



(43) International Publication Date
6 February 2014 (06.02.2014)

(10) International Publication Number
WO 2014/020502 A2

- (51) **International Patent Classification:**
G01N 33/574 (2006.01)
- (21) **International Application Number:**
PCT/IB2013/056122
- (22) **International Filing Date:**
25 July 2013 (25.07.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/677,859 31 July 2012 (31.07.2012) US
- (71) **Applicant (for all designated States except US):** **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH).
- (72) **Inventors; and**
- (71) **Applicants (for US only):** **GAULIS, Swann** [CH/CH]; Novartis Pharma AG, Werk Klybeck, Postfach, CH-4002 Basel (CH). **JEAY, Sebastien** [FR/CH]; Novartis Pharma AG, Werk Klybeck, Postfach, CH-4002 Basel (CH).
- (74) **Common Representative:** **NOVARTIS AG**; Lichtstrasse 35, CH-4056 Basel (CH).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).
- Published:**
— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) **Title:** MARKERS ASSOCIATED WITH HUMAN DOUBLE MINUTE 2 INHIBITORS

(57) **Abstract:** The invention provides methods of monitoring differential gene expression of biomarkers to determine patient sensitivity to Human Double Minute inhibitors (MDM2i), methods of determining the sensitivity of a cell to an MDM2i by measuring biomarkers and methods of screening for candidate MDM2i.



WO 2014/020502 A2

MARKERS ASSOCIATED WITH HUMAN DOUBLE MINUTE 2 INHIBITORS**FIELD OF THE INVENTION**

[001] The present invention relates to the field of pharmacogenomics, and the use of biomarkers useful in determining patient sensitivity prior to treatment, following patient response after treatment, cancer sensitivity and screening of compounds.

BACKGROUND

[002] p53, also known as tumor protein 53, is a tumor suppressor gene involved in the prevention of cancer, often referred to as the gatekeeper or guardian of the genome (Levine, Cell 1997, 88:323-331). The p53 gene encodes for a transcription factor that is normally quiescent, and becoming activated when the cell is stressed or damaged, such as when DNA damage incurred from a mutagen. If the cell is stressed or damaged, p53 acts to limit the damage, or barring that, trigger the apoptotic pathway so the damaged cell is eliminated and no longer a threat to the organism (Vogelstein et al., Nature 2000, 408:307-310). An analysis of different cancers showed that p53 is mutated in about 50% of human cancers (Hollstein et al., Nucleic Acids Res. 1994, 22:3551-3555; Hollstein et al., Science 1991, 253(5015): 49-53). Humans who are heterozygous for p53, with only a single functional copy, will develop tumors early in adulthood, a disorder known as Li-Fraumeni syndrome (Varley et al., Hum. Mutat. 2003, 21(3):313-320). However, as much as p53 regulates the cell's fate, p53 is regulated by another protein known as MDM2.

[003] Double minute 2 protein (MDM2) was discovered as a negative regulator of p53 (Fakharzadeh et al., EMBO J. 1991, 10(6):1565-1565). MDM2 encodes an E3 ligase containing a p53 binding domain and a nuclear export signal sequence, and upon complexing with p53, removes it from the nucleus and ubiquitinylates it, which promotes the degradation of the p53 protein via the ubiquitin-proteasome pathway (Haupt et al., Nature 1997, 387(6630):296-299; Piette et al., Oncogene 1997 15(9):1001-1010). In addition, MDM2 directly inhibits the activity of p53 by binding to the p53 transactivation domain, also preventing p53 mediated gene expression (Wu et al., Genes Dev. 1993, 7:1126-1132). Thus, MDM2 regulates p53 in multiple ways.

[004] MDM2 is overexpressed in a number of cancers, for example, liposarcoma, glioblastoma, and leukemia (Momand et al., Nucleic Acids Res. 1998, 26(15):3453-3459). Overexpression of MDM2 can interfere with the activities of p53, preventing apoptosis and growth arrest of the tumor (de Rozieres et al., Oncogene 2000, 19(13):1691-1697). Overexpression of MDM2 correlates with poor prognosis in glioma, and acute lymphocytic leukemia (Onel et al., Mol. Cancer Res. 2004, 2(1):1-8).

[005] As MDM2 is an inhibitor of p53, therapeutics which prevent the binding of MDM2 to p53 would prevent the degradation of p53, allowing free p53 to bind and mediate gene expression in cancer cells, resulting in cell cycle arrest and apoptosis. There are previous reports of small molecule inhibitors of the p53-MDM2 interaction (Vassilev et al., Science, 2004, 303(5659):844-888; Zhang et al., Anticancer drugs, 2009 20(6):416-424; Vu et al., Curr. Topics Microbiol. Immuno., 2011, 348:151-172). The mode of binding of these compounds and a crystal structure of the human MDM2–Nutlin complex as well as a scaffold and pockets of the p53 binding site on MDM2 are also known (Vassilev, *supra*). The first of these MDM2 inhibitors, known as the Nutlins, bind MDM2 and occupy the p53 binding pocket, preventing the formation of the MDM2-p53 complex. This leads to less degradation of the p53 protein, and expression of p53 target genes. Cancer cell lines treated with Nutlins showed growth arrest and increased apoptosis. For example, the SJSA-1 osteosarcoma line contains amplified copies of the MDM2 gene. Treatment of this line with Nutlin-3 reduced proliferation and increased apoptosis (Vassilev et al., Science, 2004, 303(5659):844-888). The SJSA-1 cell line was used in creating xenographs in mouse. Administration of Nutlin-3 reduced xenograft growth by 90%. To investigate the effect the Nutlin compounds had on non-cancerous cells, human and mouse normal fibroblasts were treated with Nutlin-3 and while the proliferation of the cells was slowed, they retained their viability (Vassilev, *supra*).

[006] Finding biomarkers which indicate which patient should receive a therapeutic is useful, especially with regard to cancer. This allows for more timely and aggressive treatment as opposed to a trial and error approach. In addition, the discovery of biomarkers which indicate that cells continue to be sensitive to the therapy after administration is also useful. These biomarkers can be used to monitor the response of those patients receiving the therapeutic. If biomarkers indicate that the patient has become insensitive to the treatment, then the dosage administered can be increased, decreased, completely discontinued or an additional therapeutic administered. As such, there is a need to develop biomarkers associated with MDM2 inhibitors. This approach ensures that the correct patients receive the appropriate treatment and during the course of the treatment the patient can be monitored for continued MDM2 inhibitor sensitivity.

[007] In the development of MDM2 inhibitors, specific biomarkers will aid in understanding the mechanism of action upon administration. The mechanism of action may involve a complex cascade of regulatory mechanisms in the cell cycle and differential gene expression. This analysis is done at the pre-clinical stage of drug development in order to determine the particular sensitivity of cancer cells to the MDM2 inhibitor candidate and the activity of the candidate. Of particular interest in the pharmacodynamic investigation is the

identification of specific markers of sensitivity and activity, such as the ones disclosed herein.

SUMMARY OF THE INVENTION

[008] The invention relates to the analysis that a number of genes identified in Table 2 act as specific biomarkers in determining the sensitivity of cells to MDM2 inhibitors (henceforth “MDM2i”). The invention relates to the analysis that at least one of the biomarkers in Table 2 provides a “gene signature” for MDM2i that has increased accuracy and specificity in predicting which cancer cells are sensitive to MDM2i. The method analyzes the gene expression or protein level of at least one of the biomarkers in Table 2 in a cancer sample taken from a patient which predicts the sensitivity of the cancer sample to an MDM2i. The pattern of expression level changes may be indicative of a favorable response or an unfavorable one. In addition, the gene signature provided in Table 2 has increased predictive value because it also indicates that the p53 pathway is functional. This is an unexpected result as many tumors contain a mutated p53 and a non-functional pathway which provides the tumor with a growth advantage. The invention is an example of “personalized medicine” wherein patients are treated based on a functional genomic signature that is specific to that individual.

[009] The predictive value of at least one biomarker in Table 2 can also be used after treatment with an MDM2i to determine if the patient remains sensitive to the treatment. Once the MDM2i therapeutic has been administered, the biomarkers are used to monitor the continued sensitivity of the patient to MDM2i treatment. The disclosure also relates to the up or down regulation of the expression of the identified genes after MDM2i treatment. This is useful in determining that patients receive the correct course of treatment. The invention comprises a method of predicting and monitoring the sensitivity of a patient to MDM2i treatment. The method includes the step of administration of an MDM2i to the patient and measurement of biomarker gene expression on a biological sample obtained from the patient. The response of the patient is evaluated based on the detection of gene expression of at least one biomarker from Table 2. Detection and/or alteration in the level of expression of at least one biomarker is indicative of the sensitivity of the patient to the treatment. The pattern of expression level can be indicative of a favorable patient response or an unfavorable one.

DESCRIPTION OF THE DRAWINGS

[0010] Figure 1A shows the in vitro potency of the MDM2i in disrupting p53-MDM2 interaction. Figure 1B shows the in vitro potency of the MDM2i on the proliferation of cancer cells.

[0011] Figure 2 shows the p53 status (mutant or wild type) and the sensitivity of the cancer cells to MDM2i(2). The X axis is the crossing point for sensitivity and the Y axis is the Amax value. Figure 2 demonstrates the sensitivity of cells to MDM2i(2) and p53 mutational status, mt (mutant) or wild type (wt). The mt panel displays the MDM2i(2) sensitivity profiles for p53 mutated CCLE cell lines, the wt panel displays the MDM2i(2) sensitivity profiles for p53 wild-type CCLE cell lines. Amax is defined as the maximal effect level (the inhibition at the highest tested MDM2i(2) concentration, calibrated to a reference inhibitor), and IC50 is defined as of the μ M concentration at which MDM2i(2) response reached an absolute inhibition of -50 with respect to the reference inhibitor. Cell line count is broken down by MDM2i(2) chemical sensitivity and p53 mutation status, and associated statistics. Data is also displayed as a contingency table with associated statistics.

[0012] Figure 3 are biomarkers showing the fold change in expression and the statistical significance of the overexpression value. Figure 3 shows selected MDM2i chemical sensitivity predictive biomarkers and their associated statistics. The gene identities are official HUGO symbols. The 'Expr' prefix indicates these biomarkers are of the transcript expression type.

[0013] Figure 4 is a Western blot of sensitive and insensitive representative cells treated with an MDM2i(1) for 4 hours at various concentrations and then probed for selected p53 target genes as pharmacodynamic biomarker representatives.

[0014] Figure 5 is a graph demonstrating the increase in predictive value of the gene signature as opposed to a larger set of biomarkers. Figure 5 shows cross-validation performances of MDM2i(2) chemical sensitivity predictive models. Performances of models derived from three predictive feature sets are evaluated using sensitivity (fraction of correctly predicted sensitive cell lines), specificity (fraction of correctly predicted insensitive cell lines), PPV (positive predicted value, fraction of sensitive cell lines predicted as such) and NPV (negative predictive value, fraction of insensitive cell lines predicted as such). Error bars are one standard deviation of the means over the 5 iterations of 5-fold cross-validations. The model using the 13 selected biomarker depicted in Table 2 achieves better predictivity of MDM2i(2) chemical sensitivity, and this independently of the performance measure, but most strikingly when PPV is considered as the performance indicator.

[0015] Figure 6 is a list of cell lines, each analyzed for the gene signature and the prediction of whether the cell line is sensitive or insensitive and the IC50 when treated with an MDM2i.

[0016] Figure 7A-C shows the dose-dependent inhibition of tumor growth in the SJSA-1 xenograft model (predicted to be sensitive by the gene signature) following treatment with MDM2i(1), and the concomitant induction of p21(CDKN1A) expression as a representative pharmacodynamic biomarker.

[0017] Figure 8 shows the expression of p21(CDKN1A) at the protein level after treatment of MDM2i(1) sensitive cells at efficacious doses.

[0018] Figure 9 is a model of tumor samples predicted to be sensitive using the gene signature in the OncExpress database and the Primary Tumor Bank. Figure 9 shows lineage specific correlations of MDM2i chemical sensitivity predictions in human primary tumor collections and CCLE cell line data. The left panel shows the correlation between the fractions of predicted sensitive samples from the human primary tumor sample collection and the observed sensitivity ratio in the cell line data. The right panel shows the correlation between the fractions of predicted sensitive samples from the human primary tumor sample collection and the predicted sensitive ratios in the primary tumor xenograft collection. Samples/Xenografts/Cell lines are organized by lineage. The dashed line is the identity line.

[0019] Figure 10 represents the Positive Predictive Values (PPV) achieved by the MDM2i(2) sensitivity predictive models, built from multiple combinations of biomarkers described in Table 2. Figure 10 shows Positive Predicted Values (PPV) achieved by MDM2i(2) sensitivity predictive models built from multiple combinations of biomarkers. The combinatorial and single-gene model PPVs are shown as box-and-whisker plots and compared to the PPVs given by the two p53 mutation status and thirteen biomarker models (the whiskers extend 1.5 times the interquartile range, the black line is the median, the notches are an estimation of the median confidence interval, the box width is proportional to the number of data points, the outliers are not shown; 'bm(s)': biomarker(s); 'allExMt': p53 all exon mutation model; 'ex5to8mt': p53 exon 5 to 8 mutation model).

[0020] Figure 11 represents the specificities achieved by the MDM2i sensitivity predictive models, built from multiple combinations of biomarkers described in Table 2. Figure 11 shows specificities achieved by MDM2i(2) sensitivity predictive models built from multiple combinations of biomarkers. The combinatorial and single-gene model specificities are shown as box-and-whisker plots and compared to the PPVs given by the two p53 mutation status and thirteen biomarker models (the whiskers extend 1.5 times the interquartile range, the black line is the median, the notches are an estimation of the median confidence interval, the box width is proportional to the number of data points, the outliers are not shown; 'bm(s)': biomarker(s); 'allExMt': p53 all exon mutation model; 'ex5to8mt': p53 exon 5 to 8 mutation model).

[0021] Figure 12 represents the sensitivities achieved by the MDM2i sensitivity predictive models, built from multiple combinations of biomarkers described in Table 2. Figure 12 shows the sensitivities achieved by MDM2i(2) sensitivity predictive models built from multiple combinations of biomarkers. The combinatorial and single-gene model sensitivities are shown as box-and-whisker plots and compared to the PPVs given by the two p53 mutation status and thirteen biomarker models (the whiskers extend 1.5 times the

interquartile range, the black line is the median, the notches are an estimation of the median confidence interval, the box width is proportional to the number of data points, the outliers are not shown; 'bm(s)': biomarker(s); 'allExMt': p53 all exon mutation model; 'ex5to8mt': p53 exon 5 to 8 mutation model).

[0022] Figure 13 represents the PPV achieved by the MDM2i sensitivity predictive models, built from multiple combinations of biomarkers described in Table 2, together with p53 mutation status. Figure 13 shows the Positive Predicted Values (PPV) achieved by MDM2i(2) sensitivity predictive models built from combining biomarkers with p53 mutation status. For a description of the plot refer to Figure 10, 11 or 12; 'bm(s)': biomarker(s); 'mt': p53 exon 5 to 8 mutations; 'allExMt': p53 all exon mutation model; 'ex5to8mt': p53 exon 5 to 8 mutation model).

[0023] Figure 14 represents the specificities achieved by the MDM2i sensitivity predictive models, built from multiple combinations of biomarkers described in Table 2, together with p53 mutation status. Figure 14 shows the specificities achieved by MDM2i(2) sensitivity predictive models built from combining biomarkers with p53 mutation status. For a description of the plot refer to Figure 10, 11 or 12; 'bm(s)': biomarker(s); 'mt': p53 exon 5 to 8 mutations; 'allExMt': p53 all exon mutation model; 'ex5to8mt': p53 exon 5 to 8 mutation model).

[0024] Figure 15 represents the sensitivities achieved by the MDM2i sensitivity predictive models, built from multiple combinations of biomarkers described in Table 2, together with p53 mutation status. Figure 15 shows the sensitivities achieved by MDM2i(2) sensitivity predictive models built from combining biomarkers with p53 mutation status. For a description of the plot refer to Figure 10, 11 or 12; 'bm(s)': biomarker(s); 'mt': p53 exon 5 to 8 mutations; 'allExMt': p53 all exon mutation model; 'ex5to8mt': p53 exon 5 to 8 mutation model).

[0025] Figure 16 is a graph showing the response of human primary xenograft models to MDM2i(1) and their predicted sensitivity/insensitivity. Figure 16 represents human primary xenograft models that are predicted to be sensitive to MDM2i using the biomarkers disclosed in Table 2 are confirmed sensitive to a daily dosing of 100 mg/kg MDM2i(1), supporting the use of the described predictive model in cancer patient populations (HMEX=melanoma; HCOX=colorectal cancer; HSAX=liposarcoma; HKIX=renal cell carcinoma; CHLI or HLIX=hepatic cancer; HBRX=breast cancer; HPAX=pancreatic cancer; HLUX=lung cancer).

[0026] Figure 17 is a graph showing the response to MDM2i(1) of human primary xenograft models that are pre-selected for wild type p53 and then predicted to be sensitive/insensitive. Figure 17 shows wild-type p53 human primary xenograft models that are predicted to be sensitive to MDM2i using the biomarkers disclosed in Table 2 are

confirmed sensitive to a daily dosing of 100 mg/kg MDM2i(1), supporting the use of the described predictive model in wild-type p53 pre-selected cancer patient populations (HMEX=melanoma; HCOX=colorectal cancer; HSAX=liposarcoma; HKIX=renal cell carcinoma; CHLI=hepatic cancer; HPAX=pancreatic cancer; HLUX=lung cancer).

DESCRIPTION OF THE INVENTION

[0027] The aspects, features and embodiments of the present invention are summarized in the following items and can be used respectively alone or in combination:

[0028] 1. A method of predicting the sensitivity of a cancer patient for treatment with a Human Double Minute 2 inhibitor (MDM2i), the method comprising: measuring gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient, and gene expression of the at least one biomarker indicates that the patient is sensitive to treatment with an MDM2i.

[0029] 2. A method of treating a cancer patient comprising: measuring gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient; determining sensitivity of the patient to an MDM2i; and administering to the patient an MDM2i.

[0030] 3. A method of treating a cancer patient comprising: assaying for gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient; the presence of at least one biomarker indicates sensitivity of the patient to an MDM2i; and administering to the patient an MDM2i.

[0031] 4. A method of predicting the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising: assaying for gene expression of at least one biomarker selected from Table 2 in the cancer cell obtained from a patient cancer sample and the gene expression of the at least one biomarker selected from Table 2 indicates that the cancer cell is sensitive to treatment with an MDM2i.

[0032] 5. A method of predicting the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising: measuring gene expression of at least one biomarker selected from Table 2 in the cell; and the gene expression of the at least one biomarker selected from Table 2 indicates that the cancer cell is sensitive to treatment with an MDM2i.

[0033] 6. A method of screening for MDM2i candidates the method comprising:
a) contacting a cell with a MDM2i candidate;
b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i candidate and expression of the at least one biomarker indicates sensitivity to an MDM2i; and

c) comparing the reduction in cell proliferation from the cell contacted with the MDM2i candidate with the reduction in cell proliferation of a cell contacted with an MDM2i taken from Table 1 and the cell proliferation of an untreated or placebo treated cell.

[0034] 7. A method of determining the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising: a) contacting a cancer cell with at least one MDM2i; b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i; c) comparing the cell proliferation of cancer cells treated with at least one MDM2i with an untreated or placebo treated cancer cell, wherein there is reduced cell proliferation of the treated cancer cell when compared with the untreated to placebo treated cancer cell.

[0035] 8. The method of any one of items 1 to 7, wherein more than one biomarker is selected from Table 2.

[0036] 9. The method of item 1 or 8, wherein at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve or all thirteen biomarkers are selected from Table 2.

[0037] 10. The method of any one of items 1 to 9, wherein p53 is selected as a biomarker in addition to any biomarker selected from Table 2.

[0038] 11. The method of any one of items 1 to 10, comprising the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and/or AEN.

