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

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Abstract

Background

Multiple Myeloma is a plasma-cell tumor with heterogeneity in molecular abnormalities and treatment response.

Design and Methods

We have assessed whether human myeloma cell lines have kept patients' heterogeneity using Affymetrix gene expression profiling of 40 myeloma cell lines obtained with or without IL6 addition and could provide a signature for stratification of patient's risk.

Results

Myeloma cell lines, especially those derived in the presence of IL6, displayed a heterogeneity that overlaps the one of the patients with MM. Myeloma cell lines segregated into 6 groups marked by overexpression of *MAF*, *MMSET*, *CCND1*, *FRZB* with or without overexpression of cancer testis antigens. Cell lines of CTA/MAF and MAF groups have a translocation involving C-MAF or MAFB, cell lines of groups CCND1-1 and CCND1-2like have a t(11;14) and cell lines of group MMSET have a t(4;14). CTA/FRZB group gathers cell lines that had none or no recurrent 14q32 translocation. Expression of 248 genes accounted for myeloma cell lines molecular heterogeneity. Myeloma cell lines heterogeneity genes comprise genes with prognostic value for survival of patients making it possible to build powerful prognostic scores.

Conclusion

Myeloma cell lines derived in the presence of IL6 recapitulate the molecular diversity of MM that made it possible the design, using myeloma cell lines heterogeneity genes, of a high-risk signature for patients at diagnosis. We propose this classification to be used when addressing physiopathology of MM with myeloma cell lines.

Introduction

Multiple Myeloma (MM) is a plasma-cell malignancy with a high degree of biological and genetic heterogeneity at presentation and a great variability with regards to the clinical outcome of the patients in response to chemotherapy(1). During the last 15 years, numerous studies have pointed out the heterogeneity of both the phenotype (CD20, CD28, CD56, CD117) and chromosomal abnormalities of multiple myeloma cells (MMC) in association with patient outcome (2-4). Chromosomal abnormalities include full or partial deletion of chromosomes 13 or 17, 1q21 amplifications, recurrent 14q32 translocations (in 40% of the patients involving either *CCND1, MMSET* and *FGFR3, CCND3, c-MAF or MAFB)* and hyperdiploidy (in 45% of patients with MM). The combined analysis of phenotype, chromosomal abnormalities and morphology has brought up the concept of “many and multiple myelomas”(5).

Bergsagel *et al.* have proposed a GEP-based molecular classification of MM taking into account the ubiquitous expression of D-type cyclins (6). They pointed out an expression of one of the three cyclin D genes as a general feature of MM and proposed a classification of patients within eight TC (translocation/cyclin D) groups(6, 7). Using GEP of 414 newly diagnosed patients, Zhan et al. have proposed a molecular classification of MM into 7 groups(8). These groups are characterized by an overexpression of genes involved in cell cycle and proliferation (PR group for proliferation), a lower expression of genes involved in bone disease (LB group for ‘‘low bone disease’’), an aberrant expression of *FGFR3* and *MMSET* genes (MS group for MMSET), a hyperdiploid signature (HY group); an overexpression of *cyclin D1* or *cyclin D3* genes (CD-1 and CD-2 groups), or an overexpression of *MAF* and *MAFB* genes (MF group) (8). HY, CD-1, CD-2, and LB groups had a longer event-free survival and overall survivals than PR, MS, and MF groups. This seven-group

GEP classification is a significant predictor for survival in MM. Using the same series of Affymetrix GEP data, Shaughnessy's group identified 70 genes whose up-regulation or down-regulation was linked with bad prognosis. A high-risk score was built delineating a subset of 13% of newly-diagnosed patients with adverse prognosis(9). This high-risk score has a strong prognostic value. The Intergroupe Français du Myélome (IFM) group reported another gene signature - IFM score - for high-risk patients(10). Finally, hyperdiploid MM were also subclassified into 4 groups with different clinical outcomes or drug sensitivity(11).

The obtainment of human myeloma cell lines (HMCLs) is an important tool for promoting our understanding myeloma pathogenesis and finding novel therapies. In particular, the biological studies of MM disease are often done with a limited set of HMCLs. General conclusions are then being drawn for explaining MM disease, without really knowing the relevance of a limited set of HMCLs to MM disease *in vivo*. The immortalization of primary MMC into HMCL remains a rare event occurring only once primary MMC do highly proliferate *in vivo* and have likely escaped their bone marrow environment dependence with extramedullary proliferation. One mechanism could be the high frequency of *Myc* gene deregulation in 93% of these HMCLs(12). Since the identification of IL-6 as a main growth factor for MMC 20 years ago (13-15), we have obtained a large cohort of HMCLs, culturing primary MMC from patients with extramedullary proliferation with IL-6(16). These HMCLs are heterogeneous based on phenotype, chromosomal abnormalities and growth factor responses(17-20). Previous evaluation on limited number of HMCLs lacking IL6-type HMCLs showed that HMCLs were not a reflect of MM diversity(21, 22)

Analyzing the gene expression profile (GEP) of 40 HMCLs including a majority of IL6-type HMCLs with Affymetrix U133 2.0 plus microarrays, we show here that HMCLs

have kept the molecular heterogeneity of primary MMC of newly diagnosed patients. A list of 248 genes makes it possible to document HMCL biological and genetic heterogeneity. Of major interest, this HMCL heterogeneity gene list comprises genes that allow the design of powerful gene based risk scores according to patients' treatment. This classification should be used when addressing physiopathology of MM with HMCLs.

Design and Methods

Human Myeloma Cell Line (HMCLs)

XGs, NANs, BCN, MDN and SBN HMCLs were derived in our laboratories from primary myeloma cells cultured in RPMI1640 medium in the presence of 10% fetal calf serum (FCS) and 3 ng/ml recombinant IL-6 as previously described(16, 23-25). ANBL-6 was kindly provided by Dr Jelinek (Rochester,USA), KMS-11, KMS12-BM, KMS12-PE and KMM1 by Dr Otsuki (Okayama, Japan), JJN3 by Dr Van Riet (Bruxelles, Belgium), JIM3 by Dr MacLennan (Birmingham, UK), Karpas620 by Dr Karpas (Cambridge UK) and MM1S by Dr S. Rosen (Chicago, USA). AMO-1, LP1, L363, NCI-H929, U266, OPM2, and SKMM2 were from DSMZ (Germany), and RPMI8226 from ATTC (USA). All HMCLs derived in our laboratories and ANBL-6 were cultured in the presence of r-IL-6. Identification of each HMCL was assessed by HLA Class I typing. Interphase FISH was performed according to our previously reported standard protocol(26). Metaphase spreads and interphase cells were evaluated using a DM RXA2 fluorescence microscope (Leica, Bensheim, Germany). Ras and TP53 mutations were identified by direct sequencing of RT-PCR products (Supplementary TableS2). HMCL microarray data have been deposited in the

ArrayExpress public database under accession numbers E-TABM-937 and E-TABM-1088.

Primary myeloma cells

Multiple Myeloma cells (MMC) were purified from 206 patients with newly-diagnosed MM after written informed consent was given at the University hospitals of Heidelberg (Germany) or Montpellier (France). The study was approved by the ethics boards of Heidelberg University and Montpellier University.

These 206 patients were treated with Vincristine, Adriamicyne and Dexamethasone (VAD), high dose Melphalan (HDM) and autologous stem cell transplantation (ASCT) (27) and were termed in the following Heidelberg-Montpellier (HM) series. The .CEL files and MAS5 files have been deposited in the ArrayExpress public database, under accession number E-MTAB-362. We also used Affymetrix data of a cohort of 345 purified MMC from previously untreated patients from the Arkansas Cancer Research Center (ACRC, Little Rock, AR). The patients were treated with total therapy 2 including HDM and ASCT (28) and termed in the following ACRC-TT2 series. These data are publicly available via the online Gene Expression Omnibus (Gene Expression Profile of Multiple Myeloma, accession number GSE2658. http://www.ncbi.nlm.nih.gov/geo/). After Ficoll-density gradient centrifugation, plasma cells were purified using anti-CD138 MACS microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany).

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(29, 30). 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)(31). 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 *Taq*Man 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.

Spiked *MMSET* expression surrogating t(4;14)

The t(4;14) translocation results in aberrant *FGFR3* expression in 70% of patients and *MMSET* spiked expression in 100% of patients(32), and spiked *MMSET* expression has been taken as surrogate for the presence of t(4;14) as previously described(19).

Gene expression profiling and statistical analyses

Gene expression data were normalized with the MAS5 algorithm and analyzed with our bioinformatics platforms: RAGE (http://rage.montp.inserm.fr/)(33) and Amazonia

(http://amazonia.montp.inserm.fr/)(34). 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 4163 unique genes. A hierarchical clustering using average linkage and centered correlation metric was used to identify subgroups. The genes that were significantly overexpressed or underexpressed in specific subgroups were identified using multiclass supervised analysis with the significance analysis of microarray software (SAM)(35) with a 1000-permutation adjustment. The event free or overall survival of subgroups of patients was compared with the log-rank test and survival curves computed with the Kaplan-Meier method. The prognostic values of parameters were compared with univariate or multivariate Cox analysis. Statistical comparisons were done with Mann-Whitney, Chi-square, or Student t-tests. Statistical tests were performed with the software package SPSS 12.0 (SPSS, Chicago, IL).

Results

Characteristics of HMCL cohort

Our cohort of 40 HMCLs comprises 24 HMCLs reproducible obtained adding exogenous IL-6 and FCS (23 from Montpellier/Nantes teams) and 16 HMCLs (collected from other laboratories and commonly used worldwide) that were obtained with FCS without adding exogenous IL-6. These HMCLs will be termed in the following HMCLs$^{serum+IL-6}$ and HMCLs^{serum} (Table 1). A major difference between HMCLs$^{serum+IL-6}$ and HMCLs^{serum} is their dependence on addition of exogenous IL-6 to grow *in vitro*. The growth of 16 of the 24 HMCLs$^{serum+IL-6}$ and that of 0 out of the 16 HMCLs^{serum} was strictly dependent on addition of exogenous IL-6 *in vitro* (IL-6

dependence ++, *P* = 6E-5). A simple explanation is that adding exogenous IL-6 made it possible to expand both exogenous IL-6 dependent and independent primary MMC. CD45 expression was associated with IL-6 dependence as 62.5% (15/24) of HMCLs$^{serum+IL-6}$ did express CD45 as compared to 25% (4/16) of HMCLs^{serum} (*P* = .027). All HMCLs but one expressed CD138, 36/40 expressed highly CD38, 38/40 did not express CD20 and 39/40 did not express CD19 (Supplementary Table S1). Cytogenetics was done in 39 HMCLs. 90% of the HMCLs had a 14q32 translocation involving *MAF* (31%), *CCND1* (28%) or *MMSET* (23%) genes (Tables 1A and 1B). Of note, HMCLs with t(4;14) translocation rarely expressed CD45 (2/9) and when it is the case at a low level (Table 1A), as reported for primary MMC(36). *TP53* abnormalities (point mutation, deletion, insertion, lack of expression, see details in Supplementary Table S2) were found in 65% of HMCLs (Table 1A & B). HMCLs$^{serum+IL-6}$ had a trend to have less TP53 abnormalities than HMCLs^{serum}, 58% versus 81% (*P*=0.1). *Ras* mutations (*N-ras* and *K-ras*) were found in 45% of HMCLs (Table 1B). Six HMCLs, five from HMCLs$^{serum+IL-6}$ cohort, had neither *N*- or *K-Ras* mutation nor TP53 abnormality (Tables 1, Supplementary Table S2). There was a 4-fold increase in the frequency of translocations involving *c-MAF* or *MAFB* genes compared with those published for primary MMC of newly diagnosed patients (*P* < .0001) (1). Thus, HMCLs are heterogeneous in term of the method of obtainment, IL-6 dependence, phenotype and gene abnormalities. The greatest heterogeneity is found for HMCLs$^{serum+IL-6}$ with 25% of HMCLs$^{serum+IL-6}$ with no myeloma specific recurrent translocation or no translocation versus 6% for HMCLs^{serum} (*P* = .0002). In order to look further for HMCL heterogeneity, whole genome transcriptome analysis was performed using Affymetrix U133 2.0 plus microarrays.

Gene expression profiling of the HMCLs

Comparing the gene expression profile of HMCLs$^{serum+IL-6}$ and HMCLs^{serum}, 23 genes were upregulated in HMCLs$^{serum+IL-6}$, but none in HMCLs^{serum} (1000 permutations, ratio ≥ 2 and FDR = 0%, Supplementary Table S3A). Among these 23 genes, three - CD45 (PTPRC), SOCS3, and BCL6 - have been reported to be induced by IL-6 in MMC or other cell lineages (37-39)(Supplementary Table S3A). The NF-κB gene index reported by Anunziata *et al*. (40) and adapted to HMCLs (41) ranged from 4.2 to 8.6 without significant difference between HMCLs$^{serum+IL-6}$ and HMCLs^{serum} (Supplementary Table S4). Only 2 genes (*MDM2 and CDKN1A*), 2 well known TP53 targets (42) were differentially expressed between HMCLs without and with TP53 mutations (data not shown). No significant differential gene expression was found between HMCLs with or without N-ras or K-ras mutations (results not shown).

