

CLAIMS

1. A method of predicting the sensitivity of tumor cell growth to inhibition by a Wnt inhibitor, comprising:
- 5 (a) detecting in a sample of tumor cells from a patient a level of a biomarker, where the biomarker is selected from the group consisting of:
- (i) DNA copy number at the RNF43 chromosomal region and/or at the ZNRF3 chromosomal region to determine a loss of heterozygosity,
- 10 (ii) sequenced genomic DNA, cDNA or RNA from cancer tissues to detect an inactivation mutation in the RNF43 gene and/or the ZNRF3 gene;
- (iii) RNF43 mRNA expression and/or ZNRF3 mRNA expression;
- (iv) RNF43 protein expression and/or ZNRF3 protein expression;
- 15 (v) the functional effect of RNF43 gene loss and/or ZNRF3 gene loss; or
- (vi) a combination of biomarkers (i) – (v);
- (b) comparing the level of the biomarker in the tumor cell sample to a control level of the biomarker selected from the group consisting of:
- 20 (i) a control level of the biomarker that has been correlated with sensitivity to the Wnt inhibitor; and
- (ii) a control level of the biomarker that has been correlated with resistance to the Wnt inhibitor; and
- (c) selecting the patient as being predicted to benefit from therapeutic administration of the Wnt inhibitor, if the patient's tumor has an
- 25 inactivating RNF43 or ZNRF3 mutation, if the patient's tumor has a decreased copy number of RNF43 or ZNRF3, or the patient tumor has a decreased expression of RNF43 mRNA or protein or a decreased expression of ZNRF3 mRNA or protein indicates that the patients tumor is
- 30 likely to be sensitive to Wnt inhibitor.

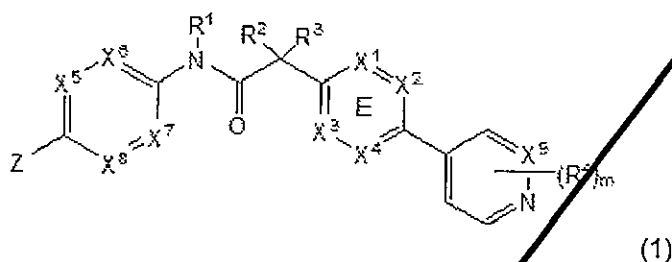
2. A method of predicting the sensitivity of a tumor cell to a Wnt inhibitor, the method comprising contacting a tumor cell with at least one Wnt inhibitor and
- (a) detecting the tumor cell a level of a biomarker, where the biomarker is selected from the group consisting of:
- 5 (i) DNA copy number at the RNF43 chromosomal region and/or at the ZNRF33 chromosomal region to determine a loss of heterozygosity,
- (ii) genomic DNA, cDNA or RNA from tumor cell to detect an inactivation mutation in the RNF43 gene and/or the ZNRF3 gene;
- 10 (iii) RNF43 mRNA expression and/or ZNRF3 mRNA expression;
- (iv) RNF43 protein expression and/or ZNRF3 protein expression;
- (v) the functional effect of RNF43 gene loss and/or ZNRF3 gene loss; or
- (vi) a combination of biomarkers (i) – (v):
- 15 (b) comparing the level of the biomarker in the tumor cell to a control level;
- (c) determining the sensitivity of the tumor cell to a Wnt inhibitor based on the difference of the biomarker level in the tumor cell and the control level.
3. The method of claim 1 or 2, wherein the detecting of DNA copy number at the RNF43 chromosomal region in step 1(a)(i) is by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 1.
- 20 4. The method of any one of claims 1 to 3, wherein the detecting of DNA copy number at the ZNRF3 chromosomal region in step 1(a)(i) is by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 2.
- 25 5. The method of any one of claim 1 to 3, wherein the step of detecting of DNA copy number at the ZNRF3 chromosomal region in step 1(a)(i) is by fluorescent *in situ* hybridization (FISH).
- 30 6. The method of claim 1 or 2, wherein the assay to measure RNF43 mRNA expression in step 1(a)(iii) is by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 1.

