

Supplementary Information  
for

**Treatment-specific Changes in Gene Expression Discriminate *in vivo* Drug Response in Human Leukemia Cells**

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<sup>1,2</sup>Meyling H. Cheok, <sup>1</sup>Wenjian Yang, <sup>3,4</sup>Ching-Hon Pui, <sup>5</sup>James R. Downing,  
<sup>6</sup>Cheng Cheng, <sup>7</sup>Clayton W. Naeve, <sup>1,4</sup>Mary V. Relling, <sup>1,4</sup>William E. Evans

The Hematological Malignancies Program, Departments of <sup>1</sup>Pharmaceutical Sciences, <sup>3</sup>Hematology-Oncology, <sup>5</sup>Pathology, <sup>6</sup>Biostatistics, and the <sup>7</sup>Hartwell Center for Bioinformatics and Biotechnology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA. The <sup>4</sup>University of Tennessee, Memphis, USA and <sup>2</sup>The University of Bonn, Germany. Correspondence and reprint requests should be addressed to W.E.E (e-mail: [william.evans@stjude.org](mailto:william.evans@stjude.org))

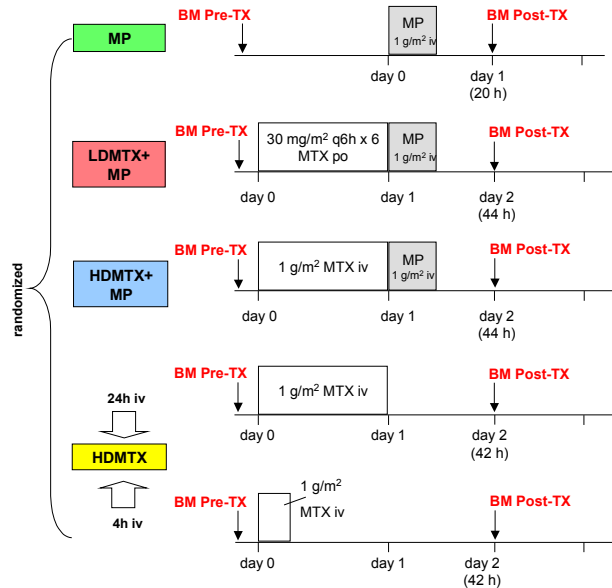
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## Patient characteristics and bone marrow sampling times

**Fig. 1: Treatment regimen and bone marrow sampling times.**

Bone marrow samples from children with acute lymphoblastic leukemia (ALL) were obtained at diagnosis (BM Pre-TX) and one day post-treatment (BM Post-TX) with mercaptopurine (MP) or high-dose methotrexate (HDMTX) given alone, or mercaptopurine in combination with either low-dose MTX (LDMTX+MP) or high-dose MTX (HDMTX+MP).



**Table 1: Patient characteristics.**

(a) The study included 60 pediatric patients with newly diagnosed ALL randomized to one of four initial treatments (“training set”). (b) Additionally, 17 new patients from the current protocol were included as an independent “test set”.

(a) treatment	n (n=60)	age at diagnosis (median in years)	sex	race	lineage	DNA ploidy	molecular translocations
MP	12	6.8	male: 7 female: 5	white: 10 black: 2 other: 0	B: 8 T: 4	hyperdiploid: 1 other: 11	MLL: 1 E2A-PBX: 0 TEL-AML: 1 BCR-ABL: 0 ND <sup>2</sup> : 10
HDMTX	22	6	male: 14 female: 8	white: 19 black: 1 other: 2	B: 19 T: 3	hyperdiploid: 4 other: 18	MLL: 0 E2A-PBX: 2 TEL-AML: 9 BCR-ABL: 0 ND <sup>2</sup> : 11
HDMTX+MP	10	5	male: 5 female: 5	white: 8 black: 1 other: 1	B: 8 T: 2	hyperdiploid: 0 other: 10	MLL: 2 E2A-PBX: 1 TEL-AML: 3 BCR-ABL: 0 ND <sup>2</sup> : 4
LDMTX+MP	16	6.1	male: 13 female: 3	white: 12 black: 1 other: 3	B: 13 T: 3	hyperdiploid: 2 other: 14	MLL: 0 E2A-PBX: 0 TEL-AML: 4 BCR-ABL: 0 ND <sup>2</sup> : 12
P value <sup>1</sup>		P=0.726	P=0.390	P=0.665	P=0.552	P=0.643	P=0.120

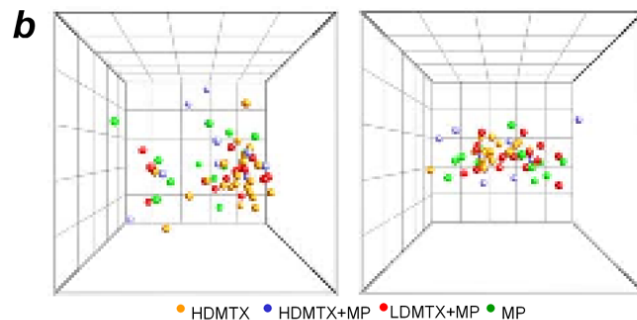
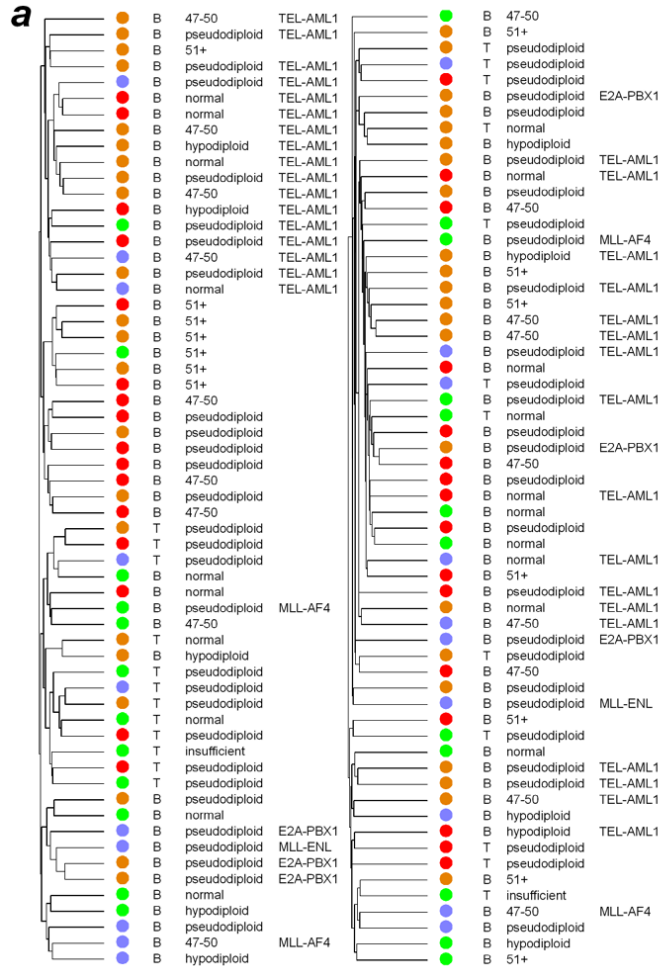
(b) treatment	n (n=17)	age at diagnosis (median in years)	sex	race	lineage	DNA ploidy	molecular translocations
HDMTX	17	5	male: 11 female: 6	white: 8 black: 5 other: 4	B: 13 T: 4	hyperdiploid: 8 other: 9	MLL: 0 E2A-PBX: 0 TEL-AML: 2 BCR-ABL: 1 ND <sup>2</sup> : 14

<sup>1</sup>P value determined by Fisher's exact test except Age by Wilcoxon's rank sum test. <sup>2</sup>ND=none detected

## Clustering of patients based on gene expression patterns

**Fig. 2: "Unsupervised" hierarchical clustering and principal component analysis (PCA).**

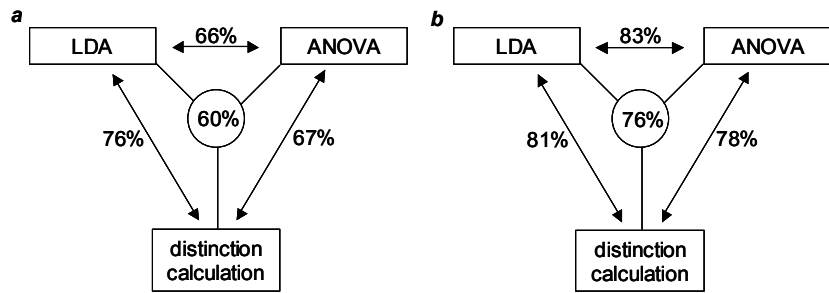
(a) We used 8222 probe sets (fold-change, referred to as "relative change" in the published manuscript) and 8002 (post-treatment) for "unsupervised" hierarchical clustering (all genes after filtering). Post-treatment expression tended to cluster samples by lineage, ploidy and molecular subtypes, whereas this was not the case for the fold-change in gene expression. (b) The top three principal components using all genes on the array, did not discriminate by treatment, based on either post-treatment gene expression or fold-change in gene expression, reflected as distances in three-dimensional-space.



## Selection of discriminating genes

**Fig. 3: Discriminating genes by different gene selection methods.**

The overlap among the 500 top-ranked probe sets identified by linear discriminant analysis (LDA), the 500 top-ranked probe sets identified by ANOVA and the 4x100 top-ranked probe sets (359 and 352 unique probe sets) identified by distinction calculation (one-versus-all) are shown as percentage overlap. The circle indicates the overlap among all three methods. **(a)** Probe sets for post-treatment expression and **(b)** probe sets for fold-change in expression.



**Table 2: Discriminating genes by fold-change and post-treatment expression.**

Shown in the following table are the 27 probe sets (26 genes, 18%) that were present in both sets of genes discriminating among the four treatments (the 150 probe sets based on fold-change and the 150 probe sets based on post-treatment expression), as determined by LDA.

