

BIOMARKERS FOR CANCER THERAPY USING MDM2 ANTAGONISTS

FIELD OF THE INVENTION

This invention relates to biomarkers for cancer therapy. In particular, the invention provides biological markers that identify a cancer cell as likely to be sensitive to an MDM2 antagonist. These biomarkers can be incorporated into methods, systems and kits for predicting response to treatment, and into personalised treatments for cancer.

BACKGROUND TO THE INVENTION

Precision medicine, or personalised medicine, is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment and lifestyle for each patient. It is often said to be the practice of administering the right dosage of the right drug at the right time.

A particular focus of precision medicine is the need to predict whether a given patient will respond to a specific drug. A test that is able to predict whether a particular drug will effectively treat an individual patient is often referred to as a companion diagnostic. Effective companion diagnostics are very desirable because of their ability to improve treatment outcomes for patients while also saving the significant economic cost of providing ineffective treatments. An effective companion diagnostic for a new therapeutic agent can also increase the chances of that therapy being trialled in the correct population and ultimately being approved.

Precision medicines and companion diagnostics often rely on biomarkers that are able to predict reliably whether a patient is likely to respond to a specific treatment. Identifying reliable biomarkers for every therapy and disease is a very significant challenge.

Iorio *et al* (Cell. 2016 Jul 28;166(3):740-75) "A Landscape of Pharmacogenomic Interactions in Cancer" report how cancer-driven alterations identified in 11,289 tumours from 29 tissues (integrating somatic mutations, copy number alterations, DNA methylation, and gene expression) can be mapped onto 1,001 molecularly annotated human cancer cell lines and correlated with sensitivity to 265 drugs. While such studies provide a resource to link genotypes with cellular phenotypes and to identify therapeutic options for selected cancer sub-populations, the development of clinically-relevant molecularly-targeted cancer therapies remains a formidable challenge.

There remains a need to identify reliable biomarkers for use in precision medicine.

SUMMARY

The disclosure is based on the identification of biomarkers that can be used to predict effective treatment of cancer using an MDM2 antagonist. Identifying one or more of these biomarkers in a cancer patient allows a determination to be made whether the patient's cancer is likely to be treated or likely to be successfully treated using an MDM2 antagonist. Accordingly, in certain examples the disclosure relates generally to a companion diagnostic for MDM2 antagonist therapy.

In particular, a biomarker identified in the present disclosure is SKP2. The SKP2 protein and the gene encoding it are known in the art, with the Entrez gene ID 6502. As used herein, SKP2 is referred to as a “biomarker of the disclosure”.

In one aspect, the disclosure provides a use of an MDM2 antagonist in the manufacture of a medicament for treating a cancer, wherein the cancer is SKP2 depleted; and wherein MDM2 receptor antagonist is (2S,3S)-3-(4-chlorophenyl)-3-[(1R)-1-(4-chlorophenyl)-7-fluoro-5-[(1S)-1-hydroxy-1-(oxan-4-yl)propyl]-1-methoxy-3-oxo-2,3-dihydro-1H-isoindol-2-yl]-2-methylpropanoic acid or a tautomer, pharmaceutically acceptable salt or solvate thereof. In particular, in one example the disclosure provides an MDM2 antagonist for use in a method of treating cancer, wherein the cancer expresses low levels of SKP2.

In a further aspect, the disclosure provides a use of a pharmaceutical composition comprising an MDM2 inhibitor, wherein the MDM2 inhibitor is (2S,3S)-3-(4-chlorophenyl)-3-[(1R)-1-(4-chlorophenyl)-7-fluoro-5-[(1S)-1-hydroxy-1-(oxan-4-yl)propyl]-1-methoxy-3-oxo-2,3-dihydro-1H-isoindol-2-yl]-2-

methylpropanoic acid or a tautomer, N-oxide, pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament for treating cancer in a patient, wherein the cancer is SKP2 depleted.

In a further aspect, the disclosure provides a use of an MDM2 antagonist in the manufacture of a medicament for treating cancer in a patient in need thereof, wherein the method comprises: (i) determining that a sample from the patient is SKP2 depleted; and (ii) administering an effective amount of the MDM2 antagonist to the patient; and wherein the MDM2 antagonist is (2S,3S)-3-(4-chlorophenyl)-3-[(1R)-1-(4-chlorophenyl)-7-fluoro-5-[(1S)-1-hydroxy-1-(oxan-4-yl)propyl]-1-methoxy-3-oxo-2,3-dihydro-1H-isoindol-2-yl]-2-methylpropanoic acid or a tautomer, N-oxide, pharmaceutically acceptable salt or solvate thereof.

Sensitivity to MDM2 antagonism can be identified by reduced SKP2 expression.

For SKP2, protein and/or nucleic acid levels may typically be measured. Protein level can be achieved using, for example, immunohistochemistry (IHC).

SKP2 nucleic acid may be DNA or RNA. RNA may be detected, typically as SKP2 mRNA. Measurement techniques can therefore include quantitative techniques such as RT-PCR or Nanostring analysis, as are known in the art.

DNA can also be measured. In some examples copy number variation (CNV) analysis and/or mutational analysis (e.g. DNA sequencing) may be used to detect SKP2 status.

