

SUPPLEMENT

Identification of genes associated with chemotherapy cross-resistance and treatment response in childhood acute lymphoblastic leukemia

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Table of content

Supplemental Table 1: Characteristics of patients within each group classified as cross-resistant and VCR-ASP discordant resistant ALL.	3
Supplemental Table 2: False discovery rate.	4
Supplemental Table 3: Cross-validation using gene expression scores for cross-resistance and VCR-ASP discordant resistance.	4
Supplemental Table 4: Transcription factor motifs over-represented in the set of genes over-expressed in cross-resistant versus cross-sensitive ALL.	4

Supplemental Table 5: Genes discriminating VCR-ASP discordant resistance in pediatric B-lineage ALL.....	6
Supplemental Table 6: Transcription factor binding sites over-represented in the sub-cluster of genes over-expressed in VCR-ASP discordant resistant ALL.....	10
Supplemental Table 7: Overlap of genes discriminating multiple-drug resistance and genes discriminating ALL subtypes	11
Supplemental Table 8: Overlap in genes discriminating multiple-drug cross-resistance and genes discriminating PRD-, VCR-, ASP- and DNR single-drug resistance.	11
Supplemental Table 9: Overlap in genes discriminating VCR-ASP discordant resistance and genes discriminating VCR- and ASP single-drug resistance.....	12
Supplemental Table 10: Proportional-hazards regression analysis.....	14
Supplemental Table 11: The multiple-drug cross-resistance gene expression score is significantly predictive of resistance of other antileukemic agents.....	15
Supplemental Figure 1: Correlation of in vitro sensitivity of primary ALL cells.	16
Supplemental Figure 2: Distribution of cross-resistance (CR-scores) and VCR-ASP discordant resistance scores (VCR-ASP-scores) in B-lineage ALL patients.	17
Supplemental Figure 3: Distribution of LC ₅₀ values for each drug tested in the CR-group and VCR-ASP group analyzed for gene expression.	18
Supplemental Figure 4: Principal component analysis plot of genes discriminating cross-resistance and VCR-ASP discordant resistance.	19
Supplemental Figure 5: Gene Ontology (GO) of genes discriminating cross-resistance and genes discriminating VCR-ASP discordant resistance.....	20
Supplemental Figure 6: Hierarchical clustering of ALL patients using genes discriminating cross-resistance and VCR-ASP discordant resistance.	21
Supplemental Figure 7: Hierarchical clustering using ALL subtype adjusted genes that discriminate VCR-ASP discordant resistance.....	22
Supplemental Figure 8: Treatment outcome among VCR-ASP discordant resistant B-lineage ALL patients using the adjusted gene expression score.....	23
Supplemental Figure 9: VCR-ASP discordant resistance adds predictive value related to relapse-free survival to single drug resistance of PRD-VCR-ASP-DNR in patients with ALL.....	23

Supplemental Table 1: Characteristics of patients within each group classified as cross-resistant and VCR-ASP discordant resistant ALL.

The ALL subtypes of the 129 patients for whom gene expression was performed included 40 with the *TEL-AML1* gene fusion t(12;21), 33 hyperdiploid (>50 chromosomes), six with the *E2A-PBX1* gene fusion t(1;19), five with the *BCR-ABL* gene fusion t(9;22) and three with the *MLL-AF4* gene fusion t(4;11). The remaining 40 B-lineage ALLs were negative for all five of these genetic subtypes. White blood cell count at diagnosis (WBC), age at diagnosis and sex in patients defined as cross-resistant (n=29) or cross-sensitive (n=38) by the CR-score. Patients with VCR-sensitive plus ASP-resistant ALL (VCR-S+ASP-R, n=42) or VCR-resistant ALL plus ASP-sensitive (VCR-R+ASP-S, n=34), as defined by the VCR-ASP discordant resistance phenotype. *TEL-AML1* translocation and hyperdiploidy showed a trend of higher prevalence in younger children, but was not statistically significant in our population (P=0.1074, ANOVA). Sixteen of 34 (47%) ALLs that were VCR-resistant plus ASP-sensitive compared to only three of 42 (7%) that were VCR-sensitive plus ASP-resistant, had the t(12;21) translocation yielding the *TEL-AML1* gene fusion (P=0.0001, Fisher's exact test). Fifteen of 34 (44%) ALLs that were VCR-resistant plus ASP-sensitive, compared to only four of 42 (10%) that were VCR-sensitive plus ASP-resistant, were hyperdiploid (P=0.001, Fisher's exact test). All 19 *TEL-AML1* positive and 19 hyperdiploid ALLs grouped together in one branch in the hierarchical clustering using the 200 probe sets discriminating VCR-ASP-discordant resistance (Supplemental Figure 6A).

	Cross-sensitive	Cross-resistant	P-value*	VCR-R+ASP-S	VCR-S+ASP-R	P-value*
Sex						
Female	15	12	1.00	16	19	1.00
Male	23	17		18	23	
Age						
<10 years	34	16	0.002	27	28	0.30
>10 years	4	13		7	14	
WBC						
<50/nL	24	20	0.80	23	27	0.81
>50/nL	14	9		11	15	
ALL subtype						
B-lineage other	9	18	0.006	3	25	<0.0001
<i>BCR-ABL</i>	2	1		0	5	
<i>E2A-PBX1</i>	5	0		0	3	
<i>MLL-AF4</i>	0	1		0	2	
<i>TEL-AML1</i>	11	6		16	3	
Hyperdiploid	11	3		15	4	

* Fisher's exact test

Supplemental Table 2: False discovery rate.

False Discovery rate (FDR) was determined for the cross-resistance group and the vincristine plus asparaginase (VCR-ASP) discordant resistant group. Corresponding number of probe sets and Spearman's rank correlation P-value (α) are shown. FDR is lower and the number of gene probe sets is higher if the PCA score is based on the in vitro sensitivity data of 441 patients compared to using the PCA score based on the in vitro sensitivity data of only 129 patients.

α	<u>In vitro sensitivity of 441 patients</u>				<u>In vitro sensitivity of 129 patients</u>			
	Cross-resistance		VCR-ASP discordant resistance		Cross-resistance		VCR-ASP discordant resistance	
	Probe Sets	FDR [%]	Probe sets	FDR [%]	Probe Sets	FDR [%]	Probe sets	FDR [%]
0.00001	12	1.0	118	0.1	0	0	0	0
0.0001	51	2.8	229	0.6	4	23	4	16
0.001	170	8.0	496	2.9	32	40	32	40
0.01	480	20.6	1240	11.7	258	51	274	49

Supplemental Table 3: Cross-validation using gene expression scores for cross-resistance and VCR-ASP discordant resistance.

Spearman's rank correlation (ρ) and P-value for top probe sets discriminating cross-resistance and vincristine-asparaginase (VCR-ASP) discordant resistance are listed. In each iteration, based on 10-fold cross-validation, genes were reselected (**A**) or were not reselected (**B**).

A

B

Probe sets	<u>Cross-resistance</u>		<u>VCR-ASP discordance</u>		<u>Cross-resistance</u>		<u>VCR-ASP discordance</u>	
	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
30	0.50	<0.000	0.45	<0.0001	0.74	<0.000	0.63	<0.0001
51	0.52	<0.000	0.45	<0.0001	0.73	<0.000	0.63	<0.0001
100	0.53	<0.000	0.46	<0.0001	0.72	<0.000	0.62	<0.0001
200	0.50	<0.000	0.53	<0.0001	0.70	<0.000	0.64	<0.0001

Supplemental Table 4: Transcription factor motifs over-represented in the set of genes over-expressed in cross-resistant versus cross-sensitive ALL.

The promoter region and the 5'UTR for specific sets of genes was used for Match™ to search the most recent TRANSFAC® Professional 8.1 database (vertebrates) (<http://www.biobase.de/>) for common regulatory elements. We determined statistical over-representation of the common motifs compared to "all" (~20,000) human promoter sequences (UCSC hg17; CLOVER) (Matys et al., 2003) and additionally we used the promoter sequences of the "other" gene set as background.

For the 10 genes that were commonly over-expressed in cross-resistant ALL (2 of 12 were excluded, *SLC4A1* because no reliable upstream region was found, and *MUC4* because the expression levels were relatively low), we identified 41 common transcription factor binding sites within 1.5kb 5' of the transcription start site, and of those eight were significantly over-represented in these 10 genes compared to all human promoter sequences and to the promoter sequences of 34 genes that were under-expressed in cross-resistant ALL ($P < 0.05$). For these eight factors, we found 14 probe sets on the array. Two of these factors that interact with one of the eight over-represented binding motifs are expressed at a significantly different level in cross-resistant versus cross-sensitive ALL cells (ELF2, lower; IRF1 higher). Different isoforms of ELF2 are known to act as either an inhibitor or a transactivator and physically interact with the AML1 (RUNX1) domain (Cho, J, *JBC*, 2004). IRF1 encodes interferon regulatory factor 1, a member of the interferon regulatory transcription factor (IRF) family. IRF1 functions as a transcription activator of interferons and interferon targets.

In addition, a transcription binding site for GATA3, a transcriptional activator which binds to the enhancer of the T-cell receptor alpha and delta genes, was identified in all 10 genes (although not over-represented compared to the human genome), and interestingly GATA3 was among the genes that were over-expressed in cross-resistant ALL (R/S ratio 3.38). The gene probe set, transcription factor name (TF), gene symbol (GS), Pearson correlation coefficient (R²) of the expression level with the CR-score, the R/S ratios comparing their expression in cross-resistant (R) versus cross-sensitive (S) ALL, and the raw score and P-values from the TRANSFAC analysis are listed.

Probe set ID	TF name	GS	R ²	R/S ratio	P-value R/S t-test	Raw score	P-value* from 'all'	P-value ⁺ from 'other'
203010_at	signal transducer and activator of transcription 5A	STAT5A	0.18	1.19	0.0743	2.5	0.032	0.017
212550_at	signal transducer and activator of transcription 5B	STAT5B	0.11	1.03	0.4314	2.15	0.046	0.022
212549_at	signal transducer and activator of transcription 5B	STAT5B	0.15	1.05	0.1746	2.15	0.046	0.022
205026_at	signal transducer and activator of transcription 5B	STAT5B	0.05	0.94	0.4379	2.15	0.046	0.022
203541_s_at	basic transcription element binding protein 1	BTEB1	0.04	1.16	0.3699	20.6	0.023	0.008
203542_s_at	basic transcription element binding protein 1	BTEB1	0.05	1.17	0.1100	20.6	0.023	0.008
203543_s_at	basic transcription element binding protein 1	BTEB1	-0.07	0.98	0.4952	20.6	0.023	0.008
209212_s_at	Kruppel-like factor 5	KLF5	0.13	1.21	0.0983	20.6	0.023	0.008
202308_at	sterol regulatory element binding transcr. factor 1	SREBF1	0.04	1.19	0.3490	5.41	0.011	0.014
202308_at	sterol regulatory element binding transcr. factor 1	SREBF1	0.04	1.19	0.3490	8.54	0.009	0.005
203822_s_at	E74-like factor 2 (ets domain transcription factor)	ELF2	-0.20	0.83	0.0036	7.46	0.01	0.011
210361_s_at	E74-like factor 2 (ets domain transcription factor)	ELF2	-0.11	0.93	0.0539	7.46	0.01	0.011
203275_at	interferon regulatory factor 2	IRF2	-0.05	1.17	0.3121	1.76	0.013	0.026
202531_at	interferon regulatory factor 1	IRF1	0.18	1.34	0.0150	4.98	0.015	0.009

* P-value from over-representation compared to "all" ~20,000 human promoter sequences

⁺ P-value from over-representation compared to promoter sequences of "other" 34 under-expressed genes

Supplemental Table 5: Genes discriminating VCR-ASP discordant resistance in pediatric B-lineage ALL.