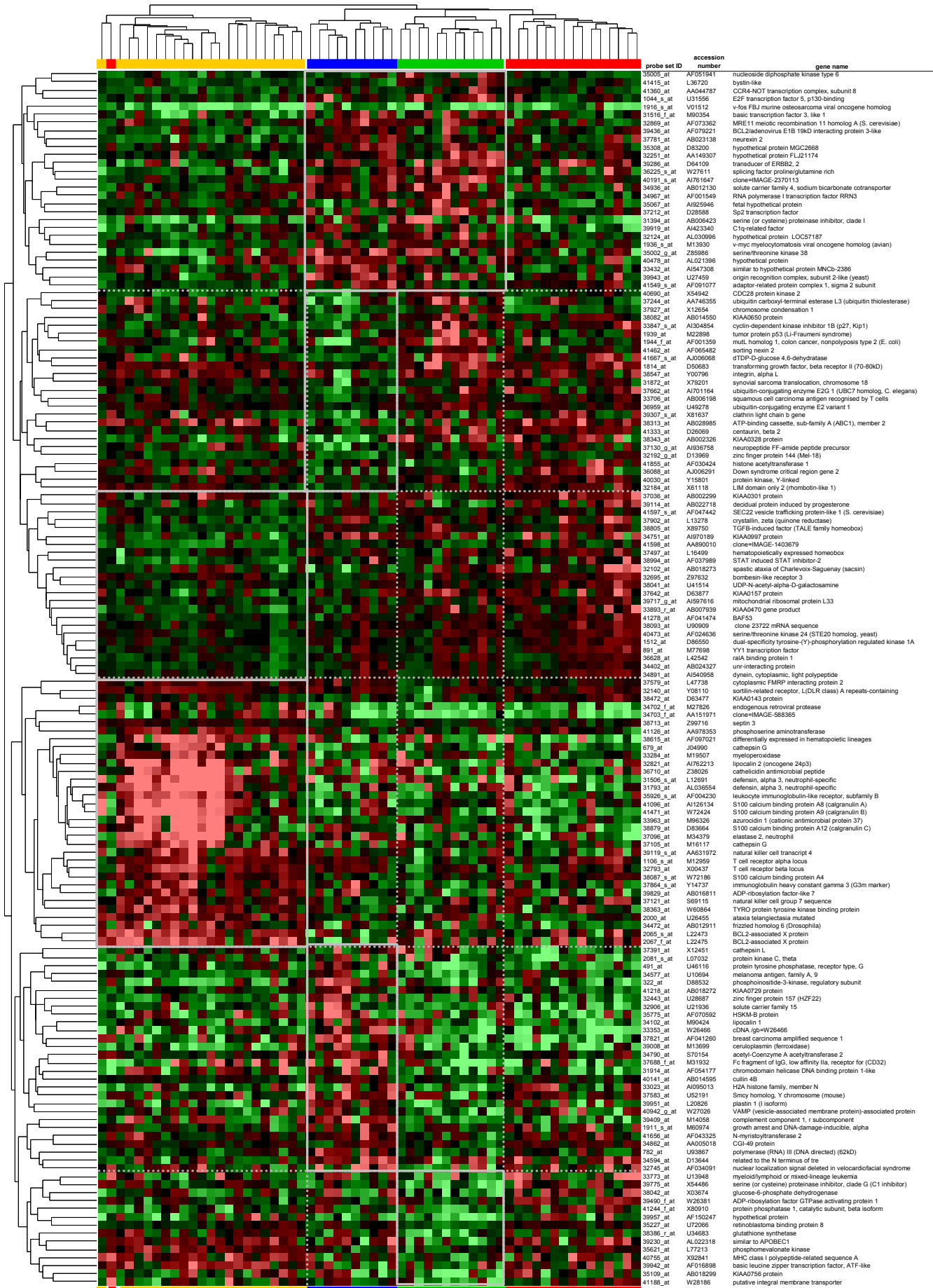
probe set ID	accession number	gene name
34967_at	AF001549	RNA polymerase I transcription factor RRN3
34577_at	U10694	melanoma antigen, family A, 9
41415_at	L36720	bystin-like
37781_at	AB023138	neurexin 2
1916_s_at	V01512	c-fos
39286_at	D64109	transducer of ERBB2, 2
37662_at	AI701164	ubiquitin-conjugating enzyme E2G 1
38082_at	AB014550	KIAA0650 protein
31872_at	X79201	synovial sarcoma translocation, chromosome 18
37244_at	AA746355	ubiquitin carboxyl-terminal esterase L3
2065_s_at	L22473	BCL2-associated X protein
38386_r_at	U34683	glutathione synthetase
2000_at	U26455	ataxia telangiectasia mutated
38615_at	AF097021	differentially expressed in hematopoietic lineages
37105_at	M16117	cathepsin G
36710_at	Z38026	cathelicidin antimicrobial peptide
37096_at	M34379	elastase 2, neutrophil
33963_at	M96326	azurocidin 1 (cationic antimicrobial protein 37)
38879_at	D83664	S100 calcium binding protein A12 (calgranulin C)
41471_at	W72424	S100 calcium binding protein A9 (calgranulin B)
41096_at	AI126134	S100 calcium binding protein A8 (calgranulin A)
32821_at	AI762213	lipocalin 2 (oncogene 24p3)
31506_s_at	L12691	defensin, alpha 3, neutrophil-specific
31793_at	AL036554	defensin, alpha 3, neutrophil-specific
37864_s_at	Y14737	immunoglobulin heavy constant gamma 3
37391_at	X12451	cathepsin L
2081_s_at	L07032	protein kinase C, theta

**Fig. 4: Hierarchical clustering of changes in expression for selected genes.**

Hierarchical clustering of fold-change in gene expression after treatment, using the 150 most discriminating probe sets (124 genes and 23 ESTs;  $P=0.017$ ) selected by LDA. Each column represents a patient ( $n=60$ ) labeled according to the treatment given (color legend at the bottom) and each row represents a gene probe set. Hierarchical clustering (top dendrogram) correctly grouped each patient into the four treatments, with one exception. Red indicates up-regulation and green indicates down-regulation in gene expression (fold-change) after treatment, according to the fold-change scale in the upper right. Solid lines enclose groups of genes showing greatest difference for the treatment indicated. These are the same data as shown in Fig. 4 of the primary manuscript, but with gene names provided. Hierarchical clustering performed after removing either one of two replicate probe sets for three genes did not cause a difference in clustering (data not shown).

# clusters of individuals

clusters of genes



HDMTX HDMTX+MP MP LDMTX+MP

treatments



**Table 3: Discriminating genes by one-versus-all distinction calculation.**

To distinguish one treatment from all other treatments, we also computed distinction values for each probe set. The 353 gene probe sets are rank-ordered according to the distinction value (DC) for each treatment [HDMTX (HD), HDMTX+MP (HDMP), LDMTX+MP (LDMP), MP], (-) indicates genes down-regulated and (+) genes up-regulated. Additionally, we performed one thousand permutations with random class labels to obtain the *P* values for each probe set. The *P* value was defined as the probability of obtaining by random assignment distinction values less than or equal to the observed. Among a large number of probe sets with *P* values <0.01, 50 probe sets with the largest distinction values were selected, listed here are the top 50 probe sets for each treatment.

probe set ID	accession number	gene name	HD DC	HDMP DC	LDMP DC	MP DC	HD <i>P</i> val.	HDMP <i>P</i> val.	LDMP <i>P</i> val.	MP <i>P</i> val.	rank
41471_at	W72424	S100 calcium binding protein A9 (calgranulin B)	0.9	-0.3	-0.5	-0.4	0.000	0.139	0.001	0.017	HD+01
35926_s_at	AF004230	leukocyte immunoglobulin-like receptor, subfamily B	0.8	-0.4	-0.4	-0.3	0.000	0.017	0.011	0.088	HD+02
31793_at	AL036554	defensin, alpha 3, neutrophil-specific	0.8	-0.3	-0.4	-0.3	0.000	0.060	0.008	0.122	HD+03
41096_at	AI126134	S100 calcium binding protein A8 (calgranulin A)	0.7	-0.4	-0.4	-0.3	0.000	0.057	0.010	0.077	HD+04
34703_f_at	AA151971	clone=IMAGE-588365	0.6	-0.1	-0.4	-0.4	0.000	0.660	0.014	0.029	HD+05
41126_at	AA978353	phosphoserine aminotransferase	0.6	-0.2	-0.3	-0.3	0.000	0.218	0.057	0.133	HD+06
37105_at	M16117	cathepsin G	0.6	-0.2	-0.4	-0.2	0.000	0.206	0.006	0.229	HD+07
38087_s_at	W72186	S100 calcium binding protein A4	0.6	-0.5	0.0	-0.3	0.000	0.008	0.712	0.059	HD+08
39119_s_at	AA631972	natural killer cell transcript 4	0.6	-0.1	-0.4	-0.2	0.000	0.737	0.012	0.133	HD+09
32793_at	X00437	T cell receptor beta locus	0.6	0.0	-0.4	-0.4	0.000	0.964	0.012	0.035	HD+10
33963_at	M96326	azurocidin 1 (cationic antimicrobial protein 37)	0.6	-0.3	-0.4	-0.2	0.001	0.095	0.008	0.430	HD+11
34702_f_at	M27826	endogenous retroviral protease	0.6	-0.1	-0.4	-0.2	0.000	0.404	0.010	0.266	HD+12
38363_at	W60864	TYRO protein tyrosine kinase binding protein	0.5	-0.2	-0.2	-0.3	0.000	0.172	0.140	0.108	HD+13
2000_at	U26455	ataxia telangiectasia mutated	0.5	-0.3	-0.4	0.0	0.000	0.071	0.038	0.570	HD+14
38615_at	AF097021	differentially expressed in hematopoietic lineages	0.5	-0.2	-0.4	0.0	0.000	0.281	0.010	0.712	HD+15
32749_s_at	AL050396	filamin A, alpha (actin binding protein 280)	0.5	-0.3	-0.2	-0.2	0.000	0.125	0.260	0.225	HD+16
2065_s_at	L22473	BCL2-associated X protein	0.5	-0.5	-0.1	-0.1	0.000	0.007	0.688	0.436	HD+17
32847_at	U48959	myosin, light polypeptide kinase	0.5	0.0	-0.3	-0.2	0.000	0.926	0.028	0.402	HD+18
39829_at	AB016811	ADP-ribosylation factor-like 7	0.5	0.1	-0.4	-0.3	0.002	0.457	0.035	0.049	HD+19
757_at	D28364	annexin II, 5 UTR	0.5	-0.2	-0.2	-0.4	0.000	0.313	0.367	0.007	HD+20
40362_at	X61498	nuclear factor of light polypeptide gene enhancer	0.5	-0.3	-0.2	-0.2	0.000	0.096	0.476	0.125	HD+21
33501_r_at	S71043	Ig alpha 2=immunoglobulin A heavy chain allotype 2	0.5	-0.1	-0.3	-0.2	0.000	0.512	0.022	0.211	HD+22
33284_at	M19507	myeloperoxidase	0.5	-0.3	-0.4	0.0	0.001	0.069	0.006	0.697	HD+23
34546_at	AI250799	defensin, alpha 4, corticostatin	0.5	-0.1	-0.3	-0.2	0.000	0.406	0.052	0.205	HD+24
40155_at	D31883	actin binding LIM protein 1	0.5	0.0	-0.3	-0.3	0.000	0.895	0.027	0.087	HD+25
38805_at	X89750	TGFB-induced factor (TALE family homeobox)	-0.8	0.2	0.3	0.3	0.000	0.395	0.003	0.402	HD-01
39286_at	D64109	transducer of ERBB2, 2	-0.6	0.2	0.2	0.5	0.000	0.218	0.154	0.021	HD-02
36628_at	L42542	ralA binding protein 1	-0.6	0.2	0.2	0.4	0.001	0.312	0.166	0.031	HD-03
34402_at	AB024327	unr-interacting protein	-0.6	0.2	0.2	0.3	0.000	0.213	0.044	0.247	HD-04
33893_r_at	AB007939	KIAA0470 gene product	-0.6	-0.1	0.4	0.3	0.000	0.569	0.004	0.117	HD-05
39024_at	AF042357	nucleoporin 98kD	-0.5	0.1	0.3	0.3	0.001	0.713	0.095	0.063	HD-06
41549_s_at	AF091077	adaptor-related protein complex 1, sigma 2 subunit	-0.5	0.6	0.2	-0.1	0.000	0.000	0.356	0.498	HD-07
41360_at	AA044787	CCR4-NOT transcription complex, subunit 8	-0.5	0.1	0.1	0.4	0.000	0.584	0.290	0.035	HD-08
37781_at	AB023138	neurexin 2	-0.5	0.6	0.1	0.2	0.000	0.002	0.528	0.243	HD-09
37642_at	D63877	KIAA0157 protein	-0.5	-0.1	0.3	0.4	0.000	0.514	0.034	0.017	HD-10
41598_at	AA890010	clone=IMAGE-1403679	-0.5	0.3	0.3	0.1	0.001	0.109	0.013	0.894	HD-11
38270_at	AF005043	poly (ADP-ribose) glycohydrolase	-0.5	0.2	0.3	0.2	0.001	0.364	0.014	0.719	HD-12
41597_s_at	AF047442	SEC22 vesicle trafficking protein-like 1	-0.5	0.3	0.3	0.2	0.000	0.180	0.079	0.427	HD-13
34751_at	AI970189	KIAA0997 protein	-0.5	0.5	0.2	0.0	0.002	0.008	0.100	0.747	HD-14
37902_at	L13278	crystallin, zeta (quinone reductase)	-0.5	0.1	0.4	0.1	0.000	0.474	0.015	0.785	HD-15
36585_at	M36341	ADP-ribosylation factor 4	-0.5	0.0	0.3	0.2	0.000	0.784	0.051	0.151	HD-16
38973_at	AB028943	HIC1-related gene on chromosome 22	-0.5	0.2	0.1	0.3	0.000	0.277	0.129	0.173	HD-17

