanine
Glutamate Metabolism	↑	Carboxyethyl-GABA
	↓	S-1-pyrroline-5-carboxylate
Histidine Metabolism	↑	N-acetylhistidine
Lysine Metabolism	↑	6-oxopiperidine-2-carboxylate
Phenylalanine Metabolism	↑	N-acetylphenylalanine
Tyrosine Metabolism	↑	N-acetyltyrosine
Tryptophan Metabolism	↓	Tryptophan
	↑	N-acetyltryptophan
	↑	Tryptophan betaine
	↓	Indoleacetate
	↓	Indoleacetylglutamine
	↓	5-bromotryptophan
Leucine, Isoleucine and Valine Metabolism	↑	Beta-hydroxyisovalerate
	↓	3-methylglutarylcarnitine (2)
	↑	Ethylmalonate
	↑	Isobutyrylcarnitine (C4)
	↑	2,3-dihydroxy-2-methylbutyrate
Methionine, Cysteine, SAM and Taurine Metabolism	↑	N-acetylmethionine
Urea cycle; Arginine and Proline Metabolism	↑	Trans-4-hydroxyproline
Peptide (n=2)		
Acetylated Peptides	↓	4-hydroxyphenylacetylglutamine
	↓	Phenylacetylglycine
Carbohydrate (n=4)		
Pentose Metabolism	↑	Ribitol
	↑	Xylose
	↑	Arabitol/xylitol
Fructose, Mannose and Galactose Metabolism	↓	Galactonate
Energy (n=1)		
TCA Cycle	↓	Succinylcarnitine (C4-DC)
Lipid (n=49)		
Medium Chain Fatty Acid	↓	Caprate (10:0)
	↓	10-undecenoate (11:1n1)
Fatty Acid, Dicarboxylate	↑	Adipate (C6-DC)
	↑	Pimelate (C7-DC)
	↑	Suberate (C8-DC)
	↓	Dodecanedioate (C12-DC)

	↓	Tetradecanedioate (C14-DC)
	↓	Hexadecenedioate (C16:1-DC)
	↓	Octadecanedioate (C18-DC)
	↓	Octadecenedioate (C18:1-DC)
	↓	Eicosanodioate (C20-DC)
	↓	3-carboxy-4-methyl-5-pentyl-2-furanpropionate (3-Cmpfp)
Fatty Acid Metabolism(Acyl Glycine)	↑	Hexanoylglycine
Fatty Acid Metabolism(Acyl Carnitine)	↑	Hexanoylcarnitine (C6)
	↑	5-dodecenoylcarnitine (C12:1)
	↑	Cis-4-decenoylcarnitine (C10:1)
	↑	Laurylcarnitine (C12)
	↑	Myristoylcarnitine (C14)
	↑	Myristoleoylcarnitine (C14:1)
	↑	Suberoylcarnitine (C8-DC)
	↑	Adipoylcarnitine (C6-DC)
	↑	Pimeloylcarnitine/3-methyladipoylcarnitine (C7-DC)
Fatty Acid, Monohydroxy	↓	2-hydroxyoctanoate
	↓	2-hydroxydecanoate
	↑	3-hydroxyhexanoate
	↑	3-hydroxysebacate
	↑	5-hydroxyvalproate
Endocannabinoid	↓	N-stearoylserine
Phosphatidylcholine (PC)	↓	1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)
Phosphatidylinositol (PI)	↑	1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)
	↑	1-stearoyl-2-linoleoyl-GPI (18:0/18:2)
Lysophospholipid	↓	1-palmitoyl-GPC (16:0)
	↓	1-palmitoleoyl-GPC (16:1)
	↓	1-stearoyl-GPC (18:0)
Diacylglycerol	↓	Diacylglycerol (14:0/18:1, 16:0/16:1) [1]
	↓	Diacylglycerol (14:0/18:1, 16:0/16:1) [2]
	↓	Palmitoyl-oleoyl-glycerol (16:0/18:1) [2]
	↓	Palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]
