

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 aspects the disclosure relates generally to a companion diagnostic for MDM2 antagonist therapy.

The biomarkers identified in the present disclosure are: (i) BAP1; and/or (ii) CDKN2A; and/or (iii) one, two, three, four, five, six, seven, eight, nine, ten or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1. These proteins and the genes encoding them are all known in the art, and the Entrez gene ID's are provided below. As used herein, these biomarkers are referred to as the "biomarkers of the disclosure".

In one aspect, the present disclosure provides a use of an MDM2 antagonist in the manufacture of a medicament for treating a cancer, wherein the cancer is BAP1 depleted, 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.

In another aspect, the present 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 as defined herein.

In particular, in one example the disclosure provides an MDM2 antagonist for use in a method of treating cancer, wherein the cancer

is BAP1 depleted; and/or

is CDKN2A depleted; and/or

shows increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1.

Sensitivity to MDM2 antagonism can be identified by: (i) reduced BAP1 expression; and/or (ii) reduced CDKN2A expression; and/or (iii) increased expression of one, two, three, four, five, six, seven, eight, nine, ten or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1.

In one example, an MDM2 antagonist is provided for use in a method of treating a cancer, wherein the cancer is BAP1 depleted. In this example, the BAP1 depleted cancer may also be CDKN2A depleted; and/or show increased expression of one, two, three, four, five or more the interferon signature genes. For CDKN2A, protein is typically measured. This can be achieved using, for example, immunohistochemistry (IHC). In some examples mutational analysis (e.g. DNA sequencing) may be used to detect CDKN2A status.

For BAP1, protein may typically be measured. This can be achieved using, for example, immunohistochemistry (IHC). Cellular location may also be measured in some examples. In some examples mutational analysis (e.g. DNA sequencing) may be used to detect BAP1 status.

CDKN2A and BAP1 are sometimes referred to herein as the protein biomarkers. The CDKN2A gene encodes the p16(INK4A) and the p14(ARF) proteins, and references to the gene CDKN2A includes the proteins encoded by CDKN2A. The CDKN2A loss can be measured by low protein expression product levels i.e. an expression level that is lower than a control expression level, of p16(INK4A) and/or the p14(ARF) i.e. a consequence of the CDKN2A gene loss is loss of p16 and/or P14.

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 a gene, mutation of the gene e.g. BAP1 or CDKN2A 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”.

The remaining biomarkers identified herein (i.e. those identified above as having increased expression) are sometimes referred to as the interferon signature, or IFN signature, biomarkers. They are also referred to by the term Type 1 interferon pathway genes. Typically, these biomarkers will be detected as mRNA. Measurement techniques for one or more IFN signature biomarkers can therefore include quantitative techniques such as rtPCR 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 biomarker gene status.

The biomarkers of the disclosure may be measured directly or indirectly. Indirect measurement typically involves detection of a molecule that is functionally upstream or downstream of the biomarker and the level of which correlates with the level of the biomarker. For example, a substrate upon which the biomarker acts can be used as an indirect measurement of the biomarker. In one example, BAP1 levels may be measured by detecting the level of histone H2A ubiquitination, with increased H2A ubiquitination typically reflecting decreased BAP1. In another example, BAP1 depletion can be assessed by determining increased EZH2 expression or activity.

The data in the Examples below indicate that depletion, for example loss (also known as total or complete loss) of CDKN2A and/or BAP1, and/or elevated levels of one or more of the IFN signature biomarkers, is predictive of sensitivity of cancer cells to an MDM2 antagonist. Accordingly, low levels of CDKN2A and/or BAP1; and/or high levels of one or more of the IFN signature biomarkers, can be used to identify a cancer suitable for treatment with an MDM2 antagonist.

