A high-risk signature for patients with multiple myeloma established from the molecular classification of human myeloma cell lines

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Supplemental Appendix A :

Preparation of complementary RNA (cRNA) and microarray hybridization

HMCLs were cultured with or without IL-6 (as described above) and RNA were prepared from exponentially growing cells. RNA was extracted using the RNeasy Kit (Qiagen, Hilden, Germany) as previously described.[1,2] Biotinylated cRNA was amplified with a double *in vitro* transcription and hybridized to the human U133 2.0 plus GeneChips, according to the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA).[3] Fluorescence intensities were quantified and analyzed using the GECOS software (Affymetrix).

Real-time RT-PCR

Total RNA was converted to cDNA using the Superscript II reverse transcriptase (Invitrogen, Cergy Pontoise, France). The assays-on-demand primers and probes and the TaqMan Universal Master Mix were used according to the manufacturer's instructions (Applied Biosystems, Courtaboeuf, France). The measurement of gene expression was performed using the ABI Prism 7000 Sequence Detection System and analyzed using the ABI PRISM 7000 SDS Software. For each primer, serial dilutions of a standard cDNA were amplified to create a standard curve to assess the PCR efficiency of each assay, only assays showing linearity across the dilution series were used for deltaCT analysis. CT values were obtained for GAPDH and the respective genes of interest during log phase of the cycle. Gene of interest levels were normalized to GAPDH for each sample (δCT = CT gene of interest – CT GAPDH) and compared with the values obtained for a known positive control using the following formula $100/2^{\delta\delta CT}$ where $\delta\delta$CT = δCT unknown – δCT positive control.

Gene expression analyses

Gene expression data were normalized with the MAS5 algorithm and analyzed with our bioinformatics platforms: RAGE (http://rage.montp.inserm.fr/)[4] and Amazonia (http://amazonia.montp.inserm.fr/).[5] Probe sets with a present call in less then 3 out of 40 HMCLs and a variation coefficient ≤ 100 were excluded from the analysis. When several probe sets interrogated a same gene, the probe set with the highest variance among HMCLs was used, yielding to 4,163 unique genes. A hierarchical clustering using average linkage and centered correlation metric was used to identify subgroups. The genes that were significantly over-expressed or under-expressed in specific subgroups were identified using multiclass supervized analysis with the significance analysis of microarray software (SAM)[6] with a 1,000-permutation adjustment.

References

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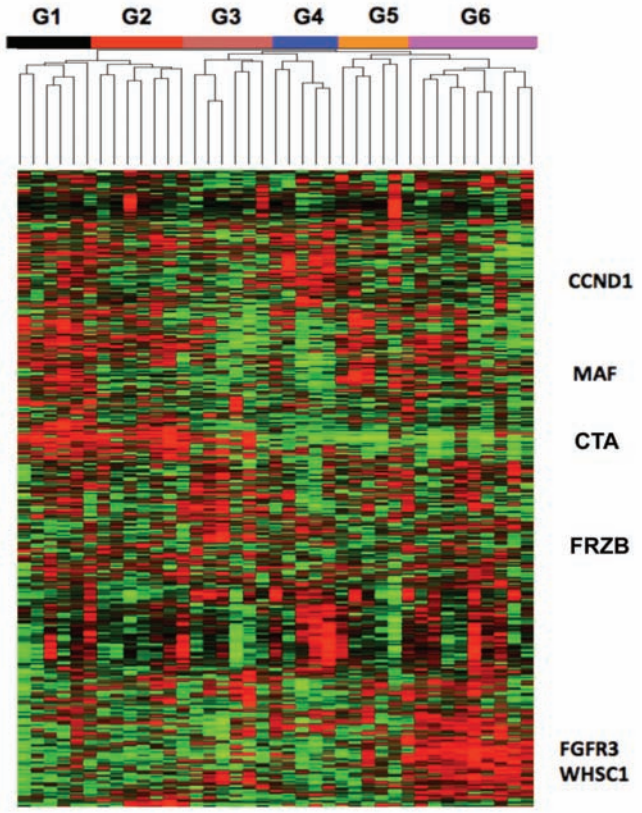
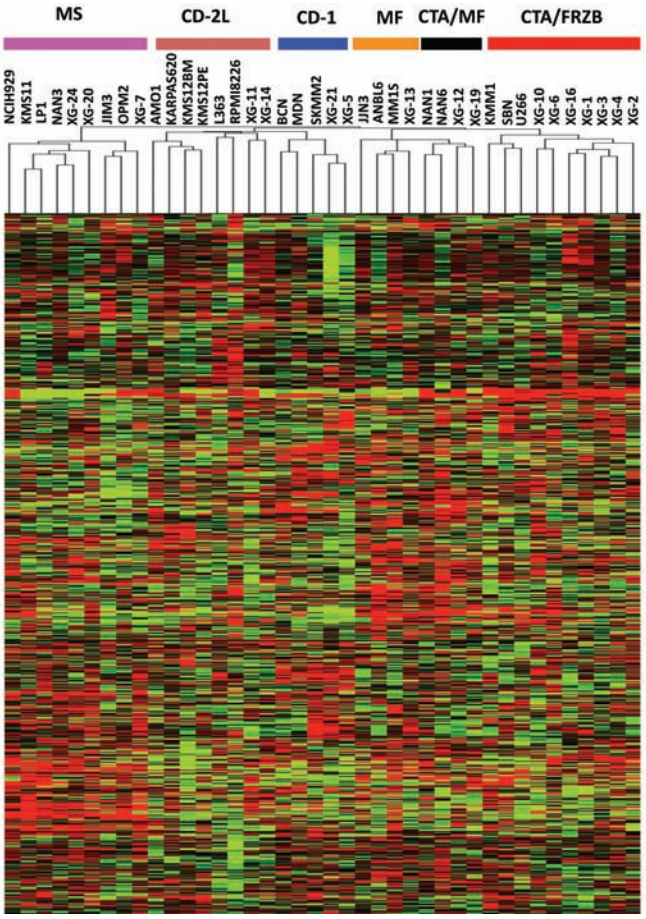
6. Cui X, Churchill GA. Statistical tests for differential expression in cDNA microarray experiments. Genome Biol. 2003;4(4):210.

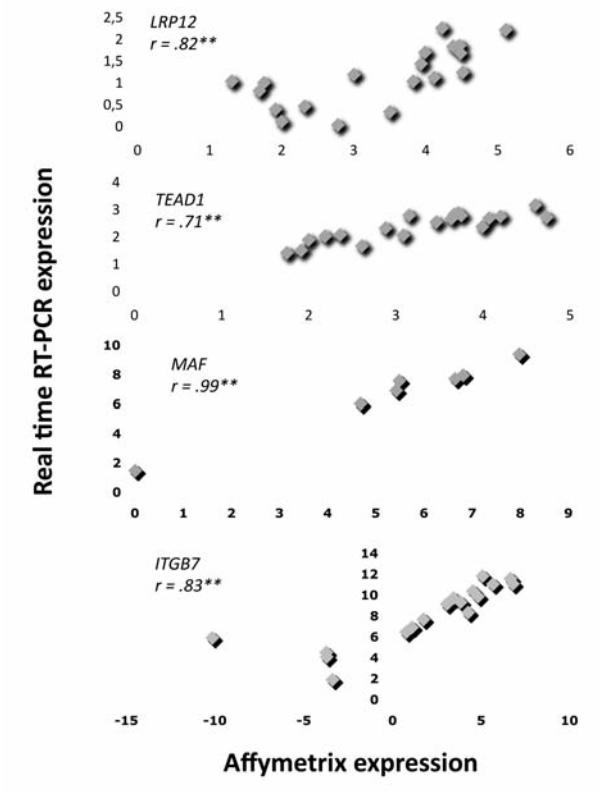
Online Supplementary Figure S1. Clustering of our cohort of 40 HMCLs using 4163 probe sets with the highest variance (lines). HMCLs (columns) were split into six groups with a significant correlation of the GEP of the HMCLs within a same group.