Sphingolipid Metabolism	↓	Sphinganine-1-phosphate
Progestin Steroids	↓	5alpha-pregnan-3beta,20alpha-diol monosulfate (2)
	↓	Pregnanediol-3-glucuronide
Corticosteroids	↑	Cortisone
Androgenic Steroids	↑	Androstenediol (3beta,17beta) disulfate (1)
	↑	Androstenediol (3beta,17beta) disulfate (2)
	↑	Andro steroid monosulfate C19H28O6S (1)
Primary Bile Acid Metabolism	↓	Glycochenodeoxycholate glucuronide (1)
Secondary Bile Acid Metabolism	↓	Isoursodeoxycholate
	↓	7-ketolithocholate
	↓	3b-hydroxy-5-cholenoic acid
Nucleotide (n=6)		
Purine Metabolism, (Hypo)Xanthine/Inosine containing	↑	N1-methylinosine
Purine Metabolism, Adenine containing	↑	N6-carbamoylthreonyladenosine

Purine Metabolism, Guanine containing	↑	7-methylguanine
Pyrimidine Metabolism, Uracil containing	↑	3-ureidopropionate
Pyrimidine Metabolism, Cytidine containing	↑	Cytidine
Pyrimidine Metabolism, Thymine containing	↑	3-aminoisobutyrate
Cofactors and Vitamins (n=4)		
Nicotinate and Nicotinamide Metabolism	↑	N1-Methyl-2-pyridone-5-carboxamide
Pantothenate and CoA Metabolism	↓	Pantothenate
Tocopherol Metabolism	↑	Gamma-CEHC
Hemoglobin and Porphyrin Metabolism	↓	Bilirubin (E,E)
Xenobiotics (n=15)		
Benzoate Metabolism	↓	4-hydroxyhippurate
	↓	Catechol sulfate
	↓	Guaiacol sulfate
	↓	4-ethylphenylsulfate
Xanthine Metabolism	↑	3-methylxanthine
Food Component/Plant	↓	Ferulylglycine (1)
	↓	Ferulylglycine (2)
	↓	Acesulfame
	↓	Thymol sulfate
	↓	4-allylphenol sulfate
Drug - Analgesics, Anesthetics	↓	Lidocaine
Drug - Cardiovascular	↓	4-hydroxycoumarin
Drug - Neurological	↑	3-hydroxyvalproate
	↑	2-propyl-2-pentenoate (2-ene-valproate)
Chemical	↑	O-sulfo-L-tyrosine
Partially Characterized Molecules (n=3)		
Partially Characterized Molecules	↑	Glucuronide of C8H16O2 (1)
	↓	Glucuronide of C10H18O2 (7)
	↓	Glucuronide of C14H22O4 (2)

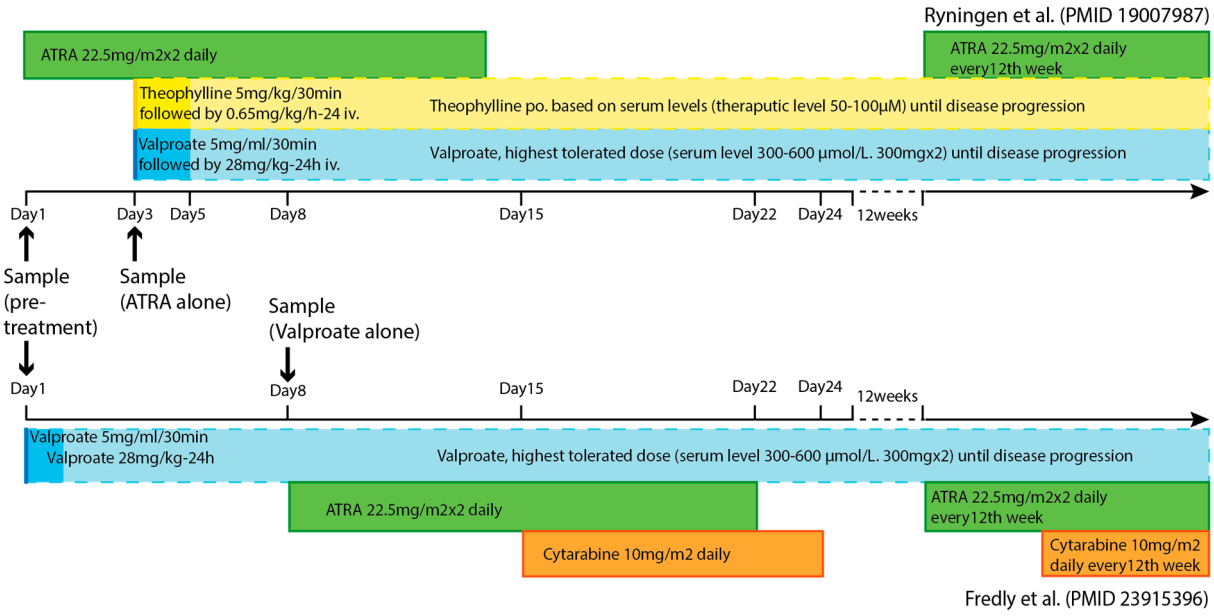


Figure S1. Timeline of treatment schedule for patients included in two clinical studies. In the first study shown at the top (Ryningen *et al.*), patients were give ATRA alone for two days before theophylline and valproic acid were given on day 3. Samples were collected pretherapy (day 1) and after 2-days of ATRA treatment. In the second study shown at the bottom (Fredly *et al.*), patients received valproic acid alone for 7 days before ATRA and then subsequently cytarabine were given. Samples were collected pretherapy (day 1) and after 7 days of valproic acid therapy.

