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(54) Title: METHODS FOR PREDICTING RESPONSE TO HDACI/DNMTI COMBINATION IN MULTIPLE MYELOMA

$$HADMS = \sum_{i=1}^{n} \beta i \times Ci \qquad (I)$$

(57) Abstract: The present invention relates to a method of testing whether a patient suffering from multiple myeloma will respond or not to a combination treatment consisting of at least one histone deacetylase inhibitor (HDACi) with at least one DNA methyltransferase inhibitors (DNMTi) comprising: i) determining the expression level (ELi) of several genes G_1-G_n selected from table A in a biological sample obtained from said patient ii) comparing the expression level (ELi) determined at step i) with a predetermined reference level (ELRi) iii) calculating the HADMS score trough the following formula (I) wherein βi represent the regression β coefficient reference value for the gene Gi and Ci = 1 if the expression of the gene Gi (ELi) is higher than the predetermined reference level (ELRi) or Ci = -1 if the expression of the gene (ELi) is lower than or equal to the predetermined reference level (ELRi) iv) comparing the score HADMS determined at step iii) with a predetermined reference value $HADMS_R$ v) and concluding that the patient will respond to the combination treatment when the HADMS score is higher than the predetermined reference value $HADMS_R$ or concluding that the patient will not respond to the combination treatment when the HADMS score is lower than the predetermined reference value $HADMS_R$.

METHODS FOR PREDICTING RESPONSE TO HDACi/DNMTi COMBINATION IN MULTIPLE MYELOMA

FIELD OF THE INVENTION:

The present invention relates to methods for predicting multiple myeloma treatment response.

BACKGROUND OF THE INVENTION:

Multiple myeloma (MM) is an almost fatal neoplasia characterized by the accumulation of malignant plasma cells (MMC) in the bone marrow. The profile of DNA methylation in MM comprises genomic global hypomethylation and simultaneous promoter hypermethylation of known or potential tumor suppressor genes (Heuck, 2013; Walker, 2010). Recently, hypermethylation of several potential suppressor genes was demonstrated to be associated with significantly shorter overall survival (Heuck, 2013).

Decitabine (5-aza-2'-deoxycytidine) or 5-azacytidine are both clinically used DNMT inhibitors for the treatment of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) (Hollenbach, 2010). In MM, clinical trials are ongoing with DNMTi as monotherapy or combined with lenalidomide or dexamethasone (Maes, 2013). Histone deacetylases (HDAC) represent also molecular targets for the treatment of different cancers including MM (Feng, 2008; Khan, 2004; Lavelle, 2001; Mitsiades, 2004; Mitsiades, 2003; Catley, 2003; Kaiser, 2006; Neri, 2012; Neri, 2008; Minami, 2013; Hideshima, 2013). Romidepsin and Vorinostat (SAHA) have been approved by the Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (Zhang, 2009) and several HDACi are evaluated in clinical trials in MM (Maes, 2013; Neri, 2012). Proteasome inhibition leading to accumulation of ubiquitinated proteins, affecting unfolded protein response (UPR) and increasing HDAC-mediated aggregosome formation indicated that HDACi and bortezomib combination could be promising in MM (Richardson, 2013 ;San-Miguel, 2013). Combination of panobinostat/bortezomib/dexamethasone (PANORAMA) and of vorinostat/bortezomib (VANTAGE 088) have been initiated in two large phase III clinical trials (Richardson, 2013; Dimopoulos, 2013). Results of VANTAGE 088 trial shown that association of vorinostat and bortezomib prolonged significantly progression free survival, compared to bortezomib and placebo, in patients with relapsed or refractory MM (Dimopoulos, 2013). However, this combination is associated with toxicity and new treatment

schedules should be investigated to increase tolerability and enhance efficacy (Dimopoulos, 2013).

It was reported that HDACi and DNMTi treatment can induce MAGE-A3 in MM, an attractive target for immunotherapy, and facilitate killing by MAGE-A3 specific cytotoxic T lymphocytes (Moreno-Bost, 2011). Recently, Matthews *et al* investigated the potential of combining HDACi with a BH3-only mimetic (ABT-737), recombinant human TNF-related apoptosis-inducing ligand (rhTRAIL) or 5-azacitidine, *in vivo*, using the Vk*MYC transgenic MM mouse model (Matthews, 2013). HADCi/rhTRAIL or HDACi/ABT-737 combinations are associated with important drug induced toxicity *in vivo*. In contrast, HDACi and DNMTi demonstrated a significant reduction of tumor load *in vivo* and prolonged survival of mice without toxicity (Matthews, 2013). In patients with solid cancers or advanced haematological malignancies, HDACi and DNMTi combination was well tolerated (Bots, 2009) and suggested promising activity in MDS, AML (Bots, 2009; Fandy, 2009; Zhang, 2009) and refractory advanced non-small cell lung cancer (Juergens, 2011). Together, these observations suggest that targeting the aberrant tumor-specific epigenetic program with DNMTi and HDACi treatment could have therapeutic interest in MM. However, identification of biomarkers predictive for sensitivity of MMCs to epigenetic therapies remains an important objective to improve clinical trials. The inventors recently reported gene expression(GEP)-based risk scores to predict the sensitivity of MMC to DNMTi (Moreaux, 2013; Moreaux, 2012) and HDACi (Moreaux, 2013). Since HDACi and DNMTi combination have potential therapeutic value in MM, the inventors searched to build a GEP-based score that could be useful to conduct epigenetic-targeted combination trials.

The identification of biomarkers predictive for sensitivity of MMCs to HDACi and DNMTi combination is an important objective for optimizing these clinical trials. In the present invention, the inventors used gene expression profiling of Multiple Myeloma Cells (MMCs) to build a novel "HDACi/DNMTi score" or "HADMS" that makes it possible identification of patients whose MMCs will be targeted by a combination treatment consisting of at least one DNA methyltransferase inhibitor (DNMTi) with at least one histone deacetylase inhibitor (HDACi).

SUMMARY OF THE INVENTION:

The present invention relates to a method of testing whether a patient suffering from multiple myeloma will respond or not to a combination treatment consisting of at least one

DNA methyltransferase inhibitor (DNMTi) with at least one histone deacetylase inhibitor (HDACi).

DETAILED DESCRIPTION OF THE INVENTION:

The multiple myeloma treatment response was investigated by the inventors using histone deacetylase inhibitor (HDACi), DNA methyltransferase inhibitors (DNMTi), human multiple myeloma cell lines (HMCLs) and primary multiple myeloma cells of patients.

Decitabine and TSA treatment resulted in a significant upregulation of 375 genes. Among the 375 genes, the 96 genes building the histone acetylation/DNA methylation score (HADM score or HADMS), include 42 genes associated with a bad prognostic value and 54 genes associated with a good prognosis in a cohort of 206 newly-diagnosed patients (HM cohort). Using maxstat analysis for overall survival, HADM score was significantly associated with high-risk myeloma in the 2 independent patients' cohorts, HM and UAMS-TT2. The inventors reported a new gene expression-based score to predict the myeloma cell sensitivity to a combination treatment consisting of at least one DNA methyltransferase inhibitor (DNMTi) with at least one histone deacetylase inhibitor (HDACi). HADM score allows identification of high-risk patients associated with MMC's higher sensitivity to a combination treatment consisting of at least one DNA methyltransferase inhibitor (DNMTi) with at least one histone deacetylase inhibitor (HDACi), which is useful in identifying patients who could benefit from combination of epigenetic therapy.

Definitions

The term "patient" denotes a mammal. In a preferred embodiment of the invention, a patient refers to any patient (preferably human) afflicted with multiple myeloma. The term "multiple myeloma" refers to multiple myeloma such as revised in the World Health Organisation Classification C90.

The term "histone deacetylase inhibitor" or "HDACi" has its general meaning in the art and refers to a multiple myeloma treatment. The term "histone deacetylase inhibitor" or "HDACi" refers to histone deacetylase inhibitor that can be grouped in four classes: hydroxamates (panobinostat (LBH-589), trichostatin-A (TSA), vorinostat (SAHA), belinostat (PXD101), NVP-LAQ824 and givinostat (ITF2357)), cyclic peptide (romidepsin (depsipeptide)), aliphatic acids (valproic acid (VPA) and sodium phenylbutyrate) and

benzamides (MS-275, MGCD0103) (10). HDACi are characterized as class I-specific HDACs inhibitors (MGCD0103, romidepsin and MS-275) or as pan-HDAC inhibitors, denoting activity against both classes I and II HDACs (TSA, panobinostat, vorinostat and belinostat) (10).

The term "DNA methyltransferase inhibitors" or "DNMTi" has its general meaning in the art and refers to a multiple myeloma treatment. The term "DNA methyltransferase inhibitors" or "DNMTi" refers to DNA methyltransferase inhibitor that can be sub-divided into nucleoside analogue (5-Azacytidine (azacytidine), 5-Aza-2'-deoxycytidine (decitabine, 5-Aza-CdR), zebularine, 5-Fluoro-2′-deoxycytidine (5-F-CdR), 5,6-Dihydro-5-azacytidine (DHAC)) and non-nucleoside analogue families (Hydralazine, Procainamide, Procaine, EGCG ((-)-epigallocatechin-3-gallate), Psammaplin A, MG98, RG108) (8).

The term "biological sample" refers to multiple myeloma cells, bone marrow or medullary cell.

All the genes pertaining to the invention are known per se, and are listed in the below Table A.

Gene	Gene ID Probeset	Gene Symbol	β coefficient	Reference Level (ELRi)
G1	225842_at	---	-0,899957319	69
G2	226725_at	---	0,818731911	131
G3	240979_at	---	-1,734769051	72
G4	209993_at	ABCB1	1,014206321	30
G5	205997_at	ADAM28	-0,895564458	57
G6	209122_at	ADFP	-0,943134148	171
G7	206385_s_at	ANK3	-0,86352778	10
G8	225283_at	ARRDC4	-0,942014898	45
G9	201243_s_at	ATP1B1	-1,157295661	102
G10	242234_at	BIRC4BP	1,09912532	103
G11	212560_at	C11orf32	-0,80719933	10
G12	210785_s_at	C1orf38	-0,923865573	138

G13	216379_x_at	CD24	-0,934005159	46
G14	221555_x_at	CDC14B	-1,008662223	139
G15	225685_at	CDC42EP3	-0,976621995	92
G16	201131_s_at	CDH1	-0,790641274	3
G17	202284_s_at	CDKN1A	-1,115018187	529
G18	213348_at	CDKN1C	-1,01782728	35
G19	213800_at	CFH	0,927589961	46
G20	213317_at	CLIC5	0,955430136	164
G21	224583_at	COTL1	-0,81118032	6
G22	235700_at	CT45-2	0,947041492	22
G23	202436_s_at	CYP1B1	-1,036466399	28
G24	208779_x_at	DDR1	-0,947438553	102
G25	222793_at	DDX58	0,962155444	133
G26	214079_at	DHRS2	1,113374737	61
G27	219313_at	DKFZp434C0328	-1,005680344	133
G28	221563_at	DUSP10	1,037759883	144
G29	200878_at	EPAS1	-1,038609726	127
G30	224657_at	ERRFI1	-0,846840786	25
G31	225328_at	FBXO32	-1,060608582	184
G32	228745_at	FLJ13611	-1,219072077	57
G33	212464_s_at	FN1	-1,072661597	1
G34	211458_s_at	GABARAPL1	-0,96494036	350
G35	231577_s_at	GBP1	-0,865861628	68
G36	226269_at	GDAP1	1,176392353	22
G37	200696_s_at	GSN	-1,035458903	50
G38	214469_at	HIST1H2AE	1,168940874	87
G39	235456_at	HIST1H2BD	1,224250233	99
G40	203932_at	HLA-DMB	-1,211306838	161
G41	212998_x_at	HLA-DQB1	-0,800807606	2
G42	208894_at	HLA-DRA	-1,122412883	143
G43	215193_x_at	HLA-DRB1	-0,883817028	47
G44	211538_s_at	HSPA2	1,029045845	45
G45	202411_at	IFI27	1,106775525	185

G46	203153_at	IFIT1	0,967220137	401
G47	229450_at	IFIT3	1,241731919	642
G48	205227_at	IL1RAP	-0,879498221	7
G49	225525_at	KIAA1671	1,103510707	18
G50	235252_at	KSR	-1,275100289	82
G51	236565_s_at	LARP6	1,131704184	57
G52	226702_at	LOC129607	0,803290573	723
G53	225407_at	MBP	-0,929928327	10
G54	235568_at	MCEMP1	-0,783211082	50
G55	214696_at	MGC14376	-1,004393637	296
G56	238430_x_at	MGC19764	0,949198229	85
G57	226066_at	MITF	0,949130851	164
G58	212509_s_at	MXRA7	-1,106064046	156
G59	203215_s_at	MYO6	-0,878410657	107
G60	203413_at	NELL2	1,101239744	55
G61	229963_at	NGFRAP1L1	1,205822872	1834
G62	205552_s_at	OAS1	1,098161459	590
G63	204972_at	OAS2	1,569325358	749
G64	218543_s_at	PARP12	1,097562753	589
G65	224701_at	PARP14	1,412504773	360
G66	223220_s_at	PARP9	0,928781518	343
G67	205380_at	PDZK1	0,944860168	5
G68	217996_at	PHLDA1	-1,065526416	242
G69	203879_at	PIK3CD	-1,353529364	74
G70	201939_at	PLK2	1,1090142	107
G71	202430_s_at	PLSCR1	1,260332375	301
G72	203680_at	PRKAR2B	-0,966689497	24
G73	202252_at	RAB13	-1,308103119	248
G74	230233_at	RASGEF1B	-1,145228745	66
G75	242625_at	RSAD2	0,993659251	89
G76	34408_at	RTN2	-1,251325387	90
G77	210592_s_at	SAT	-1,011124683	1916
G78	204030_s_at	SCHIP1	-1,020819238	21

G79	210432_s_at	SCN3A	1,158531601	21
G80	201427_s_at	SEPP1	-1,053836286	447
G81	228726_at	SERPINB1	-1,143171879	75
G82	209723_at	SERPINB9	-0,80370612	75
G83	205352_at	SERPINI1	1,274785788	305
G84	226728_at	SLC27A1	-0,93950361	94
G85	216236_s_at	SLC2A14	-0,949244583	48
G86	202497_x_at	SLC2A3	-1,029814297	12
G87	209762_x_at	SP110	1,463775754	318
G88	210394_x_at	SSX4	0,934669303	102
G89	209969_s_at	STAT1	1,014749908	394
G90	206118_at	STAT4	0,948161655	270
G91	202085_at	TJP2	-1,12983309	25
G92	223949_at	TMPRSS3	0,962769445	10
G93	213423_x_at	TUSC3	0,786424757	56
G94	219211_at	USP18	1,144102267	188
G95	228617_at	XAF1	1,175253328	686
G96	219062_s_at	ZCCHC2	-0,984374978	11

Table A: Set of predictive genes.

Methods for predicting response

The present invention relates to a method of testing whether a patient suffering from multiple myeloma will respond or not to a combination treatment consisting of at least one histone deacetylase inhibitor (HDACi) with at least one DNA methyltransferase inhibitor (DNMTi) comprising:

i) determining the expression level (ELi) of several genes G_1-G_n selected from table A in a biological sample obtained from said patient

ii) comparing the expression level (ELi) determined at step i) with a predetermined reference level (ELRi)

iii) calculating the HADMS score trough the following formula

$$HADMS = \sum_{i=1}^{n} \beta i \times Ci$$

wherein βi represent the regression β coefficient reference value for the gene G_i and Ci = 1 if the expression of the gene G_i (ELi) is higher than the predetermined reference level (ELRi) or Ci = -1 if the expression of the gene (ELi) is lower than or equal to the predetermined reference level (ELRi)

iv) comparing the score HADMS determined at step iii) with a predetermined reference value $HADMS_R$

v) and concluding that the patient will respond to the combination treatment when the HADMS score is higher than the predetermined reference value $HADMS_R$ or concluding that the patient will not respond to the combination treatment when the HADMS score is lower than the predetermined reference value $HADMS_R$.