Identification of 6 groups in human myeloma cell lines based on gene expression profiles and identification of 248 genes documenting the heterogeneity of HMCLs.

The 40 HMCLs could be clustered into six groups using unsupervised hierarchical clustering and 4163 probe sets with the highest variance. Within each group, the GEP of the HMCLs were significantly correlated each other ($P \leq .05$) but none of the six groups was significantly correlated to another one. Each of the 6 groups could be identified by genes known to be important markers of MM disease such as *c-MAF, CCND1*, *FRZB*, *MMSET*, *FGFR3* and cancer testis antigens (CTA) (Supplementary Figure S1). To further delineate the gene signature of these 6 groups, a SAM multiclass analysis was ran identifying 248 HMCL heterogeneity genes differentially expressed between the 6 groups (1000 permutations, FDR ≤ 5%) (Supplementary Tables S5 to S10). Using these 248 genes, an unsupervised hierarchical clustering grouped HMCLs into 2 major clusters and for each cluster, into 3 groups. In the first

cluster, the 2 groups are characterized by translocations involving *MMSET/FGFR3*, or *MAF* loci and were termed MS, and MF groups by analogy with ACRC molecular classification(8, 43) (Figures 1 A&B). In the second cluster, 2 groups overexpress CTA genes : one is also characterized by *MAF* translocations (termed CTA/MF) and a second one by a lack of recurrent translocations and an overexpression of FRZB (termed CTA/FRZB). This later comprises 5 HMCLs without 14q32 translocation or MM specific recurrent translocations, with an increase in odd chromosomes, and shows overexpression of *CTA* and *FRZB* genes. As 5 out of 7 HMCLs from CTA/FRZB group are of male origin, we have checked that CTA/FRZB group was not built due to Y chromosome gene expression since deleting Y chromosome genes did not change the CTA/FRZB clustering (data not shown). CTA/MF and CTA/FRZB groups comprise exogenous IL-6 dependent HMCLs only except the U266 HMCL that can grow without adding IL-6. But U266 cells produce autocrine IL-6 that drives their growth(44). 5 out of 6 HMCLs of CTA/MF group had translocations involving *c-MAF* or *MAFB* as HMCLs of MF group and SAM analysis between CTA/MF and MF groups indicated that mostly *CTA* genes were differentially expressed (Table S3B & C).

The two last groups gather HMCLs with *cyclin D1* or *D3* translocations. One group had a typical CCND1 signature and was termed CD-1. The other one had also a CCND1 signature with expression of genes that were found in CD-2 group (*RRAS2, ZDHHC14, MDK, DMD*). However, because this group didn't overexpress anchoring genes of CD-2 group like *MS41* or *PAX5*, it was termed CD-2like (CD-2L). SAM analysis between CD-1 and CD-2L groups indicated that among genes differentially expressed between the 2 groups, 6 (*WARS, DNAJC12, TM6SF1, ATF5, STOM,*

ERN1) belong to the genes differentially expressed between CD-1 and CD-2 groups of patients.

Of note, a homologous classification of HMCLs was obtained using the 700 genes of the Arkansas group(8) allowing molecular classification of patients (Supplementary Figure S2) (*P* = NS).

Expression of 4 genes - *LRP12, TEAD1, MAF* and *ITGB7* - was validated by real time RT-PCR (Supplementary Figure S3).

HMCLs have kept some of the molecular heterogeneity of newly diagnosed patients

Using the 248 genes discriminating the 6 HMCL groups, an unsupervised clustering was ran with the GEP of primary MMC of 345 newly-diagnosed patients of ACRC TT2 cohort (Figure 2). Six clusters were identified with a significant correlation between samples within a given cluster ($P \leq .05$). This clustering of primary MMC based on HMCL heterogeneity gene signature partially overlapped the ACRC 7 group molecular classification ($P = .01$)(8). Cluster 1 comprised 93% of patients of the MS (spiked MMSET expression) group, cluster 2: 71% of patients of LB (low bone disease) group, cluster 3: 100% of patients of MF (c-MAF or MAF-B overexpression) group, cluster 4: 46% of patients of PR (proliferation) group and 29% of patients of HY (hyperdiploid) group, cluster 5: 92% of patients of HY group and cluster 6: 89% of patients of CD-1 or CD-2 (cyclin D1 expression) groups (Figure 2). These data demonstrate that HMCLs have kept the molecular heterogeneity MMC of newly-diagnosed patients. Of note, 25 genes were shared between the 248 HMCL heterogeneity genes and the 350 top 50 genes making it possible to define the ACRC 7 group molecular classification (8).

Can HMCL heterogeneity genes predict for patient's survival?

Given that the 248 HMCL heterogeneity genes could be indicators of primary MMC heterogeneity, we looked for whether some of these genes could predict for patient's survival. We used the Heidelberg-Montpellier (HM) cohort of 206 patients treated within or in analogy to GMMG-HD3 trial (27) and the ACRC-TT2 cohort of 345 patients treated with total therapy 2 (Supplementary Table S11) (28). Seven out of the 248 HMCL heterogeneity genes - *TEAD1, CLEC11A, LRP12, MMSET, FGFR3, NUDT11 and KIAA1671* **-** had bad prognostic value for both EFS and OAS in the HM cohort and for OAS in the ACRC-TT2 cohort (Figure 3A & B). All 7 genes were overexpressed in the MS group – t(4;14) – and 3/7 - *LRP12, TEAD1, NUDT11* - were significantly overexpressed in the PR group of patients (Figure S4). To combine the prognostic information of these 7 genes, a simple staging was built scoring patients from 0 to 7 (patients with 0/7 genes present, 1/7, 2/7, …, 7/7), running Kaplan-Meier analyses with the 8 patients groups, grouping together groups with no prognostic difference, and thus obtaining 3 major patient groups with different EFS in HM cohort and in OAS in both HM and ACRC-TT2 series. Group 1 comprises patients whose MMC expressed none or 1 bad prognosis gene, group 2 patients expressing 2 to 4 bad prognosis genes and group 3 at least 5 (Figure 4A). Group 1 represented 71% of the HM patients and 73% of the ACRC-TT2 patients, group 2 21% and 22% of HM or ACRC-TT2 patients respectively and group 3, 8% and 5% of HM and ACRC-TT2 patients (Figure 4A and Figure S5). Group 3 was associated with the worst prognosis in the HM and the ACRC-TT2 cohorts (30.5 months and 41 months in the HM and the ACRC-TT2 cohorts respectively)(Figure 4B and 4C).

At least 2 gene scores based on microarray GEP were predictive for patients' event free or overall survival, the high risk score (HRS) with 70 genes (9) and the Intergroupe Francais du Myelome (IFM) score with 15 genes (10). The HMCL score

gene list shares no gene with HRS or IFM score gene lists. When compared together in multivariate Cox analysis, only the HMCL score was significant unlike HRS and/or IFM scores in the HM cohort. In ACRC-TT2, HMCL score and HRS were significant in multivariate Cox analysis, IFM score being no more significant (Supplementary Table S12). The prognostic value of the HMCL score is not hardly surprising since it identified 2 groups of patients which are already known to have poor prognosis for patients treated with VAD and then HDM and ASCT: patients with t(4;14) and patients with a proliferation gene signature.

To go on concerning the interest of using HMCL gene list to define prognostic patients' group, patients with t(4;14) who are already known to have a poor prognosis according to this treatment protocol were deleted and the same methodology was ran. A list of 6 prognostic genes was obtained - *FSTL5, GAGE1, GAGE12, BCHE, HOOK3 and LOC283352* – (different from the previous 7 prognostic genes) making it possible to separate patients without t(4;14) in 2 groups with a different prognosis (Figure 5A&B and Figure S6). Compared to these 2 groups, patients with t(4;14) had a worst prognosis (Supplementary Figure S6). *GAGE1* and *GAGE12* were overexpressed in the PR group, *BCHE* in the CD-1 group, *LOC283352* in the HY and the PR groups and *FSTL5* in the HY group. *HOOK3* was not significantly overexpressed by one of the 7 groups of patients (data not shown). This second HMCL gene score strengthens the interest of using HMCL gene list to define prognostic patients' group in MM.

Discussion

In this study, we have investigated the molecular heterogeneity of a large cohort of HMCLs and it reflects part of the molecular heterogeneity of primary MMC of newly-diagnosed patients. This heterogeneity of MM is evidenced by various genetic

abnormalities, in particular 5 MM specific recurrent translocations, and the finding of 7 molecular groups using unsupervised classification of high-throughput gene expression data by the ACRC group(8). This HMCL heterogeneity is in full contrast with previous finding showing that HMCLs were not representative of MM diversity (21, 22) but both previous studies used a limited number of HMCLs mainly of serum-type only. Using only HMCL information, a 248-HMCL heterogeneity gene list was found allowing to classify MMC of newly-diagnosed patients with a significant overlap with previously-reported ACRC molecular MMC classification(8). Given this overlapping, the ACRC nomenclature was mainly used to term HMCL groups. In addition, this 248 HMCL heterogeneity gene list comprises genes that can identify patients poorly responding to a given treatment. Several simple and logical conclusions can be drawn. First, the method of obtaining HMCLs greatly influences their molecular heterogeneity. The greatest heterogeneity was found within HMCLs$^{serum+IL-6}$, *i.e.* obtained with IL-6 and serum. In the presence of IL-6 and serum, MMC with or without myeloma-specific recurrent translocations have been immortalized, whereas, in the presence of serum, mostly MMC with recurrent translocations (involving *cyclin D1, MAF* or *MMSET/FGR3* genes) (14/16 HMCL^{serum}) have been immortalized. This suggests that addition of exogenous IL-6 has made it possible to expand both exogenous IL-6 dependent and independent primary MMC. An immediate conclusion is to go on obtaining HMCLs culturing primary MMC with all known myeloma growth factors (at least IGF-1, IL-6, APRIL, IL-21, HGF) to avoid selection of primary MMC responsive to one specific growth factor only(19, 20, 45, 46).

HMCLs^{serum} were mostly CD45^{-} (12/16 HMCLs^{serum}) and respond to IGF-1 or IL-21(20, 47). In contrast, HMCLs$^{serum+IL-6}$ were CD45^{+} or CD45^{-} (with a proportion close

to that reported in newly-diagnosed patients, 2/3 and 1/3, respectively(48) and $CD45^+$ HMCLs are not stimulated by IGF-1 or IL21 alone. *C-MAF* or *MAF-B* translocated HMCLs were highly represented within the $HMCLs^{serum+IL\text{-}6}$ cohort (8/24 $HMCLs^{serum+IL\text{-}6}$) suggesting that MMC overexpressing *MAF* genes are highly sensitive to paracrine IL-6. Besides the heterogeneity due to culture method of parental primary MMC, the molecular heterogeneity of HMCLs is built on Ig translocation occurring in parental primary MMC and on *CTA* gene expression. Myeloma cells frequently express CTA although the mechanisms deregulating CTA expression remain pending(49). *CTA* gene expression is epigenetically regulated in various cancers(50, 51). More recently, Walker *et al*. have shown that the transition of normal PC and MGUS stage to MM is associated with DNA hypomethylation, but the transition of intramedullary stage to PCL or HMCL stage is associated with DNA hypermethylation(52). The *CTA* signature in CTA^{pos} HMCLs could be an indicator of higher hypomethylation status than in CTA^{neg} HMCLs(51).

All HMCLs with t(4;14) translocation underexpress *CTA*. One possible explanation is that the deregulation induced by t(4;14) yields to a drug resistance and poor prognosis, making CTA deregulation not necessary. Noteworthy, primary MMC with t(4;14) overexpress IGF1R and IGF1 (19, 53), making likely easier their immortalization without IL-6. On the other hand, 4 of 7 HMCLs of the CTA/FRZB group have no myeloma-specific recurrent translocations. This CTA/FRZB group expresses *CTA* genes and *FRZB* that could fit with one of the four reported hyperdiploid groups of patients overexpressing *CTA* genes(11). Of note, all these HMCLs overexpress EDNRB which is also overexpress by hyperdiploid group of patients(43). However, they have numerous genetic abnormalities, eventually

induced secondarily at relapses or *in vitro*, making not possible to ascertain they originate from hyperdiploid MMCs.

Regarding *cyclin D* or *MAF* translocations, HMCLs belong both to two groups. HMCLs of CD-2L group were mostly IL-6 independent HMCLs (6 out of 7) and expressed some CTA in contrast to those of CD-1 group (1 out of 5 HMCLs^{IL-6}). Ingenuity analysis of genes differentially expressed between CD-1 and CD-2L groups shown an upregulation of genes related to the lipid metabolism (data not shown). CTA/MAF HMCLs are all IL-6 dependent. An explanation was that adding IL-6 could be critical to immortalize primary MMC from these patients. In agreement, we found that primary MMC from MF patients underexpressed *IL-6* gene but overexpressed both chains of IL-6R complex suggesting that MMC from MF patients are more dependent on paracrine IL-6(19).

