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Running Title: HA score and sensitivity of MM cells to HDACi.

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Abstract

Background

Multiple myeloma (MM) is a still fatal plasma cell cancer. Novel compounds are currently clinically tested as single agent in relapsing patients, but in best cases with partial response of a fraction of patients, emphasizing the need to design tools predicting drug efficacy. Histone deacetylase (HDAC) inhibitors (i) are anticancer agents targeting epigenetic regulation of gene expression and are in clinical development in MM.

Methods

In order to create a score predicting HDACi efficacy, 5 MM cell lines were treated with trichostatin A (TSA) and gene expression profiles were determined.

Results

The expression of 95 genes was found to be upregulated by TSA, using paired supervised analysis with Significance Analysis of Microarrays software. Thirty-seven of these 95 genes had prognostic value for overall survival in a cohort of 206 newly-diagnosed MM patients and their prognostic information was summed up in a histone acetylation score (HA Score), patients with the highest HA score having the shorter overall survival. Noteworthy, MM cell lines or patients' primary MMCs with a high HA Score had a significant higher sensitivity to TSA, valproic acid, Panobinostat or Vorinostat.

Conclusion

In conclusion, the HA Score allows identification of MM patients with poor survival, who could benefit from HDAC inhibitor treatment.

Introduction

The molecular events governing the onset and progression of malignant transformation involve oncogenic activation and inactivation of tumor suppressor genes, which help cancer cells overriding the normal mechanisms controlling cellular survival and proliferation (Hahn & Weinberg, 2002; Vogelstein & Kinzler, 2004). These molecular events are triggered by DNA alterations (translocations, amplifications or deletions, mutations) and also by epigenetic modifications (Baylin, 2005). Epigenetic modifications include methylation of DNA cytosine residues and histone methylation or acetylation and are critical in the initiation and progression of many cancers(Kondo, 2009). Acetylation of histone releases condensed chromatin into a more relaxed structure that is associated with greater levels of gene transcription. Histone acetylation and then gene transcription is controlled by histone acetyl transferases (HAT) and histone deacetylases (HDAC) bringing and removing acetyl groups. Eighteen HDACs have been described and classified into four classes based on cellular localization and function(Lane & Chabner, 2009). Class I HDACs are located into nucleus and comprise HDACs 1, 2, 3 and 8. Class II HDACs comprise HDACs 4, 5, 7 and 9 (class IIa), which shuttle back and forth between the nucleus and the cytoplasm and HDACs 6 and 10 (class IIb), which are expressed in the cytoplasm only. Class III HDACs include the sirtuin family, which does not act primarily on histones and class IV includes HDAC11(Lane & Chabner, 2009; Neri *et al*, 2012). HDAC inhibitors (HDACi) are now being used in the treatment of some hematologic malignancies (Kelly *et al*, 2010). HDACi are classified into four classes according to their chemical structure: aliphatic acids [valproic acid and sodium phenylbutyrate], hydroxamates [panobinostat, trichostatin-A (TSA), vorinostat, belinostat (PXD101), NVP-LAQ824 and givinostat (ITF2357)], cyclic peptide

[romidepsin (depsipeptide)], and benzamides [MS-275, MGCD0103](Neri *et al*, 2012). HDACi include inhibitors specific to class I HDACs (MGCD0103, romidepsin and MS-275) and pan-HDACi (TSA, panobinostat, vorinostat and belinostat)(Neri *et al*, 2012). Multiple myeloma (MM) is a plasma cell neoplasm characterized by the accumulation of malignant plasma cells (PCs), termed Multiple Myeloma Cells (MMCs), primarily within the bone marrow (BM). Despite the recent introduction of novel agents such as Bortezomib or Lenalidomide, MM remains an almost incurable disease(Moreau, 2012). MM arises through progressive accumulation of multiple genetic abnormalities that include primarily overexpression of a D-type cyclin gene, *CCND1* (cyclin D1) in the case of t(11;14)(q13;q32.3) translocation, *CCND3* (cyclin D3) in the case of the rare t(6p23;14q32) translocation, or *CCND2* (cyclin D2) on the background of a t(14q32;16q23) translocation involving *CMAF* or t(4;14)(p16.3;q32.3) involving WHSC1/*FGFR3*. *CCND* genes are also overexpressed in hyperdiploid MM patients due to gene amplification or downregulation of miRNAs that target *CCND* genes (Bergsagel & Kuehl, 2005; Rio-Machin *et al*, 2013). Secondary genetic abnormalities include *NRAS* or *KRAS* mutations, *TP53* monoallelic deletion and mutations, *MYC* alterations, mutations of genes coding for NF-κB pathway (Bergsagel & Kuehl, 2005; Hideshima *et al*, 2004; Morgan *et al*, 2012). HDACi have already been evaluated in MM including Trichostatin A (TSA)(Lavelle *et al*, 2001), vorinostat(Mitsiades *et al*, 2004; Mitsiades *et al*, 2003), depsipeptide(Khan *et al*, 2004), KD5170(Feng *et al*, 2008), NVP-LAQ824(Catley *et al*, 2003), valproic acid(Kaiser *et al*, 2006; Neri *et al*, 2008) and panobinostat(Neri *et al*, 2012). HDACi induce G1 cell cycle arrest in MMCs through dephosphorylation of Retinoblastoma protein and increase expression of p53 and p21 (Lavelle *et al*, 2001; Mitsiades *et al*, 2003; Neri *et al*, 2008). HDACi induce apoptosis by dowregulation of Bcl-2 family

members(Khan *et al*, 2004; Mitsiades *et al*, 2003) and overcome drug resistance mediated by the BM environment(Mitsiades *et al*, 2003). Furthermore, glucose-regulated protein 78 (GRP78) was recently identified as a novel non-histone target of HDACi(Kahali *et al*, 2011; Rao *et al*, 2010). GRP78 plays a central role in the unfolded protein response (UPR). GRP78 acetylation following HDACi treatment was described to activate UPR and contributes to the antitumor activity of HDACi. Class 1 HDACs binding to GRP78, within the endoplasmic reticulum (ER), represent a novel mode of UPR regulation and an interesting mechanism of HDACi action(Kahali *et al*, 2012). The ER of normal plasma cells and MMCs is well developed to accommodate the production and secretion of large amounts of immunoglobulins. That's why association of HDACi and proteasome inhibitors could be promising in MM treatment(Hideshima & Anderson, 2013). When used as a single agent in patients with relapsing/refractory MM, HDACi have shown modest anti-tumor activity(Niesvizky *et al*, 2011; Richardson *et al*, 2008). In combination with other anti-MM treatments, HDACi can induce durable anti-tumor responses (Badros *et al*, 2009; Harrison *et al*, 2011).

To improve the clinical testing of the efficacy of novel agents, a major stake is identify patients who could benefit from treatment by finding biomarkers predictive for sensitivity of MMCs to HDACi. We recently reported the development of a gene expression-based risk score predicting the sensitivity of MMCs to DNA methylation inhibitors(Moreaux *et al*, 2012). In the current study, we used the same strategy to build a histone acetylation (HA) score, based on genes whose expression is deregulated by HDACi in MMCs. HA score makes it possible identifying a subgroup of 42% of patients with short overall survival, whose MMCs are highly sensitive to HDAC inhibition.

Materials and methods

Human Myeloma Cell Lines (HMCLs)

Human myeloma cell lines (HMCLs, N=40) were obtained as previously described(Gu *et al*, 2000; Moreaux *et al*, 2011; Rebouissou *et al*, 1998; Tarte *et al*, 1999; Zhang *et al*, 1994). HMCLs phenotypic and molecular characteristics have been previously described (Moreaux *et al*, 2011). HMCLs microarray data have been deposited in the ArrayExpress public database (accession numbers E-TABM-937 and E-TABM-1088).