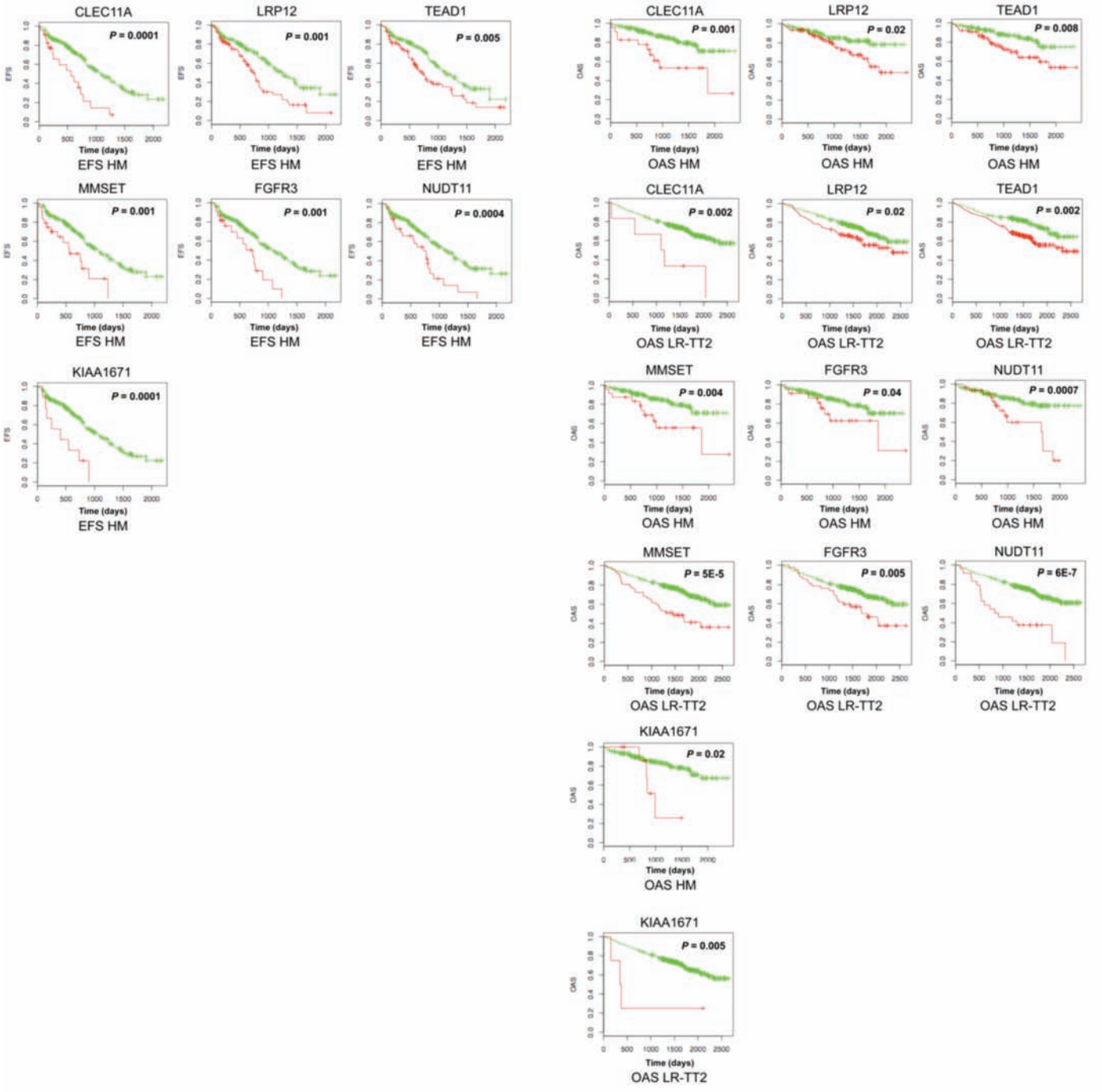
Online Supplementary Figure S2. Clustering of our cohort of 40 HMCLs using the 700 genes of the molecular classification of MM (Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, et al. The molecular classification of multiple myeloma. Blood. 2006 Sep 15;108(6):2020-8).



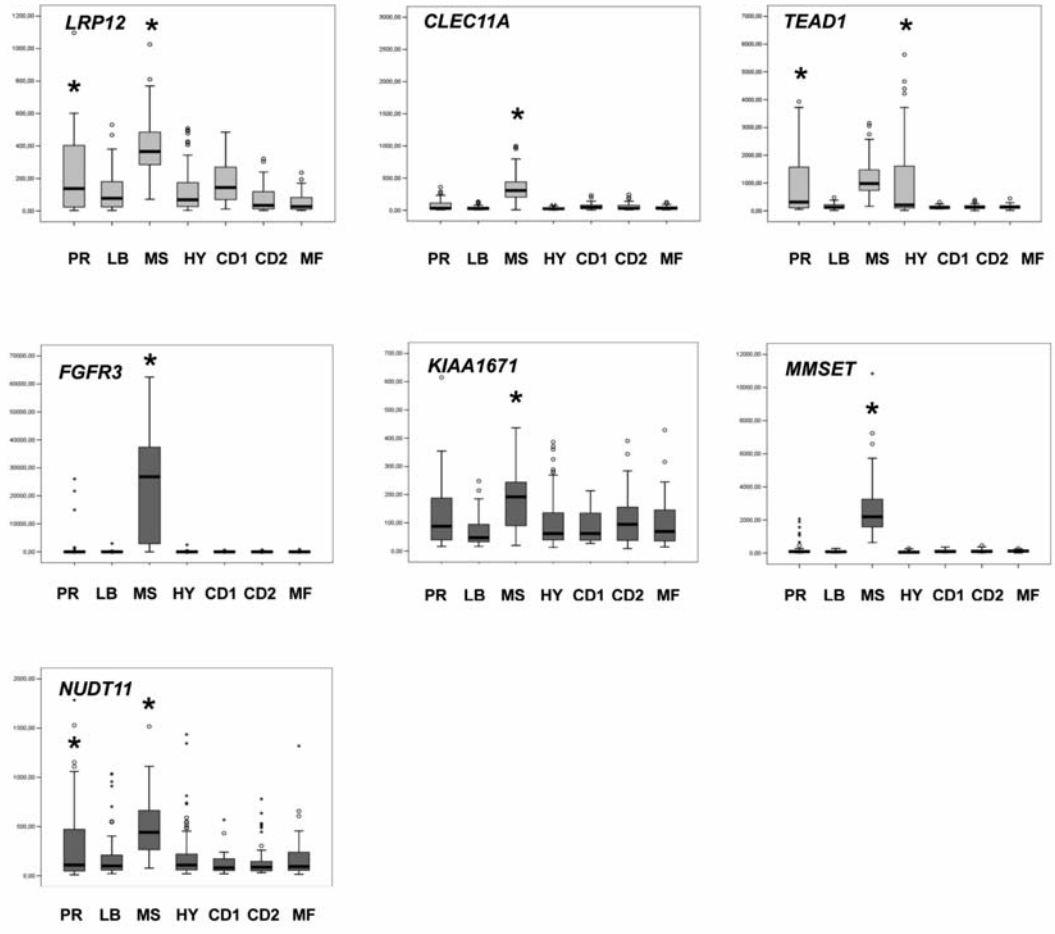
Online Supplementary Figure S3. Validation of Affymetrix data. Gene expressions of *LRP12*, TEAD1, MAF and *ITGB7* in a large panel of 20 HMCL were assayed with real time RT-PCR and normalized with GAPDH. The correlation coefficient between Affymetrix and real-time RT-PCR values were determined.