Summary of the 200 gene probe sets discriminating VCR-ASP discordant resistance (139 genes, 13 cDNA clones) with corresponding Probe Set ID, gene name, gene symbol (GS), map location (ML) and ratio of expression in the unfavorable versus favorable group for VCR-ASP discordant resistance (Ratio). The ratio of expression calculated by the median log-transformed signal in VCR-sensitive plus ASP-resistant versus VCR-resistant plus ASP-sensitive (>1 is over-expressed in VCR-sensitive plus ASP-resistant ALL). A ratio of <1 represents an under-expressed probe sets in VCR-sensitive plus ASP-resistant patients. The 64 under-expressed probe sets in VCR-sensitive plus ASP-resistant samples had a median expression ratio of 0.55, and the 136 over-expressed probe sets had a median expression ratio of 1.32.

Probe Set ID	Gene Name	GS	ML	Ratio
207645_s_at	chromodomain helicase DNA binding protein 1-like	CHD1L	1q12	2.08
200772_x_at	prothymosin, alpha (gene sequence 28)	PTMA	2q35-q36	1.37
211921_x_at	prothymosin, alpha (gene sequence 28)	PTMA	2q35-q36	1.37
214097_at	ribosomal protein S21	RPS21	20q13.3	1.42
211940_x_at	H3 histone, family 3A	H3F3A	1q41	1.22
208755_x_at	H3 histone, family 3A	H3F3A	1q41	1.26
213828_x_at	H3 histone, family 3A	H3F3A	1q41	1.30
211445_x_at	FKSG17	FKSG17	8q22.3	1.37
200094_s_at	eukaryotic translation elongation factor 2	EEF2	19pter-q12	1.30
204102_s_at	eukaryotic translation elongation factor 2	EEF2	19pter-q12	1.25
211937_at	eukaryotic translation initiation factor 4B	EIF4B	12q13.13	1.50
201892_s_at	IMP (inosine monophosphate) dehydrogenase 2	IMPDH2	3p21.2	1.81
210501_x_at	eukaryotic translation initiation factor 3 subunit k	eIF3k	19q13.2	1.33
221494_x_at	eukaryotic translation initiation factor 3 subunit k	eIF3k	19q13.2	1.35
211623_s_at	fibrillarin	FBL	19q13.1	1.40
201216_at	chromosome 12 open reading frame 8	C12orf8	12q24.13	1.59
213356_x_at	heterogeneous nuclear ribonucleoprotein A1	HNRPA1	12q13.1	1.29
200016_x_at	heterogeneous nuclear ribonucleoprotein A1	HNRPA1	12q13.1	1.29
200029_at	ribosomal protein L19	RPL19	17q11.2-q12	1.27
200081_s_at	CDNA clone IMAGE:6301163	unknown4	4q21.3	1.28
200651_at	guanine nucleotide binding protein (G protein)	GNB2L1	5q35.3	1.42
200936_at	ribosomal protein L8	RPL8	8q24.3	1.35
213414_s_at	ribosomal protein S19	RPS19	19q13.2	1.23
200010_at	ribosomal protein L11	RPL11	1p36.1-p35	1.29
213080_x_at	ribosomal protein L5	RPL5	1p22.1	1.37
200858_s_at	ribosomal protein S8	RPS8	1p34.1-p32	1.25
200034_s_at	ribosomal protein L6	RPL6	12q24.1	1.33
208635_x_at	nascent-polypeptide-assoc. complex alpha polypeptide	NACA	12q23-q24.1	1.42
200735_x_at	nascent-polypeptide-assoc. complex alpha polypeptide	NACA	12q23-q24.1	1.29
211939_x_at	basic transcription factor 3	BTF3	5q13.3	1.30
208517_x_at	basic transcription factor 3	BTF3	5q13.3	1.38
200005_at	eukaryotic translation initiation factor 3, subunit 7 zeta	EIF3S7	22q13.1	1.36
200036_s_at	ribosomal protein L10a	RPL10A	6p21.3-p21.2	1.32
212042_x_at	ribosomal protein L7	RPL7	8q13.3	1.34
200717_x_at	ribosomal protein L7	RPL7	8q13.3	1.26
217740_x_at	ribosomal protein L7a	RPL7A	9q34	1.32
200088_x_at	ribosomal protein L12	RPL12	9q34	1.29

Probe Set ID	Gene Name	GS	ML	Ratio
200809_x_at	ribosomal protein L12	RPL12	9q34	1.22
214271_x_at	ribosomal protein L12	RPL12	9q34	1.32
200715_x_at	ribosomal protein L13a	RPL13A	19q13.3	1.58
201592_at	eukaryotic translation initiation factor 3, subunit 3 gamma	EIF3S3	8q24.11	1.53
200024_at	ribosomal protein S5	RPS5	19q13.4	1.38
214167_s_at	ribosomal protein, large, P0	RPLP0	12q24.2	1.57
211972_x_at	ribosomal protein, large, P0	RPLP0	12q24.2	1.35
211720_x_at	ribosomal protein, large, P0	RPLP0	12q24.2	1.40
201033_x_at	ribosomal protein, large, P0	RPLP0	12q24.2	1.35
208856_x_at	ribosomal protein, large, P0	RPLP0	12q24.2	1.35
208692_at	ribosomal protein S3	RPS3	11q13.3-q13.5	1.28
211927_x_at	eukaryotic translation elongation factor 1 gamma	EEF1G	11q12.3	1.27
200689_x_at	eukaryotic translation elongation factor 1 gamma	EEF1G	11q12.3	1.35
211345_x_at	eukaryotic translation elongation factor 1 gamma	EEF1G	11q12.3	1.32
213588_x_at	ribosomal protein L14	RPL14	3p22-p21.2	1.44
200705_s_at	eukaryotic translation elongation factor 1 beta 2	EEF1B2	2q33-q34	1.52
215963_x_at	ribosomal protein L3	RPL3	22q13	1.57
201217_x_at	ribosomal protein L3	RPL3	22q13	1.58
211666_x_at	ribosomal protein L3	RPL3	22q13	1.54
211073_x_at	ribosomal protein L3	RPL3	22q13	1.53
212039_x_at	ribosomal protein L3	RPL3	22q13	1.50
200089_s_at	ribosomal protein L4	RPL4	15q22	1.34
211710_x_at	ribosomal protein L4	RPL4	15q22	1.47
201154_x_at	ribosomal protein L4	RPL4	15q22	1.39
211542_x_at	ribosomal protein S10, clone MGC:10943, mRNA	unknown6	---	1.23
200817_x_at	ribosomal protein S10	RPS10	6p21.31	1.30
213890_x_at	ribosomal protein S16	RPS16	19q13.1	1.27
200781_s_at	ribosomal protein S15a	RPS15A	16p	1.27
200926_at	ribosomal protein S23	RPS23	5q14.1	1.29
200909_s_at	ribosomal protein, large P2	RPLP2	11p15.5-p15.4	1.45
208904_s_at	ribosomal protein S28	RPS28	19p13.2	1.29
203034_s_at	ribosomal protein L27a	RPL27A	11p15	1.31
201492_s_at	ribosomal protein L41	RPL41	12q13	1.26
201429_s_at	ribosomal protein L37a	RPL37A	2q35	1.24
208825_x_at	ribosomal protein L23a	RPL23A	17q11	1.28
208834_x_at	cadherin 1, type 1, E-cadherin (epithelial)	CDH1	16q22.1	1.31
213377_x_at	ribosomal protein S12	RPS12	6q23.1	1.30
201049_s_at	ribosomal protein S18	RPS18	6p21.3	1.31
200963_x_at	ribosomal protein L31	RPL31	2q12.1	1.29
200763_s_at	ribosomal protein, large, P1	RPLP1	15q22	1.26
200061_s_at	ribosomal protein S24	RPS24	10q22-q23	1.22
213583_x_at	eukaryotic translation elongation factor 1 alpha 1	EEF1A1	6q14.1	1.17
204892_x_at	eukaryotic translation elongation factor 1 alpha 1	EEF1A1	6q14.1	1.24
206559_x_at	eukaryotic translation elongation factor 1 alpha 1	EEF1A1	6q14.1	1.25
213614_x_at	eukaryotic translation elongation factor 1 alpha 1	EEF1A1	6q14.1	1.15
212433_x_at	ribosomal protein S2	RPS2	16p13.3	1.27
210646_x_at	ribosomal protein L13a	RPL13A	19q13.3	1.34
212790_x_at	ribosomal protein L13a	RPL13A	19q13.3	1.35
221798_x_at	ribosomal protein S2	RPS2	16p13.3	1.26
203107_x_at	ribosomal protein S2	RPS2	16p13.3	1.17
211942_x_at	ribosomal protein L13a	RPL13A	19q13.3	1.32
200031_s_at	ribosomal protein S11	RPS11	19q13.3	1.24
200716_x_at	ribosomal protein L13a	RPL13A	19q13.3	1.32
214042_s_at	Similar to ribosomal protein L22 (LOC389175), mRNA	unknown2	3q26.31	1.48
207132_x_at	prefoldin 5	PFDN5	12q12	1.27
221700_s_at	ubiquitin A-52 residue ribosomal protein fusion product 1	UBA52	19p13.1-p12	1.21