7. The method of claim 1, 2 or 6, wherein the assay to measure ZNRF3 mRNA expression in step 1(a)(iii) is by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 2.
- 5 8. The method of claim 1 or 2, wherein the assay to measure RNF43 protein expression in step 1(a)(iv) is by immunohistochemistry.
9. The method of claim 1 or 2, wherein the assay to measure loss of function in step 1(a)(v) is by using FACS assay with anti-Frizzled antibody.
- 10 10. The method of claim 1 or 2, wherein the assay to measure loss of function in step 1(a)(v) is by using a Wnt reporter assay.
11. The method of claim 10, wherein Wnt reporter assay measures a protein activity, wherein the protein activity is selected from the group consisting of increased Frizzled protein levels, increased LRP6 protein levels, increased LRP6 phosphorylation, and increased Disheveled phosphorylation.
- 15 12. The method of any one of claims 1 to 11, wherein the step of comparing comprises comparing the biomarker level in the tumor cells to a control level of the biomarker in one or more control cells that are resistant to the Wnt inhibitor.
- 20 13. The method of any one of claims 1 to 11, wherein the step of comparing comprises comparing the biomarker level in the tumor cells to a control level of the biomarker in one or more control cells that are sensitive to the Wnt inhibitor.
- 25 14. The method of any one of claims 1 to 11, wherein the control level of the biomarker that has been correlated with sensitivity to the Wnt inhibitor has been predetermined.
- 30 15. The method of any one of claims 1 to 11, wherein the control level of the biomarker that has been correlated with resistance to the Wnt inhibitor has been predetermined.
- 35 16. An assay kit for selecting a cancer patient who is predicted to benefit or not to benefit from therapeutic administration of a Wnt inhibitor, the assay kit comprising:

- (a) a means for detecting in a sample of tumor cells a level of a biomarker or a combination of biomarkers selected from the group consisting of:
- (i) a loss of heterozygosity of the RNF43 gene and/or ZNRF3 gene;
 - (ii) a level of loss of function of the RNF43 gene and/or ZNRF3 gene;
 - (iii) a level of RNF43 mRNA and/or ZNRF3 mRNA expression;
 - (iv) a level of RNF43 protein and/or ZNRF3 protein expression; and
 - (v) a functional effect of RNF43 gene and/or ZNRF3 gene loss;
- (b) a control.
17. The assay kit of claim 16, wherein the control is selected from the group consisting of:
- (i) a control sample for detecting sensitivity to the Wnt inhibitor;
 - (ii) a control sample for detecting resistance to the Wnt inhibitor;
 - (iii) information containing a predetermined control level of the biomarker that has been correlated with sensitivity to the Wnt inhibitor; and
 - (iv) information containing a predetermined control level of the biomarker that has been correlated with resistance to the Wnt inhibitor.
18. The assay kit of claim 16 or 17, wherein the means for detecting the RNF43 gene in any of steps 15(a)(i) and 21(a)(i) comprises a nucleotide probe that hybridizes to a nucleotide having the sequence of SEQ ID NO: 1.
19. The assay kit of any one of claims 16 to 18, wherein the means for detecting the ZNRF3 gene in any of steps 15(a)(i) and 21(a)(i) comprises a nucleotide probe that hybridizes to a nucleotide having the sequence of SEQ ID NO: 2.
20. The assay kit of claim 16 or 17, wherein the means for detecting the RNF43 gene step 15(a)(i) comprises a nucleotide probe that hybridizes to genomic locus 17q22.
21. The assay kit of claim 16, 17 or 20, wherein the means for detecting the ZNRF3 gene step 16(a)(i) comprises a nucleotide probe that hybridizes to genomic locus 22q12.1.

22. The assay kit of claim 16, 17 or 20, wherein the means for detecting RNF43 protein in step 16(a)(iii) comprises an antibody or antigen binding fragment thereof that selectively binds to a polynucleotide having the sequence of SEQ ID NO: 3.
- 5
23. The assay kit of any one of claims 16 to 22, wherein the means for detecting ZNRF3 protein in step 16(a)(iii) comprises an antibody or antigen binding fragment thereof that selectively binds to a polynucleotide having the sequence of SEQ ID NO: 4.
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24. The assay kit of any one of claims 16 to 23, wherein the means for detecting comprises a detectable label.
25. The assay kit of any one of claims 16 to 24, wherein the means for detecting is immobilized on a substrate.
- 15
26. A pharmaceutical composition comprising a Wnt inhibitor for use in treatment of cancer in a patient, wherein the patient's level of the biomarker as defined in claim 1 is statistically similar to or less than a control level of the biomarker and the control biomarker level has been correlated with sensitivity to the Wnt inhibitor.
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27. The pharmaceutical composition comprising a Wnt inhibitor of claim 26, wherein the control level is the normal or baseline level of the biomarker, a level of the biomarker in the healthy cell or tissue sample or a control level of the biomarker that has been correlated with resistance to the Wnt inhibitor.
- 25
28. The pharmaceutical composition comprising a Wnt inhibitor of claim 26 or 27, wherein the cancer is pancreatic cancer.
- 30