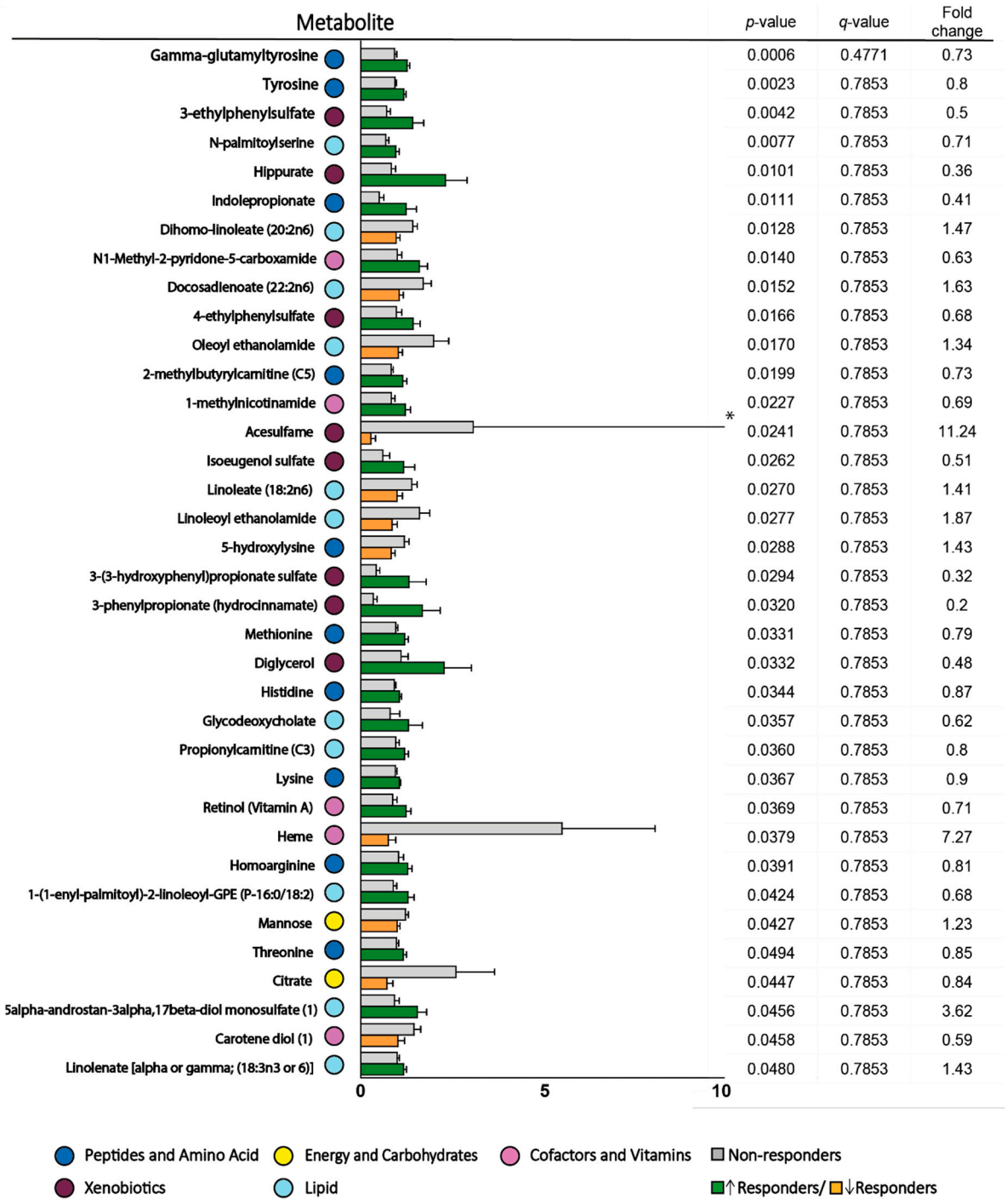


Figure S2. Identification and classification of metabolites in pretherapy serum samples that differed significantly between responders and non-responders to the antileukemic treatment of ATRA plus valproic acid. Thirty-six metabolites differed significantly between responders and non-responders (p<0.05, Welch´s two sample t-test). The p-values, q-values and mean fold change values for each metabolite are listed to the right in the figure (ranked by p-value), and a fold change >1 indicates that the levels were increased in responders compared with non-responders. Metabolite levels for non-responders are shown in grey, while increased levels in responders are shown in green (25/36 increased), and decreased levels in responders are shown in orange (11/36 decreased). Color codes for classification of metabolites are explained at the bottom of the figure. Error bars show Standard deviation (SD). * Acesulfame SD 8.963.