Probe Set ID	Gene Name	GS	ML	Ratio
200013_at	MAM domain containing 2	MAMDC2	9q21.13-q21.2	1.29
214143_x_at	ribosomal protein L24	RPL24	3q12	1.28
200082_s_at	ribosomal protein S7	RPS7	2p25	1.21
213941_x_at	ribosomal protein S7	RPS7	2p25	1.41
200012_x_at	CDNA clone MGC:71781 IMAGE:5287560	unknown3	4q12	1.28
200091_s_at	ribosomal protein S25	RPS25	11q23.3	1.22
221775_x_at	ribosomal protein L22	RPL22	1p36.3-p36.2	1.28
208768_x_at	ribosomal protein L22	RPL22	1p36.3-p36.2	1.29
213687_s_at	ribosomal protein L35a	RPL35A	3q29-qter	1.29
211487_x_at	ribosomal protein S17, clone MGC:11144, mRNA	unknown5	---	1.27
201665_x_at	ribosomal protein S17	RPS17	15q	1.21
200062_s_at	ribosomal protein L30	RPL30	8q22	1.29
208645_s_at	ribosomal protein S14	RPS14	5q31-q33	1.26
200018_at	ribosomal protein S13	RPS13	11p15	1.30
200092_s_at	ribosomal protein L37	RPL37	5p13	1.21
216380_x_at	ribosomal protein S28	RPS28	19p13.2	1.26
217807_s_at	glioma tumor suppressor candidate region gene 2	GLTSCR2	19q13.3	1.42
217747_s_at	ribosomal protein S9	RPS9	19q13.4	1.33
214317_x_at	ribosomal protein S9	RPS9	19q13.4	1.38
217313_at	AC004692 PAC clone RP5-1107K12 from 7p12-p14	unknown1	---	1.24
200022_at	ribosomal protein L18	RPL18	19q13	1.34
200003_s_at	ribosomal protein L28	RPL28	19q13.4	1.35
200869_at	ribosomal protein L18a	RPL18A	19p13	1.35
211475_s_at	BCL2-associated athanogene	BAG1	9p12	1.40
209087_x_at	melanoma cell adhesion molecule	MCAM	11q23.3	1.98
210869_s_at	melanoma cell adhesion molecule	MCAM	11q23.3	1.48
212481_s_at	tropomyosin 4	TPM4	19p13.1	1.53
209604_s_at	GATA binding protein 3	GATA3	10p15	2.74
209602_s_at	GATA binding protein 3	GATA3	10p15	8.01
217787_s_at	polypeptide UDP-N-acetylgalactosaminyltransferase 2	GALNT2	1q41-q42	2.30
204897_at	prostaglandin E receptor 4 (subtype EP4)	PTGER4	5p13.1	2.73
217523_at	CD44 antigen (homing function and Indian blood group)	CD44	11p13	2.33
212886_at	DKFZP434C171 protein	DKFZP434C171	5q33.1	1.85
205434_s_at	AP2 associated kinase 1	AAK1	2p24.3-p14	1.45
220306_at	FLJ20202 protein	FLJ20202	1p12	1.84
60528_at	phospholipase A2, group IVB (cytosolic)	PLA2G4B	15q11.2-q21.3	1.20
202315_s_at	breakpoint cluster region	BCR	22q11.23	1.48
213897_s_at	mitochondrial ribosomal protein L23	MRPL23	11p15.5-p15.4	1.70
201163_s_at	insulin-like growth factor binding protein 7	IGFBP7	4q12	1.93
201403_s_at	microsomal glutathione S-transferase 3	MGST3	1q23	1.51
217809_at	basic leucine zipper and W2 domains 2	BZW2	7p21.2	1.52
211709_s_at	stem cell growth factor; lymphocyte secreted C-type lectin	SCGF	19q13.3	3.20
205131_x_at	stem cell growth factor; lymphocyte secreted C-type lectin	SCGF	19q13.3	3.73
209073_s_at	numb homolog (Drosophila)	NUMB	14q24.3	0.71
209806_at	histone 1, H2be	HIST1H2BE	6p21.3	0.58
215245_x_at	fragile X mental retardation 1	FMR1	xq27.3	0.60
212476_at	centaurin, beta 2	CENTB2	3q29	0.56
209004_s_at	F-box and leucine-rich repeat protein 5	FBXL5	4p15.33	0.64
218951_s_at	hypothetical protein FLJ11323	FLJ11323	xp22.33; yp11.32	0.60
206219_s_at	vav 1 oncogene	VAV1	19p13.2	0.74
219131_at	transitional epithelia response protein	TERE1	1pter	0.65
212438_at	putative nucleic acid binding protein RY-1	RY1	2p13.3	0.65
200709_at	FK506 binding protein 1A, 12kDa	FKBP1A	20p13	0.72
203611_at	telomeric repeat binding factor 2	TERF2	16q22.1	0.38
205267_at	POU domain, class 2, associating factor 1	POU2AF1	11q23.1	0.39
213039_at	rho/rac guanine nucleotide exchange factor (GEF) 18	ARHGEF18	19p13.3	0.57

Probe Set ID	Gene Name	GS	ML	Ratio
39318_at	T-cell leukemia/lymphoma 1A	TCL1A	14q32.1	0.46
209995_s_at	T-cell leukemia/lymphoma 1A	TCL1A	14q32.1	0.42
208990_s_at	heterogeneous nuclear ribonucleoprotein H3 (2H9)	HNRPH3	10q22	0.58
203302_at	deoxycytidine kinase	DCK	4q13.3-q21.1	0.63
221725_at	WAS protein family, member 2	WASF2	1p36.11-p34.3	0.67
212492_s_at	jumonji domain containing 2B	JMJD2B	19p13.3	0.75
221011_s_at	likely ortholog of mouse limb-bud and heart gene	LBH	2p23.3	0.68
218642_s_at	coiled-coil-helix-coiled-coil-helix domain containing 7	CHCHD7	8q11.23	0.63
219253_at	family with sequence similarity 11, member B	FAM11B	2q14.2	0.72
204698_at	interferon stimulated gene 20kDa	ISG20	15q26	0.37
33304_at	interferon stimulated gene 20kDa	ISG20	15q26	0.58
218076_s_at	Rho GTPase activating protein 17	ARHGAP17	16p12.2	0.65
204849_at	transcription factor-like 5 (basic helix-loop-helix)	TCFL5	20q13.3-qter	0.15
219753_at	stromal antigen 3	STAG3	7q22.1	0.36
202838_at	fucosidase, alpha-L- 1, tissue	FUCA1	1p34	0.48
221747_at	tensin	TNS	2q35-q36	0.52
221748_s_at	tensin	TNS	2q35-q36	0.48
209695_at	protein tyrosine phosphatase type IVA, member 3	PTP4A3	8q24.3	0.49
44790_s_at	chromosome 13 open reading frame 18	C13orf18	13q14.11	0.38
219471_at	chromosome 13 open reading frame 18	C13orf18	13q14.11	0.38
203388_at	arrestin, beta 2	ARRB2	17p13	0.54
200696_s_at	gelsolin (amyloidosis, Finnish type)	GSN	9q33	0.57
217984_at	ribonuclease T2	RNASET2	6q27	0.59
217983_s_at	ribonuclease T2	RNASET2	6q27	0.63
204265_s_at	G-protein signalling modulator 3	GPSM3	6p21.3	0.70
221601_s_at	regulator of Fas-induced apoptosis	TOSO	1q32.1	0.49
221602_s_at	regulator of Fas-induced apoptosis	TOSO	1q32.1	0.26
219256_s_at	SH3 domain and tetratricopeptide repeats 1	SH3TC1	4p16.1	0.50
221757_at	LIM domain kinase 2	LIMK2	22q12.2	0.54
221756_at	LIM domain kinase 2	LIMK2	22q12.2	0.57
203710_at	inositol 1,4,5-triphosphate receptor, type 1	ITPR1	3p26-p25	0.54
205928_at	zinc finger protein 443	ZNF443	19p13.2	0.45
204689_at	hematopoietically expressed homeobox	HHEX	10q23.33	0.62
215933_s_at	hematopoietically expressed homeobox	HHEX	10q23.33	0.66
209431_s_at	zinc finger protein 278	ZNF278	22q12.2	0.69
214958_s_at	epidermodysplasia verruciformis 1	EVER1	17q25.3	0.56
201015_s_at	junction plakoglobin	JUP	17q21	0.78
212070_at	G protein-coupled receptor 56	GPR56	16q13	0.30
221773_at	ELK3, ETS-domain protein (SRF accessory protein 2)	ELK3	12q23	0.44
209568_s_at	ral guanine nucleotide dissociation stimulator-like 1	RGL1	1q25.2	0.48
221641_s_at	acyl-Coenzyme A thioesterase 2, mitochondrial	ACATE2	xp22.13	0.35
207571_x_at	chromosome 1 open reading frame 38	C1orf38	1p35.3	0.50
210785_s_at	chromosome 1 open reading frame 38	C1orf38	1p35.3	0.44
212956_at	KIAA0882 protein	KIAA0882	4q31.1	0.19
220230_s_at	cytochrome b5 reductase b5R.2	CYB5R2	11p15.4	0.52
204011_at	sprouty homolog 2 (Drosophila)	SPRY2	13q22.2	0.45
201663_s_at	SMC4 structural maintenance of chromosomes 4-like 1	SMC4L1	3q26.1	0.72
201260_s_at	synaptophysin-like protein	SYPL	7q22.2	0.71
203978_at	nucleotide binding protein 1 (MinD homolog, E. coli)	NUBP1	16p13.2	0.72
207390_s_at	smoothelin	SMTN	22q12.2	0.41
203680_at	protein kinase, cAMP-dependent, regulatory, type II	PRKAR2B	7q22	0.48

Supplemental Table 6: Transcription factor binding sites over-represented in the sub-cluster of genes over-expressed in VCR-ASP discordant resistant ALL.

The promoter region and the 5'UTR for specific sets of genes was used for Match™ to search the most recent TRANSFAC® Professional 8.1 database (vertebrates) (<http://www.biobase.de/>) for common regulatory elements. We determined statistical over-representation of the common motifs compared to “all” (~20,000) human promoter sequences (UCSC hg17; CLOVER) (Matys et al., 2003) and additionally we used the promoter sequences of the “other” gene set as background.

We found a total of 152 common transcription factor binding sites in the promoter regions of these 59 genes (3kb 5' of the transcription start site). Furthermore, we found significant over-representation of the same set of common transcription factor binding sites when sequences were compared against either “all” human promoter sequences or against the “other” 200 protein synthesis related genes. All these transcription factors are expressed in ALL cells except HEN1, but only ELF-1 was expressed at a significantly different level in ALL cells that were VCR-sensitive plus ASP-resistant versus VCR-resistant plus ASP-sensitive (P=0.014, t-test, R/S ratio =1.28).

Motif ID	Motif	TF	Raw score	P-value from 'all' *	P-value from 'other' †
M00069	V\$YY1_02	YY1	6.39	0	0.01
M00341	V\$GABP_B	GABP	1.41	0.001	0.044
M00971	V\$ETS_Q6	Ets	2.6	0.002	0.024
M00746	V\$ELF1_Q6	ELF-1 [§]	5.15	0.009	0.012
M00068	V\$HEN1_01	HEN1	2.28	0.017	0.003
M00264	V\$STAF_02	Staf	3.59	0.023	0.017

* P-value from over-representation compared to “all” ~20,000 human promoter sequences

† P-value from over-representation compared to promoter sequences of “other” 96 protein synthesis genes

§ P-value <0.05 in the two VCR-ASP discordant resistance phenotypes

Supplemental Table 7: Overlap of genes discriminating multiple-drug resistance and genes discriminating ALL subtypes

A. The overlap with the top 100 gene probe sets discriminating ALL genetic subtypes (*TEL-AML1* [TEL], hyperdiploid [HD], T-lineage [T]) (Ross et al., 2003; Yeoh et al., 2002) and the 200 gene probe sets discriminating VCR-ASP discordant resistance. Only 13 probe sets (11 genes) were in common. **B.** Furthermore, among the top 100 gene probe sets discriminating ALL genetic subtypes (Ross et al., 2003; Yeoh et al., 2002) and the 51 gene probe sets discriminating cross-resistance, there were only three genes in common.

