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(54) **Title:** METHODS OF TREATING COLORECTAL CANCERS HARBORING UPSTREAM WNT PATHWAY MUTATIONS

(57) **Abstract:** The present invention relates to a method of treating patients suffering from colorectal cancer, more specifically BRAF^{V600E}-mutant or BRAF wild type, but KRAS wild type and microsatellite-unstable colorectal cancer by using a Wnt pathway inhibitor or a combination comprising a Wnt pathway inhibitor and either a BRAF inhibitor, or an EGFR inhibitor, or both. The disclosure also relates to corresponding pharmaceutical formulations, uses, methods, combinations, and related disclosure embodiments.



Methods of treating colorectal cancers harboring upstream Wnt pathway mutations

Field of the Disclosure

The present invention relates to a method of treating patients suffering from *BRAF*^{V600E}-mutant and *BRAF* wild-type colorectal cancer by using a Wnt pathway inhibitor or combinations comprising a Wnt pathway inhibitor and another inhibitor. The disclosure also relates to corresponding pharmaceutical formulations, uses, methods, combinations, and related disclosure embodiments.

Background of the Disclosure

Colorectal cancer (CRC) is the fourth most frequently diagnosed cancer and the second leading cause of cancer death in the United States and the European Union. In the last 15 years, the median overall survival (OS) for patients with metastatic CRC has markedly improved from approximately 8-12 months to 18-24 months (Kopetz, S. *et al.*, *J. Clin. Oncol.* 2009, 27:3677-82). The improvement is attributed to two factors: 1) the increased utilization of hepatic resection and 2) the addition of new medical therapies (irinotecan, oxaliplatin, bevacizumab, cetuximab, and pantumumab) to the standard treatment with 5-fluorouracil plus leucovorin. However, this survival benefit has not extended to all subtypes of the disease. Approximately 5-10% of CRCs harbor an oncogenic mutation at position V600 of the *BRAF* protein (Fearon, E.R., *Annu. Rev. Pathol. Mech. Dis.* 2011, 6:479-507). Patients with metastatic *BRAF*^{V600E}-mutant CRC have a very poor prognosis, with a median OS of only 10 months (Kopetz, S. *et al.*, *J. Clin. Oncol.* 2009, 27:3677-82). Thus, there remains a critical need for more effective therapies for this subset of patients.

Summary of the Invention

Surprisingly it has been found that a Wnt inhibitor, or a combination of Wnt inhibitor and either a *BRAF* inhibitor, or EGFR inhibitor, or both, according to the present disclosure, pharmaceutical compositions or combination product of the present disclosure, are especially useful for treatment of a patient with a *BRAF*^{V600E}-mutant colorectal cancer, particularly the cancer with a mutated Wnt pathway.

In addition, it has been determined that a Wnt inhibitor, or a combination of a Wnt inhibitor and an EGFR inhibitor, can be useful for the treatment of patients with microsatellite-unstable *BRAF* wild-type (*BRAF*^{WT}) colorectal cancer with a mutated Wnt pathway, particularly upstream Wnt pathway mutation. The colorectal cancer can be *KRAS* wild-type (*KRAS*^{WT}).

Unlike colorectal cancers that develop via the conventional pathway, colorectal cancers (CRCs) that arise via the serrated pathway harbor *BRAF* or *KRAS* mutations but generally lack mutations in the Wnt pathway component *APC*. Therefore, in serrated CRCs MAPK pathway activation, not Wnt pathway activation, was thought to be the dominant oncogenic driver. It has been unexpectedly found that activation of the Wnt pathway has an important role in the pathogenesis of serrated CRC. Molecular epidemiology data showed that patient *BRAF*^{V600E}-mutant CRC samples are enriched for genetic alterations in upstream Wnt pathway regulators (e.g. *RNF43*, *ZNRF3*, *RSPO2* and *RSPO3*), strongly confirming this notion. *In vivo* experiments in two preclinical tumor xenograft models of *BRAF*^{V600E}-