Primary multiple myeloma cells and gene expression profiling

Patients presenting with previously untreated multiple myeloma (N=206) or monoclonal gammopathy of undetermined significance (N=5) at the university hospitals of Heidelberg and Montpellier as well as 7 healthy donors have been included in the study approved by the ethics committee of Montpellier and Heidelberg after written informed consent in accordance with the Declaration of Helsinki. Clinical parameters and treatment regimens of the MM patients included in the Heidelberg-Montpellier (HM) cohort were previously described(Moreaux *et al*, 2012).

Normal bone marrow plasma cells and myeloma cells were purified as previously published(Moreaux *et al*, 2012) and whole genome gene expression profiling assayed with Affymetrix U133 2.0 plus microarrays (Affymetrix, Santa Clara, CA, USA) (ArrayExpress accession number E-MTAB-372). Affymetrix data of an independent cohort of 345 MM patients from the University of Arkansas for Medical Sciences (UAMS, Little Rock, AR) were also used (Gene Expression Omnibus accession number GSE2658. http://www.ncbi.nlm.nih.gov/geo/). T(4;14) translocation was evaluated using *MMSET* spike expression(Kassambara *et al*, 2012b) and

del17p13 surrogated by *TP53* probe set signal(Xiong *et al*, 2008) for UAMS-TT2 patients.

Change in gene expression profile of myeloma cell lines by HDACi

5 HMCLs (XG-5, XG-6, XG-7, XG-20 and LP1) were treated without or with 0.33 mol/L TSA (Sigma, St Louis, MO) for 24 h in RPMI 1640, 10% fetal bovine serum supplemented with IL-6 for IL-6 dependent HMCLs(Moreaux *et al*, 2011; Moreaux *et al*, 2012). Whole genome gene expression profiling was assayed with Affymetrix U133 2.0 plus microarrays (Affymetrix).

Sensitivity of myeloma cell lines and primary myeloma cells to HDACi.

HMCLs were cultured with graded TSA, valproic acid (VPA) (Sigma), vorinostat (SAHA) (Sigma) or panobinostat (LBH-589) (Sigma) concentrations. HMCLs cell growth was quantified with a Cell Titer Glo Luminescent Assay (Promega, Madison, WI) as described(Moreaux *et al*, 2012). The half inhibitory concentration (IC50) was determined using GraphPad Prism (http://www.graphpad.com/scientific-software/prism/).

Primary myeloma cells of 13 patients were cultured with or without graded concentrations of TSA and MMCs cytotoxicity was evaluated using anti-CD138-PE mAb (Immunotech, Marseille, France) as described(Mahtouk *et al*, 2004; Moreaux *et al*, 2012).

Bioinformatics and statistics

Gene expression data were analyzed using SAM (Significance Analysis of Microarrays) software(Cui & Churchill, 2003) and our bioinformatics platforms (http://rage.montp.inserm.fr/ and http://amazonia.montp.inserm.fr/)(Reme *et al*, 2008; Tanguy Le Carrour, 2010) as published(Moreaux *et al*, 2012). All computations were performed using R 2.15.1 (http://www.r-project.org/) and bioconductor 2.0. Survival

analyzes were investigated using the Kaplan-Meier method and Cox's proportional hazards model as published(Moreaux *et al*, 2012).

The Histone acetylation risk score (termed HA Score) was built using our previously published methodologies to develop prognostic scores using set of prognostic genes coding for related proteins (Kassambara *et al*, 2012a; Kassambara *et al*, 2012b; Moreaux *et al*, 2012). HA Score is the sum of the Cox beta coefficients of each of the 37 TSA-deregulated genes with a prognostic value, weighted by ±1 if the patient MMC signal for a given gene is above or below the probeset maxstat value of this gene(Moreaux *et al*, 2012).

Results

Identification of prognostic genes whose expression is upregulated by trichostatin A treatment of multiple myeloma cells.

Genes upregulated by TSA treatment of MMCs were identified treating 5 HMCLs with 0.33 M TSA for 24h, a concentration inducing histone acetylation in mammalian cells, in particular in myeloma cells *in vitro* (Heller *et al*, 2008; Yoshida *et al*, 1990). In addition, this TSA treatment did not affect myeloma cell viability (Supplementary Table S1)(Heller *et al*, 2008). Using SAM supervised paired analysis, expression of 95 genes was found significantly upregulated by TSA treatment (FDR < 5%; Supplementary Table S2). TSA-regulated genes are significantly enriched in genes related to "Immunological disease and Inflammatory disease" pathway (P < .05; Ingenuity pathway analysis, data not shown). Noteworthy, TSA deregulated genes were also found to be upregulated by Panobinostat treatment in MMC (Supplementary Figure S1). We next investigated associations of TSA deregulated genes with overall survival using Maxstat R function. The aim was to identify genes regulated by HDAC that have potentially important disease modulating functions. The results of our analysis were corrected for multiple testing using the Benjamini-Hochberg algorithm. Investigating the expression of these 95 TSA-regulated genes in primary MMCs of a cohort of 206 newly-diagnosed patients (HM cohort), 16 genes had a bad prognostic value and 21 a good one (Table 2). The prognostic information of HDACi regulated genes was gathered within a histone acetylation score (HA Score) as indicated in Materials and Methods. The value of HA Score in normal, premalignant or malignant plasma cells is displayed in Figure 1. Cells from MGUS patients had a significant higher HA Score than normal BMPCs (P < .001), MMCs of patients a significantly higher HA Score than normal BMPCs or plasma cells from

MGUS-patients ($P < .001$), and HMCLs the highest score ($P < .001$) (Figure 1). Figure 2B shows the contribution of the 16 bad prognostic and the 21 good prognostic genes for HA score. Bad prognostic genes are highly expressed in patients with high HA score and the reverse for good prognostic genes. Investigating the HA Score in the 8 groups of the molecular classification of multiple myeloma(Zhan *et al*, 2006), HA Score was significantly higher in the proliferation subgroup ($P < .001$) associated with a poor prognosis and significantly lower in the CD2 subgroup ($P < .001$)(Zhan *et al*, 2006) (Figure 3). Among the 37 genes of the HA Score, 7 code for proteins that have been described as lysine acetylation target proteins and 18 have been identified as HDACi targets(Bantscheff *et al*, 2011; Choudhary *et al*, 2009; Iwahashi *et al*, 2011; Niesen & Blanck, 2009) (Supplementary Tables S5 and S6). Gene expression profiles of HA Score genes in purified MMC and normal bone marrow subpopulations are listed in supplementary Figure S2. Supplementary Figure S2 shows a highly variable expression of each of the 37 genes in primary MMCs of the patients, indicating they all contribute to unravel disease heterogeneity.

Prognostic value of HA score in two independent cohorts of patients.

When used as a continuous variable, HA Score had prognostic value ($P \leq 10^{-4}$, results not shown). Using Maxstat R function, a maximum difference in overall survival (OS) was obtained with a HA Score = -11.3 splitting patients in a high-risk group of 42.7 % patients (HA Score > - 11.3) with a 43.5 months median OS and a low risk group of 57.3% patients (HA Score ≤ -11.3) with not reached median survival (Figure 2A). High-risk patients are characterized by a higher expression of the 16 bad prognostic genes and a lower expression of the 21 good ones used for HA Score building (Figure 2B). Using univariate Cox analysis, HA Score, UAMS-HRS, IFM-

score and GPI had prognostic value as well as t(4;14), del17p, β2m, albumin and ISS using the HM patient cohort (Supplementary Table S3). When these parameters were compared two by two, HA Score tested with β2m and t(4;14) remained significant. When tested together, HA Score, β2m, t(4;14) and GPI kept prognostic value. The HA Score, computed using HM cohort parameters, is also prognostic in an independent cohort of 345 patients from UAMS (UAMS-TT2 cohort). The median OS of patients with high HA Score was 71.4 months and not reached for patients with low HA Score (P < .0001) (Figure 2A). Using Cox univariate analysis, UAMS-HRS, IFM and GPI scores as well as t(4;14) and del17p had prognostic value. Serum concentrations of β2m or albumin are not publicly available for this cohort. When analysed two by two, HA Score remained significant compared to UAMS-HRS, IFM, GPI, t(4;14), and del17p in the UAMS-TT2 cohort (Supplementary Table S3). When these parameters were tested together, HA Score, UAMS-HRS, t(4;14) and del17p kept prognostic value in UAMS-TT2 cohort.