There are a variety of measures of the biomarker, including the presence or absence of the gene, mutation of the gene, gene expression level and protein expression level. The term depletion may mean loss or complete loss of the SKP2 gene, mutation of the SKP2 gene and loss of function, or it may mean low gene expression and low protein expression and function, which result from the loss or mutation of the gene or otherwise. All of these depletions are encompassed by the term “depleted”.

SKP2 can be detected directly, or indirectly. Indirect measurement typically involves detection of a molecule that is functionally upstream or downstream of SKP2 and the level of which correlates with the level of the biomarker. SKP2 can be detected indirectly through the detection of one or more SKP2 substrates. Accordingly, SKP2 substrates are also referred to herein as biomarkers of the disclosure. As described below, a typical SKP2 substrate is p27. In one example of the disclosure the SKP2 level is assessed by measuring the levels of one or more, for example two or more, three or more, five or more or ten or more, of p27, p21, p57, E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43. In one example, depleted SKP2 is determined by identifying increased or high levels of one or more, for example two or more, three or more, five or more or ten or more, of p27, p21, p57, E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43. In some examples, a combination of protein and nucleic acids may be detected.

High SKP2 substrate levels are indicative of low SKP2. In one example the biomarkers of the disclosure are low SKP2, or increased or high levels of one or more, for example two, three, four, five, six, seven, eight, nine, ten or more, of p27, p21, p57, E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43.

The data in the Examples below indicate that depletion for example loss (also known as total or complete loss) of SKP2 is predictive of sensitivity of cancer cells to an MDM2 antagonist. Accordingly, low levels of SKP2 can be used to identify a cancer suitable for treatment with an MDM2 antagonist.

Low SKP2 expression is indicative of sensitivity of cancer cells to an MDM2 antagonist. Conversely, normal or high SKP2 expression is indicative of resistance to an MDM2 antagonist. When a tumour has normal or high SKP2 and is therefore indicated not to be sensitive to MDM2 antagonism, an agent to induce sensitivity to an MDM2 antagonist can be used. This may be, for example, an agent to lower the level of SKP2 in the tumour. Accordingly, one method of treating cancer in a patient is provided wherein a patient is selected on the basis of having normal or high levels of SKP2 within a biological sample obtained from said patient, and that patient is administered a therapeutically effective amount of an MDM2 antagonist and an agent to induce sensitivity to an MDM2 antagonist, for example an agent to lower the levels of SKP2 in the tumour.

In some examples, the expression of a biomarker of the disclosure is determined relative to a non-cancer cell. This cancer:non-cancer comparison may be particularly useful for assessing SKP2 loss. The non-cancer cell will typically be a cell of the same type as the cancer cell. The non-cancer cell may be from the same patient, or may be from a different patient, or may be a value known for a non-cancer cell of that type. In this way, expression can be compared relative to control levels determined in healthy individuals or relative to control levels determined in normal non-proliferative tissue.

In some examples, the expression of a biomarker of the disclosure is determined relative to cancer cell samples from MDM2 inhibitor non-responsive subjects, or in a sample of cancer cells from an MDM2 inhibitor non-responsive subject. In some examples, the biomarker is decreased relative to the cancer cell samples from MDM2 inhibitor non-responsive subjects, or in a sample of cancer cells from an MDM2 inhibitor non-responsive subject. The non-responsive cancer cells will typically be a cell of the same cancer type as the tested cancer cell. The non-responsive cancer cells will typically be from a different patient or patients from the tested sample, or may be a value known for a non-responsive cancer cell of that cancer type. In some examples, the patient can be identified as a candidate for treatment with an MDM2 antagonist when the expression level of SKP2 is low relative to the upper limit of normal (ULN). Accordingly, in some examples a cancer is identified as treatable with an MDM2 inhibitor when SKP2 expression in the cancer is below the upper limit of normal (ULN).

Optionally, the method may comprise the step of administering a therapeutically effective amount of an MDM2 antagonist to the patient.

In all examples and examples described herein, the cancer is typically a p53-wild-type cancer.

In one example, the disclosure provides an MDM2 antagonist for use in the treatment of cancer, in particular a p53 wild type cancer, wherein the cancer is characterised by the biomarker of the disclosure within a biological sample obtained from the patient.

According to another example of the disclosure, there is provided a method of treating cancer in a patient wherein said method comprises the steps of selecting a patient based on the expression profile of a biomarker of the disclosure. In certain examples, the patient is selected based on:

having decreased SKP2 expression within a biological sample obtained from said patient;

and optionally then administering a therapeutically effective amount of a MDM2 antagonist to said patient.

According to a further example of the disclosure, there is provided an MDM2 antagonist for use in the treatment of cancer in a patient, characterised in that said patient has been selected for having:

decreased or low SKP2 expression within a biological sample obtained from said patient.

In certain examples a sample of patient tissue is tested prior to treatment, to determine the cancer biomarker expression profile. The sample may typically comprise one or more cancer cells, cancer DNA, or circulating tumour DNA. The sample may be a blood sample. The sample may be a tumour sample, for example a tumour biopsy. The testing may comprise an assay to detect protein, mRNA, DNA and/or ctDNA.

In another example, the disclosure provides the use of the expression level of SKP2 in a cancer cell sample of a human patient, as a biomarker for assessing whether the cancer is susceptible to treatment with an MDM2 antagonist.