[0039] 12. The method of any one of items 1 to 11, wherein comparing the gene expression of the at least one biomarker with gene expression of a control sample indicates a functional p53 gene pathway.

[0040] 13. The method of any one of items 1 to 4 or 8 to 12, wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[0041] 14. The method of any one of items 1 to 13, wherein an mRNA or protein of at least one biomarker is measured.

[0042] 15. The method of any one of items 1, 2 or 5 to 11, wherein the expression of the at least one biomarker is increased in the cancer sample when compared to a control.

[0043] 16. The method of any one of items 1 to 15, wherein the MDM2i is selected from Table 1.

[0044] 17. The method of any one of items 1 to 16, wherein the MDM2i is a compound that binds to a p53 binding pocket of MDM2.

- [0045]** 18. The method of any one of items 1 to 16, wherein the MDM2i is a compound that binds to substantially the same p53 binding pocket of MDM2 as Nutlin-3a or the MDM2i from the Table 1.
- [0046]** 19. The method of any one of items 1 to 18, wherein the MDM2i is a compound that prevents the protein-protein interaction between p53 and MDM2.
- [0047]** 20. The method of any one of items 1 to 19, wherein the MDM2i is a compound that inhibits cell proliferation by inducing the p53 pathway activity.
- [0048]** 21. The method of any one of items 2 to 20 further comprising obtaining a biological sample from the patient prior to the administration of the MDM2i.
- [0049]** 22. The method of any one of items 2 to 21, wherein the MDM2i is administered in a therapeutically effective amount.
- [0050]** 23. The method of any one of items 4 to 14, or 16 to 22, wherein the gene expression of the at least one biomarker is increased in the cancer cell.
- [0051]** 24. The method of any one of items 3 to 5 or 7 to 23, wherein the IC₅₀ of the cancer cell contacted with at least one MDM2i is less than 1 μ M
- [0052]** 25. The method of any one of items 3 to 5, or 7 to 25, wherein the cell is contacted by the MDM2i at least at two different time points.
- [0053]** 26. The method of any one of items 3 to 5, or 7 to 26, wherein the cell is contacted by two different MDM2i.
- [0054]** 27. The method of item 26, wherein the cell is contacted by the two different MDM2i at the same time.
- [0055]** 28. The method of item 26, wherein the cell is contacted by two different MDM2i at different time points.
- [0056]** 29. The method of any one of items 1 to 28, wherein the MDM2i is an isoquinolinone.
- [0057]** 30. A method of screening for MDM2i candidates, the method comprising: a) contacting a cell with a MDM2i candidate; b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i candidate; c) the gene expression of the at least one biomarker indicates sensitivity to an MDM2i; and d) comparing the reduction in cell proliferation from the cell contacted with the MDM2i candidate with the reduction in cell proliferation of a cell contacted with an MDM2i taken from Table 1 and the cell proliferation of an untreated or placebo treated cell.
- [0058]** 31. The method of item 30, wherein the gene expression of the MDM2i candidate is compared with the gene expression of an MDM2i selected from Table 1.
- [0059]** 32. The method of item 30 or 31, wherein the MDM2i candidate increases gene expression of at least one, at least two, at least three, at least four, at least five, at least

six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve or all thirteen biomarkers from Table 2.

[0060] 33. The method of any one of items 30 to 32, wherein the cell is a cancer cell selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[0061] 34. The method of any one of items 30 to 33, wherein the expression of an mRNA or protein of at least one biomarker of Table 2 is measured.

[0062] 35. The method of any one of items 30 to 34, comprising the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[0063] 36. Composition comprising an MDM2i for use in treatment of cancer in a selected cancer patient population, wherein the cancer patient population is selected on the basis of gene expression of at least one biomarker selected from Table 2 in a cancer cell sample obtained from said patients.

[0064] 37. The composition of item 36, wherein at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve or all thirteen biomarkers are selected from Table 2.

[0065] 38. The composition of items 36 or 37, wherein p53 is selected as a biomarker in addition to any biomarker selected from Table 2.

[0066] 39. The composition of any one of items 36 to 38, wherein the biomarker is MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and/or AEN.

[0067] 40. The composition of any one of items 36 to 39, wherein the MDM2i is selected from Table 1.

[0068] 41. The composition of any one of items 36 to 40, wherein the MDM2i is a compound that binds to a p53 binding pocket of MDM2.

[0069] 42. The composition of any one of items 36 to 41, wherein the MDM2i is a compound that binds to substantially the same p53 binding pocket of MDM2 as Nutlin-3a or the MDM2i from the Table 1.

[0070] 43. The composition of any one of items 36 to 42, wherein the MDM2i is a compound that prevents the protein-protein interaction between p53 and MDM2.

[0071] 44. The composition of any one of items 36 to 43, wherein the MDM2i is a compound that inhibits cell proliferation by inducing the p53 pathway activity.

[0072] 45. The composition of any one of items 36 to 44, wherein the MDM2i is an isoquinolinone

[0073] 46. The composition of any one of items 36 to 45, wherein the cancer cell sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[0074] 47. The composition of any one of items 36 to 46, wherein the patients are selected on the basis of gene expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[0075] 48. The composition of any one of items 36, 37, or 39 to 47 for use in treatment of a cancer in a selected cancer patient population, wherein the cancer patient population is selected from a wild-type p53 pre-selected cancer patient population.

[0076] 49. A kit for predicting the sensitivity of a cancer patient for treatment with a Human Double Minute 2 inhibitor (MDM2i) comprising: i) means for detecting the expression of any one of the biomarkers from the table 2, preferably more than one, particularly at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve or all thirteen biomarkers selected from Table 2; and ii) instructions how to use said kit.

[0077] 50. The kit of item 49, wherein the biomarkers are MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B or/and AEN.

[0078] 51. The kit of item 49 or 50 further comprising means for detecting the expression of p53.

[0079] 52. Use of the kit according to item 49 to 51 for any of the methods of items 1 to 32.

[0080] In one aspect, the disclosure provides for methods of analyzing at least one of the biomarkers identified in Table 2 in a sample containing cancer cells wherein expression of at least one biomarker indicates if the cancer cell will be sensitive to MDM2i treatment. The pattern of expression changes can be indicative of a favorable patient response or of an unfavorable one and patients can be selected or rejected based on the expression of at least one biomarker from Table 2. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

[0081] After treatment with an MDM2i, the disclosure provides for methods of analyzing at least one of the biomarkers identified in Table 2 in a sample containing cancer cells wherein expression of the biomarker after MDM2i treatment indicates that the patient is still sensitive to MDM2i treatment. Detection and/or alteration in the level of expression of at least one biomarker is indicative of the MDM2i sensitivity, and this correlates with a response of the patient to the treatment. Alternatively, all of the biomarkers in Table 2 can be

assayed for as a single set. The pattern of expression can be indicative of a favorable patient response or of an unfavorable one.

[0082] Accordingly, the disclosure provides for a method of predicting the sensitivity of a cancer patient for treatment with a Human Double Minute 2 inhibitor (MDM2i), the method comprising: assaying for gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient; and the gene expression of the at least one biomarker indicates that the patient is sensitive to treatment with an MDM2i.

[0083] The method wherein more than one biomarker is selected from Table 2.

[0084] The method comprising assaying for gene expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[0085] The method wherein p53 is assayed for a mutation prior to assay of the at least one biomarker, and the presence of a p53 mutation indicates decreased sensitivity to an MDM2i.

[0086] The method wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[0087] The method wherein an mRNA or protein of at least one biomarker is measured.

[0088] The method wherein the MDM2i is an isoquinolinone.

[0089] The method wherein the MDM2i is selected from Table 1.

[0090] A method of treating a cancer patient comprising: assaying for gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient; the presence the at least one biomarker indicates sensitivity of the patient to an MDM2i; and administering to the patient an MDM2i.

[0091] The method wherein more than one biomarker is selected from Table 2.

[0092] The method comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[0093] The method wherein p53 is assayed for a mutation prior to assay of the at least one biomarker and the presence of a p53 mutation indicates decreased sensitivity to an MDM2i.

[0094] The method further comprising obtaining a biological sample from the patient prior to the administration of the MDM2i, and comparing the differential gene expression of at least one biomarker from Table 2 in the MDM2i untreated cancer sample with an MDM2i

treated cancer sample, wherein increased gene expression indicates continued patient sensitivity.

[0095] The method wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[0096] The method wherein the MDM2i is an isoquinolinone.

[0097] The method wherein the MDM2i is selected from Table 1.

[0098] The method wherein the MDM2i is administered in a therapeutically effective amount.

[0099] A method of predicting the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising: assaying for gene expression of at least one biomarker selected from Table 2 in the cancer cell obtained from a patient sample and the gene expression of the at least one biomarker selected from Table 2 indicates that the cancer cell is sensitive to treatment with an MDM2i.

[00100] The method wherein more than one biomarker is selected from Table 2.

[00101] The method comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[00102] The method wherein p53 is assayed for a mutation prior to assay of the at least one biomarker and the presence of a p53 mutation indicates decreased sensitivity to an MDM2i.

[00103] The method wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[00104] The method wherein a mRNA or protein of at least one biomarker is measured.

[00105] The method wherein the MDM2i is an isoquinolinone.

[00106] The method wherein the MDM2i is selected from Table 1.

[00107] The method wherein the MDM2i is administered in a therapeutically effective amount.

[00108] A method of determining the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising: a) contacting a cancer cell with at least one MDM2i; b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i and expression of at least one biomarker indicates sensitivity to an MDM2i; and c) comparing the cell proliferation of cancer cells treated with at

least one MDM2i with an untreated or placebo treated cancer cell; wherein there is reduced cell proliferation of at the treated cancer cell when compared with the untreated or placebo treated cancer cell.

[00109] The method wherein the reduced cell proliferation of the cancer cell contacted with at least one MDM2i has an IC₅₀ of less than 1 μ M.

[00110] The method wherein the MDM2i is an isoquinolinone.

[00111] The method wherein the MDM2i is selected from Table 1.

[00112] The method wherein the cell is contacted by the MDM2i at least two different time points.

[00113] The method wherein the cell is contacted by two different MDM2i at step a).

[00114] The method wherein the cell is contacted by the two different MDM2i at the same time.

[00115] The method wherein the cell is contacted by two different MDM2i at different time points.

[00116] The method wherein the cancer cell is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[00117] The method wherein an mRNA or protein of at least one biomarker is measured.

[00118] The method comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[00119] The method wherein the steps b) and c) are repeated at time points selected from the group consisting of: 4 hours, 8 hours, 16 hours, 24 hours, 48 hours, 3 days, 1 week, 1 month and several months after administration of each dose of MDM2i.

[00120] A method of screening for MDM2i candidates the method comprising:
a) contacting a cell with a MDM2i candidate;
b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i candidate and expression of the at least one biomarker indicates sensitivity to an MDM2i; and c) comparing the reduction in cell proliferation from the cell contacted with the MDM2i candidate with the reduction in cell proliferation of a cell contacted with an MDM2i taken from Table 1 and the cell proliferation of an untreated or placebo treated cell.

[00121] The method wherein assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[00122] The method wherein the MDM2i candidate increases gene expression of at least one biomarker of Table 2.

[00123] The method wherein the cell is a cancer cell selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[00124] The method wherein the expression of an mRNA or protein of at least one biomarker of Table 2 is measured.

[00125] The method comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[00126] Composition comprising an MDM2i for use in treatment of cancer in a selected cancer patient population, wherein the cancer patient population is selected on the basis of showing gene expression of at least one biomarker selected from Table 2 in a cancer cell sample obtained from said patients.

[00127] The composition wherein the cancer cells sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura

[00128] The composition wherein the patients are selected on the basis of gene expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

[00129] A kit for predicting the sensitivity of a cancer patient for treatment with a Human Double Minute 2 inhibitor (MDM2i) comprising
i) means for detecting the expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN; and ii) instructions how to use said kit.

[00130] Use of the kit for any of the methods of listed above.

[00131] **Definitions**

[00132] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

[00133] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about." It also is to be understood,

although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[00134] The terms “marker” or “biomarker” are used interchangeably herein. A biomarker is a nucleic acid or polypeptide and the presence, absence or differential expression of the nucleic acid or polypeptide is used to determine sensitivity to any MDM2i. For example, CDKN1A is a biomarker.

[00135] “MDM2” refers to an E3 ubiquitin-protein ligase that mediates the ubiquitination of p53, permits the nuclear export of p53 and triggers p53 degradation. Unless specifically stated otherwise, MDM2 as used herein refers to human MDM2- accession numbers NM_002392 / NP_002383 (SEQ ID NO. 1/ SEQ ID NO. 2).

[00136] “p53” refers to the tumor suppressor protein, and refers to human p53 (p53 accession numbers: DNA - NM_000546, protein - NP_000537).

[00137] A cell is “sensitive” or displays “sensitivity” for inhibition with an MDM2i when at least one of the biomarkers disclosed in Table 2 is differentially expressed. Alternatively, a cell is “sensitive” for inhibition with an MDM2i when all of the biomarkers disclosed in Table 2 as a set are differentially expressed. In one embodiment a cell is “sensitive” or displays “sensitivity”, or is “sensitive to treatment” when IC₅₀ of an MDM2i for reducing cell proliferation is less than 10 μ M, preferably less than 1 μ M.

[00138] A “control cell”, a “control tissue” and a “control sample” refer to baseline control, or a cancer cell, tissue or a sample, respectively, that is insensitive to MDM2i (i.e. IC₅₀ of a MDM2i for reducing cell proliferation is more than 10 μ M; or when “sensitive” is defined with IC₅₀ of a MDM2i for reducing cell proliferation being less than 1 μ M, then “insensitive” denotes IC₅₀ of a MDM2i for reducing cell proliferation is more than 1 μ M).

[00139] A “normal sample” refers to non-cancerous tissue or cell.

[00140] The terms “nucleic acid” and “polynucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polymer. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and

single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[00141] A "gene" refers to a polynucleotide containing at least one open reading frame (ORF) that is capable of encoding a particular polypeptide or protein after being transcribed and translated. A polynucleotide sequence can be used to identify larger fragments or full-length coding sequences of the gene with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art.

[00142] "Gene expression" or alternatively a "gene product" refers to the nucleic acids or amino acids (e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

[00143] The term "polypeptide" is used interchangeably with the term "protein" and in its broadest sense refers to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits can be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc.

[00144] As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, and both the D and L optical isomers, amino acid analogs, and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

[00145] The term "isolated" means separated from constituents, cellular and otherwise, in which the polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, are normally associated with in nature. For example, an isolated polynucleotide is separated from the 3' and 5' contiguous nucleotides with which it is normally associated within its native or natural environment, e.g., on the chromosome. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody, or fragment(s) thereof, does not require "isolation" to distinguish it from its naturally occurring counterpart. In addition, a "concentrated," "separated" or "diluted" polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, is distinguishable from its naturally occurring counterpart in that the concentration or number of molecules per volume is greater in a "concentrated" version or less than in a "separated" version than that of its naturally occurring counterpart. A polynucleotide, peptide, polypeptide, protein, antibody, or fragment(s) thereof, which differs from the naturally occurring counterpart in its primary sequence or, for example, by its glycosylation pattern, need not be present in its isolated form since it is distinguishable from its naturally occurring counterpart by its primary sequence or, alternatively, by another characteristic such as glycosylation pattern. Thus, a

non-naturally occurring polynucleotide is provided as a separate embodiment from the isolated naturally occurring polynucleotide. A protein produced in a bacterial cell is provided as a separate embodiment from the naturally occurring protein isolated from a eukaryotic cell in which it is produced in nature.

[00146] A "probe" when used in the context of polynucleotide manipulation refers to an oligonucleotide that is provided as a reagent to detect a target potentially present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes.

[00147] A "primer" is a short polynucleotide, generally with a free 3'-OH group that binds to a target or "template" potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "pair of primers" or a "set of primers" consisting of an "upstream" and a "downstream" primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and taught, for example in PCR: A Practical Approach, M. MacPherson et al., IRL Press at Oxford University Press (1991). All processes of producing replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "replication." A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition (1989)).

[00148] As used herein, "expression" refers to the process by which DNA is transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently translated into peptides, polypeptides or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[00149] "Differentially expressed" as applied to a gene, refers to the differential production of the mRNA transcribed and/or translated from the gene or the protein product encoded by the gene. A differentially expressed gene may be overexpressed or underexpressed as compared to the expression level of a control cell. However, as used herein, overexpression is an increase in gene expression and generally is at least 1.25 fold or, alternatively, at least 1.5 fold or, alternatively, at least 2 fold, or alternatively, at least 3 fold or alternatively, at least 4 fold expression over that detected in a control counterpart cell or tissue. As used herein, underexpression, is a reduction of gene expression and generally is at least 1.25 fold, or alternatively, at least 1.5 fold, or alternatively, at least 2 fold or alternatively, at least 3 fold or alternatively, at least 4 fold expression under that detected in a

control counterpart cell or tissue. The term "differentially expressed" also refers to where expression in a cancer cell or cancerous tissue is detected and present but expression in a control cell is undetectable.

[00150] A high expression level of the gene may occur because of over expression of the gene or an increase in gene copy number. The gene may also be translated into increased protein levels because of deregulation or absence of a negative regulator.

[00151] A "gene expression profile" refers to a pattern of expression of at least one biomarker that recurs in multiple samples and reflects a property shared by those samples, such as tissue type, response to a particular treatment, or activation of a particular biological process or pathway in the cells. Furthermore, a gene expression profile differentiates between samples that share that common property and those that do not with better accuracy than would likely be achieved by assigning the samples to the two groups at random. A gene expression profile may be used to predict whether samples of unknown status share that common property or not. Some variation between the levels of at least one biomarker and the typical profile is to be expected, but the overall similarity of the expression levels to the typical profile is such that it is statistically unlikely that the similarity would be observed by chance in samples not sharing the common property that the expression profile reflects.

[00152] The term "cDNA" refers to complementary DNA, i.e. mRNA molecules present in a cell or organism made into cDNA with an enzyme such as reverse transcriptase. A "cDNA library" is a collection of all of the mRNA molecules present in a cell or organism, all turned into cDNA molecules with the enzyme reverse transcriptase, then inserted into "vectors" (other DNA molecules that can continue to replicate after addition of foreign DNA). Exemplary vectors for libraries include bacteriophage (also known as "phage"), viruses that infect bacteria, for example, lambda phage. The library can then be probed for the specific cDNA (and thus mRNA) of interest.

[00153] As used herein, "solid phase support" or "solid support", used interchangeably, is not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, plastic beads, alumina gels, microarrays, and chips. As used herein, "solid support" also includes synthetic antigen-presenting matrices, cells, and liposomes. A suitable solid phase support may be selected on the basis of desired end use and suitability for various protocols. For example, for peptide synthesis, solid phase support may refer to resins such as polystyrene (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories), polyHIPE(R)[™] resin (obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene

resin grafted with polyethylene glycol (TentaGelR™, Rapp Polymere, Tubingen, Germany), or polydimethylacrylamide resin (obtained from Milligen/Bioscience, California).

[00154] A polynucleotide also can be attached to a solid support for use in high throughput screening assays. PCT WO 97/10365, for example, discloses the construction of high density oligonucleotide chips. See also, U.S. Pat. Nos. 5,405,783; 5,412,087 and 5,445,934. Using this method, the probes are synthesized on a derivatized glass surface to form chip arrays. Photoprotected nucleoside phosphoramidites are coupled to the glass surface, selectively deprotected by photolysis through a photolithographic mask and reacted with a second protected nucleoside phosphoramidite. The coupling/deprotection process is repeated until the desired probe is complete.

[00155] As an example, transcriptional activity can be assessed by measuring levels of messenger RNA using a gene chip such as the Affymetrix® HG-U133-Plus-2 GeneChips. High-throughput, real-time quantitation of RNA of a large number of genes of interest thus becomes possible in a reproducible system.