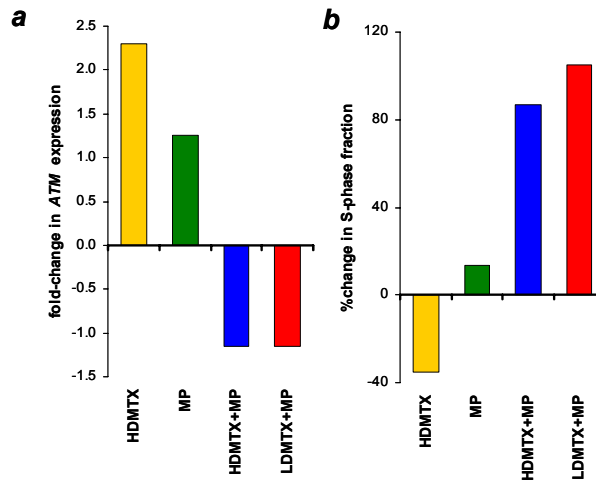
probe set ID	accession number	gene name	HD DC	HDMP DC	LDMP DC	MP DC	HD P val.	HDMP P val.	LDMP P val.	MP P val.	rank
38093_at	U90909	clone 23722 mRNA	-0.5	0.1	0.4	0.1	0.000	0.712	0.027	0.249	HD-18
31510_s_at	Z48950	H3 histone, family 3B (H3.3B)	-0.5	0.2	0.2	0.3	0.000	0.149	0.131	0.148	HD-19
40976_at	AF052432	katanin p80 (WD40-containing) subunit B 1	-0.5	0.1	0.1	0.3	0.000	0.595	0.247	0.076	HD-20
37984_s_at	M57763	ADP-ribosylation factor 6	-0.5	0.2	0.2	0.3	0.000	0.200	0.186	0.137	HD-21
39436_at	AF079221	BCL2/adenovirus E1B 19kD interacting protein	-0.5	0.5	0.1	0.2	0.000	0.012	0.753	0.148	HD-22
1512_at	D86550	tyrosine-(Y)-phosphorylation regulated kinase	-0.5	0.0	0.4	0.1	0.001	0.965	0.002	0.654	HD-23
38994_at	AF037989	STAT induced STAT inhibitor-2	-0.5	0.3	0.3	0.0	0.000	0.098	0.093	0.732	HD-24
41790_at	AL031230	glycosylphosphatidylinositol specific phospholipase	-0.5	0.2	0.3	0.0	0.001	0.213	0.005	0.829	HD-25
322_at	D88532	phosphoinositide-3-kinase, regulatory subunit	-0.3	0.8	-0.1	0.0	0.050	0.000	0.359	0.897	HDMP+01
39951_at	L20826	plastin 1 (I isoform)	-0.2	0.8	0.0	-0.2	0.112	0.000	0.878	0.173	HDMP+02
32257_f_at	AF003001	telomeric repeat binding factor (NIMA-interacting) 1	-0.2	0.7	0.0	-0.1	0.188	0.000	0.652	0.522	HDMP+03
782_at	U93867	polymerase (RNA) III (DNA directed) (62kD)	-0.1	0.7	-0.1	-0.4	0.492	0.000	0.436	0.060	HDMP+04
491_at	U46116	protein tyrosine phosphatase, receptor type, G	0.0	0.6	-0.2	-0.4	0.914	0.001	0.181	0.080	HDMP+05
37563_at	AB007871	KIAA0411 gene product	-0.1	0.6	-0.1	-0.2	0.641	0.001	0.757	0.086	HDMP+06
40478_at	AL021396	hypothetical protein	-0.1	0.6	-0.1	-0.2	0.462	0.000	0.311	0.380	HDMP+07
39489_g_at	W27720	protocadherin 9	-0.2	0.6	-0.1	0.0	0.082	0.001	0.588	0.825	HDMP+08
39008_at	M13699	ceruloplasmin (ferroxidase)	-0.1	0.6	-0.3	0.1	0.586	0.001	0.028	0.603	HDMP+09
36729_g_at	M76446	adrenergic, alpha-1D-, receptor	-0.1	0.6	-0.1	-0.2	0.614	0.002	0.607	0.182	HDMP+10
40383_at	AB023200	gene from NF2/meningioma region of 22q12	0.0	0.6	-0.2	-0.2	0.895	0.002	0.083	0.404	HDMP+11
1368_at	M27492	interleukin 1 receptor, type I	-0.1	0.6	-0.1	-0.2	0.529	0.001	0.298	0.449	HDMP+12
33353_at	W26466	32f11 Homo sapiens cDNA	0.1	0.6	-0.4	-0.1	0.330	0.002	0.005	0.573	HDMP+13
35002_g_at	Z85986	serine/threonine kinase 38	-0.4	0.6	-0.1	0.3	0.002	0.002	0.652	0.194	HDMP+14
34102_at	M90424	lipocalin 1	-0.1	0.6	-0.3	0.1	0.336	0.001	0.036	0.531	HDMP+15
36435_at	AF070670	protein phosphatase 1A	-0.1	0.6	-0.2	0.0	0.370	0.006	0.286	0.806	HDMP+16
39943_at	U27459	origin recognition complex, subunit 2-like	-0.3	0.6	-0.2	0.2	0.013	0.001	0.202	0.267	HDMP+17
39544_at	AB002351	desmuslin	-0.3	0.6	-0.1	0.2	0.030	0.001	0.255	0.089	HDMP+18
33432_at	AI547308	similar to hypothetical protein MNCb-2386	-0.1	0.6	-0.3	0.0	0.511	0.006	0.028	0.856	HDMP+19
37583_at	U52191	Smcy homolog, Y chromosome (mouse)	-0.2	0.5	0.1	-0.3	0.172	0.001	0.377	0.050	HDMP+20
33427_s_at	AF106861	attractin	-0.1	0.5	-0.1	-0.1	0.276	0.002	0.610	0.600	HDMP+21
32443_at	U28687	zinc finger protein 157 (HZF22)	-0.3	0.5	-0.1	0.0	0.062	0.007	0.493	0.981	HDMP+22
31896_at	AL050281	neuroblastoma-amplified protein	-0.2	0.5	-0.1	0.0	0.146	0.007	0.366	0.984	HDMP+23
34594_at	D13644	related to the N terminus of tre	-0.4	0.5	0.2	-0.1	0.005	0.002	0.643	0.872	HDMP+24
35308_at	D83200	hypothetical protein MGC2668	-0.4	0.5	-0.1	0.1	0.009	0.003	0.341	0.327	HDMP+25
41667_s_at	AJ006068	dTDP-D-glucose 4,6-dehydratase	-0.1	-0.7	0.2	0.3	0.313	0.000	0.063	0.137	HDMP-01
37662_at	AI701164	ubiquitin-conjugating enzyme E2G 1	-0.1	-0.6	0.3	0.2	0.281	0.000	0.058	0.135	HDMP-02
38713_at	Z99716	septin 3	0.5	-0.6	0.0	-0.1	0.000	0.002	0.714	0.786	HDMP-03
32117_at	U51698	apoptosis antagonizing transcription factor	0.0	-0.6	0.3	0.1	0.823	0.004	0.070	0.378	HDMP-04
36514_at	U66469	cell growth regulatory with ring finger domain	0.3	-0.6	-0.1	0.1	0.026	0.002	0.572	0.614	HDMP-05
38313_at	AB028985	ATP-binding cassette, sub-family A (ABC1)	0.2	-0.6	0.3	-0.2	0.121	0.003	0.084	0.272	HDMP-06
39307_s_at	X81637	clathrin light chain b gene	0.0	-0.6	0.3	0.1	0.781	0.002	0.029	0.906	HDMP-07
41826_at	W28287	KIAA1467 protein	0.1	-0.6	0.2	0.1	0.592	0.001	0.345	0.360	HDMP-08
37130_g_at	AI936758	neuropeptide FF-amide peptide precursor	0.2	-0.5	0.3	-0.1	0.208	0.006	0.247	0.901	HDMP-09
38082_at	AB014550	KIAA0650 protein	-0.1	-0.5	0.2	0.4	0.377	0.000	0.161	0.010	HDMP-10
34960_g_at	M15059	Fc fragment of IgE, low affinity II	0.1	-0.5	0.1	0.1	0.299	0.002	0.500	0.458	HDMP-11
37121_at	S69115	natural killer cell group 7 sequence	0.3	-0.5	0.1	-0.2	0.018	0.005	0.571	0.389	HDMP-12
37244_at	AA746355	ubiquitin carboxyl-terminal esterase L3	0.2	-0.5	-0.2	0.4	0.112	0.004	0.502	0.056	HDMP-13
1814_at	D50683	transforming growth factor, beta receptor II	-0.1	-0.5	0.1	0.4	0.351	0.003	0.211	0.160	HDMP-14
579_at	M95724	centromere protein C 1	0.3	-0.5	-0.1	0.1	0.035	0.007	0.671	0.670	HDMP-15
35969_at	N23137	M-phase phosphoprotein 9	0.0	-0.5	0.2	0.2	0.954	0.002	0.226	0.213	HDMP-16
33706_at	AB006198	squamous cell carcinoma antigen	0.1	-0.5	0.1	0.2	0.656	0.005	0.261	0.245	HDMP-17
41246_at	AI743134	trinucleotide repeat containing 3	0.1	-0.5	0.0	0.3	0.357	0.003	0.956	0.099	HDMP-18
31838_at	U79274	protein predicted by clone 23733	0.2	-0.5	0.1	0.0	0.196	0.004	0.583	0.854	HDMP-19
35963_at	AI201243	HLA class II region expressed gene KE2	0.2	-0.5	-0.1	0.2	0.071	0.004	0.982	0.717	HDMP-20
41398_at	AL049305	hypothetical protein FLJ21940	0.1	-0.5	0.1	0.0	0.301	0.003	0.510	0.940	HDMP-21