In a further example, the disclosure provides a method for prognosing or assessing the responsiveness of a human cancer patient to treatment with an MDM2 antagonist, comprising assessing the expression level in a sample from a cancer patient of the biomarker of the disclosure and determining whether the tested expression level indicates that the cancer should be treated with an MDM2 antagonist.

In some examples, the biomarker of the disclosure indicates that the cancer is likely to be apoptosed effectively. Therefore, in some examples the disclosure is able to identify those patients for whom treatment will be particularly effective.

In some examples, the assessment step comprises an *in vitro* assay to determine the expression level of the biomarker or biomarkers.

In some examples, the assessment step comprises comparing the expression level with the expression level known to be associated with responsiveness or non-responsiveness to treatment with an MDM2 antagonist. In some examples, the assessment step comprises comparing the observed expression level with a threshold value reflecting in the same manner the expression level associated with susceptibility to treatment with an MDM2 antagonist, to assess whether the tested expression level indicates that the cancer can be treated with an MDM2 antagonist.

In some examples, the patient is classified into a group based on the biomarker profile. This may include classifying the patient as likely to respond well (or strongly), or not, to treatment with an MDM2 antagonist.

In a further example, the disclosure provides a method of determining whether a human cancer patient is suitable for treatment with an MDM2 antagonist, comprising

detecting in a sample of cancer cells from the patient the expression the biomarker of the disclosure; and

assessing whether the cancer in the patient is likely to be treated with a MDM2 antagonist on the basis of the expression level of the biomarkers in the sample. Optionally, the method of this example comprises the further step of treating the cancer in the patient using an MDM2 antagonist.

In a further example the disclosure provides an MDM2 antagonist for use in the treatment of cancer in a patient in combination with an anticancer compound, characterised in that said cancer in said patient is a p53 wild type cancer, which has been selected for having the biomarker of the disclosure.

In a further example the disclosure provides a method of treating cancer in a patient, wherein said cancer in said patient is optionally a p53 wild type cancer, and wherein the patient has been selected as having the biomarker of the disclosure at a level that indicates that MDM2 antagonist treatment will be effective; and administering a therapeutically effective amount of a MDM2 antagonist and optionally another anticancer agent to the selected patient.

In a further example the disclosure provides a method of identifying a patient suffering from cancer suitable for treatment with an MDM2 antagonist wherein said method comprises detecting, and optionally quantifying, the expression of SKP2.

In a further example the disclosure provides a method of selecting a patient (e.g. suffering from cancer) wherein said method comprises the steps of selecting a patient by detecting, and optionally quantifying, the expression of SKP2.

In a further example the disclosure provides a method of determining the likelihood that a cancer patient will respond to therapy with an MDM2 antagonist, the method comprising:

- obtaining a measurement of decreased SKP2 expression in a cancer cell sample from the patient, compared to a corresponding non-cancer cell;

- and determining that the patient is likely to respond to therapy with an MDM2 antagonist on the basis of that measurement.

In a further example the disclosure provides a drug administration process comprising:

- determining one or more biomarkers of the disclosure

- administering a therapeutically effective amount of an MDM2 antagonist to a patient with one or more biomarkers of the disclosure.

In yet a further example, the disclosure provides a method of detecting the expression SKP2 in a human patient suffering from cancer. This method typically comprises:

- (a) obtaining a sample of cancer cells from a human patient; and

- (b) detecting whether SKP2 is expressed in the sampled cancer cells by contacting the sample with one or more reagents for detecting expression of SKP2.

In a still further example, the disclosure provides a kit or device for detecting the expression level of at least one biomarker for sensitivity to MDM2 antagonism in a sample from a human patient, said kit or device comprising a detection reagent or detection reagents for detecting the biomarker of the disclosure.

In a further example, the disclosure resides in a system for assessing whether a human cancer patient is susceptible to treatment with an MDM2 antagonist, the system comprising:

detection means able and adapted to detect in a sample from the human patient the biomarker of the disclosure

a processor able and adapted to determine from the determined biomarker or biomarkers an indication of the likelihood of the patient being treatable with an MDM2 antagonist.

The system optionally contains a data connection to an interface, particularly a graphical user interface, capable of presenting information, preferably also capable of putting in information such as the age of the subject, as well as optionally other patient information such as sex and/or medical history information, said interface being either a part of the system or a remote interface. Optionally one or more of the foregoing items, particularly the processor, are enabled to function "in the cloud", i.e., not on a fixed machine, but by means of an internet-based application.

The disclosure also provides methods of identifying and screening patients, combinations, and kits.

In a further example, the disclosure provides a method of screening or identifying a patient for treatment with an MDM2 antagonist comprising determining whether said patient has:

decreased SKP2 expression within a biological sample obtained from said patient.

In a further example, the disclosure provides a method of identifying a patient responder comprising testing a patient for:

decreased SKP2 expression within a biological sample obtained from said patient.

In a further example, the disclosure provides a method of treatment comprising:

- (a) identifying a patient in need of treatment for cancer, optionally a p53 wild type cancer such as myeloid leukemia, optionally AML;
- (b) determining that the patient has decreased SKP2 expression within a biological sample obtained from said patient; and
- (c) treating the patient with a therapeutically effective amount of an MDM2 antagonist.