Using the 248 HMCL heterogeneity genes, we have found 7 genes - *CLEC11A, FGFR3, KIAA1671, LRP12, MMSET, NUDT11, TEAD1* – that make it possible to build an HMCL score predicting survival in newly-diagnosed patients with MM treated with high-dose chemotherapy. This HMCL score segregates MM patients into 3 groups with low, intermediate and high risk. These 7 HMCL genes share no gene with those of 2 previously reported gene-based risk scores, HRS and IFM scores(9, 10). This HMCL score was shown to be more potent than IFM score in the 2 independent patient cohorts, more potent than HRS in our patient cohort and remains independent of HRS in ACRC-TT2 series (Supplementary Table S12). This is remarkable because this list of 248 HMCL heterogeneity genes has been obtained with cells cultured for many years *in vitro*, from a minor subset of patients with extramedullary proliferation. Not surprisingly, the 2 patient groups with poor prognosis comprise mainly patients with t(4;14) and patients with a proliferation gene

signature, 2 groups already identified with poor prognosis in patients treated with HDM and ASCT. To further investigate the interest of using HMCL gene list to define prognostic patients' group, the same methodology was applied to patient cohorts deleted of patients with t(4;14) already known to have a poor prognosis, making it possible to separate patients without t(4;14) in 2 different prognosis groups (Figure 5A & B). Thus, this 248 HMCL heterogeneity gene list – defined independently of primary MMC information - comprises genes that could help to define patients poorly responding to a given treatment.

Another important conclusion regarding the current data is the interest but also the danger of using HMCLs to study MM biology. Primary MMC are uneasy to obtain in sufficient amounts and poorly survive in culture *in vitro*(16, 25). This is the reason why the majority of the studies of MM disease physiopathology used a limited set of cell lines and a final validation with 1-5 samples of primary MMC, often from patients with extramedullary proliferation. Given the 6 group molecular heterogeneity of HMCLs we reported here using 248 genes, it should be important to recommend the investigators to classify the HMCLs they used in one of the groups and to indicate that the extension of HMCL-derived concepts to MM disease should be limited to patients' subgroups at least. In this study, the role and influence of microenvironment on HMCL gene expression profile was not addressed. It was demonstrated that stromal interactions could influence expression patterns of MMC(54-56). Further analysis of GEP modification by coculture should be of major interest to identify pathways activated by the microenvironment in relation to MM diversity.

In conclusion, this study has shown that HMCLs obtained with IL-6 and serum cover a large part of the molecular heterogeneity primary MMC. A 248-gene list defining HMCLs heterogeneity contains new prognostic genes. This representative cohort

could be helpful to identify common critical pathways and new therapeutic targets for molecular and chromosome based myeloma subgroups.

AUTHORSHIP AND DISCLOSURES

The authors have nothing to disclose.

Author contributions:

MJ performed research and participated in the writing of the paper.
KB participated in the design of the research and the writing of the paper.
RB participated in the design of the research and the writing of the paper.
HD, and GH collected bone marrow samples and clinical data and participated in the writing of the paper.
RT participated in the bioinformatic studies and participated in the writing of the paper.
JA provided cytogenetics analysis.
JM participated in the writing of the paper.
DG provided cytogenetics analysis
MS performed research
AM participated in the design of the research and the writing of the paper.
PDC performed research, participated in the design of the research and the writing of the paper.

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Table 1A Characteristics of the HMCL cohort

HMCL Name HMCL$^{serum+IL-6}$	IL-6 dependence[1]	Origin[2]	Disease[3]	Patient sample[4]	Gender	Isotype	t(14q32 or 22q11 ;)	Target genes	Ras	TP53	CD45	HMCL classification
ANBL6	+	CO	MM	PB	F	I	t(14;16)	*c-Maf*	*wt*	*abn*	-	MF
BCN	+	MN	MM	PB	F	Gk	t(14;16)	*c-Maf*	*wt*	*wt*	-	CD-1
MDN	+	MN	PCL	PB	M	Gk	t(11;14)	*CCND1*	*mut*	*wt*	+	CD-1
NAN1	+	MN	MM	PE	M	Ak	t(14;16)	*c-Maf*	*wt*	*abn*	-	CTA/MF
NAN3	+	MN	MM	PE	F	Ak	t(4;14)	*MMSET*	*mut*	*abn*	-	MS
NAN6	+	MN	MM	PB	F	Ak	t(14;20)	*MafB*	*wt*	*abn*	+	CTA/MF
SBN	+	MN	PCT	PE	M	Al	t(14 ;?)	*unknown*	*wt*	*wt*	-	CTA/FRZB
XG1	++	MN	MM	PB	M	Ak	t(11;14)	*CCND1*	*mut*	*abn*	+	CTA/FRZB
XG2	++	MN	MM	PE	F	Gl	t(12;14)	unknown	*mut*	*abn*	+	CTA/FRZB
XG3	++	MN	PCL	PE	F	I	t(14 ;?)	*unknown*	*mut*	*wt*	+	CTA/FRZB
XG4	++	MN	MM	PB	M	Gk	t(14 ;?)	*unknown*	wt	abn	-	CTA/FRZB
XG5	++	MN	MM	PB	F	I	t(11;14)	*CCND1*	*wt*	*abn*	-	CD-1
XG6	++	MN	MM	PB	F	Gl	t(16;22)	*c-Maf*	*wt*	*wt*	+	CTA/MF
XG7	+	MN	MM	PB	F	Ak	t(4;14)	*MMSET*	*mut*	*wt*	+/	MS
XG10	++	MN	PCT	AF	F	Ak	t(14 ;?)	*unknown*	*mut*	*wt*	+	CTA/MF
XG11	++	MN	PCL	PB	F	I	t(11;14)	*CCND1*	*wt*	*abn*	+	CD-2L
XG12	++	MN	PCL	PB	F	I	t(14;16)	*c-Maf*	*mut*	*wt*	+	CTA/MF
XG13	++	MN	PCL	PB	M	Gl	t(14;16)	*c-Maf*	*wt*	*abn*	+	MF
XG14	++	MN	PCL	PB	M	k	t(11;14)	*CCND1*	*mut*	*abn*	+	MF
XG16	++	MN	PCL	PB	M	k	none	*none*	*mut*	*abn*	+	CTA/FRZB
XG19	++	MN	PCL	PB	F	Al	t(14;16)	*c-Maf*	*wt*	*wt*	+	CTA/MF
XG20	++	MN	PCL	PB	M	I	t(4;14)	*MMSET*	*wt*	*abn*	-	MS
XG21	++	MN	MM	PE	M	I	t(11;14)	*CCND1*	*wt*	*wt*	+	CD-1
XG24	++	MN	PCL	PB	F	Al	t(4;14)	*MMSET/FGFR3*	mut	wt	-	MS
HMCL^{serum}												
AMO1	-	CO	PCT	AF	F	Ak	t(12;14)	unknown	*wt*	*wt*	+	CD-2L
JIM3	-	CO	MM	PE	F	A	t(4;14)	*MMSET/FGFR3*	*wt*	*abn*	-	MS
JJN3	-	CO	MM	PE	F	Ak	t(14;16)	*c-Maf*	*mut*	*abn*	+/-	MF
Karpas620	-	CO	PCL	PB	F	Gk	t(11;14)	*CCND1*	*mut*	*abn*	-	CD-2L
KMM1	-	CO	MM	SC	M	I	t(6;14)	*CCND3*	*mut*	*abn*	-	CD-2L
KMS11	-	CO	MM	PE	F	Gk	t(4;14)	*MMSET/FGFR3*	*wt*	*abn*	-	MS
KMS12BM	-	CO	MM	BM	F	NS	t(11;14)	*CCND1*	*wt*	*abn*	-	CD-2L
KMS12PE	-	CO	MM	PE	F	NS	t(11;14)	*CCND1*	*wt*	*abn*	-	CD-2L
L363	-	CO	PCL	PE	F	NS	t(20;22)	*MafB*	*mut*	*abn*	-	CD-2L
LP1	-	CO	MM	PB	F	Gl	t(4;14)	*MMSET/FGFR3*	*wt*	*abn*	-	MS
MM1S	-	CO	PCL	PB	F	Al	t(14;16)	*c-Maf*	*mut*	*wt*	-	MF
NCI-H929	-	CO	MM	PE	F	Ak	t(4;14)	*MMSET/FGFR3*	*mut*	*wt*	+/-	MS
OPM2	-	CO	MM	PB	F	Gl	t(4;14)	*MMSET/FGFR3*	*wt*	*abn*	-	MS
RPMI8226	-	CO	MM	PB	M	Gl	t(14;16)	*c-Maf*	*mut*	*abn*	-	MF
SKMM2	-	CO	PCL	PB	M	Gk	t(11;14)	*CCND1*	*wt*	*abn*	-	CD-1
U266	-	CO	MM	PB	M	El	t(11;14)	*CCND1*	*wt*	*abn*	+	CTA/FRZB

Table 1 continues on next page.

Table 1B. Characteristics of HMCLs$^{serum+IL6}$ and HMCLs^{serum}

Characteristics	HMCLs		HMCLs$^{serum+IL6}$		HMCLs^{serum}	
	n	%	n	%	n	%
	40		24	57	16	43
Chromosomal abnormalities						
t(4;14)	9	22.5%	4	17%	5	31%
t(14/16)(q32/q23) and other c-MAF/MAFB translocations	12	30%	8	33%	4	25%
t(11;14)	11	27.5%	6	25%	5	31%
t(6;14)	1	2.5%	0	0%	1	6%
t(14;other)	6	15%	5	21%	1	6%
no t(14;)	1	2.5%	1	4%	0	0%
CD45+ expression (including partial expression)	19	47.5%	15	62.5%	4	25%
TP53 abnormalities	26	62%	13	54%	13	81%
N-or K-Ras mutations	18	45%	11	46%	7	44%

Table 2

A. Differential probe set expression between HMCLs$^{serum+IL-6}$ and HMCLs^{serum}

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 HMCLs$^{IL-6+serum}$ and HMCLs^{serum} (SAM analysis, 1000 permutations, FDR=0%)

B. 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. 1000 permutations, FDR=0%)

C. Differential probe set expression between Groups CD-2L and CD-1

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 CT/CD-1 and CD-1 Groups (SAM analysis, 1000 permutations, FDR<5%)

Figure and Table legends:

Table 1. HMCL cohort
A Characteristics of the HMCL cohort
HMCLs were obtained culturing primary myeloma cells with culture medium supplemented with FCS alone or recombinant IL-6 and FCS as indicated by the laboratory of origin.
1. ++ if growth is strictly dependent on adding exogenous IL-6, + if dependent on adding exogenous IL-6, - if not.
2. Origin of the HMCL, MN Montpellier or Nantes, CO collected
3. Disease at diagnosis: MM multiple myeloma, PCL plasma cell leukemia, PCT plasmacytoma
4. Origin of the sample: AF ascitic fluid, BM bone marrow, PE pleural effusion, PB peripheral blood, SC subcutaneous
SBN, XG3 and XG4 have a not recurrent translocation of the 14q32 locus, XG16 has a wild-type 14q32 locus
Target genes: target genes of the translocation
Ras: mutation (codons 12 or 13 or 61) of N or K-Ras, see details in supplementary Table S2
TP53: abn abnormal (point mutations, deletion, insertion), abn$ lack of mRNA expression, see details in supplementary Table S2
Phenotype was analyzed by flow cytometry (see Supplementary Table S1)

B Compared frequencies of chromosomal abnormalities, CD45 expression, TP53 abnormalities and Ras mutations in HMCLs$^{serum+IL6}$ and HMCLs^{serum}

Figure 1. Hierarchical clustering of the 40 HMCLs using the 248 HMCL heterogeneity genes.

A. The 248 genes were identified using SAM multiclass supervised analysis of the 6 molecular groups identified in Figure 1 (1000 permutations, $P \leq .05$). Red and green indicate overexpressed and underexpressed genes, respectively. The expression of some genes is indicated on the right of the dendrogram. HMCLs were clustered into 2 major clusters. One cluster is split into 2 clusters and 4 groups named CTA/MF, CTA/FRZB, CD-1 and CD-2L. The other one is split into 2 groups termed as MF and MS. Name of groups was chosen by analogy with the ACRC molecular classification(8).

B. The Affymetrix signal for each gene is proportional to the height of each bar (representing a single HMCL). Note that *spiked* expression of *CCND1, MAF* and *MAFB,* and *FGFR3* and *MMSET* is strongly correlated with specific subgroup designations.

Figure 2. Unsupervised clustering of the gene expression profiling of primary myeloma cells of newly-diagnosed patients using the 248 HMCL heterogeneity genes.

The Affymetrix gene expression profiles of purified myeloma cells from 345 newly-diagnosed patients were publicly available from the ACRC (Gene Expression Profile of Multiple Myeloma, accession number GSE2658. http://www.ncbi.nlm.nih.gov/geo/). An unsupervised clustering of the 345 samples (columns) using the 248 HMCL heterogeneity genes (lines) makes it possible to cluster samples into 6 major groups defined by the different grey color scale horizontal histograms and arrows. The percentages above the horizontal histograms indicate the overlap of this clustering with the previously published ACRC 7 molecular group classification(8).

Figure 3. 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).

