

found that AML cell lines and 16 primary aAML samples with wild-type p53 responded to MDM2 antagonist (inhibitor) by induction of p53-dependent apoptosis. These findings support the rationale of targeting the p53-MDM2 interaction as a therapeutic strategy for AML.

[0005] Based on the proposed mechanism of action of the drug, the presence of functional p53 protein and related pathway effector molecules are required for this class of drugs to be efficacious. Not all patients will have functional p53 proteins and related pathway effector molecules. In order to better determine whether a patient can benefit from therapy, there is a need to discover predictive molecular tests for identifying patients that are most likely to respond to therapy. One approach for assessing potential response to a MDM2 antagonist is to assess whether or not the TP53 gene is mutated. However, this is complicated by the fact that a multitude of mutations can be found in TP53 in cancer. Not all of these mutations will interfere with activity of the p53 protein, further complicating interpretation of TP53 mutational tests. In addition, there is a range of responses to MDM2 antagonists in wild type TP53 cell lines and patients. Therefore, the ability to predict responsiveness to an MDM2 antagonist from an easily interpretable diagnostic tool is an unmet need in clinical development of MDM2 antagonists. To this end, the development of a gene expression signature that reflects p53 pathway activity could provide a means of selecting patients most likely to respond to MDM2 antagonist therapy.

SUMMARY OF THE INVENTION

[0006] ~~In one aspect, the present invention discloses~~ relates to a method for predicting the response of a patient having cancer to therapy, wherein the patients therapy comprises the treatment with a compound which acts as inhibitor of the MDM2-p53 interaction, said method comprising the steps of:

a) measuring a level in a sample pre-obtained from the subject to obtain a value or values representing this level; and

b) comparing the value or values from step a) to a standard value or set of standard values.

5 [0006a] In one aspect, the present disclosure relates to an *in vitro* method to predict responsiveness of a patient, with cancer, to a therapy, said method comprising detecting a level of at least one gene selected from the group consisting of BAX, RPS27L, EDA2R, XPC, DDB2, FDXR, MDM2, CDKN1A, TRIAP1, BBC3, CCNG1, TNFRSF10B, or CDKN2A and using that level as a biomarker for predicting the patient's response to a compound, wherein the compound
10 is an inhibitor of the MDM2-p53 interaction.

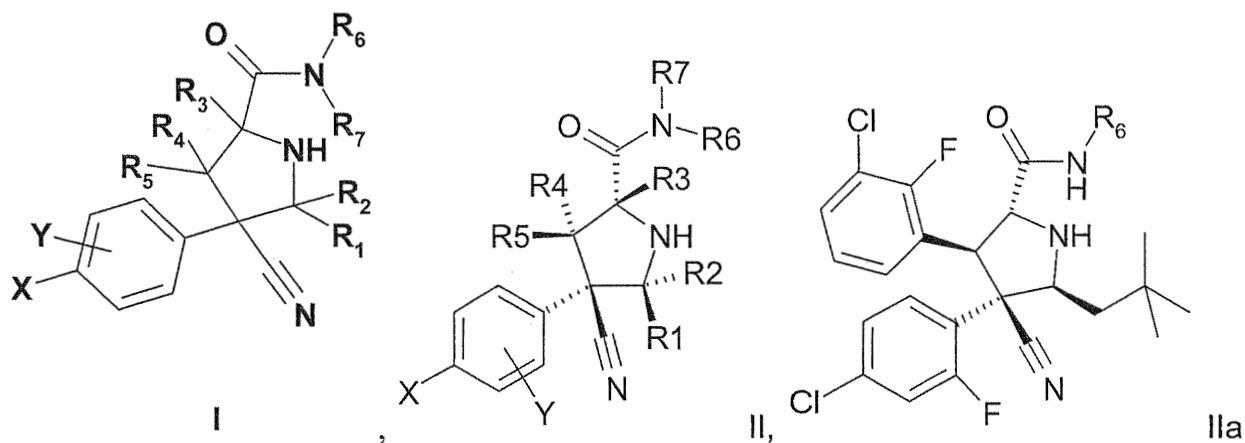
[0006b] In another aspect, the present disclosure relates to a method method for predicting the response of a patient with cancer to treatment with a compound which acts as inhibitor of the MDM2-p53 interaction, said method comprising the steps of:

15 a) measuring a level of at least one gene selected from the group consisting of BAX, RPS27L, EDA2R, XPC, DDB2, FDXR, MDM2, CDKN1A, TRIAP1, BBC3, CCNG1, TNFRSF10B, or CDKN2A in a sample pre-obtained from the patient to obtain a value or values representing this level; and

b) comparing the value or values from step a) to a standard value or set of standard values.