In a further example, the disclosure provides a method of treatment comprising:

- (a) identifying a patient in need of treatment for cancer, optionally myeloid leukemia such as AML;
- (b) determining the biomarker of the disclosure in the patient;
- (c) selecting an MDM2 antagonist as a treatment for the patient, based on the recognition that MDM2 antagonists are effective in patients who have one or more biomarkers of the disclosure;
- (d) treating the patient with a therapeutically effective amount of an MDM2 antagonist.

In a further example, the cancer is AML with a translocation t(15;17).

In a further example, the disclosure provides a method of selecting a treatment for a cancer patient comprising:

- (a) assaying one or more biological samples thereby determining the biomarker of the disclosure in the patient;
- (b) based on that determination selecting that patient for treatment with a therapeutically effective amount of an MDM2 antagonist.

In a further example, the disclosure provides a process for selecting a patient (e.g. suffering from cancer) for treatment with an MDM2 antagonist, characterised in that said patient has been selected for having:

decreased or low SKP2 expression within a biological sample obtained from said patient.

In a further example, the disclosure provides an MDM2 antagonist for use in the treatment of cancer in a patient, characterised in that said patient is known to have:

decreased SKP2 expression within a biological sample obtained from said patient.

In a further example, the disclosure provides a kit for treating cancer in a patient, wherein said kit comprises a biosensor for detection and/or quantification of SKP2, and/or reagents for the detection of SKP2, optionally together with instructions for use of the kit in accordance with the methods as defined herein.

In a further example, the disclosure provides a method of determining responsiveness of an individual with cancer to treatment with an MDM2 antagonist comprising:

decreased SKP2 expression within a biological sample obtained from said patient.

In a further example, the disclosure provides a method of determining responsiveness of an individual with cancer to treatment with an MDM2 antagonist comprising identifying a patient:

having decreased SKP2 expression within a biological sample obtained from said patient; and
then

administering a therapeutically effective amount of an MDM2 antagonist to said patient.

In a further example, the disclosure provides a method of treating cancer in a patient wherein said method comprises the steps of selecting a patient:

having decreased SKP2 expression within a biological sample obtained from said patient; and

administering to said patient selected in steps herein a therapeutically effective amount of an MDM2 antagonist *in combination with* interferon(s) (e.g. interferon alpha).

In a further example, the disclosure provides a drug administration process comprising:

- (i) ordering determination of SKP2 expression; and
- (ii) administering a therapeutically effective amount of an MDM2 antagonist to a patient with decreased levels of SKP2.

In a further example, the disclosure provides a packaged pharmaceutical product comprising:

- (i) an MDM2 antagonist;
- (ii) patient insert detailing instructions for use of the MDM2 antagonist in the treatment of patients identified using the biomarker profile described herein.

In a further example, the disclosure provides a method of treating cancer in a patient wherein said method comprises:

- (i) contacting a sample from a patient with a primer, antibody, substrate or probe, to determine the expression levels of SKP2;

- (ii) selecting a patient having decreased levels of SKP2 in a biological sample obtained from said patient;
- (iii) followed by administering a therapeutically effective amount of an MDM2 antagonist to said patient selected in step (ii).

The levels of SKP2 can be determined by measuring the levels of SKP2 expression directly. The protein or nucleic acid levels of SKP2 can be measured, as previously described.

In a further example, the levels of SKP2 may be determined by measuring the levels of SKP2 substrates. Known SKP2 substrates are disclosed in Chan *et al.* The Scientific World Journal (2010) 10, 1001-1015, in particular those described in Table 1.

Table 1 from Chan *et al*

Substrate	Function	Regulation
p27	Cell cycle inhibition	Skp2 induces the degradation of p27 and promotes cell cycle progression
p21	Cell cycle inhibition	Skp2 induces the degradation of p21 and promotes cell cycle progression
p57	Cell cycle inhibition	Skp2 induces the degradation of p57 and promotes cell cycle progression
E2F-1	Cell cycle regulation/ Apoptosis	Skp2 induces the degradation of E2F-1 and downregulation of its transcriptional activity
MEF	Cell cycle regulation	Skp2 induces the degradation of MEF at the G1/S transition
p130	Cell cycle inhibition	Skp2 induces the degradation of p130 and promotes G1/S transition
Tob1	Cell cycle inhibition	Skp2 induces the degradation of Tob1 and promotes G1/S transition
Cyclin D	Cell cycle regulation	Skp2 induces the degradation of Cyclin D and regulates cell cycle progression
Cyclin E	Cell cycle regulation	Skp2 induces the degradation of Cyclin E and regulates cell cycle progression
Smad4	Cell cycle arrest	Skp2 induces the degradation of cancer-derived Smad4 mutants
Myc	Cell cycle regulation/ Apoptosis	Skp2 triggers the degradation of Myc and enhances its transcriptional activity
Myb	Cell cycle regulation	Skp2 causes the degradation of Myc, and Myb degradation may be required for controlling the cell cycle progression
RASSF1A	Cell cycle inhibition	Skp2 induces the degradation of RASSF1A and promotes G1/S transition
Foxo1	Apoptosis	Skp2 triggers the degradation of Foxo1 and induces cell cycle arrest and apoptosis
Orc1p	DNA replication	Skp2 triggers the degradation of Orc1p to ensure that DNA replication is restricted to the S phase
Cdt1	DNA replication	Skp2 triggers the degradation of Cdt1 at S phase to prevent re-replication
Rag-2	DNA recombination	Skp2 triggers the degradation of Rag-2 and regulates V(D)J recombination
Brca2	DNA repair	Skp2 triggers the degradation of Brca2 and increases cell proliferation potential
Cdk9	Transcriptional elongation	Skp2 triggers the degradation of Cdk9 and regulates its transcriptional activity
MKP1	ERK signaling	Skp2 triggers the degradation of MKP1 and provides the a positive feedback in ERK activation
UBP43	Type 1 IFN signaling	Skp2 causes the degradation of UBP43 and may play a role in modifying type 1 IFN signaling