29. The method of any one of claims 1 to 15, or the assay of any one of claims 16 to 25, or the pharmaceutical composition of any one of claims 26 to 28, wherein a Wnt inhibitor is a compound of Formula (1):



- 5 or a physiologically acceptable salt thereof, wherein:
 X¹, X², X³ and X⁴ is selected from N and CR⁷;
 one of X⁵, X⁶, X⁷ and X⁸ is N and the others are CH;
 X⁹ is selected from N and CH;
 Z is selected from phenyl, pyrazinyl, pyridinyl, pyridazinyl and piperazinyl;
 10 wherein each phenyl, pyrazinyl, pyridinyl, pyridazinyl or piperazinyl of Z is optionally substituted with an R⁶ group;
 R¹, R² and R³ are hydrogen;
 m is 1;
 R⁴ is selected from hydrogen, halo, difluoromethyl, trifluoromethyl and methyl;
 15 R⁶ is selected from hydrogen, halo and -C(O)R¹⁰; wherein R¹⁰ is methyl;
 and
 R⁷ is selected from hydrogen, halo, cyano, methyl and trifluoromethyl.

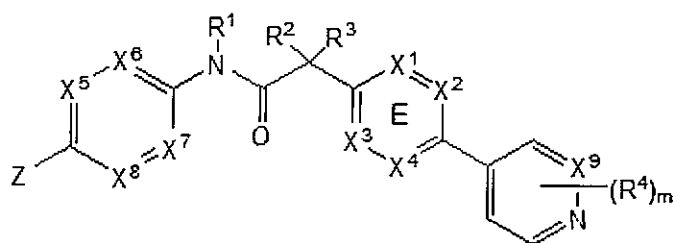
30. The method of any one of claims 1 to 15, or the assay of any one of claims 16 to 25, or the pharmaceutical composition of any one of claims 26 to 28, wherein a Wnt inhibitor is a compound selected from the group of
- 5 N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[5-methyl-6-(pyridazin-4-yl)pyridin-3-yl]acetamide;
- 2-[5-methyl-6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-[5-(pyrazin-2-yl)pyridin-2-yl]acetamide;
- 10 N-(2,3'-bipyridin-6'-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide ;
- N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-methyl-3-(trifluoromethyl)-2,4'-bipyridin-5-yl)acetamide;
- 15 N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2-fluoro-3-methyl-2,4'-bipyridin-5-yl)acetamide; and
- 2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide ;
- or a pharmaceutically acceptable salt thereof.

CLAIMS

1. A method of predicting the sensitivity of tumor cell growth to inhibition by a Wnt inhibitor, comprising:
 - (a) detecting in a sample of tumor cells from a patient a level of a biomarker, where the biomarker is selected from the group consisting of:
 - (i) DNA copy number at the RNF43 chromosomal region and/or at the ZNRF3 chromosomal region to determine a loss of heterozygosity,
 - (ii) sequenced genomic DNA, cDNA or RNA from cancer tissues to detect an inactivation mutation in the RNF43 gene and/or the ZNRF3 gene;
 - (iii) RNF43 mRNA expression and/or ZNRF3 mRNA expression;
 - (iv) RNF43 protein expression and/or ZNRF3 protein expression;or
 - (v) a combination of biomarkers (i) – (iv):
 - (b) comparing the level of the biomarker in the tumor cell sample to a control level of the biomarker selected from the group consisting of:
 - (i) a control level of the biomarker that has been correlated with sensitivity to the Wnt inhibitor; and
 - (ii) a control level of the biomarker that has been correlated with resistance to the Wnt inhibitor; and
 - (c) selecting the patient as being predicted to benefit from therapeutic administration of the Wnt inhibitor, if the patient's tumor has an inactivating RNF43 or ZNRF3 mutation, if the patient's tumor has a decreased copy number of RNF43 or ZNRF3, or the patient tumor has a decreased expression of RNF43 mRNA or protein or a decreased expression of ZNRF3 mRNA or protein indicates that the patients tumor is likely to be sensitive to Wnt inhibitor.
2. A method of predicting the sensitivity of a tumor cell to a Wnt inhibitor, the method comprising contacting a tumor cell with at least one Wnt inhibitor and
 - (a) detecting the tumor cell a level of a biomarker, where the biomarker is selected from the group consisting of:
 - (i) DNA copy number at the RNF43 chromosomal region and/or at the ZNRF3 chromosomal region to determine a loss of heterozygosity,
 - (ii) genomic DNA, cDNA or RNA from tumor cell to detect an inactivation mutation in the RNF43 gene and/or the ZNRF3 gene;