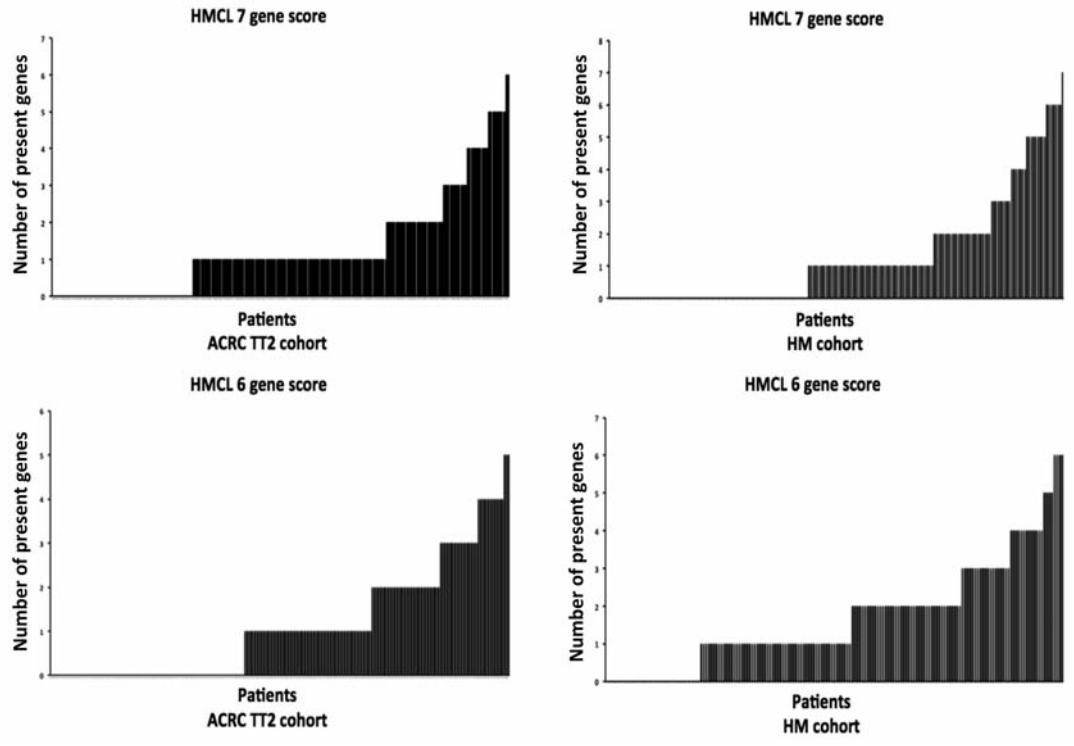
Online Supplementary Figure S4. Kaplan-Meier estimates of event-free survival (A) and overall survival (B) of patients with absent (green) or present (red) Affymetrix call for CLEC11A, LRP12, TEAD1, MMSET, FGFR3, NUDT11 and KIAA1671 in the HM cohort (206 patients) and the ACRC-TT2 cohort (345 patients).



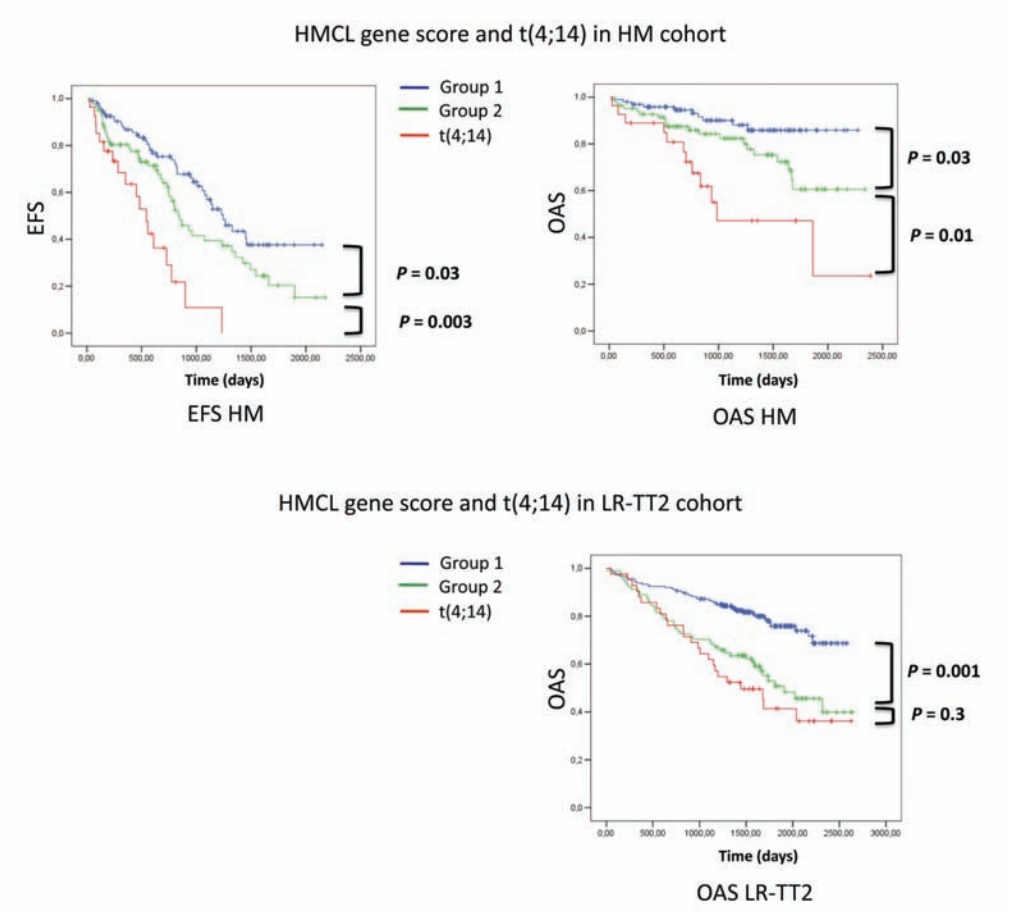
Online Supplementary Figure S5. Expression of LRP12, CLEC11A, TEAD1, FGFR3, KIAA1671, MMSET and NUDT11 in the 7 ACRC molecular groups of patents with ACRC--TT2 cohort. *The mean expression of a gene in a group is statistically significantly different from that in all groups using Student's t--test ($P \leq 0.05$).



Online Supplementary Figure S6. HMCL scores in the various datasets of patients.



Online Supplementary Figure S7. Kaplan-Meier estimates of overall survival and event-free survival of 6 HMCL gene score defined low risk patients (blue), high risk patients (green) and t(4;14) patients (red) in the HM cohort and the ACRC-TT2 cohort.



Online Supplementary TableS1. Ras and TP53 mutations in HMCLs.

	N, K-ras cd	nt Change	P53 cd	nt Change	exon
HMCL$^{serum+IL-6}$					
ANBL-6	wt		331 Q>STOP	CAG>TAG	9
BCN	wt		wt		
MDN	N13 G>D	GGT>GAT	wt		
NAN1	wt		180 E>STOP	GAG>TAG	5
NAN3	N61 Q>H	CAA>AAA	248 R>R+Q	CGG>CGG+CAG	7
NAN6	wt		Deletion exons 7-9		
SBN	wt		wt		
XG1	N12 G>R	GGT>CGT	126 Y>N	TAC>AAC	5
XG2	K12 G>A	GGT>GCT	176 C>Y	TGC>TAC	5
XG3	N61 Q>L	CAA>AAA	wt		
XG4	wt		181 R>R+C	CGC>CGC+TGC	5
XG5	wt		282 R>W	CGG>TGG	8
XG6	wt		wt		
XG7	K12 G>C	GGT>TGT	wt		
XG10	K13 S>S+C	GGC>GGC+TGC	wt		
XG11	wt		135 C>Y	TGC>TAC	5
XG12	N61 Q>L	CAA>AAA	wt		
XG13	wt		248 R>Q	CGG>CAG	7
XG14	N61 Q>Q+L	CAA>CAA+AAA	266 G>E	GGA>GAA	8
XG16	N 61Q>H	CAA>CAC	220 Y>C	TAT>TGT	6
XG19	wt		wt		
XG20	wt		Deletion (exons 7-?)		
XG21	wt		wt		
XG24	K12 G>G+V	GGT>GGT+GTC	Wt		
HMCL^{serum}					
AMO1	wt		wt		
JIM3	wt		273 R>S	CGT>TGT	8
JJN3	N12 G>D	GGT>GAT	No PCR product		
Karpas 620	K12 G>D	GGT>GAT	135 C>Y	TGC>TAC	5
KMM1	N13 G>D	GGT>GAT	135 C>C+F	TGC>TGC+TTC	5
KMS11	wt		No PCR product		
KMS12BM	Wt		337 R>L	CGC>CTC	10
KMS12PE	Wt		337 R>L	CGC>CTC	10
L363	N61 Q>H	CAA>CAC	Insertion intron 7-8		
LP1	wt		286 E>K	GAA>AAA	8
MM1S	K12 G>A	GGT>GCT	wt		
NCI-H929	N13 G>D	GGT>GAT	wt		
OPM2	wt		175 R>H	CGC>CAC	5
RPMI 8226	K12 G>A	GGT>GCT	285 E>K	GAG>AAG	8
SKMM2	wt		132 K>N	AAG>AAT	5
U266	wt		161 A>T	GCC>ACC	5

Online Supplementary TableS2. HMCL phenotype.