HA Score allows identification of human myeloma cell lines or patients' primary MMCs sensitive to trichostatin A *in vitro*.

We investigated whether HA Score could predict for the sensitivity of HMCLs to HDAC inhibitors. 10 out of 40 HMCLs(Moreaux *et al*, 2011) with the highest or lowest HA Score were selected to assay for TSA sensitivity. The 5 HMCLs with the highest HA Score displayed a significant (P = .0004) 5-fold higher sensitivity to TSA (median IC50 = 10.97 nM ; range: 6.32 to 17.4 nM) than the 5 HMCLs with low HA Score (median IC50 = 52.33 nM ; range: 29.49 to 57.74 nM) (Figure 4). No difference in recurrent genetic abnormalities were found between HMCLs with the highest or lowest HA Score (Table 1). HA Score could also predict for sensitivity of patients' primary MMCs, co-cultured with bone marrow environment, to TSA. The TSA

concentrations used to treat primary MM samples were chosen in order to cover the range of TSA concentrations yielding 50% inhibition of the growth of the 10 HMCLs displaying high and low HA Scores (Figure 4).

Primary MMCs of 8 patients with a HA Score above the Max Stat cutoff (> -11.3, Figures 1 and 2) exhibited significant ($P < .05$) 2.4-fold higher TSA sensitivity than MMCs of 5 patients with HA Score ≤ -11.3 (Figure 5).

HA Score is predictive for sensitivity of human myeloma cells to other clinical grade HDACi *in vitro*

We sought to determine whether HA Score could predict for the sensitivity of myeloma cells to other clinical grade HDAC inhibitors(Neri *et al*, 2012). The 5 HMCLs with the highest HA Score exhibited a significant higher sensitivity to Panobinostat, VPA or Vorinostat (median IC50 = 1.16 nM, 0.28 M and 528 nM respectively) than the 5 HMCLs with lowest HA Score ($P = .007$, $P = .009$ and $P = .02$; median IC50 = 3.16 nM, 0.43 M and 897 nM respectively) (Figure 6A-B-C).

Discussion

In this study, we have identified a gene expression-based histone acetylation score (HA Score) which is predictive for patients' survival and for the *in vitro* sensitivity of human myeloma cell lines or patients' primary myeloma cells to the pan-HDAC inhibitor, trichostatin A, and also to other 3 clinical-grade HDACi. HDACi have been investigated for treating patients with MM, either as single agent (Neri *et al*, 2012; Niesvizky *et al*, 2011; Richardson *et al*, 2008) or in combination with current drugs used in MM, such as Dexamethasone, Lenalidomide or Bortezomib(Badros *et al*, 2009; Harrison *et al*, 2011; Neri *et al*, 2012). Panobinostat, used as a single-agent, demonstrated limited activity in patients with MM, refractory to at least two lines of

treatment(Wolf *et al*, 2012). In association with melphalan, Panobinostat yielded a 33% overall response rate in a phase I study including 12 patients with relapsed/refractory MM (Offidani *et al*, 2012). Association of Panobinostat and Bortezomib could be promising since proteasome inhibition affects the unfolded protein response pathway leading to increased HDAC mediated aggregosome formation(Hideshima *et al*, 2011). Phase IB and II studies have shown that association of Panobinostat to Bortezomib and dexamethasone could yield to objective response in relapsing patients refractory to Bortezomib therapy (Richardson PG, 2011; San-Miguel JF, 2009; Siegel DS, 2008). Given the encouraging *in vitro* and *in vivo* data, association of Panobinostat, Bortezomib and dexamethasone is now beeing evaluated in a large phase III randomized trial(San-Miguel JF, 2011). Combination of Vorinostat and Bortezomib was also investigated in a phase II trial including patients with MM refractory or ineligible to Bortezomib or IMiD therapy (Siegel DS, 2011) and showed a 17% overall response rate and 6 months median response duration. This data also prompt investigating the interest of a combination of Vorinostat and Bortezomib in phase III trial including 637 patients with relapsed/refractory MM(Dimopoulos MA, 2011). Final results of the trial remain awaited, but interim results did not demonstrate a marked therapeutic benefit of vorinostat(Dimopoulos MA, 2011). These trials suggest that HDACi could have some benefit for MM. However, their efficacy could be underestimated because limited to a subgroup of patients. The current HA Score could be promising to investigate whether the best response to HDACi is found in patients with MMCs displaying a high HA Score.

Only upregulated genes were identified in the HMCLs treated with TSA compared to untreated HMCLs. This may be explained by the 24 h treatment of cell lines with

HDACi according to usual protocols. This short treatment makes it possible to release the transcriptional suppressor activity of HDAC yielding to overexpressed genes. But it is likely too short to get an indirect repression of genes due to the HDACi-induced overexpression of an inhibitor of these genes. Among the current 95 genes deregulated by TSA treatment in HMCLs, 24 genes were commonly identified by Heller *et al*. (Supplementary Table S4)(Heller *et al*, 2008). 7/37 HA Score genes code for proteins that have been described as lysine acetylation target proteins and 18/37 HA Score genes have been identified as HDACi targets(Bantscheff *et al*, 2011; Choudhary *et al*, 2009; Iwahashi *et al*, 2011; Niesen & Blanck, 2009) (Supplementary Tables S5 and S6).

Why HA Score which is built using 37 HDACi-upregulated and prognostic genes could predict for the sensitivity of MMCs to HDACi? Patients with high HA score, and poor survival, are characterized by a higher expression of the 16 bad prognostic genes and a lower expression of the 21 good ones in MMCs (Figure 2B). Thus, one can speculate that primary MMCs of patients with high HA score have a high tumor metabolism and growth, which can be efficiently targeted by upregulation of gene products encoded by genes upregulated by HDACi, in particular the 21 good prognostic genes. At the opposite, MMCs of patients with a low HA score could be in a more quiescent state and less sensitive to HDCAi. But a full understanding of the reason why HA score could predict for HDACi sensitivity will be provided by an extensive study of the function of the products encoded by HDACi-regulated genes in promoting MMC survival and/or proliferation. Some genes could highlight pathways involved in MM and we comment below the putative roles of *NFKBIZ* (nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor zeta)*, BASP1* (Brain acid-soluble protein 1) or *QKI* (Quaking), whose expression in MMCs is induced by HDACi

treatment and is associated with good prognosis. NFKBIZ is a member of IκB family(Totzke *et al*, 2006), localized in the nucleus where it interacts with and regulates nuclear NF-κB activity. Suppression of endogenous NFKBIZ renders cells more resistant to apoptosis, whereas its overexpression induces cell death(Totzke *et al*, 2006; Yamazaki *et al*, 2001). This is of interest because NF-κB pathway is frequently activated through various gene mutations in MM(Annunziata *et al*, 2007; Keats *et al*, 2007). More recently, it was demonstrated that NFKBIZ inhibits the transcriptional activity of STAT3 leading to cell growth inhibition and apoptosis induction mediated by down-regulation of a known STAT3 target, Mcl-1(Wu *et al*, 2009). Mcl-1 is the major anti-apoptotic protein for MMCs, involved in IL-6-mediated survival of MMCs(Derenne *et al*, 2002; Jourdan *et al*, 2003). BASP1 is repressed in Myc-transformed cells and conversely, has a strong potential to inhibit cell transformation induced by Myc(Hartl *et al*, 2009). The inhibition of Myc-induced fibroblast cell transformation by BASP1 also prevents the transcriptional activation or repression of known Myc target genes. BASP1 appears to be a potential tumor suppressor in cancer(Hartl *et al*, 2009). Myc protein is frequently highly expressed in primary MMCs(Skopelitou *et al*, 1993) and a vicious circle involving IRF4 and Myc was identified yielding to deregulation of MMC growth(Shaffer *et al*, 2008). HDAC inhibitors could be useful to target NF-κB or Myc activation in MMCs through upregulation of NFKBIBZ and BASP1 expression. The RNA binding protein QKI belongs to the evolutionarily conserved signal transduction and activator of RNA family. It has been demonstrated that overexpression of QKI induced the G1 cell cycle arrest in oligodendrocyte progenitor cells(Larocque *et al*, 2005). Furthermore, QKI inhibits colon cancer cell growth, acting as a tumor suppressor(Yang *et al*, 2010). It was demonstrated that QKI protein is directly transcribed by E2F1, which in