Figure 4. Seven-gene prognostic score.

A. Distribution of the patients from the HM and ACRC-TT2 cohorts according to the 7-gene HMCL score. B. Kaplan-Meier estimates of overall survival and event-free survival (C) of low risk patients (blue), intermediate risk patients (green) and high risk patients (red) according to our 7-gene HMCL score.

Figure 5. Kaplan-Meier estimates of overall survival and event-free survival of patients without t(4;14) according to a 6-HMCLgene score in the HM (A) or ACRC-TT2 cohort (B).

Figure 1A

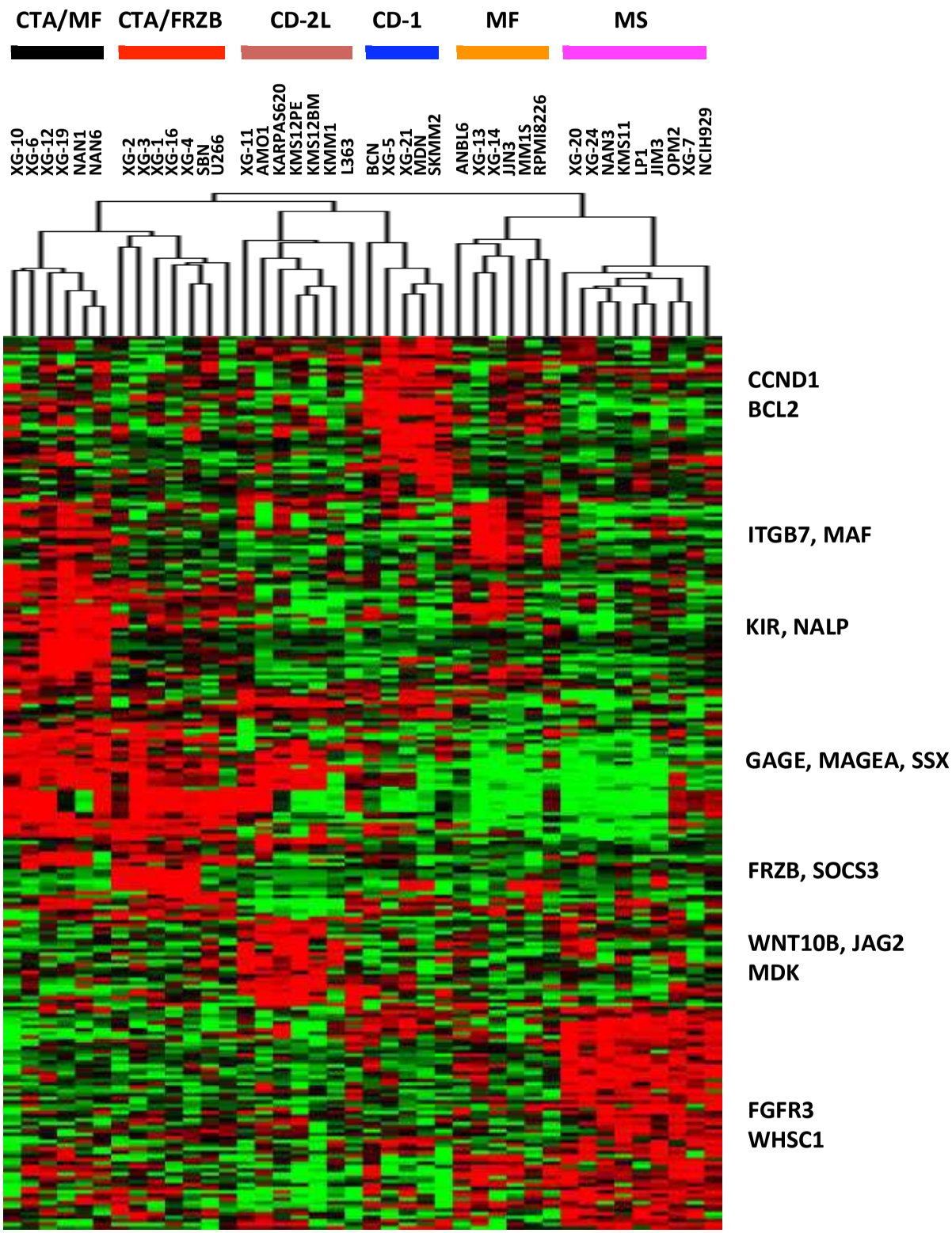

CTA/MF
CTA/FRZB
CD-2L
CD-1
MF
MS
XG-10
XG-6
XG-12
XG-19
NAN1
NAN6
XG-2
XG-3
XG-1
XG-16
XG-4
SBN
U266
XG-11
AMO1
KARPAS620
KMS12PE
KMS12BM
KMM1
L363
BCN
XG-5
XG-21
MDN
SKMM2
ANBL6
XG-13
XG-14
JJN3
MM1S
RPMI8226
XG-20
XG-24
NAN3
KMS11
LP1
JIM3
OPM2
XG-7
NCIH929
CCND1
BCL2
ITGB7, MAF
KIR, NALP
GAGE, MAGEA, SSX
FRZB, SOCS3
WNT10B, JAG2
MDK
FGFR3
WHSC1