probe set ID	accession number	gene name	HD DC	HDMP DC	LDMP DC	MP DC	HD P val.	HDMP P val.	LDMP P val.	MP P val.	rank
40821_at	M61832	S-adenosylhomocysteine hydrolase	0.1	-0.5	0.0	0.2	0.392	0.002	0.499	0.670	HDMP-22
41277_at	AW021542	sin3-associated polypeptide, 18kD	0.0	-0.5	0.3	0.0	0.862	0.004	0.103	0.716	HDMP-23
33870_at	AB029005	chromosome 5 open reading frame 7	-0.1	-0.5	0.3	0.3	0.324	0.005	0.053	0.155	HDMP-24
32102_at	AB018273	spastic ataxia of Charlevoix-Saguenay (sacsin)	-0.1	-0.5	0.3	0.2	0.635	0.005	0.021	0.538	HDMP-25
38041_at	U41514	UDP-N-acetyl-alpha-D-galactosamine	-0.2	-0.2	0.5	-0.2	0.167	0.241	0.002	0.246	LDMP+01
32695_at	Z97632	bombesin-like receptor 3	-0.1	-0.3	0.5	-0.1	0.502	0.058	0.001	0.340	LDMP+02
40377_at	AB014582	KIAA0682 gene product	-0.3	-0.1	0.5	-0.1	0.044	0.720	0.000	0.270	LDMP+03
37036_at	AB002299	KIAA0301 protein	-0.2	-0.3	0.5	-0.1	0.246	0.102	0.012	0.899	LDMP+04
39717_g_at	AI597616	mitochondrial ribosomal protein L33	-0.4	-0.1	0.5	0.1	0.001	0.429	0.005	0.428	LDMP+05
33773_at	U13948	myeloid/lymphoid or mixed-lineage leukemia	-0.2	0.0	0.5	-0.3	0.087	0.881	0.007	0.127	LDMP+06
40030_at	Y15801	protein kinase, Y-linked	-0.1	-0.4	0.5	-0.1	0.541	0.022	0.000	0.169	LDMP+07
891_at	M77698	YY1 transcription factor	-0.5	-0.1	0.5	0.1	0.002	0.532	0.000	0.738	LDMP+08
34307_at	U81006	transmembrane 9 superfamily member 2	-0.2	-0.3	0.5	-0.1	0.120	0.136	0.000	0.568	LDMP+09
32643_at	L07956	glucan (1,4-alpha-), branching enzyme 1	-0.1	-0.2	0.5	-0.2	0.412	0.227	0.025	0.539	LDMP+10
40473_at	AF024636	serine/threonine kinase 24 (STE20 homolog, yeast)	-0.5	-0.1	0.5	0.1	0.000	0.770	0.002	0.431	LDMP+11
39490_f_at	W26381	ADP-ribosylation factor GTPase activating protein 1	-0.3	0.0	0.5	-0.1	0.030	0.961	0.002	0.414	LDMP+12
38472_at	D63477	KIAA0143 protein	0.0	-0.2	0.5	-0.3	0.815	0.332	0.003	0.064	LDMP+13
1914_at	U66838	cyclin A1	-0.1	0.0	0.5	-0.3	0.560	0.793	0.074	0.323	LDMP+14
38042_at	X03674	glucose-6-phosphate dehydrogenase	-0.1	0.1	0.5	-0.5	0.617	0.524	0.001	0.002	LDMP+15
34891_at	AI540958	dynein, cytoplasmic, light polypeptide	-0.4	0.1	0.5	0.0	0.004	0.715	0.000	0.693	LDMP+16
33457_at	AB029028	KIAA1105 protein	-0.1	-0.3	0.5	-0.2	0.551	0.125	0.003	0.169	LDMP+17
37497_at	L16499	hematopoietically expressed homeobox	-0.4	0.2	0.5	0.0	0.000	0.339	0.026	0.685	LDMP+18
41750_at	D49489	protein disulfide isomerase-related protein	-0.2	-0.2	0.4	-0.1	0.221	0.245	0.001	0.316	LDMP+19
37735_at	U31383	guanine nucleotide binding protein 10	-0.2	-0.3	0.4	0.1	0.081	0.058	0.000	0.597	LDMP+20
316_g_at	D45132	PR domain containing 2, with ZNF domain	-0.1	-0.2	0.4	-0.3	0.468	0.347	0.011	0.211	LDMP+21
2044_s_at	M15400	retinoblastoma 1 (including osteosarcoma)	-0.2	-0.3	0.4	0.0	0.162	0.082	0.012	0.697	LDMP+22
41562_at	L13689	murine leukemia viral (bmi-1) oncogene homolog	-0.3	-0.1	0.4	0.0	0.071	0.430	0.000	0.160	LDMP+23
40167_s_at	AF038187	CS box-containing WD protein	-0.1	-0.3	0.4	-0.1	0.626	0.055	0.002	0.568	LDMP+24
40607_at	U97105	dihydropyrimidinase-like 2	-0.1	-0.2	0.4	-0.2	0.564	0.304	0.005	0.179	LDMP+25
37096_at	M34379	elastase 2, neutrophil	0.6	0.0	-0.5	-0.2	0.000	0.811	0.000	0.279	LDMP-01
37391_at	X12451	cathepsin L	0.1	0.2	-0.5	0.2	0.396	0.164	0.000	0.320	LDMP-02
31914_at	AF054177	chromodomain helicase DNA binding protein 1-like	0.3	0.4	-0.5	-0.2	0.063	0.015	0.001	0.274	LDMP-03
1186_at	D49493	growth differentiation factor 10	0.1	0.2	-0.5	0.2	0.284	0.306	0.006	0.436	LDMP-04
34967_at	AF001549	RNA polymerase I transcription factor RRN3	0.0	0.2	-0.5	0.4	0.966	0.328	0.004	0.052	LDMP-05
34243_i_at	U89358	lethal (3) malignant brain tumor l(3)mbt protein	0.4	0.1	-0.5	0.0	0.009	0.696	0.003	0.846	LDMP-06
39948_at	AI693307	MAX protein	0.3	0.0	-0.5	0.1	0.029	0.824	0.010	0.998	LDMP-07
41218_at	AB018272	KIAA0729 protein	-0.1	0.3	-0.5	0.4	0.385	0.111	0.002	0.009	LDMP-08
2081_s_at	L07032	protein kinase C, theta	0.1	0.3	-0.5	0.2	0.377	0.122	0.002	0.398	LDMP-09
34472_at	AB012911	frizzled homolog 6 (Drosophila)	0.5	-0.2	-0.5	0.0	0.000	0.302	0.001	0.877	LDMP-10
38879_at	D83664	S100 calcium binding protein A12 (calgranulin C)	0.5	0.0	-0.5	-0.2	0.000	0.936	0.008	0.145	LDMP-11
39369_at	AB023152	KIAA0935 protein	0.3	0.0	-0.4	0.0	0.025	0.835	0.007	0.742	LDMP-12
32821_at	AI762213	lipocalin 2 (oncogene 24p3)	0.6	-0.1	-0.4	-0.2	0.000	0.579	0.002	0.296	LDMP-13
35023_at	U00803	fyn-related kinase	0.0	0.3	-0.4	0.1	0.846	0.077	0.012	0.471	LDMP-14
37927_at	X12654	chromosome condensation 1	0.3	-0.3	-0.4	0.3	0.069	0.105	0.006	0.048	LDMP-15
39993_at	D11466	phosphatidylinositol glycan, class A	0.2	0.1	-0.4	0.2	0.131	0.729	0.002	0.326	LDMP-16
795_s_at	X66358	cyclin-dependent kinase-like 1 (CDC2-related kinase)	0.1	0.2	-0.4	0.2	0.460	0.244	0.000	0.162	LDMP-17
40684_at	U78190	GTP cyclohydrolase I feedback regulatory protein	0.3	0.1	-0.4	0.0	0.040	0.548	0.003	0.920	LDMP-18
39919_at	AI423340	C1q-related factor	-0.1	0.4	-0.4	0.4	0.417	0.056	0.022	0.044	LDMP-19
39956_at	AF041853	kinesin family member 3A	0.1	0.0	-0.4	0.4	0.340	0.966	0.007	0.042	LDMP-20
41232_at	AL050022	DKFZP564D116 protein	0.2	0.0	-0.4	0.3	0.210	0.993	0.005	0.117	LDMP-21
32869_at	AF073362	MRE11 meiotic recombination 11 homolog A	-0.1	0.4	-0.4	0.3	0.414	0.035	0.007	0.041	LDMP-22
39201_r_at	W28760	51c8 Homo sapiens cDNA	0.2	0.3	-0.4	-0.1	0.115	0.146	0.001	0.910	LDMP-23
39527_at	AF090102	KIAA1155 protein	0.3	0.0	-0.4	0.1	0.037	0.879	0.004	0.706	LDMP-24
36105_at	M18728	carcinoembryonic antigen-related cell adhesion	0.4	0.0	-0.4	-0.2	0.002	0.897	0.003	0.538	LDMP-25