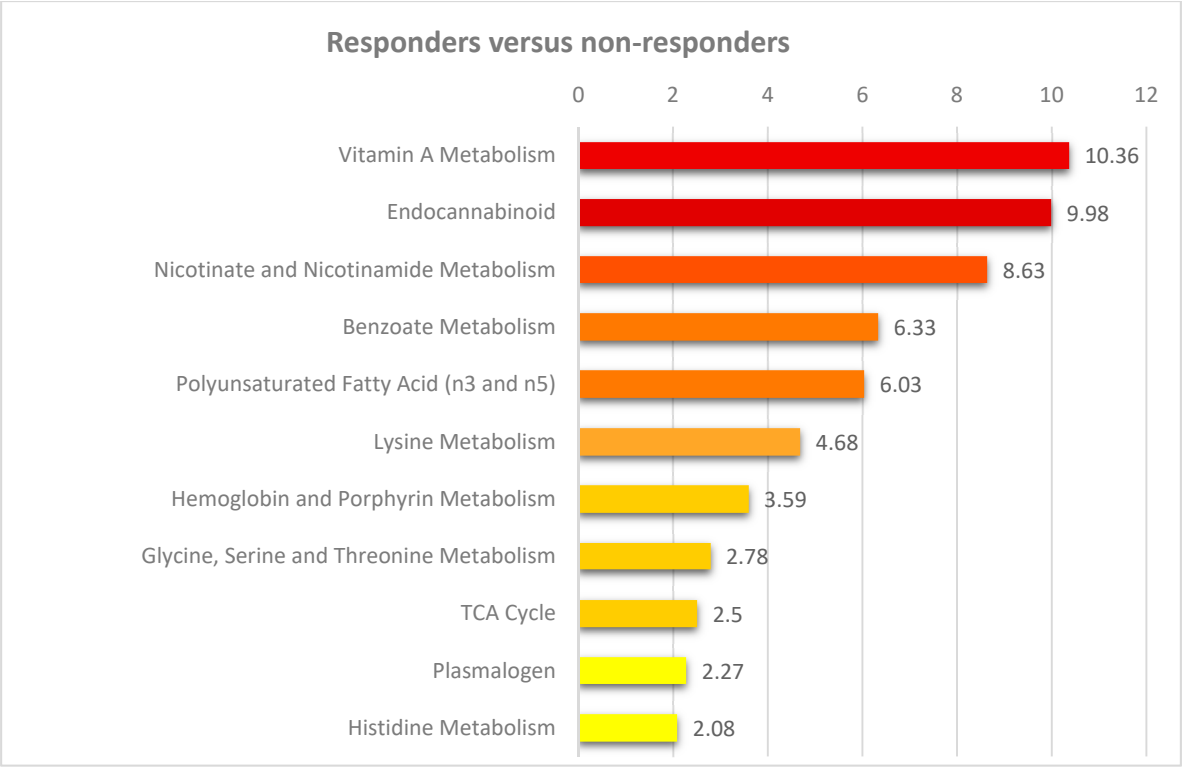


Figure S3. Pathway enrichment analysis based on pretreatment levels of metabolites that differed between responders and non-responders to antileukemic treatment based on ATRA and valproic acid. The analysis is used for visualization and biological interpretation of the metabolomics