In some embodiments, the expression levels of at least 42 genes from Table A are determined wherein said genes are: EPAS1, ATP1B1, TJP2, RAB13, IFI27, PLSCR1, CYP1B1, SLC2A3, IFIT1, SCHIP1, PDZK1, DDR1, HLA-DRA, SERPINB9, SP110, SSX4, C1orf38, FN1, MXRA7, CLIC5, HIST1H2AE, MGC14376, HLA-DRB1, SLC2A14, USP18, DKFZp434C0328, CDC14B, DDX58, PARP9, TMPRSS3, COTL1, PARP14, KIAA1671, GDAP1, LOC129607, SLC27A1, FLJ13611, KSR, HIST1H2BD, 240979_at EST, BIRC4BP and RSAD2.

In some embodiment, the expression levels of 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 or 96 genes from Table A are determined wherein every combinations of genes comprises a minimal set of 42 genes consisting of: EPAS1, ATP1B1, TJP2, RAB13, IFI27, PLSCR1, CYP1B1, SLC2A3, IFIT1, SCHIP1, PDZK1, DDR1, HLA-DRA, SERPINB9, SP110, SSX4, C1orf38, FN1, MXRA7, CLIC5, HIST1H2AE, MGC14376, HLA-DRB1, SLC2A14, USP18, DKFZp434C0328, CDC14B, DDX58, PARP9, TMPRSS3, COTL1, PARP14, KIAA1671, GDAP1, LOC129607, SLC27A1, FLJ13611, KSR, HIST1H2BD, 240979_at EST, BIRC4BP and RSAD2.

In some embodiments, the expression levels of the 96 genes of Table A are determined.

Determination of the expression level of the genes can be performed by a variety of techniques. Generally, the expression level as determined is a relative expression level. More preferably, the determination comprises contacting the biological sample with selective reagents such as probes, primers or ligands, and thereby detecting the presence, or measuring the amount, of polypeptide or nucleic acids of interest originally in the biological sample. Contacting may be performed in any suitable device, such as a plate, microtiter dish, test tube, well, glass, column, and so forth. In specific embodiments, the contacting is performed on a substrate coated with the reagent, such as a nucleic acid array or a specific ligand array. The substrate may be a solid or semi-solid substrate such as any suitable support comprising glass, plastic, nylon, paper, metal, polymers and the like. The substrate may be of various forms and sizes, such as a slide, a membrane, a bead, a column, a gel, etc. The contacting may be made under any condition suitable for a detectable complex, such as a nucleic acid hybrid or an antibody-antigen complex, to be formed between the reagent and the nucleic acids or polypeptides of the biological sample.

In a preferred embodiment, the expression level may be determined by determining the quantity of mRNA.

Methods for determining the quantity of mRNA are well known in the art. For example the nucleic acid contained in the biological sample is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA is then detected by hybridization (e. g., Northern blot analysis) and/or amplification (e.g., RT-PCR). Preferably quantitative or semi-quantitative RT-PCR is preferred. Real-time quantitative or semi-quantitative RT-PCR is particularly advantageous.

Other methods of amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of interest herein find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably 85% identical and even more preferably 90-95% identical. In certain embodiments,

it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization. A wide variety of appropriate indicators are known in the art including, fluorescent, radioactive, enzymatic or other ligands (e. g. avidin/biotin).

Probes typically comprise single-stranded nucleic acids of between 10 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are "specific" to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency hybridization conditions (corresponding to the highest melting temperature Tm, e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate).

The nucleic acid primers or probes used in the above amplification and detection method may be assembled as a kit. Such a kit includes consensus primers and molecular probes. A preferred kit also includes the components necessary to determine if amplification has occurred. The kit may also include, for example, PCR buffers and enzymes; positive control sequences, reaction control primers; and instructions for amplifying and detecting the specific sequences.

In a particular embodiment, the methods of the invention comprise the steps of providing total RNAs extracted from a biological samples and subjecting the RNAs to amplification and hybridization to specific probes, more particularly by means of a quantitative or semi-quantitative RT-PCR.

In another preferred embodiment, the expression level is determined by DNA chip analysis. Such DNA chip or nucleic acid microarray consists of different nucleic acid probes that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. A microchip may be constituted of polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, or nitrocellulose. Probes comprise nucleic acids such as cDNAs or oligonucleotides that may be about 10 to about 60 base pairs. To determine the expression level, a biological sample from a test patient, optionally first subjected to a reverse transcription, is labelled and contacted with the microarray in hybridization conditions, leading to the formation of complexes between target nucleic acids that are complementary to probe sequences attached to the microarray surface. The labelled hybridized complexes are then detected and can be quantified or semi-quantified. Labelling may be achieved by various methods, e.g. by using radioactive or

fluorescent labelling. Many variants of the microarray hybridization technology are available to the man skilled in the art (see e.g. the review by Hoheisel, Nature Reviews, Genetics, 2006, 7:200-210)

In this context, the invention further provides a DNA chip comprising a solid support which carries nucleic acids that are specific to the genes listed in Table A.

Predetermined reference values ELRi or $HADMS_R$ used for comparison may consist of "cut-off" values.

For example; each reference ("cut-off") value ELRi for each gene may be determined by carrying out a method comprising the steps of:

a) providing a collection of samples from patients suffering from multiple myeloma;

b) determining the expression level of the relevant gene for each sample contained in the collection provided at step a);

c) ranking the samples according to said expression level

d) classifying said samples in pairs of subsets of increasing, respectively decreasing, number of members ranked according to their expression level,

e) providing, for each sample provided at step a), information relating to the actual clinical outcome for the corresponding cancer patient (i.e. the duration of the disease-free survival (DFS), or the event free survival (EFS) or the overall survival (OS) or both);

f) for each pair of subsets of tumour tissue samples, obtaining a Kaplan Meier percentage of survival curve;

g) for each pair of subsets of tumour tissue samples calculating the statistical significance (p value) between both subsets

h) selecting as reference value ELR for the expression level, the value of expression level for which the p value is the smallest.

For example the expression level of a gene Gi has been assessed for 100 samples of 100 patients. The 100 samples are ranked according to the expression level of gene Gi. Sample 1 has the highest expression level and sample 100 has the lowest expression level. A first grouping provides two subsets: on one side sample Nr 1 and on the other side the 99 other samples. The next grouping provides on one side samples 1 and 2 and on the other side the 98 remaining samples etc., until the last grouping: on one side samples 1 to 99 and on the other side sample Nr 100. According to the information relating to the actual clinical outcome for the corresponding cancer patient, Kaplan Meier curves are prepared for each of the 99 groups of two subsets. Also for each of the 99 groups, the p value between both subsets was

calculated. The reference value ELRi is then selected such as the discrimination based on the criterion of the minimum p value is the strongest. In other terms, the expression level corresponding to the boundary between both subsets for which the p value is minimum is considered as the reference value. It should be noted that according to the experiments made by the inventors, the reference value ELRi is not necessarily the median value of expression levels.

The man skilled in the art also understands that the same technique of assessment of the $HADMS_R$ could be used for obtaining the reference value and thereafter for assessment of the response to the combination treatment of the present invention. However in one embodiment, the reference value $HADMS_R$ is the median value of HADMS.

In one embodiment, the reference value ELRi for the gene Gi is described in table A (right column).

The regression β coefficient reference values may be easily determined by the skilled man in the art for each gene Gi using a Cox model. The Cox model is based on a modeling approach to the analysis of survival data. The purpose of the model is to simultaneously explore the effects of several variables on survival. The Cox model is a well-recognised statistical technique for analysing survival data. When it is used to analyse the survival of patients in a clinical trial, the model allows us to isolate the effects of treatment from the effects of other variables. The logrank test cannot be used to explore (and adjust for) the effects of several variables, such as age and disease duration, known to affect survival. Adjustment for variables that are known to affect survival may improve the precision with which we can estimate the treatment effect. The regression method introduced by Cox is used to investigate several variables at a time. It is also known as proportional hazards regression analysis. Briefly, the procedure models or regresses the survival times (or more specifically, the so-called hazard function) on the explanatory variables. The hazard function is the probability that an individual will experience an event (for example, death) within a small time interval, given that the individual has survived up to the beginning of the interval. It can therefore be interpreted as the risk of dying at time **t**. The quantity h0 (**t**) is the baseline or underlying hazard function and corresponds to the probability of dying (or reaching an event) when all the explanatory variables are zero. The baseline hazard function is analogous to the intercept in ordinary regression (since exp0= 1). The regression coefficient β gives the

proportional change that can be expected in the hazard, related to changes in the explanatory variables. The coefficient β is estimated by a statistical method called maximum likelihood. In survival analysis, the hazard ratio (HR) (Hazard Ratio = exp(β)) is the ratio of the hazard rates corresponding to the conditions described by two sets of explanatory variables. For example, in a drug study, the treated population may die at twice the rate per unit time as the control population. The hazard ratio would be 2, indicating higher hazard of death from the treatment.

In one embodiment, the regression β coefficient reference values are described in Table A.

Typically, the reference value $HADMS_R$ is -21.57 for determining whether a patient suffering from multiple myeloma will respond to the combination treatment of the invention and for predicting the survival time of patient suffering from multiple myeloma.

The invention also relates to a kit for performing the methods as above described, wherein said kit comprises means for measuring the expression level of the genes listed in Table A. Typically the kit may include a primer, a set of primers, a probe, a set of probes as above described. In a particular embodiment, the probe or set of probes are labelled as above described. The kit may also contain other suitably packaged reagents and materials needed for the particular detection protocol, including solid-phase matrices, if applicable, and standards.

In a particular embodiment, the score may be generated by a computer program.

Methods of treatment

The method of the invention allows to define a subgroup of patients who will be responsive ("responder") or not ("non responder") to the treatment with the combination treatment consisting of at least one histone deacetylase inhibitor with at least one DNA methyltransferase inhibitor.

A further object of the invention relates to a method for the treatment of multiple myeloma in a patient in need thereof.

In the context of the invention, the term "treating" or "treatment", as used herein, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition.

In a particular embodiment, the method comprises the following steps:

a) testing whether the patient will respond or not to the combination treatment of the present invention by performing the method according to the invention

b) administering the combination treatment of the present invention, when the HADMS score is higher thant the reference value $HADMS_R$ (i.e. the patient will respond to the combination treatment consisting of at least one histone deacetylase inhibitor with at least one DNA methyltransferase inhibitor).

A further object of the invention relates to a combination treatment consisting of at least one histone deacetylase inhibitor with at least one DNA methyltransferase inhibitor for use in the treatment of multiple myeloma in a patient in need thereof, wherein the patient was being classified as responder by the method as above described.

A further object of the invention relates to a combination treatment consisting of trichostatin-A (TSA) or vorinostat (SAHA) with decitabine (5-aza-2'-deoxycytidine) or 5-azacytidine for use in the treatment of multiple myeloma in a patient in need thereof, wherein the patient was being classified as responder by the method as above described.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Clustergram of the signals of the 96 genes used to build HADMS score in myeloma cells of 206 previously untreated patients.

The signals of the 96 probe sets in MMCs of 206 patients, ordered by increasing HADMS score, are displayed from low (deep blue) to high (deep red) expression.

Figure 2: HADMS score in normal and malignant plasma cells.

HADMS 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).

Figure 3: Prognostic value of HADMS score in multiple myeloma.

(A) Patients of the HM cohort were ranked according to increased HADMS score and a maximum difference in OS was obtained with HADMS score = -21.57 splitting patients in high risk (23.7%) and low risk (76.3%) groups. The prognostic value of HADMS score was validated using an independent cohort of 345 patients from UAMS treated with TT2 therapy (UAMS- TT2 cohort). The parameters to compute the HADMS score of patients of UAMS-TT2 cohort and the proportions delineating the 2 prognostic groups were those defined with HM cohort.

(B) The HADMS score could also predict for event free survival (EFS) in the HM and UAMS-TT2 cohorts.

Figure 4: HADMS score predicts for sensitivity of primary myeloma cells of patients to HDACi/DNMTi combined treatment.

(A) Mononuclear cells from tumour samples of 10 patients with MM were cultured for 4 days in the presence of IL-6 (2 ng/ml) with or without graded decitabine and TSA concentrations. At day 4 of culture, the count of viable $CD138^+$ MMCs was determined using flow cytometry. The grey columns represent the mean ± SD of primary myeloma cell counts (expressed as the percentage of the count without adding drugs) of the 5 patients with a low HADMS score and the white columns that of the 5 patients with a high HADMS score.

(B) 5-azacitidine and SAHA combination was also investigated using samples of 12 myeloma patients. The grey columns represent the mean ± SD of primary myeloma cell counts (expressed as the percentage of the count without adding drugs) of the 7 patients with a low HADMS score and the white columns that of the 5 patients with a high HADMS score.

Figure 5: HADMS score in normal plasma cell differentiation.

HADMS score in normal memory B cells (MB, n = 5), normal preplasmablasts (PrePB, n = 5), normal plasmablasts (PB, n = 5), normal early plasma cells (Early PC, n = 5), 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).

Figure 6: HADMS in MMCs of patients using UAMS-TT2 cohort.

The HADMS 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 ($P < .05$).

EXAMPLE:

Material & Methods

Human Myeloma Cell Lines (HMCLs) and primary multiple myeloma cells of patients.

Human myeloma cell lines HMCLs, N=40 were obtained as previously described (Gu, 2000; Moreaux, 2011; Rebouissou, 1998; Tarte, 1999; Zhang, 1994) or purchased from DSMZ and American Type Culture Collection. Microarray data are deposited in the ArrayExpress public database (accession numbers E-TABM-937 and E-TABM-1088). 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 (Hose, 2011). Gene expression profiling (GEP) of purified MMCs was assayed using Affymetrix U133 2.0 plus microarrays (Affymetrix, Santa Clara, CA, USA) as described (De Vos, 2002) and data normalized using the MAS5 Affymetrix algorithm. The .CEL and MAS5 files are deposited in the ArrayExpress public database (http://www.ebi.ac.uk/arrayexpress/), under accession number E-MTAB-362. We also used publicly available MAS5 normalized GEP data (GEO, http://www.ncbi.nlm.nih.gov/geo/, accession number GSE2658) from purified MMCs of a cohort of 345 patients treated with total therapy 2 protocol (UAMS-TT2 cohort) at the University of Arkansas for Medical Sciences (UAMS, Little Rock, USA) (Barlogie, 2006). T(4;14) translocation was evaluated using *MMSET* spike expression

(Kassambara, 2012) and del17p13 surrogated by *TP53* probe set signal (Xiong, 2008) for UAMS-TT2 patients. Gene expression data of normal memory B cells (MB), preplasmasts, plasmablasts and early plasma cells (Jourdan, 2009; Jourdan, 2011) are deposited in the ArrayExpress databases under accession numbers E-MEXP-2360 and E-MEXP-3034.

Identification of genes deregulated by HDACi + DNMTi combination.

5 HMCLs (XG-5, XG-6, XG-7, XG-20 and LP1) were treated with 0.5 µmol/L Decitabine (Sigma, St Louis, MO) for 7 days in RPMI 1640, 10% fetal bovine serum supplemented with IL-6 for IL-6 dependent HMCLs. During the last 24 hours, 0.33 µmol/L TSA (Sigma) was added as described by Heller *et al* (Heller, 2008). Whole genome gene expression profiling was assayed with Affymetrix U133 2.0 plus microarrays (Affymetrix).

Sensitivity of primary myeloma cells to HDACi + DNMTi combination.

Primary myeloma cells of 10 patients were cultured with or without graded concentrations of Decitabine and TSA. Primary myeloma cells of 12 patients were cultured with or without graded concentrations of 5-azacitidine (Sigma) and vorinostat (SAHA) (Sigma). MMCs cytotoxicity was evaluated using anti-CD138-PE mAb (Immunotech, Marseille, France) as described (Mahtouk, 2004; Moreaux, 2012). Results were analyzed using GraphPad Prism (http://www.graphpad.com/scientific-software/prism/).