[00156] The terms "stringent hybridization conditions" refers to conditions under which a nucleic acid probe will specifically hybridize to its target subsequence, and to no other sequences. The conditions determining the stringency of hybridization include: temperature, ionic strength, and the concentration of denaturing agents such as formamide. Varying one of these factors may influence another factor and one of skill in the art will appreciate changes in the conditions to maintain the desired level of stringency. An example of a highly stringent hybridization is: 0.015M sodium chloride, 0.0015M sodium citrate at 65-68 °C or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42 °C (see Sambrook, *supra*). An example of a "moderately stringent" hybridization is the conditions of: 0.015M sodium chloride, 0.0015M sodium citrate at 50-65 °C or 0.015M sodium chloride, 0.0015M sodium citrate, and 20% formamide at 37-50 °C. The moderately stringent conditions are used when a moderate amount of nucleic acid mismatch is desired. One of skill in the art will appreciate that washing is part of the hybridization conditions. For example, washing conditions can include 0.2X-0.1X SSC/0.1% SDS and temperatures from 42-68 °C, wherein increasing temperature increases the stringency of the wash conditions.

[00157] When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary." A double-stranded polynucleotide can be "complementary" or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. "Complementarity" or "homology" (the degree that one polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to form hydrogen bonding with each other, according to generally accepted base-pairing rules.

[00158] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 80%, 85%, 90%, 95%, 98% or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols in Molecular Biology, Ausubel et al., eds., (1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant.

[00159] The term "cell proliferative disorders" shall include dysregulation of normal physiological function characterized by abnormal cell growth and/or division or loss of function. Examples of "cell proliferative disorders" include but are not limited to hyperplasia, neoplasia, metaplasia, and various autoimmune disorders, e.g., those characterized by the dysregulation of T cell apoptosis.

[00160] As used herein, the terms "neoplastic cells," "neoplastic disease," "neoplasia," "tumor," "tumor cells," "cancer," and "cancer cells," (used interchangeably) refer to cells which exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation (i.e., de-regulated cell division). Neoplastic cells can be malignant or benign. A metastatic cell or tissue means that the cell can invade and destroy neighboring body structures.

[00161] The term "cancer" refers to cancer diseases including, for example, breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

[00162] The term "PBMC" refers to peripheral blood mononuclear cells and includes "PBL" - peripheral blood lymphocytes.

[00163] "Suppressing" tumor growth indicates a reduction in tumor cell growth when contacted with an MDM2i compared to tumor growth without contact with an MDM2i compound. Tumor cell growth can be assessed by any means known in the art, including, but not limited to, measuring tumor size, determining whether tumor cells are proliferating using a 3H-thymidine incorporation assay, measuring glucose uptake by FDG-PET (fluorodeoxyglucose positron emission tomography) imaging, or counting tumor cells. "Suppressing" tumor cell growth means any or all of the following states: slowing, delaying and stopping tumor growth, as well as tumor shrinkage.

[00164] A "composition" is a combination of active agent and another carrier, e.g., compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Carriers also include pharmaceutical excipients and additives, for example; proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Carbohydrate excipients include, for example; monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

[00165] Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like.

[00166] The term "carrier" further includes a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Additional carriers include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl-quadrature-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as TWEEN 20™ and TWEEN 80™), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

[00167] As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives and any of the above noted carriers with the additional proviso that they be acceptable for use in vivo. For examples of carriers, stabilizers and adjuvants, see Remington's Pharmaceutical Science., 15th Ed. (Mack Publ. Co., Easton (1975) and in the Physician's Desk Reference, 52nd ed., Medical Economics, Montvale, N.J. (1998).

[00168] An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

[00169] A "subject," "individual" or "patient" is used interchangeably herein, which refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, mice, simians, humans, farm animals, sport animals, and pets.

[00170] An "inhibitor" of MDM2 as used herein reduces the association of p53 and MDM2. This inhibition may include, for example, reducing the association of p53 and MDM2 before they are bound together, or reducing the association of p53 and MDM2 after they are bound together, thus freeing both molecules.

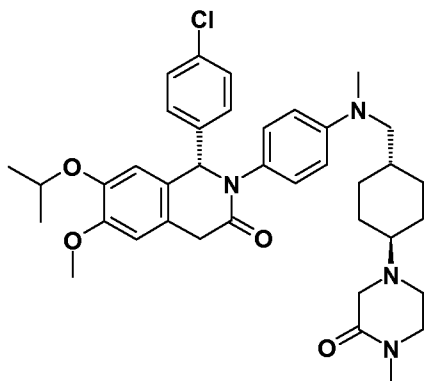
[00171] A number of genes have now been identified as biomarkers for MDM2i. The presence or decrease or increase of gene expression of one or more of the biomarkers identified herein and in Table 2 can be used to determine patient sensitivity to any MDM2i, for example, the expression of a biomarker indicates that a cancer patient is sensitive to and would favorably respond to administration of an MDM2i. As another example, after treatment with an MDM2i, a patient sample can be obtained and the sample assayed for sensitivity to discover if the patient is still sensitive to the MDM2i treatment. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

[00172] MDM2 inhibitors (MDM2i) are compounds which are inhibitors of the p53 - MDM2 association, and are useful in conjunction with the methods or uses of the invention. MDM2i are useful in pharmaceutical compositions for human or veterinary use where inhibition of the p53 - MDM2 association is indicated, e.g., in the treatment of tumors and/or cancerous cell growth. In particular, such compounds are useful in the treatment of human cancer, since the progression of these cancers may be at least partially dependent upon overriding the "gatekeeper" function of p53, for example the overexpression of MDM2. MDM2i compounds are useful in treating, for example, carcinomas (e.g., breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura). A listing of exemplary MDM2i compounds is found in Table 1 (see WO 2011076786). The MDM2i compounds found in Table 1 are of the isoquinolinone class of molecules. Other MDM2i that bind to a p53 binding pocket of MDM2, particularly to substantially the same p53 binding pocket of MDM2 as Nutlin-3a or substantially where the exemplary MDM2i from the Table 1 binds, can also be applied in the methods or uses of the invention. MDM2i used according to present embodiments can be structurally related to the one described in Table 1 (i.e. MDM2i(1) and MDM2i(2)) or to Nutlin 3a, such as, for example, substituted isoquinolinones, or quinazolinones. The methods included herein can also be used with other compounds such as the spiro-oxindoles,

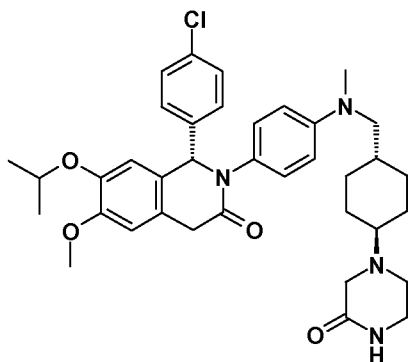
imidazolyl indole and cis-imidazoline (see Shangary et al., Mol. Cancer Ther. 2008 7(6): 1533-1542; Furet et al., BioOrg.Med.Chem. Let. 2012 22:3498-3502 and Carol et al., Pediatr. Blood Cancer 2012 pages 1-9, published online July 2, 2012, prior to inclusion into journal). MDM2i as used herein prevents the protein-protein interaction between p53 and MDM2 and/or inhibits cell proliferation by inducing the p53 pathway activity.

[00173] Table 1 - MDM2i compounds

[00174] MDM2i(1)



[00175] MDM2i(2)



[00176] Measurement of Gene Expression

[00177] Detection of gene expression can be by any appropriate method, including for example, detecting the quantity of mRNA transcribed from the gene or the quantity of cDNA produced from the reverse transcription of the mRNA transcribed from the gene or the quantity of the polypeptide or protein encoded by the gene. These methods can be performed on a sample by sample basis or modified for high throughput analysis. For example, using Affymetrix™ U133 microarray chips.

[00178] In one aspect, gene expression is detected and quantitated by hybridization to a probe that specifically hybridizes to the appropriate probe for that biomarker. The probes also can be attached to a solid support for use in high throughput screening assays using

methods known in the art. WO 97/10365 and U.S. Pat. Nos. 5,405,783, 5,412,087 and 5,445,934, for example, disclose the construction of high density oligonucleotide chips which can contain one or more of the sequences disclosed herein. Using the methods disclosed in U.S. Pat. Nos. 5,405,783, 5,412,087 and 5,445,934, the probes of this invention are synthesized on a derivatized glass surface. Photoprotected nucleoside phosphoramidites are coupled to the glass surface, selectively deprotected by photolysis through a photolithographic mask, and reacted with a second protected nucleoside phosphoramidite. The coupling/deprotection process is repeated until the desired probe is complete.

[00179] In one aspect, the expression level of a gene is determined through exposure of a nucleic acid sample to the probe-modified chip. Extracted nucleic acid is labeled, for example, with a fluorescent tag, preferably during an amplification step. Hybridization of the labeled sample is performed at an appropriate stringency level. The degree of probe-nucleic acid hybridization is quantitatively measured using a detection device. See U.S. Pat. Nos. 5,578,832 and 5,631,734.

[00180] Alternatively any one of gene copy number, transcription, or translation can be determined using known techniques. For example, an amplification method such as PCR may be useful. General procedures for PCR are taught in MacPherson et al., PCR: A Practical Approach, (IRL Press at Oxford University Press (1991)). However, PCR conditions used for each application reaction are empirically determined. A number of parameters influence the success of a reaction. Among them are annealing temperature and time, extension time, Mg²⁺ and /or ATP concentration, pH, and the relative concentration of primers, templates, and deoxyribonucleotides. After amplification, the resulting DNA fragments can be detected by agarose gel electrophoresis followed by visualization with ethidium bromide staining and ultraviolet illumination.

[00181] In one embodiment, the hybridized nucleic acids are detected by detecting one or more labels attached to the sample nucleic acids. The labels can be incorporated by any of a number of means well known to those of skill in the art. However, in one aspect, the label is simultaneously incorporated during the amplification step in the preparation of the sample nucleic acid. Thus, for example, polymerase chain reaction (PCR) with labeled primers or labeled nucleotides will provide a labeled amplification product. In a separate embodiment, transcription amplification, as described above, using a labeled nucleotide (e.g. fluorescein-labeled UTP and/or CTP) incorporates a label in to the transcribed nucleic acids.

[00182] Alternatively, a label may be added directly to the original nucleic acid sample (e.g., mRNA, polyA, mRNA, cDNA, etc.) or to the amplification product after the amplification is completed. Means of attaching labels to nucleic acids are well known to those of skill in the art and include, for example nick translation or end-labeling (e.g. with a labeled RNA) by

kinasing of the nucleic acid and subsequent attachment (ligation) of a nucleic acid linker joining the sample nucleic acid to a label (e.g., a fluorophore).

[00183] Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads (e.g., Dynabeads™), fluorescent dyes (e.g., fluorescein, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P) enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

[00184] Detection of labels is well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and calorimetric labels are detected by simply visualizing the colored label.

[00185] The detectable label may be added to the target (sample) nucleic acid(s) prior to, or after the hybridization, such as described in WO 97/10365. These detectable labels are directly attached to or incorporated into the target (sample) nucleic acid prior to hybridization. In contrast, "indirect labels" are joined to the hybrid duplex after hybridization. Generally, the indirect label is attached to a binding moiety that has been attached to the target nucleic acid prior to the hybridization. For example, the target nucleic acid may be biotinylated before the hybridization. After hybridization, an avidin-conjugated fluorophore will bind the biotin bearing hybrid duplexes providing a label that is easily detected. For a detailed review of methods of labeling nucleic acids and detecting labeled hybridized nucleic acids see Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 24: Hybridization with Nucleic Acid Probes, P. Tijssen, ed. Elsevier, N.Y. (1993).

[00186] **Detection of polypeptides**

[00187] Expression level of the biomarker can also be determined by examining protein expression or the protein product at least one of the biomarkers listed in Table 2. Determining the protein level involves measuring the amount of any immunospecific binding that occurs between an antibody that selectively recognizes and binds to the polypeptide of the biomarker in a sample obtained from a patient and comparing this to the amount of immunospecific binding of at least one biomarker in a control sample. The amount of protein

expression of the biomarker can be increased or reduced when compared with control expression. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

[00188] A variety of techniques are available in the art for protein analysis. They include but are not limited to radioimmunoassays, ELISA (enzyme linked immunosorbent assays), "sandwich" immunoassays, immunoradiometric assays, in situ immunoassays (using e.g., colloidal gold, enzyme or radioisotope labels), western blot analysis, immunoprecipitation assays, immunofluorescent assays, flow cytometry, immunohistochemistry, confocal microscopy, enzymatic assays, surface plasmon resonance and PAGE-SDS.

[00189] Assaying for biomarkers and MDM2i treatment

[00190] Once a patient has been predicted to be sensitive to an MDM2i, administration of any MDM2i to a patient can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents may be empirically adjusted.

[00191] At least one of the biomarkers provided in Table 2 can be assayed for after MDM2i administration in order to determine if the patient remains sensitive to the MDM2i treatment. In addition, at least one biomarker can be assayed for in multiple time points after a single MDM2i administration. For example, an initial bolus of an MDM2i is administered, at least one biomarker from Table 2 is assayed for at 1 hour, 2 hours, 3 hours, 4 hours, 8 hours, 16 hours, 24 hours, 48 hours, 3 days, 1 week or 1 month or several months after the first treatment. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

[00192] The at least one biomarker in Table 2 can be assayed for after each MDM2i administration, so if there are multiple MDM2i administrations, then at least one biomarker can be assayed for after each administration to determine continued patient sensitivity. The patient could undergo multiple MDM2i administrations and the biomarkers then assayed at different time points. For example, a course of treatment can require administration of an initial dose of MDM2i, a second dose a specified time period later, and still a third dose hours after the second dose. At least one biomarker of Table 2 could be assayed for at 1 hour, 2 hours, 3 hours, 4 hours, 8 hours, 16 hours, 24 hours, 48 hours, 3 days, 1 week or 1 month or several months after administration of each dose of MDM2i. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

[00193] It is also within the scope of the invention that different biomarkers are assayed for at different time points. Without being bound to any one theory, due to mechanism of action of the MDM2i or of the biomarker, the response to the MDM2i is delayed and at least one biomarker from Table 2 is assayed for at any time after administration to determine if the patient remains sensitive to MDM2i administration. An assay for at least one biomarker in Table 2 after each administration of MDM2i will provide guidance as to the means, dosage and course of treatment. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

[00194] Finally, there is administration of different MDM2is and followed by assaying for at least one biomarker in Table 2. In this embodiment, more than one MDM2i is chosen and administered to the patient. At least one biomarker from Table 2 can then be assayed for after administration of each different MDM2i. This assay can also be done at multiple time points after administration of the different MDM2i. For example, a first MDM2i could be administered to the patient and at least one biomarker assayed at 1 hour, 2 hours, 3 hours, 4 hours, 8 hours, 16 hours, 24 hours, 48 hours, 3 days, 1 week or 1 month or several months after administration. A second MDM2i could then be administered and at least one biomarker could be assayed for again at 1 hour, 2 hours, 3 hours, 4 hours, 8 hours, 16 hours, 24 hours, 48 hours, 3 days, 1 week or 1 month or several months after administration of the second MDM2i. In each case, all of the biomarkers in Table 2 can be assayed for as a single set.

[00195] Another aspect of the invention provides for a method of assessing for suitable dose levels of an MDM2i, comprising monitoring the differential expression of at least one of the genes identified in Table 2 after administration of the MDM2i. For example, after administration of a first bolus of MDM2i, at least one biomarker of Table 2 is analyzed and based on this result, an increase or decrease in MDM2i dosage is recommended. After administration of the adjusted dosage of MDM2i the analysis of at least one biomarker will determine whether the patient is still sensitive to the adjusted dose and that the adjusted dose is providing the expected benefit, e.g., suppressing tumor growth. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set for assessing sensitivity to the dose of the MDM2i.

[00196] Kits for assessing the activity of any MDM2i can be made. For example, a kit comprising nucleic acid primers for PCR or for microarray hybridization for the biomarkers listed in Table 2 can be used for assessing MDM2i sensitivity. Alternatively, a kit supplied with antibodies for at least one of the biomarkers listed in Table 2 would be useful in assaying for MDM2i sensitivity.

[00197] It is well known in the art that cancers can become resistant to chemotherapeutic treatment, especially when that treatment is prolonged. Assaying for

differential expression of at least one of the biomarkers in Table 2 can be done after prolonged treatment with any chemotherapeutic to determine if the cancer is sensitive to the MDM2i. For example, kinase inhibitors such as Gleevec® will strongly inhibit a specific kinase, but may also weakly inhibit other kinases. There are also other MDM2i, for example, the Nutlin family of compounds. If the patient has been previously treated with another chemotherapeutic or another MDM2i, it is useful information for the patient to assay for at least one of the biomarkers in Table 2 to determine if the tumor is sensitive to an MDM2i. This assay can be especially beneficial to the patient if the cancer goes into remission and then re-grows or has metastasized to a different site.

[00198] Screening for MDM2 inhibitors

[00199] It is possible to assay for at least one biomarker listed in Table 2 to screen for other MDM2i. This method comprises assaying a cell with at least one biomarker from Table 2, which predicts if the cell is sensitive to an MDM2i candidate inhibitor, the cell is then contacted with the candidate MDM2i and the IC₅₀ of the treated cell is compared with a known MDM2i contacting a sensitive cell. For example, for cells predicted to be sensitive to any MDM2i as determined by the differential expression of at least one biomarker in Table 2, the candidate MDM2i will have an IC₅₀ ≤ 3 μM. The measurement of at least one biomarker from Table 2 expression can be done by methods described previously, for example, PCR or microarray analysis. Alternatively, all of the biomarkers in Table 2 can be assayed for as a single set.

TABLE 2

Gene Name	Accession number	SEQ ID NO. (nucleotide/protein)
MDM2	NM_002392 / NP_002383	SEQ ID NO. 1/ SEQ ID NO. 2
CDKN1A	NM_000389 / NP_000380	SEQ ID NO. 3/ SEQ ID NO. 4
ZMAT3	NM_022470 / NP_071915	SEQ ID NO. 5/ SEQ ID NO. 6
DDB2	NM_000107 / NP_000098	SEQ ID NO. 7/ SEQ ID NO. 8
FDXR	NM_004110 / NP_004101	SEQ ID NO. 9/ SEQ ID NO. 10
RPS27L	NM_015920 / NP_057004	SEQ ID NO. 11/ SEQ ID NO. 12
BAX	NM_004324 / NP_004315	SEQ ID NO. 13/ SEQ ID NO. 14
RRM2B	NM_015713 / NP_056528	SEQ ID NO. 15/ SEQ ID NO. 16

SESN1	NM_014454 / NP_055269	SEQ ID NO. 17/ SEQ ID NO. 18
CCNG1	NM_004060 / NP_004051	SEQ ID NO. 19/ SEQ ID NO. 20
XPC	NM_004628 / NP_004619	SEQ ID NO. 21/ SEQ ID NO. 22
TNFRSF10B	NM_003842 / NP_003833	SEQ ID NO. 23/ SEQ ID NO. 24
AEN	NM_022767 / NP_073604	SEQ ID NO. 25/ SEQ ID NO. 26

EXAMPLES

[00200] Example 1: Both MDM2i(1) and MDM2i(2) are equally potent p53-MDM2 inhibitors in biochemical and cellular assays.

[00201] *TR-FRET Assay for IC₅₀ determination:* standard assay conditions consisted of 60 µL total volume in white 384-well plates (Greiner Bio-One: Frickenhausen, Germany), in PBS buffer containing 125 mM NaCl, 0.001% Novexin, 0.01% Gelatin, 0.2% Pluronic F-127, 1 mM DTT and 1.7% final DMSO). Both MDM2i(1) and MDM2i(2) were added at different concentrations to 0.1 nM biotinylated MDM2 (human MDM2 amino acids 2-188, internal preparations), 0.1 nM Europium-labeled streptavidin (Perkin Elmer: Waltham, MA, USA) and 10 nM Cy5-p53 peptide (Cy5-p53 aa18-26, internal preparation). After incubation at room temperature for 15 minutes, samples were measured on a GeniosPro reader (Tecan: Mannedorf, Germany). FRET assay readout was calculated from the raw data of the two distinct fluorescence signals measured in time resolved mode (fluorescence 665 nm/fluorescence 620 nm x 1000). IC₅₀ values are calculated by curve fitting using XLfit® (Fit Model #205). This data is shown in Figure 1A.

