

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

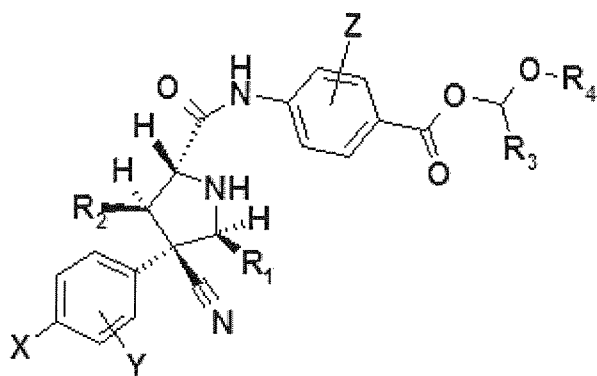
b) comparing the value or values from step a) to a standard value or set of standard values.

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 levels of a four-gene signature selected from the group consisting of MDM2, XPC, BBC3 and CDKN2A, and using those levels as biomarkers for predicting the patient's response to a compound, wherein the compound is an inhibitor of the MDM2-p53 interaction.

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[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

15 MDM2-inhibitors are disclosed in WO 2013/135648, which is incorporated in its entirety herein.



III

[0008] The present 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 5 2010/031713, WO 2011/098398 and WO 2013/135648.

The 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 10 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,

- 15 a) where the mRNA expression level of MDM2, XPC, BBC3 and CDKN2A had been measured from a sample obtained from that patient prior to treatment;
- b) a value designated as the patient's signature score had been calculated using the expression levels obtained in a);
- c) said patient's signature score obtained from b) had been compared to a reference level; and

d) said patient has been identified as more likely to respond to the therapy comprising said MDM2 inhibitor when the patient's signature score is above said reference 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
5 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
10 lung cancer. In yet another embodiment, the cancer is acute myeloid leukemia (AML), or Myelodysplastic Syndrome (MDS), or Myeloproliferative Neoplasms (MPN).

[0009] The 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
5 of response in the sample is compared. The kit may also comprise a captive reagent.

[0010] 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.

10 [0011] The present 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
15 inhibitor 2A).

[0012] The present disclosure identifies a 4-gene expression signature (mRNA) for identifying responses to MDM2 antagonist.

[0013] The present disclosure also identifies a 4-gene expression signature (mRNA) for monitoring treatment of cancer in a patient with an MDM2 antagonist.

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.

[00015a] In yet another aspect, the disclosure relates to an *in vitro* method for monitoring
5 efficacy of therapy comprising an MDM2 inhibitor, in a patient suffering from cancer, the method comprising a) measuring the mRNA expression levels 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 further provides the predictive signature utilizing mRNA values in determining the effectiveness of MDM2 antagonist therapy to cancers, in particular,
15 AML.

[00017] The present disclosure 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 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.

CLAIMS

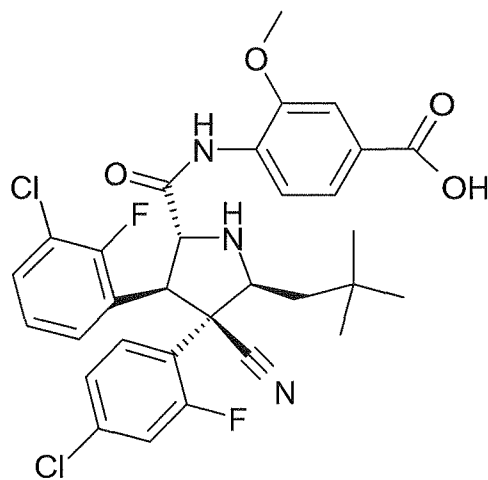
1. An *in vitro* method to predict responsiveness of a patient, with cancer, to a therapy, said method comprising detecting levels of a four-gene signature consisting of MDM2, XPC, BBC3 and CDKN2A, and using those levels as biomarkers for predicting the patient's response to a compound, wherein the compound is an inhibitor of the MDM2-p53 interaction.