mutant CRC (one with an *RNF43* mutation and the other with a *RSPO3* fusion) demonstrate anti-tumor activity with the Wnt pathway inhibitor 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (**compound A**), as a single agent and in combination with other targeted therapies. In both models, regression of tumor xenografts was achieved with the novel combination of (i) a Wnt pathway inhibitor 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide, (ii) a BRAF inhibitor (S)-methyl 1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate (**compound B**), and (iii) an EGFR inhibitor cetuximab. This data allows one to deduce a general concept and teaching that a combination of a Wnt inhibitor, and either a mutant BRAF inhibitor or an EGFR inhibitor, or both, will enable the effective treatment of *BRAF*^{V600E}-mutant CRCs, particularly those that harbor Wnt pathway mutations, especially upstream Wnt pathway mutations.

As mentioned, *RNF43* mutations are enriched in *BRAF*^{V600E} CRC. But *BRAF*^{V600E} CRCs are also frequently microsatellite unstable due to hypermethylation of DNA repair genes (Lubomierski, N. *et al.*, *Cancer* 2005, 104:952-61). The mismatch repair deficiency in these tumors may directly contribute to *RNF43* mutagenesis, as *RNF43* mutations tend to be small insertions/deletions in homopolymeric tracts. By testing if microsatellite-unstable, *BRAF*^{WT}, *KRAS*^{WT} CRCs with *RNF43* mutations may also benefit from COMPOUND A and/or COMPOUND A+cetuximab, we selected a pre-clinical microsatellite-unstable, *BRAF*^{WT}, *KRAS*^{WT}, *RNF43*^{W200L, E278delE}, *ZNRF3*^{Q226*, C230fs} CRC model (T70) for testing and further found that Wnt inhibitor compound A, optionally in combination with an EGFR inhibitor cetuximab significantly decrease cell proliferation in a patient-derived microsatellite-unstable, upstream Wnt mutant CRC xenograft model that is wild type for BRAF and KRAS. This is a good indication that a Wnt inhibitor, or a combination of a Wnt inhibitor and an EGFR inhibitor, can be useful for the treatment of patients with microsatellite-unstable, BRAF wild-type (*BRAF*^{WT}), KRAS wild-type (*KRAS*^{WT}) colorectal cancer harboring upstream Wnt pathway mutations.

Majority of microsatellite unstable colorectal cancer is expected to be BRAF mutant. However, also BRAF wild-type colorectal cancer was found to be sensitive to a Wnt inhibitor, either alone or in combination with another drug. Particularly as microsatellite instable tumors cause *RNF43* mutation it would be possible to treat BRAF wt colorectal cancer as long it harbors *RNF43* or other WNT upstream pathway mutation. Because *ZNRF3* and *RNF43* are both homologous cell surface transmembrane E3 ubiquitin ligases for Wnt receptor Frizzled it is expected that the Wnt inhibitor could be effectively applied in the treatment of colorectal cancer, *BRAF*^{V600E} mutated or BRAF wt, which is *ZNRF3* mutated (but not *RNF43* mutated). In the event that the cancer is BRAF wt, there is no need to use a BRAF inhibitor together with the Wnt inhibitor.

Specifically, the present disclosure provides the following aspects, advantageous features and specific embodiments, respectively alone or in combination, as listed in the following items:

1. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer.
2. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to item 1, wherein the cancer is microsatellite-unstable.

3. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to item 1 or 2, wherein the cancer is *KRAS* wild-type.
4. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to any one of items 1 to 3 in combination with a BRAF inhibitor.
5. A Wnt inhibitor for use in the treatment of microsatellite-unstable, BRAF wild-type and KRAS wild-type colorectal cancer.
6. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 5 in combination with an EGFR inhibitor.
7. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 6, wherein a Wnt pathway in the colorectal cancer is activated compared to a control.
8. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 7, wherein *AXIN2* expression in the colorectal cancer is higher compared to a control.
9. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 8, wherein the colorectal cancer comprises mutated *RNF43*, mutated *RSPO2* or mutated *RSPO3*.
10. A Wnt inhibitor for use in the treatment of colorectal cancer according to item 9, wherein the colorectal cancer comprises mutated *RNF43*.
11. A Wnt inhibitor for use in the treatment of colorectal cancer according to item 9, wherein the colorectal cancer comprises *RSPO* fusion.
12. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 8, wherein the colorectal cancer comprises mutated ZNRF3, RSPO1, RSPO4, LGR4-6, Frizzled1-10, LRP5, LRP6, WISP3, Wise, SFRP1-3, WIF-1, DKK1, DKK4, LKB1/STK11, Keap-1 and/or NRF2, preferably ZNRF3.
13. A Wnt inhibitor for use in the treatment of microsatellite-unstable colorectal cancer according to any one of items 2 to 12, wherein at least one mismatch repair system gene is inactivated in the colorectal cancer compared to a control.
14. A Wnt inhibitor for use in the treatment of microsatellite-unstable colorectal cancer according to item 13, wherein the mismatch repair system gene is MLH1, MSH2, MSH6 or PMS2.
15. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of previous items, wherein the Wnt inhibitor is the inhibitor of Porcupine or Frizzled.
16. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of previous items, wherein the Wnt inhibitor is a Porcupine inhibitor.
17. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 4 to 16, wherein the Wnt inhibitor and the inhibitor in the combination are to be administered simultaneously or sequentially.
18. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 4 to 17, wherein the Wnt inhibitor and the inhibitor in the combination are used in the form of a fixed combination.
19. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 18, further comprising at least one pharmaceutically acceptable carrier.
20. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 19 in a form of a pharmaceutical composition.
21. A pharmaceutical combination comprising (i) a Wnt inhibitor and (ii) a BRAF inhibitor.
22. The pharmaceutical combination according to item 21 further comprising an EGFR inhibitor.

23. A pharmaceutical combination comprising a Wnt inhibitor and an EGFR inhibitor.
24. The pharmaceutical combination according to any one of items 21 to 23, wherein the pharmaceutical combination comprises the inhibitors separately or together.
25. The pharmaceutical combination according to any one of items 21 to 24 for simultaneous or sequential use of the inhibitors.
26. The pharmaceutical combination according to any one of items 21 to 25, further comprising at least one pharmaceutically acceptable carrier.
27. The pharmaceutical combination according to any one of items 21 to 26 in the form of a fixed combination.
28. The pharmaceutical combination according to any one of items 21, 22, or 24 to 27, in the form or a kit of parts for the combined administration, wherein the Wnt inhibitor and the BRAF inhibitor are administered independently at the same time or separately within time intervals, and the time intervals allow the Wnt inhibitor and the BRAF inhibitor to be jointly active.
29. The pharmaceutical combination according to any one of items 21 to 27 in the form or a kit of parts for the combined administration, wherein the Wnt inhibitor and the BRAF inhibitor and/or EGFR inhibitor are administered independently at the same time or separately within time intervals, and the time intervals allow the Wnt inhibitor and the BRAF inhibitor and/or EGFR inhibitor to be jointly active.
30. The pharmaceutical combination according to any one of items 23 to 27 in the form or a kit of parts for the combined administration, wherein the Wnt inhibitor and the EGFR inhibitor are administered independently at the same time or separately within time intervals, and the time intervals allow the Wnt inhibitor and the EGFR inhibitor to be jointly active.
31. The pharmaceutical combination according to any one of items 21 to 30, wherein the inhibitors are in a quantity which is jointly therapeutically effective for the treatment of cancer.
32. The pharmaceutical combination according to any one of items 21 to 30 for use in the treatment of cancer.
33. The pharmaceutical combination according to items 31 or 32, wherein the cancer is colorectal cancer.
34. The pharmaceutical combination according to any one of items 31 to 33, wherein the cancer is microsatellite-unstable.
35. The pharmaceutical combination according to any one of items 33 or 34, wherein the cancer is *KRAS* wild-type.
36. The pharmaceutical combination according to any one of items 31 or 35, wherein the cancer is *BRAF*^{V600E}-mutant colorectal cancer.
37. The pharmaceutical combination according to any one of items 33 or 35, wherein the cancer is *BRAF* wild-type.
38. The pharmaceutical combination according to any one of items 31 to 35 or 37, wherein the cancer is microsatellite-unstable, *BRAF* wild-type and *KRAS* wild-type colorectal cancer.
39. The pharmaceutical combination according to any one of items 33 to 38, wherein a Wnt pathway in the colorectal cancer is activated compared to a control.
40. The pharmaceutical combination according to any one of items 33 to 39, wherein *AXIN2* expression in the colorectal cancer is higher compared to a control.