probe set ID	accession number	gene name	HD DC	HDMP DC	LDMP DC	MP DC	HD P val.	HDMP P val.	LDMP P val.	MP P val.	rank
41415_at	L36720	bystin-like	-0.3	-0.2	0.0	0.6	0.020	0.242	0.973	0.000	Mp+01
36225_s_at	W27611	splicing factor proline/glutamine rich	-0.4	0.1	0.0	0.6	0.005	0.495	0.751	0.003	Mp+02
35005_at	AF051941	nucleoside diphosphate kinase type 6	-0.2	-0.2	-0.2	0.6	0.100	0.354	0.205	0.000	Mp+03
40690_at	X54942	CDC28 protein kinase 2	-0.3	-0.2	0.1	0.6	0.014	0.296	0.274	0.003	Mp+04
39643_at	U94703	polymerase (DNA directed), gamma 2	-0.1	-0.1	-0.2	0.6	0.346	0.683	0.241	0.001	Mp+05
35943_s_at	D13317	GA binding protein transcription factor	-0.1	-0.1	-0.2	0.5	0.416	0.522	0.242	0.000	Mp+06
39637_at	U14528	solute carrier family 26 (sulfate transporter)	-0.2	-0.2	-0.1	0.5	0.155	0.277	0.721	0.000	Mp+07
41462_at	AF065482	sorting nexin 2	-0.2	-0.4	0.1	0.5	0.063	0.028	0.171	0.008	Mp+08
1199_at	D13748	eukaryotic translation initiation factor 4A, isoform 1	-0.2	-0.1	-0.1	0.5	0.279	0.635	0.587	0.006	Mp+09
39368_at	AL031668	eukaryotic translation initiation factor 2	-0.3	-0.1	0.1	0.5	0.022	0.448	0.265	0.017	Mp+10
40349_at	AL049442	clone DKFZp586N1720	-0.3	-0.1	-0.1	0.5	0.039	0.581	0.657	0.000	Mp+11
32251_at	AA149307	hypothetical protein FLJ21174	-0.4	0.0	-0.1	0.5	0.003	0.793	0.287	0.000	Mp+12
40191_s_at	AI761647	clone=IMAGE-2370113	-0.5	0.3	-0.3	0.5	0.000	0.084	0.262	0.007	Mp+13
1939_at	M22898	tumor protein p53 (Li-Fraumeni syndrome)	-0.2	-0.2	-0.1	0.5	0.093	0.262	0.936	0.008	Mp+14
35432_at	AF074723	RNA polymerase II transcriptional regulation mediator	-0.1	-0.3	0.0	0.5	0.333	0.103	0.636	0.011	Mp+15
2025_s_at	M80261	APEX nuclease (multifunctional DNA repair enzyme)	-0.1	-0.1	-0.1	0.5	0.331	0.531	0.649	0.010	Mp+16
37535_at	M27691	cAMP responsive element binding protein 1	-0.3	0.1	-0.1	0.5	0.062	0.490	0.515	0.006	Mp+17
37722_s_at	U26266	deoxyhypusine synthase	-0.3	-0.1	0.0	0.5	0.013	0.718	0.835	0.004	Mp+18
40122_at	AF037448	NS1-associated protein 1	-0.2	-0.1	-0.1	0.5	0.124	0.551	0.979	0.011	Mp+19
1936_s_at	M13930	v-myc myelocytomatosis viral oncogene homolog	-0.5	0.2	0.0	0.5	0.001	0.292	0.827	0.001	Mp+20
1944_f_at	AF001359	mutL homolog 1, colon cancer, nonpolyposis type 2	0.0	-0.4	-0.1	0.5	0.980	0.013	0.789	0.012	Mp+21
628_at	L37882	frizzled homolog 2 (Drosophila)	-0.1	-0.3	0.0	0.5	0.449	0.077	0.979	0.003	Mp+22
38414_at	U05340	CDC20 cell division cycle 20 homolog	-0.1	-0.3	0.0	0.5	0.635	0.087	0.895	0.007	Mp+23
39731_at	Z23064	RNA binding motif protein, X chromosome	-0.4	-0.1	0.2	0.5	0.003	0.626	0.123	0.011	Mp+24
32129_at	AL079314	hypothetical protein, similar to PRAJA1	-0.1	-0.1	-0.2	0.5	0.459	0.505	0.354	0.008	Mp+25
35227_at	U72066	retinoblastoma binding protein 8	0.1	0.1	0.4	-0.8	0.370	0.659	0.063	0.001	MP-01
39775_at	X54486	serine (or cysteine) proteinase inhibitor, clade G	0.2	0.0	0.4	-0.7	0.184	0.796	0.021	0.000	MP-02
38386_r_at	U34683	glutathione synthetase	0.4	0.1	0.0	-0.7	0.001	0.684	0.820	0.000	MP-03
39957_at	AF150247	hypothetical protein	0.2	-0.2	0.3	-0.6	0.103	0.293	0.108	0.000	MP-04
1106_s_at	M12959	T cell receptor alpha locus	0.4	-0.1	0.1	-0.6	0.014	0.703	0.535	0.000	MP-05
39230_at	AL022318	similar to APOBEC1	0.3	0.0	0.2	-0.6	0.053	0.999	0.436	0.001	MP-06
40755_at	X92841	MHC class I polypeptide-related sequence A	0.4	-0.2	0.1	-0.6	0.003	0.238	0.517	0.001	MP-07
32904_at	M28393	perforin 1 (pore forming protein)	0.2	0.1	0.2	-0.6	0.217	0.634	0.177	0.002	MP-08
34660_at	AI142565	ribonuclease, RNase A family, k6	0.3	0.2	-0.1	-0.6	0.029	0.319	0.382	0.004	MP-09
41188_at	W28186	putative integral membrane transporter	0.1	0.0	0.3	-0.6	0.588	0.807	0.038	0.003	MP-10
35621_at	L77213	phosphomevalonate kinase	0.6	-0.2	0.0	-0.6	0.000	0.158	0.810	0.004	MP-11
37980_at	U03644	CBF1 interacting corepressor	0.1	0.1	0.2	-0.5	0.378	0.572	0.374	0.002	MP-12
41855_at	AF030424	histone acetyltransferase 1	0.1	-0.1	0.4	-0.5	0.559	0.638	0.018	0.000	MP-13
35109_at	AB018299	KIAA0756 protein	0.2	-0.1	0.2	-0.5	0.106	0.745	0.289	0.008	MP-14
37624_at	M29458	carbonic anhydrase III, muscle specific	0.2	0.2	0.0	-0.5	0.231	0.329	0.864	0.001	MP-15
40942_g_at	W27026	VAMP (vesicle-associated membrane protein)	0.0	0.3	0.2	-0.5	0.869	0.059	0.494	0.009	MP-16
37967_at	AF000424	lymphocyte antigen 117	0.3	-0.1	0.1	-0.5	0.040	0.477	0.780	0.003	MP-17
36155_at	D87465	KIAA0275 gene product	0.3	0.3	-0.2	-0.5	0.022	0.164	0.185	0.001	MP-18
31556_at	X61072	T cell receptor, clone IGRA17	0.3	0.0	0.1	-0.5	0.078	0.799	0.612	0.014	MP-19
32140_at	Y08110	sortilin-related receptor	0.4	0.2	0.0	-0.5	0.006	0.345	0.723	0.003	MP-20
36710_at	Z38026	cathelicidin antimicrobial peptide	0.5	-0.1	-0.2	-0.5	0.001	0.622	0.076	0.031	MP-21
41400_at	K02581	thymidine kinase 1, soluble	0.2	0.1	0.1	-0.5	0.150	0.777	0.632	0.005	MP-22
40141_at	AB014595	cullin 4B	0.0	0.4	0.1	-0.5	0.931	0.019	0.653	0.003	MP-23
38968_at	AB005047	SH3-domain binding protein 5	0.1	0.1	0.2	-0.5	0.591	0.523	0.200	0.004	MP-24
34965_at	AF031824	cystatin F (leukocystatin)	0.4	-0.1	0.0	-0.5	0.003	0.462	0.605	0.018	MP-25

**Fig. 5: Treatment-induced changes in percentage of ALL cells in S-phase.**

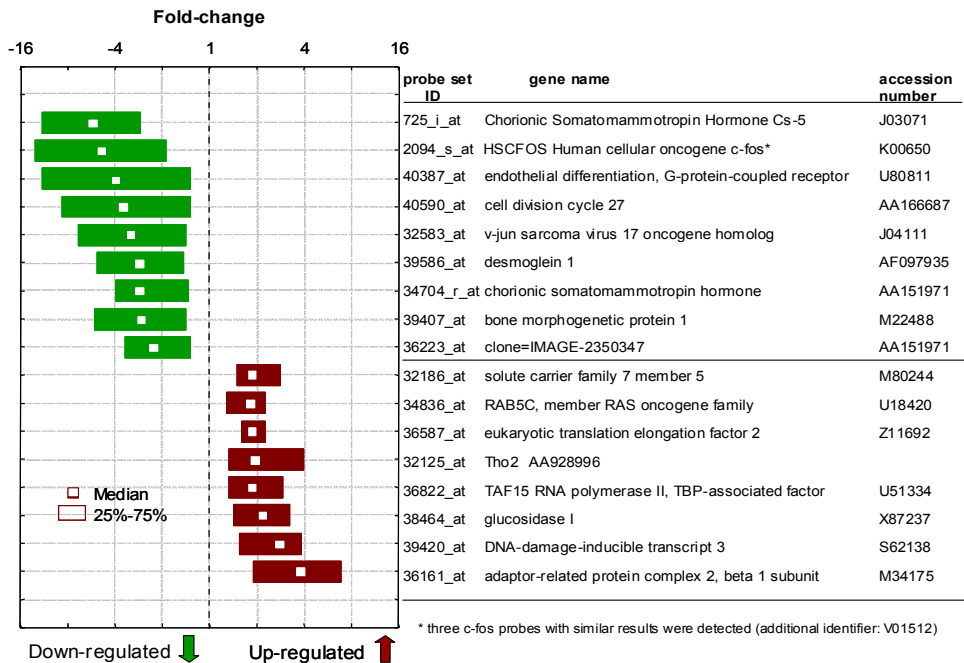
Treatment-induced changes in percentage of ALL cells in S-phase of the cell cycle. Percentages of bone marrow leukemia cells in S-phase from bone marrow leukemia blast at diagnosis and post-treatment were available for 23 of the 60 patients analyzed in this study. Propidium-iodide stained DNA content was measured by flow cytometry with the Coulter EPICS-V flow cytometer (Coulter Electronics, Inc., Hialeah, Florida). The computer program PEAK was used to calculate the percentages of cells in the G0/G1, S, G2+M phase. **(a)** The median fold-change of *ATM* expression in the four treatment groups: HDMTX, n=7, median= +2.3, range (-1.2 to 3.2); MP, n=6, median= +1.3, range (-3.0 to 2.6); HDMTX+MP, n=5, median= -1.1, range (-2.3 to 1.3); LDMTX+MP, n=5, median= -1.1, range (-2.0 to 1.3). *ATM* induction was significantly greater after HDMTX as compared to the other three treatments ( $P=0.007$ , Wilcoxon). **(b)** The median change in %S-phase of ALL cells from pre- to post-treatment in the four treatment groups: HDMTX, median= -35, range (-67 to 102); MP, median= +13.6, range (-27 to 95); HDMTX+MP, median= +87, range (-88 to 386); LDMTX+MP, median= +105, range (-38 to 187). The difference in change of %S-phase was statistically significant when the HDMTX group was compared to the other three treatments combined ( $P=0.033$ , Wilcoxon).



## Common gene expression changes after all treatments

**Fig. 6: Commonly regulated genes after all four initial treatments.**

Displayed are the median and the inter-quantile range of the 17 genes that exhibited higher expression after treatment (red) or lower expression after treatment (green), in at least 70% of patients, across all four treatments (Wilcoxon's rank sum test  $P<0.01$ ).



**Table 4 (a) and (b): Changes in gene expression after single agent chemotherapy versus the same agents in combination**

**(a)** Of the 97 gene probe sets that changed by more than 50% in at least 70% of patients after treatment with HDMTX alone, 38 increased and 59 decreased. As shown in Table 4 (a) below, only 21.1% of genes (8/38) that were up-regulated after HDMTX alone were also up-regulated when HDMTX was given with MP. Further, only 11.9% of genes (7/59) were down-regulated after HDMTX alone and also down-regulated after HDMTX when given with MP. The genes that concordantly changed after HDMTX alone and after HDMTX+MP are listed below. Negative numbers indicate a decrease and positive an increase in expression.