data and was based on significantly altered metabolites ($p<0.05$). Only signaling pathways with an enrichment value greater than two and at least two metabolites within each pathway are shown. The most significant pathway is shown in red and less significant pathways in light yellow.

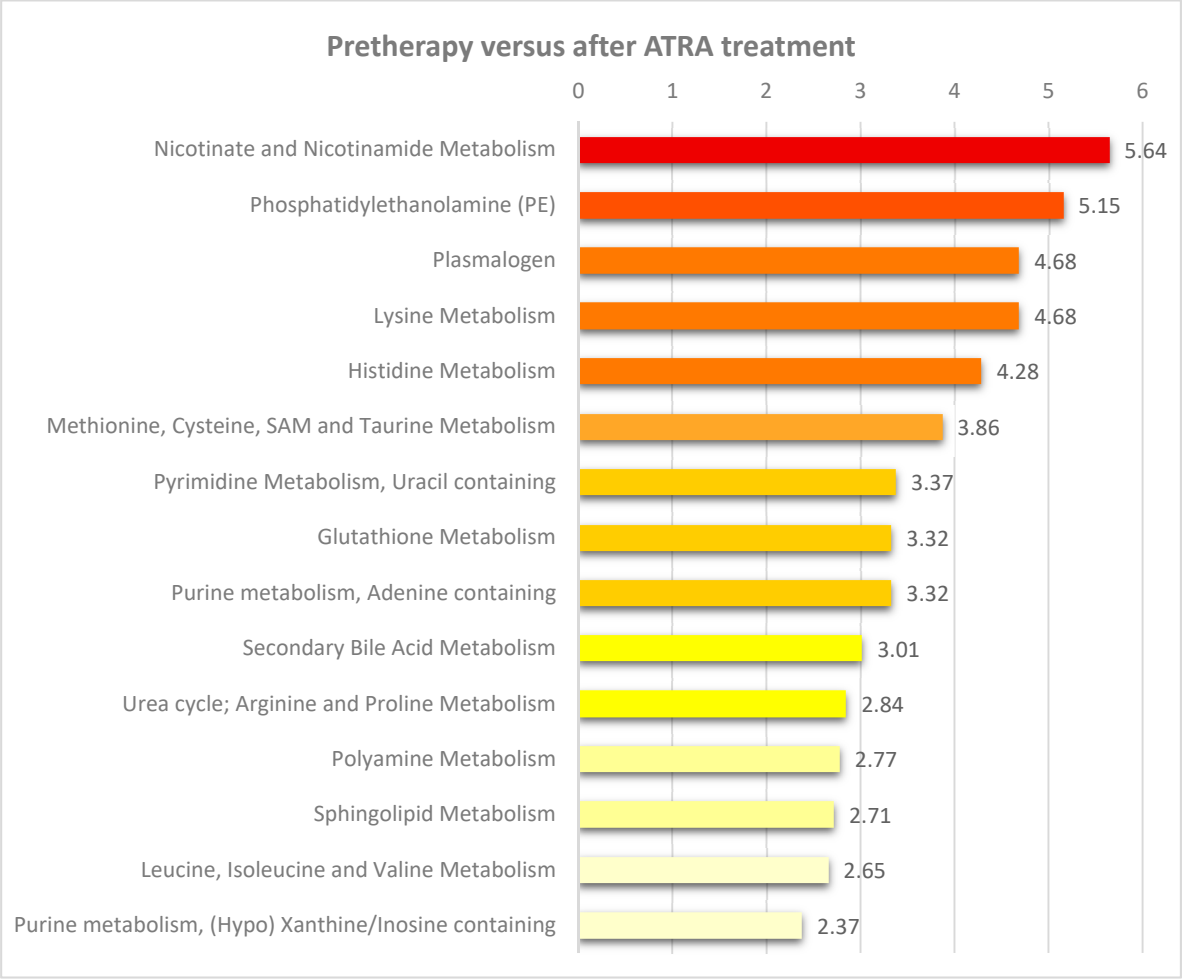