- (iii) RNF43 mRNA expression and/or ZNRF3 mRNA expression;
 - (iv) RNF43 protein expression and/or ZNRF3 protein expression;
 - or
 - (v) a combination of biomarkers (i) – (iv):
- (b) comparing the level of the biomarker in the tumor cell to a control level;
 - (c) determining the sensitivity of the tumor cell to a Wnt inhibitor based on the difference of the biomarker level in the tumor cell and the control level.
3. The method of claim 1 or 2, wherein the detecting of DNA copy number at the RNF43 chromosomal region is by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 1.
 4. The method of any one of claims 1 to 3, wherein the detecting of DNA copy number at the ZNRF3 chromosomal region is by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 2.
 5. The method of any one of claim 1 to 3, wherein the step of detecting of DNA copy number at the ZNRF3 chromosomal region is by fluorescent *in situ* hybridization (FISH).
 6. The method of claim 1 or 2, wherein RNF43 mRNA expression is measured by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 1.
 7. The method of claim 1, 2 or 6, wherein ZNRF3 mRNA expression is measured by hybridization using a probe that hybridizes with a nucleotide having a sequence of SEQ ID NO: 2.
 8. The method of claim 1 or 2, wherein RNF43 protein expression is measured by immunohistochemistry.
 9. The method of any one of claims 1 to 8, wherein the step of comparing comprises comparing the biomarker level in the tumor cells to a control level of the biomarker in one or more control cells that are resistant to the Wnt inhibitor.
 10. The method of any one of claims 1 to 8, wherein the step of comparing comprises comparing the biomarker level in the tumor cells to a control level of the biomarker in one or more control cells that are sensitive to the Wnt inhibitor.

11. The method of any one of claims 1 to 8, wherein the control level of the biomarker that has been correlated with sensitivity to the Wnt inhibitor has been predetermined.
12. The method of any one of claims 1 to 8, wherein the control level of the biomarker that has been correlated with resistance to the Wnt inhibitor has been predetermined.
13. Use of a pharmaceutical composition comprising a Wnt inhibitor for the manufacture of a medicament for the treatment of cancer in a patient, wherein the patient's level of the biomarker as defined in claim 1 is statistically similar to or less than a control level of the biomarker and the control biomarker level has been correlated with sensitivity to the Wnt inhibitor.
14. The use of claim 13, wherein the control level is the normal or baseline level of the biomarker, a level of the biomarker in the healthy cell or tissue sample or a control level of the biomarker that has been correlated with resistance to the Wnt inhibitor.
15. The use of claim 13 or 14, wherein the cancer is pancreatic cancer.
16. The method of any one of claims 1 to 12, the use of any one of claims 13, 14 or 15, wherein a Wnt inhibitor is a compound of Formula (1):



(1)

or a physiologically acceptable salt thereof, wherein:

X^1 , X^2 , X^3 and X^4 is selected from N and CR^7 ;

one of X^5 , X^6 , X^7 and X^8 is N and the others are CH;

X^9 is selected from N and CH;

Z is selected from phenyl, pyrazinyl, pyridinyl, pyridazinyl and piperazinyl; wherein each phenyl, pyrazinyl, pyridinyl, pyridazinyl or piperazinyl of Z is optionally substituted with an R^6 group;

R^1 , R^2 and R^3 are hydrogen;

m is 1;

R⁴ is selected from hydrogen, halo, difluoromethyl, trifluoromethyl and methyl;

R⁶ is selected from hydrogen, halo and -C(O)R¹⁰; wherein R¹⁰ is methyl; and

R⁷ is selected from hydrogen, halo, cyano, methyl and trifluoromethyl.

17. The method of any one of claims 1 to 12, the use of any one of claims 13 or 14 or 15, wherein a Wnt inhibitor is a compound selected from the group of
- N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[5-methyl-6-(pyridazin-4-yl)pyridin-3-yl]acetamide;
- 2-[5-methyl-6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-[5-(pyrazin-2-yl)pyridin-2-yl]acetamide;
- N-(2,3'-bipyridin-6'-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide ;
- N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-methyl-3-(trifluoromethyl)-2,4'-bipyridin-5-yl)acetamide;
- N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)acetamide; and
- 2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide ;
- or a pharmaceutically acceptable salt thereof.