Traditional modes of therapy (radiation therapy, chemotherapy, and hormonal therapy), while useful, have been limited by the emergence of treatment-resistant cancer cells. Clearly, new approaches are needed to identify targets for treating metastatic breast cancer and cancer generally.

SUMMARY ~~OF THE INVENTION~~

In an aspect, there is provided the use of a CXR1 antagonist or a IL8-CXCR1 signaling pathway antagonist in association with a chemotherapeutic in the preparation of a medicament for treating a subject having a tumor.

The present ~~invention~~disclosure provides methods of treating cancer by administering an IL8-CXCR1 pathway inhibitor (e.g., an anti-CXCR1 antibody or Repertaxin) alone or in combination with an additional chemotherapeutic agent such that non-tumorigenic and tumorigenic cancer cells in a subject are killed. The present ~~invention~~disclosure also provides compositions and methods for treating and diagnosing the presence of solid tumor stem cells in a patient (e.g., based on the presence of CXCR1 or FBXO21).

In some embodiments, the present ~~invention~~disclosure provides methods of treating cancer comprising: administering an IL8-CXCR1 pathway antagonist and an additional chemotherapeutic agent to a subject. In certain embodiments, the present ~~invention~~disclosure provides methods of reducing or eliminating cancer stem cells and non-tumorigenic cancer cells in a subject comprising: administering Repertaxin or derivative thereof to a subject under conditions such that at least a portion of the cancer stem cells and at least a portion of the non-tumorigenic cancer cells are killed. In other embodiments, the present ~~invention~~disclosure provides methods of reducing or eliminating cancer stem cells and non-tumorigenic cancer cells in a subject comprising: administering an IL8-CXCR1 pathway antagonist and an additional chemotherapeutic agent to a subject under conditions such that at least a portion of the cancer stem cells and at least a portion of the non-tumorigenic cancer cells are killed. In particular embodiments, the present ~~invention~~disclosure provides compositions or kits comprising an IL8-CXCR1 pathway antagonist and an additional chemotherapeutic agent.

In certain embodiments, the IL8-CXCR1 pathway antagonist comprises an agent that specifically blocks the binding of IL8 to CXCR1. In some embodiments, the agent binds to (is specific for) CXCR1, but does not bind to CXCR2. In other embodiments, the agent binds to CXCR1. In particular embodiments, the agent comprises an anti-CXCR1 antibody or antibody fragment. In additional embodiments, the agent comprises

Repertaxin or a derivative thereof. In further embodiments, the additional chemotherapeutic agent comprises an anti-mitotic compound. In certain embodiments, the anti-mitotic compound is selected from the group consisting of: docetaxel, doxorubicin, paclitaxel, fluorouracil, vincristine, vinblastine, nocodazole, colchicine, podophyllotoxin, steganacin, and combretastatin. In other embodiments, the anti-mitotic compound is a catharalthus alkaloids (e.g., vincristine and vinblastine); or a benzimidazole carbamates such as nocodazole; or colchicine or related compounds such as podophyllotoxin, steganacin or combretastatin; or a taxane such as paclitaxel and docetaxel. In certain embodiments, the additional chemotherapeutic agent comprises docetaxel.

In particular embodiments, the subject has a type of cancer that, when treated with a chemotherapeutic, has increased levels of IL-8 production (e.g., which causes an increase in cancer stem cell number of motility). In some embodiments, the subject has a type of cancer selected from the group consisting of: prostate cancer, ovarian cancer, breast cancer, melanoma, non-small cell lung cancer, small-cell lung cancer, and esophageal adenocarcinoma.

In other embodiments, the present ~~invention~~disclosure provides methods of detecting solid tumor stem cells comprising; a) providing: i) a sample taken from a tumor of a subject, and ii) an antibody, or antibody fragment (or other binding molecule), specific for the CXCR1 protein or FBXO21 protein (or another protein from Table 1); and b) contacting the tissue sample with the antibody, or antibody fragment, under conditions such that the presence or absence of CXCR1+ or FBXO21+ solid tumor stem cells are detected.

In particular embodiments, the antibody, or antibody fragment, is conjugated to a signal molecule. In further embodiments, the signal molecule comprises a fluorescent molecule. In other embodiments, the signal molecule comprises an enzyme that can catalyze a color producing reaction in the presence of a colorimetric substrate. In certain embodiments, the method further comprises contacting the sample with a secondary antibody, or secondary antibody fragment, specific for the antibody or antibody fragment. In other embodiments, the secondary antibody, or secondary antibody fragment, comprises a signal molecule. In particular embodiments, no other proteins or nucleic acids are assayed in order to determine the presence or absence of the CXCR1 or

FBXO21+ solid tumor stem cells. In additional embodiments, the tumor is selected from the group consisting of: a prostate cancer tumor, an ovarian cancer tumor, a breast cancer tumor, a melanoma, a non-small cell lung cancer tumor, a small-cell lung cancer tumor, and an esophageal adenocarcinoma tumor.