In some examples, the decreased or increased expression of the biomarker or biomarkers of the disclosure are determined relative to a non-cancer cell. This cancer:non-cancer comparison may be particularly useful for assessing BAP1 loss and/or CDKN2A 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 other examples, the decreased or increased expression of the biomarker or biomarkers of the disclosure are 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 one or more IFN signature biomarkers are increased or elevated relative to the amount of RNA determined in 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 BAP1 and/or CDKN2A is low relative to the upper limit of normal (ULN), and/or the expression level of at least one of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 is high relative to 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 aspects 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 one or more of the biomarkers 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 one more of the biomarkers of the disclosure. In certain examples, the patient is selected based on:

having decreased BAP1 expression within a biological sample obtained from said patient;
and/or

having decreased CDKN2A expression within a biological sample obtained from said patient;
and/or

having increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5,

MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 BAP1 expression within a biological sample obtained from said patient; and/or

decreased or low CDKN2A expression within a biological sample obtained from said patient; and/or

increased or high expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 levels of one or more biomarkers of the disclosure in a cancer cell sample of a human patient, as biomarkers 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 one or more biomarkers 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 one or more biomarkers of the disclosure indicate 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 of one or more biomarkers 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 one or more biomarkers 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 one or more biomarkers 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 one or more biomarkers of the disclosure.

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 one or more biomarkers of the disclosure.

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 BAP1 expression in a cancer cell sample from the patient, compared to a corresponding non-cancer cell; and/or

- obtaining a measurement of decreased CDKN2A expression in a cancer cell sample from the patient, compared to a corresponding non-cancer cell; and/or

- obtaining a measurement that demonstrates increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74,

IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1;

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 of one or more biomarkers of the disclosure 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 said biomarkers are expressed in the sampled cancer cells by contacting the sample with one or more reagents for detecting expression of the biomarkers.

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 one or more biomarkers 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 one or more biomarkers 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 BAP1 expression within a biological sample obtained from said patient; and/or

decreased CDKN2A expression within a biological sample obtained from said patient; and/or

increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 BAP1 expression within a biological sample obtained from said patient; and/or
decreased CDKN2A expression within a biological sample obtained from said patient; and/or
increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 mesothelioma;
- (b) determining that the patient has
decreased BAP1 expression within a biological sample obtained from said patient; and/or
decreased CDKN2A expression within a biological sample obtained from said patient; and/or
increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 mesothelioma;
- (b) determining one or more biomarkers 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 disclosure provides a method of selecting a treatment for a cancer patient comprising:

- (a) assaying one or more biological samples thereby determining one or more biomarkers 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 BAP1 expression within a biological sample obtained from said patient; and/or
decreased or low CDKN2A expression within a biological sample obtained from said patient;
and/or

increased or high expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 BAP1 expression within a biological sample obtained from said patient; and/or
decreased CDKN2A expression within a biological sample obtained from said patient; and/or
increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 one or more biomarkers of the disclosure, and/or reagents for the detection of one or more biomarkers of the disclosure, 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 BAP1 expression within a biological sample obtained from said patient; and/or
decreased CDKN2A expression within a biological sample obtained from said patient; and/or
increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5,

MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 BAP1 expression within a biological sample obtained from said patient;
and/or
having decreased CDKN2A expression within a biological sample obtained from said patient;
and/or
having increased expression of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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 BAP1 expression within a biological sample obtained from said patient;
and/or
having decreased CDKN2A 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) to elevate the expression levels one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1.

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

- (i) ordering determination of BAP1 expression; and/or
- (ii) ordering determination of CDKN2A expression; and/or
- (iii) ordering determination of expression levels of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1; and

- (iv) administering a therapeutically effective amount of an MDM2 antagonist to a patient with decreased levels of BAP1 and/or CDKN2A and/or increased levels of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1.

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 BAP1 and/or CDKN2A and/or one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1;
- (ii) selecting a patient having decreased levels of BAP1 and/or CDKN2A and/or increased levels of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 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).

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 any one or more of the following: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3,

PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1;

- (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 said at least one gene is high 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 BAP1 and/or CDKN2A is low relative to (e.g. below) the upper limit of normal (ULN), and/or the expression level of at least one of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 is high relative to (e.g. above) the upper limit of normal (ULN).