A

Probe ID	ALL Subtype	Title	Gene Symbol
200709_at	TEL ¹	FK506 binding protein 1A, 12kDa	FKBP1A
202838_at	TEL ¹	fucosidase, alpha-L- 1, tissue	FUCA1
203611_at	TEL ¹	telomeric repeat binding factor 2	TERF2
203710_at	TEL ¹	inositol 1,4,5-triphosphate receptor, type 1	ITPR1
204849_at	TEL ¹	transcription factor-like 5 (basic helix-loop-helix)	TCFL5
205267_at	TEL ¹	POU domain, class 2, associating factor 1	POU2AF1
209695_at	TEL ¹	protein tyrosine phosphatase type IVA, member 3	PTP4A3
221494_x_at	HD ¹	muscle specific gene	M9
203680_at	HD ²	protein kinase, cAMP-dependent, regulatory, type II, beta	PRKAR2B
204849_at	TEL ²	transcription factor-like 5 (basic helix-loop-helix)	TCFL5
221748_s_at	TEL ²	Homo sapiens cDNA FLJ32766 fis, clone TESTI2001862	
200709_at	TEL ²	FK506 binding protein 1A, 12kDa	FKBP1A
221747_at	TEL ²	Homo sapiens cDNA FLJ32766 fis, clone TESTI2001862	

B

Probe ID	ALL Subtype	Title	Gene Symbol
200709_at	HD ¹	transcription factor 4	TCF4
202838_at	T ²	CD79B antigen (immunoglobulin-associated beta)	CD79B
203611_at	T ²	immunoglobulin heavy constant mu	IGHM

¹ Ross et al., 2003; ² Yeoh et al., 2002

Supplemental Table 8: Overlap in genes discriminating multiple-drug cross-resistance and genes discriminating PRD-, VCR-, ASP- and DNR single-drug resistance.

Summary of the overlapping gene probe sets with corresponding gene symbol (GS), map location (ML) and ratio of the median log-transformed expression signal in cross-resistant versus cross-sensitive ALL for multiple-drug cross-resistance (Ratio). A gene probe set with a ratio of >1 is over-expressed and with a ratio of <1 is under-expressed probe sets in cross-resistant ALL compared to cross-sensitive ALL. The probe sets in overlap between multiple-drug cross-resistance and single-drug resistance to PRD (**A**), ASP (**B**) and DNR (**C**) alone are shown, highlighted in orange and yellow are genes that are in common, no overlap was found with VCR alone. (**D**) Rows indicate the overlapping genes (n) for single-drug resistance PRD, VCR, ASP, DNR; and columns overlapping genes (n) and percentages for cross-resistance (CR) and VCR-

ASP discordant resistance (VA). Overlap occurs in cases the total is not matching the sum of the genes. (1) shows the numbers and the percentages if the significant set of genes identified were compared and (2) shows the results if the analysis is done using the same number of genes for multiple-drug resistance versus single-drug resistance.

A

Probe Set ID	Gene Name	GS	ML	R/S ratio
213061_s_at	N-terminal asparagine amidase	NTAN1	16p13.13	0.60
203274_at	coagulation factor VIII-associated (intronic transcript)	F8A	xq28	0.60
202521_at	CCCTC-binding factor (zinc finger protein)	CTCF	16q21-q22.3	0.66
218438_s_at	endothelial-derived gene 1	EG1	4p16	0.67
219679_s_at	WW domain containing adaptor with coiled-coil	WAC	10p12.1	0.67
208739_x_at	SMT3 suppressor of mif two 3 homolog 2 (yeast)	SUMO2	17q25	0.69
221547_at	PRP18 pre-mRNA processing factor 18 homolog	PRPF18	10p14	0.77
208620_at	poly(rC) binding protein 1	PCBP1	2p13-p12	0.81
217729_s_at	amino-terminal enhancer of split	AES	19p13.3	0.82
205193_at	v-maf musculoaponeurotic fibrosarcoma oncogene	MAFF	22q13.1	1.76
209795_at	CD69 antigen (p60, early T-cell activation antigen)	CD69	12p13-p12	2.14
36711_at	v-maf musculoaponeurotic fibrosarcoma oncogene	MAFF	22q13.1	2.25
218589_at	purinergic receptor P2Y, G-protein coupled, 5	P2RY5	13q14	2.69

B

Probe Set ID	Gene Name	GS	ML	Ratio
203274_at	coagulation factor VIII-associated (intronic transcript)	F8A	xq28	0.60
201140_s_at	RAB5C, member RAS oncogene family	RAB5C	17q21.2	0.63
201156_s_at	RAB5C, member RAS oncogene family	RAB5C	17q21.2	0.76
209604_s_at	GATA binding protein 3	GATA3	10p15	3.38

C

Probe Set ID	Gene Name	GS	ML	Ratio
213061_s_at	N-terminal asparagine amidase	NTAN1	16p13.13	0.60
202777_at	soc-2 suppressor of clear homolog (C. elegans)	SHOC2	10q25	0.61
202521_at	CCCTC-binding factor (zinc finger protein)	CTCF	16q21-q22.3	0.66
218438_s_at	endothelial-derived gene 1	EG1	4p16	0.67
200059_s_at	ras homolog gene family, member A	RHOA	3p21.3	0.82

D

		1) multiple resistance				
		Overlap	CR (51)	VA (200)	% of CR	% of VA
single resistance	PRD (42)		13	0	25%	0%
	VCR (59)		0	15	0%	8%
	ASP (54)		4	36	8%	18%
	DNR (22)		5	0	10%	0%
	Total (146)		18	51	35%	26%

		2) multiple resistance				
		Overlap	CR (100)	VA (100)	% of CR	% of VA
single resistance	PRD (100)		21	0	21%	0%
	VCR (100)		1	10	1%	10%
	ASP (100)		14	36	14%	36%
	DNR (100)		19	1	19%	1%
	Total (382)		45	46	45%	47%

Supplemental Table 9: Overlap in genes discriminating VCR-ASP discordant resistance and genes discriminating VCR- and ASP single-drug resistance.

Summary of the overlapping gene probe sets with corresponding gene symbol (GS), map location (ML) and ratio of expression in the unfavorable versus favorable group for VCR-ASP discordant resistance (Ratio). The ratio was calculated using the median log-transformed signal in VCR-

sensitive plus ASP-resistant versus VCR-resistant plus ASP-sensitive (>1 is a gene probe set over-expressed in VCR-sensitive plus ASP-resistant ALL cells). A ratio of <1 is a gene probe set under-expressed probe sets in VCR-sensitive plus ASP-resistant ALL cells. The probe sets in overlap between VCR-ASP discordant resistance and single-drug resistance to ASP (A) and single-drug resistance to VCR (B) no overlap was found with PRD or DNR single-drug resistance. See also Supplemental Table 8D.

A

Probe Set ID	Gene Name	GS	ML	Ratio
203388_at	arrestin, beta 2	ARRB2	17p13	0.54
202315_s_at	breakpoint cluster region	BCR	22q11.23	1.48
212886_at	DKFZP434C171 protein	DKFZP434C171	5q33.1	1.85
200705_s_at	eukaryotic translation elongation factor 1 beta 2	EEF1B2	2q33-q34	1.52
200689_x_at	eukaryotic translation elongation factor 1 gamma	EEF1G	11q12.3	1.35
211927_x_at	eukaryotic translation elongation factor 1 gamma	EEF1G	11q12.3	1.27
210501_x_at	eukaryotic translation initiation factor 3 subunit k	eIF3k	19q13.2	1.33
200005_at	eukaryotic translation initiation factor 3, subunit 7 zeta	EIF3S7	22q13.1	1.36
221773_at	ELK3, ETS-domain protein (SRF accessory protein 2)	ELK3	12q23	0.44
211623_s_at	fibrillarin	FBL	19q13.1	1.40
220306_at	FLJ20202 protein	FLJ20202	1p12	1.84
209602_s_at	GATA binding protein 3	GATA3	10p15	8.01
209604_s_at	GATA binding protein 3	GATA3	10p15	2.74
217807_s_at	glioma tumor suppressor candidate region gene 2	GLTSCR2	19q13.3	1.42
200651_at	guanine nucleotide binding protein (G protein)	GNB2L1	5q35.3	1.42
212070_at	G protein-coupled receptor 56	GPR56	16q13	0.30
213828_x_at	H3 histone, family 3A	H3F3A	1q41	1.30
208755_x_at	H3 histone, family 3A	H3F3A	1q41	1.26
211940_x_at	H3 histone, family 3A	H3F3A	1q41	1.22
209806_at	histone 1, H2be	HIST1H2BE	6p21.3	0.58
201163_s_at	insulin-like growth factor binding protein 7	IGFBP7	4q12	1.93
201015_s_at	junction plakoglobin	JUP	17q21	0.78
200010_at	ribosomal protein L11	RPL11	1p36.1-p35	1.29
200715_x_at	ribosomal protein L13a	RPL13A	19q13.3	1.58
201217_x_at	ribosomal protein L3	RPL3	22q13	1.58
215963_x_at	ribosomal protein L3	RPL3	22q13	1.57
211666_x_at	ribosomal protein L3	RPL3	22q13	1.54
211073_x_at	ribosomal protein L3	RPL3	22q13	1.53
212039_x_at	ribosomal protein L3	RPL3	22q13	1.50
200089_s_at	ribosomal protein L4	RPL4	15q22	1.34
213080_x_at	ribosomal protein L5	RPL5	1p22.1	1.37
200034_s_at	ribosomal protein L6	RPL6	12q24.1	1.33
217740_x_at	ribosomal protein L7a	RPL7A	9q34	1.32
214167_s_at	ribosomal protein, large, P0	RPLP0	12q24.2	1.57
208692_at	ribosomal protein S3	RPS3	11q13.3-q13.5	1.28
200024_at	ribosomal protein S5	RPS5	19q13.4	1.38

B

Probe Set ID	Gene Name	GS	ML	Ratio
219471_at	chromosome 13 open reading frame 18	C13orf18	13q14.11	0.38
44790_s_at	chromosome 13 open reading frame 18	C13orf18	13q14.11	0.38
217523_at	CD44 antigen	CD44	11p13	2.33
218642_s_at	coiled-coil-helix-coiled-coil-helix domain containing 7	CHCHD7	8q11.23	0.63
218951_s_at	hypothetical protein FLJ11323	FLJ11323	xp22.33; yp11.32	0.60
212492_s_at	jumonji domain containing 2B	JMJD2B	19p13.3	0.75
212956_at	KIAA0882 protein	KIAA0882	4q31.1	0.19
221011_s_at	likely ortholog of mouse limb-bud and heart gene	LBH	2p23.3	0.68
200088_x_at	ribosomal protein L12	RPL12	9q34	1.29
200909_s_at	ribosomal protein, large P2	RPLP2	11p15.5-p15.4	1.45
200031_s_at	ribosomal protein S11	RPS11	19q13.3	1.24
213377_x_at	ribosomal protein S12	RPS12	6q23.1	1.30
200781_s_at	ribosomal protein S15a	RPS15A	16p	1.27
212438_at	putative nucleic acid binding protein RY-1	RY1	2p13.3	0.65
204849_at	transcription factor-like 5 (basic helix-loop-helix)	TCFL5	20q13.3-qter	0.15

Supplemental Table 10: Proportional-hazards regression analysis.