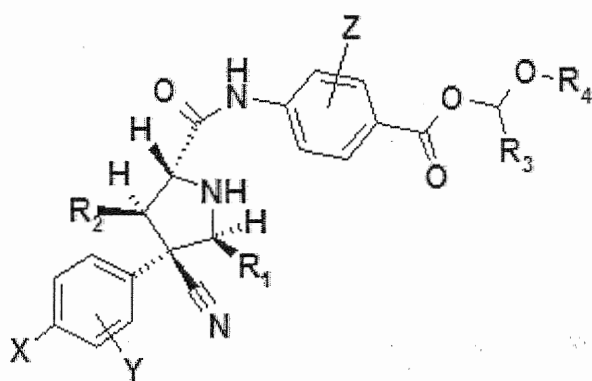
20 **[0007]** Compounds which act as inhibitor of the MDM2-p53 interaction (MDM2-inhibitors, MDM2-antagonists) are known in the art. MDM2-inhibitors and methods for making them are for example disclosed in U.S. Patent 8,354,444 B2, as well as in WO 2007/063013, WO 2010/031713 and WO 2011/098398, which are incorporated in their entirety herein. Additional MDM2-inhibitors are disclosed in WO 2013/135648, which is incorporated in its entirety herein.

U.S. Patent 8,354,444 B2, as well as WO 2010/031713, WO 2011/098398 disclose for example compounds of formula I, II and IIa



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WO 2013/135648 discloses for example compounds of formula III



III

[0008] ~~In one aspect, the present invention~~ disclosure relates to a method for predicting the response of a patient having cancer to therapy, wherein the patients therapy comprises the treatment with a compound which acts as inhibitor of the MDM2-p53 interaction, preferably an MDM2-inhibitor as specifically disclosed in the working examples of U.S. Patent 8,354,444 B2, or in WO 2007/063013, WO 2010/031713, WO 2011/098398 and WO 2013/135648.

~~In a further aspect, the invention~~ disclosure also relates to a method of treating a neoplastic disease, cancer, in a patient in need thereof, comprising measuring a level in a sample from the patient to obtain a value or values representing this level, and treating the subject with a compound of general formula I or a pharmaceutically acceptable derivative thereof as defined above.

In a further aspect, the disclosure relates to use of a compound which acts as an inhibitor of MDM2-p53 interaction in the manufacture of a medicament for the treatment of cancer of a patient, wherein the patient is one whose level of MDM2 had been measured from a sample obtained from the patient in order to obtain a value or values representing this level.

A "neoplastic disease" that is potentially treatable based on the MDM2 response is a cancer. In one embodiment, the cancer is selected from the group consisting of breast cancer, prostate cancer, cervical cancer, ovarian cancer, gastric cancer, colorectal cancer (i.e. including colon cancer and rectal cancer), pancreatic cancer, liver cancer, brain cancer, neuroendocrine cancer,

lung cancer, kidney cancer, hematological malignancies, melanoma and sarcomas. In another embodiment, the cancer is selected from the group consisting of hematological malignancies, prostate cancer, breast cancer, cervical cancer, ovarian cancer, colorectal cancer, melanoma and lung cancer. In yet another embodiment, the cancer is acute myeloid leukemia (AML), or

5 Myelodysplastic Syndrome (MDS), or Myeloproliferative Neoplasms (MPN).

[0009] ~~In yet another aspect, the invention~~ disclosure also relates to a kit for predicting the response to a compound of formula or a pharmaceutically acceptable derivative thereof, as defined above, comprising reagents necessary for measuring the level in a sample. More preferably the kit also comprises a comparator module which comprises a standard value or set of standard values to which the level of response in the sample is compared. The kit may also comprise a captive reagent. In yet another aspect, the disclosure relates to a kit for predicting the response to treatment with a compound which acts as inhibitor of the MDM2-p53 interaction, comprising a) reagents for measuring a level of at least one gene selected from the group consisting of BAX, RPS27L, EDA2R, XPC, DDB2, FDXR, MDM2, CDKN1A, TRIAP1, BBC3, CCNG1, TNFRSF10B, or CDKN2A in a sample; and b) a comparator module.

[00010] More preferably the kit comprises an MDM2 inhibitor, preferably an MDM2-inhibitor as specifically disclosed in U.S. Patent 8,354,444 B2, or in WO 2007/063013, WO 2010/031713, WO 2011/098398 and WO 2013/135648; or a compound according to formulae IIa or III; or a specific MDM2-inhibitor as disclosed herein.

[00011] The present ~~invention~~ disclosure identifies a gene expression signature (mRNA) panel based on *in vitro* and clinical data that identify patients most likely to respond to MDM2 antagonist therapy. The mRNA signature is characterized by up-regulations of at least three genes, specifically MDM2, XPC (xeroderma pigmentosum, complementation group C), BBC3 (BCL2 binding component 3) and down-regulation of CDKN2A (cyclin-dependent kinase inhibitor 2A).

