Claims

1. A multiplexed amplification reaction kit for identifying a subject suffering from a malignancy sensitive to treatment with an inhibitor of the Wnt signaling pathway comprising:

a primer pair capable of amplifying a PTPRK(e13)-RSPO3(e2) R-Spondin gene-fusion,

control primer pairs capable of amplifying wild type PTPRK and/or wild-type RSPO3 sequences, and/or

a synthetic oligonucleotide internal control template sequence and internal control primer pair capable of amplifying the synthetic internal control template sequence.

2. The kit of claim 1, further comprising,

one or more additional multiplexed amplification reactions comprising:

primer pairs capable of amplifying one or more R-Spondin gene-fusions, wherein the R-Spondin gene-fusion is a PTPRK(e1)-RSPO3(e2) fusion, a PTPRK(e7)-RSPO3(e2) fusion, or a EIF3E(e1)-RSPO2(e2) fusion,

control primer pairs capable of amplifying wild type PTPRK, wild-type EIF3E, wild-type RSPO2, and/or wild-type RSPO3 sequences, and/or

synthetic oligonucleotide internal control template sequences and internal control primer pairs capable of amplifying one or more synthetic internal control template sequences.

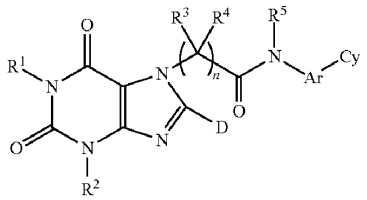
3. The kit of claims 1 or 2, further comprising one or more probe sequences complementary to a sequence in the one or more R-Spondin gene-fusions, the one or more wild type genes, or the one or more internal control sequences.

4. The kit of claim 3, wherein the one or more probe sequences further comprise a fluorophore moiety.

5. The kit of claim 4, wherein the fluorophore moiety is FAM, HEX, JOE, Cy3, Cy5, TAMRA, Tye 563, Tye 556, TEX 615, Cal Red 610 or LCRED640.

6. The kit of claim 4, further comprising reagents for performing real-time, quantitative polymerase chain reaction.

7. The kit of claim 1 or 2, wherein the Wnt inhibitor has the structure (I):



(I),

wherein

R^1, R^2, R^3, R^4 and R^5 are each independently, H or an alkyl group;

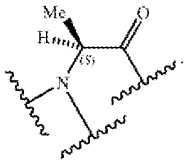
D is selected from the group consisting of H, halogen, alkyl, cycloalkyl, aryl, and dialkylamino, each (other than H and halogen) being optionally substituted;

Ar is an aryl or heteroaryl group, optionally substituted;

Cy is an aryl, heteroaryl or a saturated ring containing at least one heteroatom, each being optionally substituted; and

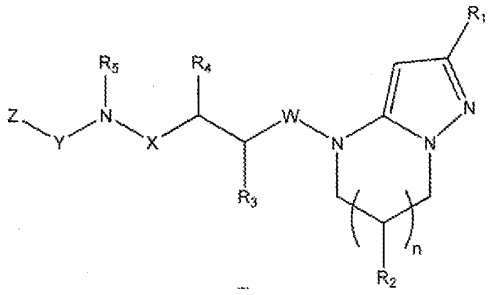
n is an integer from 1 to 3,

wherein, if n=1 and one of R^3 and R^4 is methyl and the other is H, the stereochemistry of the compound is as shown in partial structure (II):



(II).

8. The kit of claims 1 or 2, wherein the Wnt inhibitor has the structure (III):



(III)

wherein:

R_1 represents H; optionally substituted alkyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkoxy, NH_2, —NHC_{1-3}alkyl and —$N(C_{1-3}alkyl)_2$); —C(O)Oalkyl; haloalkyl; haloalkoxy; or -alkylaryl;

each R_2 independently represents H; optionally substituted alkyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkoxy, NH_2, —NHC_{1-3}alkyl and —$N(C_{1-3}alkyl)_2$); -alkylaryl; optionally substituted carbocyclyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkyl, C_{1-6}haloalkoxy and halo); optionally substituted heterocyclyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, —$C(O)OC_{1-6}$alkyl, —$C(O)C_{1-6}$alkyl and —$C(O)NHC_{1-6}$alkyl); —NHalkyl; —$N(alkyl)_2$; amino; hydroxyl; alkoxy or halo;

n represents 0, 1 or 2;