Statistical analysis

Gene expression data were analyzed using SAM (Significance Analysis of Microarrays) software (Cui, 2003) as published (Kassambara, 2012). The statistical significance of differences in overall survival between groups of patients was calculated by the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. Survival curves were plotted using the Kaplan-Meier method. All these analyses have been done with R.2.10.1 (http://www.r-project.org/) and bioconductor version 2.5. Histone acetylation/DNA methylation risk score (termed HADMS Score) was built using our previously published methodology with the Decitabine/TSA combination deregulated prognostic genes (Moreaux, 2012; Moreaux, 2013). Briefly, HADMS Score was constructed as the sum of the Cox model beta coefficients of each of the Decitabine/TSA combination deregulated genes with a prognostic value, weighted by ±1 according to the patient MMC signal above or below the probeset maxstat value (Kassambara, 2012; Moreaux, 2012; Moreaux, 2013). Significantly enriched pathways were identified using Reactome functional interaction map. Gene set enrichment analysis was carried out by computing overlaps with canonical pathways and gene ontology gene sets obtained from the Broad Institute (Subramanian, 2005).

Results

Identification of genes whose expression is deregulated by decitabine and trichostatin A combination and associated with a prognostic value in multiple myeloma.

Using gene expression microarrays, the inventors analyzed gene expression changes in 5 HMCLs after sublethal (Table 1) treatment of decitabine and TSA combination (Heller, 2008). Decitabine and TSA treatment resulted in a significant upregulation of 375 genes (SAM supervised paired analysis, FDR < 5%; Table 2). REACTOME analysis revealed that decitabine/TSA-regulated genes are significantly enriched in genes related to interferon signaling ($P < 0.0001$; FDR = 1E-3), cell adhesion molecules ($P < 0.0001$; FDR = 1.6E-4), antigen processing and presentation ($P < 0.0001$; FDR = 7.6E-5) and EGF receptor signaling ($P = 0.0004$; FDR = 4.3E-3) pathways (Table 3). In order to identify genes deregulated by HDAC and DNMT linked with important function in MM pathophysiology, the inventors researched the genes deregulated by decitabine and TSA treatment whose expression is associated with a prognostic value using Maxstat R function and Benjamini-Hochberg multiple testing correction (Kassambara, 2012). Among the 375 genes, 42 genes had a bad prognostic value and 54 a good one in our cohort of 206 newly-diagnosed patients (HM cohort) (Table A). The prognostic information of decitabine and TSA combination regulated genes was gathered in a HADMS score as described in Materials and Methods section (Figure 1). HADMS score values in normal, premalignant or malignant plasma cells are displayed in Figure 2. HADMS score value was significantly higher in MMC from MGUS patients compared to normal BMPCs ($P = 0.009$; Figure 2). MMCs of patients have a significantly higher HADMS score than plasma cells from MGUS-patients ($P = 0.003$) and HMCLs the highest score ($P < 0.001$) (Figure 2). Investigating the HADMS score in the 8 groups of the molecular classification of multiple myeloma, HADMS score was significantly higher in the proliferation, t(4;14) and hyperdiploid subgroups ($P < 0.001$; $P = 0.001$ and $P < 0.001$ respectively) and significantly lower in the low bone disease and CD2 subgroups ($P = 0.002$ and $P < 0.001$) (Zhan, 2006) (Figure 6).

	Cell viability (%)				
	Day 0	Day 3		Day 7	
HMCLS		Control	5-aza	Control	5-aza and TSA during the last 24h
XG-5	70±2	70±1	65±5	81±5	69±7
XG-6	90±2	90±2	90±1	93±5	79±2
XG-7	100±0	90±2	90±2	92±4	83±6
XG-20	100±0	91±3	91±3	95±5	80±5
LP1	100±0	91±2	91±2	94±5	87±3

Table 1: Cell viability of HMCLs treated with 0.5 µM decitabine for 7 days and 0.33 µM during the last 24 hours. Data are the mean percentages ± SD of viable cells evaluated by trypan blue exclusion (3 experiments).

Probeset	Gene	Ratio	Banding
Intercellular communication and membrane proteins			
205997_at	ADAM28	27.0	8p21.2
201952_at	ALCAM	16.4	3q13.1
209462_at	APLP1	24.5	19q13.1
211404_s_at	APLP2	2.5	11q23-q25|11q24
205239_at	AREG	24.1	4q13-q21
217767_at	C3	10.1	19p13.3-p13.2
209906_at	C3AR1	17.9	12p13.31
204103_at	CCL4	3.7	17q12
226545_at	CD109	6.3	6q13
216379_x_at	CD24	15.5	6q21
229221_at	CD44	3.2	11p13
213958_at	CD6	8.8	11q13
203904_x_at	CD82	3.2	11p11.2
204440_at	CD83	3.8	6p23

201005_at	CD9	15.2	12p13.3
201131_s_at	CDH1	107.5	16q22.1
213800_at	CFH	7.9	1q32
221698_s_at	CLEC7A	9.5	12p13.2-p12.3
1556499_s_at	COL1A1	11.6	17q21.3-q22.1
202403_s_at	COL1A2	5.4	7q22.1
205898_at	CX3CR1	5.2	3p21|3p21.3
208779_x_at	DDR1	2.7	6p21.3
226281_at	DNER	15.3	2q36.3
202668_at	EFNB2	5.2	13q33
225078_at	EMP2	7.4	16p13.2
213506_at	F2RL1	158.2	5q13
201579_at	FAT	14.1	4q35
212464_s_at	FN1	79.4	2q34
204222_s_at	GLIPR1	3.2	12q21.1
231166_at	GPR155	4.2	2q31.1
229055_at	GPR68	5.8	14q31
200696_s_at	GSN	7.6	9q33
217478_s_at	HLA-DMA	2.6	6p21.3
203932_at	HLA-DMB	4.2	6p21.3
211990_at	HLA-DPA1	7.6	6p21.3
201137_s_at	HLA-DPB1	5.5	6p21.3
212671_s_at	HLA-DQA1	6.5	6p21.3
212998_x_at	HLA-DQB1	18.5	6p21.3
208894_at	HLA-DRA	7.9	6p21.3
215193_x_at	HLA-DRB1	4.1	6p21.3
208306_x_at	HLA-DRB5	3.6	6p21.3
217362_x_at	HLA-DRB6	4.4	6p21.3
219403_s_at	HPSE	5.6	4q21.3
210095_s_at	IGFBP3	5.3	7p13-p12
206172_at	IL13RA2	32.9	Xq13.1-q28
203233_at	IL4R	3.5	16p11.2-12.1
216331_at	ITGA7	5.5	12q13

207509_s_at	LAIR2	4.9	19q13.4
205569_at	LAMP3	16.4	3q26.3-q27
221581_s_at	LAT2	4.3	7q11.23
200923_at	LGALS3BP	18.2	17q25
208933_s_at	LGALS8	14.0	1q42-q43
225060_at	LRP11	40.4	6q25.1
235568_at	MCEMP1	12.3	19p13.2
210605_s_at	MFGE8	4.5	15q25
212473_s_at	MICAL2	11.3	11p15.3
205959_at	MMP13	221.2	11q22.3
212509_s_at	MXRA7	15.0	17q25.1
203413_at	NELL2	6.2	12q13.11-q13.12
204105_s_at	NRCAM	3.2	7q31.1-q31.2
214617_at	PRF1	36.7	10q22
212646_at	RAFTLIN	3.3	3p25.1-p24.3
212158_at	SDC2	26.3	8q22-q23
202071_at	SDC4	5.3	20q12
204563_at	SELL	4.1	1q23-q25
201427_s_at	SEPP1	104.7	5q31
228726_at	SERPINB1	6.1	6p25
211474_s_at	SERPINB6	8.2	6p25
209723_at	SERPINB9	33.2	6p25
202283_at	SERPINF1	51.0	17p13.1
205352_at	SERPINI1	8.4	3q26.1
209848_s_at	SILV	12.6	12q13-q14
206310_at	SPINK2	22.3	4q12
205016_at	TGFA	6.0	2p13
226625_at	TGFBR3	3.9	1p33-p32
202085_at	TJP2	4.7	9q13-q21
218113_at	TMEM2	3.0	9q13-q21
202688_at	TNFSF10	9.9	3q26
207426_s_at	TNFSF4	3.4	1q25
206907_at	TNFSF9	7.2	19p13.3

203476_at	TPBG	8.1	6q14-q15
200931_s_at	VCL	3.4	10q22.1-q23
227530_at	AKAP12	29.7	6q24-q25
Signal transduction			
218501_at	ARHGEF3	5.6	3p21-p13
227915_at	ASB2	4.6	14q31-q32
209682_at	CBLB	2.4	3q13.11
213385_at	CHN2	2.7	7p15.3
201041_s_at	DUSP1	3.6	5q34
221563_at	DUSP10	2.1	1q41
207111_at	EMR1	10.9	19p13.3
202609_at	EPS8	3.6	12q13
224657_at	ERRFI1	2.5	1p36.12-36.33
226269_at	GDAP1	3.6	8q21.11
204472_at	GEM	5.3	8q13-q21
227692_at	GNAI1	4.0	7q21
214022_s_at	IFITM1	5.9	11p15.5
205227_at	IL1RAP	5.1	3q28
231779_at	IRAK2	3.6	3p25.3
235252_at	KSR	3.3	17q11.2
202086_at	MX1	8.5	21q22.3
223218_s_at	NFKBIZ	6.1	3p12-q12
203964_at	NMI	3.6	2p24.3-q21.3
225626_at	PAG1	13.8	8q21.13
203879_at	PIK3CD	4.5	1p36.2
201939_at	PLK2	5.2	5q12.1-q13.2
203680_at	PRKAR2B	3.9	7q22
203355_s_at	PSD3	2.9	8pter-p23.3
202252_at	RAB13	22.7	1q21.2
219622_at	RAB20	7.7	13q34
217764_s_at	RAB31	5.9	18p11.3
217762_s_at	RAB31	2.9	18p11.3
212561_at	RAB6IP1	5.1	11p15.4

1553185_at	RASEF	2.9	9q21.32
230233_at	RASGEF1B	3.4	4q21.3
225946_at	RASSF8	3.3	12p12.3
203485_at	RTN1	8.0	14q23.1
34408_at	RTN2	5.1	19q13.32
226549_at	SBK1	3.8	16p11.2
209969_s_at	STAT1	6.3	2q32.2
206118_at	STAT4	8.3	2q32.2-q32.3
202695_s_at	STK17A	3.2	7p12-p14
220260_at	TBC1D19	4.1	4p15.2
213107_at	TNIK	4.9	3q26.2
Cytoskeleton			
224694_at	ANTXR1	4.3	2p13.1
225524_at	ANTXR2	6.4	4q21.21
212077_at	CALD1	24.8	7q33
212554_at	CAP2	6.8	6p22.3
224583_at	COTL1	3.2	16q24.1
212730_at	DMN	3.6	15q26.3
225855_at	EPB41L5	3.4	2q14.2
217892_s_at	EPLIN	2.7	12q13
208614_s_at	FLNB	7.5	3p14.3
203854_at	IF	3.8	4q25
226968_at	KIF1B	2.6	1p36.2
203130_s_at	KIF5C	7.1	2q23.1
201596_x_at	KRT18	2.3	12q13
225540_at	MAP2	23.1	2q34-q35
225407_at	MBP	2.9	18q23
201976_s_at	MYO10	4.8	5p15.1-p14.3
203215_s_at	MYO6	13.7	6q13
218678_at	NES	6.6	1q23.1
210986_s_at	TPM1	4.2	15q22.1
204141_at	TUBB2	6.3	6p25
Cell cycle			

221555_x_at	CDC14B	3.9	9q22.33
225685_at	CDC42EP3	2.6	2p21
202284_s_at	CDKN1A	2.9	6p21.2
213348_at	CDKN1C	2.8	11p15.5
31874_at	GAS2L1	7.0	22q12.2
1553599_a_at	SYCP3	22.4	12q
Metabolism			
209459_s_at	ABAT	6.5	16p13.2
209993_at	ABCB1	12.2	7q21.1
209122_at	ADFP	4.6	9p22.1
226325_at	ADSSL1	27.9	14q32.33
209160_at	AKR1C3	22.1	10p15-p14
201243_s_at	ATP1B1	6.4	1q24
213106_at	ATP8A1	2.7	4p14-p12
206633_at	CHRNA1	39.2	2q24-q32
213317_at	CLIC5	4.8	6p12.1-21.1
231265_at	COX7B2	54.1	4p12
201116_s_at	CPE	13.1	4q32.3
202295_s_at	CTSH	3.1	15q24-q25
210074_at	CTSL2	4.7	9q22.2
203475_at	CYP19A1	3.9	15q21.1
202436_s_at	CYP1B1	22.7	2p21
228391_at	CYP4V2	3.9	4q35.1-q35.2
214079_at	DHRS2	11.0	14q11.2
219532_at	ELOVL4	18.3	6q14
209392_at	ENPP2	76.3	8q24.1
202838_at	FUCA1	4.2	1p34
211458_s_at	GABARAPL1	25.4	12p13.2
231577_s_at	GBP1	10.0	1p22.2
202748_at	GBP2	3.2	1p22.2
223434_at	GBP3	18.2	1p22.2
213343_s_at	GDPD5	7.3	11q13.4-q13.5
226160_at	H6PD	2.4	1p36

1552767_a_at	HS6ST2	8.9	Xq26.2
205404_at	HSD11B1	16.8	1q32-q41
230966_at	IL4I1	4.4	19q13.3-q13.4
203710_at	ITPR1	3.4	3p26-p25
204179_at	MB	25.5	22q13.1
204059_s_at	ME1	2.4	6q12
225782_at	MSRB3	3.3	12q14.3
214440_at	NAT1	2.5	8p23.1-p21.3
211685_s_at	NCALD	2.5	8q22-q23
210519_s_at	NQO1	2.8	16q22.1
219369_s_at	OTUB2	2.6	14q32.13
202430_s_at	PLSCR1	5.4	3q23
204286_s_at	PMAIP1	3.8	18q21.32
206345_s_at	PON1	5.0	7q21.3
201876_at	PON2	2.9	7q21.3
202458_at	PRSS23	10.6	11q14.1
238017_at	RDHE2	48.7	8q12.1
204730_at	RIMS3	2.6	1pter-p22.2
217983_s_at	RNASET2	2.5	6q27
242625_at	RSAD2	56.7	2p25.2
210592_s_at	SAT	6.4	Xp22.1
210432_s_at	SCN3A	3.6	2q24
223391_at	SGPP1	4.6	14q23.2
226728_at	SLC27A1	2.4	19p13.11
216236_s_at	SLC2A14	4.3	12p13.31
202497_x_at	SLC2A3	28.1	12p13.3
202219_at	SLC6A8	6.3	Xq28
216370_s_at	TKTL1	56.6	Xq28
223949_at	TMPRSS3	6.8	21q22.3
204140_at	TPST1	3.2	7q11.21
213423_x_at	TUSC3	13.7	8p22
219211_at	USP18	30.6	22q11.21
Protein binding			

206385_s_at	ANK3	14.7	10q21
208792_s_at	CLU	3.3	8p21-p12
203695_s_at	DFNA5	9.2	7p15
200606_at	DSP	2.5	6p24
200878_at	EPAS1	3.5	2p21-p16
225328_at	FBXO32	8.3	8q24.13
200799_at	HSPA1A	63.1	6p21.3
211538_s_at	HSPA2	14.0	14q24.1
228153_at	IBRDC2	5.6	6p22.3
201315_x_at	IFITM2	4.9	11p15.5
209270_at	LAMB3	12.5	1q32
203186_s_at	S100A4	13.7	1q21
204030_s_at	SCHIP1	3.7	3q25.32-q25.33
33323_r_at	SFN	4.7	1p36.11
218404_at	SNX10	3.6	7p15.2
205573_s_at	SNX7	24.1	1p21.3
209198_s_at	SYT11	9.7	1q21.2
232914_s_at	SYTL2	13.8	11q14
232692_at	TDRD6	9.2	6p12.3
213361_at	TDRD7	3.3	9q22.33
228285_at	TDRD9	14.7	14q32.33
Cancer testis antigens			
235700_at	CT45-2	30.9	Xq26.3
214603_at	MAGEA2	30.6	Xq28
210437_at	MAGEA9	5.4	Xq28
204086_at	PRAME	12.2	22q11.22
220922_s_at	SPANXA1	55.2	Xq27.1
220217_x_at	SPANXC	4.9	Xq27.1
210394_x_at	SSX4	12.1	Xp11.23
207281_x_at	VCX	9.5	Xp22
Nuclear proteins and transcription factors			
238825_at	ACRC	9.2	Xq13.1
202672_s_at	ATF3	3.4	1q32.3