turn negatively regulates the cell cycle by targeting multiple cell cycle regulators including p27, cyclin D1 and c-fos(Yang *et al*, 2011). These results demonstrated that a better understanding of the cellular response to epigenetic-targeted treatments will increase our knowledge of MM development and progression and will provide potential therapeutic advances. Epigenetic therapies could be combined with conventional therapies to develop personalized treatments in MM and render resistant tumors responsive to treatment. These advances may limit the side effects of treatment, improving compliance with dosing regimens and overall quality of life. Our methodology could be extended to other anti-MM treatments.

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Author contributions:

MJ designed the research and wrote the paper.
HD, and GH collected bone marrow samples and clinical data and participated in the writing of the paper.
RT and VJL participated in the bioinformatics studies.
LW and RG provided with technical assistance.
KB designed and supervised the research and wrote the paper.

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Figure legends:

Figure 1: Histone acetylation Score in normal and malignant plasma cells.

HA Score in normal bone marrow plasma cells (N = 7), in pre-malignant plasma cells of patients with monoclonal gammopathy of undetermined significance (MGUS, N = 5), in multiple myeloma cells of patients with intramedullary MM (N = 206) and in human myeloma cell lines (N = 40). ** Indicate that the score value is significantly different with a *P* value < .01.

Figure 2: Prognostic value of HA Score in multiple myeloma.

(A) Patients of the HM cohort were ranked according to increased HA Score and a maximum difference in OS was obtained with HA Score = -11.3 splitting patients in high risk (42.7%) and low risk (57.3%) groups. HA Score had also prognostic value on an independent cohort of 345 patients from UAMS treated with TT2 therapy (UAMS- TT2 cohort). The parameters to compute the HA Score of patients of UAMS-TT2 cohort and the proportions delineating the 2 prognostic groups were those defined with HM cohort.

(B) Clustergram of HA Score genes ordered from best to worst prognosis. The level of the probe set signal is displayed from low (deep blue) to high (deep red) expression. MM patients (N = 206) were ordered by increasing GE-based risk score.

Figure 3: HA Score in MM molecular subgroups.

The HA Score was computed for MMCs of patients belonging to the 8 groups of the UAMS molecular classification of multiple myeloma, using UAMS-TT2 cohort. PR: proliferation, LB: low bone disease, MS: MMSET, HY: hyperdiploid, CD1: Cyclin D1, CD2: Cyclin D2, MF: MAF, MY: myeloid. * Indicate that the score value is significantly higher in the group compared to all the patients of the cohort (*P* < .05). ** Indicate

that the score value is significantly lower in the group compared to all the patients of the cohort ($P < .05$).

Figure 4: HA Score predicts for sensitivity of human myeloma cell lines to trichostatin A.

HMCLs with high HA Score (N = 5) exhibit significant higher TSA sensitivity compared to HMCLs with low HA Score (N = 5). HMCLs were cultured for 4 days in 96-well flat-bottom microtiter plates in RPMI 1640 medium, 10% FCS, 2 ng/ml IL-6 culture medium (control), and graded TSA concentrations. Data are mean values plus or minus standard deviation (SD) of 5 experiments determined on sextuplet culture wells.

Figure 5: HA Score predicts for trichostatin A sensitivity of primary myeloma cells of patients.

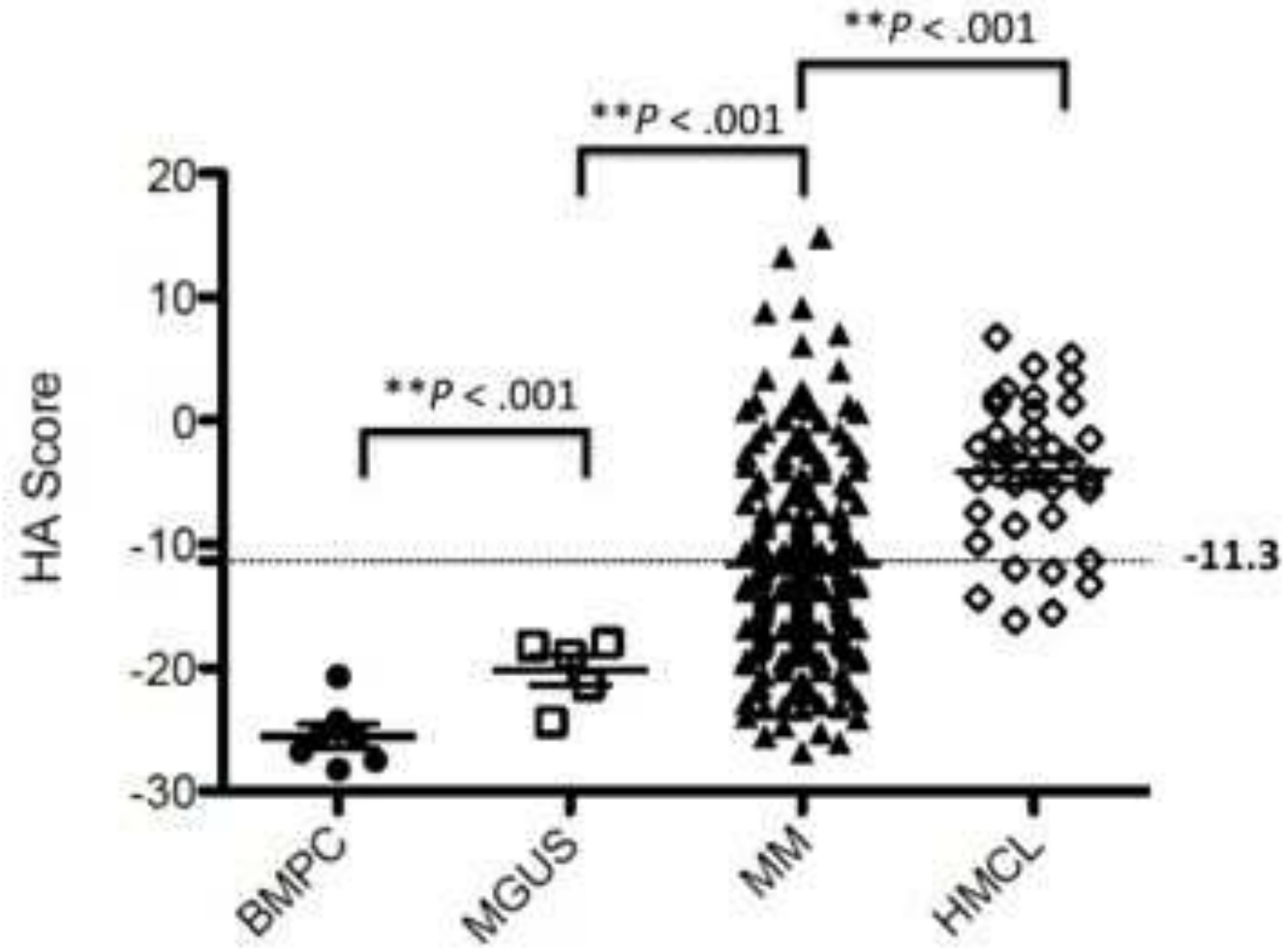
Mononuclear cells from tumor samples of 13 patients with MM were cultured for 4 days in the presence of IL-6 (2 ng/ml) with or without graded TSA concentrations. At day 4 of culture, the count of viable MMCs was determined using CD138 staining by flow cytometry. Black color represents patients with high HA Score (N = 8) and white represents patients with low HA Score values (N = 5).