In one example of the disclosure the SKP2 level is assessed by measuring the levels of one or more of p27, p21, p57, E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43. In one example, depleted SKP2 is determined by identifying increased or high levels of one or more of p27, p21, p57, E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43. In certain examples, the levels of two, three, four, five, six, seven, eight, nine, ten or more of these substrates are measured or identified. In one example, the levels of all these

substrates are measured or identified. In another example, levels of one of these substrates is measured or identified.

Typically the level is detected, determined or known to reflect low SKP2. One example would be a level of the SKP2 substrate above the ULN.

In one example of the disclosure, depleted SKP2 is determined by measuring the levels of p27 in a tumour sample. Depleted SKP2 is determined by identifying increased or high p27 levels. Typically, the levels of the p27 protein are measured. Typically the p27 protein levels are measured using immunobased assays (e.g. ELISA, MSD, WES, Western blot, IHC etc.). In another example, p27 RNA levels are measured (by, for example, PCR, nanostring, RNA seq.).

The levels of SKP2 substrates may be measured in the same samples as disclosed for SKP2 measurement. Typically, SKP2 substrates are measured in tumour samples.

In a further example, the disclosure provides a method for identifying a patient for treatment with an MDM2 antagonist, the method comprising:

- (a) contacting a sample from the patient with a plurality of oligonucleotide primers, said plurality of primers comprising at least one pair of oligonucleotide primers for SKP2 and/or a SKP2 substrate (for example, p27), as disclosed above;
- (b) performing PCR on said sample to amplify gene expression products/transcripts in the sample;
- (c) determining the level of an expression product of at least one of said genes; and
- (d) identifying the patient as a candidate for treatment with an MDM2 antagonist when the expression level of SKP2 is low relative to the upper limit of normal (ULN).

The patient may optionally be identified as a candidate for treatment with an MDM2 antagonist when the expression level of SKP2 is low relative to (e.g. below) the upper limit of normal (ULN). In certain examples, the expression level of at least one other biomarker for MDM2 antagonist treatment is also determined. Accordingly, a panel of biomarkers can be tested, wherein the panel comprises SKP2.

In a further example, the disclosure provides a method for identifying a patient for treatment with an MDM2 antagonist, the method comprising:

- (a) contacting a sample from the patient with an antibody against SKP2;
- (b) performing an assay on said sample;
- (c) determining the level of SKP2; and
- (d) identifying the patient as a candidate for treatment with an MDM2 antagonist when the level of SKP2 is or reduced relative to the upper limit of normal (ULN).

The assay in part (b) may be or comprise an immunohistochemical assay. In some examples, the assay may be or comprise an ELISA. When the sample from the patient is contacted with an antibody against SKP2, an immunohistochemical assay is typically performed on said sample, and the patient is identified as a candidate for treatment with an MDM2 antagonist when the level of SKP2 is low (or absent) relative to the upper limit of normal (ULN).

Once a patient has been identified for treatment, the methods described herein can further comprise treating cancer in the patient with an MDM2 antagonist.

In a further example, the disclosure provides a method of selecting a cancer patient for receiving an MDM2 antagonist therapy for a cancer, comprising:

- (a) determining the level of SKP2 in a biological sample from the patient; and
- (b) selecting the patient who has a level of SKP2 in the biological sample from the patient that is lower than a predetermined value.

In a further example, the disclosure provides a method for predicting efficacy of MDM2 antagonist for a cancer in a patient, or for predicting response of a cancer patient to an MDM2 antagonist for a cancer, comprising determining the level of SKP2 in a biological sample from the patient, where a biological sample level of SKP2 equal to or typically less than a predetermined value is predictive of efficacy in the patient.

In a further example, the disclosure provides a method of selecting a patient having cancer in need of treatment with an MDM2 antagonist which comprises testing a tumour sample obtained from the patient for low level of SKP2.

In a further example, the disclosure provides a method of treating cancer comprising (i) testing a tumour sample obtained from a patient suffering from or likely to suffer from cancer for SKP2 loss and (ii) administering an MDM2 antagonist to the patient from which the sample was taken.

In a further example, the disclosure provides a method of identifying a patient having cancer most likely to benefit from treatment with an MDM2 antagonist comprising measuring the level of one or more of the biomarkers of the disclosure in a tumour sample obtained from the patient and identifying whether or not the patient is likely to benefit from treatment with an MDM2 antagonist according to the levels present.

Some examples of the disclosure comprise detecting the presence of mutation of SKP2 indicative of SKP2 loss. These mutations may be compared to control levels determined in normal non-proliferative tissue or absence of mutation.