219870_at	ATF7IP2	2.9	16p13.13
206588_at	DAZL	138.5	3p24.3
222793_at	DDX58	9.7	9p12
201694_s_at	EGR1	3.4	5q31.1
205249_at	EGR2	14.8	10q21.1
225645_at	EHF	8.1	11p12
228260_at	ELAVL2	4.0	9p21
210827_s_at	ELF3	3.7	1q32.2
203349_s_at	ETV5	4.7	3q28
209603_at	GATA3	5.2	10p15
208886_at	H1F0	3.4	22q13.1
214469_at	HIST1H2AE	9.0	6p22.2-p21.1
235456_at	HIST1H2BD	7.6	6p21.3
210387_at	HIST1H2BG	6.7	6p21.3
211597_s_at	HOP	13.8	4q11-q12
208937_s_at	ID1	4.2	20q11
207826_s_at	ID3	44.6	1p36.13-p36.12
219209_at	IFIH1	4.1	2p24.3-q24.3
202597_at	IRF6	7.3	1q32.3-q41
208436_s_at	IRF7	7.6	11p15.5
225798_at	JAZF1	2.7	7p15.2-p15.1
1555420_a_at	KLF7	2.7	2q32
236565_s_at	LARP6	5.2	15q23
221011_s_at	LBH	6.7	2p23.1
229475_at	MAEL	51.3	1q24.1
235457_at	MAML2	4.2	11q21
242794_at	MAML3	6.9	4q28
238430_x_at	MGC19764	3.2	17q12
224917_at	MIRN21	6.3	---
226066_at	MITF	2.4	3p14.2-p14.1
223484_at	NMES1	204.9	15q21.1
205552_s_at	OAS1	6.9	12q24.1
204972_at	OAS2	3.2	12q24.2

210797_s_at	OASL	3.8	12q24.2
218543_s_at	PARP12	9.4	7q34
224701_at	PARP14	10.5	3q21.1
223220_s_at	PARP9	12.5	3q13-q21
204082_at	PBX3	2.1	9q33-q34
209598_at	PNMA2	4.2	8p21.2
212636_at	QKI	3.1	6q26-27
223394_at	SERTAD1	3.4	19q13.1-q13.2
225123_at	SESN3	10.7	11q21
201416_at	SOX4	6.4	6p22.3
209762_x_at	SP110	5.6	2q37.1
209306_s_at	SWAP70	3.0	11p15
227279_at	TCEAL3	2.6	Xq22.2
212761_at	TCF7L2	4.5	10q25.3
203313_s_at	TGIF	4.4	18p11.3
228988_at	ZNF6	3.5	Xq21.1-q21.2
Apoptosis			
201012_at	ANXA1	10.9	9q12-q21.2|9q12-q21.2
210538_s_at	BIRC3	7.9	11q22
210026_s_at	CARD10	7.2	22q13.1
205483_s_at	G1P2	11.9	1p36.33
204415_at	G1P3	10.4	1p35
201631_s_at	IER3	4.4	6p21.3
202411_at	IFI27	9.3	14q32
221690_s_at	NALP2	20.4	19q13.42
237461_at	NALP7	7.2	19q13.42
228617_at	XAF1	26.2	17p13.1
Others			
1559336_at	---	18.4	---
211781_x_at	---	7.5	---
222184_at	---	3.1	---
225842_at	---	27.9	---
226725_at	---	6.1	---

227193_at	---	4.1	---
227290_at	---	2.8	---
227503_at	---	5.8	---
229968_at	---	17.0	---
230383_x_at	---	5.9	---
230499_at	---	3.7	---
230860_at	---	2.9	---
234250_at	---	5.1	---
235072_s_at	---	8.1	---
235276_at	---	18.0	---
236856_x_at	---	2.3	---
238725_at	---	2.6	---
240979_at	---	4.4	---
241262_at	---	2.3	---
241763_s_at	---	7.2	---
241898_at	---	2.7	---
212543_at	AIM1	11.3	6q21
203404_at	ARMCX2	17.9	Xq21.33-q22.2
225283_at	ARRDC4	4.7	15q26.3
212599_at	AUTS2	3.8	7q11.22
215440_s_at	BEXL1	10.2	Xq22.1-q22.3
212560_at	C11orf32	7.7	---
221260_s_at	C12orf22	2.9	12q13.11-q13.12
1559584_a_at	C16orf54	29.9	16p11.2
230000_at	C17orf27	2.6	17q25.3
229973_at	C1orf173	16.8	1p31.1
210785_s_at	C1orf38	2.8	1p35.3
238480_at	chromosome 18 open reading frame 50	5.5	---
207030_s_at	CSRP2	7.9	12q21.1
219313_at	DKFZp434C0328	7.9	3q13.31
226000_at	DKFZp547A023	8.3	1p13.2
224952_at	DKFZP564D166	2.9	17q23.3

225355_at	DKFZP761M1511	9.6	5q35.2
235085_at	DKFZp761P0423	5.7	8p23.1
203498_at	DSCR1L1	4.3	6p21.1-p12.3
235759_at	EFCBP1	4.2	8q21.3
227609_at	EPSTI1	6.9	13q13.3
227410_at	FAM43A	3.5	3q29
228745_at	FLJ13611	3.6	5q12.3
218986_s_at	FLJ20035	30.1	4q32.3
228423_at	FLJ21159	6.2	4q32.1
228152_s_at	FLJ31033	58.4	4q32.3
230012_at	FLJ34790	7.0	17p13.1
228937_at	FLJ38725	4.9	13q14.11
229559_at	FLJ40125	3.8	19q13.32
214453_s_at	IFI44	46.8	1p31.1
203153_at	IFIT1	66.9	10q25-q26
226757_at	IFIT2	13.9	10q23-q25
229450_at	IFIT3	21.2	10q24
203595_s_at	IFIT5	85.3	10q23.31
235048_at	KIAA0888	3.7	5q13.3
200897_s_at	KIAA0992	7.1	4q32.3
212906_at	KIAA1201	5.6	11q24.1
225525_at	KIAA1671	2.5	---
226702_at	LOC129607	26.4	2p25.2
241353_s_at	LOC202775	4.2	7q34
239624_at	LOC440885	27.2	2p11.1
224480_s_at	MGC11324	4.2	4q21.23
214696_at	MGC14376	2.8	17p13.3
227038_at	MGC26963	4.9	4q25
236595_at	MGC4677	19.7	2p11.2
207738_s_at	NCKAP1	3.3	2q32
229963_at	NGFRAP1L1	20.1	Xq22.1
205380_at	PDZK1	9.3	1q21
212094_at	PEG10	23.9	7q21

217996_at	PHLDA1	8.6	12q15
225688_s_at	PHLDB2	8.7	3q13.2
231131_at	RP1-32F7.2	49.9	Xq21.33
220167_s_at	TP53TG3	3.3	16p13
213293_s_at	TRIM22	11.9	11p15
227174_at	WDR72	7.0	15q21.3
224894_at	YAP1	3.7	11q13
219062_s_at	ZCCHC2	4.7	18q21.33

TABLE 2: Genes overexpressed in decitabine/TSA treated HMCLs. Five HMCLs were cultured with or without 0.5 μM decitabine for 7 days and with or without 0.33 μM TSA for the last 24 hours. Gene expression was profiled with Affymetrix U133 plus 2.0 microarray. Genes significantly differentially expressed between control and decitabine + TSA treated cells were identified using SAM supervised paired analysis with a 5% false discovery rate.

Gene Set	Ratio of protein in gene set	Number of protein in gene set	Protein from network	P value	FDR	Nodes
Interferon Signaling	0.0174	161	26	0	<1.00e-03	CD44,MX1,HLA-DQA1,HLA-DPA1,GBP2,GBP1,HLA-DRA,IFITM1,HLA-DRB1,IFITM2,OAS1,OAS2,HLA-DRB5,HLA-DPB1,DDX58,IFI27,OASL,USP18,EGR1,FLNB,HLA-DQB1,STAT1,IFIT3,IFIT2,IFIT1,IRF7
Cell adhesion molecules (CAMs)	0.0144	133	15	0	<1.67e-04	NRCAM,HLA-DQA1,HLA-DPA1,HLA-DRA,HLA-DRB1,ALCAM,HLA-DRB5,HLA-DPB1,HLA-DMB,SDC4,HLA-DMA,SDC2,HLA-DQB1,CDH1,CD6
Antigen processing and presentation	0.0082	76	11	0	<7.69e-05	HLA-DQA1,HLA-DPA1,HLA-DRA,HLA-DRB1,HSPA1A,HLA-DRB5,HLA-DPB1,HLA-DMB,HLA-DMA,HLA-DQB1,HSPA2
EGF receptor signaling pathway	0.0089	82	6	0.0004	4.44e-03	PIK3CD,CBLB,AREG,TGFA,STAT4,STAT1

TABLE 3: REACTOME analysis revealed that decitabine/TSA-regulated genes are significantly enriched in genes related to interferon signaling (P < 0.0001; FDR = 1E-3), cell adhesion molecules (P < 0.0001; FDR = 1.6E-4), antigen processing and presentation (P < 0.0001; FDR = 7.6E-5) and EGF receptor signaling (P = 0.0004; FDR = 4.3E-3) pathways.

Evaluation of the prognostic significance of HADMS score in two independent cohorts of patients.

Using maxstat analysis for overall survival, HADMS score was significantly associated with high-risk myeloma in the 2 independent patients' cohorts, HM and UAMS-TT2 (Figure 3A). Maxstat statistic test split the HM-patient cohort within 2 groups: a high-risk group of 23.7 % patients (HADMS score > - 21.57) with a 27 months median OS and a low risk group of 76.3% patients (HADMS score ≤ -21.57) with not reached median survival (*P* = 7E-33; Figure 3A). In the UAMS-TT2 cohort, a HADMSs score > -21.57 is associated with a high risk (*P* = 0.007; Figure 3A) in 20.8% of the patients. The HADMS score could also predict for event free survival (EFS). The high-risk group had a median EFS of 13 and 34 months in HM and UAMS-TT2 cohorts respectively and the low-risk group had a median EFS of 40 and 62 months (*P* = 8.3E-15 and 0.003 respectively; Figure 3B).

The prognostic value of the HADMS score was compared to usual prognostic factors (β2M, ISS, t(4;14) and del17p) and published GEP-based risk scores: UAMS-HRS (Shaughnessy, 2007), IFM score (Decaux, 2008), GPI (Hose, 2011), RS score (Reme, 2013), DM score (Moreaux, 2012) and HA score (Moreaux, 2013). In univariate COX analysis, all of these factors had prognostic value (Table 4). Compared two by two or all together in multivariate COX analysis, HADMS score and β2M remained independent in the HM cohort. In UAMS-TT2 cohort, when compared two by two, HADMS score tested with IFM score, t(4;14), del17p, GPI and DM score remained independent prognostic factors. When tested all together, UAMS-HRS, t(4;14), del17p and HA score remained independent (Table 4).

		HM Cohort		TT2 Cohort	
		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P*-value	Proportional hazard ratio	*P*-value
Univariate COX analysis – Overall survival	HADMS Score	31.87	<0.0001	1.73	0.008
	β2m	1.1	<0.0001	NA	NA
	ISS	1.84	0.002	NA	NA
	HRS	2.37	0.01	4.67	<0.0001
	IFM score	2.49	0.01	1.78	0.004
	t(4 ;14)	3.32	<0.0001	2.21	0.001
	del17p	3.44	0.02	2.46	<0.0001
	GPI	2.54	<0.0001	1.75	<0.0001
	RS	4.16	<0.0001	1.91	<0.0001
	DM Score	6.02	<0.0001	1.89	0.001
	HA Score	7.43	<0.0001	1.96	<0.0001

		HM Cohort		TT2 Cohort	
		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P*-value	Proportional hazard ratio	*P*-value
Multivariate COX analysis – Overall survival	HADMS Score	29.21	<0.0001	NA	NA
	ISS	1.42	NS	NA	NA
	HADMS Score	35.72	<0.0001	NA	NA
	β2m	1.1	<0.0001	NA	NA
	HADMS Score	31.01	<0.0001	1.07	NS
	HRS	1.66	NS	4.52	<0.0001
	HADMS Score	31.49	<0.0001	1.65	0.01
	IFM score	2.16	NS	1.70	0.008

	HADMS Score t(4 ;14)	29.98 1,33	<0.0001 .NS	1.63 2.11	0.01 0.001
	HADMS Score del17p	32.87 0.86	<0.0001 NS	1.63 2.31	0.01 0.001
	HADMS Score GPI	29.04 1.36	<0.0001 NS	1.53 1.66	0.04 0.001
	HADMS Score RS	23.85 1.64	<0.0001 NS	1.40 1.81	NS <0.0001
	HADMS Score DM Score	25.49 1.56	<0.0001 NS	1.50 1.72	0.05 0.007
	HADMS Score HA Score	25.52 1.49	<0.0001 NS	1.33 1.76	NS 0.006

		HM Cohort		TT2 Cohort	
		OAS		OAS	
	Pronostic variable	Proportional hazard ratio	*P*-value	Proportional hazard ratio	*P*-value
Multivariate COX analysis – Overall survival	HADMS Score	32.90	<0.0001	0.73	NS
	β2m	1.1	<0.0001	NA	NA
	ISS	1.1	NS	NA	NA
	HRS	1.12	NS	3.75	<0.0001
	IFM score	1.1	NS	0.88	NS
	t(4 ;14)	1.42	NS	2.05	.004
	del17p	0.44	NS	2.31	.001
	GPI	0.73	NS	1.20	NS

	RS	1.44	NS	0.98	NS
	DM	1.1	NS	1.20	NS
	Score				
	HA Score	0.71	NS	1.62	0.02

Table 4: Cox univariate and multivariate analysis of OS in HM and TT2 patients' cohorts.

The prognostic factors were tested as single variable or multi variables using Cox-model. P-values and the hazard ratios (HR) are shown. NS, Not significant at a 5% threshold; GPI, gene expression based proliferation index; ISS, International Staging System; HRS, high-risk score; IFM, Intergroupe Francophone du Myélome; DM score, DNA Methylation score, HA score, Histone Acetylation score, Serum concentration of β2m and albumin are not available for UAMS TT2 patients. NA, Not available.

HADMS score is predictive of myeloma cell sensitivity to DNMTi and HDACi combination.

The efficacy of HADMS score to predict sensitivity of myeloma cells sensitivity to DNMTi and HDACi combination treatment was investigated using primary MMC of patients co-cultured with their bone marrow microenvironment *in vitro* (Mahtouk, 2004; Moreaux, 2013; Moreaux, 2012; Moreaux, 2013). MMC of patients with a high HADMS score (n = 5) were significantly more sensitive (3.4 fold) to decitabine and TSA combination than MMC of patients with a low HADMS score (n = 5) (Figure 4A). The inventors confirmed these results using another DNMTi and HDACi association. Primary MMCs of patients with a high HADMS score (n = 5) exhibited a significant 1.7 higher sensitivity to clinical grade inhibitors 5-azacitidine/SAHA combination than MMC of patients with a low HADMS score (n = 7) (Figure 4B).

MMC of patients with low HADMS score value are characterized by mature BMPC gene signature whereas patients with high HADMS score have a proliferating plasmablastic gene signature.