Single factor proportional-hazards regression analysis of the risk of relapse in relation to known prognostic factors (i.e., white blood cell count (WBC), age at diagnosis) and cross-resistance and VCR-ASP discordant resistance gene expression scores are shown in Table (A) for COALL/DCOG patients and in Table (B) for St. Jude patients. Multiple proportional-hazards regression analysis of known prognostic factors (i.e., WBC, age) and gene expression scores are shown in Table (C) for COALL/DCOG patients and in Table (D) for St. Jude patients.

Variable	A				B			
	n	HR	95% C.I.	P-value	n	HR	95% C.I.	P-value
Age								
<10 years	96	1.0*			57	1.0*		
>10 years	33	2.36	1.10-5.05	0.03	26	3.46	1.12-10.64	0.03
WBC								
<49/nL	91	1.0*			50	1.0*		
50-100/nL	20	0.88	0.26-3.03	0.84	16	3.05	0.76-12.2	0.12
>100/nL	18	3.82	1.69-8.67	0.0013	17	3.7	0.92-14.9	0.065
ALL subtype								
B-other	42	1.0*			27	1.0*		
<i>BCR-ABL</i>	5	2.49	0.72-8.64	0.15	8	11.65	2.50-54.0	0.0017
<i>E2A-PBX1</i>	6	0.93	0.21-4.07	0.92	12	0.76	0.08-6.83	0.81
Hyperdiploid	33	0.22	0.06-0.77	0.017	15	0.58	0.06-5.32	0.63
<i>MLL-AF4</i>	3	28.4	6.30-128.9	<0.0001	5	5.13	0.77-34.1	0.09
<i>TEL-AML1</i>	40	0.11	0.03-0.50	0.0038	16	0.57	0.06-5.33	0.63
GE score (CR)								
Low [†]	33	1.0*			(8)	**	**	**
Intermediate	63	2.31	0.65-8.18	0.2	73	1.0*		
High [‡]	33	5.99	1.7-21.09	0.0053	10	7.76	2.44-24.7	0.0005
GE score (VA)								
Low [†]	33	1.0*			16	1.0*		
Intermediate	64	3.59	0.81-15.9	0.09	52	1.16	0.24-5.57	0.86
High [‡]	32	9.04	2.04-40.1	0.0038	15	1.67	0.28-10.03	0.57
Table C								
Variable	C	D						
	n	HR	95%	P-value	n	HR	95% C.I.	P-value
Age								
<10 years	96	1.0*			57	1.0*		
>10 years	33	2.23	0.93-5.38	0.074	26	2.99	0.71-12.6	0.14
WBC								
<49/nL	91	1.0*			50	1.0*		
50-100/nL	20	0.98	0.27-3.5	0.98	16	4.70	1.0-22.1	0.05
>100/nL	18	5.06	2.05-12.5	0.0005	17	4.87	1.08-21.93	0.039
GE score CR								
Low [†]	33	1.0*			(8)	**	**	**
Intermediate	63	2.09	0.58-7.6	0.26	73	1.0*		
High [‡]	33	3.82	0.95-15.4	0.06	10	5.06	1.28-20.03	0.021
GE score VA								
Low [†]	33	1.0*			16	1.0*		
Intermediate	64	2.76	0.61-12.47	0.19	52	0.36	0.06-2.24	0.27
High [‡]	32	4.31	0.89-20.8	0.069	15	0.61	0.09-4.12	0.61

*reference group, **no relapses in this group, hazard ratio cannot be computed

[†]top quartile; CR, cross-resistant ALL; VA, VCR-sensitive +ASP-resistant ALL

[‡]bottom quartile; CR, cross-sensitive ALL; VA, VCR-resistant+ASP-sensitive ALL

Supplemental Table 11: The multiple-drug cross-resistance gene expression score is significantly predictive of resistance of other antileukemic agents.

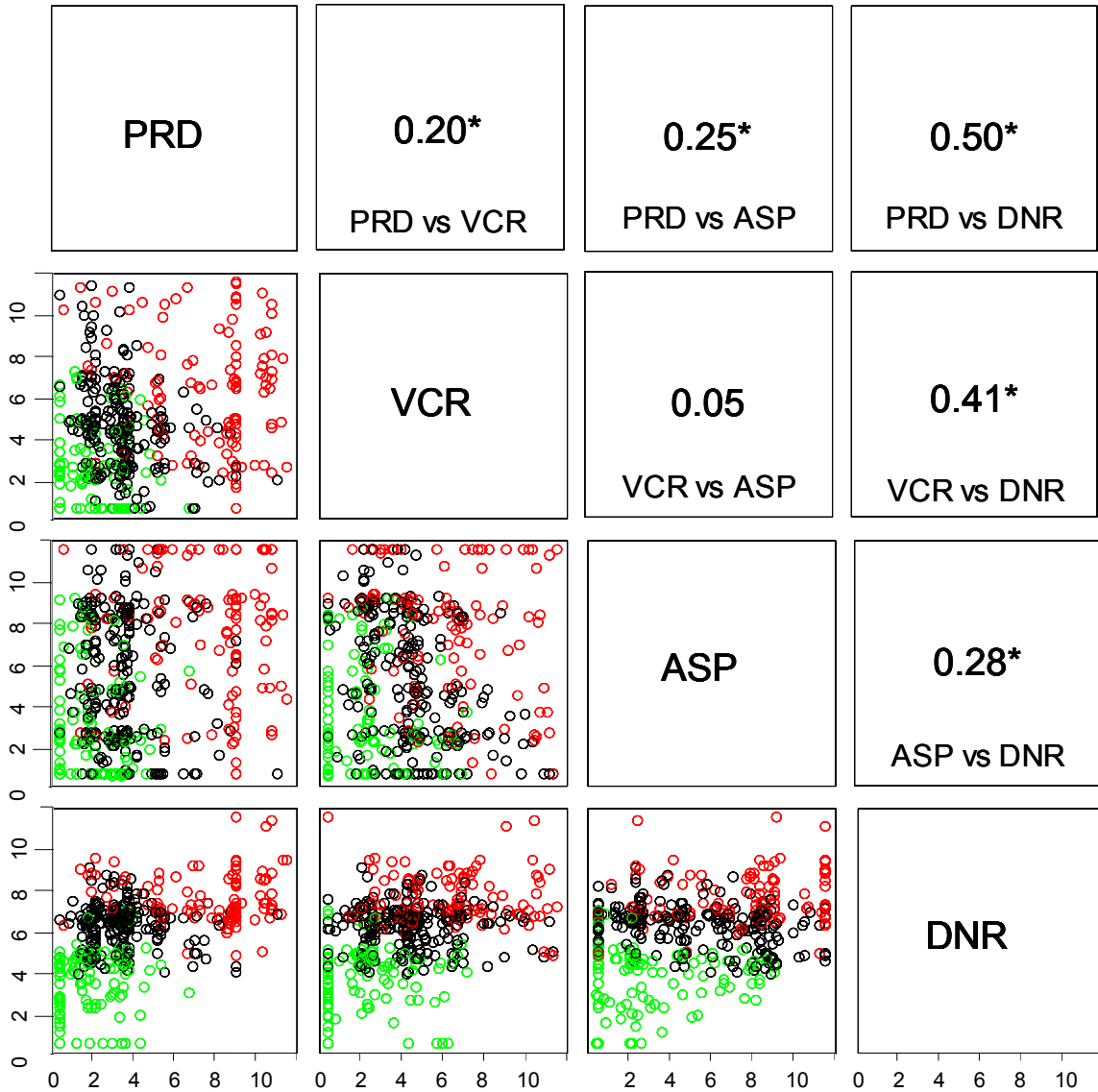
Mercaptopurine (MP) in vitro resistance, tested at St. Jude cells of additional patients enrolled on Total Therapy XV protocol (SJCRH) and MP in vitro resistance tested in a subset of patients of the COALL/DCOG patients, were significantly related to the new cross-resistance gene expression score (GE CR). Additionally, resistance to MP was not related to the VCR-ASP discordant resistance gene expression score (GE VA) and more importantly, no association was found for MP resistance with the combined single-drug resistance gene expression score (GE PDAV) (Holleman, *NEJM*, 2004). This indicates that cross-resistance indeed reflects a common resistance mechanism that relates to multiple antileukemic drugs, beyond the four drugs we studied, that is distinct from our prior combined single-drug resistance gene expression signature (sum of PDAV).

	COALL/DCOG MP n=29		SJCRH MP n=51	
	P-value*	rho*	P-value*	rho*
GE CR	<u>0.0002</u>	0.64	<u>0.007</u>	0.37
GE VA	0.54	0.12	0.13	0.22
GE PDAV	0.99	0.002	0.06	0.27

*Spearman rank correlation, P-value, coefficient (rho)

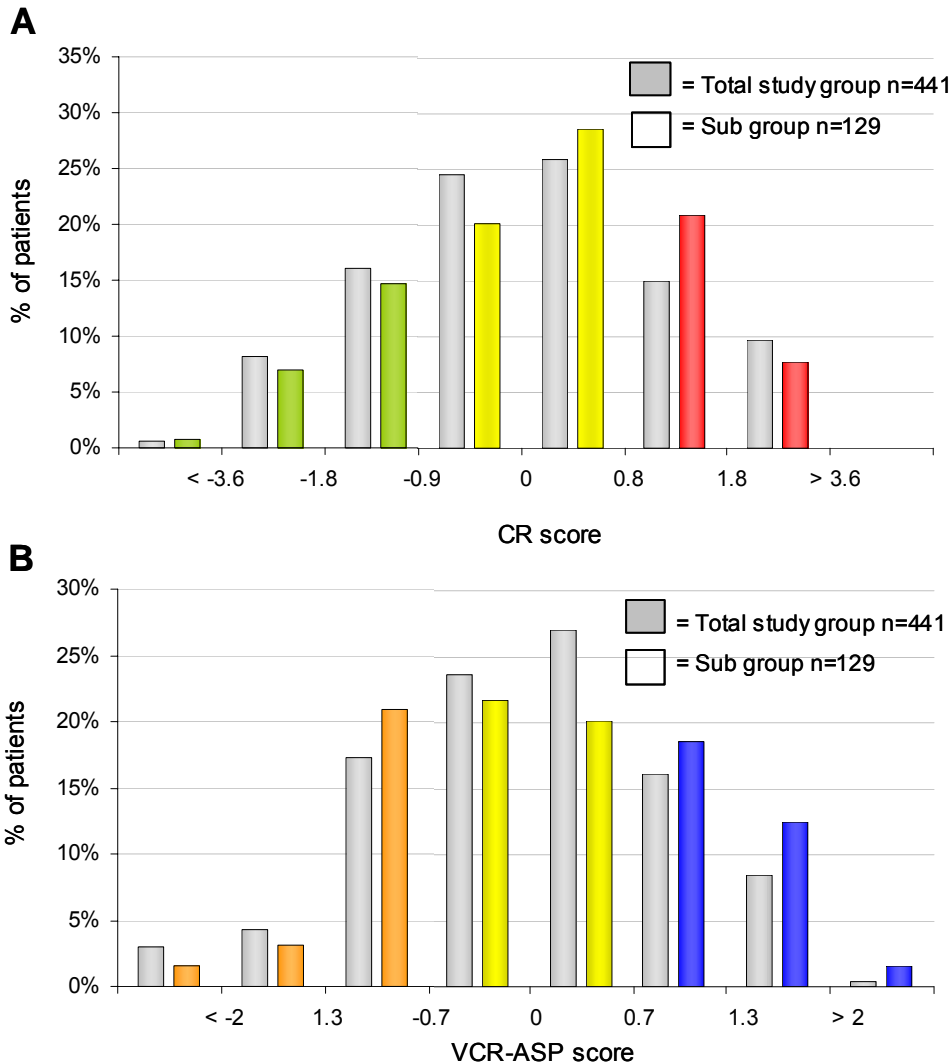
Supplemental Figure 1: Correlation of in vitro sensitivity of primary ALL cells.