(a) probe set ID	accession number	gene name	median FC HDMTX	median FC HDMTX+MP
36161_at	M34175	adaptor-related protein complex 2, beta 1 subunit	5.7	3.6
37277_at	U80017	baculoviral IAP repeat-containing 1	2.6	2.4
38819_at	U33635	PTK7 protein tyrosine kinase 7	2.5	2.4
36651_at	X15525	acid phosphatase 2, lysosomal	2.5	1.9
40123_at	D87435	golgi-specific brefeldin A resistance factor 1	2.2	2.0
34279_at	AL050141	hypothetical protein FLJ20719	2.0	2.5
32125_at	AA928996	Tho2	1.9	3.0
38464_at	X87237	glucosidase I	1.9	3.2
36432_at	AL079298	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	-1.8	-2.0
35074_at	AF004715	jerky homolog-like (mouse)	-1.9	-2.5
36246_at	Z35309	adenylate cyclase 8 (brain)	-2.2	-2.8
32413_at	M13934		-2.8	-2.4
32583_at	J04111	v-jun sarcoma virus 17 oncogene homolog (avian)	-3.5	-2.3
725_i_at	J03071		-4.0	-5.5
1915_s_at	V01512		-5.7	-2.4

**(b)** Of the 197 probe sets that changed by more than 50% in at least 70% of patients after treatment with MP alone 40 increased and 157 decreased. As shown in Table 4 (b) below, only 17.5% of genes (7/40) that were up-regulated after MP alone were also up-regulated when MP was given with HDMTX and only 11.4% of genes (18/157) that were down-regulated after MP alone were also down-regulated after MP when given with HDMTX. The genes that concordantly changed after MP alone and after HDMTX+MP are listed below. Negative numbers indicate a decrease and positive an increase in expression.

(b) probe set ID	accession number	gene name	median FC MP	median FC HDMTX+MP
36161_at	M34175	adaptor-related protein complex 2, beta 1 subunit	4.0	3.6
32125_at	AA928996	Tho2	2.5	3.0
35436_at	L06147	golgi autoantigen, golgin subfamily a, 2	2.1	2.5
34836_at	U18420	RAB5C, member RAS oncogene family	2.0	1.9
36822_at	U51334	TAF15 RNA polymerase II, TATA box binding protein	2.0	1.9
37277_at	U80017	baculoviral IAP repeat-containing 1	2.0	2.4
38915_at	AB011135	KIAA0563 gene product	2.0	1.9
31652_at	AB023217	KIAA1000 protein	-1.9	-2.2
41117_s_at	AB016243	solute carrier family 9 (sodium/hydrogen exchanger)	-2.0	-1.7
38146_at	AB011107	zinc finger protein 387	-2.1	-3.5
940_g_at	D12625	neurofibromin 1	-2.1	-1.7
31785_f_at	U92817	unnamed HERV-H protein	-2.3	-2.5
34702_f_at	M27826	chorionic somatomammotropin hormone 2	-2.3	-2.9

(b) probe set ID	accession number	gene name	median FC MP	median FC HDMTX+MP
41303_r_at	AI378632	Homo sapiens mRNA; cDNA DKFZp564P233	-2.5	-3.6
450_g_at	U66469	cell growth regulatory with ring finger domain	-2.5	-1.9
40590_at	AA166687	cell division cycle 27	-2.8	-7.7
31529_at	X99141	keratin, hair, basic, 3	-3.0	-2.1
39407_at	M22488	bone morphogenetic protein 1	-3.0	-4.3
33047_at	AI971169	ESTs, Highly similar to BCL2-like 11	-3.2	-2.1
34704_r_at	AA151971	chorionic somatomammotropin hormone 2	-3.5	-3.0
40387_at	U80811	endothelial differentiation,G-protein-coupled receptor	-3.5	-3.7
32583_at	J04111	v-jun sarcoma virus 17 oncogene homolog (avian)	-3.7	-2.3
39586_at	AF097935	desmoglein 1	-4.0	-3.0
1915_s_at	V01512		-4.9	-2.4
725_i_at	J03071		-9.2	-5.5

**Table 5 (a) and (b): Human leukemia cell lines differ from primary ALL cells.**

(a) When the treatment with HDMTX alone (12 nM x 24 h plus 18 h drug-free media) was recapitulated (i.e., comparable level of cytotoxicity), with two human ALL cell lines *in vitro* (i.e., B-lineage Nalm6 [N.MTX] and T-lineage CEM [C.MTX]), we found very little overlap in the genes that changed after treatment in the cell lines compared to the primary leukemia cells in patients. Specifically, only seven out of the 97 genes (7.2%) that changed by more than 50% in at least 70% of patients after HDMTX also changed on average by more than 50% in the cell lines. Table 5 (a) lists the genes that concordantly change after HDMTX in cell lines and in patients, negative numbers indicate a decrease and positive an increase in expression.

(a) probe set ID	accession number	gene name	median FC N.MTX	median FC C.MTX	median FC HDMTX
32264_at	L23134	granzyme M (lymphocyte met-ase 1)	1.9	1.6	2.9
36591_at	X06956	tubulin, alpha 1 (testis specific)	1.9	1.2	1.9
33143_s_at	U81800	solute carrier family 16 (monocarboxylic acid transporters),	1.5	1.7	3.6
2067_f_at	L22475	BCL2-associated X protein	7.0	1.0	3.7
2001_g_at	U26455	ataxia telangiectasia mutated	2.1	1.6	2.5
35692_at	AL080235	Ras-induced senescence 1	-2.6	-1.0	-2.5
1916_s_at	V01512	c-fos FBJ murine osteosarcoma viral oncogene homolog	-1.4	-1.9	-11.3

(b) When the treatment with MP alone (10  $\mu$ M x 24 h) was recapitulated with two human ALL cell lines *in vitro* (i.e., B-lineage Nalm6 [N.MP] and T-lineage CEM [C.MP]), we found very little overlap in the genes that changed after treatment in the cell lines compared to the primary leukemia cells in patients. Only 27 of the 197 genes (13.7%) changed in a consistent manner by more than 50% after MP treatment of cell lines and primary cells *in vivo*. Table 5 (b) lists the genes that concordantly changed after MP in cell lines and in patients as negative numbers indicate a decrease and positive an increase in expression.

(b) Probe set ID	accession number	Gene name	Median FC N.MP	Median FC C.MP	Median FC MP
37881_at	AF100907	growth differentiation factor 11	2.6	3.7	2.1
38547_at	Y00796	integrin, alpha L (antigen CD11A (p180), lymphocyte function	4.9	-1.4	1.7
39286_at	D64109	transducer of ERBB2, 2	1.9	3.0	3.0
40329_at	AL031228	ring finger protein 1	1.3	3.2	1.7
41743_i_at	AF061034	tumor necrosis factor alpha-inducible cellular protein	1.7	1.5	2.3
34335_at	AI765533	ephrin-B2	1.6	1.5	1.7

(b) Probe set ID	accession number	Gene name	Median FC N.MP	Median FC C.MP	Median FC MP
34818_at	X96381	ets variant gene 5 (ets-related molecule)	2.5	1.5	2.0
40951_at	AL049250		1.7	1.7	1.9
292_s_at	L29219	CDC-like kinase 1	1.1	2.5	1.7
31777_at	AF006464	muscle, skeletal, receptor tyrosine kinase	1.2	-5.7	-2.6
33069_f_at	U06641	UDP glycosyltransferase 2 family, polypeptide B15	-2.5	1.1	-1.7
34068_f_at	X86174	synovial sarcoma, X breakpoint 1	-1.1	-2.5	-2.8
35081_at	D14838	fibroblast growth factor 9 (glia-activating factor)	-1.0	-4.9	-2.3
35109_at	AB018299	KIAA0756 protein	-5.3	-1.0	-2.6
37871_at	X68830	islet amyloid polypeptide	-3.0	-0.9	-2.0
40322_at	D12763	interleukin 1 receptor-like 1	-1.1	-4.6	-2.1
40387_at	U80811	endothelial differentiation, lysophosphatidic acid	-4.3	1.4	-3.5
32083_at	AF027826	transmembrane 7 superfamily member 1 (upregulated in kidney)	-1.0	-10.6	-3.2
35178_at	W27944	Wnt inhibitory factor-1	-6.5	1.1	-5.3
39407_at	M22488	bone morphogenetic protein 1	-1.2	-3.7	-3.0
32834_r_at	AF013591	sudD (suppressor of bimD6, Aspergillus nidulans) homolog	-3.7	-1.1	-3.0
39448_r_at	W27095	B7 protein	-1.9	-1.3	-1.6
41244_f_at	X80910	protein phosphatase 1, catalytic subunit, beta isoform	-2.6	-1.7	-2.1
32531_at	X52947	gap junction protein, alpha 1, 43kD (connexin 43)	-1.2	-5.7	-3.2
32583_at	J04111	v-jun sarcoma virus 17 oncogene homolog (avian)	2.0	-16.0	-3.7
1152_i_at	J00117	chorionic gonadotropin, beta polypeptide	-2.8	-2.3	-6.1
618_at	M26167	platelet factor 4 variant 1	-2.1	-4.3	-4.0

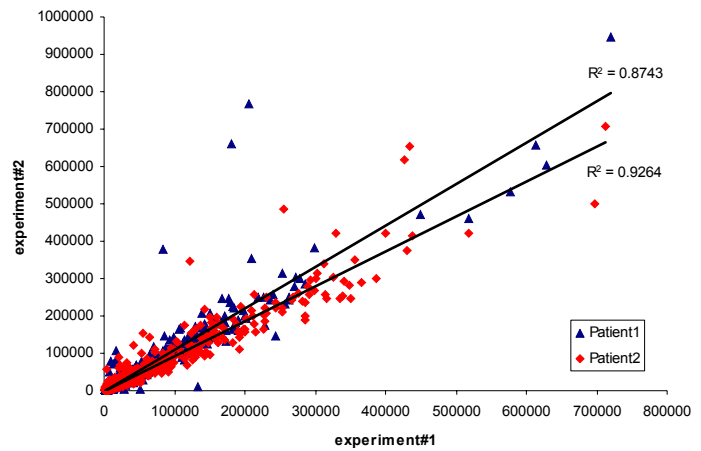
## RNA source, RNA quality and array reproducibility

We assessed total RNA integrity by electrophoresis using the Agilent Bioanalyzer (Agilent, Palo Alto, California). Additional inclusion criteria for accepting expression array results that were at least 10% of genes on an array were expressed ("present call" by MAS 5.0), GAPDH and Actin 3'/5' ratio less than 5, and a scaling factor within four standard deviations of the mean of all 120 chips analyzed in this study. We isolated mononuclear (leukemia) cells from each bone marrow aspirate by centrifugation over Ficoll. The median percent blasts (post-Ficoll) was 97% in the diagnostic bone marrow samples and 95% in post-treatment samples. All post-treatment samples, except four, had greater than 65% blasts. The four with less than 65% blasts (i.e. 41%, 49%, 60%, 62%) had 78%, 94%, 94% and 98% blasts in their diagnostic samples. The percent blast was not determined in three post-treatment samples, but all had greater than 98% blasts at diagnosis. While differences in post-treatment expression could be influenced by selection of cells that favored survival after treatment, the delayed cytotoxicity of these antimetabolites, coupled with the modest drop in circulating blast counts at the time the post-treatment samples were obtained (median 9.7% decrease across all samples), indicate that selection could not explain the magnitude of changes in gene expression that we observed. Furthermore, the percentage of ALL blasts in the pre- and post-treatment samples did not differ (median of 97% at diagnosis versus 95% post-treatment).