In order to identify if different gene signatures could be identified comparing high HADMS score and low HADMS score groups, the inventors performed a GSEA analysis. MMC of patients with a low HADMS score displayed a significant enrichment in genes associated with normal mature BMPCs (gene set: ZHAN MULTIPLE MYELOMA DN, P =

0.01, Table 5) and bone microenvironment dependence (gene sets: VILIMAS NOTCH1 TARGETS UP, ZHENG IL22 SIGNALING UP, AMIT EGF RESPONSE 120 HELA and RUTELLA RESPONSE TO HGF, $P < 0.02$, Tables 6, 7, 8 and 9). At the opposite, MMCs of patients with a high HADMS score exhibited a significant enrichment in genes associated with proliferating plasmablastic progenitors (gene sets: MOREAUX MULTIPLE MYELOMA BY TACI DN, WHITFIELD CELL CYCLE S, $P < 0.01$, Tables 10 and 11), IFN regulated genes (gene sets: REACTOME INTERFERON ALPHA BETA SIGNALING, RADAEVA RESPONSE TO IFNA1 UP and DER IFN BETA RESPONSE UP, $P < 0.01$, Table 12, 13 and 14) and transcription (gene set: REACTOME TRANSCRIPTION, $P < 0.0001$, Table 15). Investigating the HADMS score in normal plasma cell differentiation, HADMS score value was significantly higher in preplasmablasts (PrePB, $P = 0.05$) and plasmablasts (PB, $P = 0.01$) compared to memory B (MB) cells (Figure 5). Early plasma cells have the highest score ($P < 0.001$) and the HADMS score decreased drastically to the lowest value in mature BMPC ($P < 0.001$) (Figure 5).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
PYGL	PYGL	0.3619278371334076	0.07115942	Yes
ITGB2	ITGB2	0.3106897175312042	0.12719311	Yes
S100A9	S100A9	0.2768969237804413	0.1726828	Yes
S100A12	S100A12	0.25916585326194763	0.2160441	Yes
WNT10B	WNT10B	0.25076597929000854	0.26003012	Yes
LST1	LST1	0.24418431520462036	0.30363056	Yes
AIF1	AIF1	0.23883675038814545	0.34571996	Yes
CXCL12	CXCL12	0.23033247888088226	0.38202515	Yes
CEBPD	CEBPD	0.22803567349910736	0.42358118	Yes
PRKAR2B	PRKAR2B	0.21592438220977783	0.4545348	Yes
LYZ	LYZ	0.20603586733341217	0.48262537	Yes
CD24	CD24	0.2056848704814911	0.5227389	Yes
DPYSL2	DPYSL2	0.15517657995224	0.4734923	Yes
IGF2BP3	IGF2BP3	0.15381208062171936	0.5013209	Yes
LCN2	LCN2	0.15194584429264069	0.52604353	Yes
ALDH1A1	ALDH1A1	0.1436985582113266	0.53681916	Yes

HNMT	HNMT	0.1416354924440384	0.5599644	Yes
A2M	A2M	0.13225819170475006	0.56392044	Yes
CTSH	CTSH	0.13221798837184906	0.5897713	Yes
APOC1	APOC1	0.13114511966705322	0.6124445	Yes
PF4	PF4	0.12961214780807495	0.63504356	Yes
PLA2G7	PLA2G7	0.12095429003238678	0.6436146	Yes
PF4V1	PF4V1	0.1105586364865303	0.6471691	Yes
APOE	APOE	0.10622140765190125	0.65785354	Yes
VCAM1	VCAM1	0.09820647537708282	0.65988433	Yes

Table 5: Genes set enrichment analysis revealed a significant overrepresentation of the ZHAN MULTIPLE MYELOMA DN set in low HADMS score patients compared to high HADMS score patients (P=0.01).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
GATA3	GATA3	0.30586645007133484	0.0816429	Yes
RRAS2	RRAS2	0.28479474782943726	0.15764606	Yes
CD74	CD74	0.26544249057769775	0.22896256	Yes
DTX1	DTX1	0.26188379526138306	0.30266726	Yes
BCL2A1	BCL2A1	0.24331431090831757	0.3600639	Yes
THY1	THY1	0.24279563128948212	0.4300697	Yes
CD80	CD80	0.2079179733991623	0.45193675	Yes
LCK	LCK	0.18203873932361603	0.46791258	Yes
BIRC3	BIRC3	0.17756520211696625	0.51153034	Yes
CCR7	CCR7	0.17335079610347748	0.5546125	Yes
GZMA	GZMA	0.17217615246772766	0.60259527	Yes

Table 6: Genes set enrichment analysis revealed a significant overrepresentation of the VILIMAS NOTCH1 TARGETS UP set in low HADMS score patients compared to high HADMS score patients (P=0.007).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
CFD	CFD	0.3332250714302063	0.08130651	Yes
ARG1	ARG1	0.3094353675842285	0.15615763	Yes
PTX3	PTX3	0.2970482110977173	0.22765689	Yes
S100A9	S100A9	0.2768969237804413	0.29247943	Yes
HP	HP	0.24530348181724548	0.33726114	Yes
OLR1	OLR1	0.23671993613243103	0.3889897	Yes
RTN1	RTN1	0.222052663564682	0.42836308	Yes
CD14	CD14	0.21896854043006897	0.48130947	Yes
S100A8	S100A8	0.19915771484375	0.50761944	Yes
TTC9	TTC9	0.19320820271968842	0.54746306	Yes
ARRDC4	ARRDC4	0.15338373184204102	0.5182652	Yes
STXBP5L	STXBP5L	0.15230797231197357	0.55394405	Yes
SLC25A30	SLC25A30	0.14586560428142548	0.57820314	Yes
PF4	PF4	0.12961214780807495	0.5708005	Yes
CXCL6	CXCL6	0.12492252886295319	0.5925112	Yes

Table 7: Genes set enrichment analysis revealed a significant overrepresentation of the ZHENG IL22 SIGNALING UP set in low HADMS score patients compared to high HADMS score patients (P=0.007).

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PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
CYP1B1	CYP1B1	0.34851354360580444	0.14713836	Yes
PLAUR	PLAUR	0.24194176495075226	0.21557675	Yes
IL8	IL8	0.23468290269374847	0.30890372	Yes
CHST3	CHST3	0.22654478251934052	0.39625484	Yes
TGFA	TGFA	0.1808186024427414	0.41380748	Yes
IRS2	IRS2	0.1508953720331192	0.4240509	Yes
PHLDA1	PHLDA1	0.14758902788162231	0.48134747	Yes
SAT1	SAT1	0.14673903584480286	0.5423769	Yes

ANKRD57	ANKRD57	0.14143554866313934	0.58999395	Yes

Table 8: Genes set enrichment analysis revealed a significant overrepresentation of the AMIT EGF RESPONSE 120 HELA set in low HADMS score patients compared to high HADMS score patients (P=0.01).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
C5AR1	C5AR1	0.358140766620636	0.02953049	Yes
CFD	CFD	0.3332250714302063	0.055799164	Yes
FPR1	FPR1	0.3005441427230835	0.0749054	Yes
SLC2A3	SLC2A3	0.2995496690273285	0.100186415	Yes
S100A9	S100A9	0.2768969237804413	0.11799232	Yes
FYB	FYB	0.27504757046699524	0.14074183	Yes
FCN1	FCN1	0.2612757384777069	0.15699755	Yes
S100A12	S100A12	0.25916585326194763	0.17771128	Yes
SORL1	SORL1	0.2563846707344055	0.19865389	Yes
FNBP1	FNBP1	0.24261964857578278	0.20754002	Yes
AIF1	AIF1	0.23883675038814545	0.22468361	Yes
LMO2	LMO2	0.23302534222602844	0.23762788	Yes
MS4A6A	MS4A6A	0.22917360067367554	0.25279692	Yes
CEBPD	CEBPD	0.22803567349910736	0.27041975	Yes
CD14	CD14	0.21896854043006897	0.28078684	Yes
ADAM19	ADAM19	0.20949435234069824	0.28734094	Yes
SIGIRR	SIGIRR	0.19608718156814575	0.28557757	Yes
DAPP1	DAPP1	0.1886170208454132	0.29036966	Yes
TCF7L2	TCF7L2	0.18315166234970093	0.29771394	Yes
NDE1	NDE1	0.17771971225738525	0.30320895	Yes
NCOA1	NCOA1	0.1746505349874496 5	0.3126174	Yes
F2RL1	F2RL1	0.17231358587741852	0.32391486	Yes
CDKN1C	CDKN1C	0.16997265815734863	0.33455113	Yes
SPTLC2	SPTLC2	0.16576433181762695	0.341587	Yes

KLF10	KLF10	0.16473393142223358	0.3545628	Yes
CFP	CFP	0.16449259221553802	0.36775002	Yes
LRRK1	LRRK1	0.16356340050697327	0.3808588	Yes
TREM1	TREM1	0.15977086126804352	0.38831607	Yes
DPYSL2	DPYSL2	0.15517657995224	0.3916767	Yes
NR4A2	NR4A2	0.1533132642507553	0.39974797	Yes
NRG1	NRG1	0.14798858761787415	0.40157476	Yes
LTB4R	LTB4R	0.1447547972202301	0.4066057	Yes
SLC16A5	SLC16A5	0.13600145280361176	0.39768505	Yes
CHPT1	CHPT1	0.13519573211669922	0.40608168	Yes
LILRB2	LILRB2	0.13153131306171417	0.40767854	Yes
MAFB	MAFB	0.12620945274829865	0.40674004	Yes
SEPT9	SEPT9	0.12541623413562775	0.41570213	Yes
MAP4K4	MAP4K4	0.12064750492572784	0.41823488	Yes
RASGRP2	RASGRP2	0.12002833187580109	0.42766947	Yes
CD163	CD163	0.11745651066303253	0.43340993	Yes
ACPP	ACPP	0.11506164073944092	0.4387165	Yes
FCER1A	FCER1A	0.11289910972118378	0.44523138	Yes
ZNF395	ZNF395	0.11033166944980621	0.45037052	Yes
HLA-DQA1	HLA-DQA1	0.10554853826761246	0.44838372	Yes
PLEKHA5	PLEKHA5	0.10223544389009476	0.44889894	Yes
DPEP2	DPEP2	0.10112230479717255	0.4558107	Yes

Table 9: Genes set enrichment analysis revealed a significant overrepresentation of the RUTELLA RESPONSE TO HGF set in low HADMS score patients compared to high HADMS score patients (P=0.01).

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PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
MATR3	MATR3	-0.07275018095970154	-0.6754449	Yes
NUDCD2	NUDCD2	-0.08143609762191772	-0.6673385	Yes

NASP	NASP	-0.09491787105798721	-0.6665999	Yes
PAPD4	PAPD4	-0.10570180416107178	-0.6483204	Yes
RNF111	RNF111	-0.1149410605430603	-0.62537616	Yes
DDX31	DDX31	-0.12075252830982208	-0.5895196	Yes
ZNF567	ZNF567	-0.1243547722697258	-0.5481924	Yes
FLJ39632	FLJ39632	-0.13411782681941986	-0.51337713	Yes
DENND4A	DENND4A	-0.1658760905265808	-0.48896423	Yes
IREB2	IREB2	-0.17003612220287323	-0.4272409	Yes
MET	MET	-0.17687758803367615	-0.3654088	Yes
MYLIP	MYLIP	-0.1833156794309616	-0.30020893	Yes
TYMS	TYMS	-0.19542764127254486	-0.23516361	Yes
MCM2	MCM2	-0.20087213814258575	-0.16144156	Yes
CHEK1	CHEK1	-0.23729786276817322	-0.08723888	Yes
PAPOLA	PAPOLA	-0.26489123702049255	0.007963604	Yes

Table 10: Genes set enrichment analysis revealed a significant overrepresentation of the MOREAUX MULTIPLE MYELOMA BY TACI DN set in high HADMS score patients compared to low HADMS score patients (P<0.0001).

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PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
HIST1H2BC	HIST1H2BC	-0.12150858342647552	-0.60519737	Yes
PILRB	PILRB	-0.12402009218931198	-0.58559793	Yes
MAN1A2	MAN1A2	-0.1417795717716217	-0.5867501	Yes
ATAD2	ATAD2	-0.14950694143772125	-0.56747854	Yes
ESCO2	ESCO2	-0.1507851630449295	-0.54134536	Yes
PHIP	PHIP	-0.1666911095380783	-0.52446014	Yes
RRM2	RRM2	-0.18613113462924957	-0.5047358	Yes
TYMS	TYMS	-0.19542764127254486	-0.47409338	Yes
CPNE8	CPNE8	-0.19561581313610077	-0.43657154	Yes
HIST3H2A	HIST3H2A	-0.19778208434581757	-0.39954406	Yes
HELLS	HELLS	-0.20591014623641968	-0.3639134	Yes

BRIP1	BRIP1	-0.2078172266483307	-0.3244931	Yes
HIST1H4C	HIST1H4C	-0.21614468097686768	-0.28734356	Yes
UBE2T	UBE2T	-0.22249646484851837	-0.24714345	Yes
TOP2A	TOP2A	-0.23342227935791016	-0.20506296	Yes
IFIT1	IFIT1	-0.3525208830833435	-0.15163203	Yes
HIST1H2AM	HIST1H2AM	-0.3690810799598694	-0.080634885	Yes
HIST1H4H	HIST1H4H	-0.4202048182487488	2.0023435E-8	Yes

Table 11: Genes set enrichment analysis revealed a significant overrepresentation of the WHITFIELD CELL CYCLE S set in high HADMS score patients compared to low HADMS score patients (P=0.002).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
OAS3	OAS3	-0.22055576741695404	-0.7068872	Yes
USP18	USP18	-0.26444268226623535	-0.63302946	Yes
IFIT3	IFIT3	-0.2971442639827728	-0.5408215	Yes
IFI27	IFI27	-0.30257943272590637	-0.4429843	Yes
OAS1	OAS1	-0.3027102053165436	-0.34465006	Yes
IFI6	IFI6	-0.3176177442073822	-0.24238227	Yes
IFIT1	IFIT1	-0.3525208830833435	-0.12832178	Yes
ISG15	ISG15	-0.39782193303108215	4.545678E-4	Yes

Table 12: Genes set enrichment analysis revealed a significant overrepresentation of the REACTOME INTERFERON ALPHA BETA SIGNALING set in high HADMS score patients compared to low HADMS score patients (P=0.002).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
IFI44L	IFI44L	-0.24639743566513062	-0.68106884	Yes
IFI44	IFI44	-0.2476646453142166	-0.60571516	Yes

IFI27	IFI27	-0.30257943272590637	-0.52133197	Yes
OAS1	OAS1	-0.3027102053165436	-0.4289525	Yes
IFI6	IFI6	-0.3176177442073822	-0.33293292	Yes
RSAD2	RSAD2	-0.3443400263786316	-0.2280764	Yes
IFIT1	IFIT1	-0.3525208830833435	-0.12049597	Yes
ISG15	ISG15	-0.39782193303108215	4.5454444E-4	Yes

Table 13: Genes set enrichment analysis revealed a significant overrepresentation of the RADAEVA RESPONSE TO IFNA1 UP set in high HADMS score patients compared to low HADMS score patients (P=0.004).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
TEAD1	TEAD1	-0.1300404965877533	-0.5568645	Yes
PLOD2	PLOD2	-0.13836008310317993	-0.5350312	Yes
MAP1B	MAP1B	-0.13904088735580444	-0.503463	Yes
TRIM22	TRIM22	-0.16718308627605438	-0.49792445	Yes
HIF1A	HIF1A	-0.2176475077867508	-0.4826493	Yes
B2M	B2M	-0.23652824759483337	-0.4340078	Yes
IFI44	IFI44	-0.2476646453142166	-0.37934482	Yes
IFIT3	IFIT3	-0.2971442639827728	-0.3172273	Yes
OAS1	OAS1	-0.3027102053165436	-0.24719761	Yes
IFI6	IFI6	-0.3176177442073822	-0.1739135	Yes
IFIT1	IFIT1	-0.3525208830833435	-0.09201994	Yes
ISG15	ISG15	-0.39782193303108215	4.560307E-4	Yes

Table 14: Genes set enrichment analysis revealed a significant overrepresentation of the DER IFN BETA RESPONSE UP set in high HADMS score patients compared to low HADMS score patients (P<0.0001).