[00202] *Determination of binding rate constants (K_{on}, K_{off}):* the rapid mixing tool of GeniosPro reader (Tecan: Mannedorf, Germany) was used to study fast binding kinetics (single well mode). Microplates containing the inhibitor and 20 nM Cy5-labeled p53 peptide in 50 µl assay buffer were placed in the reader. After 10 min equilibration at 25°C, binding reactions were initiated by injecting 50 µl of buffer containing 0.2 nM biotinylated MDM2 and 0.2 nM europium-streptavidin at 475 µl/s. Fluorescence was measured at 665 nm and at various time intervals, the first one 0.6 sec after injection. In the absence of inhibitor, Cy5 fluorescence was maximal already at 0.6 sec and remained stable for at least 15 min. In the presence of MDM2i(1) and MDM2i(2) fluorescence decreased slowly and measurements were made until steady-state was achieved. Control fluorescence was taken as the difference between wells containing 1% DMSO and wells containing 10 µM Nutlin-3 as a control. The inhibitory effect at each time point was calculated as percent of the corresponding control. Progress curves obtained in the presence of different concentrations

of inhibitor were combined and fitted as a whole. Nonlinear regression was performed with XLfit® using a novel fit methodology that was designed to obtain precise K_{on} and K_{off} values, based on the following respective equations: $Fit = [I_{min} + ((I_{max} - (((K_i^{nH}) * I_{max}) / ((y^{nH}) + (K_i^{nH})))) * (1 - \exp(((-1) * (k_{off} + ((y * k_{off}) / K_i))) * x)))]$ and $Fit = [I_{min} + ((I_{max} - (((K_i^{nH}) * I_{max}) / ((y^{nH}) + (K_i^{nH})))) * (1 - \exp(((-1) * (k_{on} * (K_i + y))) * x)))]$, where K_i represents the constant of inhibition, I_{min} represents the minimum inhibition (in %), I_{max} represents the maximum inhibition (in %), nH represents the Hill coefficient, x represents the time and y represents the inhibitor concentration). This data is shown in Figure 1A.

[00203] *Cell proliferation inhibition and GRIP p53 translocation assay:* Effects of MDM2i(1) and MDM2i(2) on cellular growth and loss of viability is measured in both p53 wild-type (SJSA-1 and HCT116 p53^{wt/wt} cells) and p53 mutant cell lines (SAOS2 and HCT116 p53^{-/-} cells) using a standard proliferation assay based on the DNA-interacting fluorescent dye YOPRO (Invitrogen: Lucern, Switzerland). Briefly, cells are plated in 96-well plates overnight at 37°C and are treated with increasing concentrations of MDM2i(1) or MDM2i(2) for 72 hours. Cell concentration in each well is then determined using the DNA-interacting fluorescent dye YOPRO according to the manufacturer's instructions and the fluorescent signal is measured using a Gemini-EM standard plate reader (Molecular Devices: Sunnyvale, CA, USA). IC₅₀ values are calculated by curve fitting using XLfit® (Fit Model #201) and this data is shown in Figure 1A.

[00204] The mechanistic p53-MDM2 Redistribution assay (GRIP assay) is used to directly monitor in cells the ability of compounds to modulate the p53-MDM2 protein-protein interaction. In this fully engineered assay, the p53 protein is tagged with a fluorescent GFP-label and is bound to MDM2 protein which is anchored in the cytoplasm of the cells. The treatment of the cells with specific compounds causes the dissociation of the interaction between the two proteins and the translocation of the released p53-GFP protein from the cytoplasm to the nuclei. This effect is detected and quantified using a high content imaging platform using the ArrayScan-VTi (Cellomics), following the fluorescent signal over time (see Figure 1B, GRIP p53 translocation assay).

[00205] Altogether, using both *in vitro* and cellular assays, the results presented in Figures 1A and 1B show that both MDM2i(1) and MDM2i(2) are comparable potent p53-MDM2 protein-protein interaction inhibitors *in vitro*, inhibiting the p53-MDM2 protein-protein interaction, hampering tumor cell proliferation in a p53-dependent manner, and inducing p53 accumulation and translocation to the nucleus. This data is shown in Figure 1B; note that there are large discrepancies in the IC₅₀ between the cell lines. This is also an indication that there are differences in the sensitivity between the two cell lines to an MDM2i, and thus a determination of sensitivity can be useful in determining which patients receive the therapeutic.

[00206] Example 2: The p53 mutational status is associated with MDM2i chemical sensitivity in cell lines

[00207] The association of p53 mutation to MDM2i(2) chemical sensitivity in a panel of cancer-relevant cell lines was tested by Fisher's Exact test. The cell line panel is the one covered by the Cancer Cell Line Encyclopedia (CCLE) initiative (Barretina J., Caponigro G., Stransky N., Venkatesan K., *et al.* The Cancer Cell Line Encyclopedia enables predictive modeling of anticancer drug sensitivity. *Nature* 483:603-7, 2012). A detailed genomic, genetic and pharmacologic characterization was conducted on the CCLE cell lines.

[00208] p53 mutation status in CCLE cell lines is taken from a data source of gene-level genetic alterations, for example, point-mutations, insertions, deletions and complex genetic alterations, compiled from the Sanger center COSMIC data and internal sources including Exome Capture Sequencing. This comprises data from approximately 1,600 cancer-related genes over the CCLE cell lines. Analysis of the CCLE panel revealed 244 cell lines containing a mutant p53, and 112 cell lines that expressed wild type p53 (p53 accession numbers: DNA- NM_000546, protein - NP_000537).

[00209] The MDM2i(2) chemical sensitivity was determined from the pharmacologic characterization of the CCLE cell lines. The cell lines were separated in two groups according to MDM2i(2) sensitivity. One group contains the cell lines sensitive to MDM2i(2) compound, while the other group encompasses those being chemically insensitive to the MDM2i(2) compound. Such stratification resulted in two groups of 47 sensitive and 309 insensitive cell lines, respectively. From such *in vitro* chemical sensitivity data, the prediction that a cell would be sensitive to an MDM2i treatment was estimated to be 13%.

The statistical testing of the p53 mutation to sensitivity groups association shows an association between p53 mutation (mt) and the chemical sensitivity to MDM2i(2) (Figure 2). The mt panel in Figure 2 displays the MDM2i(2) sensitivity profiles for p53 mutated CCLE cell lines, the wild type (wt) panel displays the MDM2i(2) sensitivity profiles for p53 wild-type CCLE cell lines. Amax is defined as the maximal effect level (the inhibition at the highest tested MDM2i(2) concentration, calibrated to MG132, a proteasome inhibitor used as a reference, as described in the CCLE publication referenced above, and IC50 is defined as of the μ M concentration at which MDM2i(2) response reached an absolute inhibition of -50 with respect to the reference inhibitor.

Cell line count broken down by MDM2i(2) chemical sensitivity and p53 mutation status, and associated statistics. Data is also displayed as a contingency table with associated statistics.

[00210] This data indicates it is more likely for a cell line to show sensitivity to MDM2i(2) if its p53 status is wild type. Indeed, the majority of p53 mutated cell lines are found insensitive to the compound, whereas more than two-third of p53 wild type cell lines are sensitive.

[00211] From this data we can conclude a p53 wild type genotype is the first indication of MDM2i sensitivity, and therefore it is the first stratification biomarker to be considered for selecting cancer patients responsive to an MDM2i.

[00212] Example 3: Prediction of cell line chemical sensitivity to MDM2i from genomic data and clinical implication.

[00213] The two cell line sensitivity groups, given by MDM2i(2) treatment, are compared with the aim of identifying the biomarkers differentiating the sensitive cell lines from the insensitive cell lines, prior to any MDM2i treatment. Such biomarkers are used to predict the sensitivity of any MDM2i treatment. The biomarkers analyzed are the following types: 1) gene-level expression values generated by the Affymetrix GeneChip™ technology with the HG-U133 plus 2 array, summarized according to the RMA normalization method; 2) gene-level chromosome copy number values, obtained with the Affymetrix SNP6.0 technology (Affymetrix Santa Clara, CA, USA) and processed using the Affymetrix apt software, and expressed as log2 transformed ratios to a collection of HapMap reference normal samples; 3) gene-level genetic alterations or mutations, as described above in Example 2; 4) pathway-level expression values, summarizing pathway expression levels by a standardized average approach over the genes contributing to the pathways, as referenced in the GeneGo Metacore® knowledge base; 5) cell line lineage (cell line tissue of origin); 6) gene-level Tumor suppressor status, summarizing the activation status of a selection of tumor suppressor genes, by integrating the genetic alteration, copy number and expression information. Such genomic data was generated in the context of the CCLE cell line genomic and genetic characterization, and covers a total of about 45,000 genomic features.

[00214] Wilcoxon signed-rank tests or Fisher's exact tests are used to compare the two cell line group genomic features, depending on the feature type. The features having continuous values (gene expression and copy number, pathway expression features) are subjected to Wilcoxon signed-ranked test, those having discrete values (genetic alteration, tumor suppressor status and lineage features), to Fisher's exact test, for differential profile evaluation between sensitive and insensitive cell line groups. The significant features, discriminating the sensitive cell line group from the insensitive one, are the ones passing a false discovery rate-controlled p-value cutoff. Irrespective of the p-value limit, a minimum or maximum number of features per feature type are also required. To minimize the impact of the high degree of correlation among the features on the feature selection step, the feature data is clustered before the statistical tests as a pre-processing step. This step is performed at the feature type-level using the Frey's and Dueck's Affinity Propagation method (Clustering by Passing Messages Between Data Points. Frey B. and Dueck D. Science 315:972-6, 2007), and retrieves a set of features representing the most variability.

[00215] The cell line sensitivity groups or classes are defined as follows for this two-class comparison aiming at biomarker identification: a sensitive group of 47 sensitive cell lines, and an insensitive group of 204 insensitive cell lines. The 204 cell lines making this insensitive group are the most insensitive ones from the 309 insensitive cell line set mentioned in Example 2. This feature selection step yielded a total of about 200 significant features, having a significant differential profile to differentiate the sensitive cell line group from the insensitive one, and thus having the required properties to be considered as markers to predict the chemical sensitivity of samples to an MDM2i. As described above in Example 2, a relevant biomarker is the p53 mutation status itself. The statistics of the feature selection step (p-value $1.17\text{E-}21$) confirmed its role in predicting sensitivity to an MDM2i. Furthermore, the odds-ratio associated with p53 status (0.024) indicates p53 mutation is more represented in MDM2i insensitive cell lines. Still noteworthy and still according to the statistics of the feature selection step, most of the predictive biomarkers are found to be a subset of p53 transcriptional target genes. These are shown in Table 2/Figure 3. Their fold-changes indicate the transcripts of these biomarkers are more expressed in the MDM2i sensitive cell line population, as shown in Figure 3. This is likely indicative of a level of p53 functional activity pre-existing before any treatment in cell lines that are sensitive to an MDM2i.

[00216] That the biomarkers in Table 2/Figure 3 are reflective of a functional p53 pathway in MDM2i sensitive cells is verified in Figure 4. In Figure 4, cells have been treated with increasing concentrations of MDM2i(1) for 4 hours, prior to cell lysis. Whole cell lysates were prepared using a cell lysis buffer containing 50 mM Tris-HCl pH 7.5, 120 mM NaCl, 1 mM EDTA, 6 mM EGTA pH 8.5, 1% NP-40, 20 mM NaF, 1 mM PMSF and 0.5 mM Na-Vanadat, proteins were separated on NuPAGE 4-12% Bis-Tris Gel (Invitrogen # NP0322BOX, Lucerne; Switzerland), transferred onto Nitrocellulose Protran® BA 85 membranes (Whatman # 10 401 261; Piscataway, NJ, USA) at 1.5 mA/cm^2 membrane for 2 h using a semi-dry blotting system, and immunoblotted with either an anti-phospho-p53 (Ser¹⁵) (1/1000; Cell Signaling Technology #9284; Beverly, MA, USA) rabbit polyclonal antibody, or an anti-p53 (Ab-6) (Pantropic, clone DO-1,) (1/1000; Calbiochem # OP43 San Diego, CA, USA), an anti-MDM2 (Ab-1, clone IF2) (1/1000; Calbiochem # OP46), an anti-p21^{WAF1} (Ab-1, clone EA10) (1/500; Calbiochem # OP64; San Diego, CA, USA) or an anti- α -Tubulin (Sigma # T5168; St. Louis, MO, USA) mouse monoclonal antibodies, as indicated. As shown in Figure 4, increasing concentrations of MDM2i(1) induces stabilization of p53 protein levels, with no significant increase of phospho-p53 in both C3A and COLO 792 cells. Interestingly, treatment with MDM2i(1) also induces a strong *de novo* expression of both p53 target genes p21(CDKN1A) and MDM2 in C3A sensitive cells, but not in COLO-792 insensitive cell line. Altogether, this data indicates that sensitivity to an MDM2i inhibitor is

directly related to the presence of an intrinsic functional p53 pathway, that the biomarkers in Table 2/Figure 3, taken together as a set or alone, point directly to p53 pathway functionality before treatment, and indicates these biomarkers have a strong ability to predict patient sensitivity that correlates with the mechanism of action of p53.

[00217] The significant genomics features are used as the basis features for naïve Bayes probabilistic modeling of the two MDM2i chemical sensitivity groups, or classes. The goal of the modeling step is to derive a classification scheme or classifier that predicts the patient's response (either sensitive or insensitive) of an unknown sample with a certain confidence. The predictive model is defined by training a naïve Bayes algorithm over the entire chemically characterized CCLE cell line population stratified into the two above-mentioned sensitivity classes. The performance of the classifier is evaluated through 5 repeats of 5-fold cross-validations of the data used to train the model. The model performance is summarized with the following class-level measures: sensitivity, specificity, positive predictive value, and negative predictive value. The sensitive class is used as the reference for the sensitivity and positive predicted value calculations. The default output of the naïve Bayes algorithm is a score or probability, for each predicted sample to be assigned to one class or the other. A probability threshold is defined to transform the probability scores into a sensitive or insensitive class-level prediction. The probability threshold is defined as the probability maximizing the sensitivity and specificity calculated over all predicted samples. The entire and nearly-identical procedure is described in more details in the CCLE publication referenced in Example 2.

[00218] To demonstrate a better predictive power can be achieved from at least one biomarker found in Table 2, and alternatively, the biomarkers in Table 2 as a set, than from p53 mutation status alone, or from the entire predictive feature set of about 200 genes, naïve Bayes models from each of these feature sets were trained, and their performance assessed by cross-validation, as above mentioned, and compared (Figure 5). Figure 5 demonstrates the selected biomarkers found in Table 2 outperform both the p53 mutation status and the larger group of about 200 significant features, and provide a substantial improvement in predicting patient responsiveness to an MDM2i. This is particularly striking when performances are evaluated by positive predictive value (PPV).

[00219] A positive predicted value (PPV) of 76% suggests that 76% of the predicted sensitive cell lines will be sensitive to MDM2i treatment. As an extrapolation to a clinical setting, a PPV of 76% also suggests that 76% of cancer patients, predicted as sensitive to MDM2i(2) from tumor biopsies, would show clinical response upon MDM2i(2) treatment. This enrichment of clinical response by patient stratification, using the biomarkers in Table 2 and associated with the naïve Bayes predictive model, was compared to the baseline clinical response rate without prior patient stratification. The baseline clinical response rate

estimated from the chemical sensitivity data is 19%. Thus, in a clinical perspective; the biomarkers of Table 2 have a clear increase of the clinical response in the predicted sensitive patient population. This increase in prediction is greater and more specific than assaying for p53 status alone or all of the approximately 200 genomic features initially selected. This can be seen in Figure 5, where the "All 200 biomarkers" feature bar is the PPV of the approximately 200 genomic features initially selected. The PPV reported for "All 200 biomarkers" feature is 59%. The PPV for using p53 alone is 56% (Figure 5). The PPV of the set of biomarkers disclosed in Table 2 is 76% ("Table 2 selected biomarkers"). This is a surprising result, as in general, the larger data set of 200 genomic features would provide more data points and more insight into prediction of MDM2i sensitivity. However, this is not the case, as the biomarkers in Table 2, when taken as a set, provide a 17% increase in predictive value. This is important in the clinic, as now 17 additional patients out of 100 would be predicted to receive the correct treatment.

[00220] In order to test the predictive value of the biomarkers of Table 2, 52 cell lines that were not previously examined in the pharmacologic characterization as part of the CCLE project, were assayed for their sensitivity to MDM2i in proliferation assays. Briefly, cells are plated in 96-well plates overnight at 37°C and are treated with increasing concentrations of an MDM2i for 72 hours. Cell concentration in each well is then determined using the CellTiter-Glo Luminescent Cell Viability Assay® (Promega Cat. # G7571/2/3: Madison WI, USA), according to the manufacturer's instructions and the luminescent signal is measured using a SYNERGY HT plate reader (BioTek: Winooski, VT, USA). IC₅₀ values are calculated by curve fitting using XLfit® (Figure 6). Sensitivity of cell lines to an MDM2i is determined by comparing the observed IC₅₀ of all cells that were tested in cell proliferation inhibition assay as described in Example 1. The cut-off for sensitivity was determined at IC₅₀ ≤ 3 μM for both MDM2i(1) and MDM2i(2). Predictions of sensitivity for every cell lines are performed using the predictive model as described above, to confirm the values disclosed in Figure 5. The cell lines are from a variety of tumors. For example; melanoma (COLO-829, COLO-849, IGR-1, MEL-JUSO, SK-MEL-1, SK-MEL-31, UACC-62, UACC-257), leukemia (BV173, EOL-1, GDM-1, HuNS1, L-540, MV-4-11, OCI-LY3, RS4:11, SUP-B15, HDLM2, JM1), breast cancer (CAL51, EFM-192, HCC202), pancreatic cancer (DAN-G), hepatic cancer (JHH-5) and lung cancer (RERF-LCK-KJ). This assay was done with the all of the biomarkers disclosed in Table 2 as a single set. Overall, 36/52 cell lines were predicted to be sensitive to an MDM2i, and of these 36 cell lines 24 were sensitive, resulting in a positive predictive value of 66%, again a significant increase in predictive value (PPV) over assaying for p53 alone. With regard to screening out the non-responding cells, 16/52 cell lines were predicted to be insensitive to a p53-MDM2 inhibitor, and 13/16 were found to indeed be insensitive, leading to a significant negative predictive value (NPV) of 81%. Overall, these

data are similar to the predictive model performances described in Figure 5. The actual *in vitro* testing of unrelated cell lines allowed testing of the MDM2i chemical sensitivity predictive model, hence validating the biomarkers disclosed in Table 2.

[00221] Example 4: MDM2i treatment inhibits tumor growth inhibition *in vivo* which is correlated to a dose-dependent increase of p21(CDKN1A) mRNA and protein levels in tumors.