Figure 1B

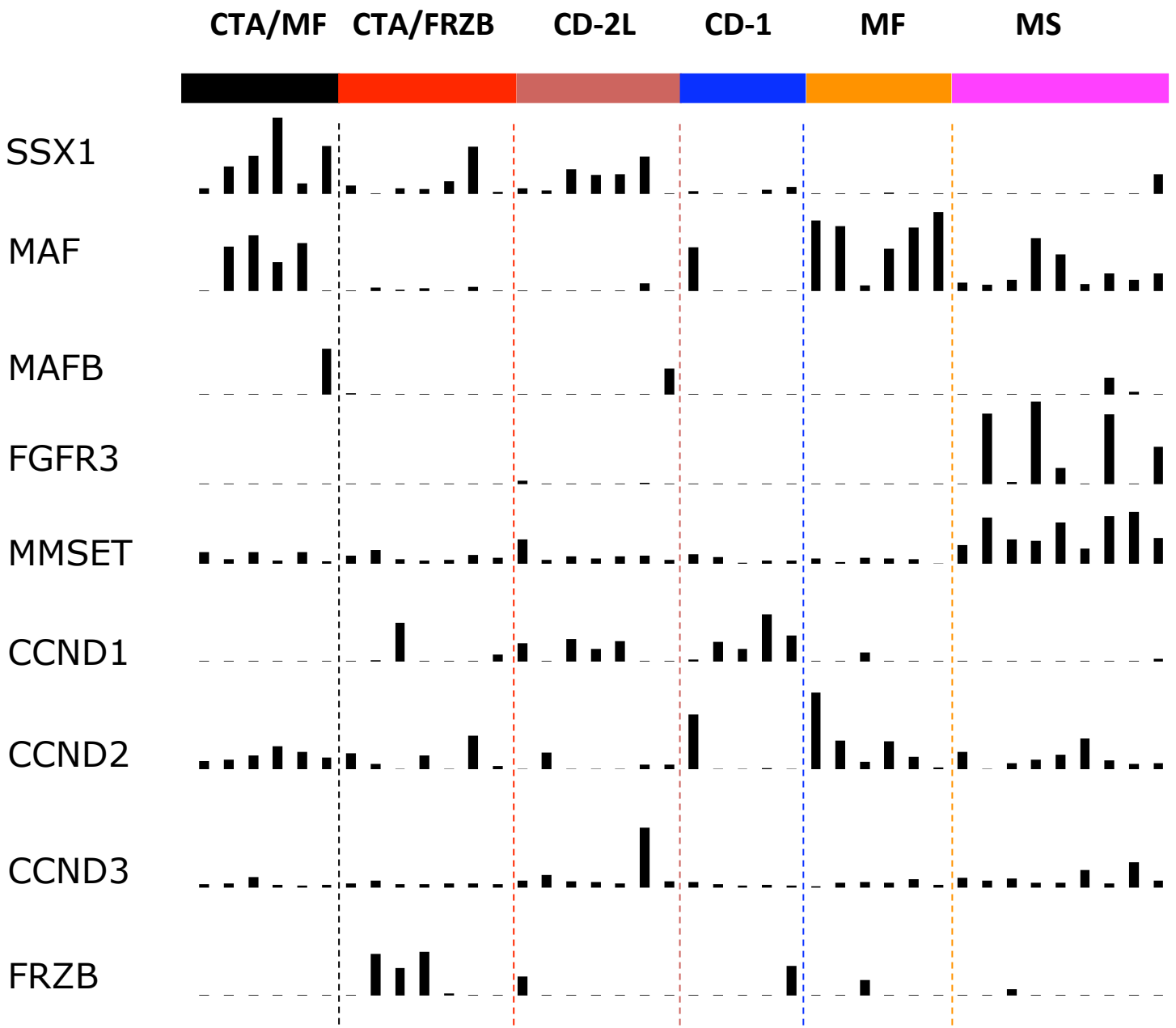

CTA/MF
CTA/FRZB
CD-2L
CD-1
MF
MS
SSX1
MAF
MAFB
FGFR3
MMSET
CCND1
CCND2
CCND3
FRZB

Figure 2

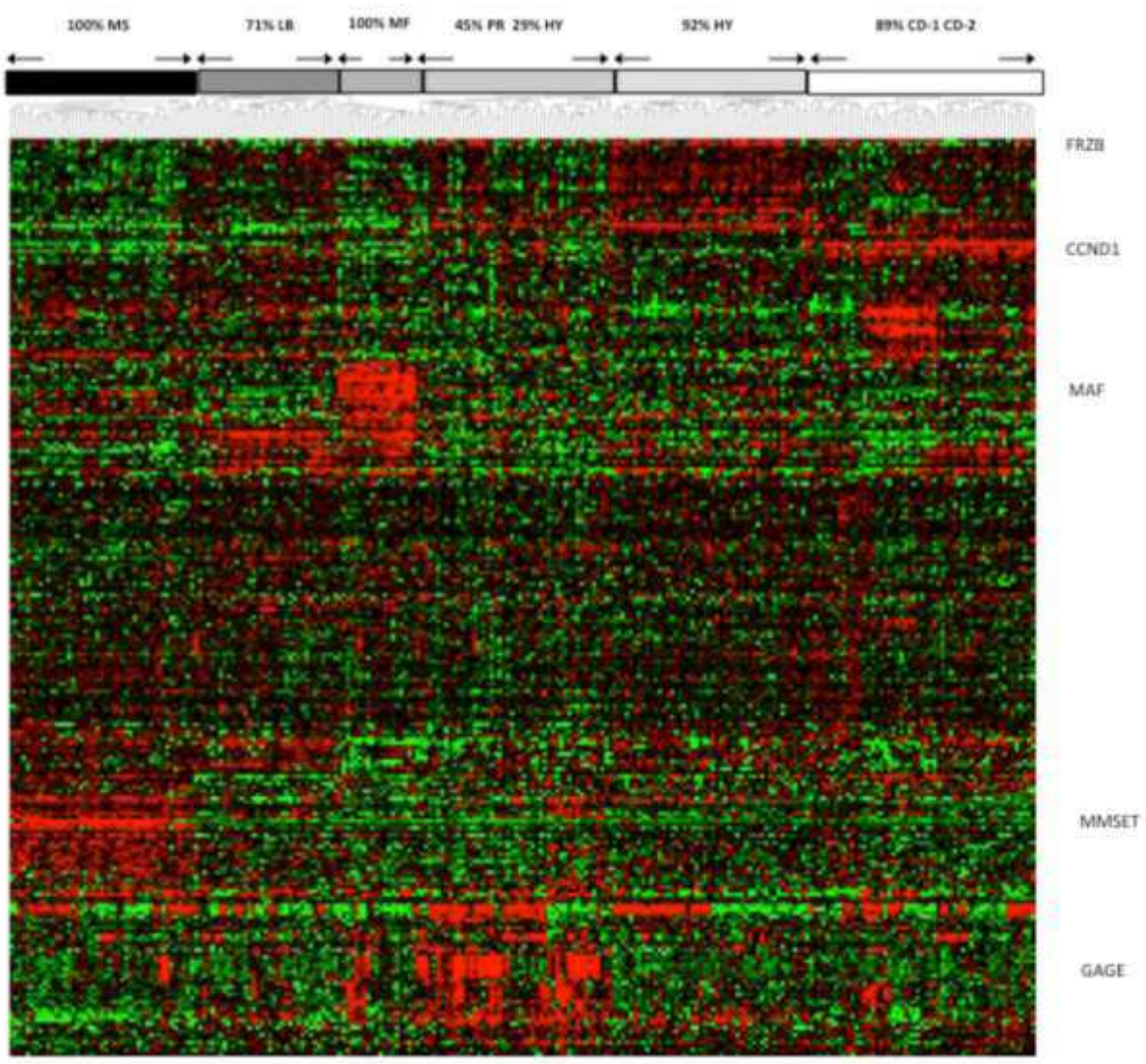

100% MS
71% LB
100% MF
45% PR 29% HY
92% HY
89% CD-1 CD-2
FRZB
CCND1
MAF
MMSET
GAGE

Figure 3A

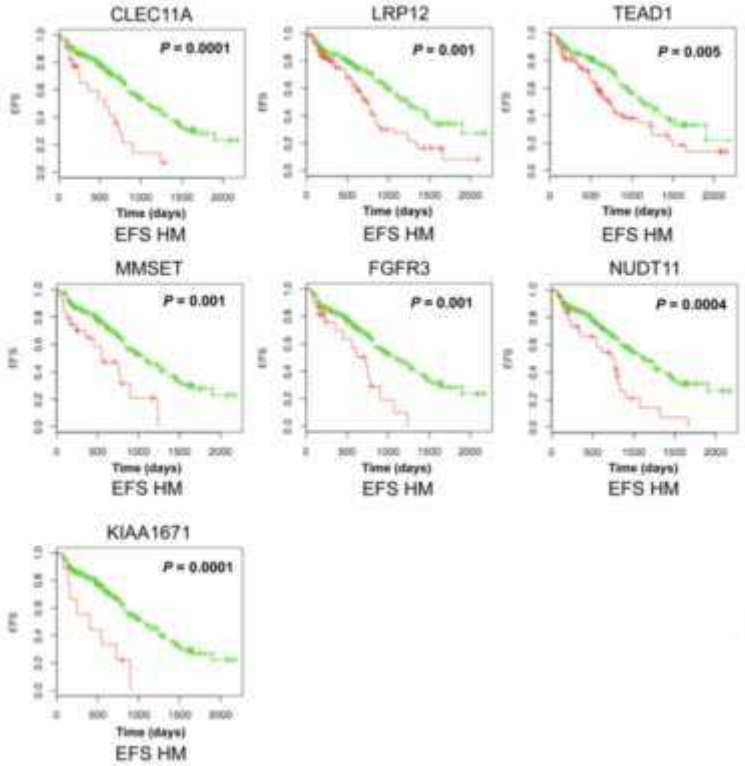


Figure 3B

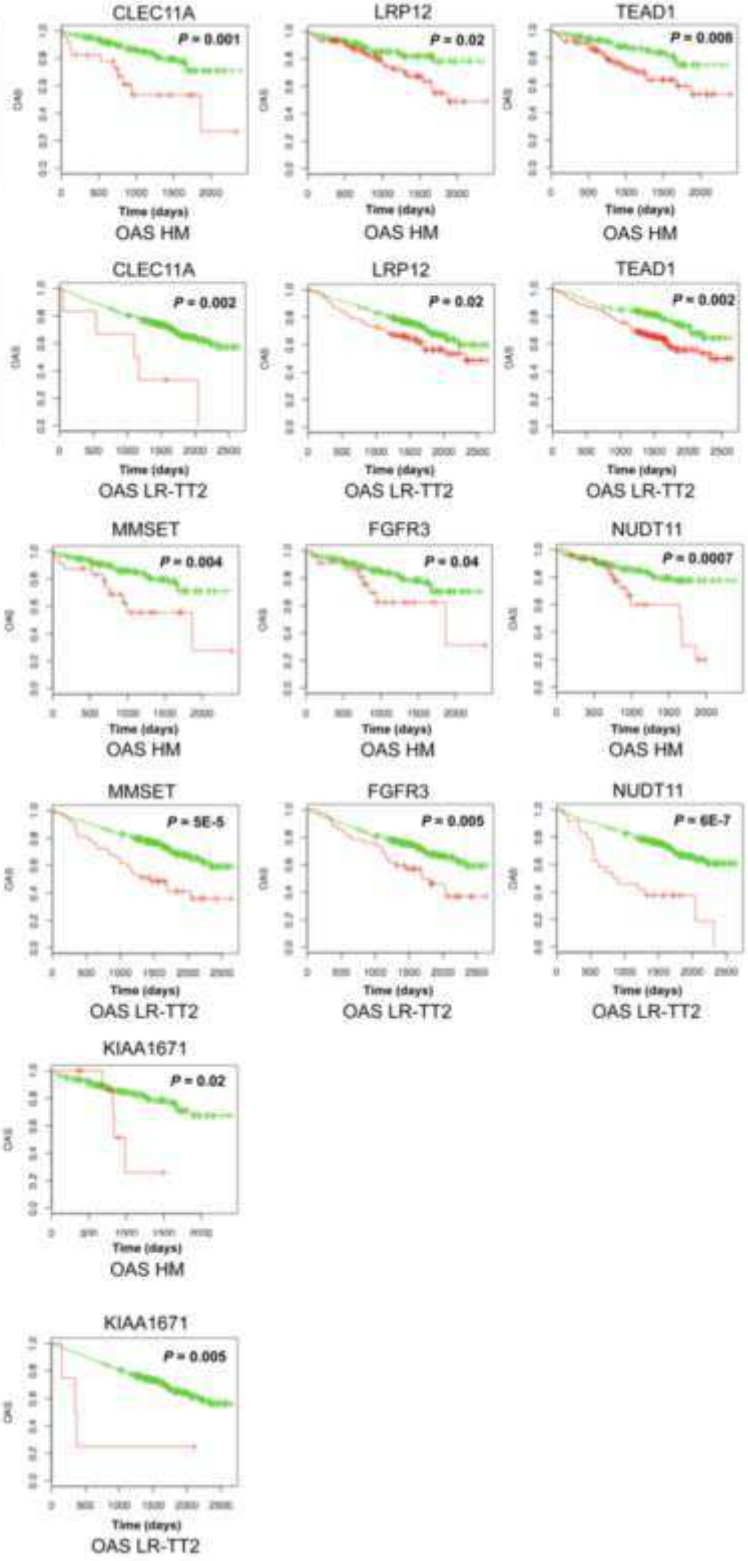


Figure 4A

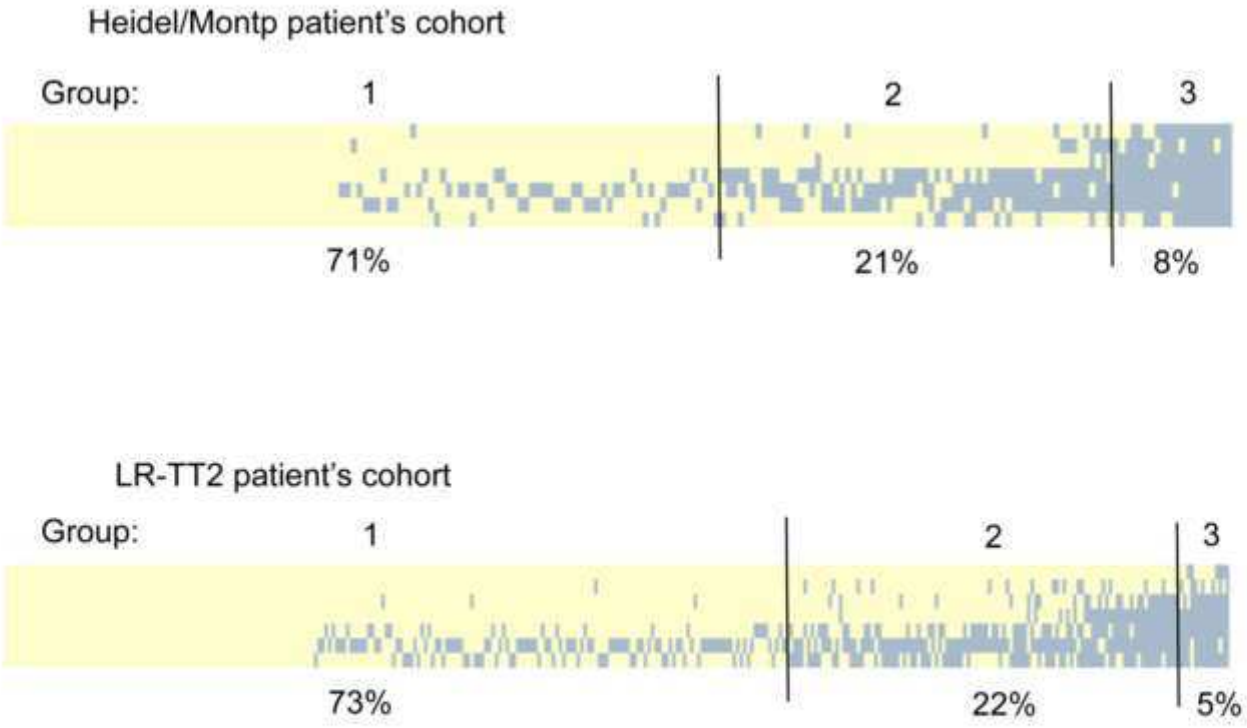


Figure 4B

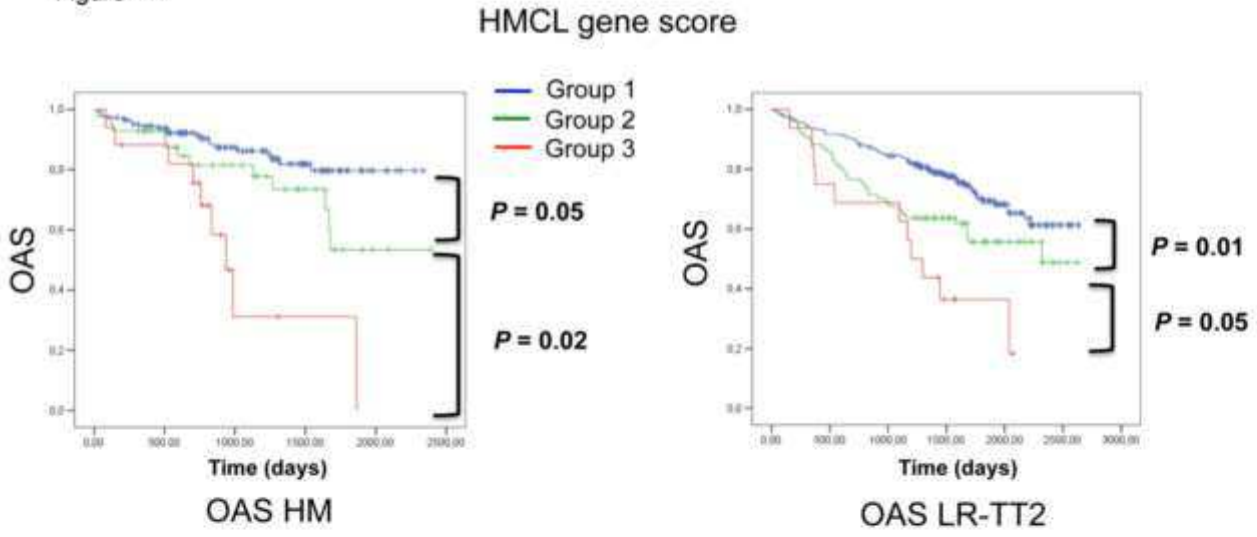


Figure 4C

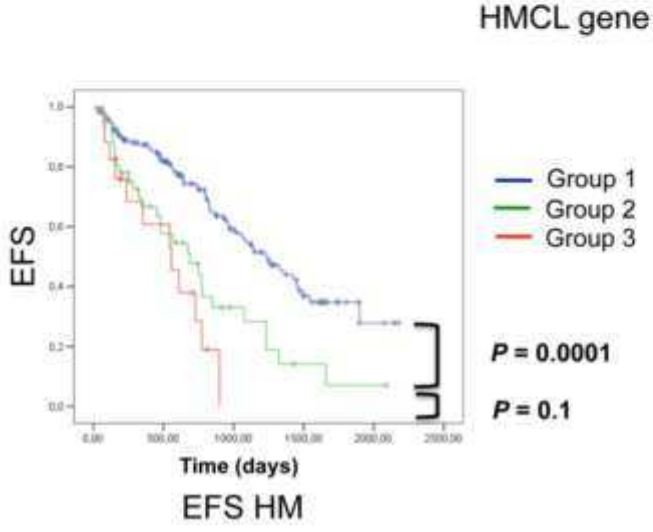
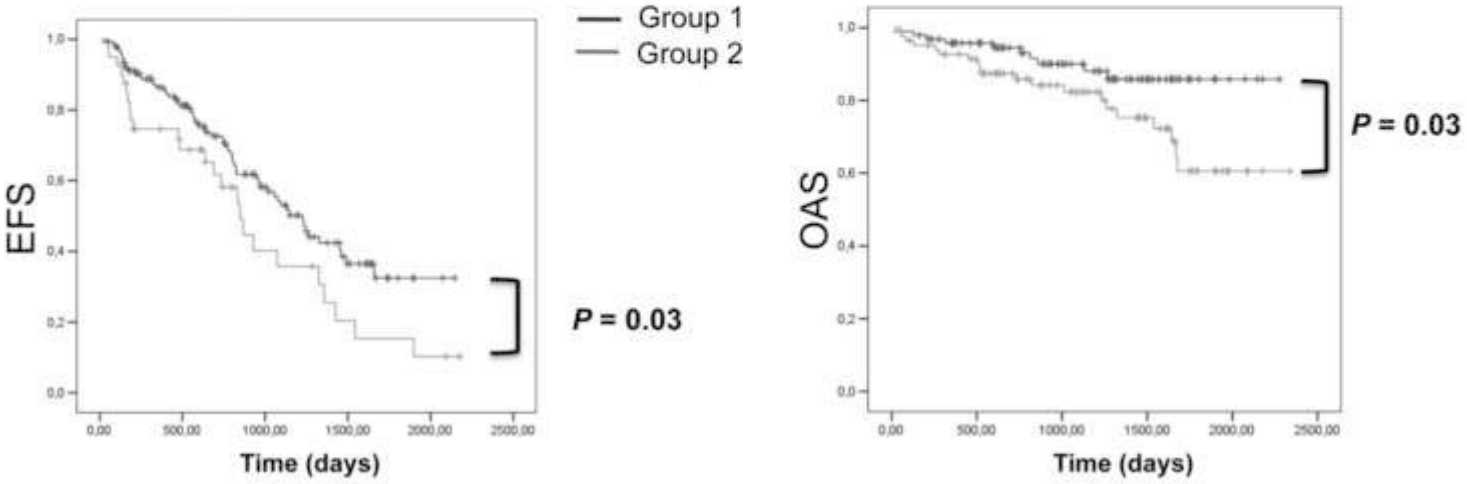