HMCL Name	CD19	CD20	CD38	CD45	CD138
HMCL$^{serum+IL-6}$					
ANBL6	0%	0%	100%, r=155	0%	100% r=381
BCN	0%	0%	100%, r=84	0%	100%, r=200
MDN	0%	0%	100%, r=120	100%, r=6	100%, r=239
NAN1	0%	16%, r=12	100%, r=282	0%	100%, r=362
NAN3	0%	0%	100%, r=96	0%	100%, r=399
NAN6	0%	0%	18%, r=10	70%, r=18	100%, r= 272
SBN	0%	0%	100%, r=9	0%	100%,r= 489
XG1	0%	0%	100%, r=169	100% r=20	100% r= 180
XG2	0%	0%	100%, r=108	100% r=5	100% r=515
XG3	0%	0%	100%, r=119	100% r=60	100% r=427
XG4	0%	0%	100%, r=98	0%	100% r=158
XG5	0%	0%	100%, r=55	0%	100% r=5
XG6	0%	0%	0%	100% r=109	100% r=401
XG7	0%	0%	100%, r=154	100% r=2	100% r=100
XG10	0%	0%	100%, r=86	100% r=95	0%
XG11	0%	0%	100%, r=53	100% r=30	100% r=53
XG12	0%	0%	100%, r=208	100% r=384	100% r=120
XG13	0%	0%	100%, r=31	100% r=2	100% r=89
XG14	0%	0%	100%, r=89	100% r=5	100% r=71
XG16	0%	0%	100%, r=159	100% r=27	100% r=131
XG19	0%	0%	100%, r=42	100% r=121	100% r=272
XG20	0%	0%	100%, r=81	0%	100% r=243
XG21	0%	0%	100%, r=27	100% r= 28	100% r=306
XG24	0%	0%	100%, r=1050	0%	100% r=281
HMCL^{serum}					
AMO1	0%	0%	100%, r=37	100%, r=12	100%, r=569
JIM3	0%	0%	100%, r=270	0%	100%, r=174
JJN3	0%	0%	100%, r=32	65%, r=4	100%, r=2.5
Karpas620	40%, r=13	50%, r=17	0%	0%	100%, r=300
KMM1	0%	0%	30%, r=12	0%	100%, r=76
KMS11	0%	0%	100%, r=116	0%	100%, r=50
KMS12BM	0%	0%	100%, r=200	0%	100%, r=49
KMS12PE	0%	0%	100%, r=459	0%	100%, r=142
L363	0%	0%	100%, r=50	0%	100%, r=221
LP1	0%	0%	100%, r=179	0%	100, r=250
MM1S	0%	0%	100%, r=126	0%	100%, r=557
NCI-H929	0%	0%	100%, r=97	18%, r=4	100%, r=150
OPM2	0%	0%	100%, r=30	0%	100%, r=681
RPMI8226	0%	0%	100%, r=200	0%	100%, r=118
SKMM2	0%	0%	100%, r=22	0%	100% r=194
U266	0%	0%	12%, r=16	80%, r=8	100%, r=137

Cells were stained with anti-controlPE or anti-CD19PE, or anti-CD20-PE or anti-CD38PE, or anti-CD45PE or anti-CD138PE then analyzed on FAcsCalibur. Ratio of fluorescence was defined as MFI of specific CD divided by MFI of control staining.

Online Supplementary Table S3A. Differential probe set expression between HMCLsserum+IL-6 and HMCLsserum.

Affymetrix Probe Set	Gene Name/ Probe Set	Chromosome Map Position	Fold change
Probe sets upregulated in HMCLs$^{serum+IL-6}$ versus HMCLs^{serum}			
203854_at	CFI	4q25	29.36
207238_s_at	PTPRC	1q31.3	21.56
208451_s_at	C4A	6p21.32	16.99
204529_s_at	TOX	9q12.1	8.75
235856_at	235856_at	6p21.32	8.24
1559316_at	1559316_at	5p14.3	8.22
219944_at	CLIP4	2p23.2	8.11
223220_s_at	PARP9	3q21.1	7.73
209109_s_at	TSPAN6	Xq22.1	6.84
219667_s_at	BANK1	4q24	6.18
203140_at	BCL6	3q27.3	5.71
227697_at	SOCS3	17q25.3	5.59
220603_s_at	MCTP2	15q26.2	5.59
203052_at	C2	6p21.32	4.97
232027_at	SYNE1	6q25.2	4.89
205903_s_at	KCNN3	1q21.3	3.78
225929_s_at	RNF213	17q25.3	3.71
206574_s_at	PTP4A3	8q24.3	3.47
228461_at	SH3RF3	2q13	3.39
225415_at	DTX3L	3q21.1	3.35
227792_at	ITPRIPL2	16p12.3	2.91
207777_s_at	SP140	2q37.1	2.72
238914_at	238914_at	18q21.2	2.55
Probe sets upregulated in HMCLs^{serum} versusHMCLs$^{serum+IL-6}$			
None			

List of genes differentially expressed between HMCLsIL-6+serum and HMCLsserum (SAM analysis, 1,000 permutations, FDR=0%)

Online Supplementary Table S3B. Differential probe set expression between Groups CT/MAF and MAF.

Affymetrix Probe Set	Gene Name/ Probe Set	Chromosome Map Position	Fold change
Probe sets upregulated in CT/MAF HMCLs versus MAF HMCLs			
227952_at	ZNF595	4p11	219.26
207493_x_at	SSX2	Xp11.22-23	209.93
207666_x_at	SSX3	SSX3	131.81
206626_x_at	SSX1	Xp11.23-p11.22	114.56
242334_at	NALP4	19q13.43	113.84
211425_x_at	SSX4	SSX3	109.58
220057_at	XAGE1	Xp11.22-21	93.45
207281_x_at	VCX2	Xp22.32	75.17
216462_at	---	Xp11.22-23	64.86
207534_at	MAGEB1	Xp21.2	58.41
232010_at	FSTL5	4q32.3	56.82
210603_at	ARD1B	4q21.21	55.51
209616_s_at	CES1	16q13-q22.1	54.56
221690_s_at	NALP2	19q13.43	45.36
221185_s_at	IQCG	3q29	28.93
1568933_at	LOC646627	1q44	27.56
241224_x_at	DSCR8	21q22.2	27.54
208528_x_at	SSX5	Xp11.22-23	23.69
1559316_at	---	5p14.3	23.53
229349_at	LIN28B	6q21	21.72
209550_at	NDN	15q11.2	20.76
211737_x_at	PTN	7q33-q34	20.50
231131_at	FAM133A	Xq21.32	20.37
207712_at	BAGE	21p11.1	19.35
236840_at	C12orf56	12q14.2	16.82
203921_at	CHST2	3q23	13.18
204749_at	NAP1L3	Xq21.32	11.34
223977_s_at	C18orf2	18p11.32	8.29
242276_at	---	18p11.32	8.26
211382_s_at	TACC2	10q26.13	8.06
209993_at	ABCB1	7q21.12	8.06
241074_at	C12orf32	14q32.33	7.24
230959_at	---	---	5.72
1561433_at	LOC285103	2q21.1	5.13
1562216_at	---	2p12	4.78
1557765_at	LOC340109	5p14.1	4.73
217388_s_at	KYNU	2q22.2	4.72
241675_s_at	---	14q12	4.18
206922_at	VCY /// VCY1B	---	4.03
239250_at	ZNF542	19q13.43	3.71
205656_at	PCDH17	13q21.1	2.91

List of genes differentially expressed between CT/MAF and MAF Groups (SAM analysis. 1,000 permutations, FDR=0%)

Online Supplementary Table S3C. Differential probe set expression between Groups CD-1 and CD-2L.