Figure 6: HA Score predicts for sensitivity of human myeloma cell lines to Panobinostat, Valproic acid or SAHA HDACi.

HMCLs with a high HA Score (N = 5) exhibit significant higher sensitivity to Panobinostat, Valproic acid or SAHA compared to HMCLs with a low HA Score (N = 5). HMCLs were cultured for 4 days in 96-well flat-bottom microtiter plates in RPMI 1640 medium, 10% FCS, 2 ng/ml IL-6 culture medium (control), and graded concentration of Panobinostat (A), VPA (B) or SAHA (Vorinostat) (C). Data are mean

values plus or minus standard deviation (SD) of 5 experiments determined on sextuplet culture wells.

Figure 1



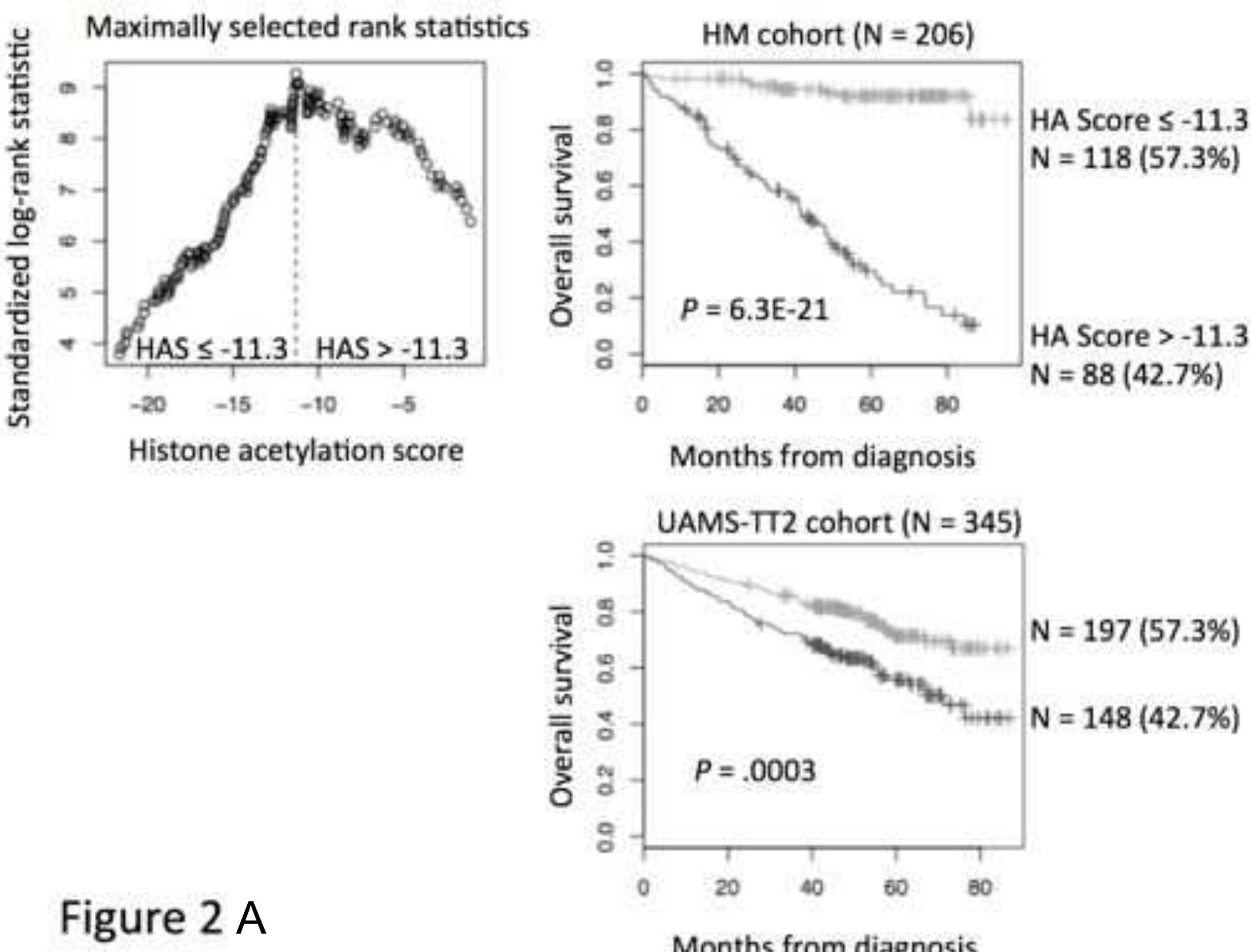

Maximally selected rank statistics
Standardized log-rank statistic
HAS ≤ -11.3
HAS > -11.3
Histone acetylation score
HM cohort (N = 206)
Overall survival
P = 6.3E-21
HA Score ≤ -11.3
N = 118 (57.3%)
HA Score > -11.3
N = 88 (42.7%)
Months from diagnosis
UAMS-TT2 cohort (N = 345)
Overall survival
P = .0003
N = 197 (57.3%)
N = 148 (42.7%)
Months from diagnosis