Figure 5A

HMCL gene score in HM cohort without t(4;14) patients

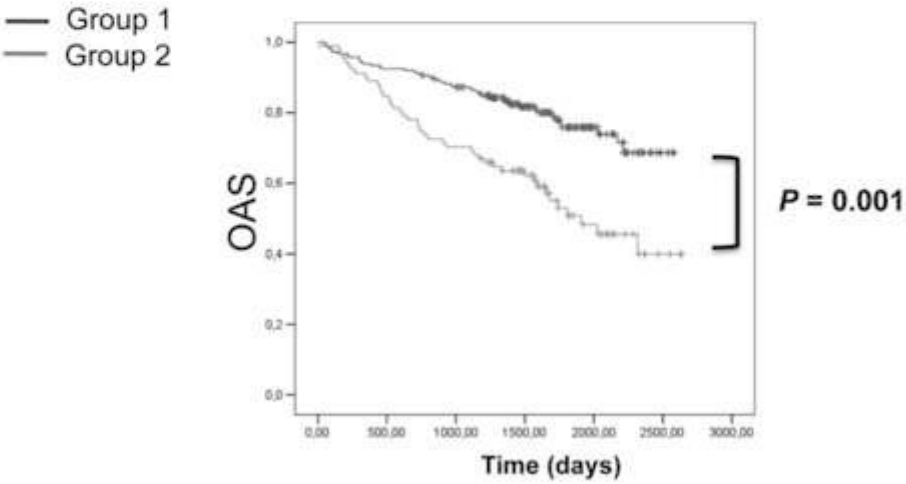


EFS HM

OAS HM

Figure 5B

HMCL gene score in LR-TT2 cohort without t(4;14) patients



OAS LR-TT2

Supplementary Table S1. 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=
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=
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.

Supplementary Table S2: 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

Supplementary Table S3

A. Differential probe set expression between HMCLs$^{serum+IL-6}$ and HMCLs^{serum}

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 HMCLs$^{IL-6+serum}$ and HMCLs^{serum} (SAM analysis, 1000 permutations, FDR=0%)

B. 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. 1000 permutations, FDR=0%)

C. 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, 1000 permutations, FDR<5%)

Supplementary Table S4

NF-κB index of the 40 HMCLs

HMCL	NF-κB index	HMCL classification group	IL-6 dependence
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	+

TABLE S5: Genes overexpressed 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

TABLE S6: Genes overexpressed 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

TABLE S7: Genes overexpressed 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)

TABLE S8: Genes overexpressed 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

TABLE S9: Genes overexpressed 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 transl 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

TABLE S10: Genes overexpressed 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

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]

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.

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	P-value	Proportional hazard ratio	P-value
Univariate COX analysis	HMCL score IFM score	2.03 1.87	0.0001 0.01	2.22 2.47	0.0001 0.02	1.78 1.77	0.0001 0.004
Multivariate Cox analysis	HMCL score IFM score	1.98 1.70	0.0001 0.04	2.11 2.10	0.0001 0.06	1.72 1.66	0.0001 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 ISS B2M Alb HRS IFM score	1.84 1.16 1.1 1.76 1.19 2 .14	0.006 0.61 0.02 0.13 0.69 0.09	1.55 1.13 1.1 1.1 3.15 1.01	0.005 0.45 0.03 0.71 0.0001 0.95

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

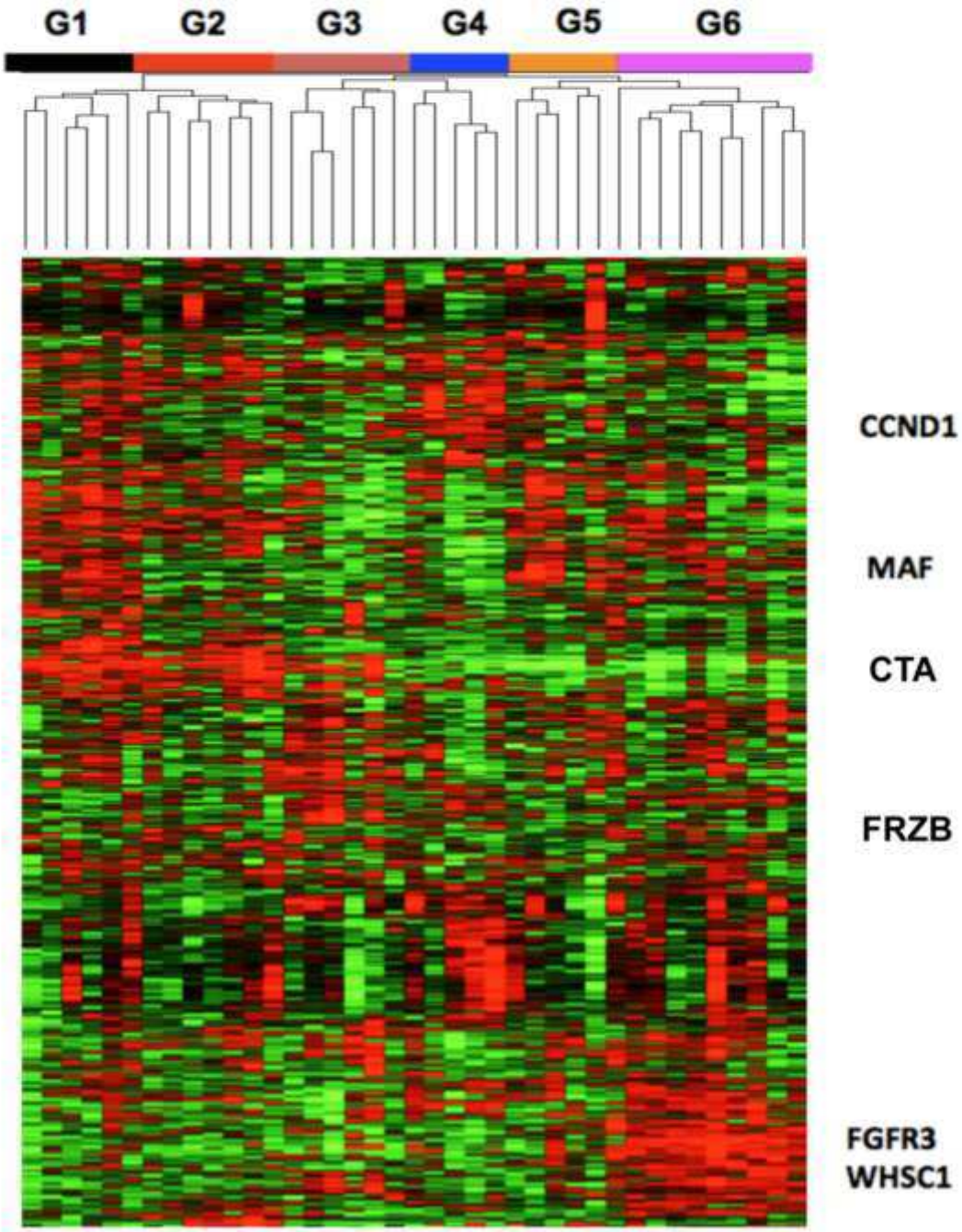
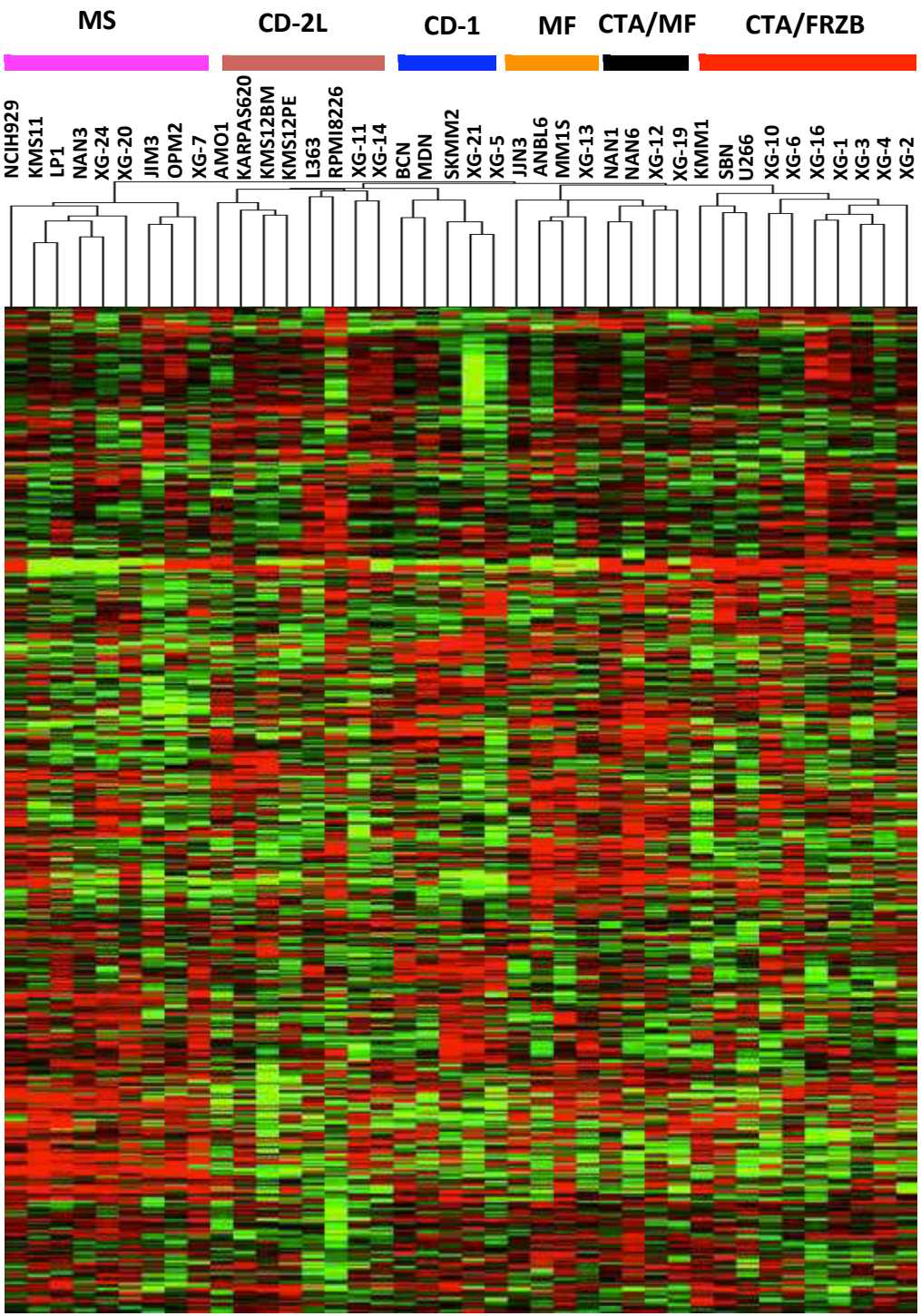
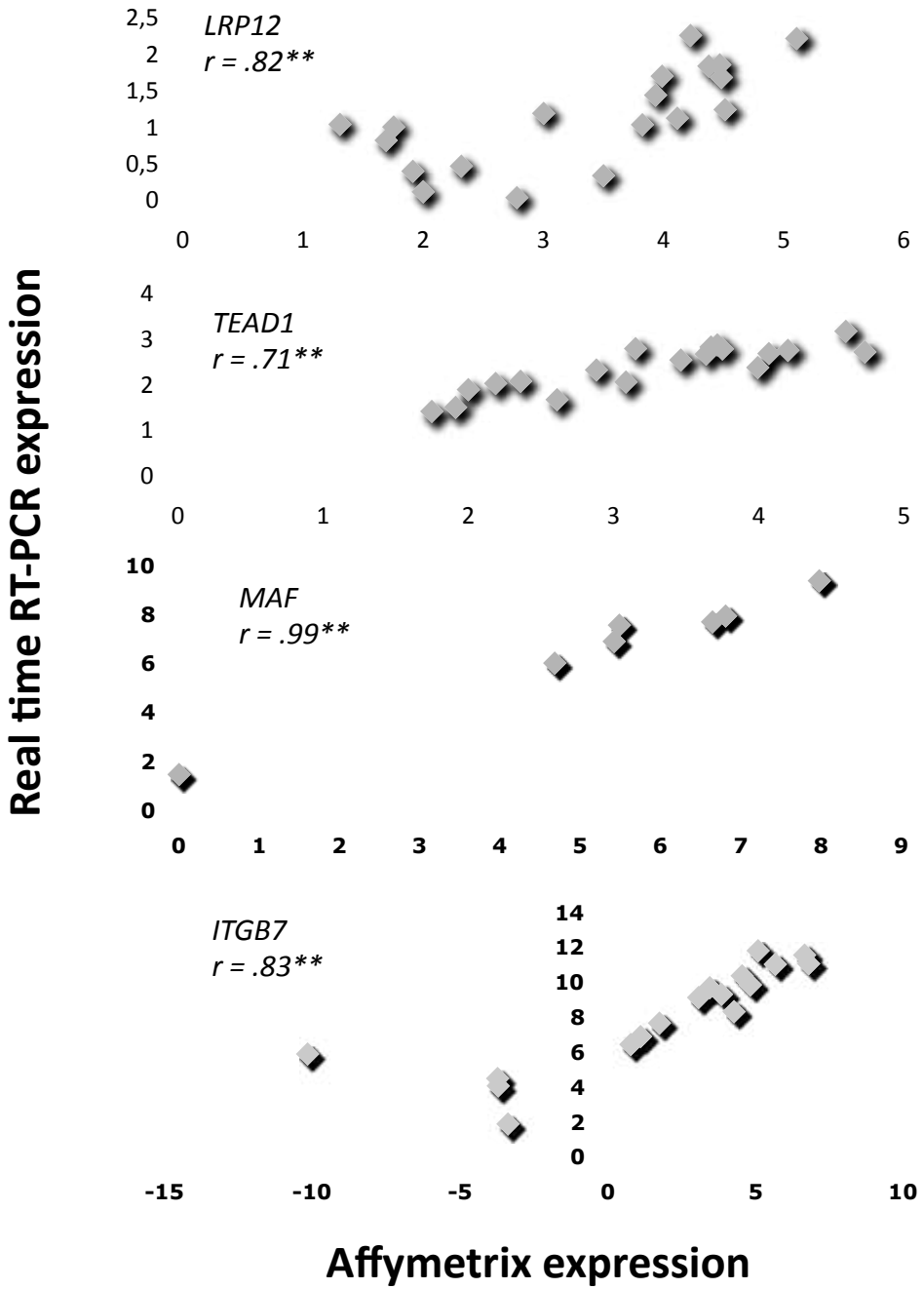