[00222] To further validate the predictive biomarkers disclosed in Figure 3, *in vivo* human xenograft models either from human primary samples or from cell lines were directly injected and grown in tumors subcutaneously in mice and then assessed for MDM2i sensitivity. All the animals were allowed to adapt for 4 days and housed in a pathogen-controlled environment (5 mice/Type III cage) with access to food and water *ad libitum*. Animals were identified with transponders. Studies were performed according to procedures covered by permit number 1975 issued by the Kantonales Veterinäramt Basel-Stadt and strictly adhered to the Eidgenössisches Tierschutzgesetz and the Eidgenössische Tierschutzverordnung. Subcutaneous tumors were induced by concentrating 3.0×10^6 SJSA-1 osteosarcoma cells in 100 μ l of PBS (without Ca^{2+} and Mg^{2+}) and injecting in the right flank of Harlan nude mice. The administration of MDM2i began 12-14 days post cell injection. MDM2i was prepared immediately before each administration. MDM2i compounds were dissolved in 0.5% HPMC (hydroxypropylmethylcellulose) and were injected daily (q24h) at 25, 50 or 100 mg/kg. Tumor volumes (TVol), determined from caliper measurements (using the formula $l \times w \times h \times \pi / 6$) were measured three times per week. Tumor response was quantified by the change in tumor volume (endpoint minus starting

value in mm^3) as the T/C, *i.e.* $\left(\frac{\Delta \text{TVol}_{\text{drug}}}{\Delta \text{TVol}_{\text{vehicle}}} \times 100 \right)$. In the case of a tumor regression, the

tumor response was quantified by the percentage of regression of the starting TVol, *i.e.*

$\left(\frac{\Delta \text{TVol}_{\text{drug}}}{\text{TVol}_{\text{Day0}}} \times 100 \right)$. The body-weight (BW) of the mice was measured three times per week

allowing calculation at any particular time-point relative to the day of initiation of treatment (day 0) of both the percentage change in BW ($\Delta\% \text{BW}$). As shown in Figure 7A, a 10-day treatment of SJSA-1 xenografted tumors with MDM2i(1) led to a dose-dependent tumor growth inhibition with a significant T/C of 50% at 25 mg/kg q24h and of 3% (stasis) at 50 mg/kg q24h. At 100 mg/kg q24h for 10 days, MDM2i(1) treatment induced a significant tumor regression of 65% (Figure 7B). All doses were well tolerated at q24h schedule, as indicated by the mean body weight curves over time.

[00223] Anti-tumor activity of MDM2i(1) was correlated with a significant dose-dependent induction of p21(CDKN1A) mRNA levels in tumors (Figure 7C). Briefly, total RNA was purified from cell pellets using the QIAshredder® (79654, Qiagen:Valencia CA, USA) and RNeasy Mini Kit® (74106, Qiagen: Valencia CA, USA) according to the manufacturer's instructions, with the exception that no DNA digestion was performed. Total RNA was eluted with 50 µL of RNase-free water. Total RNA was quantitated using the spectrophotometer ND-1000 Nanodrop® (Wilmington DE, USA). The qRT-PCR (Quantitative Reverse Transcriptase Polymerase Chain Reaction) was set up in triplicate per sample using the One-Step RT qPCR Master Mix Plus (RT-QPRT-032X, Eurogentec: Seraing, Belgium), with either control primers and primers for human p21(CDKN1A) (Hs00355782_m1, Applied Biosystems: Carlsbad CA, USA) or mouse p21(CDKN1A) (Mm00432448_m1, Applied Biosystems: Carlsbad CA, USA), namely TaqMan Gene Expression kit assays (20x probe dye FAM™ (or VIC)-TAMRA (or MGB); Applied Biosystems: Carlsbad CA, USA). More specifically, a master mix was prepared on ice for a final concentration of: 1x Master Mix buffer, 1x primer solution, and 1x Euroscript reverse transcriptase, combined with H₂O, total volume: 8 µL/well. A MicroAmp Optical 384-well Reaction Plate (4309849, Applied Biosystems) was fixed on the bench, and 2 µL of mRNA (concentration: 10 or 20 ng/µl) (or water for negative control) were pipetted in triplicate, followed by addition of 8 µL/well of master mix. The plate was then covered with a MicroAmp Optical Adhesive film kit (4313663, Applied Biosystems: Carlsbad CA, USA), centrifuged for 5 min at 1000 rpm at 4°C and placed in a 7900 HT Fast Real-Time PCR System (Applied Biosystems: Carlsbad CA, USA). The program was run with one cycle of 48°C for 30 min, one cycle of 95°C for 10 min, and finally 40 cycles of alternating 95°C for 15 sec and 60°C for 1 min. The number of cycles (CT) was determined, 2^{-CT} values were calculated, and the value normalized by dividing with the 2^{-CT} value obtained from the GAPDH control. Fold increase over control (*i.e.* DMSO- or vehicle-treated animals) was calculated and plotted in the bar graph.

[00224] In addition, the anti-tumor activity of MDM2i(1) was correlated with a significant dose-dependent induction of p21(CDKN1A) protein levels in tumors, as judged by immunohistochemistry (Figure 8). SJSA-1 xenograft tumors were collected and a 3-4 mm slice out of the middle of the tumor was removed, transferred into pre-labelled histo-cassettes and immersion-fixed in neutral buffered formalin (NBF) 10 % (v/v) (pH 6.8-7.2) (J.T. Baker, Winter Garden, FL, USA), pre-cooled at 4°C. Tumors were then fixed at room temperature for 24 hours, followed by processing in the TPC 15Duo (Tissue Processing Center, Medite) for paraffinization. Subsequently, the tumor slices were embedded in paraffin and from each paraffin block several 3 µm thick sections were cut on a rotary microtome (Mikrom International AG, Switzerland), spread in a 48°C water-bath, mounted on glass slides (SuperFrost Plus, Thermo Scientific:Waltham MA, USA), and dried in an oven

either at 37°C overnight or at 60°C for 30 min. Dry tissue section were processed for immunohistochemistry (IHC) staining. p21(CDKN1A) immunohistochemistry has been performed using the mouse monoclonal antibody clone SX118 from Dako (Cat. No. M7202 Dako: Carpinteria CA, USA) at a dilution of 1:50. Immunohistochemistry has been performed on a Ventana Discovery XT automated immunostainer using the N-Histofine Mousestain Kit (Nichirei Bioscience Inc, Japan) in combination with the DABMap Kit chromogen system, omitting the SA-HRP solution (Ventana/Roche Diagnostics GmbH, Mannheim, Germany). Antigen retrieval was done by using Cell Conditioning ULTRA® (Ventana/Roche Diagnostics GmbH, Mannheim, Germany) at mild (95°C for 8 min + 100°C for 20 min) conditions. Mouse cross reactivities were blocked by using Blocking Reagents A and B from the N-Histofine Mousestain Kit® (Nichirei Bioscience Inc, Japan) before and after primary antibody incubation, following the manufacturer instructions. The primary antibody was applied manually at the desired dilution in Dako antibody diluent (AbD), followed by incubation for 1 hour at ambient temperature. Corresponding negative controls were incubated with AbD only. Sections were subsequently stained using the labeled polymer system Simple Stain Mouse MAX PO (M) from the N-Histofine Mousestain Kit® (Nichirei) and DAB substrate from the DABMap Kit (Ventana/Roche Diagnostics). Counterstaining of sections was done using hematoxylin (Ventana/Roche Diagnostics). After the automated staining run, slides were dehydrated in a graded series of ethanol, cleared in xylene and mounted with Pertex® mounting medium.

[00225] Example 5: Prediction of MDM2i(2) sensitivity in human primary tumor mouse xenograft models and in human primary tumors.

[00226] The biomarkers in Table 2, were used in association with a naïve Bayes predictive model to predict MDM2i sensitivity in a collection of human primary tumor samples and xenograft models, to demonstrate whether the biomarkers, and their associated predictive power, exists outside of *in vitro* cell line systems.

[00227] The gene-level expression values of all the biomarkers of Table 2 were used as the feature basis for naïve Bayes probabilistic modeling. They were generated as described in Example 3, the only difference being in the RMA summarization step where the normalization was targeted to a reference set of normal & tumor samples. The naïve Bayes modeling is conducted as described in Example 3.

[00228] The human primary tumor samples and xenograft models submitted for sensitivity prediction were a collection of about 18,000 and 503 samples, respectively, for which gene expression profiles, generated with Affymetrix technology (Human Genome U133 plus 2.0 array), are available. The samples of the collection were internally annotated with controlled vocabulary for sample ontology including pathology, histology and primary

site. The associated gene chip data was gathered from both public and internal sources, and normalized as described above to the same reference sample set for consistency.

[00229] Ratios of MDM2i predicted sensitive samples from the collection were compared to the proportions of sensitive cell lines, as given by the MDM2i(2) chemical sensitivity data described in Example 3 above. A good correlation is expected to demonstrate the ability of the biomarkers disclosed in Figure 3 to be predictive for MDM2i sensitivity in human primary tumor samples. For clarity, as well as to potentially identify lineages in which sensitive cell line proportions are underestimated in the cell line chemical sensitivity data, sensitive prediction ratio to sensitive cell line ratio comparison is broken down by tissue of origin.

[00230] Figure 9 (left panel) shows a correlation between predicted sensitive human primary tumor samples from the collection and the sensitive cell lines from MDM2i chemical sensitivity data. It indicates the biomarkers disclosed in Table 2 and its use for predicting sensitivity outside of *in vitro* cell line samples is valid. It also indicates that the biomarkers disclosed in Table 2 can be used to predict MDM2i chemical sensitivity in human primary tumor samples. It reveals new tumor indications which have not been investigated previously and confirms results found in the current study. The new indications, for example, liver (hepatocellular carcinoma) and kidney (renal cell carcinoma), represent potentially new disease indications to be pre-clinically and clinically evaluated with the biomarkers disclosed in Table 2 for treatment with an MDM2i. Figure 9A also indicates that the biomarkers disclosed in Table 2 can be used to predict MDM2i chemical sensitivity in primary melanoma tumors, consistent with the results found in the current study.

[00231] Figure 9 (right panel) shows a correlation between the fractions of predicted sensitive human primary tumor samples and the predicted sensitive ratios in the primary tumor xenograft collection. The tumor samples/xenografts/cell lines are organized by lineage. The dashed line in both panels is the identity line. It shows the data generated from the *in vivo* mouse xenograft models, in which the exemplified signature and associated predictive classifier can be studied and validated, is in line with the data from the rest of the *in vivo* collection samples. It confirms the mouse xenograft models as a source of material to validate the p53 downstream target gene based classifier approach to predict clinical outcome of cancer patients and diseases indications, in an *in vivo* pre-clinical setting.

[00232] **Example 6: single biomarkers and any combinations of the identified thirteen biomarkers predict chemical sensitivity to MDM2i.**

[00233] The thirteen biomarkers depicted in Table 2, when used in association with a naïve Bayes predictive modeling framework, predict MDM2i sensitivity in both *in vitro* systems and *in vivo*, as exemplified in Examples 3 and 5. To investigate whether subsets of these thirteen biomarkers would also predict for MDM2i sensitivity, single biomarkers and

multiple combinations of them are employed as feature basis for predictive modeling. Their prediction performances are then compared to the ones achieved with either the full thirteen biomarkers or with p53 mutation status when used as a predictive feature for MDM2i sensitivity prediction.

[00234] Two instances of p53 mutation status are considered in Example 6. These two instances are defined from the Exome Capture Sequencing data of the CCLE cell lines, as mentioned in Example 2, and are meant to be surrogates of clinical settings where the p53 gene is sequenced for stratification or clinical annotation of patients.

[00235] The first instance of p53 mutation status is defined from the mutations spanning exons 5 to 8 of p53. Exons 5 to 8 encompass the DNA binding domain of p53, which contains the majority of described p53 mutations, and are the p53 exons commonly targeted for sequencing in clinical settings (for example, Rapid sequencing of the p53 gene with a new automated DNA sequencer. Bharaj B., Angelopoulou K., and Diamandis E., Clinical Chemistry 44:7 1397-1403, 1998). The second instance considers the complete open reading frame of the main p53 transcript, and is therefore defined from all coding exon mutations.

[00236] Multiple biomarker combinations can be generated from the list of 13 biomarkers disclosed in Table 2. All combination types from 2 to 12 biomarkers are evaluated as feature basis for predictive modeling of MDM2i chemical sensitivity. When more than 50 different combinations exist for a given combination type, the number of evaluated combinations is restricted to 50. All 50 combinations in each combination type were randomly picked.

[00237] All predictive models associated to the above described feature sets (single biomarkers, 2-to-12 biomarker combinations, p53 mutation status instances) were trained and evaluated mostly as described in Example 3. What differs from Example 3 is as follows: The training data was slightly larger than the one used in Example 3 and encompasses 264 cell lines (47 from the sensitive class, and 217 from the insensitive class); A p-value threshold of 0.5 was used upon the naïve Bayes probabilistic modeling to call a cell line either sensitive or insensitive in the 5-fold cross-validation scheme. Moreover, all sample strata generated by the cross-validation processes were randomly selected and independent from one-another. The performances of the combinatorial predictive models and their comparisons to the p53 mutation status instance and 13 biomarker models are shown in figures 10 to 12.

[00238] Figure 10 depicts the positive predicted values (PPV) achieved by the single biomarker, the combinatorial, the thirteen biomarker and the p53 mutation models. The PPV is an estimate of the clinical efficacy one would expect in a clinical trial upon patient selection

with the considered modeling process. For convenience, the data is depicted as box-and-whisker plots when there are more than five data points to plot per feature set.

[00239] Figure 10 shows that combinations from as few as two and three biomarkers outperform exon 5-to-8 p53 mutation ("ex5to8mt") and all-exon p53 mutation features ("allExMt"), respectively. Indeed, the upper and lower boundaries defined by the ends of the whiskers encompass about 99% of the data points, assuming a normal distribution of the data. Therefore, the majority of the evaluated 2- and 3-biomarker combinations show a higher PPV than the ones achieved by the p53 mutation status instances. Moreover, even if all single gene models do not outperform the two p53 mutations instances, a majority of them (around 75%, the box plus the upper whisker) outperforms the p53 all-exon mutations. Noteworthy, all single gene models give rise to PPVs that are higher than the sensitive cell line ratio (~18%) in the considered sample population, indicating that MDM2i sensitivity prediction models, built from as few as one biomarker, are capable of enriching the selected samples in sensitive ones.

[00240] Additionally, under this modeling exercise, as plotted in Figure 10, the PPV given by the p53 exons 5-to-8 mutation status model, averaged over the 5 cross-validation repeats, is 48%. It is significantly lower than the p53 mutation PPV disclosed in Example 3 (56%). This indicates that, in a clinical setting where p53 exon 5-to-8 sequencing is employed for patient selection, which is common practice, the exemplified 13 biomarker-based patient selection has even higher added value than anticipated from Example 3.

[00241] Figure 11 shows the specificities achieved by the several evaluated models. As for PPV in Figure 10, every combination made from as few as 2 biomarkers is sufficient to achieve specificity higher than the ones obtained from the mutations instances only. All single biomarker models outperform the mutations, when specificity is used to monitor the model performances.

[00242] Figure 12 shows the sensitivities. Sensitivity is also called recall, and is an estimate of the truly sensitive patient population retained upon patient selection. Combinations of 9 biomarkers as the feature basis for MDM2i sensitivity prediction models are sufficient to obtain sensitivities comparable to the one achieved the full 13 biomarker list. However, only a few 9-biomarker combinations would achieve sensitivities higher than the ones given by the 2 p53 mutation status predictive models. But noteworthy, all evaluated combinations, from as few as 2 biomarkers, and a majority of single biomarker models, display sensitivities higher than the one which is expected by chance upon random classification (~18%).

[00243] In conclusion from Figures 10, 11 and 12, single biomarkers, when used as feature basis in models predicting chemical sensitivity to MDM2i, are sufficient to achieve sample sensitivity predictions that would result in a significant enrichment of potentially

MDM2i responding patient in a clinical setting. Furthermore, combinations made from any 2 biomarkers, or more, increase the expected clinical efficacy with respect to the one obtained with p53 mutation-based patient selection. Assembling 8 biomarkers, from any of the biomarkers listed in Table 2, are sufficient to achieve a patient recall equivalent to the one given by the 13 biomarker model. The predictive model performance metrics, obtained for the 13 gene signature and associated combinations, can be further optimized by optimizing the class assignment p-value threshold used in the naïve Bayes probabilistic step of the model, as it was done in Example 3.

[00244] In a further embodiment, it is investigated whether the biomarkers depicted in Table 2 could predict MDM2i chemical sensitivity in collaboration with p53 mutation status. Single biomarkers and combinations of 2 biomarkers and above are combined with p53 mutation status in feature lists. The feature lists are then utilized as basis for sensitivity predictive modeling, as previously and described above. The performances of the multiple resulting models are evaluated as described above.

[00245] The p53 mutation status instance which is used as an example is the p53 exon 5-to-8 mutations. Figures 13, 14 and 15 depict the PPVs, specificities and sensitivities of those models combining p53 mutation with biomarkers, respectively, and are compared to the results given by mutation only models and the full 13-biomarker model.

[00246] Figure 13 shows that at least a single biomarker from the list of 13, in collaboration with p53 mutation status, is sufficient to achieve a PPV higher than the basal sensitivity rate (18%) in the data. It also shows that a single biomarker at minimum, still in combination with p53 mutation status, achieves a higher PPV than the ones obtained with the two above mentioned p53 mutation status instances, when employed as features in a predictive model. And finally, any 5 biomarkers in combination with p53 mutation recapitulate the PPV which is achieved by the 13 biomarker model.

[00247] The same conclusions are drawn from Figure 14 and 15 when specificities and sensitivities are taken into account as model performance metrics. Noteworthy from Figure 15, a high sensitivity can be obtained from as few as one biomarker, when modeled along with p53 mutation.

[00248] In conclusion, combining a single or multiple biomarkers from Table 2 with p53 mutation status enables the prediction of MDM2i sensitivity, and would result, when applied for patient selection in a therapeutic or clinical setting, in a significant enrichment with a limited loss of potential MDM2i responding patients.

[00249] **Example 7: The identified thirteen biomarkers predict chemical sensitivity to MDM2i *in vivo* and in pre-selected wild-type p53 human primary xenograft models.**

[00250] To further validate the predictive biomarkers disclosed in Figure 3, human primary tumors were directly transplanted and grown subcutaneously in mice and then assessed for MDM2i sensitivity. All the animals were allowed to adapt for 4 days and housed in a pathogen-controlled environment (5 mice/Type III cage) with access to food and water *ad libitum*. Animals were identified with transponders. Studies were performed according to procedures covered by permit number 1975 issued by the Kantonal Veterinärämter Basel-Stadt and strictly adhered to the Eidgenössisches Tierschutzgesetz and the Eidgenössische Tierschutzverordnung. Subcutaneous tumors were induced by transplanting tumor fragments (3x3x3 mm³) of human patient tumor in the right flank of Harlan nude mice. The administration of MDM2i(1) began when tumors were at the size of 150-200 mm³ for a treatment period up to 4 weeks. MDM2i(1) was made-up fresh for each administration. MDM2i(1) was dissolved in 0.5% HPMC (hydroxypropylmethylcellulose) and injected daily (q24h) at 100 mg/kg. Tumor volumes (TVol), determined from caliper measurements (using the formula $l \times w \times h \times \pi / 6$), were measured three times per week. Tumor response was

quantified by the percentage of change in tumor volume, *i.e.* $\left(\frac{\Delta \text{TVol}_{\text{drug}}}{\text{TVol}_{\text{Day0}}} \times 100 \right)$. The body-

weight (BW) of the mice was measured three times per week allowing calculation at any particular time-point relative to the day of initiation of treatment (day 0) of both the percentage change in BW ($\Delta\% \text{BW}$).