[00012] The present disclosure ~~invention~~ identifies a 4-gene expression signature (mRNA) for identifying responses to MDM2 antagonist.

[00013] The present disclosure ~~invention~~ also identifies a 4-gene expression signature (mRNA) for monitoring treatment of cancer in a patient with an MDM2 antagonist.

[00014] The baseline expression levels of MDM2, BBC3, CDKN2A, and XPC yield a composite score that discriminates between cell lines and patient-derived clinical specimens that are resistant to therapy, and identifies those that are sensitive (responsive) to the therapy.

5 [00015] As such, the present disclosure ~~invention~~ relates to a method for identifying sensitivity to MDM2 antagonist therapy. Furthermore, the present disclosure ~~invention~~ relates to a method for treating a

cancer patient with an MDM2 antagonist by testing the sensitivity of the patient before hand by the gene signature (mRNA) panel, more specifically a panel including MDM2, and more specifically a 4-gene expression system.

5 [00015a] In yet another aspect, the disclosure relates to an *in vitro* method for monitoring efficacy of therapy comprising an MDM2 inhibitor, in a patient suffering from cancer, the method comprising a) measuring the mRNA expression level of MDM2, XPC, BBC3 and CDKN2A in a sample obtained from that patient prior to treatment; b) using the expression levels obtained in a) to calculate a value designated as the patient's signature score prior to treatment; c) repeating step a) and b) after start of treatment with said MDM2 inhibitor; and d)
10 comparing the signature scores obtained after start of treatment with those obtained prior to treatment, whereas higher signature scores after treatment indicate a response of the patient to the treatment, and thus a recommendation to continue the treatment.

[00016] The present ~~disclosure~~ ~~invention~~ further provides the predictive signature utilizing mRNA values in determining the effectiveness of MDM2 antagonist therapy to cancers, in
15 particular, AML.

[00017] The present ~~disclosure~~ ~~invention~~ relates to the use of a gene panel containing at least the MDM2 gene as predictive mechanism for determining a patient's response to a disease, particularly cancer, more particularly acute myeloid leukemia (AML), when a patient is to be treated with an MDM2 antagonist.

20 [00018] More particularly, the present ~~disclosure~~ ~~invention~~ relates to the use of a four-gene panel in order to determine a patient's response to a disease, particularly AML, when a patient is to be treated with an MDM2 antagonist.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: This outline provides the sequence of work that forms the invention.

Figure 2: MDM2 antagonist response mRNA signature *in vitro*.

Top: The heatmap of the four gene mRNAs (in the original, colored version green corresponds
5 to lower expression, red to higher expression) associated with MDM2 antagonist response for the
most sensitive ($IC_{50} \leq 1$) and resistant ($IC_{50} \geq 10$) cell lines. For the most sensitive cell lines,
MDM2, XPC and BBC3 show high expression, and CDKN2A shows low expression. For each
cell line TP53 mutation features are at the top of the heatmap (white = wild type). The natural
log IC_{50} values are represented at the bottom.

CLAIMS

1. An *in vitro* method to predict responsiveness of a patient, with cancer, to a therapy, said method comprising detecting a level of at least one gene selected from the group consisting of BAX, RPS27L, EDA2R, XPC, DDB2, FDXR, MDM2, CDKN1A, TRIAP1, BBC3, CCNG1, TNFRSF10B, or CDKN2A and using that level as a biomarker for predicting the patient's response to a compound, wherein the compound is an inhibitor of the MDM2-p53 interaction.

2. A method according to claim 1, said method comprising using the MDM2 gene as a biomarker.

3. The method according to claim 1 or 2, wherein the response is a response to cancer therapy in a patient and the biomarker is measured *in vitro* in a sample or samples taken from the patient.

4. The method according to claim 1, said method comprising:

~~a) taking a sample from the patient;~~

~~b) measuring a level of said at least one gene according to claim 1 in the a sample that had been taken from the patient; and~~

~~c) comparing the level from the patient to standard values from a patient with the same cancer; and~~

~~d) administering a compound which acts as inhibitor of the MDM2-p53 interaction.~~

5. The method according to any one of claims 1 to 4, wherein the compound is 4-
{[2R,3S,4R,5S]-4-(4-Cholor-2-fluoro-phenyl)-3-(3-chloro-2-fluoro-phenyl)-4-cyano-5-(2,2-dimethyl-propyl)-pyrrolidine-2-carbonyl]-amino}-3-methoxy-benzoic acid of the formula

15. The method according to claim 10, wherein higher MDM2 levels in a sample or samples

i) relative to a standard value or set of standard values from a patient with the same cancer; or

ii) taken after treatment initiation and compared to a sample or samples taken from the same patient before treatment initiation; or

iii) relative to a standard value or set of standard values from normal cells or tissues;

are predictive of sensitivity to treatment with a compound which acts as inhibitor of the MDM2-p53 interaction.