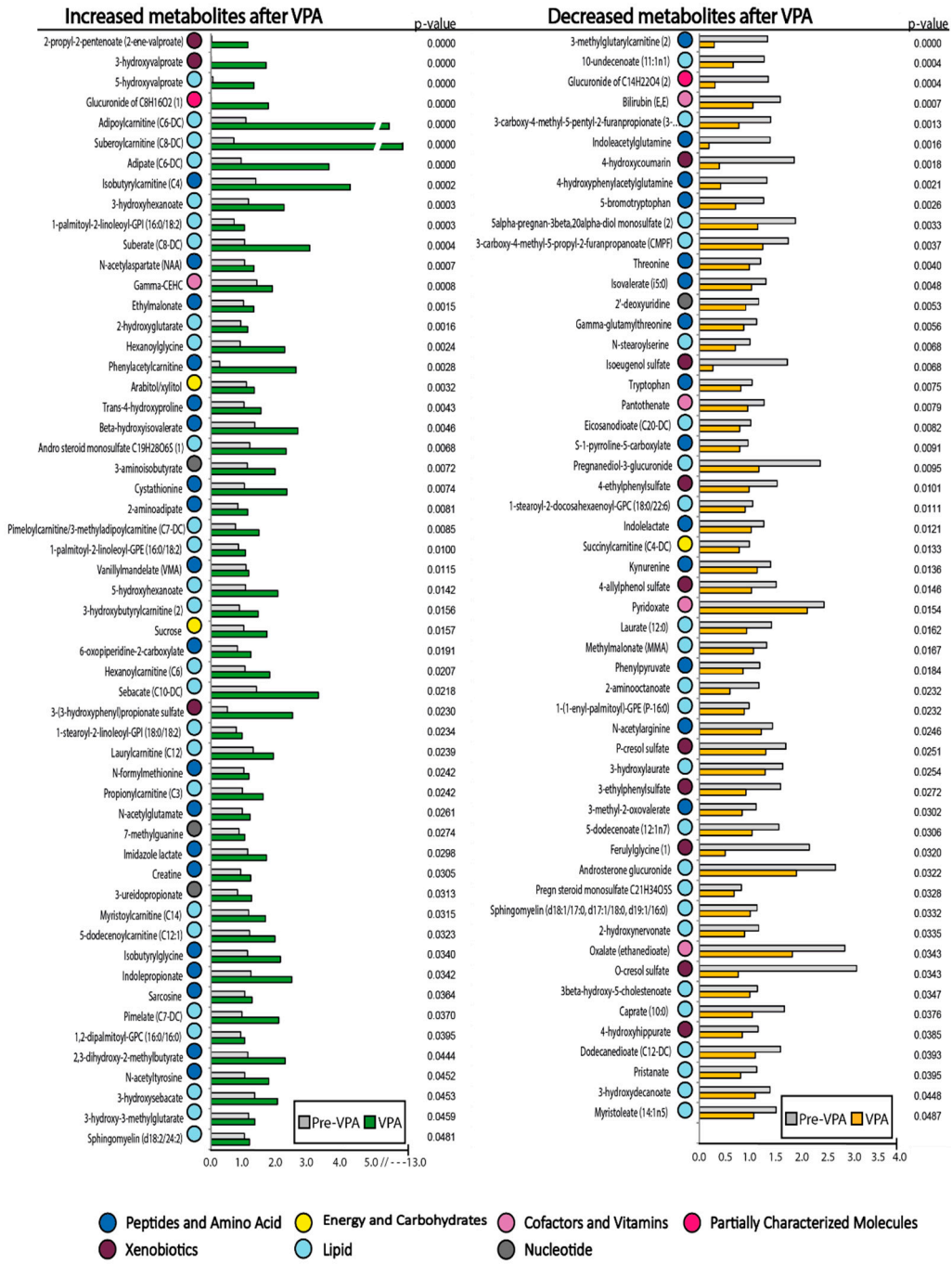
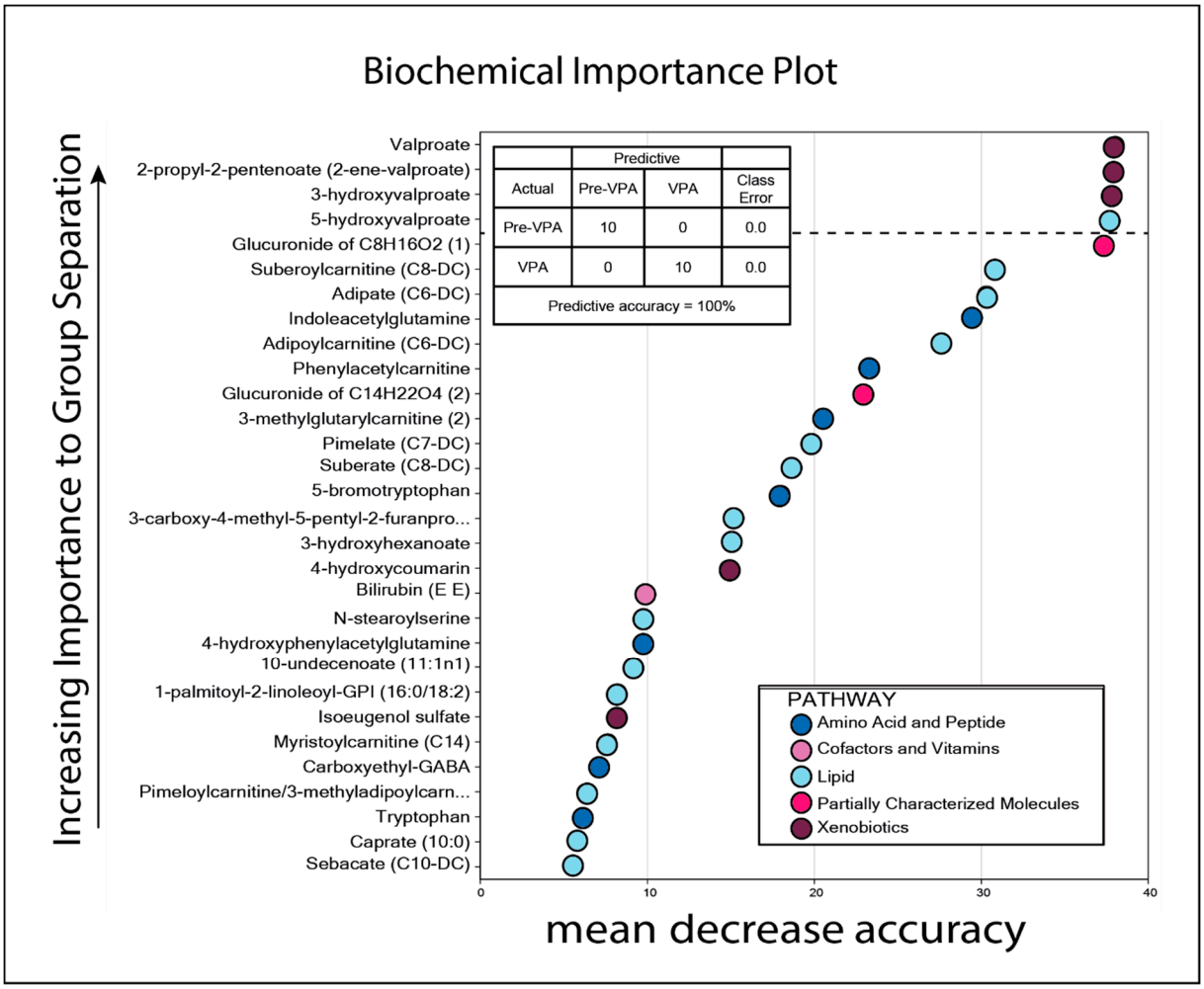


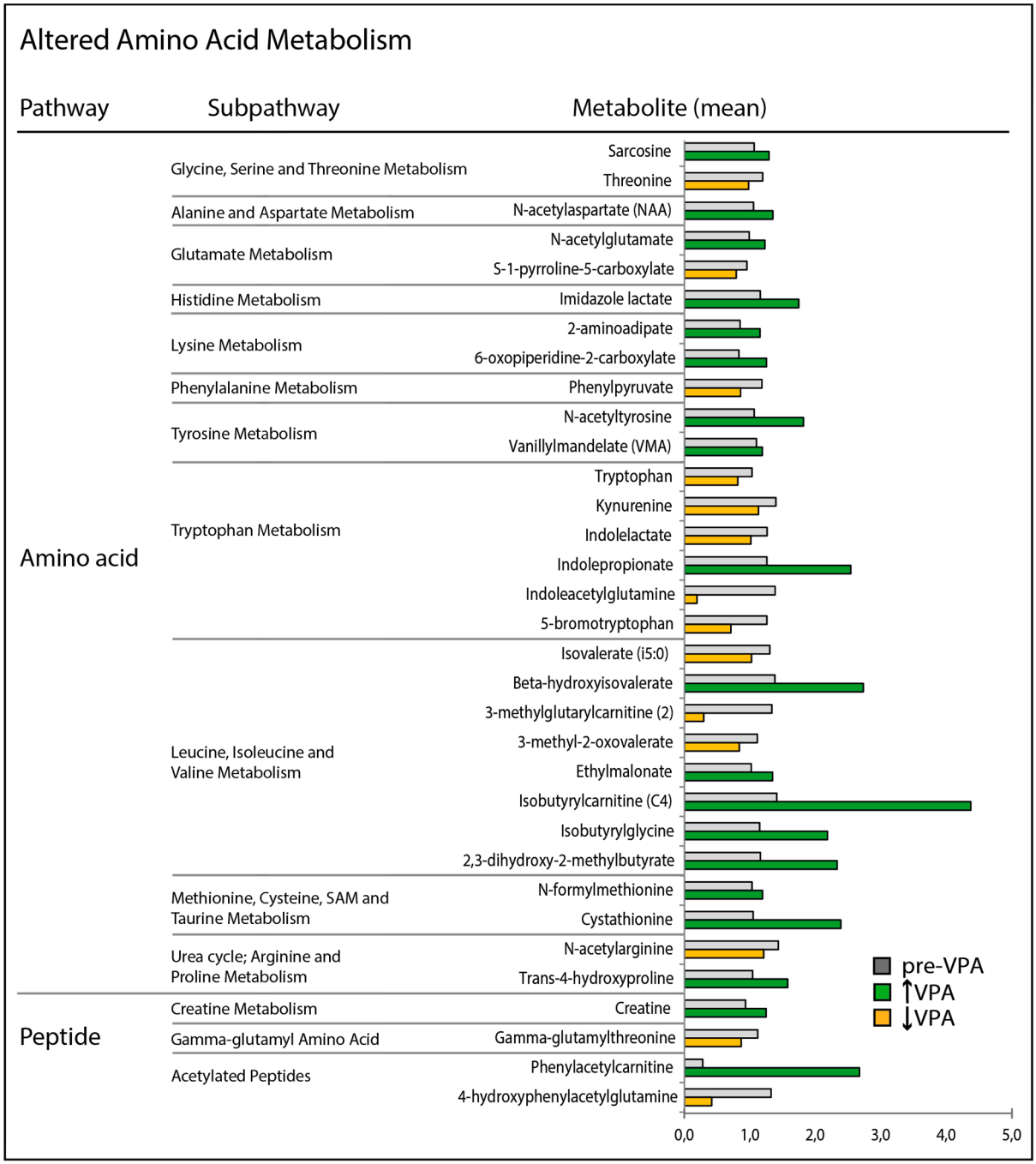
Figure S4. Pathway enrichment analysis based on metabolite levels that differed in patients pretherapy compared to during ATRA treatment for responders and non-responders to antileukemic treatment. The analysis was based on significantly altered metabolites ($p<0.05$), and only signaling pathways with an enrichment value greater than two and at least two metabolites within each pathway are shown in the figure. The most significant pathway is shown in red and less significant pathways in light yellow.