[00251] The cut-offs used for sensitivity to MDM2i were based on RECIST adapted for full tumor volume measurement (instead of the sum of longest diameters), *i.e.* models considered to be sensitive or responsive either displayed a complete response (full regression), or a partial response (>50% decrease in tumor volume) or a stable disease (between 50% decrease and 35% increase in tumor volume). *In vivo* models showing a progressive disease (>35% increase in tumor volume) were considered as non-responsive to the MDM2i treatment. The sensitivity call was made at the maximum effect time-point during the treatment period, to avoid any misleading call that could be due, for example, to the appearance of resistance mechanism(s) following a sustained treatment period with MDM2i. The human primary xenograft tumor models used for the study are from a variety of tumors, and lineage composition was chosen according to Figure 9, for example, melanoma (19), colorectal cancer (17), liposarcoma (3), renal cell carcinoma (3), hepatic cancer (7), breast cancer (1), pancreatic cancer (2) and lung cancer (3). Predictions of sensitivity for every human primary xenograft tumor model were performed using all the biomarkers disclosed in Table 2 as a single set and a predictive model mostly as exemplified in Examples 3 and 5. In this particular example, the naive Bayes probabilistic rule was trained by comparing 77 MDM2i(2) sensitive cell lines to 557 insensitive cell lines, and the naive

Bayes predictive model probability threshold, above which a xenograft tumor model is predicted as sensitive to MDM2 inhibition, was set to 0.2. Additionally, the chemical sensitivity assignment of the training cell lines into sensitive and insensitive classes was done as exemplified in Example 2, with the only difference being, when the pharmacological characterization was replicated for a given cell line, the median value of the cell line sensitivity metric was used for summarization of the replicated sensitivities.

[00252] As shown in Figure 16, 27/55 human primary xenograft tumor models were predicted to be sensitive to an MDM2i, and of these 27 *in vivo* models, 19 were experimentally confirmed as sensitive, resulting in a positive predictive value of 70.5%, improving the basal response rate of 49%. Moreover, 28/55 *in vivo* models were predicted to be insensitive to an MDM2i, and 20/28 were experimentally confirmed as insensitive, leading to a significant negative predictive value (NPV) of 71.5%. Overall, these data are comparable to the predictive model performances described in Figure 5. The assessment of human primary xenograft tumor model response to MDM2i treatment allowed the validation of the MDM2i chemical sensitivity predictive model *in vivo*, hence confirming the validity of the biomarkers disclosed in Table 2, using patient-derived xenografted tumors in mice (Figure 16).

[00253] The p53 mutation call is available for all 55 human primary xenograft tumor models used in this study. Thirty four of them are wild-type p53. As shown in Figure 17, 22/34 wild-type p53 human primary xenograft tumor models were predicted to be sensitive to an MDM2i, and of these 22 *in vivo* models, 18 were experimentally confirmed as sensitive, resulting in a positive predictive value of 82%, again significantly improving the basal response rate of 65%. Twelve out of 34 models were predicted to be insensitive to an MDM2i, and 8/12 were experimentally confirmed as insensitive, leading to a significant negative predictive value (NPV) of 66.5%. Overall, these data are equally comparable to the predictive model performances described in Figure 5, again validating the biomarkers disclosed in Table 2, using wild-type p53 selected patient-derived xenografted tumors in mice (Figure 17).

[00254] In conclusion, the *in vitro* predictive model performances described in Figure 5 were confirmed using human primary xenograft tumor models, further validating the biomarkers disclosed in Table 2 in an *in vivo* setting. Importantly, the findings described here support the use of the MDM2i chemical sensitivity predictive model in either non-selected cancer patient or wild-type p53 pre-selected cancer patient populations

CLAIMS

1. A method of predicting the sensitivity of a cancer patient for treatment with a Human Double Minute 2 inhibitor (MDM2i), the method comprising:
assaying for gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient; and the gene expression of the at least one biomarker indicates that the patient is sensitive to treatment with an MDM2i.
2. The method of claim 1, wherein more than one biomarker is selected from Table 2.
3. The method of claim 1, comprising assaying for gene expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.
4. The method of claim 1, wherein p53 is assayed for a mutation prior to assay of the at least one biomarker, and the presence of a p53 mutation indicates decreased sensitivity to an MDM2i.
5. The method of claim 1, wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.
6. The method of claim 1, wherein an mRNA or protein of at least one biomarker is measured.
7. The method of claim 1, wherein the MDM2i is an isoquinolinone.
8. The method of claim 1, wherein the MDM2i is selected from Table 1.
9. A method of treating a cancer patient comprising:
assaying for gene expression of at least one biomarker selected from Table 2 in a cancer sample obtained from the patient; and the presence of at least one biomarker indicates sensitivity of the patient to an MDM2i; and administering to the patient an MDM2i.
10. The method of claim 9, wherein more than one biomarker is selected from Table 2.

11. The method of claim 9, comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

12. The method of claim 9, wherein p53 is assayed for a mutation prior to assay of the at least one biomarker and the presence of a p53 mutation indicates decreased sensitivity to an MDM2i.

13. The method of claim 9 further comprising obtaining a biological sample from the patient prior to the administration of the MDM2i and comparing the differential gene expression of at least one biomarker from Table 2 in the MDM2i untreated cancer sample with an MDM2i treated cancer sample, wherein increased gene expression indicates continued patient sensitivity.

14. The method of claim 9, wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

15. The method of claim 9, wherein the MDM2i is an isoquinolinone.

16. The method of claim 9, wherein the MDM2i is selected from Table 1.

17. The method of claim 9, wherein the MDM2i is administered in a therapeutically effective amount.

18. A method of predicting the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising:
assaying for gene expression of at least one biomarker selected from Table 2 in the cancer cell obtained from a patient sample and the gene expression of the at least one biomarker selected from Table 2 indicates that the cancer cell is sensitive to treatment with an MDM2i.

19. The method of claim 18, wherein more than one biomarker is selected from Table 2.

20. The method of claim 18, comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.
21. The method of claim 18, wherein p53 is assayed for a mutation prior to assay of the at least one biomarker and the presence of a p53 mutation indicates decreased sensitivity to an MDM2i.
22. The method of claim 18, wherein the cancer sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.
23. The method of claim 18, wherein an mRNA or protein of at least one biomarker is measured.
24. The method of claim 18, wherein the MDM2i is an isoquinolinone.
25. The method of claim 18, wherein the MDM2i is selected from Table 1.
26. The method of claim 18, wherein the MDM2i is administered in a therapeutically effective amount.
27. A method of determining the sensitivity of a cancer cell to a Human Double Minute 2 inhibitor (MDM2i), the method comprising:
- a) contacting a cancer cell with at least one MDM2i;
 - b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i and expression of at least one biomarker indicates sensitivity to an MDM2i; and
 - c) comparing the cell proliferation of cancer cells treated with at least one MDM2i with an untreated or placebo treated cancer cell, wherein there is reduced cell proliferation of the treated cancer cell when compared with the untreated or placebo treated cancer cell.
28. The method of claim 27, wherein the reduced cell proliferation of the cancer cell contacted with at least one MDM2i has an IC₅₀ of less than 1 μ M
29. The method of claim 27, wherein the MDM2i is an isoquinolinone.

30. The method of claim 27, wherein the MDM2i is selected from Table 1.
31. The method of claim 27, wherein the cell is contacted by the MDM2i at least two different time points.
32. The method of claim 27, wherein the cell is contacted by two different MDM2i at step a).
33. The method of claim 27, wherein the cell is contacted by the two different MDM2i at the same time.
34. The method of claim 27, wherein the cell is contacted by two different MDM2i at different time points.
35. The method of claim 27, wherein the cancer cell is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.
36. The method of claim 27, wherein an mRNA or protein of at least one biomarker is measured.
37. The method of claim 27, comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.
38. The method of claim 27, wherein the steps b) and c) are repeated at a time points selected from the group consisting of: 4 hours, 8 hours, 16 hours, 24 hours, 48 hours, 3 days, 1 week, 1 month and several months after administration of each dose of MDM2i.
39. A method of screening for MDM2i candidates the method comprising:
- a) contacting a cell with a MDM2i candidate;
 - b) measuring gene expression of at least one biomarker selected from Table 2 in the cell contacted with the MDM2i candidate and expression of the at least one biomarker indicates sensitivity to an MDM2i; and

c) comparing the reduction in cell proliferation from the cell contacted with the MDM2i candidate with the reduction in cell proliferation of a cell contacted with an MDM2i taken from Table 1 and the cell proliferation of an untreated or placebo treated cell.

40. The method of claim 39, wherein assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

41. The method of claim 39, wherein the MDM2i candidate increases gene expression of at least one biomarker of Table 2.

42. The method of claim 39, wherein the cell is a cancer cell selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura.

43. The method of claim 39, wherein the expression of an mRNA or protein of at least one biomarker of Table 2 is measured.

44. The method of claim 39, comprising assaying for the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

45. Composition comprising an MDM2i for use in treatment of cancer in a selected cancer patient population, wherein the cancer patient population is selected on the basis of showing gene expression of at least one biomarker selected from Table 2 in a cancer cell sample obtained from said patients.

46. The composition of claim 45, wherein the cancer cells sample is selected from the group consisting of breast, lung, pancreas, ovary, central nervous system (CNS), endometrium, stomach, large intestine, colon, esophagus, bone, urinary tract, hematopoietic, lymphoid, liver, skin, melanoma, kidney, soft tissue sarcoma and pleura

47. The composition of claims 45 or 46, wherein the patients are selected on the basis of an gene expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN.

48. A kit for predicting the sensitivity of a cancer patient for treatment with a Human Double Minute 2 inhibitor (MDM2i) comprising

- i) means for detecting the expression of the biomarkers MDM2, CDKN1A, ZMAT3, DDB2, FDXR, RPS27L, BAX, RRM2B, SESN1, CCNG1, XPC, TNFRSF10B and AEN; and
- ii) instructions how to use said kit.

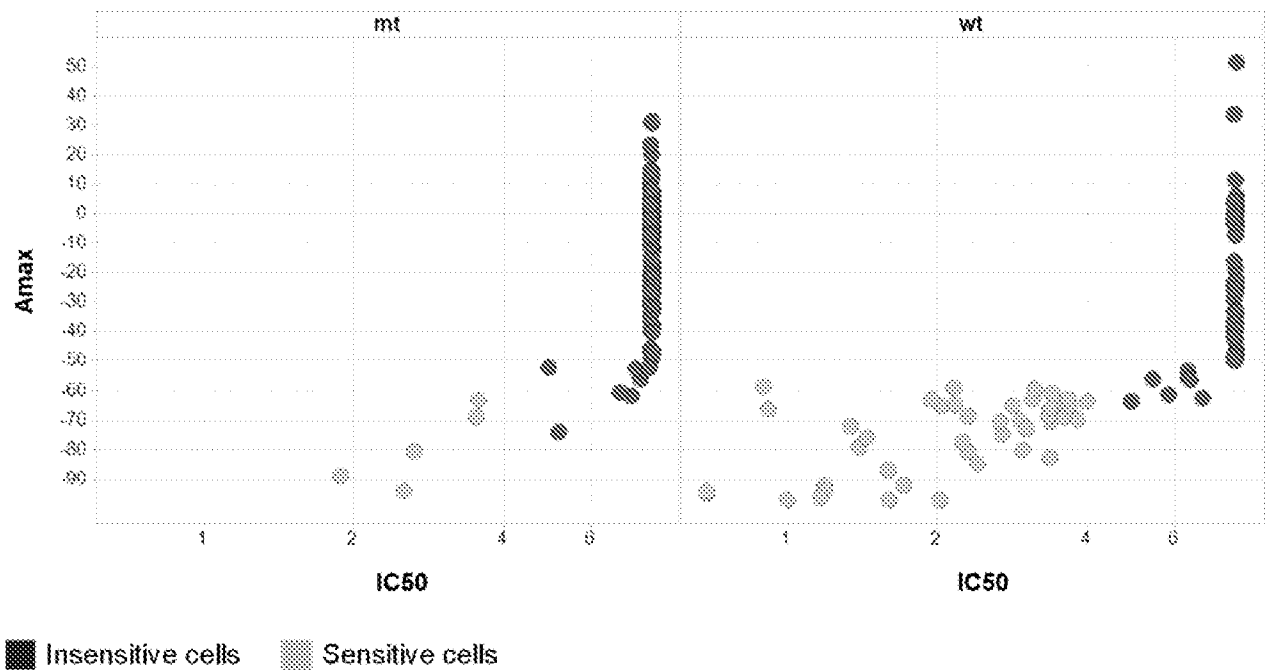
49. Use of the kit according to claim 48 for any of the methods of claims 1 to 37.

FIGURE 1Figure 1A

TR-FRET	MDM2i(1)	MDM2i(2)
IC ₅₀ (nM)	1.7	1.3
K _i (nM)	1.3	1.1
K _{on} (M ⁻¹ .s ⁻¹) × 10 ⁶	37	70
K _{off} (s ⁻¹)	0.071	0.084
T _{1/2} (s)	10	8

Figure 1B

Assay	MDM2i(1)	MDM2i(2)
Cell proliferation inhibition:		
- IC ₅₀ in SJSA-1 (p53 ^{WT/WT}) (μM)	0.29	0.34
- IC ₅₀ in SAOS2 (p53 ^{-/-}) (μM) [Fold difference]	19.75 [68x]	17.06 [50x]
- IC ₅₀ in HCT116 (p53 ^{WT/WT}) (μM)	0.35	0.44
- IC ₅₀ in HCT116 (p53 ^{-/-}) (μM) [Fold difference]	13.37 [38x]	10.60 [24x]
GRIP p53 translocation assay:		
- IC ₅₀ with 10% FCS (μM)	0.27	0.36

FIGURE 2

<u>MDM2i(2)</u>	<u>wt</u>	<u>mt</u>
<u>Insensitive</u>	<u>70</u>	<u>239</u>
<u>Sensitive</u>	<u>42</u>	<u>5</u>

P-value = 6.3e-19 (Fisher's Exact Test)

Odds ratio estimate = 28.38

FIGURE 3

Biomarkers	Fold_change	Wilcoxon_pval	Wilcoxon_pval_adjust
Expr MDM2	2.18	1.71E-18	3.09E-14
Expr CDKN1A	3.88	4.16E-15	3.76E-11
Expr ZMAT3	2.81	1.98E-14	1.20E-10
Expr DDB2	2.55	2.71E-14	1.22E-10
Expr FDXR	2.42	2.98E-13	1.08E-09
Expr RPS27L	1.97	4.08E-13	1.23E-09
Expr BAX	2.12	7.12E-13	1.84E-09
Expr RRM2B	2.06	1.88E-12	4.25E-09
Expr SESN1	2.27	5.17E-12	1.04E-08
Expr CCNG1	1.69	2.65E-11	4.81E-08
Expr XPC	1.62	9.47E-11	1.56E-07
Expr TNFRSF10B	1.91	2.42E-09	3.30E-06
Expr AEN	1.46	2.55E-09	3.30E-06

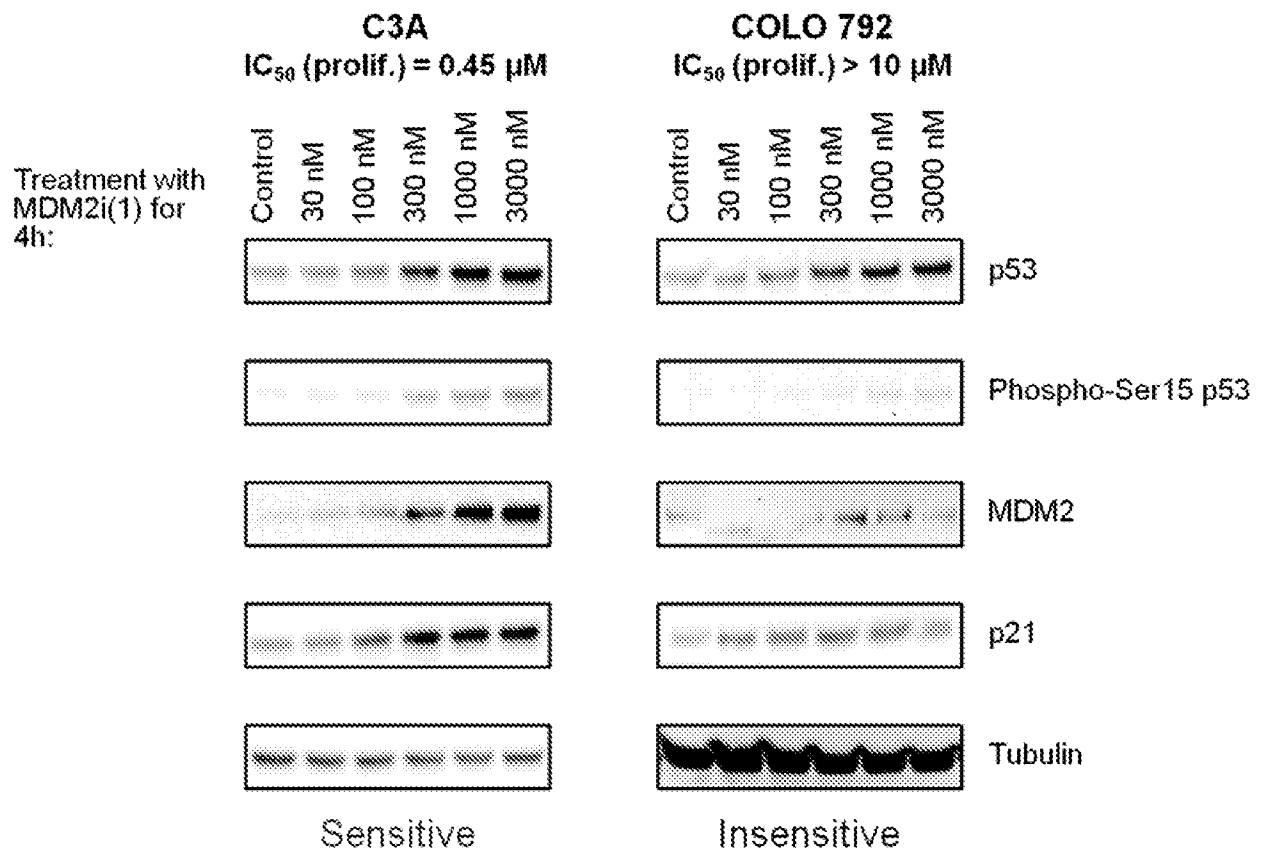
FIGURE 4

FIGURE 5

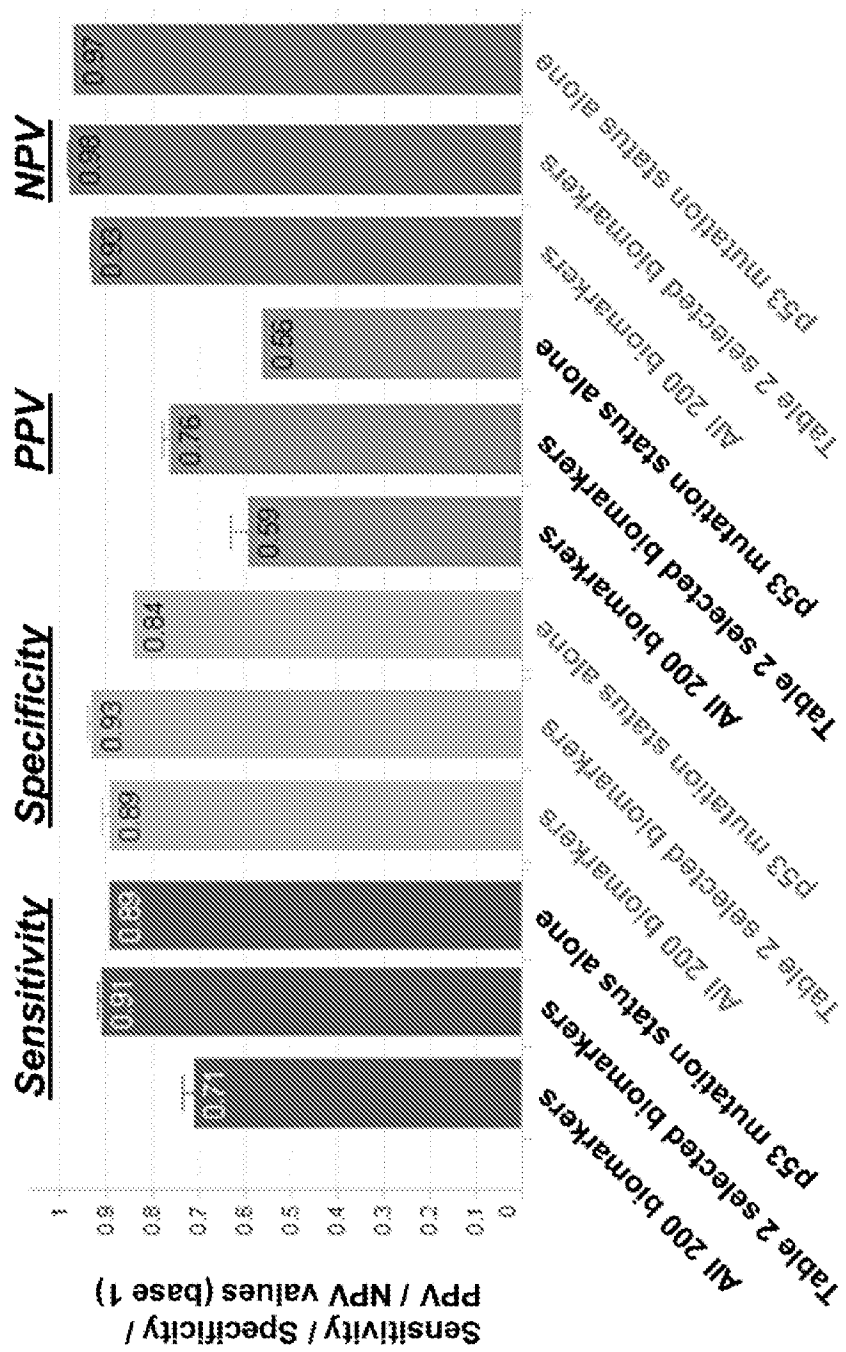
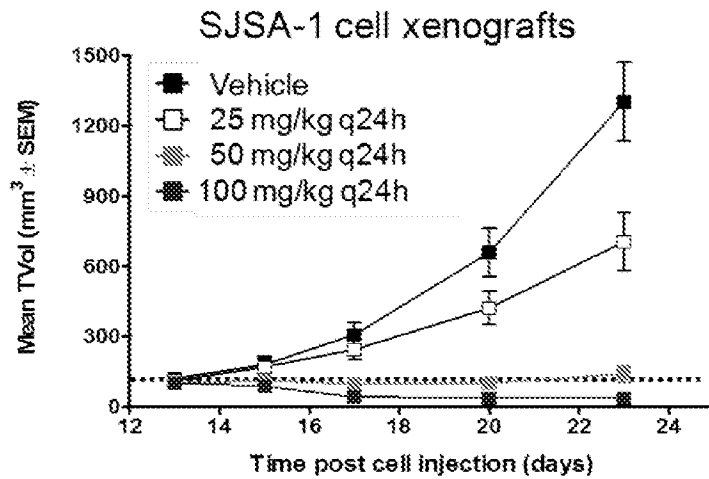