Figure 2 A

Figure 2B

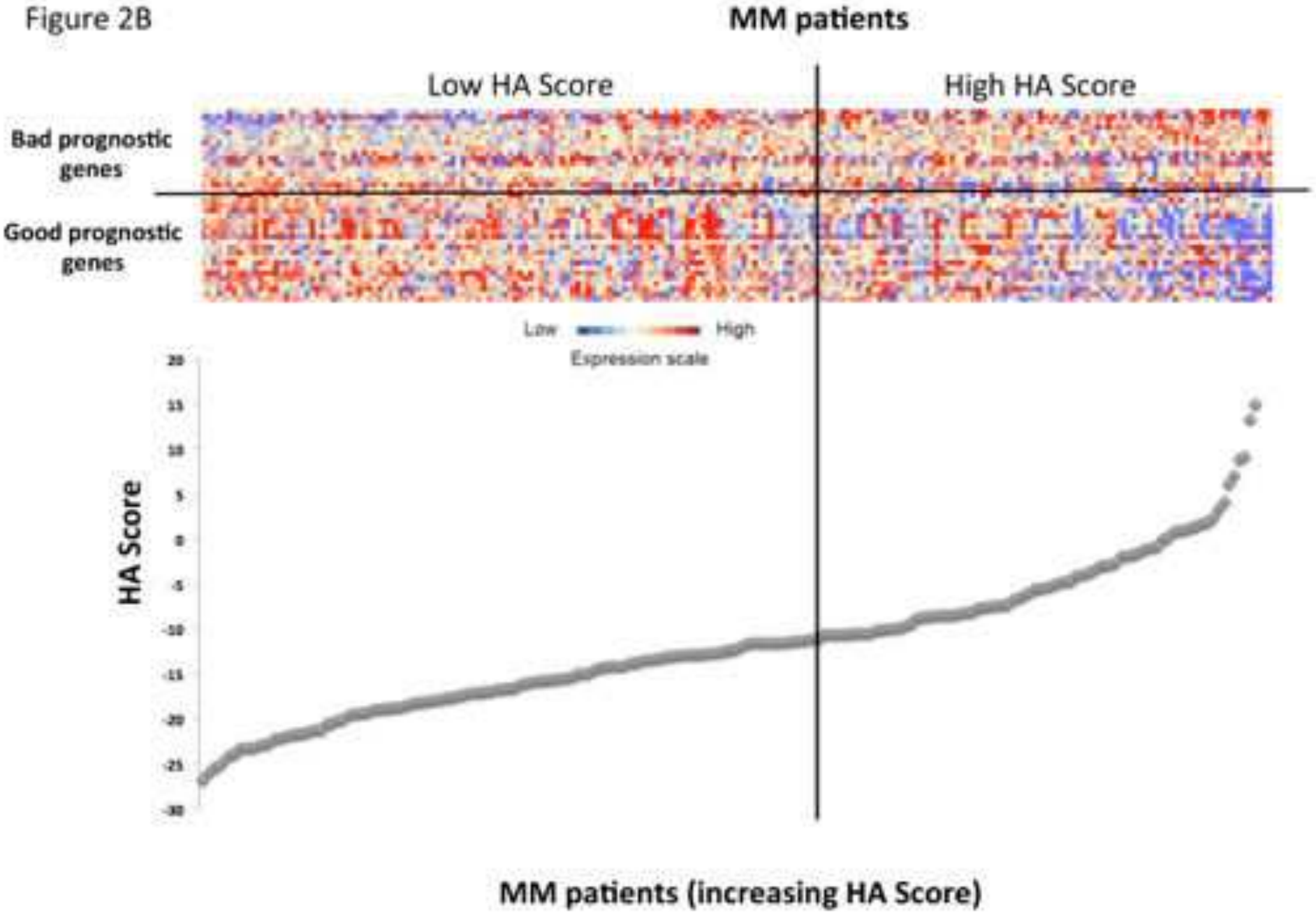

MM patients
Low HA Score
High HA Score
Bad prognostic genes
Good prognostic genes
Low
High
Espression scale
HA Score
20
15
10
5
0
-5
-10
-15
-20
-25
-30
MM patients (increasing HA Score)

Figure 3

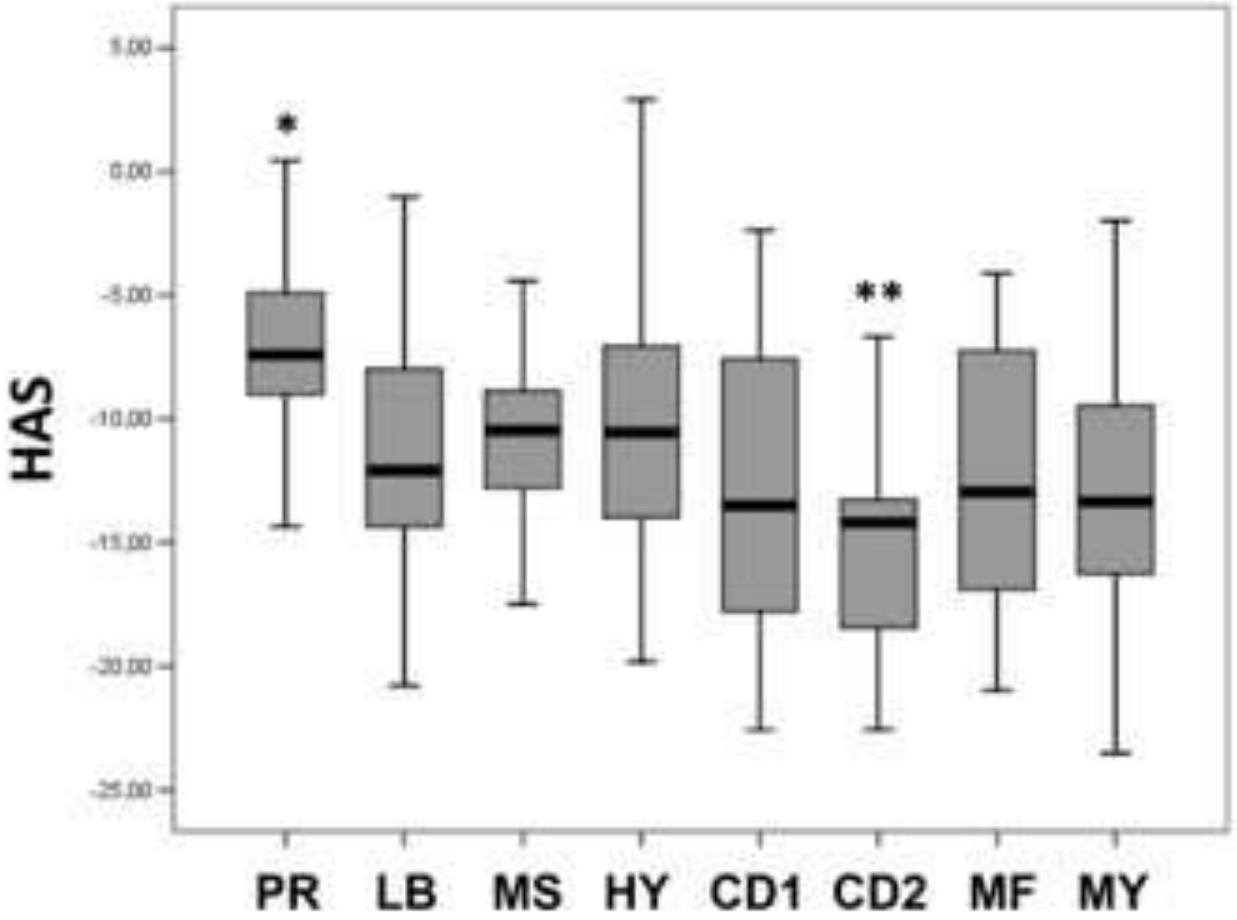
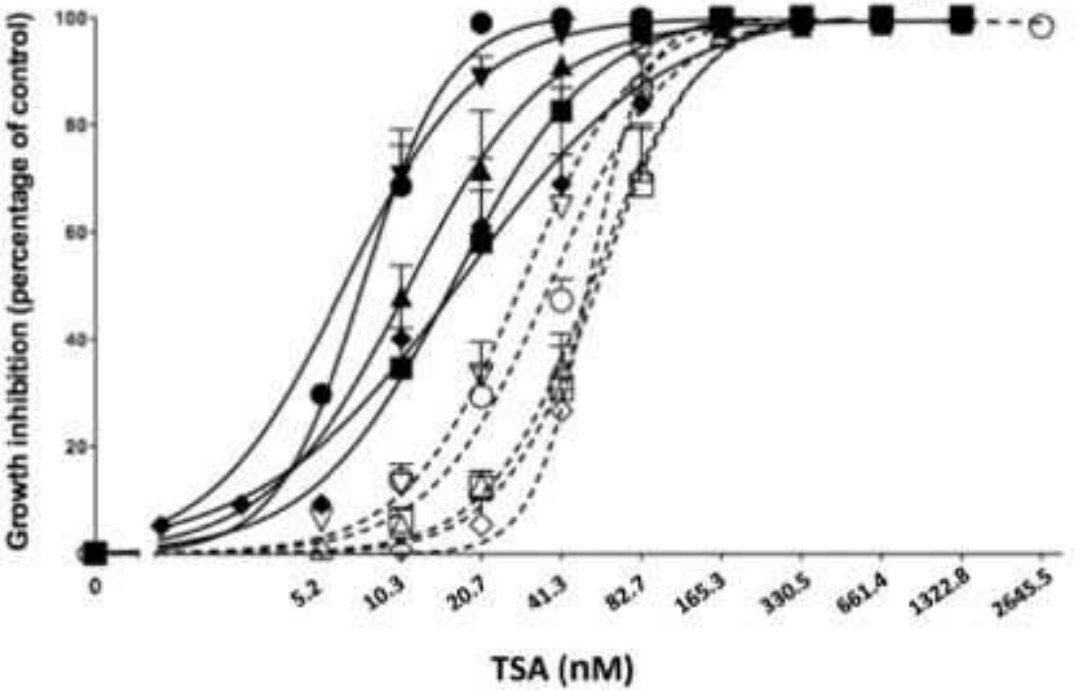


Figure 4



HMCLs	IC50 nM	HA Score	
XG20	57.74	-7.77	
AMO-1	54.20	-11.37	Low HA Score
XG7	52.33	-12.03	
LP1	37.65	-16.17	
JJN3	29.49	-9.88	
XG16	17.40	0.74	
XG6	16.28	2.46	High HA Score
XG5	10.97	1.94	
XG13	7.45	1.33	
XG21	6.32	5.18	