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

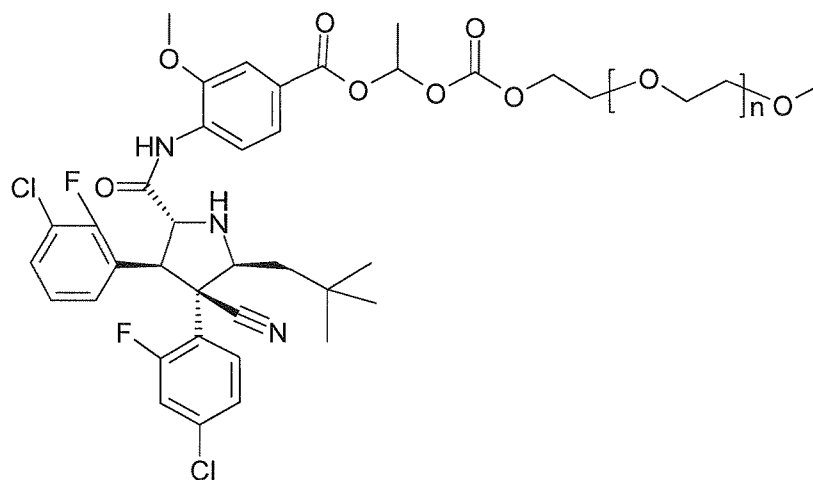
3. The method according to claim 1, said method comprising:

- a) measuring a level of said four-gene signature according to claim 1 in a sample that had been taken from the patient; and
- b) comparing the level from the patient to standard values from a patient with the same cancer.

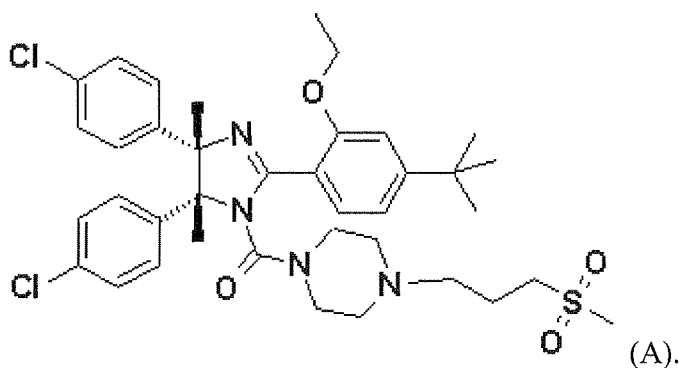
4. The method according to any one of claims 1 to 3, wherein the compound is 4-
{[2R,3S,4R,5S)-4-(4-Cholor-2-fluoro-phenyl)-3-(3-chloro-2-fluoro-phenyl)-4-cyano-5-(2,2-
dimethyl-propyl)-pyrrolidine-2-carbonyl]-amino}-3-methoxy-benzoic acid of the formula



5. The method according to any one of claims 1 to 3, wherein the compound is 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) of the formula:



6. The method according to any one of claims 1 to 3, wherein the compound is



7. The method according to any one of claims 1 to 3, wherein the level of said four-gene signature is the mRNA expression level

8. The method according to claim 1, characterized in that the four-gene signature is composed of up-regulations of three genes including MDM2, XPC, BBC3 and down-regulation of CDKN2A.

9. The method according to claim 1, wherein the cancer is selected from the group consisting of breast cancer, prostate cancer, cervical cancer, ovarian cancer, gastric cancer, colorectal cancer, pancreatic cancer, liver cancer, brain cancer, neuroendocrine cancer, lung cancer, kidney cancer, hematological malignancies, melanoma and sarcomas.

10. The method according to claim 9, wherein the cancer is acute myeloid leukemia (AML).

11. 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,

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

12. The use of claim 11 wherein said compound which acts as inhibitor of the MDM2-p53 interaction is the compound 4-{{[2R,3S,4R,5S]-4-(4-Chloro-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 4.

13. The use of claim 11 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 5.

14. The use of claim 11 wherein said compound which acts as inhibitor of the MDM2-p53 interaction is the compound (A), according to claim 6.

15. 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 levels 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.

16. The method of claim 15, wherein the MDM2 inhibitor is the compound used in the methods according to claims 4, 5 or 6.