FIGURE 6**Tumor cells displaying the p53 target gene signature are sensitive to MDM2i**

Cell Line Name	Primary Site	MDM2 Sensitivity Prediction	MDM2i(1) IC50, μ M	MDM2i(2) IC50, μ M	MDM2i(1)&(2) Consensus Sensitivity Call
SJSA-1	bone	sensitive	0.223	0.241	sensitive
CCF-STTG1	central nervous system	sensitive	0.97	0.76	sensitive
D283 Med	central nervous system	sensitive	0.192	0.191	sensitive
BV-173	haematopoietic and lymphoid tissue	sensitive	0.085	0.051	sensitive
EOL-1	haematopoietic and lymphoid tissue	sensitive	0.002	0.003	sensitive
GDM-1	haematopoietic and lymphoid tissue	sensitive	0.232	0.285	sensitive
HdS1	haematopoietic and lymphoid tissue	sensitive	0.562	0.392	sensitive
L540	haematopoietic and lymphoid tissue	sensitive	0.039	0.035	sensitive
MH-CALL-4	haematopoietic and lymphoid tissue	sensitive	0.109	0.079	sensitive
MV-4-11	haematopoietic and lymphoid tissue	sensitive	0.89	0.991	sensitive
OCL Y3	haematopoietic and lymphoid tissue	sensitive	0.553	1.579	sensitive
RS4-11	haematopoietic and lymphoid tissue	sensitive	0.177	0.297	sensitive
SUP-B15	haematopoietic and lymphoid tissue	sensitive	0.135	0.167	sensitive
A-498	kidney	sensitive	3.943	NA	insensitive
Caki-1	kidney	sensitive	4.721	NA	insensitive
CAL-51	kidney	sensitive	0.544	NA	sensitive
KMRC-2	kidney	sensitive	5.239	NA	insensitive
OS-RC-2	kidney	sensitive	10	NA	insensitive
A549	lung	sensitive	1.198	NA	sensitive
DM3	pleura	sensitive	10	NA	insensitive
IST-MES1	pleura	sensitive	4.083	NA	insensitive
JL-1	pleura	sensitive	1.421	NA	sensitive
MSTO-211H	pleura	sensitive	0.454	NA	sensitive
A101D	skin	sensitive	6.729	7.784	insensitive
COLO-829	skin	sensitive	4.238	2.175	insensitive
COLO-783	skin	sensitive	10	10	insensitive
COLO-849	skin	sensitive	2.729	2.934	sensitive
Hs 688A1T	skin	sensitive	10	10	insensitive
Hs 034.T	skin	sensitive	10	10	insensitive
Hs 940.T	skin	sensitive	1.434	2.665	sensitive
IGR-1	skin	sensitive	5.995	3.689	insensitive
MEL-JUSO	skin	sensitive	0.586	0.801	sensitive
SK-MEL-1	skin	sensitive	1.613	1.509	sensitive
SK-MEL-31	skin	sensitive	1.222	1.275	sensitive
UACC-257	skin	sensitive	2.143	2.96	sensitive
UACC-62	skin	sensitive	0.626	0.74	sensitive

Cell Line Name	Primary Site	MDM2 Sensitivity Prediction	MDM2i(1) IC50, μ M	MDM2i(2) IC50, μ M	MDM2i(1)&(2) Consensus Sensitivity Call
CAL-51	breast	insensitive	1.038	0.552	sensitive
EFM192A	breast	insensitive	10	7.43	insensitive
HCC202	breast	insensitive	7.386	7.386	insensitive
GA-10	haematopoietic and lymphoid tissue	insensitive	0.061	5.294	insensitive
HDM-2	haematopoietic and lymphoid tissue	insensitive	10	9.059	insensitive
JM1	haematopoietic and lymphoid tissue	insensitive	0.997	1.102	sensitive
KASUM-1	haematopoietic and lymphoid tissue	insensitive	5.271	7.137	insensitive
VMRC-RCW	kidney	insensitive	10	NA	insensitive
JHH-5	liver	insensitive	8.788	9.012	insensitive
HCC-95	lung	insensitive	7.129	NA	insensitive
NCH1568	lung	insensitive	6.202	NA	insensitive
REF-1C-KJ	lung	insensitive	8.753	10	insensitive
DANG	pancreas	insensitive	10	9.734	insensitive
NCH23	pleura	insensitive	2.827	NA	sensitive
COLO-818	skin	insensitive	10	10	insensitive
IGR-37	skin	insensitive	10	10	insensitive

FIGURE 7A-7CFigure 7AFigure 7B

SJSA-1 tumor xenografts – 10 days of treatment		
MDM2i(1) treatment	T/C (%)	Regression (%)
25 mg/kg q24h	50	-
50 mg/kg q24h	3	-
100 mg/kg q24h	-	65

Figure 7C

MDM2i(1)-induced CDKN1A mRNA levels in tumor correlates with tumor growth inhibition