Affymetrix Probe Set	Gene Name/ Probe Set	Chromosome Map Position	Fold change
Probe sets upregulated CD-1 HMCLs versus CD-2L HMCLs			
213293_s_at	TRIM22	11p15.4	504.31
1569040_s_at	---	2p11.2	378.00
209524_at	HDGFRP3	15q25.2	108.27
206403_at	ZNF536	19q12	51.78
1563721_at	---	2p12	39.2
1556183_at	---	2p11.2	38.81
202820_at	AHR	7p21.1	38.55
244413_at	CLECL1	12p13.1	32.96
219667_s_at	BANK1	4q24	29.29
201645_at	TNC	9q33.1	26.56
1552943_at	GABRG1	4p12	22.99
204998_s_at	ATF5	19q13.3	18.95
206584_at	LY96	8q21.11	17.72
214428_x_at	C4A	6p21.32	14.42
203595_s_at	IFIT5	10q23.31	13.77
210997_at	HGF	7q21.11	12.25
227279_at	TCEAL3	Xq22.2	11.95
205433_at	BCHE	3q26.1	11.03
208022_s_at	CDC14B	9q22.33	11.00
239045_at	ERN1	17q24.2	10.70
232226_at	LRRC4C	11p12	10.54
231956_at	KIAA1618	17q25.3	10.00
214719_at	SLC46A3	13q12.3	9.74
211709_s_at	CLEC11A	19q13.33	8.61
220448_at	KCNK12	2p16.3	8.16
219892_at	TM6SF1	15q25.2	8.13
222272_x_at	SCIN	7p21.3	8.00
223721_s_at	DNAJC12	10q22.1	7.88
239331_at	---	7q32.3	7.53
205934_at	PLCL1	2q33.1	7.20
235201_at	FOXP2	7q31.1	7.13
201060_x_at	STOM	9q34.1	7.02
236451_at	---	2q24.2	6.92
235308_at	ZBTB20	3q13.31	6.68
243963_at	SDCCAG8	1q43	6.32
225931_s_at	RNF213	17q25.3	6.22
218400_at	OAS3	12q24.13	6.19
203641_s_at	COBLL1	2q24.3	5.85
238619_at	---	7q32.3	5.85
243278_at	---	11q22.1	5.80
243846_x_at	---	11q22.1	3.80
207057_at	SLC16A7	12q14.1	5.73
200628_s_at	WARS	14q32.2	5.01
1557987_at	LOC641298	16p11/12	4.88
243808_at	---	7q21.2	3.73
204469_at	PTPRZ1	7q31.32	3.35
227443_at	C9orf150	9p23	3.33
231954_at	DKFZP434I0714	4q31.3	3.09
204793_at	GPRASP1	Xq22.1	2.01

List of genes differentially expressed between CD-1 and CD-2L Groups (SAM analysis, 1,000 permutations, FDR<5%)

Online Supplementary Table S4.

NF-κB index of the 40 HMCLs

HMCL	NF-κB index	HMCL classification group	IL-6 dependenc
AMO1	8,554343595	CD-2L	-
KMM1	8,228503797	CD-2L	-
MDN	8,209001703	CD-1	++
MM1S	8,078775196	MF	-
XG2	7,945600762	CTA/FRZB	++
L363	7,921561317	CD-2L	-
JJN3	7,889111283	MF	-
NAN6	7,594212472	CTA/MF	+
NAN1	7,550733922	CTA/MF	+
LP1	7,521504938	MS	-
KMS12BM	7,365005551	CD-2L	-
KMS12PE	7,313887715	CD-2L	-
U266	7,288816676	CTA/FRZB	-
KMS11	7,234394524	MS	-
ANBL6	6,850559115	MF	+
XG-19	6,754617308	CTA/MF	++
XG-13	6,733275595	MF	++
XG-20	6,402794142	MS	++
XG-5	6,348948064	CD-1	++
RPMI8226	6,346490619	MF	-
XG-16	6,336781746	CTA/FRZB	++
KARPAS620	5,969981107	CD-2L	-
XG-10	5,69517926	CTA/MF	++
XG-1	5,500405194	CTA/FRZB	++
SBN	5,402965152	CTA/FRZB	+
XG-4	5,373166097	CTA/FRZB	++
SKMM2	5,32757921	CD1	-
NCIH929	5,271000618	MS	-
NAN3	5,265127145	MS	+
JIM3	5,243430441	MS	-
XG-21	5,224952261	CD-1	++
OPM2	4,808323735	MS	-
XG-3	4,791794712	CTA/FRZB	++
XG-6	4,774751927	CTA/MF	++
XG-7	4,734274077	MS	+
XG-14	4,538037202	MF	++
XG-11	4,440806397	CD-2L	+
XG-12	4,417820627	CTA/MF	++
XG-24	4,371709386	MS	++
BCN	4,269586931	CD-1	+

Online Supplementary Table S5. Genes over-expressed in group CT/FRZB of HMCL.

Gene ID	Chip	Gene Name	Localization	Description
Intercellular communication signals				
U133P	219295_s_at	PCOLCE2	3q21-q24	procollagen C-endopeptidase enhancer 2
U133P	219410_at	TMEM45A	3q12.2	transmembrane protein 45A
U133P	203698_s_at	FRZB	2qter	frizzled-related protein
Transduction signals				
U133P	227697_at	SOCS3	17q25.3	suppressor of cytokine signaling 3
Cytoskeleton				
U133P	216323_x_at	TUBA3C	2q21.1	alpha-tubulin isotype H2-alpha
Protein synthesis and regulation				
U133P	209550_at	NDN	15q11.2-q12	necdin homolog (mouse)
U133P	201909_at	LOC100133662	Yp11.3	hypothetical protein LOC100133662
Cancer testis antigens				
U133P	207086_x_at	GAGE2	Xp11.23	G antigen 2
U133P	208155_x_at	GAGE6	Xp11.4-p11.2	G antigen 6
U133P	206640_x_at	GAGE12B	Xp11.23	G antigen 12B
U133P	207739_s_at	GAGE1	Xp11.4-p11.2	G antigen 1
U133P	208235_x_at	GAGE12F	Xp11.4-p11.2	G antigen 12F
U133P	207663_x_at	GAGE3	Xp11.4-p11.2	G antigen 3
U133P	206609_at	MAGEC1	Xq26	melanoma antigen family C, 1
Nuclear functions				
U133P	207912_s_at	DAZ1	Yq11.223	deleted in azoospermia 1
U133P	205000_at	DDX3Y	Yq11	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3; Y-linked
U133P	1569669_at	FOXR2	Xp11.22	forkhead box R2
U133P	208307_at	RBMY1A1	Yq11.223	RNA binding motif protein; Y-linked; family 1; member A1
U133P	206700_s_at	JARID1D	Yq11|Yq11	jumonji, AT rich interactive domain 1D
U133P	227279_at	TCEAL3	Xq22.2	transcription elongation factor A (SII)-like 3
U133P	207281_x_at	VCX	Xp22	variable charge; X-linked
U133P	211403_x_at	VCX2		variable charge, X-linked 2
Others				
U133P	239250_at	ZNF542	19q13.43	zinc finger protein 542
U133P	216039_at	LOC100132832	7q22.1	hypothetical protein LOC1001328
U133P	213122_at	LOC728137 ///	8q22.1	LOC
U133P	213122_at	TSPYL5	8q22.1	TSPY-like 5

Online Supplementary Table S6. Genes over-expressed in group CT/MF of HMCL.