P =.0004

Figure 5

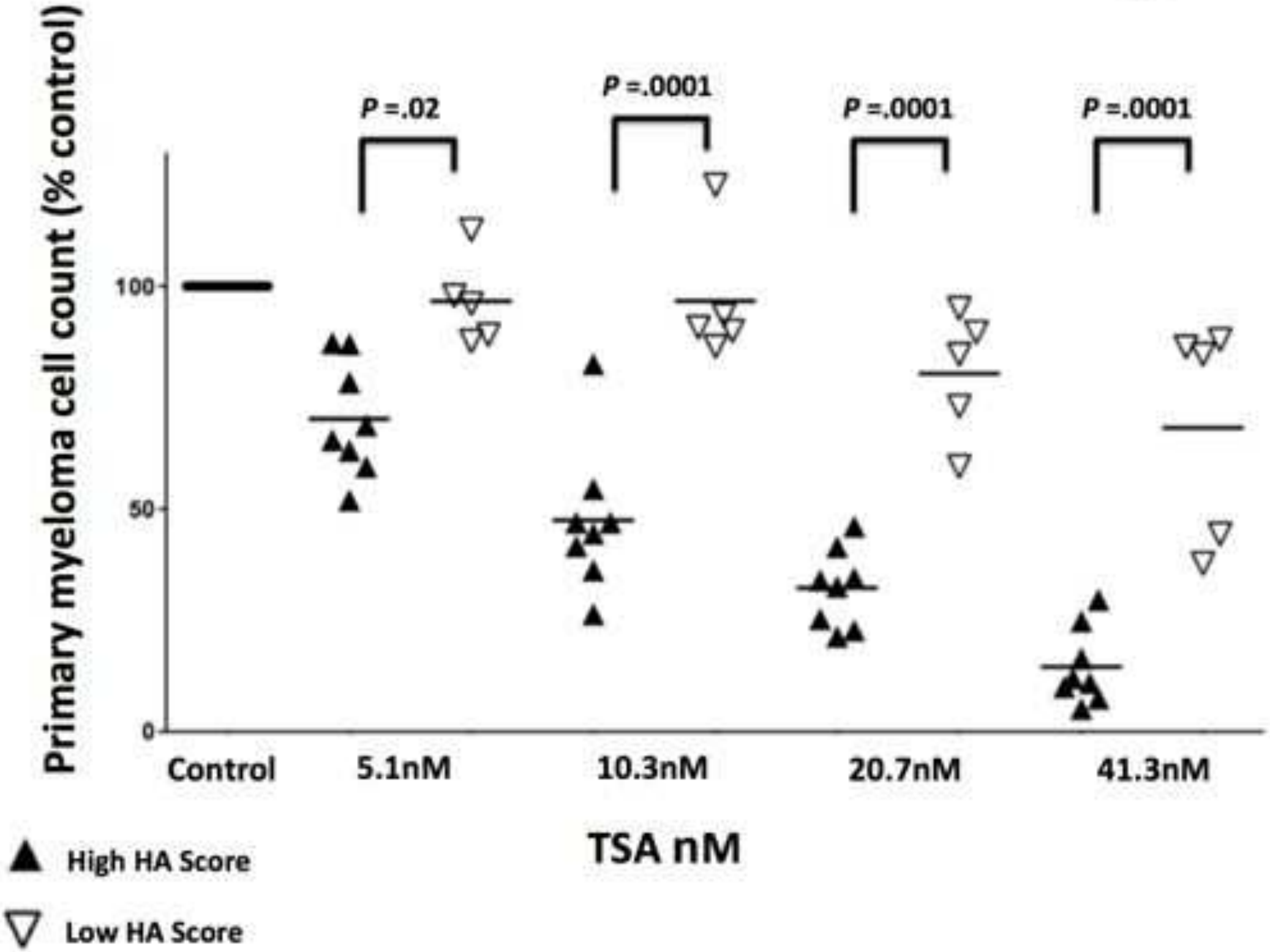
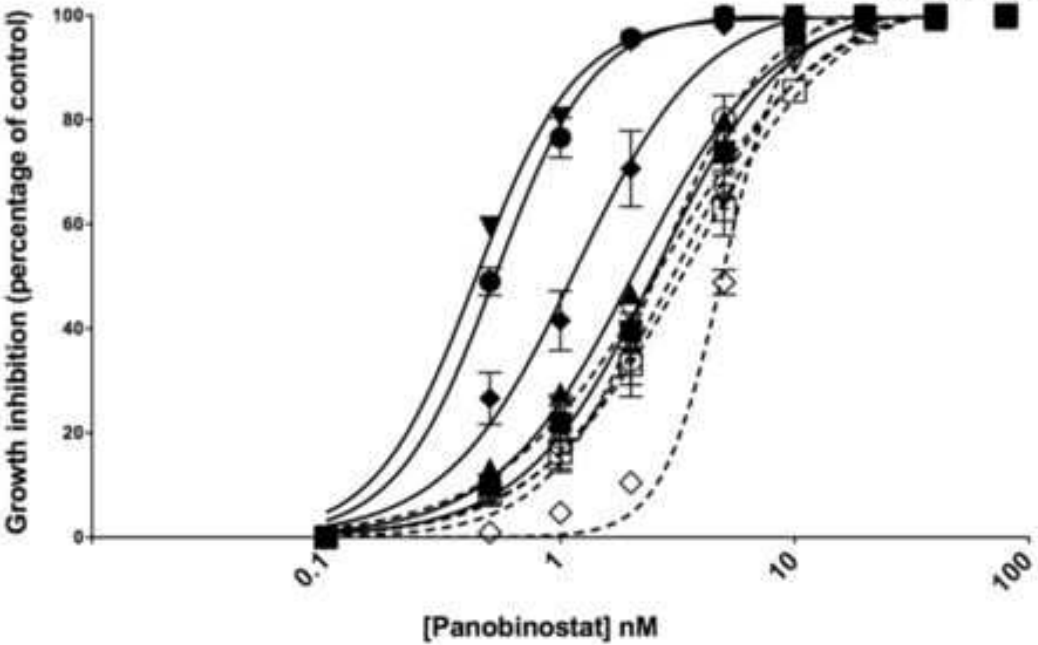

Primary myeloma cell count (% control)
P =.02
P =.0001
P =.0001
P =.0001
100
50
0
Control
5.1nM
10.3nM
20.7nM
41.3nM
TSA nM
High HA Score
Low HA Score