Pair-wise correlation of log-transformed LC_{50} values of prednisolone (PRD), vincristine (VCR), asparaginase (ASP) and daunorubicin (DNR) with corresponding rho by Spearman's Rank correlation (* $P < 0.0001$). Cross-sensitive (green) and cross-resistant (red) patients correspond to the top and bottom quartile by first component of the PCA; illustrated in black are the patients categorized as intermediates.



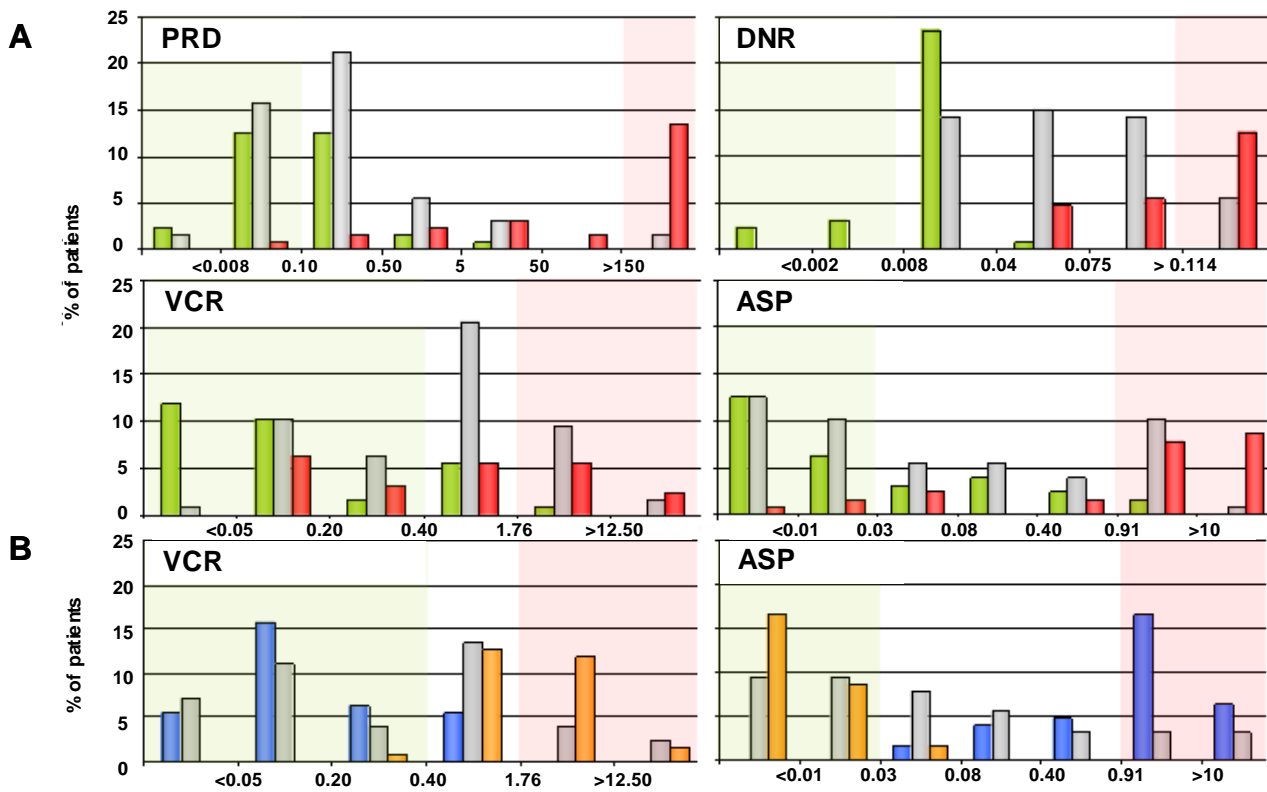
Supplemental Figure 2: Distribution of cross-resistance (CR-scores) and VCR-ASP discordant resistance scores (VCR-ASP-scores) in B-lineage ALL patients.

Shown are the distributions of CR- and VCR-ASP scores among the 441 B-lineage ALL patients in the LC₅₀ analysis (gray) and in the subgroup of 129 patients (in color) included in the gene expression analysis. The background is highlighted to indicate the LC₅₀ values that define resistance (red) and sensitive (green) ALL for each drug (Pieters et al., 1990). **A.** Distribution of cross-resistant scores of the first component. Depicted in green are cross-sensitive ALL with low CR-scores (n=38), in yellow are ALL cells with intermediate CR-scores (n=62) and in red cross-resistant ALL with high CR-scores (n=29). **B.** Distribution of VCR-ASP-scores of the second component. Depicted in blue VCR-sensitive plus ASP-resistant ALL with high VCR-ASP-scores (n=42), in yellow ALL that does not exhibit discordant sensitivity to ASP and VCR with intermediate VCR-ASP-scores (n=53) and in orange are VCR resistant plus ASP sensitive ALL with low VCR-ASP-scores (n=34).



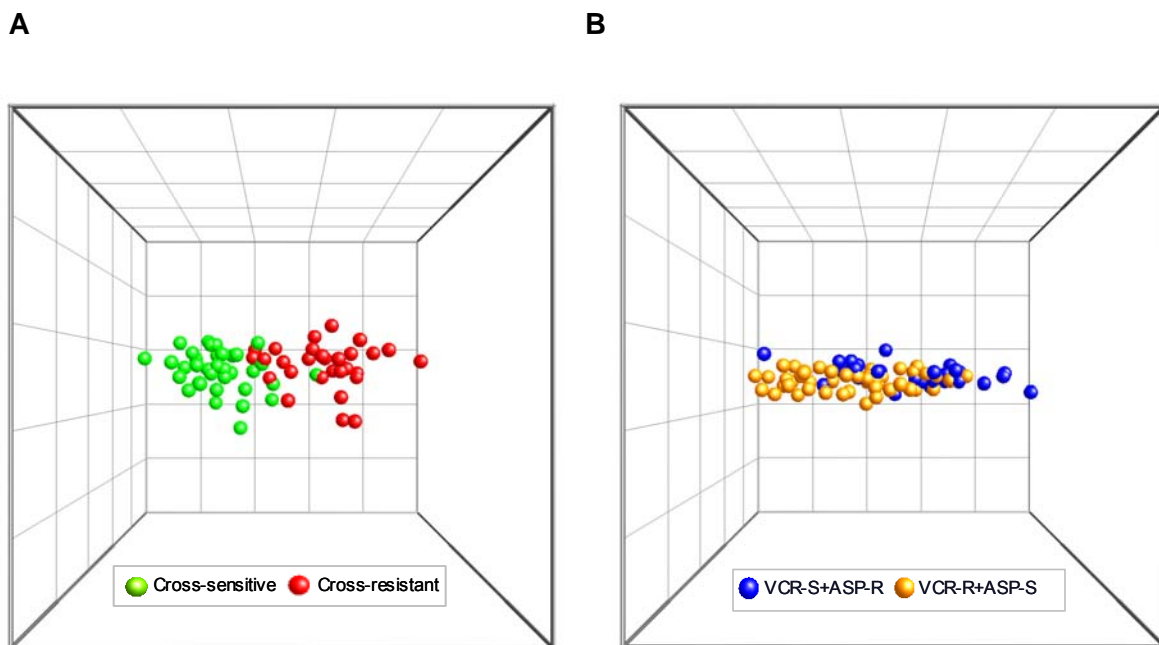
Supplemental Figure 3: Distribution of LC₅₀ values for each drug tested in the CR-group and VCR-ASP group analyzed for gene expression.

In each plot, the LC₅₀ values (n=129) for each drug are plotted on the x-axis [prednisolone (PRD), vincristine (VCR) and daunorubicin (DNR) in µg/ml; asparaginase (ASP) in IU/ml] and the percentage of patients in each range of LC₅₀ values is plotted on the y-axis. **A.** The distribution of LC₅₀ values for each drug; red indicates ALL classified based on CR-score as cross-resistant (n=29), gray as intermediate (n=62) and green as cross-sensitive (n=38). **B.** The distribution of LC₅₀ values for VCR and ASP; blue indicates VCR-sensitive plus ASP-resistant ALL based on the VCR-ASP-score (n=42), orange indicates VCR-resistant plus ASP-sensitive ALL (n=34), and the remaining patients are indicated in gray (n=53).