41. The pharmaceutical combination according to any one of items 33 to 40, wherein the colorectal cancer comprises mutated *RNF43*, mutated *RSPO2* or mutated *RSPO3*.
42. The pharmaceutical combination according to any one of items 33 to 41, wherein the colorectal cancer comprises mutated *RNF43*.
43. The pharmaceutical combination according to any one of items 33 to 41, wherein the colorectal cancer comprises *RSPO* fusion.
44. The pharmaceutical combination according to any one of items 33 to 43, wherein the colorectal cancer comprises mutated ZNRF3, RSP01, RSP04, LGR4-6, Frizzled1-10, LRP5, LRP6, WISP3, Wise, SFRP1-3, WIF-1, DKK1, DKK4, LKB1/STK11, Keap-1 and/or NRF2, preferably ZNRF3.
45. The pharmaceutical combination for use in the treatment of microsatellite-unstable colorectal cancer according to any one of items 34 to 44, wherein at least one mismatch repair system gene is inactivated in the colorectal cancer compared to a control.
46. The pharmaceutical combination for use in the treatment of microsatellite-unstable colorectal cancer according to item 45, wherein the mismatch repair system gene is MLH1, MSH2, MSH6 or PMS2.
47. The pharmaceutical combination according to any one of items 21 to 46, wherein the Wnt inhibitor is an inhibitor of Porcupine or Frizzled.
48. The pharmaceutical combination according to any one of items 21 to 47, wherein the Wnt inhibitor is the inhibitor of Porcupine.
49. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 20 or a pharmaceutical combination according to any one of items 21 to 48, wherein the Wnt inhibitor is a compound selected from the group consisting of: tert-butyl 4-(5-{2-[4-(2-methylpyridin-4-yl)phenyl]acetamido}pyridin-2-yl)-1,2,3,6-tetrahydropyridine-1-carboxylate; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[4-(pyridin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[6-(morpholin-4-yl)pyridin-3-yl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[4-(quinolin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[6-(quinolin-4-yl)pyridin-3-yl]acetamide; N-(6-methanesulfonyl-1,3-benzothiazol-2-yl)-2-[4-(pyridin-4-yl)phenyl]acetamide; N-(6-fluoro-1,3-benzothiazol-2-yl)-2-[4-(pyridin-4-yl)phenyl]acetamide; N-(1,3-benzothiazol-2-yl)-2-[4-(pyridin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[6-(pyridin-4-yl)pyridin-3-yl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[3-methyl-4-(2-methylpyridin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[4-(2-methylpyrimidin-4-yl)phenyl]acetamide; N-[4-(pyridin-2-yl)-1,3-thiazol-2-yl]-2-[4-(pyridin-4-yl)phenyl]acetamide; N-[4-(pyridin-4-yl)-1,3-thiazol-2-yl]-2-[4-(pyridin-4-yl)phenyl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[2-methyl-4-(2-methylpyridin-4-yl)phenyl]acetamide; 2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-(4-phenyl-1,3-thiazol-2-yl)acetamide; N-(isoquinolin-3-yl)-2-[4-(pyridin-4-yl)phenyl]acetamide; N-(6-fluoro-1,3-benzothiazol-2-yl)-2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; 2-[4-(pyridin-4-yl)phenyl]-N-(quinolin-2-yl)acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[5-(2-methylpyridin-4-yl)pyrimidin-2-yl]acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[5-(2-methylpyridin-4-yl)pyridin-2-yl]acetamide; 2-[3-methyl-4-(2-methylpyridin-4-yl)phenyl]-N-(4-phenyl-1,3-thiazol-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(4-phenyl-1,3-thiazol-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(5-phenylpyridin-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-