The disclosure variously provides: a method of determining if a cancer patient is amenable to treatment with an MDM2 antagonist; a method of predicting the sensitivity of tumour cell growth to inhibition by a MDM2 antagonist; a method of predicting responsiveness of a cancer in a subject to a cancer therapy including an MDM2 antagonist; a method of developing a treatment plan for a subject with cancer; an *in vitro* method for the identification of a patient responsive to or sensitive to treatment with an MDM2 antagonist regimen. The methods typically comprise comparing the levels of SKP2 in the sample, typically a tumour sample, to a reference level and predicting the responsiveness of the cancer to treatment with the cancer therapy including an MDM2 antagonist.

In a further example, the disclosure provides an *in vitro* method for predicting the likelihood that a patient suffering from a tumour, who is a candidate for treatment with an MDM2 antagonist, will respond to the treatment with the compound, comprising the step of: (a) determining the levels of SKP2 in one or more tissue samples taken from the patient, wherein (i) SKP2 depletion or loss (e.g compared to a

reference value of at least one normal non-proliferative tissue) indicates that the patient is likely to respond to the treatment and/or (ii) normal or high levels of SKP2 indicates that the patient is less likely to respond to the treatment.

In a further example, the disclosure provides an assay comprising: (a) measuring or quantifying the level of SKP2; (b) comparing the level of SKP2 (e.g. relative to control levels determined in healthy individuals) (e.g. relative to control levels determined in normal non-proliferative tissue), and if the level of SKP2 (e.g. relative to control levels determined in healthy individuals) is decreased, and/or a loss of SKP2 (e.g. relative to control levels determined in normal non-proliferative tissue) identifying the patient as suitable for treatment with an MDM2 antagonist.

In a further example, the disclosure provides an assay comprising:

- (i) contacting a biological sample obtained from a patient with an antibody (e.g. antibody specific against SKP2);
- (ii) washing the sample to remove unbound antibody;
- (iii) measuring the intensity of the signal from the bound antibody;
- (iv) comparing the measured intensity of the signal with a reference value and if the measured intensity is increased relative to the reference value;
- (v) contacting a biological sample obtained from a patient with
 - a. a primer (e.g. at least one oligonucleotide primer pairs for the SKP2 gene),
 - b. an antibody (e.g. antibody specific against SKP2), and/or
 - c. a primer for a gene or mutant indicative of loss of SKP2;
- (vi) performing PCR, RT-PCR or next generation sequencing on said sample to amplify gene expression products/transcripts in the sample;
- (vii) determining the level of an expression product of at least one of said genes; and
- (viii) identifying the subject as having an increased probability of being suitable for treatment with an MDM2 antagonist.

In a further example, the disclosure provides a method of treating cancer comprising administering an MDM2 antagonist to a subject with loss of SKP2 in a tumour sample as determined by sequencing or immunoassay.

In a further example, the disclosure provides a method of administering an MDM2 antagonist to a patient in need thereof comprising:

- (1) determining the patient levels of SKP2;
- (2) assigning a phenotype to the patient based on the levels of the genes listed above and genotype of the tumour as determined in (1), wherein the phenotype is selected from poor (P), intermediate (I), and sensitive (S), and said phenotype is assigned based upon the level of the genes in the tumour; and
- (3) administering to the patient with phenotype S an MDM2 antagonist.

In a further example, the disclosure provides use of an MDM2 antagonist in the manufacture of a medicament for use in the treatment of cancer in a patient wherein the cancer tumour has SKP2 loss.

In a further example, the disclosure provides use of an MDM2 antagonist in the manufacture of a medicament for use in the treatment of cancer in a patient identified as likely to be responsive to treatment with an MDM2 antagonist according to the method described herein.

In a further example, the disclosure provides an article of manufacture comprising, packaged together, an MDM2 antagonist medicament in a pharmaceutically acceptable carrier and a package insert indicating that the cancer medicament is for treating a patient with cancer (e.g. myeloid leukemia, optionally AML) based on the level of SKP2 as determined by an assay method used to measure the levels.

In a further example, the disclosure provides a method for advertising an MDM2 antagonist medicament comprising promoting, to a target audience, the use of the MDM2 antagonist medicament for treating a cancer patient with SKP2 loss.

In a further example, the disclosure provides apparatus configured to identify a tumour (e.g. myeloid leukemia, optionally AML) in a cancer patient as being likely to benefit from treatment with a therapeutic agent or a combination of therapeutic agents targeting MDM2 or not likely to benefit from treatment with the therapeutic agent or combination of therapeutic agents. The apparatus may comprise a storage device storing sequencing data or immunoassay data from tumour or blood-based samples for SKP2 loss to identify the patient as being either likely or not likely to benefit from the therapeutic agent or a combination of therapeutic agents targeting MDM2.

In one example of the method described here when the SKP2 levels are low or absent (e.g. SKP2 loss) then the patient is administered an MDM2 antagonist.

In another example of the method described here when the SKP2 levels are high (or present) then the patient is not administered an MDM2 antagonist.

Where expression of the biomarker of the disclosure is high, an MDM2 antagonist may be administered in combination with an additional treatment, for example an additional treatment to reduce SKP2 levels.