Gene ID	Chip	Gene Name	Localization	Description
Intercellular communication signals				
U133P	205609_at	ANGPT1	8q22.3-q23	angiopoietin 1
U133P	206978_at	CCR2	3p21	chemokine (C-C motif) receptor 2 /// chemokine (C-C motif) receptor 2
U133P	206206_at	CD180	5q12	CD180 antigen
U133P	206280_at	CDH18	5p15.2-p15.1	cadherin 18; type 2
U133P	219505_at	CECR1	22q11.2	cat eye syndrome chromosome region; candidate 1
U133P	209473_at	ENTPD1	10q24	ectonucleoside triphosphate diphosphohydrolase 1
U133P	224406_s_at	FCRL5	1q21	Fc receptor-like 5 /// Fc receptor-like 5
U133P	204834_at	FGL2	7q11.23	fibrinogen-like 2
U133P	210890_x_at	KIR2DL1	19q13.4	killer cell immunoglobulin-like receptor; two domains; long cytoplasmic tail; 1
U133P	211397_x_at	KIR2DL2	19q13.4	killer cell immunoglobulin-like receptor; two domains; long cytoplasmic tail; 2
U133P	208179_x_at	KIR2DL3	19q13.4	killer cell immunoglobulin-like receptor; two domains; long cytoplasmic tail; 3
U133P	208198_x_at	KIR2DS1	19q13.4	killer cell immunoglobulin-like receptor; two domains; short cytoplasmic tail; 1
U133P	207314_x_at	KIR3DL2	19q13.4	killer cell immunoglobulin-like receptor; three domains; long cytoplasmic tail; 2
U133P	211532_x_at	KIR2DS1	19q13.4	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 1
U133P	211688_x_at	KIR3DL2	19q13.4	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 2
U133P	227238_at	MUC15	11p14.3	mucin 15
U133P	211737_x_at	PTN	7q33-q34	pleiotrophin (heparin binding growth factor 8; neurite growth-promoting factor 1)
U133P	204563_at	SELL	1q23-q25	selectin L (lymphocyte adhesion molecule 1)
Transduction signals				
U133P	218870_at	ARHGAP15	2q22.3	Rho GTPase activating protein 15
U133P	204882_at	ARHGAP25	2p13.3	Rho GTPase activating protein 25
U133P	205590_at	RASGRP1	15q15	RAS guanyl releasing protein 1 (calcium and DAG-regulated)
U133P	220330_s_at	SAMSN1	21q11	SAM domain; SH3 domain and nuclear localisation signals; 1
U133P	217147_s_at	TRAT1	3q13	T cell receptor associated transmembrane adaptor 1
U133P	223553_s_at	DOK3	5q35.3	docking protein 3
U133P	221942_s_at	GUCY1A3	4q31.3-q33	guanylate cyclase 1, soluble, alpha 3
U133P	205270_s_at	LCP2	5q33.1-qter	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
U133P	209737_at	MAGI2	7q21	membrane associated guanylate kinase, WW and PDZ domain containing 2
Cytoskeleton				
U133P	213733_at	MYO1F	19p13.3	myosin IF
U133P	205348_s_at	DNCI1	7q21.3-q22.1	dynein; cytoplasmic; intermediate polypeptide 1
Cell cycle				
U133P	203725_at	GADD45A	1p31.2-p31.1	growth arrest and DNA-damage-inducible; alpha
Apoptosis				
U133P	221601_s_at	FAIM3	1q32.1	Fas apoptotic inhibitory molecule 3 /// Fas apoptotic inhibitory molecule 3
U133P	1552531_a_at	NALP11	19q13.42	NACHT; leucine rich repeat and PYD containing 11
U133P	221690_s_at	NALP2	19q13.42	NACHT; leucine rich repeat and PYD containing 2
U133P	242334_at	NALP4	19q13.43	NACHT; leucine rich repeat and PYD containing 4
U133P	237461_at	NALP7	19q13.42	NACHT; leucine rich repeat and PYD containing 7
U133P	218297_at	C10orf97	10p13	chromosome 10 open reading frame 97
Protein synthesis and regulation				
U133P	205141_at	ANG	14q11.1-q11.2	angiogenin, ribonuclease, RNase A family, 5
U133P	218935_at	EHD3	2p21	EH-domain containing 3
U133P	203761_at	SLA	8q22.3-qter|8	Src-like-adaptor /// Src-like-adaptor
Cancer testis antigens				
U133P	220062_s_at	MAGEC2	Xq27	melanoma antigen family C; 2
U133P	206626_x_at	SSX1	Xp11.23-p11.2	synovial sarcoma; X breakpoint 1
U133P	210497_x_at	SSX2	Xp11.23-p11.2	synovial sarcoma; X breakpoint 2
U133P	207666_x_at	SSX3	Xp11.23	synovial sarcoma; X breakpoint 3
U133P	210394_x_at	SSX4	Xp11.23	synovial sarcoma; X breakpoint 4
U133P	233514_x_at	TEX11	Xq13.1	testis expressed sequence 11
U133P	220057_at	XAGE1A /// XAGE	Xp11.22-p11.2	X antigen family; member 1
Metabolism				
U133P	203060_s_at	PAPSS2	10q23-q24	3prime-phosphoadenosine 5prime-phosphosulfate synthase 2
U133P	227791_at	SLC9A9	3q24	solute carrier family 9 (sodium/hydrogen exchanger); member 9
U133P	223832_s_at	CAPNS2	16q12.2	calpain; small subunit 2
U133P	209616_s_at	CES1	16q13-q22.1	carboxylesterase 1 (monocyte/macrophage serine esterase 1)
U133P	203921_at	CHST2	3q24|7q31	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2
U133P	231265_at	COX7B2	4p12	cytochrome c oxidase subunit VIIb2
Others				
U133P	229070_at	C6orf105	6p24.1	chromosome 6 open reading frame 105
U133P	241224_x_at	DSCR8	21q22.2	Down syndrome critical region gene 8
U133P	1569139_s_at	FAM53A	4p16.3	family with sequence similarity 53; member A
U133P	235913_at	LOC400713	19q13.41	zinc finger-like
U133P	210603_at	ARD1B	4q21.21	ARD1 homolog B (S. cerevisiae)
U133P	242135_at	MGC72104	20q11.1	Chromosome 20 open reading frame 80
U133P	244740_at	MGC9913	19q13.43	hypothetical protein MGC9913
U133P	231131_at	FAM133A	Xq21.33	family with sequence similarity 133, member A
U133P	226425_at	CLIP4	2p23.2	CAP-GLY domain containing linker protein family, member 4
U133P	218815_s_at	TMEM51	1p36.21	transmembrane protein 51
U133P	227194_at	FAM3B	21q22.3	family with sequence similarity 3, member B
U133P	219895_at	FAM70A	Xq24	family with sequence similarity 70, member A
U133P	242135_at	LOC100131707 /,	9q12	hypothetical LOC100131707 /// similar to FRG1 protein (FSHD region gene 1 protein)
U133P	232010_at	FSTL5	4q32.3	follistatin-like 5
U133P	220330_s_at	SAMSN1	21q11	SAM domain, SH3 domain and nuclear localization signals 1

Online Supplementary Table S7. Genes over-expressed in group CD-1 of HMCL.

Gene ID	Chip	Gene Name	Localization	Description
Intercellular communication signals				
U133P	206508_at	CD70	19p13	CD70,tumor necrosis factor (ligand) superfamily; member 7
U133P	233500_x_at	CLEC2D	12p13	C-type lectin domain family 2; member D
U133P	228496_s_at	CRIM1	2p21	Cysteine rich transmembrane BMP regulator 1 (chordin-like)
U133P	241805_at	GABRG1	4p12	gamma-aminobutyric acid (GABA) A receptor; gamma 1
U133P	231166_at	GPR155	2q31.1	G protein-coupled receptor 155
U133P	212203_x_at	IFITM3	11p15.5	interferon induced transmembrane protein 3 (1-8U)
U133P	210587_at	INHBE	12q13.3	inhibin; beta E
U133P	201645_at	TNC	9q33	tenascin C (hexabrachion)
U133P	225524_at	ANTXR2	4q21.21	anthrax toxin receptor 2
U133P	219892_at	TM6SF1	15q24-q26	transmembrane 6 superfamily member 1
U133P	203439_s_at	STC2	5q35.2	stanniocalcin 2
Transduction signals				
U133P	219667_s_at	BANK1	4q24	B-cell scaffold protein with ankyrin repeats 1
U133P	210999_s_at	GRB10	7p12-p11.2	growth factor receptor-bound protein 10
U133P	1556037_s_at	HHIP	4q28-q32	hedgehog interacting protein
U133P	212912_at	RPS6KA2	6q27	ribosomal protein S6 kinase; 90kDa; polypeptide 2
U133P	222668_at	INPP1	2q32	inositol polyphosphate-1-phosphatase
Cytoskeleton				
U133P	232381_s_at	DNAH5	5p15.2	dynein; axonemal; heavy polypeptide 5
U133P	209210_s_at	FERMT2	14q22.1	fermitin family homolog 2 (Drosophila)
U133P	201061_s_at	STOM	9q34.1	stomatin
Cell cycle				
U133P	208711_s_at	CCND1	11q13	cyclin D1
U133P	221556_at	CDC14B	9q22.33	CDC14 cell division cycle 14 homolog B (S. cerevisiae)
U133P	202284_s_at	CDKN1A	6p21.2	cyclin-dependent kinase inhibitor 1A (p21; Cip1)
Apoptosis				
U133P	203685_at	BCL2	18q21.33|18q	B-cell CLL/lymphoma 2
U133P	201315_x_at	IFITM2	11p15.5	interferon induced transmembrane protein 2 (1-8D)
Protein synthesis and regulation				
U133P	218976_at	DNAJC12	10q22.1	DnaJ (Hsp40) homolog; subfamily C; member 12
U133P	205124_at	MEF2B	19p12	MADS box transcription enhancer factor 2; polypeptide B (myocyte enhancer factor 2B)
U133P	243582_at	SH3RF2	5q32	SH3 domain containing ring finger 2
U133P	227134_at	SYTL1	1p36.11	synaptotagmin-like 1
U133P	213293_s_at	TRIM22	11p15	tripartite motif-containing 22
U133P	236175_at	TRIM55	8q13.1	tripartite motif-containing 55
U133P	229337_at	USP2	11q23.3	ubiquitin specific peptidase 2
U133P	213294_at	EIF2AK2		eukaryotic translation initiation factor 2-alpha kinase 2
Metabolism				
U133P	201425_at	ALDH2	12q24.2	aldehyde dehydrogenase 2 family (mitochondrial)
U133P	225285_at	BCAT1	12pter-q12	branched chain aminotransferase 1; cytosolic
U133P	205433_at	BCHE	3q26.1-q26.2	butyrylcholinesterase
U133P	209726_at	CA11	19q13.3	carbonic anhydrase XI
U133P	239045_at	ERN1	17q24.2	Endoplasmic reticulum to nucleus signalling 1
U133P	203710_at	ITPR1	3p26-p25	inositol 1;4;5-triphosphate receptor; type 1
U133P	244455_at	KCNT2	1q31.3	potassium channel; subfamily T; member 2
U133P	224918_x_at	MGST1	12p12.3-p12.1	microsomal glutathione S-transferase 1
U133P	203423_at	RBP1	3q23	retinol binding protein 1; cellular
U133P	207057_at	SLC16A7	12q13	solute carrier family 16 (monocarboxylic acid transporters); member 7
U133P	207076_s_at	ASS1	9q34.1	argininosuccinate synthetase 1
Nuclear functions				
U133P	204998_s_at	ATF5	19q13.3	activating transcription factor 5
U133P	200628_s_at	WARS	14q32.31	tryptophanyl-tRNA synthetase
Others				
U133P	239468_at	MKX	10p12.1	mohawk homeobox
U133P	238581_at	GBP5	1p22.2	Guanylate binding protein 5
U133P	1558080_s_at	LOC144871	13q32.1	hypothetical protein LOC144871
U133P	226382_at	LOC283070	10p14	hypothetical protein LOC283070
U133P	1561757_a_at	LOC283352	12q24.33	hypothetical protein LOC283352
U133P	206394_at	MYBPC2	19q13.33	myosin binding protein C, fast type
U133P	207057_at	PMAIP1	18q21.32	phorbol-12-myristate-13-acetate-induced protein 1
U133P	208451_s_at	C4A	6p21.3	complement component 4A (Rodgers blood group)