We extensively assessed reproducibility of gene expression within our facility, as previously described (*Cancer Cell* 1, 133-143 (2002)). Additionally, we tested duplicate cryopreserved bone marrow samples from two patients obtained after treatment; RNA was extracted approximately six months apart. Replicate analysis of the same sample produced similar results, as depicted in Fig. 7 (i.e.,  $R^2=0.87$  and  $0.92$ ).

**Fig. 7: Concordance between replicate gene expression experiments.**

Expression level of two separate aliquots of post-treatment bone marrow, obtained from two different patients, were extracted and analyzed independently, revealing a high level of concordance between replicate analyses.



### TaqMan real-time RT-PCR (RT-PCR)

To further establish the validity of gene expression determined by microarray analyses we performed two additional experiments to validate the microarray analysis. (1) We determined gene expression by real-time RT-PCR (reverse transcription polymerase chain reaction) in four randomly selected post-treatment patient samples for three genes (*AP1S2*, *BAX*, and *RBBP8*) and (2) we determined the fold-change in gene expression by both RT-PCR for three genes in five paired patient samples (in both pre- and post treatment sample).

(1) We used TaqMan Universal PCR Master Mix Kit and the 7900-sequence-detection system (Applied Biosystems, Foster City, California). We designed primers and probes (Table 6) with Primer Express 2.0 software (Applied Biosystems, Foster City, California) and used the housekeeping gene *RNase P* (Applied Biosystems, Foster City, California) for normalization. One  $\mu$ g of total RNA was treated with DNase I and reverse transcribed using Superscript II RNase H<sup>-</sup> reverse transcriptase and oligo dT primers (Invitrogen, Carlsbad, California). Additionally, we included controls that contained either no template or no reverse transcriptase, as negative controls in each run. We used aliquots (0.5  $\mu$ l) of RT reaction mixture (20  $\mu$ l) for quantification of *AP1S1*, *BAX*, *RBBP8* and *RNase P* gene expression.

**Table 6: Primers and probes used for real-time (TaqMan) RT-PCR**

(1)	sequence	start	length
<b><i>AP1S2</i> (NM_003916)</b>			
Forward Primer	5'-AAGCAATTGAGCAGGCTGATC-3'	395	21
Reverse Primer	5'-TCAGTCCAATTTCTTCAAGAACACTAC-3'	469	27
Probe	5'-6FAM-ACTGCAGGAGGAAGCTGAAACCCCA-TAMRA-3'	417	25
<b><i>BAX</i> (NM_004f324)</b>			
Forward Primer	5'-TGGAGCTGCAGAGGATGATTG-3'	221	21
Reverse Primer	5'-GAAGTTGCCGTCAGAAAACATG-3'	315	22
Probe	5'-6FAM-AGAGGTCTTTTTCCGAGTGGCAGCTG-TAMRA-3'	267	26
<b><i>RBBP8</i> (NM_002894)</b>			
Forward Primer	5'-GAACCCCATGTCCGATACA-3'	838	20
Reverse Primer	5'-GGATGAGTTGAAGACTTGAAAACCTTT-3'	936	26
Probe	5'-6FAM-ACATACTAAATTGGAGCACTCTGTGTGCAAATG-TAMRA-3'	868	35

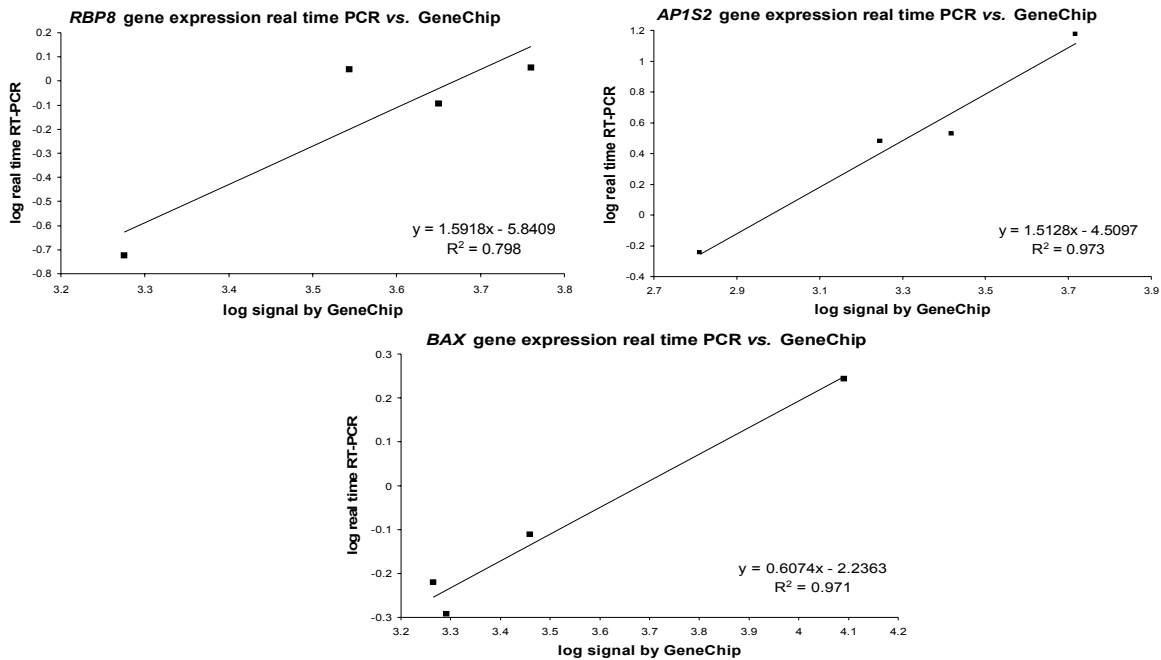
(2)	sequence	assay-on-demand ID
<b><i>ATM</i></b> Probe	5'-6FAM-CAACGCGCAGGACTTCTGCACGGAC-TAMRA-3'	Hs00175892_m1
<b><i>BAX</i></b> Probe	5'-6FAM-AAACTGGTGCTCAAGGCCCTGTGCA-TAMRA-3'	Hs00180269_m1
<b><i>FOS</i></b> Probe	5'-6FAM-TATCAACCAAAGGCCTCTTGTATC-TAMRA-3'	Hs00170630_m1

We checked primer quality by conventional PCR for amplification of the correct size and sequence of all transcripts. The total volume of the PCR reaction was 50  $\mu$ l, containing 0.5  $\mu$ l of RT-product, 400 nM each of the forward and reverse primers, 250 nM of probe, and 1X master mix. We used following thermal cycling parameters: two minutes at 50°C (activation of UNG enzyme to remove the carry-over PCR products), ten minutes at 95°C to activate AmpliTaq Gold DNA polymerase, 15 seconds at 95°C to denature and one minute at 60°C for annealing and extension, for a total of 45 cycles.

To estimate the amount of each of the three mRNAs in the four patient samples, we used linear regression analysis based on a standard curve representing six serial dilutions of cDNA made from the CEM human leukemia cells (American Type Culture Collection, Rockville, Maryland). In the standard curve, we plotted fluorescent signal intensities against the number of PCR cycles on a semi-logarithmic scale. Using CEM cDNA as standard, we achieved a high degree of linearity. We analyzed all unknown samples in triplicates in parallel with a standardization series using CEM cDNA. Based on the  $C_T$  value and the corresponding standard curve the relative quantity of the specific mRNA for each sample was calculated. We plotted normalized and log-transformed real-time RT-PCR gene expression level (*AP1S2*, *BAX*, and *RBBP8*) to the log-transformed signal from the Affymetrix MAS 5.0 output. The correlation between real-time RT-PCR and Affymetrix GeneChip was statistically significant (overall  $P=0.017$ , spearman rank test), as shown in Fig. 8 below and  $R^2$  of 0.973, 0.798, 0.971 for *AP1S2*, *BAX*, and *RBBP8*, respectively, thereby confirming expression levels determined by the gene expression array.

**Fig. 8: Real-time (TaqMan) RT-PCR vs. Affymetrix GeneChip®**

Real-time (TaqMan) RT-PCR results are plotted versus expression levels determined by Affymetrix GeneChip® results for *AP1S2*, *BAX* and *RBBP8*, in four patients.

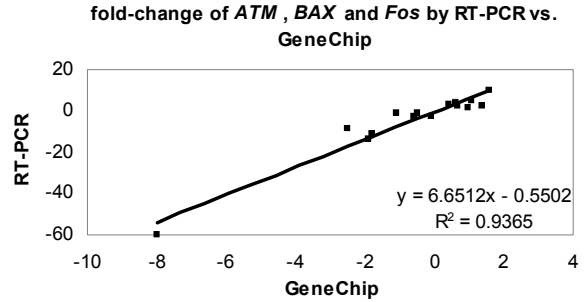


(2) We utilized the Assay-on-demand™ (part number 4331182, assay ID: ATM, Hs00175892\_m1; BAX, Hs00180269\_m1; FOS, Hs00170630\_m1; Applied Biosystems, Foster City, California) according to the manufactures protocol (Table 6). To determine the relative quantity of gene expression of *ATM*, *BAX*, *FOS* in five pre- and five corresponding post-treatment patient samples

we used the standard curve method and beta-actin, 18S-ribosomal RNA and RNase P served as internal controls. We computed fold-change in expression for each gene based on post- to pre-treatment ratio as determined by RT-PCR, and we compared these fold-change values to the fold-change as determined by microarray analysis. As depicted in Fig. 9 below, the fold-change values determined by the two independent methods were highly correlated ( $R^2=0.937$ ).

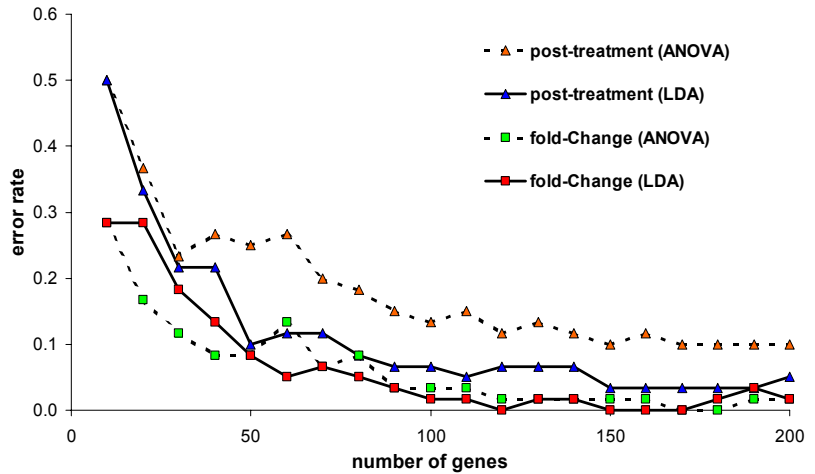
**Fig. 9: Fold-change RT-PCR versus Affymetrix GeneChip®**

RT-PCR results are plotted versus fold-change determined by Affymetrix GeneChip® results for *ATM*, *BAX* and *FOS* in five patients



**Fig. 10: Leave-one-out cross-validation of treatment classification.**

Leave-one-out cross-validation using support-vector-machine (SVM) as the classifier, determined that prediction error rates were the smallest using linear discriminant analysis with variance (LDA) compared to ANOVA as the gene selection method. We constructed SVMs using the top-ranked discriminating genes selected by LDA or ANOVA. Leave-one-out cross-validation shows that classification error rate decreased with increasing number of genes, reaching zero only for fold-change in gene expression.