SKP2 levels may be impacted by reducing gene expression, for example Notch1 signalling induces Skp2 gene expression and inhibiting Notch1 pathway will reduce Skp2 levels. The IKK-NF- κ B pathway also regulates Skp2 gene expression through p52/RelA or p52/RelB binding to the Skp2 promoter and reduction of signalling to NF κ B transcription factors will decrease SKP2 expression. Another pathway regulating Skp2 gene expression is the phosphoinositol 3-kinase (PI3K)/Akt pathway, thus inhibition of PI3K activity by PI3Ki or AKTi reduces Skp2 mRNA levels. Upstream regulators of the PI3K/AKT pathway, such as BCR-ABL and HER2 also regulate SKP2 expression.

In addition to regulation at the expression level, skp2 may be regulated via protein stability. SKP2 phosphorylation, for example by AKT or CDK2, attenuates SKP2 ubiquitination and degradation. Therefore, inhibition of SKP2 phosphorylation is another route to reduce SKP2 protein levels.

In certain examples, an MDM2 antagonist may be administered to a patient in combination with an additional cancer treatment that is not an MDM2 antagonist.

In one example a biomarker of the disclosure, in particular SKP2 and/or the SKP2 substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with an agent described in (i) –(xlix) below.

In one example the patient's tumour is determined not to be suitable for treatment with single agent MDM2 inhibitor due to presence of high or increased levels of SKP2 ("SKP2 high") and hence treatment with an additional agent can be used to trigger low or decreased levels of SKP2 ("SKP2 low") in combination with MDM2 inhibitor. In one example the patient's tumour is determined to be SKP2 high and is treated with an MDM2 antagonist in combination with an additional anticancer agent. In one example the patient's tumour is determined to be SKP2 high and is treated with an MDM2 antagonist in combination with one or more of the agents listed in (i) –(xlix) below.

In one example the biomarkers of the disclosure, in particular SKP2 and/or the SKP substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with inhibitors of AKT, inhibitors of CDK2, inhibitors of Notch1 pathway, inhibitors of the phosphoinositol 3-kinase (PI3K)/Akt pathway, inhibitors of the IKK-NF-kB pathway, inhibitors of BCR-ABL and/or inhibitors of HER2.

In one example the patient's tumour is determined to be SKP2 high and is treated with an MDM2 antagonist in combination with inhibitors of AKT, inhibitors of CDK2, inhibitors of Notch1 pathway, inhibitors of the phosphoinositol 3-kinase (PI3K)/Akt pathway, inhibitors of the IKK-NF-kB pathway, inhibitors of BCR-ABL and/or inhibitors of HER2.

In one example the biomarkers of the disclosure, in particular SKP2 and/or the SKP substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with inhibitors of CDK2.

In one example the biomarkers of the disclosure, in particular SKP2 and/or the SKP substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with inhibitors of Notch1 pathway.

In one example the biomarkers of the disclosure, in particular SKP2 and/or the SKP substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with inhibitors of the phosphoinositol 3-kinase (PI3K)/Akt pathway, in particular inhibitors of PI3K activity by PI3Ki or AKTi.

In one example the biomarkers of the disclosure, in particular SKP2 and/or the SKP substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with inhibitors of the IKK-NF-kB pathway for example through p52/RelA or p52/RelB binding to the Skp2 promoter and reduction of signalling to NFkB transcription factors.

In one example the IKK-NF-kB pathway inhibitor is an IRAK inhibitor.

In one example the biomarkers of the disclosure, in particular SKP2 and/or the SKP substrates listed herein can be used to select a patient to treat with an MDM2 antagonist in combination with inhibitors of BCR-ABL and/or inhibitors of HER2.

The term "PI3K/AKT pathway inhibitor" is used herein to define a compound which inhibits the activation of AKT, the activity of the kinase itself or modulate downstream targets, blocking the proliferative and cell survival effects of the pathway (including one or more of the target enzymes in the pathway as described herein, including phosphatidylinositol-3 kinase (PI3K), AKT, mammalian target of rapamycin (mTOR), PDK-1, p70 S6 kinase and forkhead translocation).

PI3K/AKT pathway inhibitors include PKA/B and/or PKB (akt) inhibitors, PI3K inhibitors, mTOR inhibitors, and/or calmodulin inhibitors (forkhead translocation inhibitors)

Examples of PI3K/AKT pathway inhibitors include PI3K Inhibitors.

Examples of PI3K/AKT pathway inhibitors also include mTOR.

Examples of PI3K/AKT pathway inhibitors include forkhead translocation inhibitors.

Examples of PI3K/AKT pathway inhibitors include AKT Inhibitors.

In one example the PI3K/AKT Pathway inhibitor is a PI3K inhibitor selected from one or more of the specific compounds described above. In one example the PI3K/AKT Pathway inhibitor is PI-103 or LY294002.

In one example is provided a combination of an MDM2 antagonist as described herein and a PI3K/AKT pathway inhibitor.

In another example there is provided a method of treating cancer in a patient wherein said method comprises the steps of selecting a patient:

- (a) having high levels of SKP2 (or low levels of SKP2 substrates) within a biological sample obtained from said patient; and
- (b) administering a therapeutically effective amount of an MDM2 antagonist and an agent to lower the levels of SKP2 to said patient selected in step (a).

In one example the agent or treatment to decrease SKP2 levels is an anticancer agent or treatment. In one example the agent or treatment to decrease SKP2 levels is a PI3K/AKT Pathway inhibitor.