In some embodiments, the present ~~invention~~disclosure provides methods of enriching for a population of solid tumor stem cells comprising: a) disassociating a solid tumor to generate disassociated cells; b) contacting the disassociated cells with a reagent that binds CXCR1 or FBXO21 (or other protein from Table 1); and c) selecting cells that bind to the reagent under conditions such that an a population enriched for solid tumor stem cells is generated.

In certain embodiments, no additional reagents are employed in order to generate the population enriched for solid tumor stem cells. In some embodiments, the tumor is selected from the group consisting of: a prostate cancer tumor, an ovarian cancer tumor, a breast cancer tumor, a melanoma, a non-small cell lung cancer tumor, a small-cell lung cancer tumor, and an esophageal adenocarcinoma tumor. In further embodiments, the reagent is an antibody or antibody fragment (e.g., Fab fragment). In additional embodiments, the reagent is conjugated to a fluorochrome or magnetic particles. In other embodiments, the selecting cells is performed by flow cytometry, fluorescence activated cell sorting, panning, affinity column separation, or magnetic selection.

In particular embodiments, the present ~~invention~~disclosure provides an enriched population of solid tumor stem cells isolated by the methods described herein.

In some embodiments, the present ~~invention~~disclosure provides isolated populations of cancer stem cells that are: a) tumorigenic; and b) CXCR1+ or FBXO21+. In certain embodiments, the cancer stem cells are cancer stem cells selected from the group consisting of: prostate cancer stem cells, ovarian cancer stem cells, breast cancer stem cells, skin cancer stem cells, non-small cell lung cancer stem cells, small-cell lung cancer stem cells, and esophageal adenocarcinoma stem cells. In other embodiments, the population comprises at least 60% cancer stem cells and less than 40% non-tumorigenic tumor cells. In further embodiments, the cancer stem cells: are enriched at least two-fold compared to unfractionated non-tumorigenic tumor cells (e.g., 2-fold, 3-fold, 4-fold, 5-fold, ..., 10-fold, ... 100-fold, ... 1000-fold).

In some embodiments, the present ~~invention~~disclosure provides methods for obtaining from a tumor a cellular composition comprising cancer stem cells and non-tumorigenic tumor cells, wherein at least 60% are tumorigenic stem cells and 40% or less are non-tumorigenic tumor cells, the method comprising: a) obtaining a dissociated mixture of tumor cells from a tumor; b) separating the mixture of tumor cells into a first fraction comprising at least 60% cancer stem cells and 40% or less non-tumorigenic tumor cells and a second fraction of tumor cells depleted of cancer stem cells wherein the separating is by contacting the mixture with a reagent against CXCR1 or FBXO21; and c) demonstrating the first fraction to be tumorigenic by: i) serial injection into a first host animal and the second fraction to be non-tumorigenic by serial injection into a second host animal. In certain embodiments, the separating is performed by flow cytometry, fluorescence activated cell sorting (FACS), panning, affinity chromatography or magnetic selection. In some embodiments, the separating is performed by fluorescence activated cell sorters (FACS) analysis.

In particular embodiments, the present ~~invention~~disclosure provides methods for selecting a treatment for a patient having a solid tumor, comprising: (a) obtaining a sample from the patient; (b) identifying the presence of CXCR1+ or FBXO21+ solid tumor stem cell in the sample; and (c) selecting a treatment for the patient that targets CXCR1+ or FBXO21+ solid tumor stem cells (e.g., selecting the use of an anti-CXCR1 antibody or antibody fragment). In certain embodiments, the CXCR1+ or FBXO21+ solid tumor stem cells are cancer stem cells selected from the group consisting of: prostate cancer stem cells, ovarian cancer stem cells, breast cancer stem cells, skin cancer stem cells, non-small cell lung cancer stem cells, small-cell lung cancer stem cells, and esophageal adenocarcinoma stem cells.

In some embodiments, the present ~~invention~~disclosure provides methods for screening a compound, comprising: a) exposing a sample comprising a CXCR1+ or FBXO21+ cancer stem cell to a candidate anti-neoplastic compound, wherein the candidate anti-neoplastic compound comprises a CXCR1 or FBXO21 antagonist or a IL8-CXCR1 signaling pathway antagonist; and b) detecting a change in the cell in response to the compound.

In certain embodiments, the sample comprises a non-adherent mammosphere. In further embodiments, the CXCR1 or FBXO21 antagonist, or IL8-CXCR1 signaling pathway antagonist comprises an antibody or antibody fragment. In some embodiments, the CXCR1 antagonist is a derivative of Repartaxin. In other embodiments, the detecting comprises detecting cell death of the tumorigenic breast cell. In further embodiments, the methods further comprise identifying the candidate anti-neoplastic agent as capable of killing tumorigenic cells as well as non-tumorigenic cancer cells.