Online Supplementary Table S8. Genes over-expressed in group MF of HMCL.

Gene ID	Chip	Gene Name	Localization	Description
Intercellular communication signals				
U133P	205098_at	CCR1	3p21	chemokine (C-C motif) receptor 1
U133P	205898_at	CX3CR1	3p21|3p21.3	chemokine (C-X3-C motif) receptor 1
U133P	209541_at	IGF1	12q22-q23	insulin-like growth factor 1 (somatomedin C)
U133P	217235_x_at	IGLC2	22q11.2	Immunoglobulin lambda joining 3
U133P	217148_x_at	IGLV2-14	22q11.2	immunoglobulin lambda variable 2-14
U133P	205718_at	ITGB7	12q13.13	integrin; beta 7
U133P	201721_s_at	LAPTM5	1p34	lysosomal associated multispanning membrane protein 5
U133P	205016_at	TGFA	2p13	transforming growth factor; alpha
U133P	219423_x_at	TNFRSF25	1p36.2	tumor necrosis factor receptor superfamily; member 25
Transduction signals				
U133P	209576_at	GNAI1	7q21	guanine nucleotide binding protein (G protein); alpha inhibiting activity polypeptide 1
U133P	216250_s_at	LPXN	11q12.1	leupaxin
U133P	209348_s_at	MAF	16q22-q23	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
U133P	212724_at	RND3	2q23.3	Rho family GTPase 3
U133P	1555812_a_at	ARHGDIB	12p12.3	Rho GDP dissociation inhibitor (GDI) beta
Cell cycle				
U133P	1553599_a_at	SYCP3	12q	synaptonemal complex protein 3
Protein synthesis and regulation				
U133P	218729_at	LXN	3q25.32	latexin
U133P	211474_s_at	SERPINB6	6p25	serpin peptidase inhibitor; clade B (ovalbumin); member 6
Cytoskeleton				
	223130_s_at	MYLIP	6p23-p22.3	myosin regulatory light chain interacting protein
Metabolism				
U133P	207638_at	PRSS7	21q21|21q21.	protease; serine; 7 (enterokinase)
Others				
U133P	220998_s_at	UNC93B1	11q13	unc-93 homolog B1 (C. elegans)
U133P	201876_at	PON2	7q21.3	paraoxonase 2

Online Supplementary Table S9. Genes over-expressed in group MS of HMCL.

Gene ID	Chip	Gene Name	Localization	Description
Intercellular communication signals				
U133P	224694_at	ANTXR1	2p13.1	anthrax toxin receptor 1
U133P	211709_s_at	CLEC11A	19q13.3	C-type lectin domain family 11; member A /// C-type lectin domain family 11; member A
U133P	204379_s_at	FGFR3	4p16.3	fibroblast growth factor receptor 3 (achondroplasia; thanatophoric dwarfism)
U133P	204912_at	IL10RA	11q23	interleukin 10 receptor; alpha
U133P	201124_at	ITGB5	3q21.2	integrin; beta 5
U133P	205206_at	KAL1	Xp22.32	Kallmann syndrome 1 sequence
U133P	234985_at	LDLRAD3	11p13	low density lipoprotein receptor class A domain containing 3
U133P	220253_s_at	LRP12	8q22.2-q23.1	low density lipoprotein-related protein 12
U133P	202011_at	TJP1	15q13	tight junction protein 1 (zona occludens 1)
U133P	205542_at	STEAP1	7q21	six transmembrane epithelial antigen of the prostate 1
U133P	222450_at	PMEPA1	20q13.31-q13	prostate trans TMEPAI
U133P	204944_at	PTPRG	3p21-p14	protein tyrosine phosphatase, receptor type, G
Transduction signals				
U133P	208373_s_at	P2RY6	11q13.5	pyrimidinergic receptor P2Y; G-protein coupled; 6
U133P	59697_at	RAB15	14q23.3	RAB15; member RAS onocogene family
U133P	222777_s_at	WHSC1	4p16.3	Wolf-Hirschhorn syndrome candidate 1
Cytoskeleton				
U133P	227372_s_at	BAIAP2L1	7q21.3-q22.1	BAI1-associated protein 2-like 1
U133P	212681_at	EPB41L3	18p11.32	erythrocyte membrane protein band 4.1-like 3
U133P	220161_s_at	EPB41L4B	9q31-q32	erythrocyte membrane protein band 4.1 like 4B
U133P	201910_at	FARP1	13q32.2	FERM; RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)
U133P	235114_x_at	HOOK3	8p11.21	hook homolog 3 (Drosophila)
U133P	212372_at	MYH10	17p13	myosin; heavy polypeptide 10; non-muscle
U133P	205652_s_at	TTLL1	22q13.1	tubulin tyrosine ligase-like family; member 1
U133P	204042_at	WASF3	13q12	WAS protein family; member 3
Apoptosis				
U133P	218775_s_at	WWC2	4q35.1	WW and C2 domain containing 2
Protein synthesis and regulation				
U133P	221207_s_at	NBEA	13q13	neurobeachin
Metabolism				
U133P	204497_at	ADCY9	16p13.3	adenylate cyclase 9
U133P	222446_s_at	BACE2	21q22.3	beta-site APP-cleaving enzyme 2
U133P	214608_s_at	EYA1	8q13.3	eyes absent homolog 1 (Drosophila)
U133P	229139_at	JPH1	8q21	junctophilin 1
U133P	214039_s_at	LAPTM4B	8q22.1	lysosomal associated protein transmembrane 4 beta
U133P	219855_at	NUDT11	Xp11.22	nudix (nucleoside diphosphate linked moiety X)-type motif 11
U133P	227434_at	WBSCR17	7q11.23	Williams-Beuren syndrome chromosome region 17
Nuclear functions				
U133P	201417_at	SOX4	6p22.3	SRY (sex determining region Y)-box 4
U133P	214600_at	TEAD1	11p15.4	TEA domain family member 1 (SV40 transcriptional enhancer factor)
U133P	213943_at	TWIST1	7p21.2	twist homolog 1 (acrocephalosyndactyly 3; Saethre-Chotzen syndrome) (Drosophila)
U133P	225589_at	SH3RF1	4q32.3-q33	SH3 domain c SH3MD2
Others				
U133P	226313_at	C10orf35	10q22.1	chromosome 10 open reading frame 35
U133P	218820_at	C14orf132	14q32.2	chromosome 14 open reading frame 132
U133P	52975_at	FAM125	9q33.3	family with sequence similarity 125, member B
U133P	221591_s_at	FAM64A	17p13.2	family with sequence similarity 64; member A
U133P	225525_at	CTA-221G9.4	---	KIAA1671 protein
U133P	238497_at	TMEM136	11q23.3	transmembrane protein 136
U133P	227195_at	ZNF503	10q22.2	zinc finger protein 503
U133P	226905_at	FAM101B	17p13	family with se MGC45871
U133P	212504_at	DIP2C	10p15.3	DIP2 disco-int KIAA0934

Online Supplementary Table S10. Genes over-expressed in group CD-2L of HMCL.

