for poor prognosis and BAP1 mutations in uveal melanoma patients predict for a higher risk group and metastasis.

Enhancer of zeste homolog 2 (EZH2), is a member of the Polycomb-group (PcG) family, members of which are involved in the regulation of the transcriptional state of genes by methylation of histone proteins. Drugs have been developed that specifically target EZH2 and the effect of such drugs in patients with a variety of tumors has been an active area of investigation. EZH2 inhibitors are currently being tested clinically in lymphoma patients with EZH2-activating mutations. Accordingly, there is a need in the art for treatments for patients with BAP1-mutations and biomarkers that will be useful to determine when an EZH2 inhibitor should be used to treat a cancer.

3. SUMMARY OF THE INVENTION

In one aspect, there is provided use of an EZH2 inhibitor in the manufacture of a medicament for the treatment of a cancer in a subject in need thereof, wherein the medicament is to be administered to the subject when the expression of a BAP1 biomarker in the cancer is determined to be absent or expressed at a lower level than a BAP1 reference control level, wherein the cancer is selected from the group consisting of malignant mesotheliomas, uveal melanomas, renal cell carcinoma, cutaneous melanomas, lung cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomyosarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes, and bladder cancer.The present invention relates to the use of one or more biomarkers to evaluate the likelihood that an EZH2 inhibitor would produce an anti-cancer effect in a subject. It is based, at least in part, on the discovery that the loss of BAP1 activity results in the upregulation of EZH2 expression and activity.

Accordingly, in non-limiting embodiments, the present invention provides for assay methods and kits for determining the presence of one or more biomarkers, *e.g.*, BAP1 biomarkers, in a sample from a patient, and methods of using such determinations in selecting a therapeutic regimen for a cancer patient and in methods of treating cancer patients.

The present invention provides for a method for determining whether an anti-cancer effect is likely to be produced in a cancer by an EZH2 inhibitor. In a non-limiting embodiment, the method comprises determining the expression of a BAP1 biomarker in one or more cells of the cancer, where if the BAP1 biomarker is absent or expressed at lower levels in the cancer, as compared to a reference control level, a therapeutically effective amount of an EZH2 inhibitor is administered to produce an anti-cancer effect. In certain non-limiting embodiments, the expression of the BAP1 biomarker can be determined by immunofluorescence, Western Blot, in situ hybridization or polymerase chain reaction. In certain embodiments, the method can further include determining the expression level of EZH2, SUZ12 and/or L3MBTL2 in the sample.

The present invention further provides for a method for treating a subject having a cancer. In certain non-limiting embodiments, the method comprises obtaining a sample of the cancer from the subject, and determining, in the sample, the expression level of a BAP1 biomarker and/or the expression level of EZH2 and/or SUZ12, where if the BAP1 biomarker is absent or expressed at a lower level than a BAP1 reference control level and/or if the expression of EZH2 and/or SUZ12 is increased compared to an EZH2 and/or SUZ12 reference control level, then treatment of the subject with a therapeutically effective amount of an EZH2 inhibitor is initiated.

The present invention further provides for a method for determining whether an anti-cancer effect is likely to be produced in a cancer by an EZH2 inhibitor. In a non-limiting embodiment, the method comprises obtaining a sample of the cancer from a subject, and determining, in the sample, the expression level of an BAP1 biomarker, where if the BAP1 biomarker is absent or expressed at lower level in the cancer, as compared to a reference control level, it is more likely that the EZH2 inhibitor would have an anti-cancer effect on the cancer. In certain embodiments, the BAP1 biomarker is a BAP1 protein biomarker. In certain embodiments, the BAP1 biomarker is a BAP1 nucleic acid biomarker.

In one aspect, there is provided a method for determining whether an anti-cancer effect is likely to be produced in a cancer by an EZH2 inhibitor, comprising determining the expression level of a BAP1 biomarker in a sample of the cancer, wherein if the expression of the BAP1 biomarker in the sample is absent or expressed at lower level than a BAP1 reference control level, it is more likely that the EZH2 inhibitor would have an anti-cancer effect on the cancer, wherein the cancer is selected from the group consisting of malignant mesotheliomas, uveal melanomas, renal cell carcinoma, cutaneous melanomas, lung

cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomyosarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes and bladder cancer.

The present invention further provides for a method of predicting the sensitivity of a cancer in a patient to an EZH2 inhibitor. In a non-limiting embodiment, the method comprises obtaining a sample of the cancer from the patient and determining the expression level of a BAP1 protein biomarker in the cells comprising the sample, wherein if the BAP1 biomarker is absent or reduced in expression level compared to a reference control level, then the cancer is predicted to be sensitive to an EZH2 inhibitor.

In one aspect, there is provided a method of predicting the sensitivity of a cancer to an EZH2 inhibitor, comprising determining the expression level of a BAP1 biomarker in a sample of the cancer, wherein if the expression of the BAP1 biomarker in the sample is absent or expressed at a lower level than a BAP1 reference control level, the cancer is predicted to be sensitive to the EZH2 inhibitor, wherein the cancer is selected from the group consisting of malignant mesotheliomas, uveal melanomas, renal cell carcinoma, cutaneous melanomas, lung cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomyosarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes and bladder cancer.

In certain embodiments, the cancer can be malignant mesothelioma, uveal melanoma, renal cell carcinoma, cutaneous melanoma, lung cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomysarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes or bladder cancer.