R_3 represents H or alkyl;

R_4 represents H or alkyl;

R_5 represents H or alkyl;

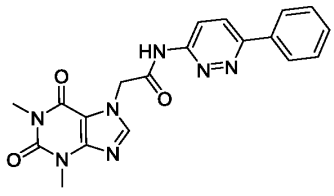
W and X each independently represent C=O; C=S; or CH_2;

Y represents aryl; heteroaryl; optionally substituted carbocyclyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkyl, C_{1-6}haloalkoxy and halo); or optionally substituted heterocyclyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, —$C(O)OC_{1-6}$alkyl, —$C(O)C_{1-6}$alkyl and —$C(O)NHC_{1-6}$alkyl); and

Z represents optionally substituted alkyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkoxy, NH_2, -NHC_{1-3}alkyl and –$N(C_{1-3}alkyl)_2$); aryl; heteroaryl; -alkylaryl;-alkylheteroaryl; optionally substituted carbocyclyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkyl, C_{1-6}haloalkoxy and halo); optionally substituted heterocyclyl (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, -$C(O)OC_{1-6}$alkyl, -$C(O)C_{1-6}$alkyl and-$C(O)NHC_{1-6}$alkyl); -alkylcarbocyclyl wherein carbocyclyl is optionally substituted (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy and halo); -alkylheterocyclyl wherein heterocyclyl is optionally substituted (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, -$C(O)OC_{1-6}$alkyl, -$C(O)C_{1-6}$alkyl and-$C(O)NHC_{1-6}$alkyl); -arylcarbocyclyl wherein carbocyclyl is optionally substituted (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy and halo); or -arylheterocyclyl wherein heterocyclyl is optionally substituted (wherein optional substituents include one or more substituents each independently selected from C_{1-6}alkyl, -$C(O)OC_{1-6}$alkyl, -$C(O)C_{1-6}$alkyl and-$C(O)NHC_{1-6}$alkyl).

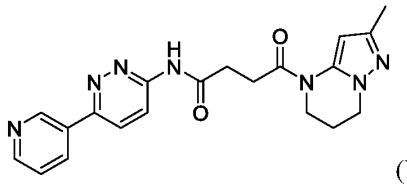
9. The kit of claims 1 or 2, wherein the inhibitor of the Wnt signaling pathway is a porcupine inhibitor.

10. The kit of claim 9, wherein the porcupine inhibitor is,
2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(6-phenylpyridazin-3-yl) acetamide, 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)-N-(6-phenyl pyridazin-3-yl)acetamide, or a compound of the structure (IV):



(IV); or

4-(2-methyl-6,7-dihydropyrazolol [1,5-a]pyrimidin-4(5H)-yl)-4-oxo-N-(6-(pyridin-3-yl)pyradizin-3-yl)butanamide, or a compound of the structure (V):



(V).

11. The kit of claims 1 or 2, wherein the malignancy is characterized by an elevated level of Wnt activity.

12. The kit of claim 11, wherein the elevated level of Wnt activity is transcriptionally or post-transcriptionally elevated.

13. The kit of claims 1 or 2, wherein the malignancy is a solid malignancy.

14. The kit of claim 1 or 2, wherein the malignancy is a gastrointestinal cancer of the esophagus, gallbladder, liver, pancreas, stomach, small intestine, large intestine, colon, rectum, or anus.

15. A method of detecting a PTPRK(e13)-RSPO3(e2) R-Spondin gene-fusion in a subject suffering from a malignancy, the method comprising,
extracting nucleic acid from a sample from the subject, and
amplifying the nucleic acid with one or more primer pairs,
wherein the primer pairs are capable of amplifying a PTPRK(e13)-RSPO3(e2) gene-fusion, and
detecting amplified nucleic acid.

16. The method of claim 15, further comprising amplifying the nucleic acid with one or more primer pairs capable of amplifying a PTPRK(e1)-RSPO3(e2) fusion, a PTPRK(e7)-RSPO3(e2) fusion, and/or a EIF3E(e1)-RSPO2(e2) fusion.

17. The method of claims 15 or 16, wherein the sample is formalin-fixed and paraffin embedded (FFPE)-fixed tumor tissue or tissue from fresh-frozen tumor.