(4-phenylpyridin-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[6-(trifluoromethoxy)-1,3-benzothiazol-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(5-phenyl-1,3-thiazol-2-yl)acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-2-[4-(2-methoxypyridin-4-yl)phenyl]acetamide; 2-[4-(2-ethylpyridin-4-yl)phenyl]-N-(6-methoxy-1,3-benzothiazol-2-yl)acetamide; 2-[2-methyl-4-(2-methylpyridin-4-yl)phenyl]-N-(4-phenyl-1,3-thiazol-2-yl)acetamide; N-[4-(4-methoxyphenyl)-1,3-thiazol-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[4-(4-fluorophenyl)-1,3-thiazol-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[4-(3,4-difluorophenyl)-1,3-thiazol-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(6-phenylpyridin-3-yl)acetamide; N-(5-phenylpyridin-2-yl)-2-[4-(pyridazin-4-yl)phenyl]acetamide; N-[5-(4-methylphenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[5-(3-methoxyphenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[5-(2-methoxyphenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[5-(4-methoxyphenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(5-phenylpyrazin-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridin-2-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)-3-(trifluoromethyl)phenyl]-N-(4-phenyl-1,3-thiazol-2-yl)acetamide; N-[5-(3-methylphenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)-3-(trifluoromethyl)phenyl]-N-(5-phenylpyridin-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridin-3-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridin-4-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(6-phenylpyridazin-3-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(4-phenylphenyl)acetamide; 2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-(5-phenylpyridin-2-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(2-phenylpyrimidin-5-yl)acetamide; 2-[4-(1H-imidazol-1-yl)phenyl]-N-(5-phenylpyridin-2-yl)acetamide; N-(6-phenylpyridin-3-yl)-2-[4-(pyridazin-4-yl)phenyl]acetamide; N-[5-(4-fluorophenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[5-(4-ethylpiperazin-1-yl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[5-[(2R,6S)-2,6-dimethylmorpholin-4-yl]pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[4-(pyridin-3-yl)phenyl]acetamide; N-(6-fluoro-1,3-benzothiazol-2-yl)-2-[4-(pyridazin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-(5-phenylpyrimidin-2-yl)acetamide; N-(6-methoxy-1,3-benzothiazol-2-yl)-N-methyl-2-[4-(pyridin-4-yl)phenyl]acetamide; 2-[4-(pyridazin-4-yl)phenyl]-N-[4-(pyridin-3-yl)phenyl]acetamide; N-(6-phenylpyridazin-3-yl)-2-[4-(pyridazin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(1H-pyrazol-4-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyrazin-2-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyrimidin-5-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[4-(pyridazin-4-yl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(1,2,3,6-tetrahydropyridin-4-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridazin-3-yl)pyridin-2-yl]acetamide; 2-[5-methyl-6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-(6-phenylpyridin-3-yl)acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridazin-4-yl)pyridin-2-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[6-(morpholin-4-yl)pyridin-3-yl]acetamide; N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[5-methyl-6-(pyridazin-4-yl)pyridin-3-yl]acetamide; 2-[3-methyl-4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridin-2-yl)pyridin-2-yl]acetamide; 2-[3-methyl-4-(pyridazin-4-yl)phenyl]-N-[5-(pyridin-2-yl)pyridin-2-yl]acetamide; 2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-[5-(pyridazin-3-yl)pyridin-2-yl]acetamide; N-[5-(pyridazin-3-yl)pyridin-2-yl]-2-[6-(pyridazin-4-yl)pyridin-3-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)-3-(trifluoromethyl)phenyl]-N-[5-(pyrazin-2-