In some embodiments, the present ~~invention~~disclosure provides methods for determining the capability of a test compound to inhibit tumorigenesis of solid tumor stem cells comprising: a) obtaining enriched solid tumor stem cells, wherein the solid tumor stem cells: i) are enriched at least two-fold compared to unfractionated tumor cells; and ii) express CXCR1 or FBXO21; b) exposing a first set, but not a second set, of the solid tumor stem cells to a test compound; c) injecting the first set of the solid tumor stem cells into a first host animal and injecting the second set of solid tumor stem cells into a second host animal; and d) comparing a tumor, if present, in the first animal with a tumor formed in the second animal in order to determine if the test compound inhibits tumor formation. In particular embodiments, the test compound is a CXCR1 or FBXO21 inhibitor, or a IL8-CXCR1 inhibitor pathway inhibitor.

In further embodiments, the present ~~invention~~disclosure provides methods for determining the capability of a test compound to inhibit tumorigenesis of solid tumor stem cells comprising: a) obtaining a sample comprising at least 60% solid tumor stem cells, wherein the solid tumor stem cells express CXCR1 or FBXO21; b) injecting the solid tumor stem cells into first and second host animals; c) treating the first host animal with a test compound, and not treating the second host animal with the test compound; and d) comparing a tumor, if present, in the first animal with a tumor formed in the second animal in order to determine if the test compound inhibits tumor formation. In other embodiments, the test compound is a CXCR1 or FBXO21 inhibitor or an IL8-CXCR1 pathway inhibitor.

CLAIMS

We claim:

1. A method of detecting solid tumor stem cells comprising: detecting CXCR1+ or FBXO21+ solid tumor stem cells from a tissue sample taken from a tumor of a subject.

2. The method of Claim 1, wherein said detecting comprising contacting said tissue sample with an antibody or antibody fragment.

3. The method of Claim 2, wherein said antibody, or antibody fragment comprises Repertaxin.

4. The method of Claim 2, wherein said antibody or antibody fragment comprises a signal molecule.

5. The method of Claim 4, wherein said signal molecule comprises a floursecent molecule or an enzyme that can catalyze a color producing reaction in the presence of a colorimetric substrate.

6. The method of Claim 1, wherein no other proteins or nucleic acids are assayed in order to determine the presence or absence of said CXCR1 or FBXO21+ solid tumor stem cells.

7. The method of Claim 1, wherein said tumor is selected from the group consisting of: a prostate cancer tumor, an ovarian cancer tumor, a breast cancer tumor, a melanoma, a non-small cell lung cancer tumor, a small-cell lung cancer tumor, and an esophageal adenocarcinoma tumor.

8. An isolated population of cancer stem cells that are:

a) tumorigenic; and

b) CXCR1+ or FBXO21+.

9. The isolated population of cancer stem cells of Claim 8, wherein said cancer stem cells are cancer stem cells selected from the group consisting of: prostate cancer stem cells, ovarian cancer stem cells, breast cancer stem cells, skin cancer stem cells, non-small cell lung cancer stem cells, small-cell lung cancer stem cells, and esophageal adenocarcinoma stem cells.

10. The isolated population of cancer stem cells of Claim 8, wherein the population comprises at least 60% cancer stem cells and less than 40% non-tumorigenic tumor cells.

11. The isolated population of cancer stem cells of Claim 8, wherein the cancer stem cells are enriched at least two-fold compared to unfractionated non-tumorigenic tumor cells.

12. The isolated population of cancer stem cells of Claim 8, wherein the cancer stem cells are enriched at least four-fold compared to unfractionated non-tumorigenic tumor cells.

13. A method for treating a subject having a tumor, comprising: administering a compound to said subject, wherein said compound is a CXCR1 or FBXO21 antagonist or an IL8-CXCR1 signaling pathway antagonist.

14. The method of claim 13, wherein said compound is Repartaxin or a Repartaxin derivative.

15. The method of claim 13, wherein said CXCR1 or FBXO21 antagonist, or IL8-CXCR1 signaling pathway antagonist comprises an antibody or antibody fragment.

16. The method of claim 13, wherein said tumor comprises cancer stem cells selected from the group consisting of: prostate cancer stem cells, ovarian cancer stem cells, breast

cancer stem cells, skin cancer stem cells, non-small cell lung cancer stem cells, small-cell lung cancer stem cells, and esophageal adenocarcinoma stem cells.

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CLAIMS

We claim:

1. Use of a CXR1 antagonist or a IL8-CXCR1 signaling pathway antagonist in association with a chemotherapeutic in the preparation of a medicament for treating a subject having a tumor.

2. Use as claimed in claim 1, wherein said tumor comprises cancer stem cells selected from the group consisting of: prostate cancer stem cells, ovarian cancer stem cells, breast cancer stem cells, skin cancer stem cells, non-small cell lung cancer stem cells, small-cell lung cancer stem cells, and esophageal adenocarcinoma stem cells.

3. Use as claimed in claim 1 or claim 2, wherein said CXCR1 antagonist or IL8-CXCR1 signaling pathway antagonist is Repertaxin or a Repertaxin derivative.

4. Use as claimed in any one of the preceding claims, wherein said CXCR1 antagonist or IL8-CXCR1 signaling pathway antagonist comprises an antibody or an antibody fragment.