PROBE	GENE SYMBOL	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
HIST1H2AE	HIST1H2AE	-0.11173597723245621	-0.6660398	Yes
HIST1H2BC	HIST1H2BC	-0.12150858342647552	-0.63995767	Yes
POU2F1	POU2F1	-0.131466805934906	-0.6092625	Yes
POLR3B	POLR3B	-0.14802579581737518	-0.5762758	Yes
HIST1H2AD	HIST1H2AD	-0.19305670261383057	-0.547694	Yes
HIST1H3D	HIST1H3D	-0.1993476301431656	-0.47697288	Yes
HIST1H4C	HIST1H4C	-0.21614468097686768	-0.40569103	Yes
HIST2H2BE	HIST2H2BE	-0.2228573113679886	-0.32577235	Yes
HIST1H3H	HIST1H3H	-0.2398541271686554	-0.24203466	Yes
PAPOLA	PAPOLA	-0.26489123702049255	-0.14853144	Yes
HIST1H4H	HIST1H4H	-0.4202048182487488	1.2529199E-8	Yes

Table 15: Genes set enrichment analysis revealed a significant overrepresentation of the REACTOME TRANSCRIPTION set in high HADMS score patients compared to low HADMS score patients (P<0.0001).

Discussion

In this study, the inventors reported a GEP-based HADMS score that allows identification of high-risk patients associated with MMC's higher sensitivity to HDACi/DNMTi combination *in vitro*. Since HDACi/DNMTi combination was well tolerated (Bots, 2009), shown promising activity in cancers including haematological malignancies (Bots, 2009; Fandy, 2009; Zhang, 2009; Juergens, 2011) and have potential therapeutic value in MM (Matthews, 2013), the HADMS score could enable the identification of MM patients who could benefit from this treatment.

Among the 375 genes deregulated by decitabine and TSA in myeloma cell lines, 48 genes were also found to be deregulated after TSA treatment (Moreaux, 2013). 16 genes were communally deregulated by decitabine (Moreaux, 2012) and decitabine/TSA treatment. The inventors identified an overlap of 5 genes whose expression was affected by decitabine, TSA or decitabine/TSA (Tables 16, 17 and 18). Mainly deregulation of IFN-regulated genes was shared between decitabine and decitabine/TSA combined treatment (Moreaux, 2012). 85 genes were identified in common in our study and the study of Heller *et al* (Table 19). Thus,

80% of the decitabine/TSA combination deregulated genes were not found to be impacted by decitabine or TSA treatment alone in MMC. Cooperation between histone modifications and DNA methylation is important for the establishment of global epigenetic patterns as well as loci-specific gene regulation (Cedar, 2009). This crosstalk can be mediated by biochemical interactions between SET domain histone methyltransferases and DNA methyltransferases (Cedar, 2009). Interestingly, HADMS score is significantly upregulated in the t(4; 14) subgroup characterized by the overexpression of the SET domain histone methyltransferase MMSET (Figure 6).

The 96 genes, building HADMS score, include 42 genes associated with a bad prognosis and 54 associated with a good prognosis (Figure 1). Among these genes, some of them could highlight pathways involved in MM biology and sensitivity to DNMTi/HDACi combination. Since a significant enrichment in genes associated with proliferation was identified in MMC of patients with high HADMS score value, the higher sensitivity of high HADMS score patients to DNMTi/HDACi combination could be explained by the fact that incorporation of DNMTi into DNA is restricted to cell cycling cells (Hollenbach, 2010). Furthermore, HDACi have been shown to induce G1 cell cycle arrest through dephosphorylation of retinoblastoma protein and increase expression of p53 and p21 (Lavelle, 2001; Mitsiades, 2003; Neri, 2012). Using methylation-specific PCR, several studies have identified hypermethylation of tumor suppressor genes including cyclin dependent kinases inhibitors (CDKI, p15 and p16) and p14 (Braggio, 2010; Takada, 2005; Mateos, 2002). The inventors reported DNMTi/HDACi treatment induced p21 and p57 CDKI expression in MMC (Table A) and their expression is associated with a good prognosis in MM patients (Table A). The inventors identified also an induction of Cdc14b expression, another good prognostic gene. Cdc14b has been proposed to play multiple functions during the cell cycle (Mocciaro, 2010). In yeast, Cdc14 protein phosphatase is essential for the inactivation of mitotic CDK and mitotic exit (Mocciaro, 2010; Wei, 2011). Cells of Cdc14b-deficient mice displayed proliferative defects and increased senescence both *in vitro* and *in vivo* (Wei, 2011). More recently, it was reported that the lack of Cdc14b results in a significant increased transcription of cell cycle specific genes including A and B-type cyclins. At the opposite, ectopic expression of Cdc14b results in a significant repression of cell cycle genes (Guillamot, 2011). Among the good prognostic genes induced by these epigenetic drugs, the inventors identify EGF negative regulator (ERRFI1 also known as Mig-6). ERRFI1 deletion in mice has been reported to activate EGFR and sustain MAPK signaling, resulting in tumor development (Zhang, 2007; Anastasi, 2007; Ferby, 2006). ERRFI1 deletion, mutation or downregulation

have been frequently identified in glioblastoma, lung and breast cancers (Anastasi, 2005; Ichimura, 2008; Ying, 2010). In glioblastoma, ERRFI1 overexpression was shown to decrease proliferation, the binding of EGFR with STX8 and drive internalized EGFR to endosomes for degradation. In contrast, ERRFI1 depletion resulted in increased tumor invasion (Ying, 2010). Furthermore, a recent study demonstrated that ERRFI1 expression is upregulated during the senescence process (Xie, 2013). The inventors have previously demonstrated that the EGF/EGF-receptor family is involved in the biology of MM (Mahtouk, 2006; Mahtouk, 2005; Mahtouk, 2004) acting as myeloma cell growth factors. A pan-ErbB inhibitor induced strong apoptosis of MMC co-cultured with their bone marrow microenvironment *in vitro* and combination with dexamethasone or anti-IL-6 antibody demonstrated additive effects (Mahtouk, 2004). Thus, DNMTi/HDACi combination could be useful to induce the expression of major tumor suppressor genes in MMC.

According to the proliferation gene signature, high HADMS score patients are characterized by an overexpression of genes related to transcription including histone cluster genes (Table 15). Core histone proteins must be synthetized rapidly during the brief S-phase when a cell is dividing (Harris, 1991). As a result, the histone mRNAs are highly cell-cycle regulated, increasing 35-fold as cell enter S-phase and decreasing again at the end of S-phase (Harris, 1991). Al together, these data could clarify why high HADMS score patients, distinguished by an active growth, can be efficiently targeted by the upregulation of HDACi/DNMTi targeted genes and especially the 54 with a good prognostic value.

At the opposite, MMC of patients with a low HADMS score could be in a more quiescent stage. GSEA analysis revealed that MMC of patients with a low HADMS score showed a signature resembling mature BMPC associated with bone marrow microenvironment dependence underlined by a significant enrichment in intercellular communication signal pathways (Tables 5 to 9). In contrast, MMC of patients with a high HADMS score are characterized by a signature sharing similarities with les differentiated proliferating plasmablastic progenitors (Tables 10 to 15). Recently, it was described, within the bone marrow of MM patients, the existence of a progenitor organization recapitulating the different maturation stages of plasma cell differentiation (Jourdan, 2011; Jourdan, 2009) and associated with proteasome inhibitor resistance (Leung-Hagesteijn, 2013; Chaidos, 2013). MMC progenitors including B cells and preplasmablasts were found to survive to proteasome inhibitors and to be significantly enriched in myeloma patients refractory to bortezomib treatment. These Xbp1s negative preblasmablastic cells are characterized by a diminished endoplasmic reticulum (ER) stress and thus resistance to proteasome inhibitors since they

have not committed to high Ig production (Leung-Hagesteijn, 2013; Orlowski, 2013). Furthermore, plasmablastic progenitors have been described to overexpress epigenetic regulators, compared to mature plasma cells, suggesting that MMC transitions in plasma cell differentiation stages could be linked to epigenetic plasticity (Chaidos, 2013). According to the GSEA results, HADMS score was significantly higher in preplasmablasts, plasmablasts and early plasma cells compared to normal mature BMPC (Figure 5). Thus, HDACi/DNMTi combined treatment could have a therapeutic interest to target tumor progenitors that contribute to treatment failure in MM.

Recent clinical trials suggested promising activity of HDACi/DNMTi combination in MDS, AML (Bots, 2009; Fandy, 2009; Zhang, 2009) and refractory advanced non-small cell lung cancer (Juergens, 2011). In MM, clinical trials evaluating DNMTi or HDACi are ongoing and their combination resulted in a significant results in Vk*MYC transgenic MM mouse model (Matthews, 2013). In the current study, the invetors reported a new score to predict the MM cell sensitivity to DNMTi and HDACi combination that could be useful identifying patients who could benefit from combination of epigenetic therapies.

UNIQID	Gene	Banding
200696_s_at	GSN	9q33
201243_s_at	ATP1B1	1q24
201631_s_at	IER3	6p21.3
202086_at	MX1	21q22.3
202411_at	IFI27	14q32
203964_at	NMI	2p24.3-q21.3
204141_at	TUBB2	6p25
205483_s_at	G1P2	1p36.33
205552_s_at	OAS1	12q24.1
209122_at	ADFP	9p22.1
209969_s_at	STAT1	2q32.2
210387_at	HIST1H2BG	6p21.3
210437_at	MAGEA9	Xq28
211990_at	HLA-DPA1	6p21.3
218543_s_at	PARP12	7q34
223484_at	NMES1	15q21.1

224917_at	MIRN21	---
227609_at	EPSTI1	13q13.3
230000_at	C17orf27	17q25.3
235700_at	CT45-2	Xq26.3
238825_at	ACRC	Xq13.1

Table 16: Genes communally overexpressed in decitabine and decitabine/TSA treated HMCLs

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UNIQID	Gene	Banding
200696_s_at	GSN	9q33
201012_at	ANXA1	9q12-q21.2|9q12-q21.2
201137_s_at	HLA-DPB1	6p21.3
202838_at	FUCA1	1p34
203355_s_at	PSD3	8pter-p23.3
203413_at	NELL2	12q13.11-q13.12
203695_s_at	DFNA5	7p15
203854_at	IF	4q25
204415_at	G1P3	1p35
204563_at	SELL	1q23-q25
205249_at	EGR2	10q21.1
205352_at	SERPINI1	3q26.1
205552_s_at	OAS1	12q24.1
206310_at	SPINK2	4q12
206385_s_at	ANK3	10q21
208894_at	HLA-DRA	6p21.3
209198_s_at	SYT11	1q21.2
209462_at	APLP1	19q13.1
209848_s_at	SILV	12q13-q14
209906_at	C3AR1	12p13.31
209969_s_at	STAT1	2q32.2
210432_s_at	SCN3A	2q24

210538_s_at	BIRC3	11q22
211685_s_at	NCALD	8q22-q23
211990_at	HLA-DPA1	6p21.3
212464_s_at	FN1	2q34
212636_at	QKI	6q26-27
212998_x_at	HLA-DQB1	6p21.3
213106_at	ATP8A1	4p14-p12
213317_at	CLIC5	6p12.1-21.1
213361_at	TDRD7	9q22.33
214079_at	DHRS2	14q11.2
215193_x_at	HLA-DRB1	6p21.3
216331_at	ITGA7	12q13
218501_at	ARHGEF3	3p21-p13
218678_at	NES	1q23.1
219209_at	IFIH1	2p24.3-q24.3
223218_s_at	NFKBIZ	3p12-q12
223484_at	NMES1	15q21.1
224701_at	PARP14	3q21.1
225123_at	SESN3	11q21
225688_s_at	PHLDB2	3q13.2
225842_at	---	---
226269_at	GDAP1	8q21.11
226281_at	DNER	2q36.3
226725_at	---	---
228152_s_at	FLJ31033	4q32.3
228260_at	ELAVL2	9p21
228726_at	SERPINB1	6p25
229973_at	C1orf173	1p31.1
230233_at	RASGEF1B	4q21.3
238430_x_at	MGC19764	17q12
34408_at	RTN2	19q13.32

Table 17: Genes communally overexpressed in TSA and decitabine/TSA treated HMCLs

UNIQID	Gene	Banding
200696_s_at	GSN	9q33
205552_s_at	OAS1	12q24.1
209969_s_at	STAT1	2q32.2
211990_at	HLA-DPA1	6p21.3
223484_at	NMES1	15q21.1

5

Table 18: Genes communally overexpressed in TSA, decitabine and decitabine/TSA treated HMCLs

UNIQID	Gene	Banding
200696_s_at	GSN	9q33
200799_at	HSPA1A	6p21.3
200878_at	EPAS1	2p21-p16
201005_at	CD9	12p13.3
201041_s_at	DUSP1	5q34
201131_s_at	CDH1	16q22.1
201137_s_at	HLA-DPB1	6p21.3
201243_s_at	ATP1B1	1q24
201416_at	SOX4	6p22.3
201427_s_at	SEPP1	5q31
201596_x_at	KRT18	12q13
201631_s_at	IER3	6p21.3
201694_s_at	EGR1	5q31.1
201939_at	PLK2	5q12.1-q13.2
201952_at	ALCAM	3q13.1
202071_at	SDC4	20q12
202219_at	SLC6A8	Xq28

202252_at	RAB13	1q21.2
202283_at	SERPINF1	17p13.1
202284_s_at	CDKN1A	6p21.2
202411_at	IFI27	14q32
202436_s_at	CYP1B1	2p21
202668_at	EFNB2	13q33
202838_at	FUCA1	1p34
203130_s_at	KIF5C	2q23.1
203153_at	IFIT1	10q25-q26
203186_s_at	S100A4	1q21
203404_at	ARMCX2	Xq21.33-q22.2
203680_at	PRKAR2B	7q22
203879_at	PIK3CD	1p36.2
204141_at	TUBB2	6p25
204415_at	G1P3	1p35
205239_at	AREG	4q13-q21
205249_at	EGR2	10q21.1
205352_at	SERPINI1	3q26.1
205569_at	LAMP3	3q26.3-q27
206310_at	SPINK2	4q12
206588_at	DAZL	3p24.3
207030_s_at	CSRP2	12q21.1
208614_s_at	FLNB	3p14.3
208886_at	H1F0	22q13.1
208937_s_at	ID1	20q11
209160_at	AKR1C3	10p15-p14
209198_s_at	SYT11	1q21.2
209306_s_at	SWAP70	11p15
209392_at	ENPP2	8q24.1
209459_s_at	ABAT	16p13.2
209462_at	APLP1	19q13.1
209603_at	GATA3	10p15
209848_s_at	SILV	12q13-q14

209969_s_at	STAT1	2q32.2
209993_at	ABCB1	7q21.1
210074_at	CTSL2	9q22.2
210095_s_at	IGFBP3	7p13-p12
210387_at	HIST1H2BG	6p21.3
210437_at	MAGEA9	Xq28
210538_s_at	BIRC3	11q22
210592_s_at	SAT	Xp22.1
210986_s_at	TPM1	15q22.1
211404_s_at	APLP2	11q23-q25|11q24
211538_s_at	HSPA2	14q24.1
211990_at	HLA-DPA1	6p21.3
212094_at	PEG10	7q21
212464_s_at	FN1	2q34
212473_s_at	MICAL2	11p15.3
212561_at	RAB6IP1	11p15.4
213348_at	CDKN1C	11p15.5
213800_at	CFH	1q32
214079_at	DHRS2	14q11.2
214440_at	NAT1	8p23.1-p21.3
214469_at	HIST1H2AE	6p22.2-p21.1
214696_at	MGC14376	17p13.3
215193_x_at	HLA-DRB1	6p21.3
215440_s_at	BEXL1	Xq22.1-q22.3
216331_at	ITGA7	12q13
216370_s_at	TKTL1	Xq28
216379_x_at	CD24	6q21
217767_at	C3	19p13.3-p13.2
219403_s_at	HPSE	4q21.3
219870_at	ATF7IP2	16p13.13
221555_x_at	CDC14B	9q22.33
224583_at	COTL1	16q24.1
227530_at	AKAP12	6q24-q25

228423_at	FLJ21159	4q32.1
34408_at	RTN2	19q13.32

Table 19: Genes overexpressed in decitabine/TSA treated HMCLs in the study conducted by Heller G et al. and the current study

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method of testing whether a patient suffering from multiple myeloma will respond or not to a combination treatment consisting of at least one histone deacetylase inhibitor (HDACi) with at least one DNA methyltransferase inhibitor (DNMTi) comprising:

i) determining the expression level (ELi) of several genes G_1-G_n selected from table A in a biological sample obtained from said patient

ii) comparing the expression level (ELi) determined at step i) with a predetermined reference level (ELRi)

iii) calculating the HADMS score trough the following formula

$$HADMS = \sum_{i=1}^{n} \beta i \times Ci$$

wherein βi represent the regression β coefficient reference value for the gene G_i and Ci = 1 if the expression of the gene G_i (ELi) is higher than the predetermined reference level (ELRi) or Ci = -1 if the expression of the gene (ELi) is lower than or equal to the predetermined reference level (ELRi)

iv) comparing the score HADMS determined at step iii) with a predetermined reference value $HADMS_R$

v) and concluding that the patient will respond to the combination treatment when the HADMS score is higher than the predetermined reference value $HADMS_R$ or concluding that the patient will not respond to the combination treatment when the HADMS score is lower than the predetermined reference value $HADMS_R$.