In one aspect, there is provided a kit when used in the method as described herein, comprising a means for detecting a BAP1 biomarker.

In one aspect, there is provided a kit when used in the method as described herein comprising (i) a means for detecting a BAP1 biomarker; and (ii) one or more primers, probe, arrays/microarray, biomarker-specific antibody, and/or bead for detecting EZH2 expression, L3MBTL2 expression and/or SUZ12 expression.

The present invention provides a kit for determining whether an anti-cancer effect is likely to be produced in a cancer by an EZH2 inhibitor. In a non-

WE CLAIM:

1. Use of an EZH2 inhibitor in the manufacture of a medicament for the treatment of a cancer in a subject in need thereof, wherein the medicament is to be administered to the subject when the expression of a BAP1 biomarker in the cancer is determined to be absent or expressed at a lower level than a BAP1 reference control level, wherein the cancer is selected from the group consisting of malignant mesotheliomas, uveal melanomas, renal cell carcinoma, cutaneous melanomas, lung cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomyosarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes, and bladder cancer.

2. The use of claim 1, wherein the expression level of EZH2 is increased in the cancer compared to an EZH2 reference control level and/or the expression level of SUZ12 is increased in the cancer compared to a SUZ12 reference control level.

3. The use of claim 1 or 2, wherein the cancer is a malignant mesothelioma, an uveal melanoma, or a renal cell carcinoma.

4. The use of any one of claims 1-3, wherein the expression of the BAP1 biomarker, SUZ12 and/or EZH2 is determined by immunofluorescence, polymerase chain reaction, *in situ* hybridization, or Western Blot.

5. The use of any one of claims 1-3, wherein the expression of the BAP1 biomarker is determined by using a reagent which specifically binds to the BAP1 biomarker.

6. The use of any one of claims 1-5, wherein the BAP1 biomarker is a BAP1 nucleic acid biomarker or a BAP1 protein biomarker.

7. The use of any one of claims 1-6, wherein the EZH2 inhibitor is selected from the group consisting of UNC1999, 3-deazaneplanocin A, EI1, EPZ-6438, GSK343, EPZ005687, EPZ011989, and GSK126.

8. The use of any one of claims 1-7, wherein the EZH2 inhibitor is EPZ-6438.

9. A method for determining whether an anti-cancer effect is likely to be produced in a cancer by an EZH2 inhibitor, comprising determining the expression level of a BAP1 biomarker in a sample of the cancer, wherein if the expression of the BAP1 biomarker in the sample is absent or expressed at lower level than a BAP1 reference control level, it is more likely that the EZH2 inhibitor would have an anti-cancer effect on the cancer,

wherein the cancer is selected from the group consisting of malignant mesotheliomas, uveal melanomas, renal cell carcinoma, cutaneous melanomas, lung cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomyosarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes, and bladder cancer.

10. A method of predicting the sensitivity of a cancer to an EZH2 inhibitor, comprising determining the expression level of a BAP1 biomarker in a sample of the cancer, wherein if the expression of the BAP1 biomarker in the sample is absent or expressed at a lower level than a BAP1 reference control level, the cancer is predicted to be sensitive to the EZH2 inhibitor,

wherein the cancer is selected from the group consisting of malignant mesotheliomas, uveal melanomas, renal cell carcinoma, cutaneous melanomas, lung cancer, breast cancer, ovarian cancer, non-melanoma skin cancer, meningioma, cholangiocarcinoma, leiomyosarcoma, neuroendocrine tumors, pancreatic cancer, paraganglioma, malignant fibrous histiocytoma, melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), acute myeloid leukemia, myelodysplastic syndromes, and bladder cancer.

11. The method of claim 9 or 10, wherein the BAP1 biomarker is a BAP1 protein biomarker or a BAP1 nucleic acid biomarker.

12. The method of any one of claims 9-11, wherein the expression of the BAP1 biomarker is determined by immunofluorescence, Western Blot, polymerase chain reaction, or *in situ* hybridization.

13. The method of any one of claims 9-12, wherein the expression of the BAP1 biomarker is determined by using a reagent which specifically binds to the BAP1 biomarker.

14. The method of any one of claims 9-13, wherein the EZH2 inhibitor is selected from the group consisting of UNC1999, 3-deazaneplanocin A, EI1, EPZ-6438, GSK343, EPZ005687, EPZ011989, and GSK126.

15. The method of any one of claims 9-14, wherein the EZH2 inhibitor is EPZ-6438.

16. The method of any one of claims 9-15, wherein the cancer is a malignant mesothelioma, a uveal melanoma, or a renal cell carcinoma.

17. A kit when used according to any one of claims 1-16, comprising a means for detecting a BAP1 biomarker.

18. A kit when used according to any one of claims 1-16, comprising a means for detecting a BAP1 biomarker, wherein the means for detecting the BAP1 biomarker comprises one or more packaged primers, probe, arrays/microarray, biomarker-specific antibody and/or bead, for detecting the BAP1 biomarker.

19. A kit when used according to any one of claims 1-16, comprising a means for detecting a BAP1 biomarker, wherein the means for detecting the BAP1 biomarker comprises one or more antibodies, or antigen binding fragment thereof, for detecting the BAP1 biomarker.

20. A kit when used according to any one of claims 1-16, comprising (i) a means for detecting a BAP1 biomarker; and (ii) one or more primers, probe, arrays/microarray, biomarker-specific antibody, and/or bead for detecting EZH2 expression, L3MBTL2 expression and/or SUZ12 expression.