Figure S1. Six molecular groups of HMCLs identified using unsupervised clustering of gene expression profiles. An unsupervised clustering was ran using 4163 probe sets with the highest variance (lines). HMCLs (columns) were split into six groups with a significant correlation of the GEP of the HMCLs within a same group. Probe set expression within HMCLs was mean-centered and depicted by a normalized-signal pseudocolor scale. Red and green indicate overexpressed and underexpressed genes, respectively. The expression of some genes is indicated on the right of the dendrogram.



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).



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 coefficient of correlations between Affymetrix and real-time RT-PCR values were determined.

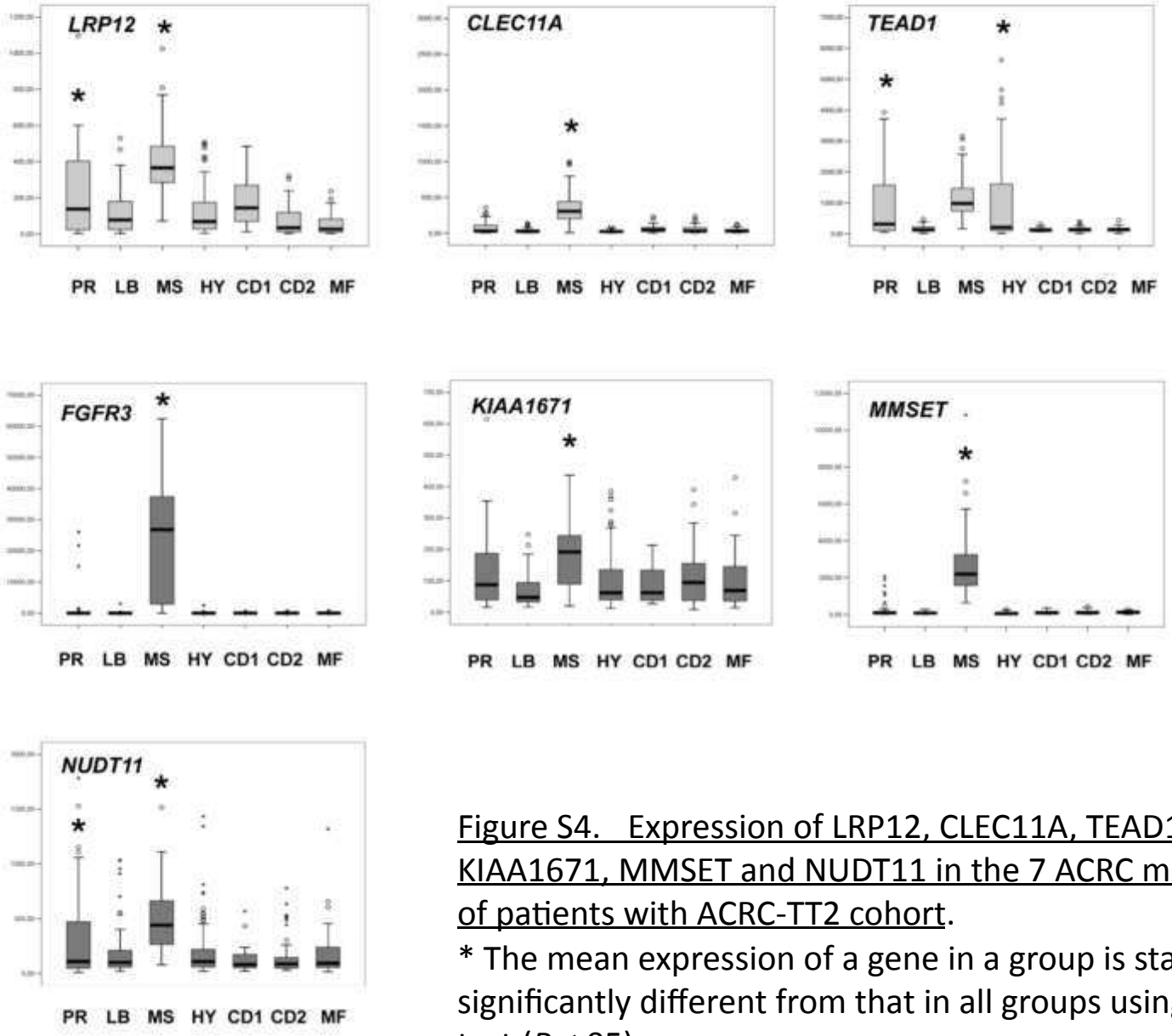
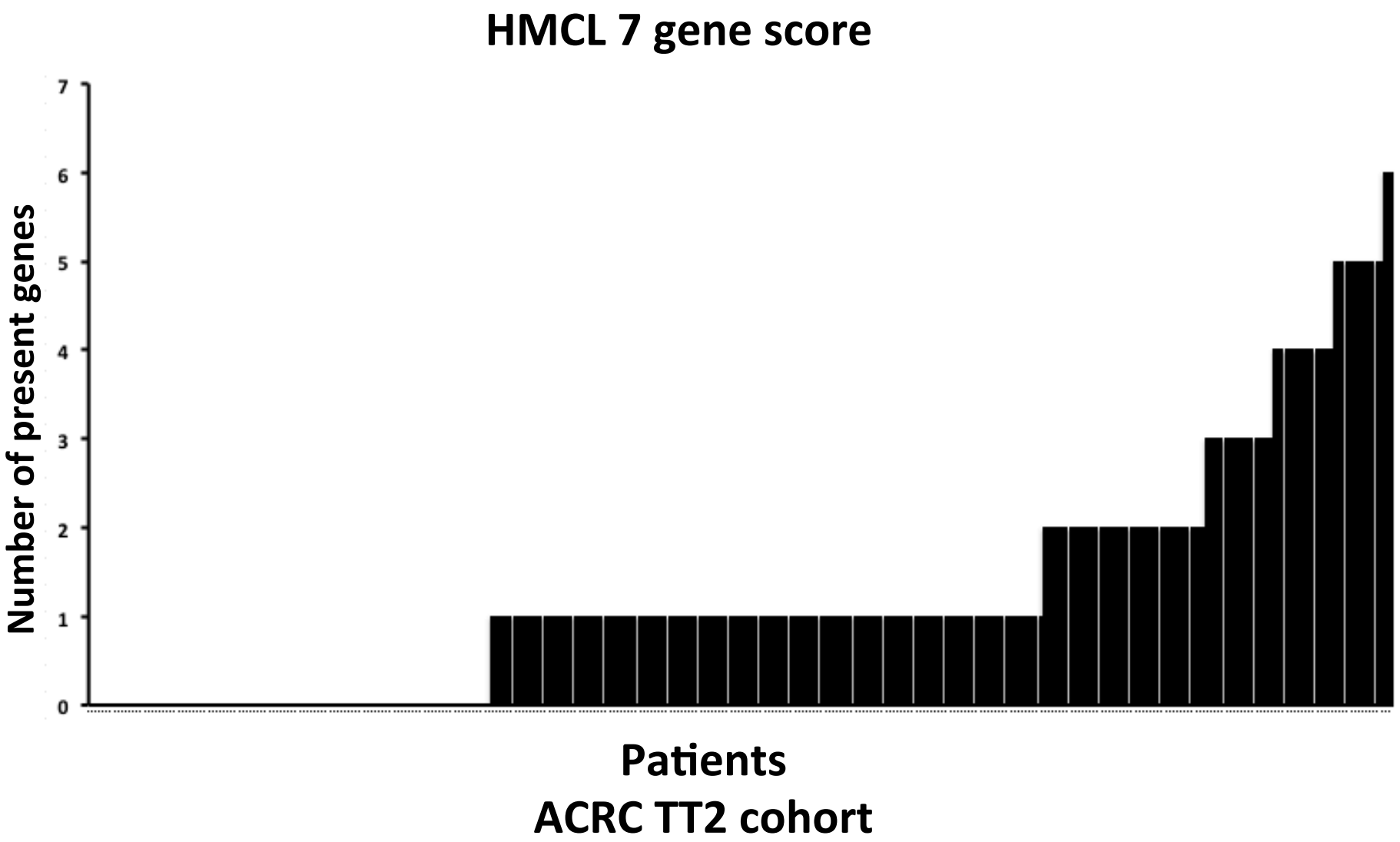
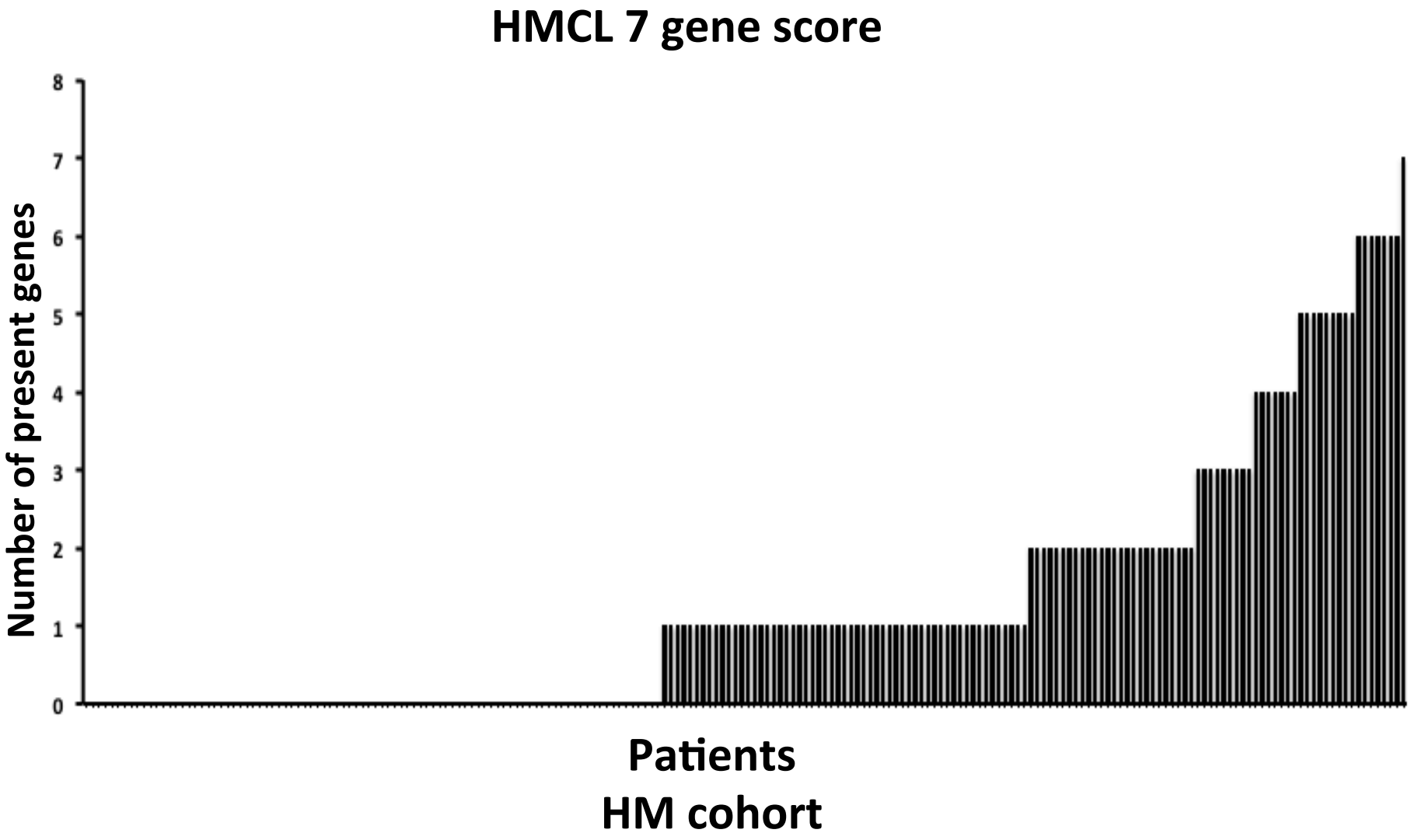