2. The method of testing whether a patient suffering from multiple myeloma will respond or not to a combination treatment consisting of at least one histone deacetylase inhibitor (HDACi) with at least one DNA methyltransferase inhibitor (DNMTi) according to claim 1 wherein the step i) comprises determining the expression levels (ELi) of 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95 or 96 genes from Table A are determined wherein every combinations of genes comprises a minimal set of 42 genes consisting of: EPAS1, ATP1B1, TJP2, RAB13, IFI27, PLSCR1, CYP1B1, SLC2A3, IFIT1, SCHIP1, PDZK1, DDR1, HLA-DRA, SERPINB9, SP110, SSX4, C1orf38, FN1, MXRA7, CLIC5, HIST1H2AE, MGC14376, HLA-DRB1, SLC2A14, USP18,

DKFZp434C0328, CDC14B, DDX58, PARP9, TMPRSS3, COTL1, PARP14, KIAA1671, GDAP1, LOC129607, SLC27A1, FLJ13611, KSR, HIST1H2BD, 240979_at EST, BIRC4BP and RSAD2.

3. A method for the treatment of multiple myeloma in a patient in need thereof comprising the steps of:

a) testing whether the patient will respond or not to the combination treatment of the present invention by performing the method according to any one of claims 1 or 2

b) administering the combination treatment of at least one histone deacetylase inhibitor with at least one DNA methyltransferase inhibitor, when the HADMS score is higher than the reference value $HADMS_R$.

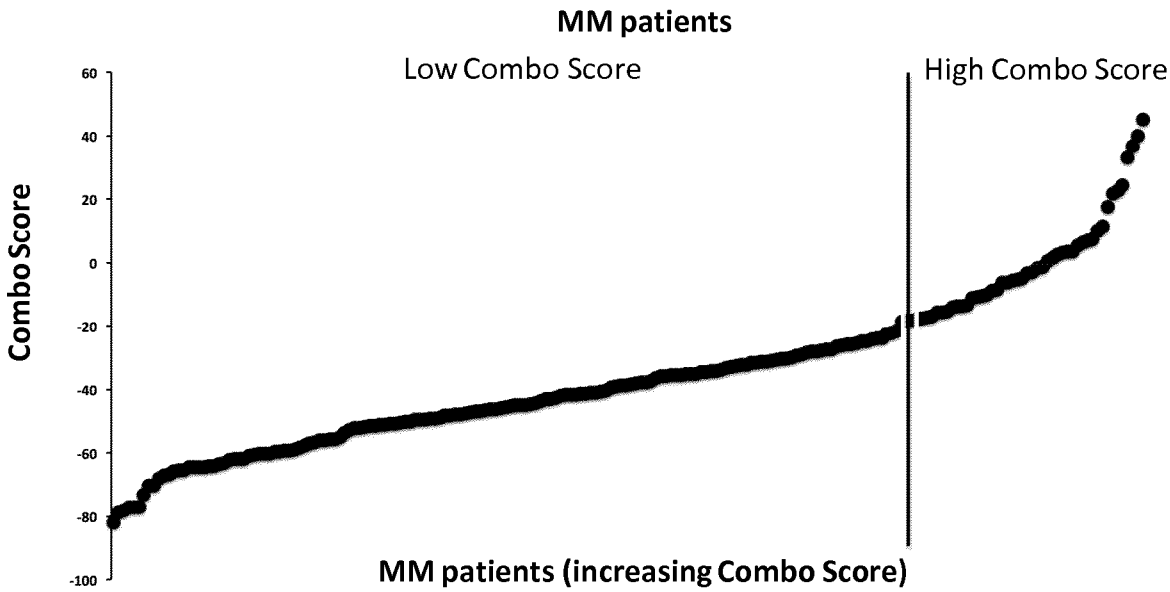
MM patients
Low Combo Score
High Combo Score
Combo Score
60
40
20
0
-20
-40
-60
-80
-100
MM patients (increasing Combo Score)

Figure 1

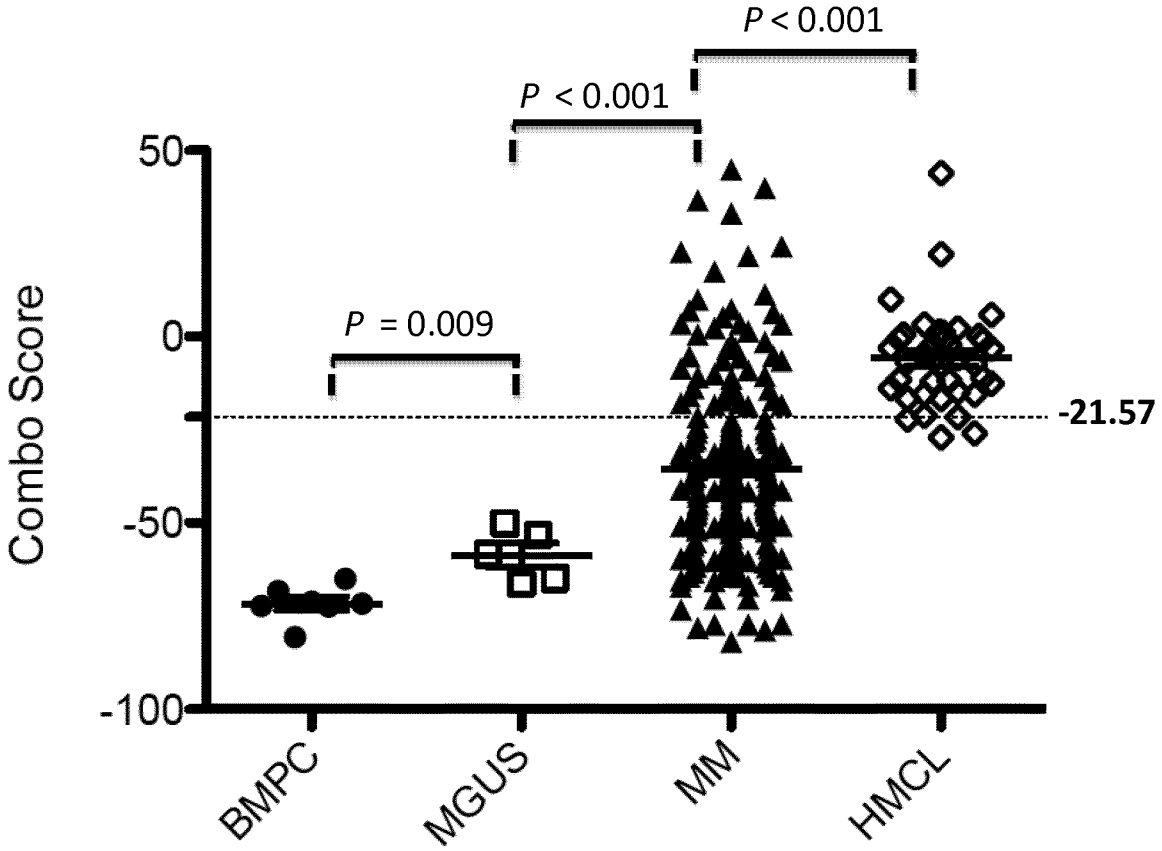
P < 0.001
P < 0.001
P = 0.009
50
0
-50
-100
Combo Score
-21.57
BMPC
MGUS
MM
HMCL

Figure 2

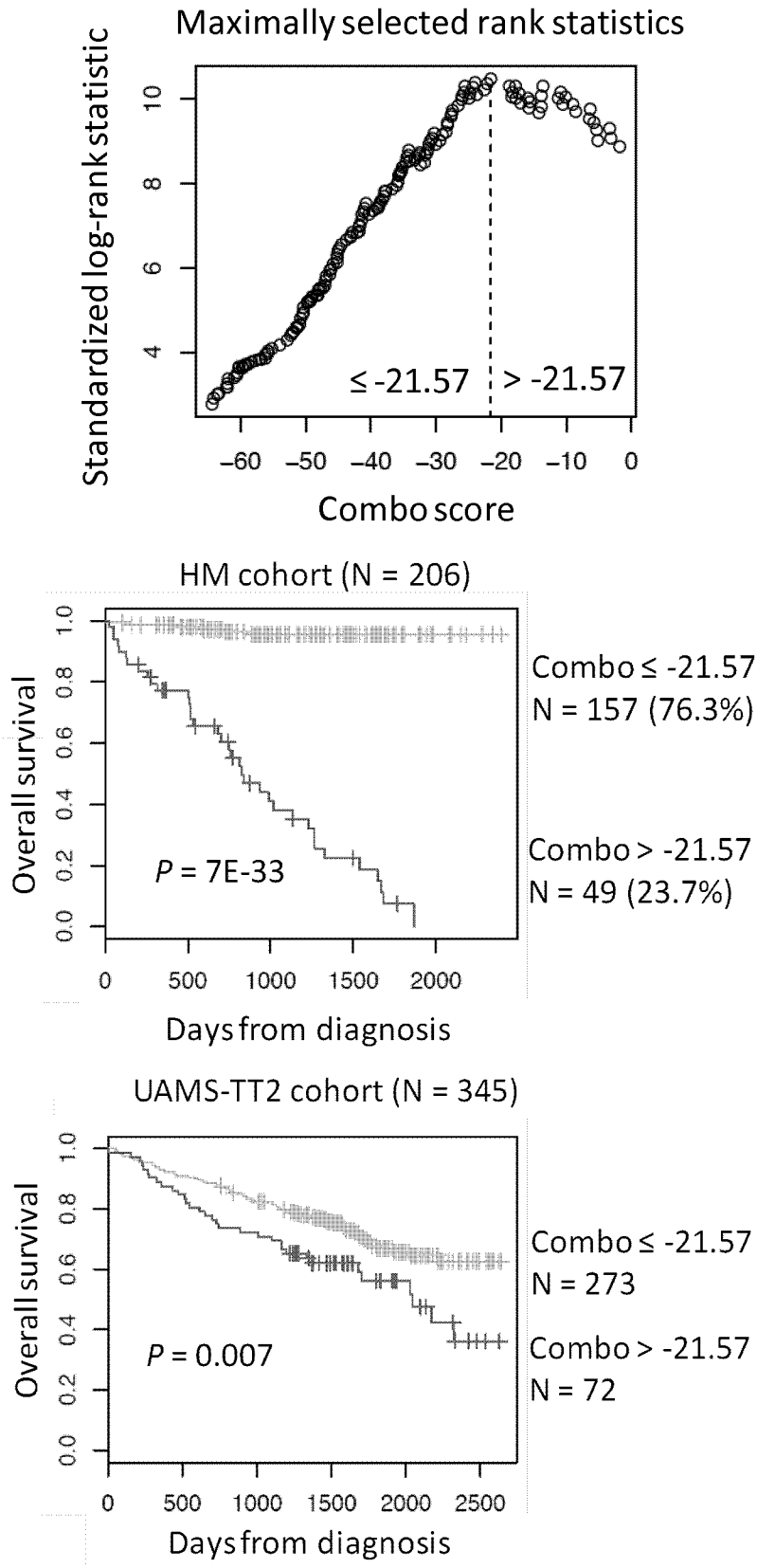
Maximally selected rank statistics
Standardized log-rank statistic
4
6
8
10
≤ -21.57
> -21.57
−60 −50 −40 −30 −20 −10 0
Combo score
HM cohort (N = 206)
Overall survival
0.0 0.2 0.4 0.6 0.8 1.0
P = 7E-33
0 500 1000 1500 2000
Days from diagnosis
Combo ≤ -21.57
N = 157 (76.3%)
Combo > -21.57
N = 49 (23.7%)
UAMS-TT2 cohort (N = 345)
Overall survival
0.0 0.2 0.4 0.6 0.8 1.0
P = 0.007
0 500 1000 1500 2000 2500
Days from diagnosis
Combo ≤ -21.57
N = 273
Combo > -21.57
N = 72

Figure 3A

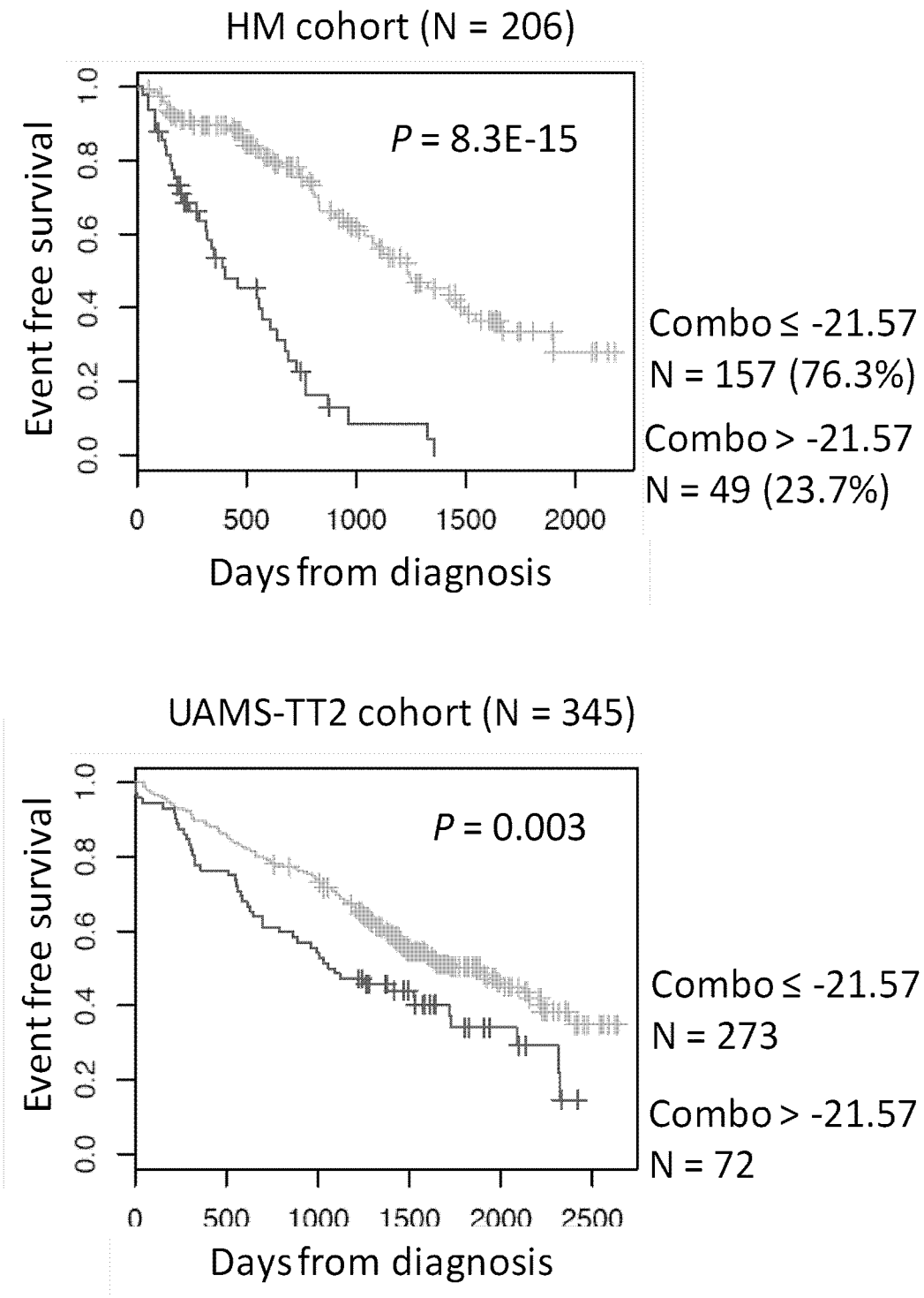
HM cohort (N = 206)
P = 8.3E-15
Event free survival
0.0
0.2
0.4
0.6
0.8
1.0
0
500
1000
1500
2000
Days from diagnosis
Combo ≤ -21.57
N = 157 (76.3%)
Combo > -21.57
N = 49 (23.7%)
UAMS-TT2 cohort (N = 345)
P = 0.003
Event free survival
0.0
0.2
0.4
0.6
0.8
1.0
0
500
1000
1500
2000
2500
Days from diagnosis
Combo ≤ -21.57
N = 273
Combo > -21.57
N = 72