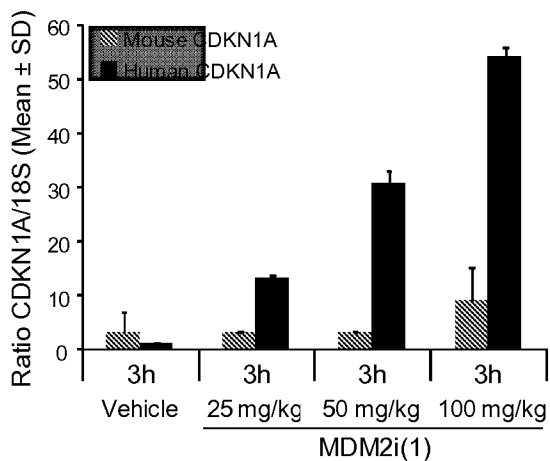


FIGURE 8

MDM2i(1) significantly increases p21 expression in SJSA-1 tumor bearing mice

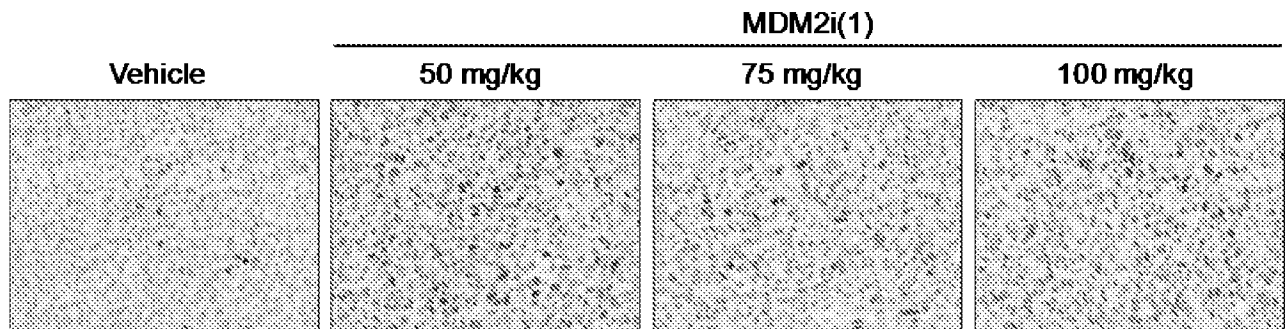


FIGURE 9

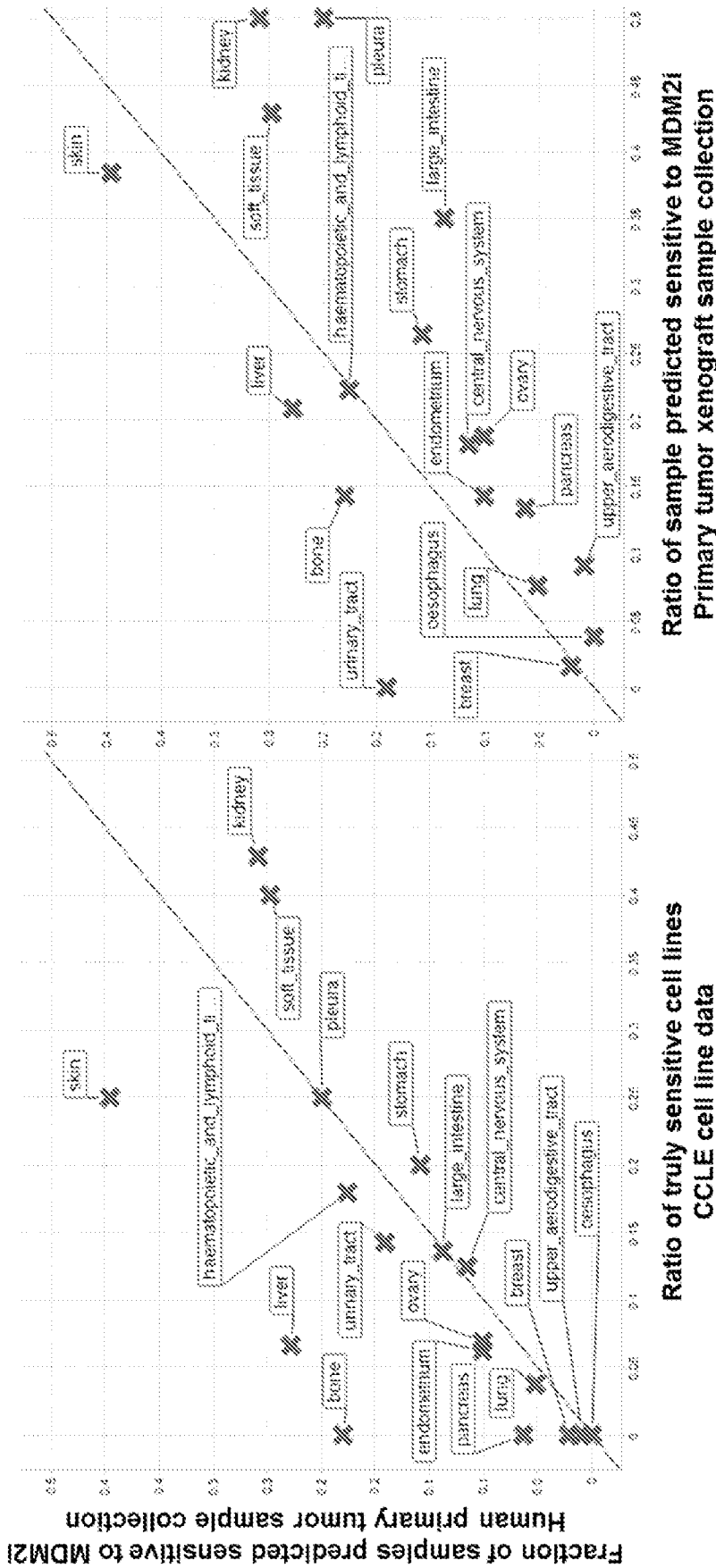


FIGURE 10

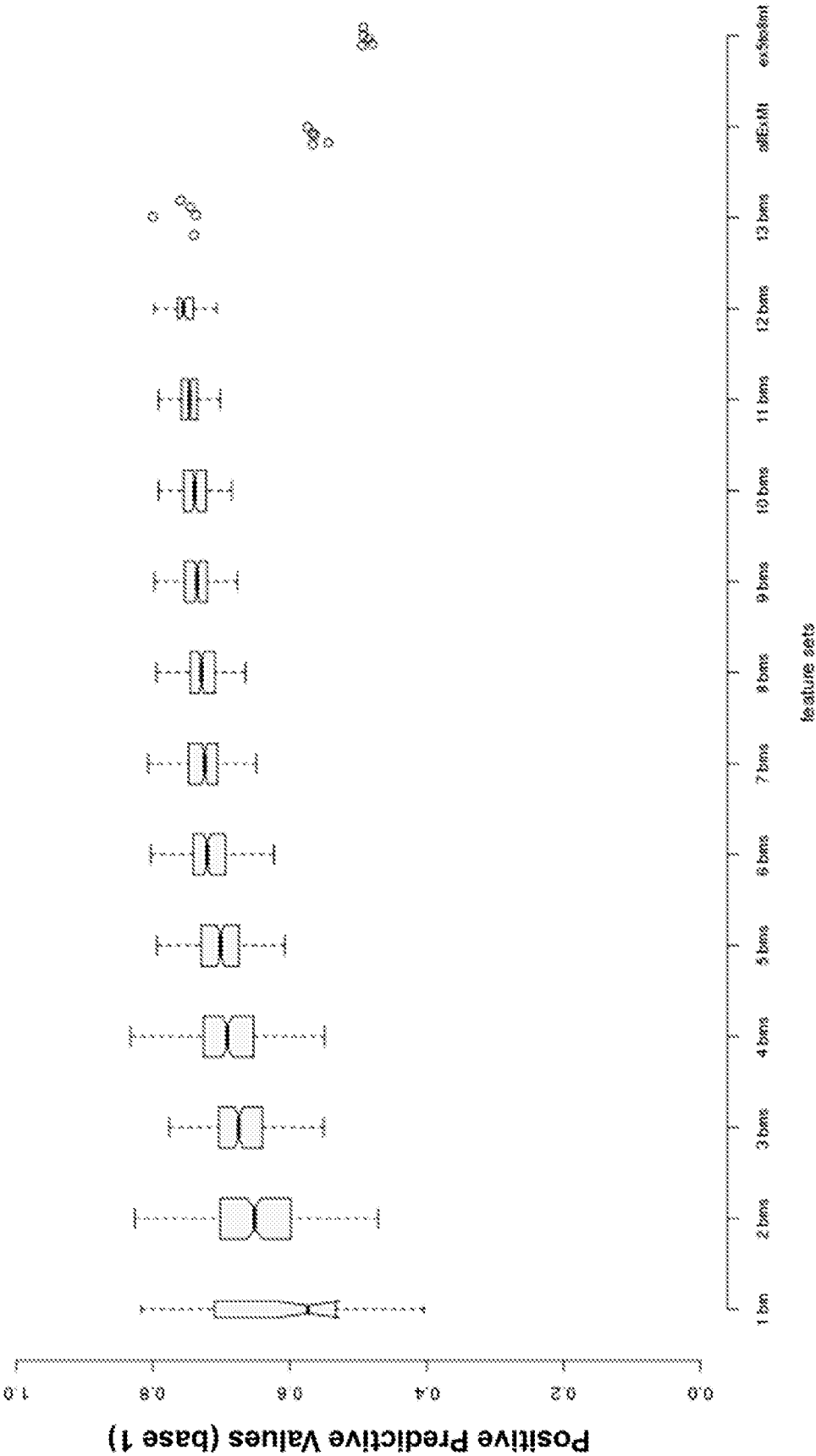


FIGURE 11

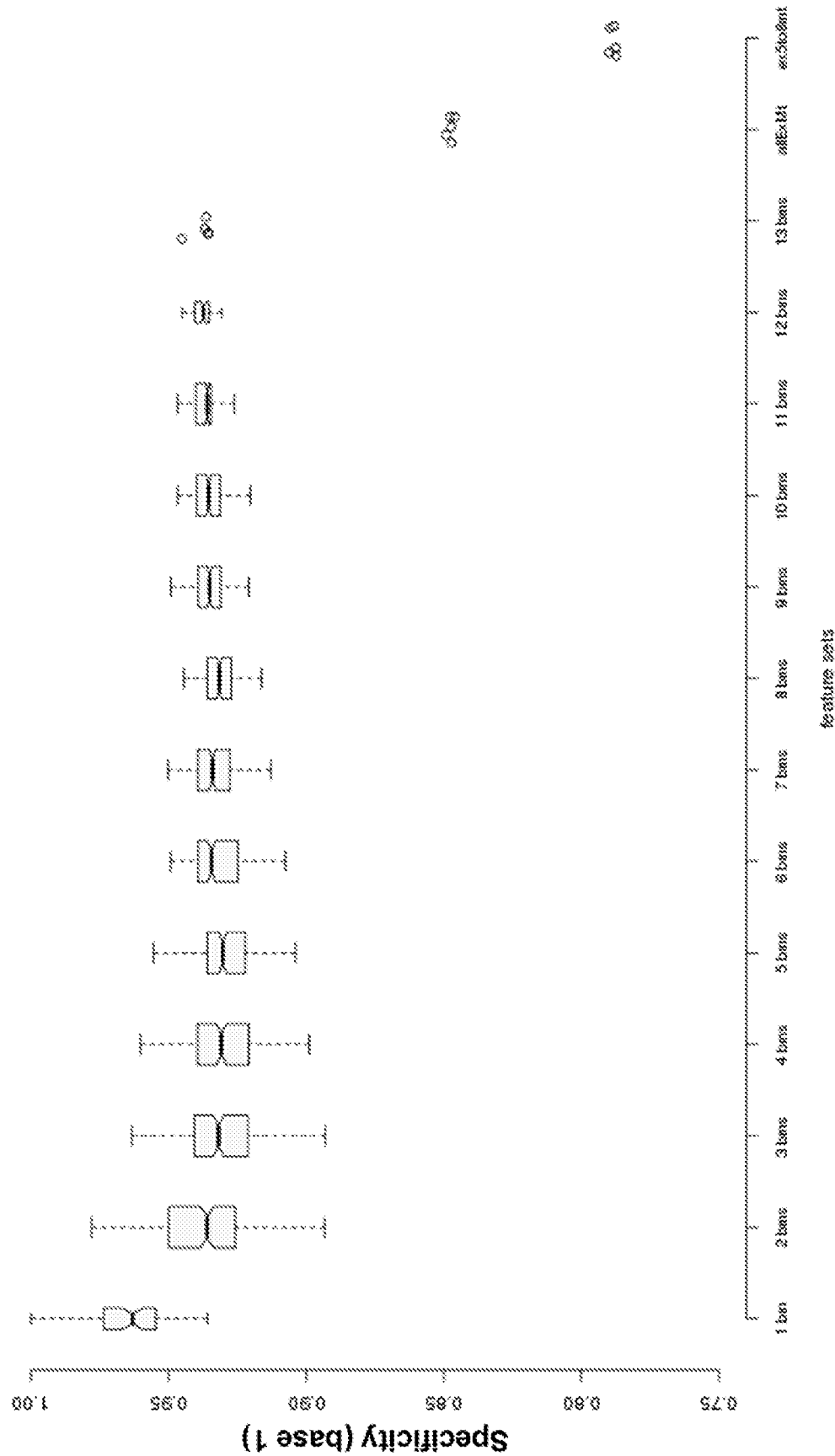


FIGURE 12

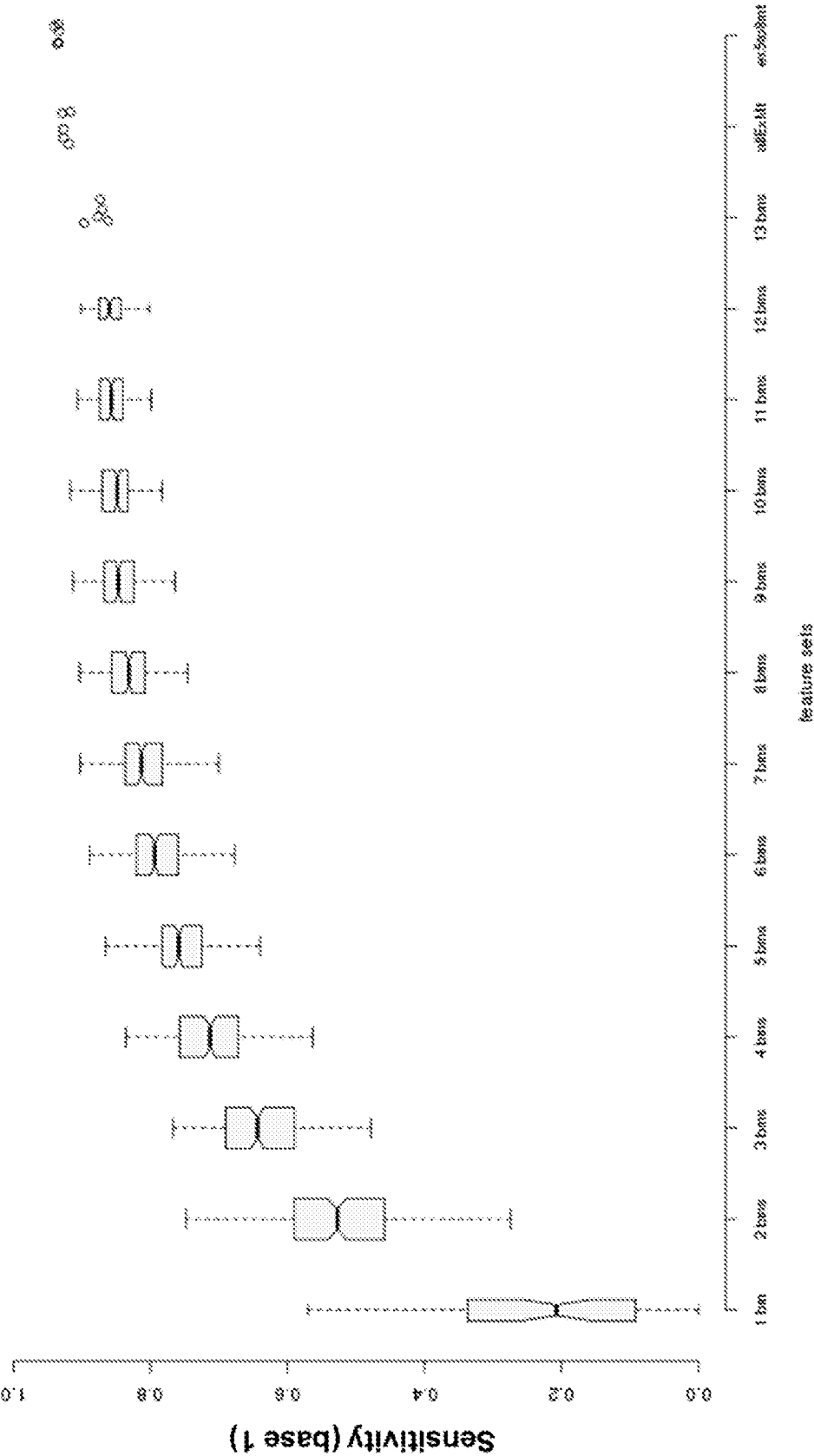


FIGURE 13

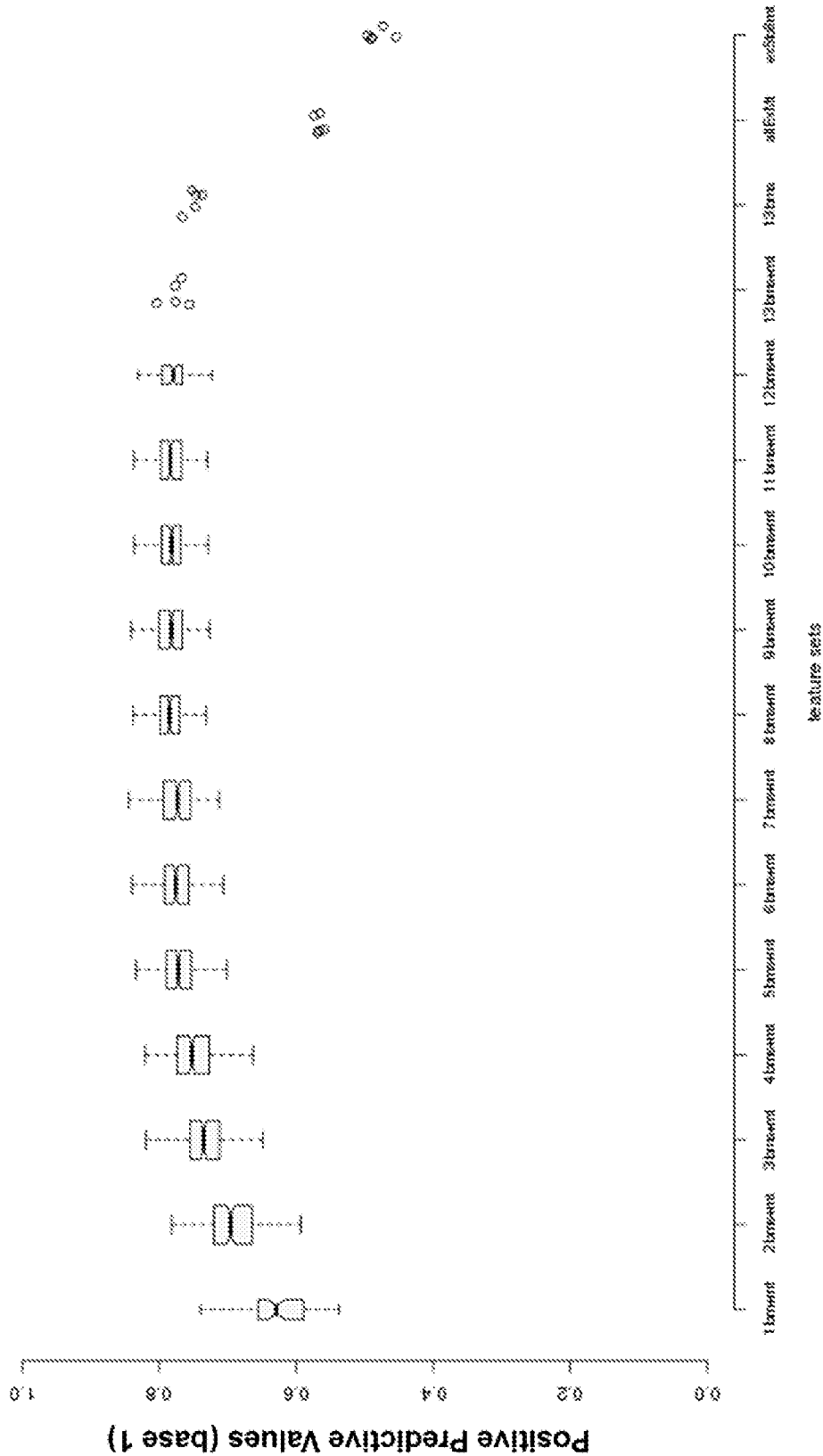


FIGURE 14

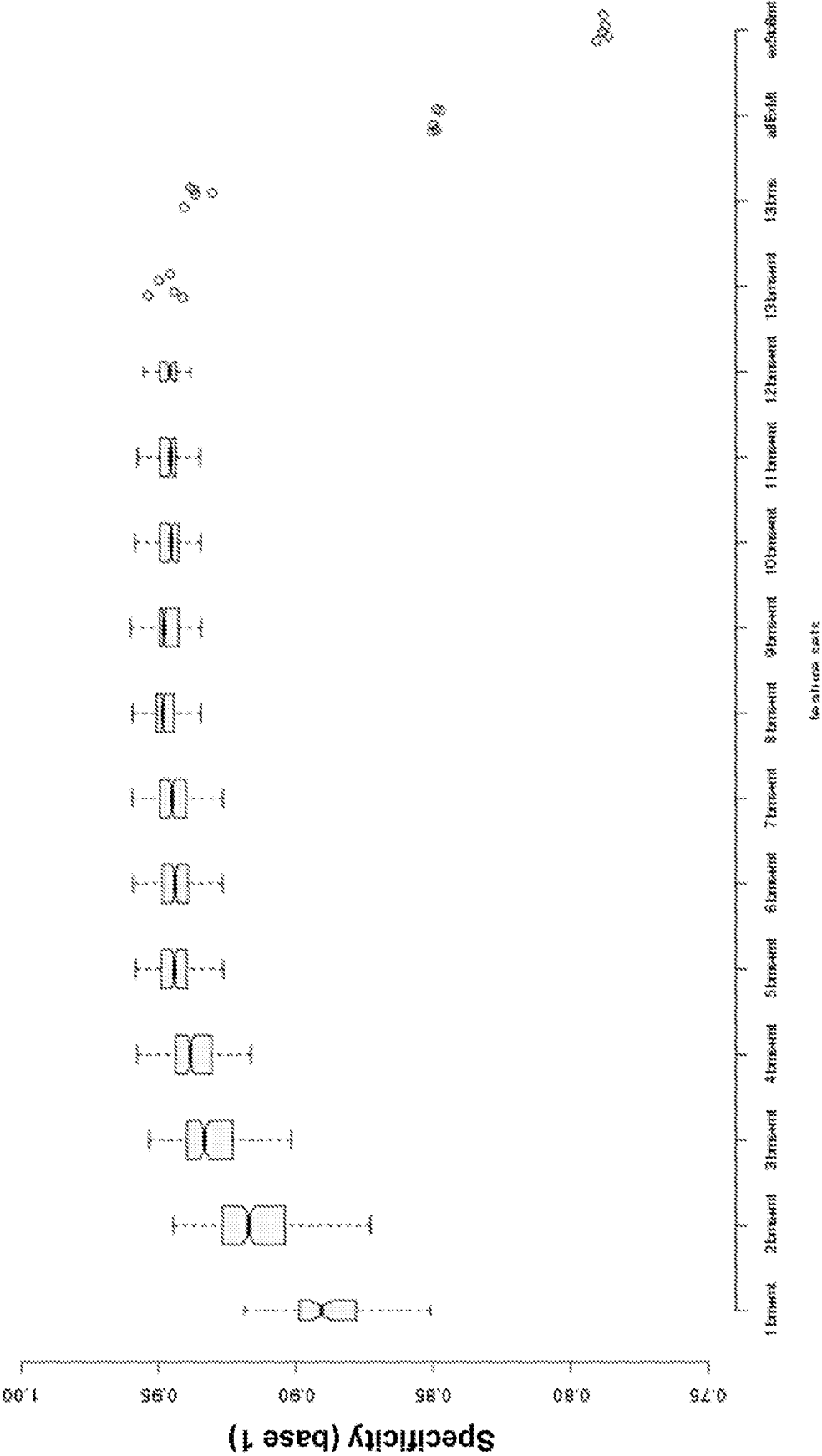


FIGURE 15

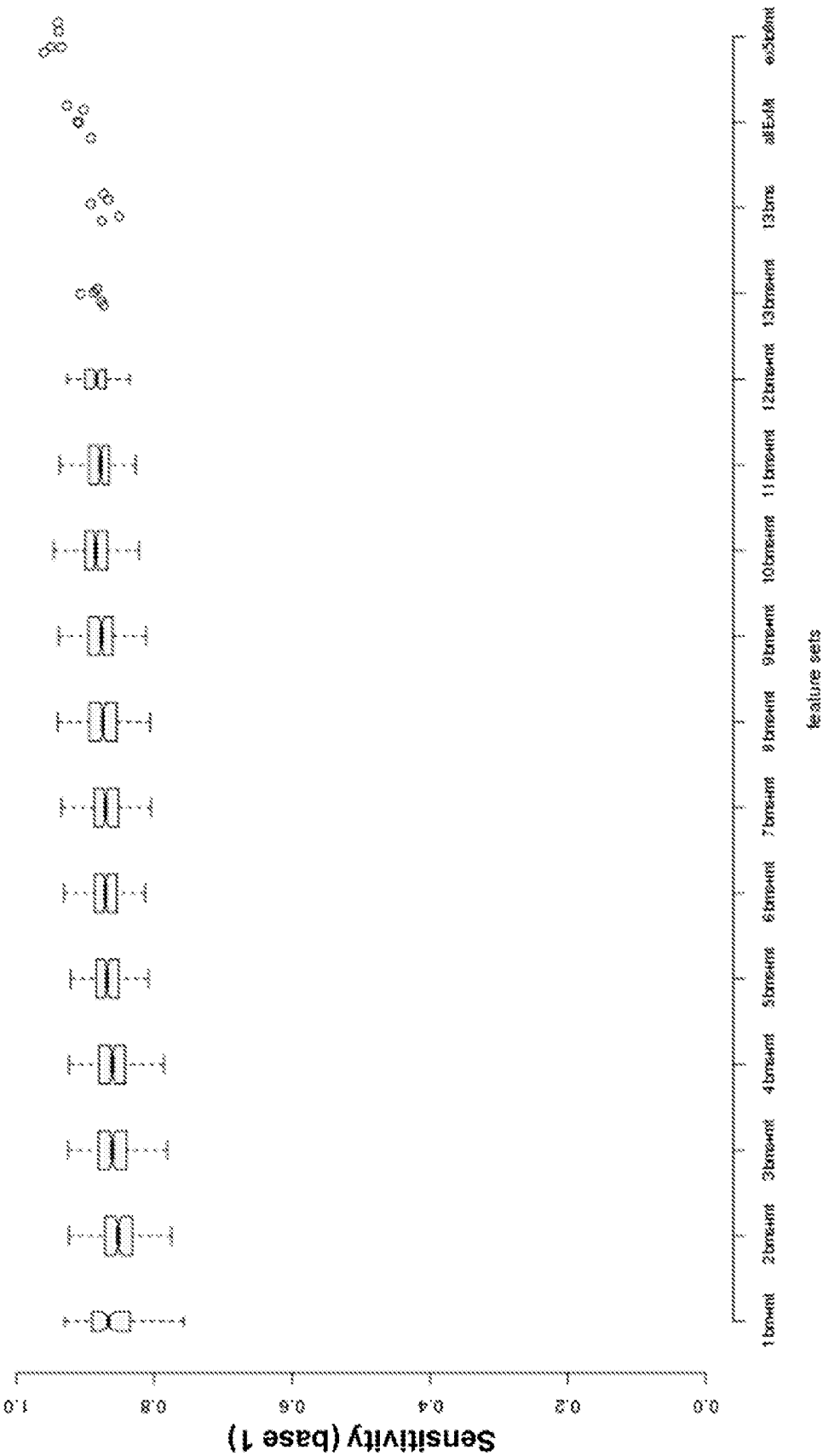


FIGURE 16

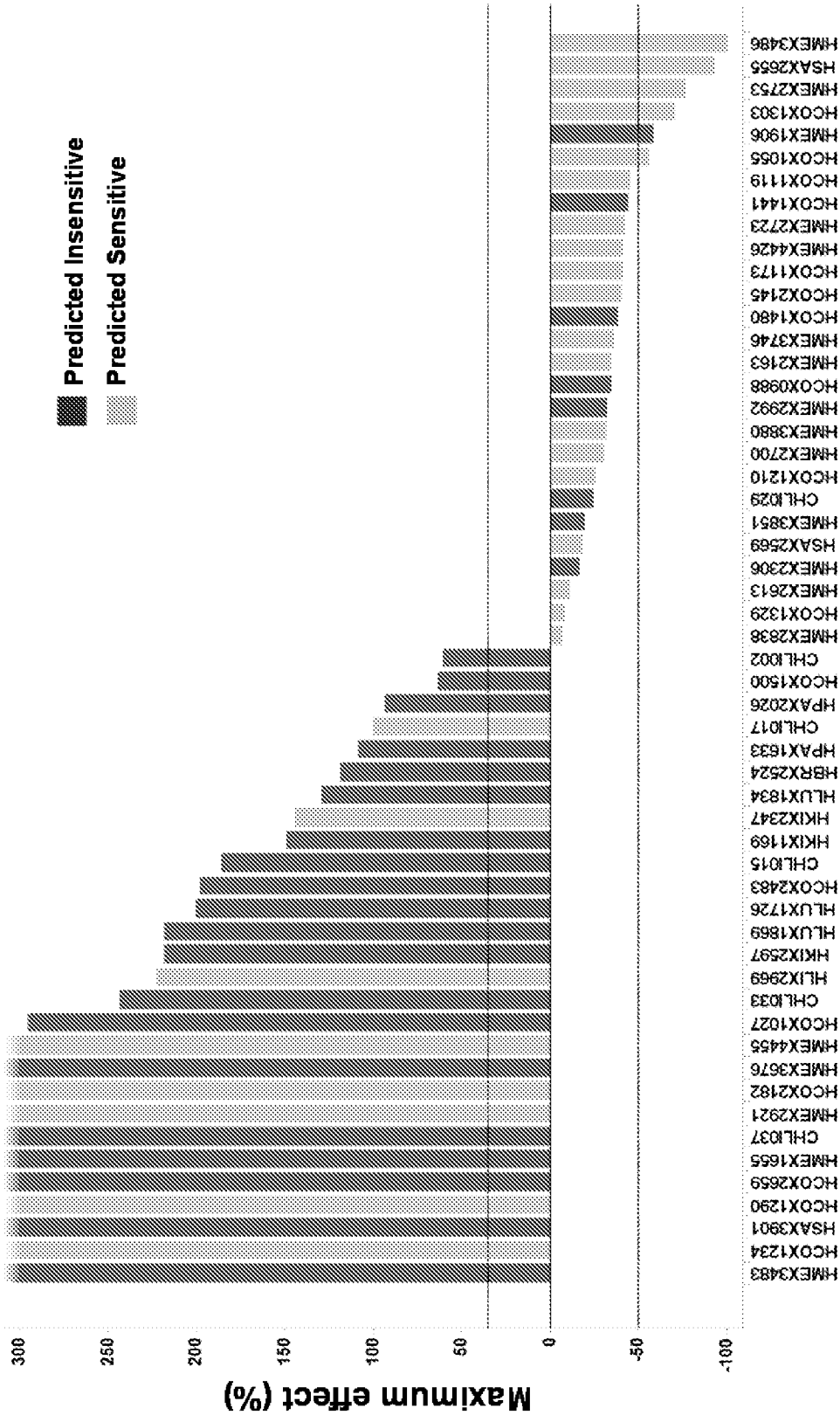


FIGURE 17