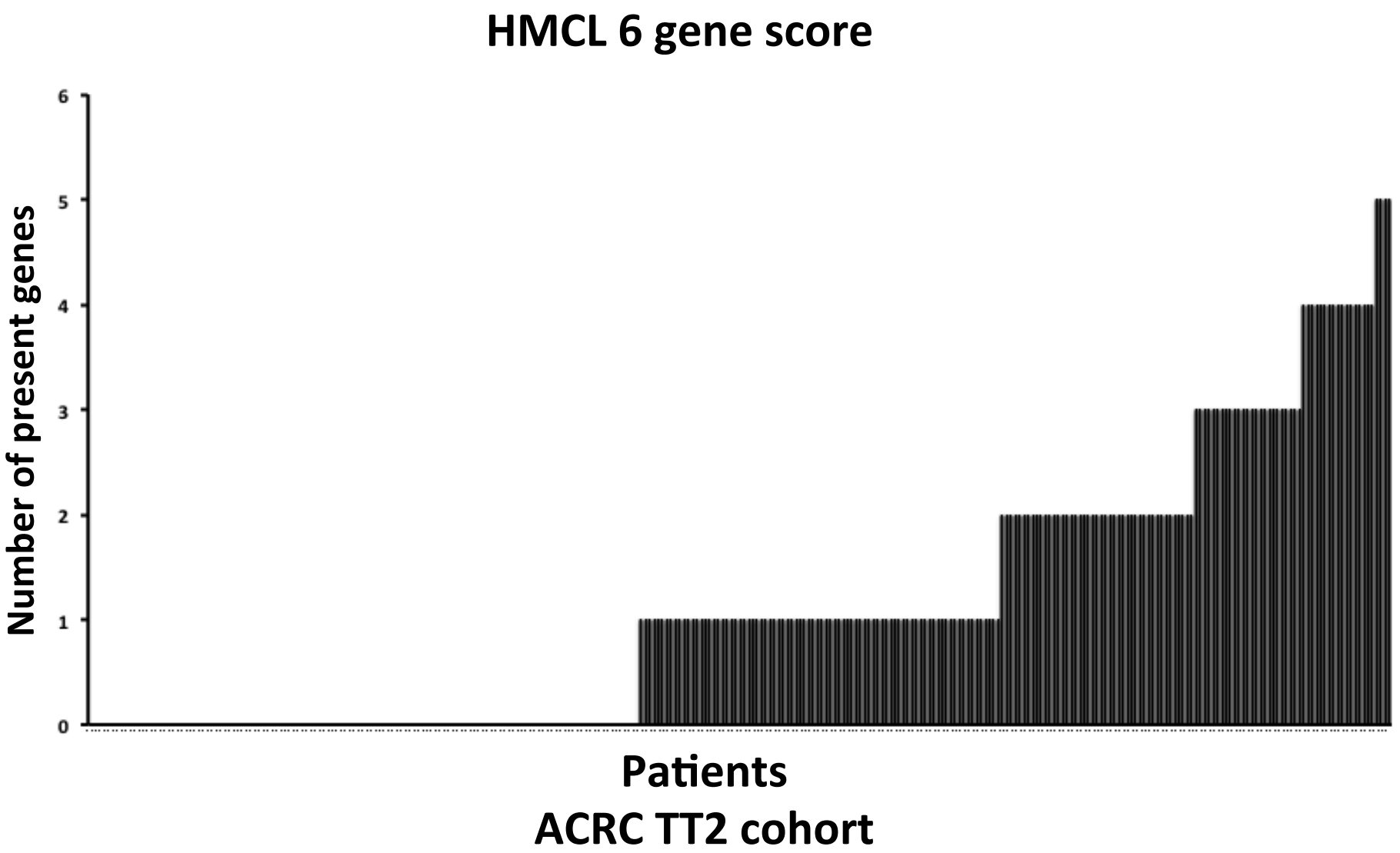
Figure S4. Expression of LRP12, CLEC11A, TEAD1, FGFR3, KIAA1671, MMSET and NUDT11 in the 7 ACRC molecular groups of patients with ACRC-TT2 cohort.

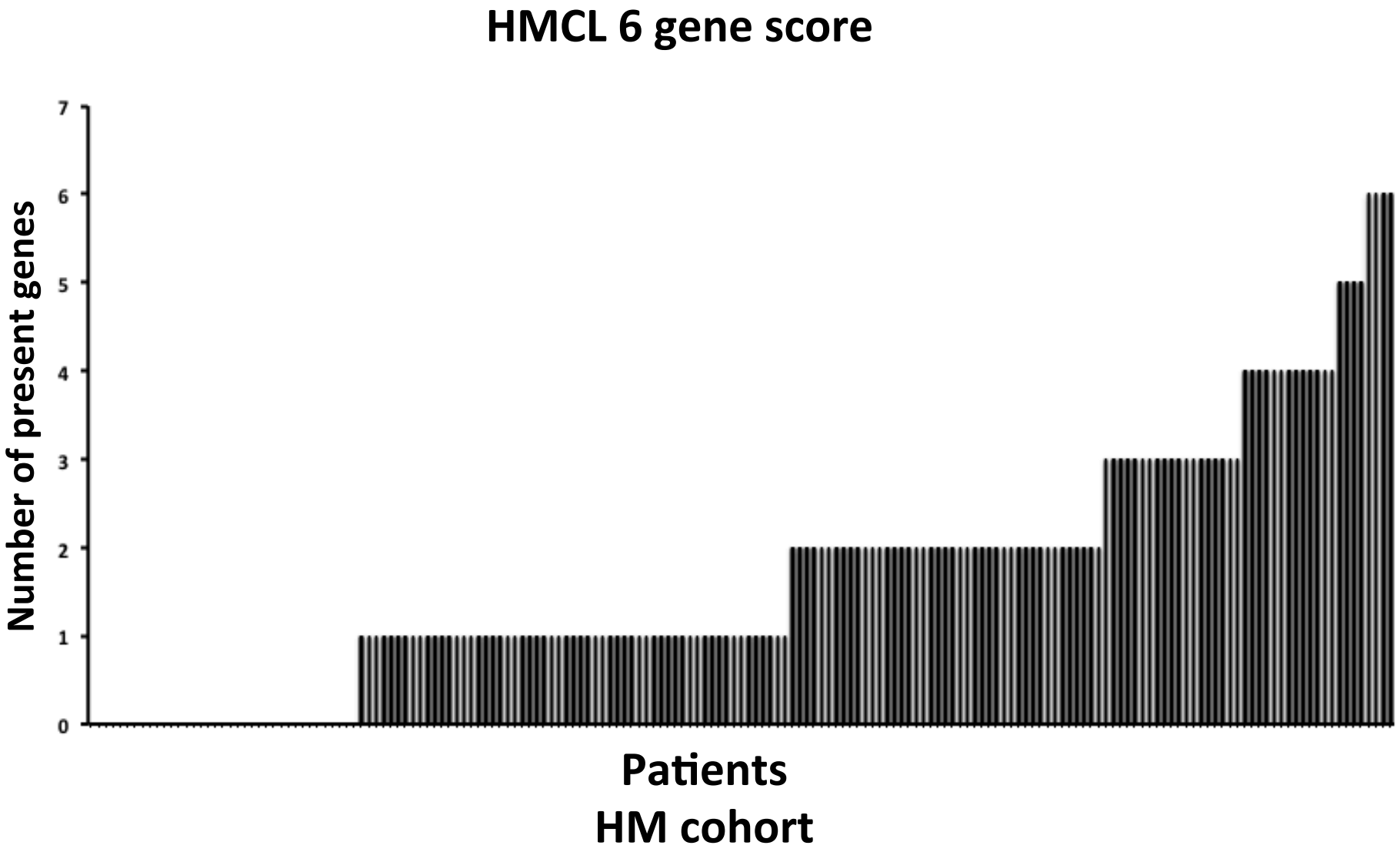
* The mean expression of a gene in a group is statistically significantly different from that in all groups using Student's *t*-test ($P \leq .05$).

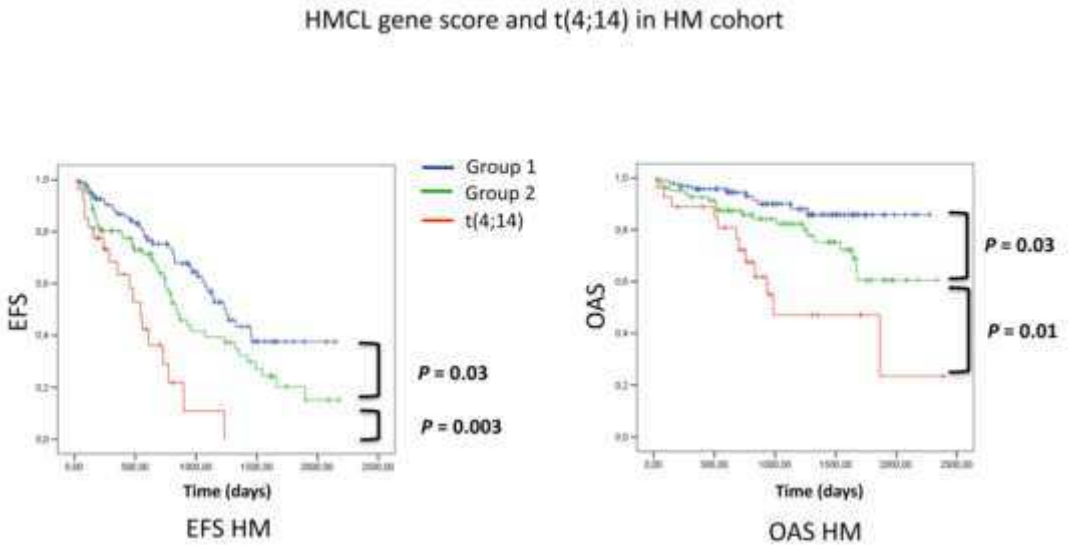
Supplementary Figure S5: HMCL scores in the various datasets of patients




HMCL 7 gene score
Number of present genes
0
1
2
3
4
5
6
7
8
Patients
HM cohort


HMCL 6 gene score
Number of present genes
6
5
4
3
2
1
0
Patients
ACRC TT2 cohort


HMCL 6 gene score
Number of present genes
0
1
2
3
4
5
6
7
Patients
HM cohort



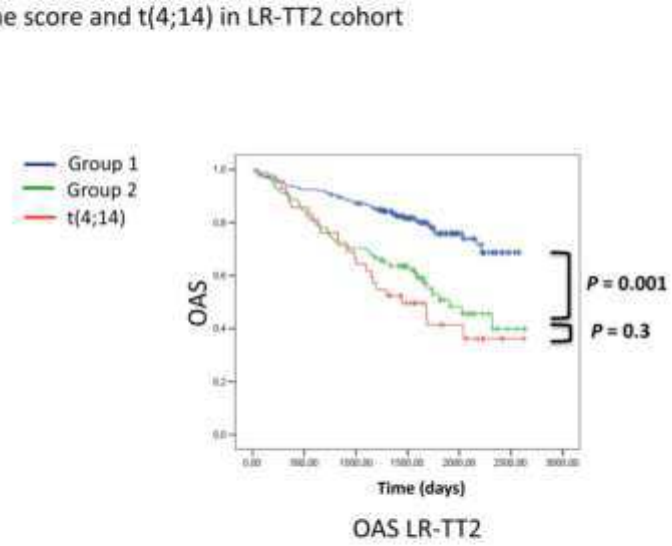


Figure S6. Kaplan-Meier estimates of overall survival and event-free survival of 6 HMCL gene score defined low risk patients (blue), high risk patients (green) and t(4;14) patients (red) in the HM cohort and the ACRC-TT2 cohort.