**Table 7: Genes discriminating each treatment from all others.**

Listed are the genes that were present in all three pair wise distinction calculations for each treatment group (all genes,  $P<0.05$ ). There were 37 genes selected in common in each of the three pair wise comparisons of HDMTX vs. MP, HDMTX vs. HDMTX+MP and HDMTX vs. LDMTX+MP. There were 21 probe sets in common in the three pair wise comparisons for HDMTX+MP, 29 probe sets for MP alone and 9 probe sets for LDMTX+MP. The number assigned within each treatment group (e.g., HDMTX01) represents the rank of each gene by its distinction value in discriminating each treatment from the other treatments. The median fold-change in the individual treatment group (FC TX group) and the median fold-change in the other treatments combined (FC other TX groups) are shown for each gene, with minus (-) indicating genes that exhibited a decrease in expression, whereas a positive number indicates those genes that exhibited an increase in expression after treatment.

rank <sup>1</sup> by TX group	probe set ID	accession number	gene name	median FC TX group	median FC other TX groups
HDMTX01	37781_at	AB023138	neurexin 2	-1.7	1.1
HDMTX02	35926_s_at	AF004230	leukocyte immunoglobulin-like receptor	2.4	-1.7
HDMTX03	34751_at	AI970189	KIAA0997 protein	-1.4	1.5
HDMTX04	2067_f_at	L22475	BCL2-associated X protein	2.5	1.1
HDMTX05	41471_at	W72424	S100 calcium-binding protein A9 (calgranulin B)	4.6	-1.9
HDMTX06	38994_at	AF037989	STAT induced STAT inhibitor-2	-1.6	1.0
HDMTX07	31793_at	AL036554	defensin, alpha 3, neutrophil-specific	5.4	-1.4
HDMTX08	41096_at	AI126134	S100 calcium-binding protein A8 (calgranulin A)	4.2	-1.3
HDMTX09	38363_at	W60864	TYRO protein tyrosine kinase binding protein	1.9	-1.1
HDMTX10	41598_at	AA890010	SEC22, vesicle trafficking protein	-1.7	-1.2
HDMTX11	32749_s_at	AL050396	filamin A, alpha (actin-binding protein-280)	2.1	1.4
HDMTX12	31506_s_at	L12691	defensin, alpha 3, neutrophil-specific	9.4	-1.4
HDMTX13	39286_at	D64109	transducer of ERBB2, 2	-1.4	2.0
HDMTX14	35621_at	L77213	phosphomevalonate kinase	1.6	1.0
HDMTX15	41126_at	AA978353	phosphoserine aminotransferase	1.7	1.0
HDMTX16	402_s_at	X69819	intercellular adhesion molecule 3	1.8	1.1
HDMTX17	40362_at	X61498	nuclear factor of kappa light polypeptide gene enhancer	2.4	1.1
HDMTX18	36789_f_at	AF025534	leukocyte immunoglobulin-like receptor	1.8	-1.7
HDMTX19	37984_s_at	M57763	ADP-ribosylation factor 6	-1.9	-1.1
HDMTX20	38973_at	AB028943	HIC1-related gene on chromosome 22	-1.2	1.6
HDMTX21	679_at	J04990	cathepsin G	1.7	-1.5
HDMTX22	32227_at	X17042	proteoglycan 1, secretory granule	1.8	-1.4
HDMTX23	38999_s_at	M86707	N-myristoyltransferase 1	-1.1	1.5
HDMTX24	34965_at	AF031824	cystatin F (leukocystatin)	1.5	-1.2
HDMTX25	40877_s_at	AF041080	D15F37 (pseudogene)	1.1	1.5
HDMTX26	39119_s_at	AA631972	natural killer cell transcript 4	2.1	-1.3
HDMTX27	1403_s_at	M21121	small inducible cytokine A5 (RANTES)	1.7	-1.2
HDMTX28	34702_f_at	M27826	endogenous retroviral protease	1.1	-2.7
HDMTX29	33371_s_at	U59877	RAB31, member RAS oncogene family	1.4	-1.6
HDMTX30	37105_at	M16117	cathepsin G	2.4	-1.1
HDMTX31	31510_s_at	Z48950	H3 histone, family 3B (H3.3B)	-1.5	-1.2
HDMTX32	33661_at	U66589	ribosomal protein L5	-1.8	1.0
HDMTX33	40450_at	L09561	polymerase (DNA directed), epsilon	-1.6	1.7
HDMTX34	41360_at	AA044787	CCR4-NOT transcription complex, subunit 8	-1.5	1.0
HDMTX35	1402_at	M16038	v-yes-1 Yamaguchi sarcoma viral related oncogene	1.7	-1.1
HDMTX36	2000_at	U26455	ataxia telangiectasia mutated	2.6	1.2
HDMTX37	40520_g_at	Y00638	protein tyrosine phosphatase, receptor type, C	1.7	1.1
HDMTX+MP01	782_at	U93867	polymerase (RNA) III (DNA directed)	1.7	-1.2
HDMTX+MP02	40478_at	AL021396	hypothetical protein	2.3	1.1
HDMTX+MP03	39489_g_at	W27720	protocadherin 9	1.4	-1.5
HDMTX+MP04	37662_at	AI701164	ubiquitin-conjugating enzyme E2G 1	-1.8	1.1
HDMTX+MP05	491_at	U46116	HSPTPRG28 Human receptor tyrosine phosphatase	1.3	-2.1
HDMTX+MP06	33870_at	AB029005	chromosome 5 open reading frame 7	-1.7	-1.1
HDMTX+MP07	32257_f_at	AF003001	telomeric repeat binding factor (NIMA-interacting) 1	2.9	-1.1
HDMTX+MP08	322_at	D88532	phosphoinositide-3-kinase, regulatory subunit,	2.0	-2.1
HDMTX+MP09	40383_at	AB023200	gene from NF2/meningioma region of 22q12	2.2	-2.2
HDMTX+MP10	41667_s_at	AJ006068	dTDP-D-glucose 4,6-dehydratase	-1.6	1.3
HDMTX+MP11	39307_s_at	X81637	clathrin light chain b	-2.1	1.0
HDMTX+MP12	36729_g_at	M76446	adrenergic, alpha-1D-, receptor	2.2	-1.1
HDMTX+MP13	1814_at	D50683	transforming growth factor, beta receptor II	1.1	1.6
HDMTX+MP14	37563_at	AB007871	KIAA0411 gene product	2.3	-1.1
HDMTX+MP15	1368_at	M27492	interleukin 1 receptor, type I	2.5	-1.4
HDMTX+MP16	579_at	M95724	centromere protein C 1	-1.7	1.1
HDMTX+MP17	36514_at	U66469	cell growth regulatory with ring finger domain	-3.5	-1.4
HDMTX+MP18	41826_at	W28287	KIAA1467 protein	-2.3	1.1
HDMTX+MP19	33353_at	W26466	cDNA /gb=W26466	1.6	-2.3
HDMTX+MP20	33427_s_at	AF106861	Attractin	2.6	1.0
HDMTX+MP21	33958_at	T06733	cDNA /clone=HFBDX74	1.8	-1.7
HDMTX+MP22	32443_at	U28687	zinc finger protein 157 (HZF22)	1.4	-1.7

rank <sup>1</sup> by TX group	probe set ID	accession number	gene name	median FC TX group	median FC other TX groups
LDMTX+MP01	37036_at	AB002299	KIAA0301 protein	1.9	1.1
LDMTX+MP02	41218_at	AB018272	KIAA0729 protein	-1.5	1.1
LDMTX+MP03	37391_at	X12451	cathepsin L	-2.2	1.0
LDMTX+MP04	2081_s_at	L07032	protein kinase C, theta	-2.7	-1.1
LDMTX+MP05	38859_at	AL080141	secretory pathway component Sec31B-1	2.7	1.5
LDMTX+MP06	795_s_at	X66358	cyclin-dependent kinase-like 1 (CDC2-related kinase)	-2.6	-1.1
LDMTX+MP07	1186_at	D49493	growth differentiation factor 10	-2.7	-1.1
LDMTX+MP08	40607_at	U97105	dihydropyrimidinase-like 2	1.4	1.0
LDMTX+MP09	40377_at	AB014582	KIAA0682 gene product	2.8	1.3
MP01	35432_at	AF074723	RNA polymerase II transcriptional regulation mediator	2.4	-1.0
MP02	37244_at	AA746355	ubiquitin carboxyl-terminal esterase L3	1.8	-1.1
MP03	40942_g_at	W27026	vesicle-associated membrane protein-associated protein	-2.4	1.0
MP04	38390_at	Z34975	low density lipoprotein receptor defect C	1.7	1.0
MP05	38414_at	U05340	CDC20 (cell division cycle 20)	-1.1	-1.9
MP06	33134_at	AB011083	adenylate cyclase 3	1.5	1.0
MP07	35116_at	X80821	KIAA0874 protein	-1.8	1.1
MP08	39927_at	U17032	Rho GTPase activating protein 5	1.5	-1.2
MP09	1944_f_at	AF001359	DNA mismatch repair protein (hMLH1) alternatively spliced	2.3	-1.2
MP10	39199_at	W28661	cDNA /gb=W28661	-2.4	-1.4
MP11	34886_at	L02320	Radixin	-1.6	1.1
MP12	36694_at	AF043472	potassium voltage-gated channel, delayed-rectifier	1.8	-1.2
MP13	36297_at	X55544	activating transcription factor 1	1.4	-1.2
MP14	35227_at	U72066	retinoblastoma-binding protein 8	-1.5	1.0
MP15	36225_s_at	W27611	splicing factor proline/glutamine rich	1.7	-1.4
MP16	37077_at	D13243	pyruvate kinase L	-2.7	-1.3
MP17	39637_at	U14528	solute carrier family 26 (sulfate transporter)	2.5	-1.3
MP18	37624_at	M29458	Human carbonic anhydrase III	-2.9	1.1
MP19	37967_at	AF000424	lymphocyte antigen 117	-1.0	1.5
MP20	35005_at	AF051941	nucleoside diphosphate kinase type 6	1.6	-1.3
MP21	1106_s_at	M12959	T cell receptor alpha locus	-1.3	1.5
MP22	39775_at	X54486	serine (or cysteine) proteinase inhibitor,	-3.2	1.0
MP23	37553_at	D50863	testis-specific kinase 1	2.0	1.3
MP24	41188_at	W28186	putative integral membrane transporter	-2.4	1.0
MP25	41244_f_at	X80910	protein phosphatase 1, catalytic subunit, beta isoform	-2.1	-1.2
MP26	39674_r_at	AB011792	extracellular matrix protein 2	-2.4	-1.2
MP27	34927_at	M28826	CD1B antigen, b polypeptide	1.5	-1.6
MP28	32904_at	M28393	perforin 1 (pore forming protein)	-1.9	1.2
MP29	33490_at	L27071	TXK tyrosine kinase	1.8	-1.2

<sup>1</sup>rank ordered by distinction values