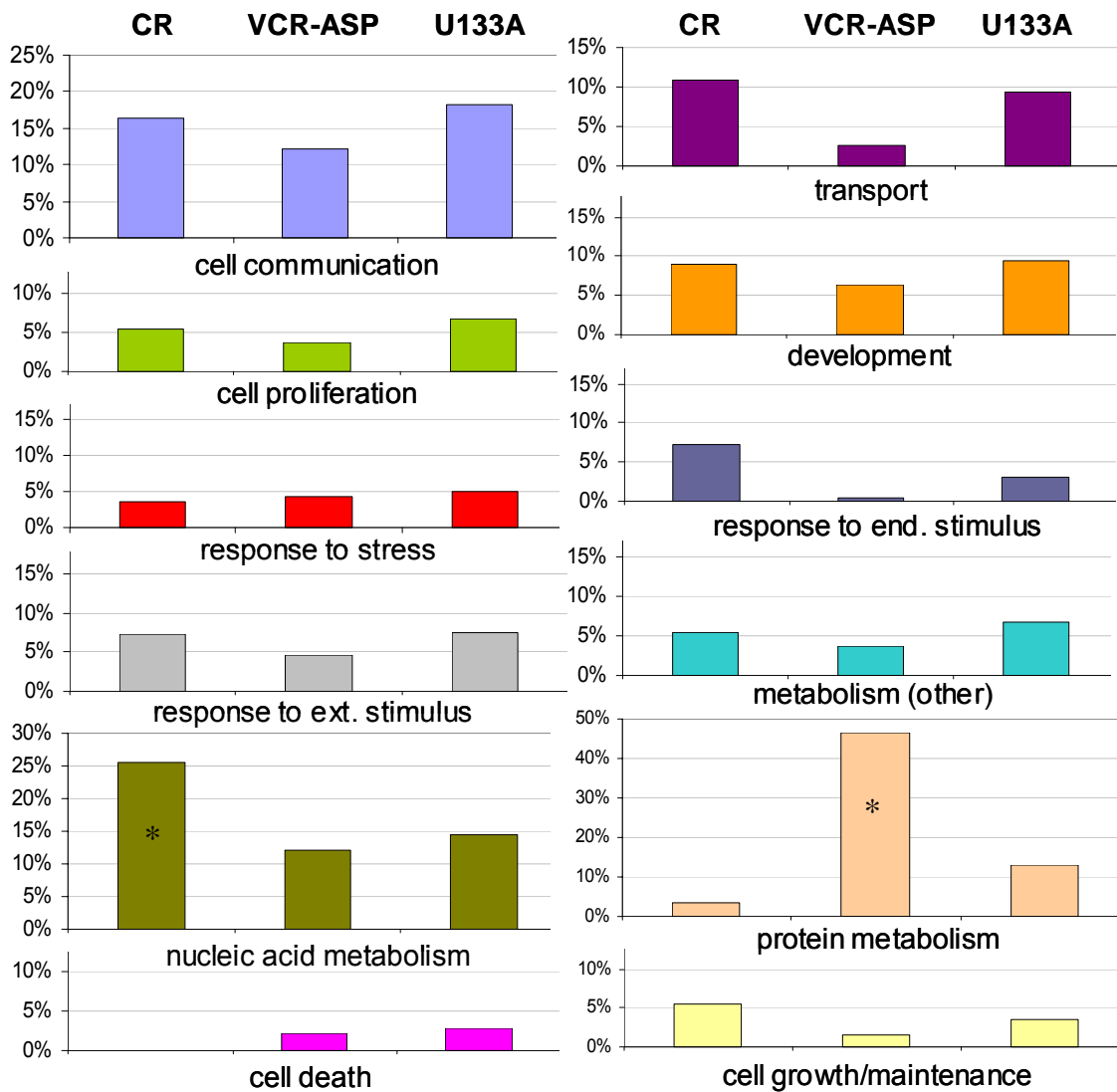
Supplemental Figure 4: Principal component analysis plot of genes discriminating cross-resistance and VCR-ASP discordant resistance.

Illustrated are the first three components of the principal component analysis (PCA) to visualize differences among ALL cells using the gene expression signatures identified. **A.** Expression of 51 gene probe sets that significantly discriminate cross-resistant (red; n=29) from cross-sensitive (green; n=38) B-lineage ALL patients ($P < 0.0001$) in three-dimensional space. **B.** Gene expression of 200 gene probe sets ($P < 0.00006$) that significantly discriminate VCR-resistant (VCR-R) plus ASP-sensitive (ASP-S) (orange; n=34) patients from VCR-sensitive plus ASP-resistance patients (blue; n=42) were used.



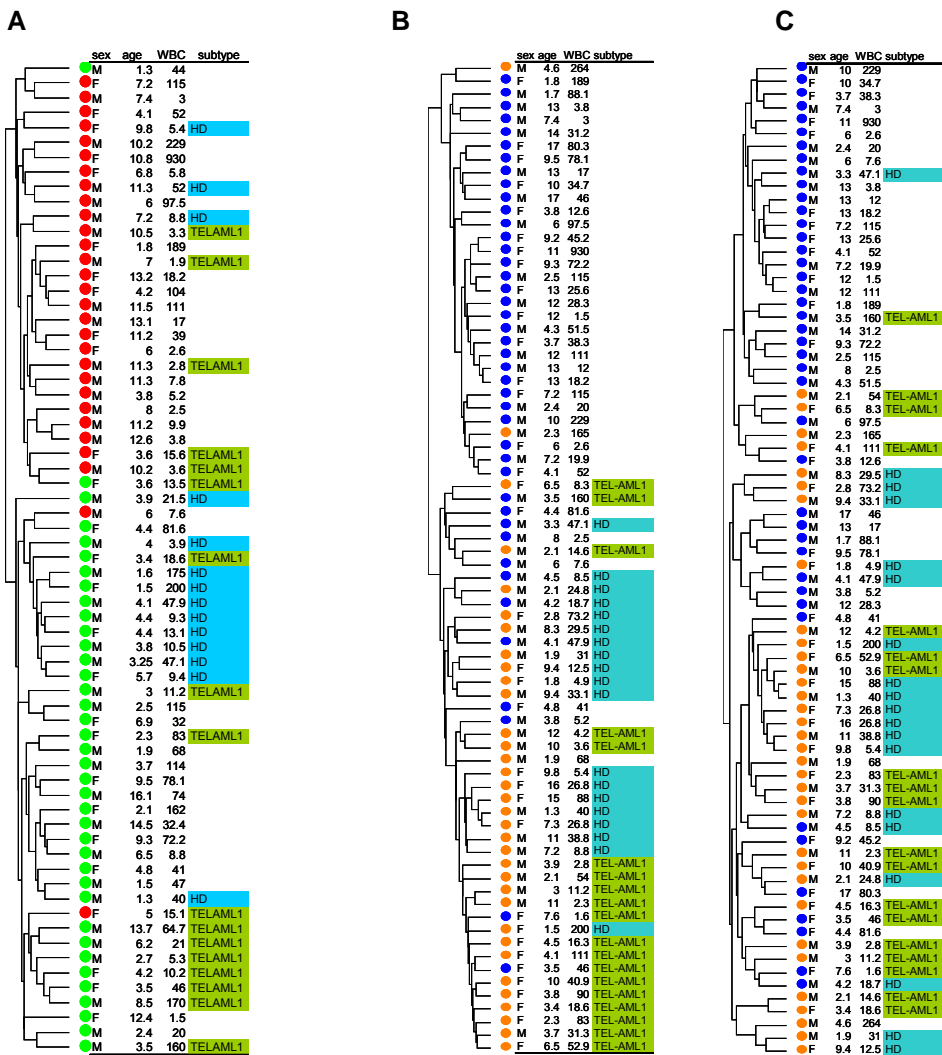
Supplemental Figure 5: Gene Ontology (GO) of genes discriminating cross-resistance and genes discriminating VCR-ASP discordant resistance.

Gene probe sets discriminating cross-resistance (CR) and VCR-ASP discordant resistance (VA) in B-lineage ALL patients were grouped according to 12 major functional categories. Each diagram shows the percentage of probe sets with GO annotation in the list of discriminating genes versus all genes represented on the U133A GeneChip. The entire genome, as represented by the 12,304 probe sets annotated in the GO database, has a total of 20,113 annotations. 51 probe sets discriminating CR (32 probe sets with GO annotation) and 200 probe sets discriminating VCR and ASP discordant resistance (142 probe sets with GO annotation) were used. Fisher's exact test (*P<0.05) was used to estimate significance of over- or under-representation of the discriminating probe sets.



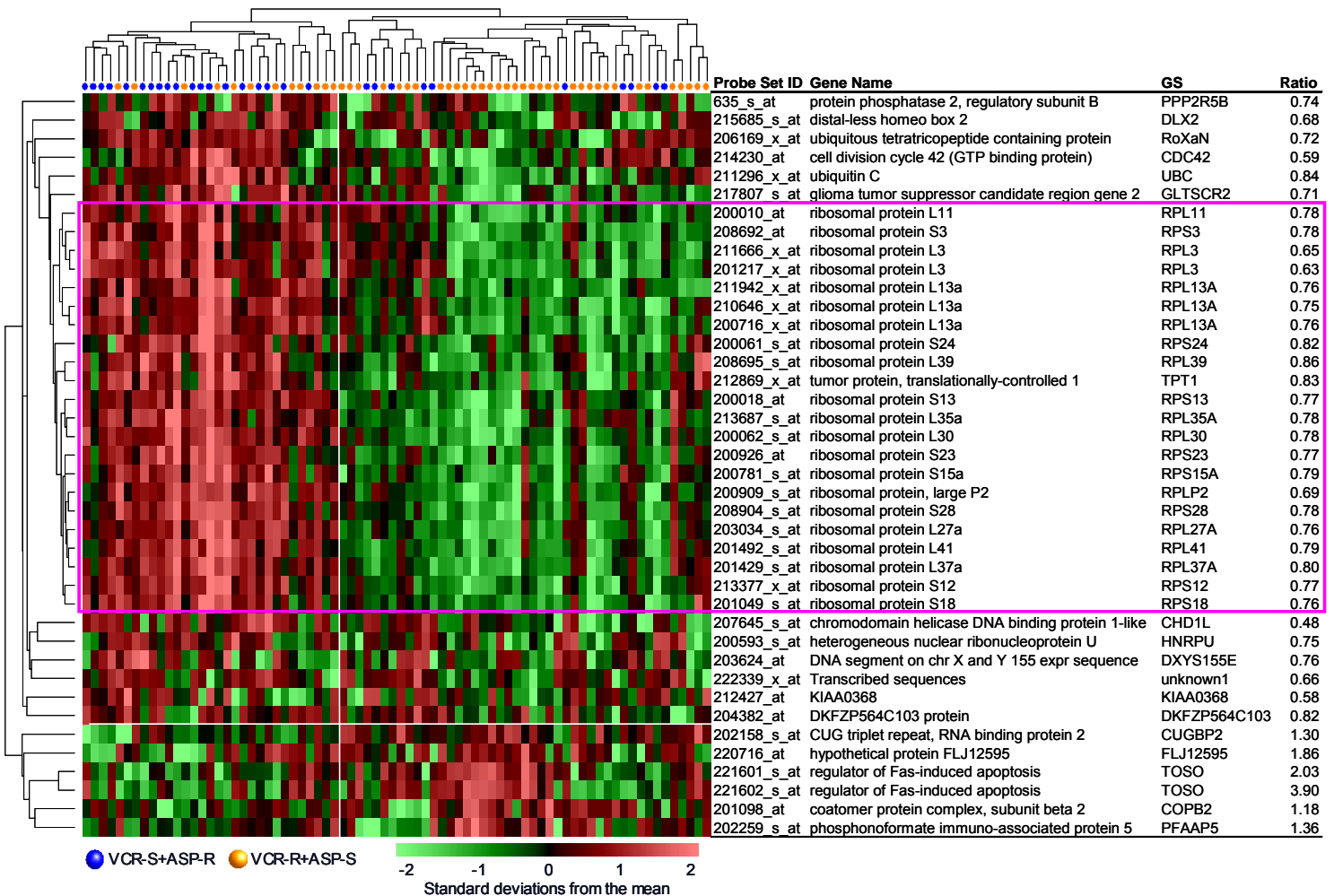
Supplemental Figure 6: Hierarchical clustering of ALL patients using genes discriminating cross-resistance and VCR-ASP discordant resistance.