Figure 6A

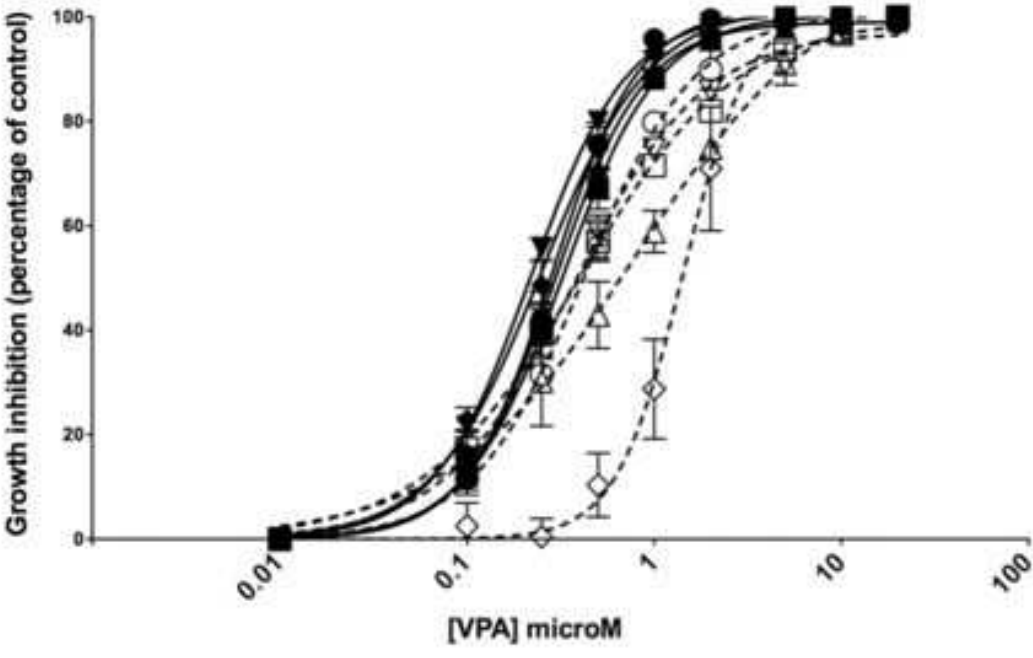


Panobinostat

HMCLs	IC50 nM	HA Score	
XG20	3.51	-7.77	
AMO-1	3.16	-11.37	Low HA Score
XG7	4.96	-12.03	
LP1	2.61	-16.17	
JJN3	2.72	-9.88	
XG16	1.16	0.74	
XG6	2.50	2.46	High HA Score
XG5	2.01	1.94	
XG13	0.52	1.33	
XG21	0.43	5.18	

***P* =.007**

Figure 6 B

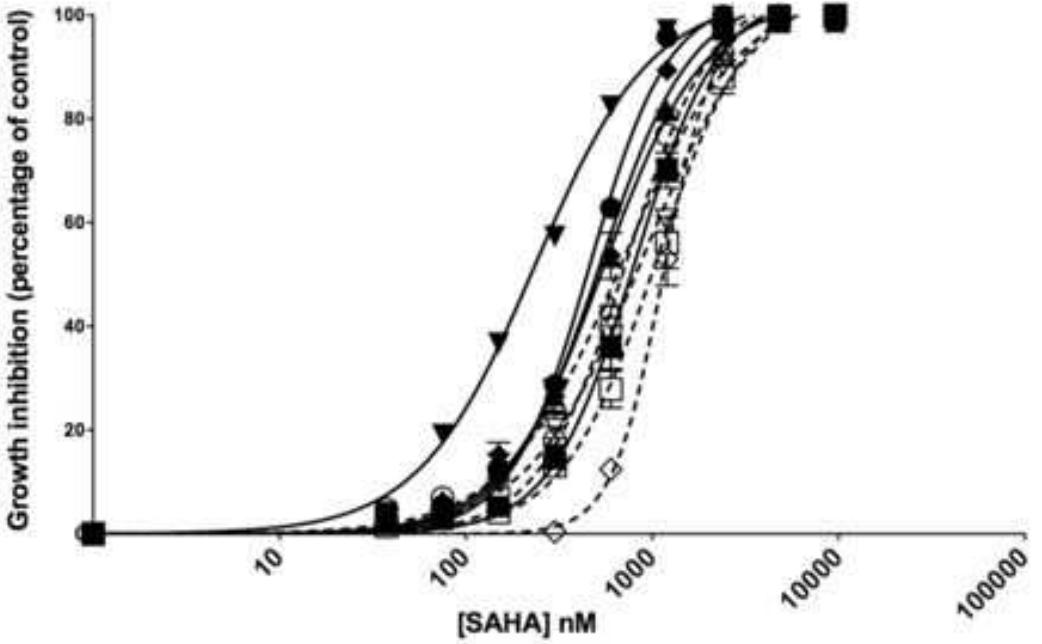


Valproic Acid (VPA)

HMCLs	IC50 µM	HA Score	
XG20	0.40	-7.77	
AMO-1	0.81	-11.37	Low HA Score
XG7	1.44	-12.03	
LP1	0.43	-16.17	
JJN3	0.37	-9.88	
XG16	0.24	0.74	
XG6	0.32	2.46	High HA Score
XG5	0.29	1.94	
XG13	0.28	1.33	
XG21	0.21	5.18	

P =.009

Figure 6C



SAHA (Vorinostat)

HMCLs	IC50 nM	HA Score	
XG20	1070	-7.77	
AMO-1	695	-11.37	Low HA Score
XG7	1154	-12.03	
LP1	728	-16.17	
JJN3	897	-9.88	
XG16	528	0.74	
XG6	805	2.46	
XG5	548	1.94	High HA Score
XG13	462	1.33	
XG21	236	5.18	

***P* =.02**

HMCL Name	IL-6 dependence[1]	Origin[2]	Disease[3]	Patient sample[4]	Gender	Isotype	t(14q32 or 22q11)	Target genes	Ras	TP53	CD45	HMCL classification
TSA Resistant HMCLs												
XG7	+	MN	MM	PB	F	Ak	t(4;14)	*MMSET*	*mut*	*wt*	+/-	MS
XG20	++	MN	PCL	PB	M	I	t(4;14)	*MMSET*	*wt*	*abn*	-	MS
AMO1	-	CO	PCT	AF	F	Ak	t(12;14)	unknown	*wt*	*wt*	+	CD-2L
JJN3	-	CO	MM	PE	F	Ak	t(14;16)	*c-Maf*	*mut*	*abn*	+/-	MF
LP1	-	CO	MM	PB	F	GI	t(4;14)	*MMSET/FGFR3*	*wt*	*abn*	-	MS
TSA Sensitive HMCLs												
XG5	++	MN	MM	PB	F	I	t(11;14)	*CCND1*	*wt*	*abn*	-	CD-1
XG6	++	MN	MM	PB	F	GI	t(16;22)	*c-Maf*	*wt*	*wt*	+	CTA/MF
XG13	++	MN	PCL	PB	M	GI	t(14;16)	*c-Maf*	*wt*	*abn*	+	MF
XG16	++	MN	PCL	PB	M	k	none	*none*	*mut*	*abn*	+	CTA/FRZB
XG21	++	MN	MM	PE	M	I	t(11;14)	*CCND1*	*wt*	*wt*	+	CD-1

Table 1. Characteristics of HDACi resistant or sensitive HMCLs

[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

Probeset	NAME	Ajusted P value (Benjamini hochberg multiple testing correction)	Hazard ratio
Bad prognostic genes			
204563_at	SELL	.04	1.94
203567_s_at	TRIM38	.04	1.96
201012_at	ANXA1	.02	2.01
205352_at	SERPINI1	.04	2.03
204944_at	PTPRG	.01	2.12
222651_s_at	TRPS1	.03	2.17
214875_x_at	APLP2	.01	2.19
203854_at	IF	.03	2.34
209958_s_at	PTHB1	.01	2.35
209969_s_at	STAT1	.009	2.37
205552_s_at	OAS1	.01	2.50
226269_at	GDAP1	.008	2.69
210432_s_at	SCN3A	.007	2.71
224701_at	PARP14	.01	2.94
214079_at	DHRS2	4.76e-05	3.11
226158_at	KLHL24	.01	3.44
Good prognostic genes			
34408_at	RTN2	2.42e-05	.28
225842_at	---	9.96e-05	.32
208894_at	HLA-DRA	.01	.36
212464_s_at	FN1	.01	.37
202391_at	BASP1	7.01e-05	.37
228726_at	SERPINB1	.009	.38
235301_at	KIAA1324L	.01	.39
206385_s_at	ANK3	.007	.40
230233_at	RASGEF1B	.04	.42
215193_x_at	HLA-DRB1	.01	.43
212636_at	QKI	.02	.44
212998_x_at	HLA-DQB1	.01	.47
223218_s_at	NFKBIZ	.03	.47
209198_s_at	SYT11	.03	.48
211990_at	HLA-DPA1	.02	.49
218918_at	MAN1C1	.04	.49
215388_s_at	CFH	.04	.52
228152_s_at	FLJ31033	.03	.53
216834_at	RGS1	.04	.54
203695_s_at	DFNA5	.04	.54
219833_s_at	EFHC1	.04	.55

Table 2: Prognostic value of TSA deregulated genes in primary MMC of newly-diagnosed patients.