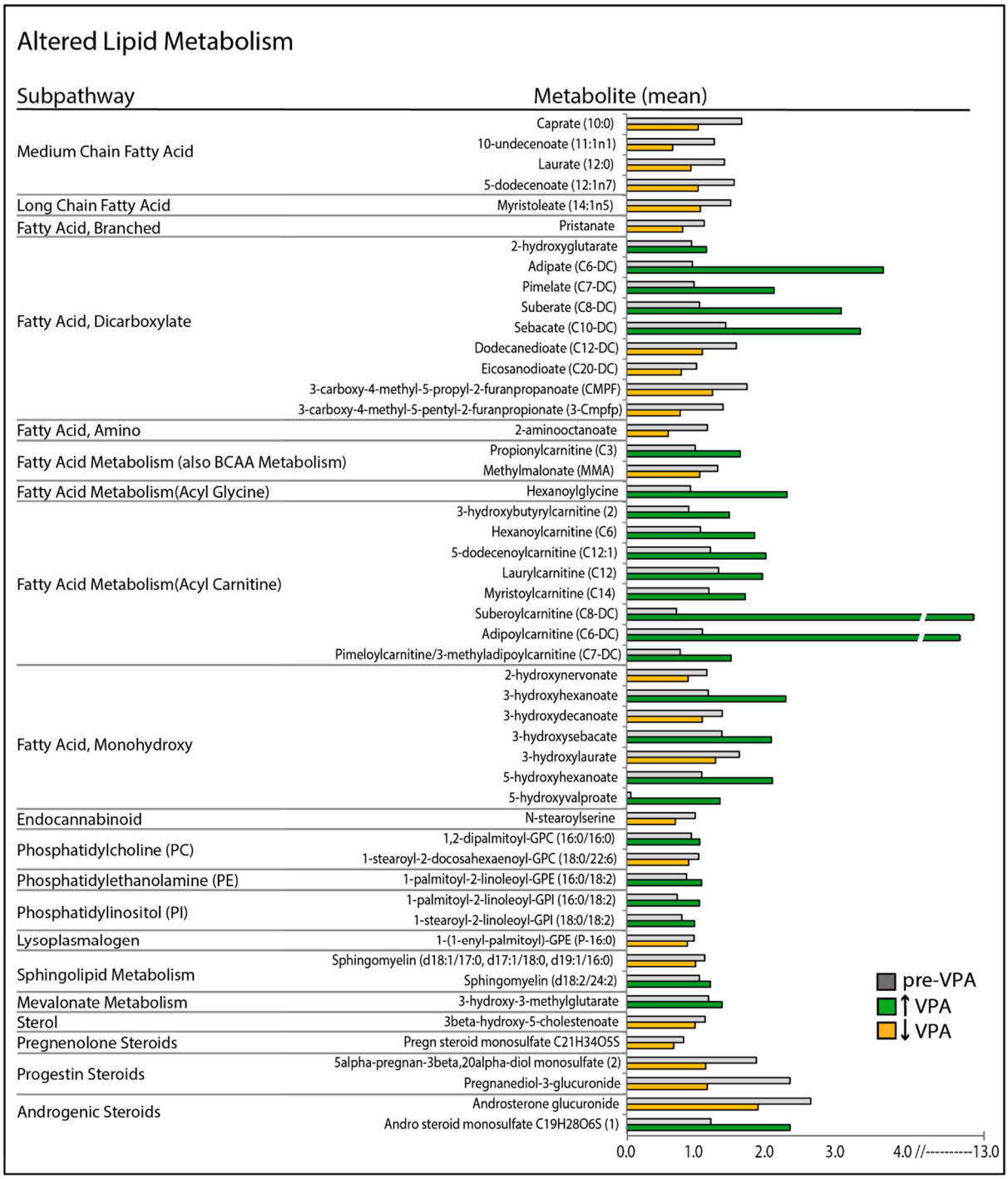
Supplementary Figure 5. The effect of 7-day valproic acid (VPA) monotherapy on the serum metabolomic profiles of AML patients. A total of 109 metabolites were significantly altered after VPA treatment ($p < 0.05$). The *p*-values for each individual metabolite are listed to the right, ranked according to *p*-values. Pretherapy systemic levels of metabolites are presented in light grey, increased levels during VPA treatment are presented in green and decreased levels in yellow. Color codes for classification of individual metabolites are explained at the bottom of the figure.



Supplementary Figure 6. The effect of valproic acid monotherapy for seven days on the serum metabolomics profiles for 10 patients (5 responders and 5 non-responders; patients included in the study described in PMID 23915396, valproic acid metabolites included in the study). The random forest analysis was based on the identification of all 886 metabolites in pretherapy samples and samples collected after seven days of treatment. The analysis showed a predictive accuracy of 100% (insert table). The importance plot shows the metabolites listed after their importance for separation of the two sets of samples. The figure shows the top-30 ranked metabolites. Color codes for classification of metabolites are shown to the lower right.



Supplementary Figure 7. The effect of 7-day valproic acid monotherapy on serum metabolomic profiles; significantly altered amino acid and peptide metabolites when comparing samples derived from 10 patients (5 responders and 5 non-responders; all patients included in the study by Fredly et al. PMID 23915396). The pretreatment samples are presented in grey; metabolites increased during valproic acid therapy are presented in green and decreased levels after valproic acid therapy are presented in yellow.



Supplementary Figure 8. The effect of 7-day valproic acid monotherapy on serum metabolomic profiles; significantly altered lipid metabolites when comparing samples derived from 10 patients (5 responders and 5 non-responders; all patients included in the study by Fredly et al. PMID 23915396). The pretreatment samples are presented in grey; metabolites increased during valproic acid therapy are presented in green and decreased levels after valproic acid treatment are presented in yellow.