16. A method for predicting the response of a patient with cancer to treatment with a compound which acts as inhibitor of the MDM2-p53 interaction, said method comprising the steps of:

a) measuring a level of at least one gene selected from the group consisting of BAX, RPS27L, EDA2R, XPC, DDB2, FDXR, MDM2, CDKN1A, TRIAP1, BBC3, CCNG1, TNFRSF10B, or CDKN2A in a sample pre-obtained from the patient to obtain a value or values representing this level; and

b) comparing the value or values from step a) to a standard value or set of standard values.

17. Use of a compound which acts as an inhibitor of MDM2-p53 interaction in the manufacture of a medicament for the treatment of cancer of a patient, wherein the patient is one whose ~~A method of treating cancer, in a patient in need thereof, said method comprising:~~ measuring a level of MDM2 had been measured from in a sample obtained from the patient in order to obtain a value or values representing this level, and treating the patient with a compound which acts as inhibitor of the MDM2-p53 interaction.

18. The ~~method-use~~ of claim 17 wherein the patient is one whose further ~~comprising measuring levels of MDM2, XPC, BBC3 and CDKN2A~~ had been measured from ~~in a sample obtained from the patient in order to obtain a value or values representing these~~ levels, and treating the patient with a compound which acts as inhibitor of the MDM2-p53 ~~interaction.~~

19. The ~~method-use~~ of claim 18, ~~comprising~~ wherein the patient is one,
a) ~~measuring the where the mRNA expression level of MDM2, XPC, BBC3 and CDKN2A~~ had been measured from ~~in a sample obtained from that patient prior to treatment;~~
b) ~~using the expression levels obtained in a) to calculate a value designated as the patient's~~ signature score had been calculated using the expression levels obtained in a);
c) ~~comparing said patient's signature score obtained from b)~~ had been compared to a ~~reference level; and~~
d) ~~identifying said patient~~ has been identified as more likely to respond to the therapy ~~comprising said MDM2 inhibitor when the patients's signature score is above said reference~~ level.

20. The ~~method-use~~ of claim 17, 18 or 19 wherein said compound which acts as inhibitor of the MDM2-p53 interaction is the compound 4- $\{[(2R,3S,4R,5S)-4-(4-Cholor-2-fluoro-phenyl)-3-(3-chloro-2-fluoro-phenyl)-4-cyano-5-(2,2-dimethyl-propyl)-pyrrolidine-2-carbonyl]-amino\}$ -3-methoxy-benzoic acid, according to claim 5.

21. The ~~method-use~~ of claim 17, 18 or 19 wherein said compound which acts as inhibitor of the MDM2-p53 interaction is the compound 4- $\{[(2R,3S,4R,5S)-3-(3-Chloro-2-fluoro-phenyl)-4-(4-chloro-2-fluoro-phenyl)-4-cyano-5-(2,2-dimethyl-propyl)-pyrrolidine-2-carbonyl]-amino\}$ -3-methoxy-benzoic acid 1-mPEG-carbonyloxy-ethyl ester (mPEG, average MW, ~2000), according to claim 6.

22. The ~~method~~use of claim 17, 18 or 19 wherein said compound which acts as inhibitor of the MDM2-p53 interaction is the compound (A), according to claim 7.

23. An *in vitro* method for monitoring efficacy of therapy comprising an MDM2 inhibitor, in a patient suffering from cancer, the method comprising

- a) measuring the mRNA expression level of MDM2, XPC, BBC3 and CDKN2A in a sample obtained from that patient prior to treatment;
- b) using the expression levels obtained in a) to calculate a value designated as the patient's signature score prior to treatment;
- c) repeating step a) and b) after start of treatment with said MDM2 inhibitor; and
- d) comparing the signature scores obtained after start of treatment with those obtained prior to treatment, whereas higher signature scores after treatment indicate a response of the patient to the treatment, and thus a recommendation to continue the treatment.

24. The method of claim 23, wherein the MDM2 inhibitor is the compound used in the methods according to claims 5, 6 or 7.

25. A kit for predicting the response to treatment with a compound which acts as inhibitor of the MDM2-p53 interaction, comprising;

- a) reagents for measuring a level of at least one gene selected from the group consisting of BAX, RPS27L, EDA2R, XPC, DDB2, FDXR, MDM2, CDKN1A, TRIAP1, BBC3, CCNG1, TNFRSF10B, or CDKN2A in a sample; and
- b) a comparator module.

26. The kit according to claim 25, wherein the level of said at least one gene is the mRNA expression level.