In another example there is provided an MDM2 antagonist for use in a method of treating a cancer having normal or high SKP2 expression, in combination with an agent to induce sensitivity to an MDM2 antagonist for example an agent to lower the level of SKP2.

Another example provides a method of treating cancer in a patient wherein said method comprises the steps of selecting a patient:

- (a) having normal or high levels of SKP2 within a biological sample obtained from said patient; and
- (b) administering a therapeutically effective amount of an MDM2 antagonist and an agent to induce sensitivity to an MDM2 antagonist for example an agent to lower the levels of SKP2 to said patient selected in step (a).

The agent to induce sensitivity to an MDM2 antagonist may in some examples be ASTX660, also known as tolinapant.

CLAIMS:

1. Use of an MDM2 antagonist in the manufacture of a medicament for treating a cancer, wherein the cancer
is SKP2 depleted;
5 and wherein MDM2 receptor antagonist is (2S,3S)-3-(4-chlorophenyl)-3-[(1R)-1-(4-chlorophenyl)-7-fluoro-5-[(1S)-1-hydroxy-1-(oxan-4-yl)propyl]-1-methoxy-3-oxo-2,3-dihydro-1H-isindol-2-yl]-2-methylpropanoic acid or a tautomer, pharmaceutically acceptable salt or solvate thereof.
2. The use according to claim 1, wherein the SKP2 depletion is determined by:
10 determining SKP2 levels directly; or
determining increased or high levels of one, two, three, four or more of p27, p21, p57, E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43; or
determining increased or high levels of p27 and optionally one or more of p21, p57,
15 E2F-1, MEF, P130, Tob1, cyclin D, cyclin E, Smad4, Myc, Mcb, RASSF1A, Foxo1, Orc1p, Cdt1, Rag2, Brca2, CDK9, MPK1, and/or UBP43.
3. The use according to claim 1 or claim 2, wherein a sample of patient tissue is tested to determine the cancer expression profile prior to treatment.
4. The use according to claim 3, wherein the sample comprises cancer DNA, ctDNA, or cancer
20 cells.
5. The use according to claim 3 or claim 4, wherein the testing comprises an assay to detect protein, mRNA and/or ctDNA.
6. The use according to claim 5, wherein (i) protein is detected using an immunoassay, a protein-binding assay, an antibody-based assay, an antigen-binding protein-based assay, a protein-based array, an enzyme-linked immunosorbent assay (ELISA), flow cytometry, a protein array,
25 a blot, a Western blot, nephelometry, turbidimetry, chromatography, mass spectrometry, enzymatic activity, a radioimmunoassay, immunofluorescence, immunochemiluminescence, immunoelectrochemiluminescence, immunoelectrophoretic, a competitive immunoassay, or immunoprecipitation; and/or (ii) wherein mRNA is detected using RT-PCR or a quantitative gene
30 expression assay.
7. The use according to any of claims 3 to 6 wherein the patient is selected for treatment based on the determined expression profile.
8. The use according to any preceding claim, wherein the cancer is a blood cancer, a leukemia, a lymphoma, a myeloid leukemia or an acute myeloid leukemia.
- 35 9. The use according to claim 8, wherein the cancer is acute myeloid leukemia with a translocation t(15;17).
10. The use according to any preceding claim, wherein the cancer is P53 wild-type.
11. The use according to any preceding claim, wherein the cancer cells undergo apoptosis following the treatment step.

12. The use according to any preceding claim, wherein activated caspase-3 is induced by the MDM2 antagonist in at least a proportion of the cancer cells.
13. The use according to claim 11, wherein activated caspase-3 is induced by the MDM2 antagonist in at least 40% of the cancer cells or at least 60% of the cancer cells.
- 5 14. The use according to any preceding claim, wherein the cancer shows SKP2 loss.
15. The use according to any of claims 1 to 13, wherein the MDM2 antagonist is part of a combination therapy with a second therapeutic agent.
17. The use according to claim 15, wherein the second therapeutic agent or the agent to induce sensitivity to an MDM2 antagonist is ASTX660.
- 10 18. Use of a pharmaceutical composition comprising an MDM2 inhibitor, wherein the MDM2 inhibitor is (2S,3S)-3-(4-chlorophenyl)-3-[(1R)-1-(4-chlorophenyl)-7-fluoro-5-[(1S)-1-hydroxy-1-(oxan-4-yl)propyl]-1-methoxy-3-oxo-2,3-dihydro-1H-isoindol-2-yl]-2-methylpropanoic acid or a tautomer, N-oxide, pharmaceutically acceptable salt or solvate thereof, in the manufacture of a medicament for treating cancer in a patient, wherein the cancer is SKP2 depleted.
- 15 19. Use of an MDM2 antagonist in the manufacture of a medicament for treating cancer in a patient in need thereof, wherein the method comprises:
 - (i) determining that a sample from the patient is SKP2 depleted; and
 - (ii) administering an effective amount of the MDM2 antagonist to the patient;and wherein the MDM2 antagonist is (2S,3S)-3-(4-chlorophenyl)-3-[(1R)-1-(4-chlorophenyl)-7-fluoro-5-[(1S)-1-hydroxy-1-(oxan-4-yl)propyl]-1-methoxy-3-oxo-2,3-dihydro-1H-isoindol-2-yl]-2-methylpropanoic acid or a tautomer, N-oxide, pharmaceutically acceptable salt or solvate thereof.
- 20