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 one or more biomarkers of the disclosure;
- (b) performing an assay on said sample;
- (c) determining the level of one or more biomarkers of the disclosure; and
- (d) identifying the patient as a candidate for treatment with an MDM2 antagonist when the level of one or more biomarkers of the disclosure is elevated 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 BAP1 and/or CDKN2A, 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 BAP1 or CDKN2A 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 one or more of BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 in a biological sample from the patient; and

(b) selecting the patient who has a level of BAP1 and/or CDKN2A in the biological sample from the patient that is lower than a predetermined value, and/or a level of one or more of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 in the biological sample from the patient that is equal to or greater 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 one or more of BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and/or BRCA1 in a biological sample from the patient, where a biological sample level of BAP1 and/or CDKN2A equal to or typically less than a predetermined value and/or a level of one or more of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 equal to or typically greater 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) a tumour sample obtained from the patient for elevated CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and/or BRCA1 and/or (b) a tumour sample obtained from the patient for low level of BAP1 and/or CDKN2A.

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 elevated IFN signature biomarkers and/or for BAP1 loss and/or CDKN2A 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 BAP1 and/or CDKN2A indicative of BAP1 loss and/or CDKN2A 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 one or more biomarkers of the disclosure 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 one example the methods comprise analysing one or more, for example, two or more, or three or more, or four or more, or five or more, or six or more, or seven or more, or eight or more, or nine or more, or ten or more, or fifteen or more biomarkers described herein. In example the one or more biomarkers include BAP1. In example the two or more biomarkers include BAP1 and CDKN2A, In one example the two or more biomarkers include BAP1 and one or more biomarkers selected from CDKN2A, CXCL10, CXCL11, IRF7, IFITM1, IRF9, MX1 or IFI35.

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 one or more of BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1, in one or more tissue samples taken from the patient, wherein (i) an elevated level of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 (e.g. compared to a reference value of at least one healthy reference person), and/or BAP1 loss and/or CDKN2A 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) a lower level of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1, and/or normal or high levels of BAP1 and/or CDKN2A 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 one or more of BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1; (b) comparing the level of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 (e.g. relative to control levels determined in healthy individuals), and/or a BAP1 and/or CDKN2A (e.g. relative to control levels determined in normal non-proliferative tissue), and if the level of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 (e.g. relative to control levels determined in healthy individuals) is elevated, and/or there is a loss of BAP1 and/or CDKN2A (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 one or more of BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1;
- (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 any one or more of the following genes: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1),

- b. an antibody (e.g. antibody specific against one or more of BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1), and/or
- c. a primer for a gene or mutant indicative of loss of BAP1 or loss of CDKN2A;
- (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 elevated expression of one or more of the following genes: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA, and/or loss of BAP1 and/or CDKN2A 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 BAP1, CDKN2A, CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and/or BRCA1;
- (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 BAP1 loss and/or CDKN2A loss, and/or the patient has elevated expression of one or more of the following genes: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58,

TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1.

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 (e.g. mesothelioma, renal, or glioblastoma) medicament is for treating a patient with cancer based on levels of a biomarker or biomarkers identified herein 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 elevated levels of one or more of the following genes: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 and/or BAP1 loss and/or CDKN2A loss.

In a further example, the disclosure provides apparatus configured to identify a tumour (e.g. mesothelioma) of 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 the levels of one or more of the following genes: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1, and/or BAP1 loss and/or CDKN2A 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 BAP1 and/or CDKN2A levels are low or absent (e.g. BAP1 loss or CDKN2A loss) and/or the levels of one or more of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 is/are not normal (e.g. increased or high) then the patient is administered an MDM2 antagonist.

In another example of the method described here, when the BAP1 and/or CDKN2A levels are high (or present) and/or the levels of one or more of CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1, WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1 is/are normal or low then the patient is not administered an MDM2 antagonist.

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 the at least one biomarker of the disclosure can be used to select a patient to treat with an MDM2 antagonist in combination with an agent described in (i) –(xlix) below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Cancer cell lines with CDKN2A loss showed increased sensitivity to Compound 1 compared to those with wild-type CDKN2A across all tumour types tested (A) and in specific indications such as non-small-cell lung carcinoma (NSCLC) (B).

Figure 2: Percentages of activated caspase-3 positive cells following 72-hour treatment with DMSO and 1µM Compound 1 in human patient-derived mesothelioma cell lines.