18. The method of claim 15 or 16, wherein the nucleic acid is RNA.

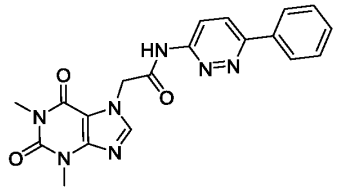
19. The method of claims 15 or 16, wherein the amplification step comprises a real time-PCR amplification reaction.

20. The method of claims 15 or 16, wherein the malignancy is a gastrointestinal cancer of the esophagus, gallbladder, liver, pancreas, stomach, small intestine, large intestine, colon, rectum, or anus.

21. The method of claims 15 or 16, further comprising treating the subject with an inhibitor of the Wnt signaling pathway when one or more R-Spondin gene fusions are detected.

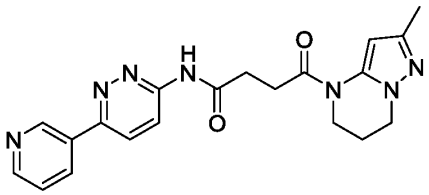
22. The method of claim 21, wherein the inhibitor of the Wnt-signaling pathway is a porcupine inhibitor.

23. The method of claim 22, wherein the porcupine inhibitor is, 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(6-phenylpyridazin-3-yl) acetamide, 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)-N-(6-phenyl pyridazin-3-yl)acetamide, or a compound of the structure (IV):



(IV); or

4-(2-methyl-6,7-dihydropyrazolol [1,5-a]pyrimidin-4(5H)-yl)-4-oxo-N-(6-(pyridin-3-yl)pyradizin-3-yl)butanamide, or a compound of the structure (V):



(V).

24. A method of identifying sensitivity to an inhibitor of Wnt signaling in a subject suffering from a malignancy comprising,
extracting nucleic acid from a sample from the subject,
amplifying the nucleic acid with a primer pair capable of amplifying a PTPRK(e13)-RSPO3(e2) R-Spondin gene-fusion, and
identifying sensitivity to an inhibitor of Wnt signaling when a PTPRK(e13)-RSPO3(e2) R-Spondin gene-fusion is amplified.

25. The method of claim 24, further comprising amplifying the nucleic acid with one or more additional primer pairs capable of amplifying a PTPRK(e1)-RSPO3(e2) fusion, a PTPRK(e7)-RSPO3(e2) fusion, or a EIF3E(e1)-RSPO2(e2) fusion, and
identifying sensitivity to an inhibitor of Wnt signaling when a PTPRK(e1)-RSPO3(e2) fusion, a PTPRK(e7)-RSPO3(e2) fusion, a PTPRK(e13)-RSPO3(e2) fusion, or a EIF3E(e1)-RSPO2(e2) fusion is amplified.

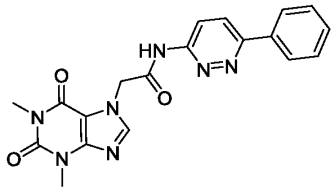
26. A method of treating a subject with a malignancy, wherein said malignancy has been identified according to the method of claims 25 or 26.

27. The method of claim 26, wherein the subject is treated with an inhibitor of the Wnt signaling pathway.

28. The method of claim 27, wherein the subject is treated with a Wnt-porcupine inhibitor.

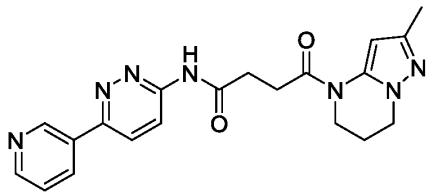
29. The method of claim 28, wherein the Wnt-porcupine inhibitor is,

2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(6-phenylpyridazin-3-yl) acetamide, 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)-N-(6-phenyl pyridazin-3-yl)acetamide, or a compound of the structure (IV):



(IV); or

4-(2-methyl-6,7-dihydropyrazolol [1,5-a]pyrimidin-4(5H)-yl)-4-oxo-N-(6-(pyridin-3-yl)pyradizin-3-yl)butanamide, or a compound of the structure (V):



(V).

30. The method of claims 24 or 25, wherein the malignancy is a gastrointestinal cancer of the esophagus, gallbladder, liver, pancreas, stomach, small intestine, large intestine, colon, rectum, or anus.