A. Hierarchical clustering of cross-resistant (red dots, n=29) and cross-sensitive (blue dots, n=38) patients using genes discriminating cross-resistance. Listed for each patient are gender (sex), age and white blood cell count at diagnosis (WBC) and ALL subtype colored in green *TEL-AML1*-positive and in blue hyperdiploid (HD) ALL. **B.** Hierarchical clustering of VCR-sensitive plus ASP-resistant (blue dots, n=42) patients and VCR-resistant plus ASP-sensitive (orange dots, n=34) patients using genes discriminating VCR-ASP discordance resistance. **C.** Hierarchical clustering branch of patients using ALL subtype adjusted gene expression of VCR-ASP discriminating genes.



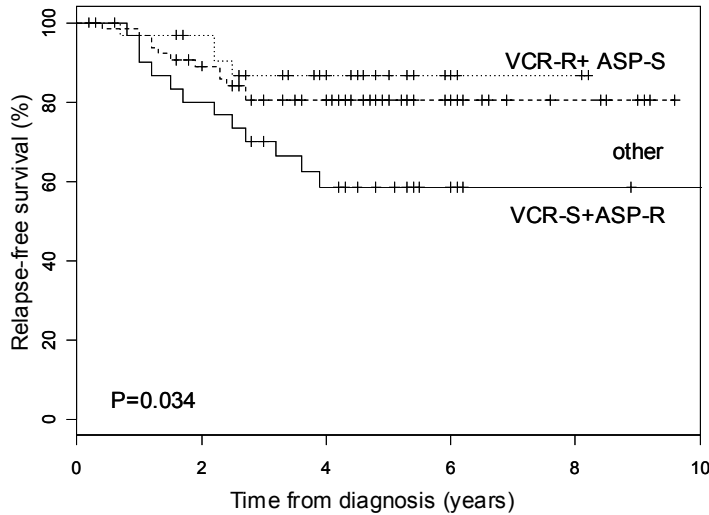
Supplemental Figure 7: Hierarchical clustering using ALL subtype adjusted genes that discriminate VCR-ASP discordant resistance.

40 gene probe sets in rows with $P < 0.0008$ discriminating VCR-sensitive plus ASP-resistant (blue, $n=42$) and VCR-resistant plus ASP-sensitive (orange, $n=34$) ALL cells in columns adjusted for *TEL-AML1* and hyperdiploidy. Probe set ID, gene name and gene symbol (GS) are listed. The genes involved in protein biosynthesis ($n=22$) are highlighted with a magenta colored box. The heat map indicates high (red) or low (green) level of expression relative to scale shown. Notably, genes expressed at a low level commonly change by more than 2-fold whereas genes that are expressed at high levels typically change by less than 2-fold. ALL VCR-ASP discordant resistance genes related to protein synthesis are expressed at a relatively high level.



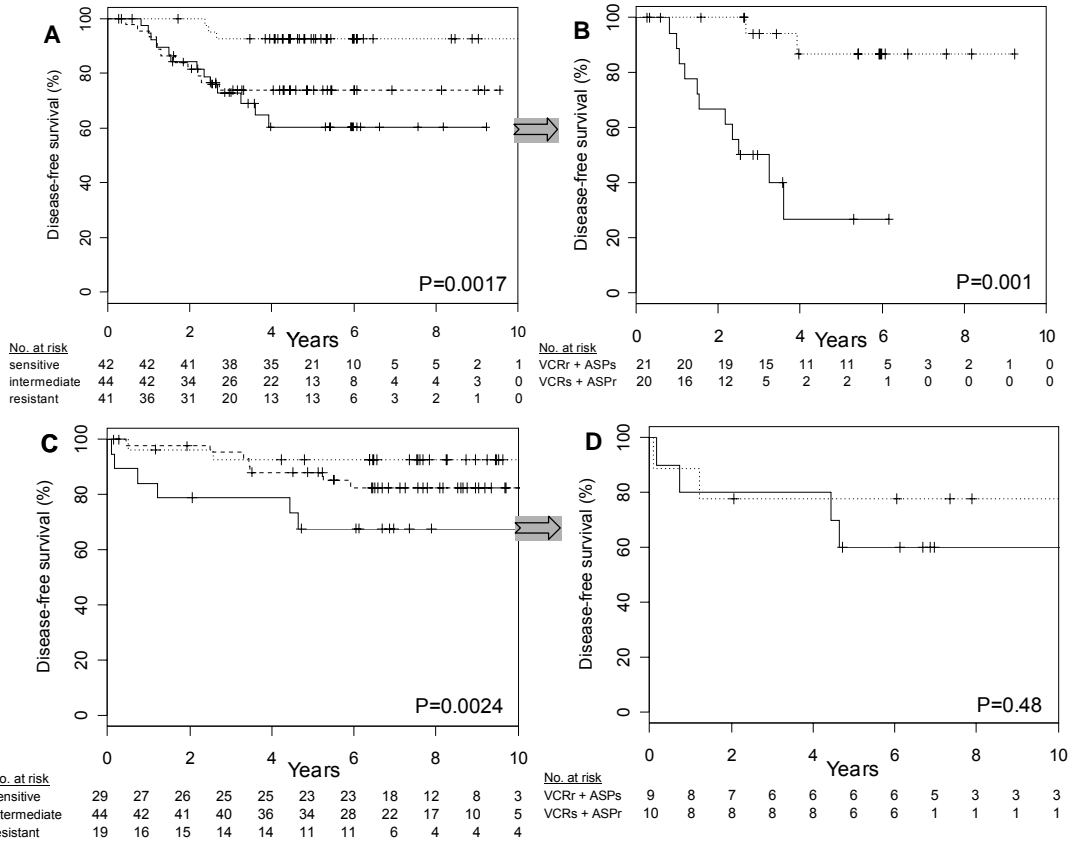
Supplemental Figure 8: Treatment outcome among VCR-ASP discordant resistant B-lineage ALL patients using the adjusted gene expression score.

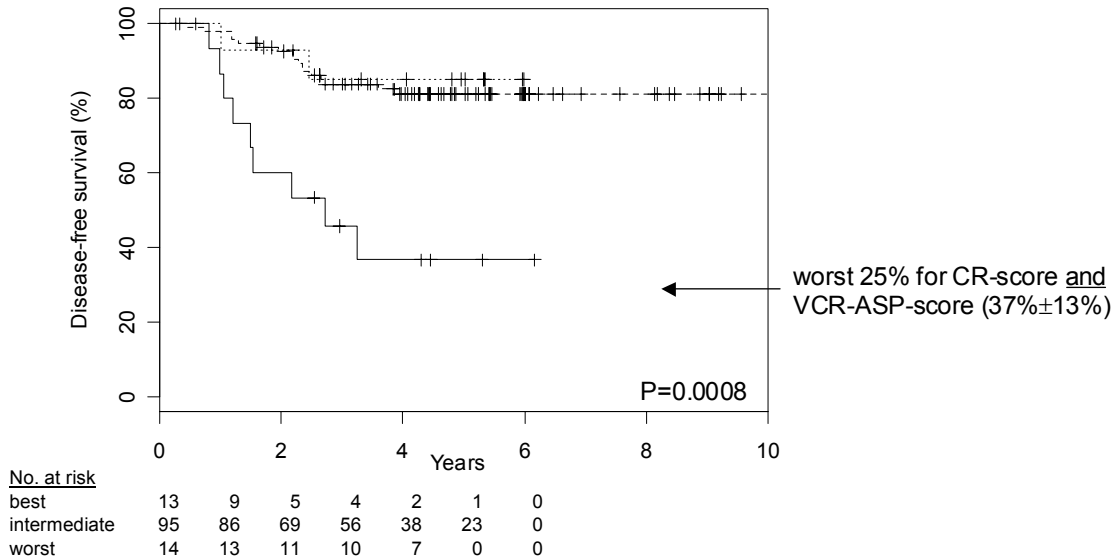
Survival curve of the probability of relapse-free survival among 129 patients using gene expression score adjusted for ALL subtypes to define VCR-sensitive plus ASP-resistance (VCR-S+ASP-R), other and VCR-resistance plus ASP-sensitive patients (VCR-R+ASP-S), are shown.



Supplemental Figure 9: VCR-ASP discordant resistance adds predictive value related to relapse-free survival to single-drug resistance of PRD-VCR-ASP-DNR in patients with ALL.

Disease-free survival using the COALL/DCOG cohort (n=127) subdivided into equal groups (1/3) using the single PVAD resistance score (panel A). After stratification by PVAD, the worst group (n=41) was further divided into equal groups (1/2) by the VCR-ASP discordant resistance gene expression (VCR-ASP) score (panel B), revealing significant further discrimination by the VCR-ASP discordant resistance gene expression pattern. Shown is the disease-free survival in St. Jude patients (n=92), divided by the single PVAD resistance score defined by the COALL/DCOG cohort (panel C). After stratification by PDAV gene expression score, the worst group (n=19) was further divided into equal groups (1/2) by the multiple drug resistance (CR) gene expression score (panel D). After stratification by PVAD gene expression score, the worst group (n=19) was further sub-grouped (1/2) by the VCR-ASP discordant resistance gene expression score (panel D). Patients whose ALL cells exhibit a gene expression pattern indicative of both worst cross-resistance and worst VCR-ASP discordant resistance scores (i.e., the worst 25% of both CR- and VCR-ASP-scores are depicted in panel E). The 5-year treatment outcome of patients with both types of multiple-drug resistance (most unfavorable quartile for both CR and VCR-ASP) was $37\% \pm 13\%$, compared to $91\% \pm 9\%$ in patients in the best quartile for both scores.





Supplemental Discussion GLUT3

A gene significantly related to cross-resistance was SLC2A3 (GLUT3), and three additional probe sets represent the SLC2A3 (GLUT3) transporter were significantly correlated with the first component of the PCA (CR-score) and over-expressed in cross-resistant ALL (P=0.003, 0.01, 0.01; R/S ratio=1.4, 1.5, 1.7). SLC2A3 is a member of the solute carrier family 2, which functions as a facilitated glucose transporter and over-expression of SLC2A3 was previously detected in the murine erythroleukemia PC-V160 subline selected for vincristine resistance (which over-expresses ABCB1 (MDR1/PgP) and ABCC1 (MRP1). These cells exhibited an increased rate of facilitative glucose transport and glucose transport protein level in the plasma paralleled active VCR-efflux and increased VCR-resistance (Martell et al., 1997). GLUT3 up-regulation has been linked to malignant transformation and postulated to be a fundamental component of the carcinogenic process (Macheda et al., 2004). Also, GLUT3 over-expression was detected in various cancers (e.g., lung, ovarian, gastric) but not in the corresponding normal tissues, and has been associated with tumor progression, worse outcome and metastasis (Younes et al., 1997a; Younes et al., 1997b). It is conceivable that more efficient glucose metabolism serves as a general survival advantage (Burgman et al., 2001; Zelzer et al., 1998).

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