Figure 3: Heatmap of the significantly differentially expressed genes in the comparison of apoptotic and non-apoptotic mesothelioma cell lines. Columns are cell lines and rows are genes. The key on the top left indicates the log fold change of genes.

Figure 4: GSEA enrichment plot of the Interferon alpha signalling pathway. The x-axis are genes (vertical black lines) and y-axis represents enrichment score (ES), which represents the enrichment of interferon alpha signalling pathway at the top of the ranked gene list. Genes with a distinct peak at the beginning of the plot are highly positively correlated with the apoptotic phenotype.

Figure 5: Ingenuity pathway analysis (IPA)-generated Interferon signalling pathway. Both up-regulated and down-regulated genes were used for the analysis. Genes significantly up-regulated in apoptotic cell lines are highlighted in grey background.

Figure 6: Interferon signature genes upregulated also in renal tumours. For each gene, the bars left to right represent GTEx (normal tissues), TCGA-GBM (glioblastoma), TCGA-KIRC (kidney renal clear cell carcinoma) and TCGA-MESO (mesothelioma).

Figure 7: Western blot showing protein levels of BAP1 and β-Actin in total lysates of 12 patient-derived mesothelioma cell lines. Cell lines are grouped as Apoptotic vs. Non-Apoptotic as shown in Figure 2 (* non-specific band) (A). Tukey boxplot shows quantification of BAP1 protein expression normalised to β-Actin from Figure 7A . **P <0.005, Mann–Whitney test (B).

CLAIMS:

1. Use of an MDM2 antagonist in the manufacture of a medicament for treating a cancer, wherein the cancer is BAP1 depleted, wherein the MDM2 antagonist is (2S,3S)-3-(4-chlorophenyl)-3-
5 [(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.
2. The use according to claim 1, wherein the cancer:
is CDKN2A depleted; and/or
shows increased expression of one, two, three, four, five or more interferon signature
10 genes.
3. The use according to claim 2, wherein the interferon signature genes are CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1,
15 WARS, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, FLI1 and BRCA1.
4. The use according to any of claims 1 to 3, wherein a sample of patient tissue is tested to determine the cancer expression profile prior to treatment.
5. The use according to claim 4, wherein the sample comprises cancer DNA, ctDNA, or cancer
20 cells.
6. The use according to claim 4 or claim 5, wherein the testing comprises an assay to detect protein, mRNA and/or ctDNA.
7. The use according to claim 6, 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 expression assay.
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8. The use according to any of claims 4 to 7 wherein the patient is selected for treatment based on the determined expression profile.
9. The use according to any preceding claim, wherein the cancer is:
(i) non-small-cell lung carcinoma, mesothelioma, glioblastoma or kidney renal
35 clear Cell carcinoma; or
(ii) brain, clear cell renal cell carcinoma (ccRCC), esophageal cancer or melanoma.
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
40 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 12, 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 increased expression, relative to a control, of one, two, three, four, five or more of: CXCL10, CXCL11, RSAD2, MX1, BATF2, IFI44L, IFITM1, ISG15, CMPK2, IFI27, CD74, IFIH1, CCRL2, IFI44, HERC6, ISG20, IFIT3, HLA-C, OAS1, IFI35, IRF9, EPSTI1, USP18, BST2, CSF1, C1S, DHX58, TRIM14, OASL, IRF7, LGALS3BP, DDX60, LAP3, LAMP3, PARP12, PARP9, SP110, PLSCR1 and WARS.
- 10 15. The use according to claim 14, wherein the cancer shows increased expression of CXCL10 or CXCL11.
16. The use according to any preceding claim, wherein the cancer shows increased expression of one, two, three, four, five or more of IRF7, STAT1, IRF3, IRF5, MSC, JUN, SPI1, IRF1, COMMD3-BMI1, STAT2, RUNX3, SREBF1, IRF9, FLI1 and BRCA1.
- 15 17. The use according to any preceding claim, wherein the cancer shows BAP1 loss.
18. The use according to any preceding claim, wherein the cancer shows CDKN2A loss.
19. The use according to any of claims 1 to 18, wherein the MDM2 antagonist is part of a combination therapy with a second therapeutic agent.
20. 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 as defined in any of claims 1 to 3.

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