Figure 3B

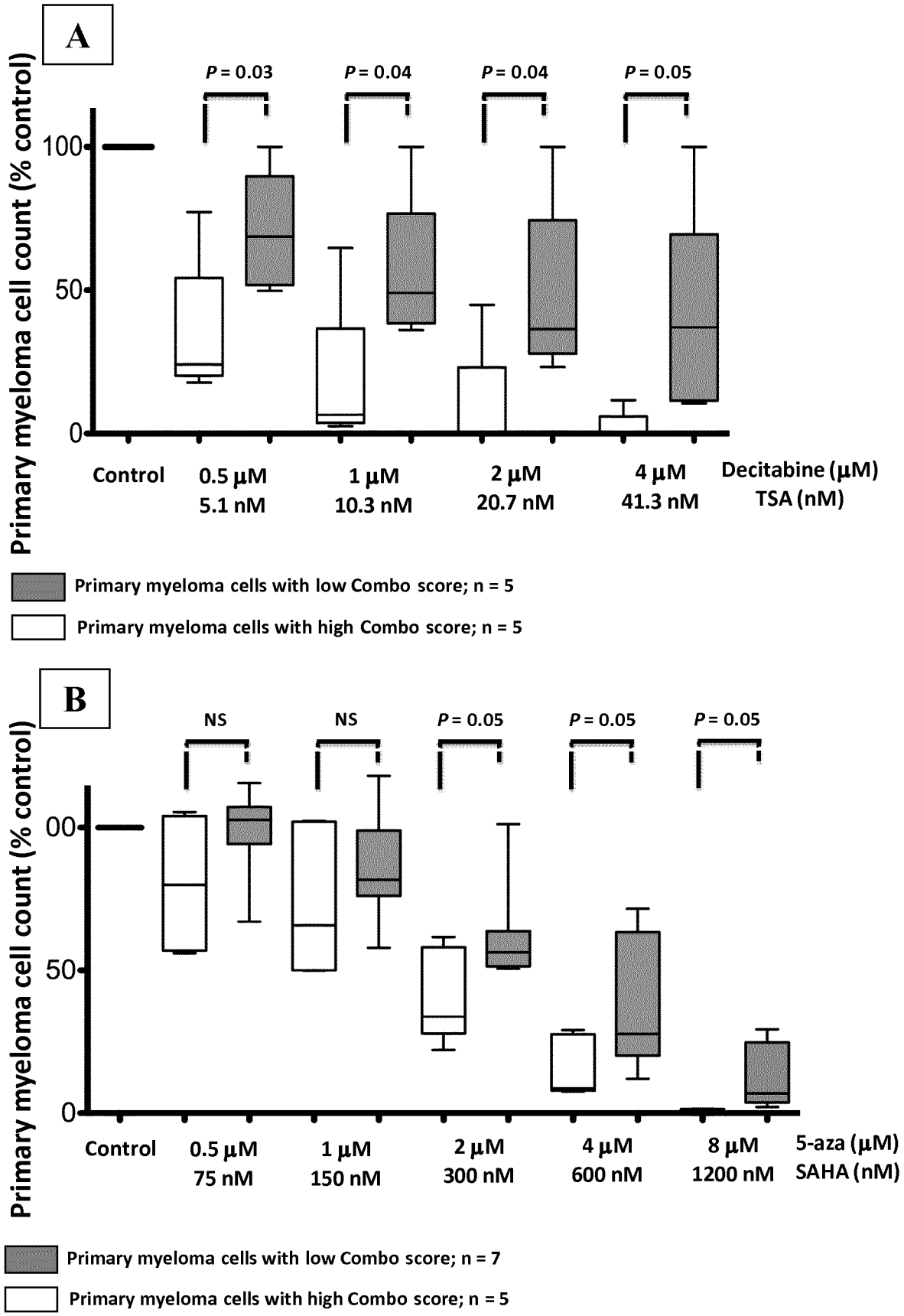
A
P = 0.03
P = 0.04
P = 0.04
P = 0.05
Primary myeloma cell count (% control)
100
50
0
Control
0.5 µM
5.1 nM
1 µM
10.3 nM
2 µM
20.7 nM
4 µM
41.3 nM
Decitabine (µM)
TSA (nM)
Primary myeloma cells with low Combo score; n = 5
Primary myeloma cells with high Combo score; n = 5
B
NS
NS
P = 0.05
P = 0.05
P = 0.05
Primary myeloma cell count (% control)
00
50
0
Control
0.5 µM
75 nM
1 µM
150 nM
2 µM
300 nM
4 µM
600 nM
8 µM
1200 nM
5-aza (µM)
SAHA (nM)
Primary myeloma cells with low Combo score; n = 7
Primary myeloma cells with high Combo score; n = 5

Figure 4

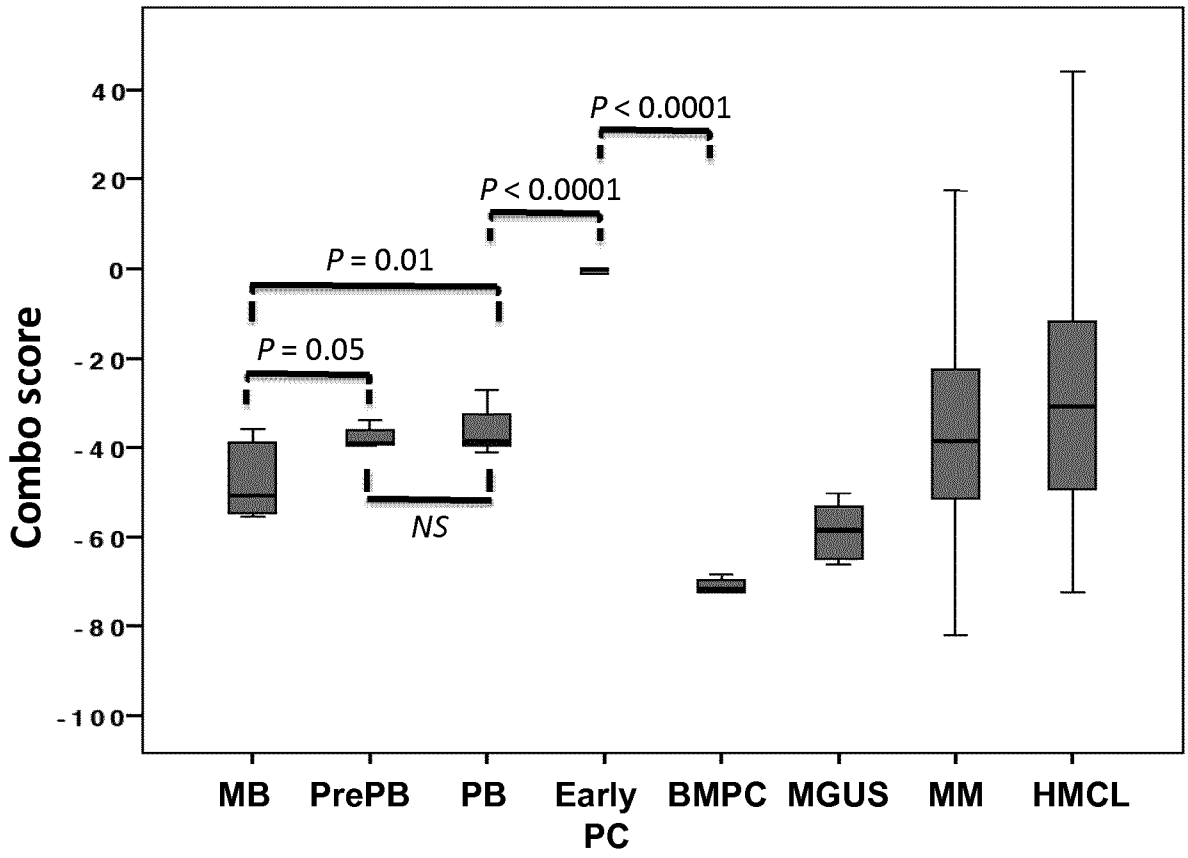
Combo score
40
20
0
-20
-40
-60
-80
-100
P < 0.0001
P < 0.0001
P = 0.01
P = 0.05
NS
MB
PrePB
PB
Early PC
BMPC
MGUS
MM
HMCL

Figure 5

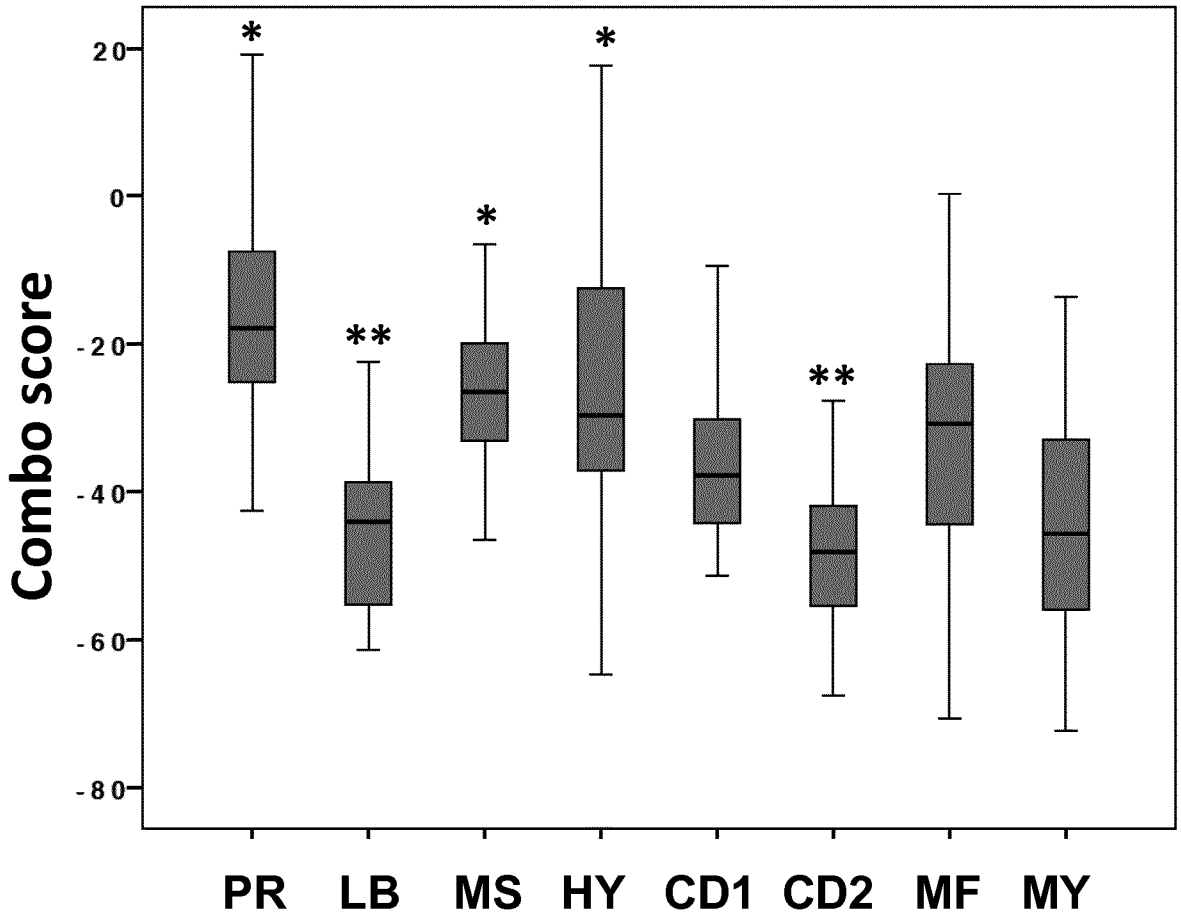

Figure 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/055992

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 A61K31/7068
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. MOREAUX ET AL: "A high-risk signature for patients with multiple myeloma established from the molecular classification of human myeloma cell lines", HAEMATOLOGICA, vol. 96, no. 4, 20 December 2010 (2010-12-20), pages 574-582, XP055050703, ISSN: 0390-6078, DOI: 10.3324/haematol.2010.033456 cited in the application pages 575,580; table S8 page 578 abstract -/--	1,2

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 2015	01/09/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Reuter, Uwe

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& J. Moreaux ET AL: "A high-risk signature for patients with multiple myeloma established from the molecular classification of human myeloma cell lines- supplementary appendix", Haematologica, 20 December 2010 (2010-12-20), pages 1-14, XP055051624, DOI: 10.3324/haematol.2010.033456 Retrieved from the Internet: URL:http://www.haematologica.org/content/suppl/2011/04/01/haematol.2010.033456.DC2/033456.Moreaux_suppl.pdf [retrieved on 2013-01-29]	

X	K. Vanderkerken ET AL: "EPIGENETIC CHANGES OF MYELOMA CELLS WITHIN THE BONE MARROW MICROENVIRONMENT", Haematologica: Abstract Book 13th International Myeloma Workshop, Paris, France, May 3-6, 2011, vol. 96, no. supplement 1 1 May 2011 (2011-05-01), pages s8-s9, XP055051689, ISSN: 0390-6078 Retrieved from the Internet: URL:http://www.haematologica.org/content/96/supplement_1/S1.full.pdf+html [retrieved on 2013-01-30]	3
Y	abstract	1,2

X	G. HELLER ET AL: "Genome-Wide Transcriptional Response to 5-Aza-2'-Deoxycytidine and Trichostatin A in Multiple Myeloma Cells", CANCER RESEARCH, vol. 68, no. 1, 1 January 2008 (2008-01-01), pages 44-54, XP055051386, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-07-2531	3
Y	abstract page 44 - page 45 page 53	1,2

X	EMMA M. SMITH ET AL: "The potential role of epigenetic therapy in multiple myeloma", BRITISH JOURNAL OF HAEMATOLOGY, vol. 148, no. 5, 1 March 2010 (2010-03-01), pages 702-713, XP055051410, ISSN: 0007-1048, DOI: 10.1111/j.1365-2141.2009.07976.x page 706 - page 708	3

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/055992

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. MOREAUX ET AL: "Development of Gene Expression-Based Score to Predict Sensitivity of Multiple Myeloma Cells to DNA Methylation Inhibitors", MOLECULAR CANCER THERAPEUTICS, vol. 11, no. 12, 1 December 2012 (2012-12-01), pages 2685-2692, XP055050719, ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-12-0721 cited in the application page 2686 page 2688	1,2
Y	J MOREAUX ET AL: "Gene expression-based prediction of myeloma cell sensitivity to histone deacetylase inhibitors", BRITISH JOURNAL OF CANCER, vol. 109, no. 3, 18 July 2013 (2013-07-18) , pages 676-685, XP055085883, ISSN: 0007-0920, DOI: 10.1038/bjc.2013.392 cited in the application page 679	1,2

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