Gene ID	Chip	Gene Name	Localization	Description
Intercellular communication signals				
U133P	209728_at	HLA-DRB4	6p21.3	major histocompatibility complex; class II; DR beta 4
U133P	217362_x_at	HLA-DRB6	6p21.3	major histocompatibility complex; class II; DR beta 6 (pseudogene)
U133P	212671_s_at	HLA-DQA1	6p21.3	major histocompatibility complex, class II, DQ alpha 1
U133P	212850_s_at	LRP4	11p11.2-p12	low density lipoprotein receptor-related protein 4
U133P	209035_at	MDK	11p11.2	midkine (neurite growth-promoting factor 2)
U133P	231725_at	PCDHB2	5q31	protocadherin beta 2
U133P	223629_at	PCDHB5	5q31	protocadherin beta 5
U133P	205479_s_at	PLAU	10q24	plasminogen activator; urokinase
U133P	206213_at	WNT10B	12q13	wingless-type MMTV integration site family; member 10B
U133P	32137_at	JAG2	14q32	jagged 2
U133P	200665_s_at	SPARC	5q31.3-q32	secreted protein, acidic, cysteine-rich (osteonectin)
Transduction signals				
U133P	212611_at	DTX4	11q12.1	deltex 4 homolog (Drosophila)
U133P	1552846_s_at	RAB42	1p35.3	RAB42; member RAS homolog family
U133P	34408_at	RTN2	19q13.32	reticulon 2
U133P	212589_at	RRAS2	11p15.2	related RAS viral (r-ras) oncogene homolog 2
Cytoskeleton				
U133P	203881_s_at	DMD	Xp21.2	dystrophin (muscular dystrophy; Duchenne and Becker types)
U133P	208614_s_at	FLNB	3p14.3	filamin B; beta (actin binding protein 278)
U133P	227084_at	DTNA	18q12	dystrobrevin, alpha
Cell cycle				
U133P	224428_s_at	CDCA7	2q31	cell division cycle associated 7
U133P	205418_at	FES	15q26.1	feline sarcoma oncogene
Protein synthesis and regulation				
U133P	213610_s_at	KLHL23	2q31.1	kelch-like 23 (Drosophila)
U133P	202283_at	SERPINF1	17p13.1	serpin peptidase inhibitor, clade F
Metabolism				
U133P	204044_at	QPRT	16p11.2	quinolinate phosphoribosyltransferase
Cancer testis antigens				
U133P	210295_at	MAGEA10	Xq28	melanoma antigen family A; 10
Nuclear functions				
U133P	207030_s_at	CSRP2	12q21.1	cysteine and glycine-rich protein 2
U133P	206140_at	LHX2	9q33-q34.1	LIM homeobox 2
U133P	229349_at	LIN28B	6q21	lin-28 homolog B (C. elegans)
U133P	211105_s_at	NFATC1	18q23	nuclear factor of activated T-cells; cytoplasmic; calcineurin-dependent 1
U133P	226610_at	CENPV	17p11.2	centromere protein V
Others				
U133P	1553138_a_at	ANKLE1	19p13.11	ankyrin repeat and LEM domain containing 1
U133P	229437_at	BIC	21q21.3	BIC transcript
U133P	222761_at	BIVM	13q32-q33.1	basic; immunoglobulin-like variable motif containing
U133P	1555538_s_at	FAM9B	Xp22.32	family with sequence similarity 9; member B
U133P	213058_at	TTC28	22q12.1	tetratricopeptide repeat domain 28
U133P	212646_at	RAFTLIN	3p25.1-p24.3	raft-linking protein
U133P	219247_s_at	ZDHHC14	6q25.3	zinc finger; DHHC-type containing 14
U133P	218974_at	SOBP	6q21	sine oculis bin FLJ10159

Online Supplementary Table S11. Clinical patient data for age, serum-β2-microglobulin, and plasma cell infiltration in the Heidelberg/Montpellier-group (HM) and the Arkansas cohort. Median value and range are given. NA, not available. ISS, International Staging System.

Charateristic	HM cohort (n=206)	Arkansas cohort (n=345)
Age	58.5 [27 – 73]	57 [25 – 77]
Monoclonal protein		
IgG	120	193
IgA	46	93
Bence Jones	35	47
Asecretory	4	6
IgD	1	3
NA	0	3
Myeloma in Durie and Salmon stage		
I	22	NA
II	31	NA
III	153	NA
Myeloma in ISS stage		
I	97	189
II	73	86
III	33	70
NA	3	0
Serum-β2-microglobulin	2.99 [1.3 – 53.6]	2.9 [1.0 – 38.7]
Plasma cells in bone marrow	42 [1 – 100]	42 [4 – 98]

Online Supplementary Table S12. Univariate and multivariate proportional hazards analysis.

		HM Cohort				LR-TT2 Cohort	
		EFS		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value
Univariate COX analysis	HMCL score	2.03	0.0001	2.22	0.0001	NA	NA
	ISS	1.34	0.02	1.97	0.001		
Multivariate Cox analysis	HMCL score	1.94	0.0001	2.05	0.0001	NA	NA
	ISS	1.26	0.08	1.79	0.005		

		HM Cohort				LR-TT2 Cohort	
		EFS		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value
Univariate COX analysis	HMCL score	2.03	0.0001	2.22	0.0001	NA	NA
	B2M	1.10	0.007	1.1	0.0001		
	Alb	1.51	0.04	2.06	0.02		
Multivariate Cox analysis	HMCL score	1.94	0.0001	2.06	0.001	NA	NA
	B2M	1.1	0.02	1.1	0.002		
	Alb	1.24	0.3	1.78	0.08		

		HM Cohort				LR-TT2 Cohort	
		EFS		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value
Univariate COX analysis	HMCL score	2.03	0.0001	2.22	0.0001	1.78	0.0001
	HRS	1.91	0.006	2.37	0.01	4.67	0.0001
Multivariate Cox analysis	HMCL score	1.90	0.0001	2.03	0.001	1.51	0.009
	HRS	1.42	0.1	1.62	0.2	4.04	0.0001

		HM Cohort				LR-TT2 Cohort	
		EFS		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value
Univariate COX analysis	HMCL score	2.03	0.0001	2.22	0.0001	1.78	0.0001
	MS group	3.07	0.0001	3.32	0.0001	2.21	0.001
Multivariate Cox analysis	HMCL score	1.89	0.006	1.98	0.04	1.58	0.02
	MS group	1.19	0.6	1.28	0.6	1.30	0.4

		HM Cohort				LR-TT2 Cohort	
		EFS		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P* value	Proportional hazard ratio		Proportional hazard ratio	*P* value
Univariate COX analysis	HMCL score	2.03	0.0001	2.22	0.0001	1.78	0.0001
	IFM score	1.87	0.01	2.47	0.02	1.77	0.004
Multivariate Cox analysis	HMCL score	1.98	0.0001	2.11	0.0001	1.72	0.0001
	IFM score	1.70	0.04	2.10	0.06	1.66	0.01

		OAS			
		HM Cohort		LR-TT2 Cohort	
	Pronostic variable	Proportional hazard ratio	*P* value	Proportional hazard ratio	*P* value
Multivariate Cox analysis	HMCL score	1.84	0.006	1.55	0.005
	ISS	1.16	0.61	1.13	0.45
	B2M	1.1	0.02	1.1	0.03
	Alb	1.76	0.13	1.1	0.71
	HRS	1.19	0.69	3.15	0.0001
	IFM score	2 .14	0.09	1.01	0.95

Univariate analyses were made to screen for prognostic variables by using Cox's proportional hazards regression. The Cox's model was also used for multivariate analysis to identify the most significant variables related to survival. NA: Not available