yl)pyridin-2-yl]acetamide; N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[5-methyl-6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[4-(2-methylpyridin-4-yl)-3-(trifluoromethyl)phenyl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[5-(pyridazin-4-yl)pyridin-2-yl]acetamide; N-[5-(3-fluorophenyl)pyridin-2-yl]-2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[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; 2-[5-methyl-6-(pyridazin-4-yl)pyridin-3-yl]-N-(6-phenylpyridin-3-yl)acetamide; 2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-(6-phenylpyridin-3-yl)acetamide; N-(6-phenylpyridin-3-yl)-2-[6-(pyridazin-4-yl)pyridin-3-yl]acetamide; N-[6-(1-acetyl-1,2,3,6-tetrahydropyridin-4-yl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; methyl 4-(5-{2-[4-(2-methylpyridin-4-yl)phenyl]acetamido}pyridin-2-yl)-1,2,3,6-tetrahydropyridine-1-carboxylate; N-[6-(1-methanesulfonyl-1,2,3,6-tetrahydropyridin-4-yl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[6-(1-methylpiperidin-4-yl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; 2-[5-methyl-6-(pyridazin-4-yl)pyridin-3-yl]-N-[5-(pyridin-2-yl)pyridin-2-yl]acetamide; 2-[6-(pyridazin-4-yl)pyridin-3-yl]-N-[5-(pyridin-2-yl)pyridin-2-yl]acetamide; 2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-(5-phenylpyrimidin-2-yl)acetamide; N-[5-(3-fluorophenyl)pyrimidin-2-yl]-2-[6-(pyridazin-4-yl)pyridin-3-yl]acetamide; ethyl 4-(5-{2-[4-(2-methylpyridin-4-yl)phenyl]acetamido}pyridin-2-yl)-1,2,3,6-tetrahydropyridine-1-carboxylate; propan-2-yl 4-(5-{2-[4-(2-methylpyridin-4-yl)phenyl]acetamido}pyridin-2-yl)-1,2,3,6-tetrahydropyridine-1-carboxylate; 1-methylcyclopropyl 4-(5-{2-[4-(2-methylpyridin-4-yl)phenyl]acetamido}pyridin-2-yl)-1,2,3,6-tetrahydropyridine-1-carboxylate; 2-[4-(2-methylpyridin-4-yl)phenyl]-N-[6-(1,2,3,6-tetrahydropyridin-4-yl)pyridin-3-yl]acetamide; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[5-methyl-6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[5-methyl-6-(pyridazin-4-yl)pyridin-3-yl]acetamide; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)-3-(trifluoromethyl)phenyl]acetamide; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[4-(pyridazin-4-yl)-3-(trifluoromethyl)phenyl]acetamide; N-[6-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[6-(1-ethyl-1,2,3,6-tetrahydropyridin-4-yl)pyridin-3-yl]-2-[4-(2-methylpyridin-4-yl)phenyl]acetamide; N-[6-(3-fluorophenyl)pyridin-3-yl]-2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]acetamide; N-(6-phenylpyridazin-3-yl)-2-[6-(pyridazin-4-yl)pyridin-3-yl]acetamide; 2-[6-(2-methylpyridin-4-yl)pyridin-3-yl]-N-(6-phenylpyridazin-3-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-cyano-4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(2,3'-bipyridin-6'-yl)-2-(4-(pyridazin-4-yl)-3-(trifluoromethyl)phenyl)acetamide; N-(5-(pyridazin-3-yl)pyridin-2-yl)-2-(4-(pyridazin-4-yl)phenyl)acetamide; N-(5-(3-fluorophenyl)pyridin-2-yl)-2-(6-(pyridazin-4-yl)pyridin-3-yl)acetamide; N-(6-(3-fluorophenyl)pyridin-3-yl)-2-(6-(pyridazin-4-yl)pyridin-3-yl)acetamide; N-(6-(1-(2-amino-2-oxoethyl)-1,2,3,6-tetrahydropyridin-4-yl)pyridin-3-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(6-(3-fluorophenyl)pyridin-3-yl)-2-(4-(pyridazin-4-yl)phenyl)acetamide; N-(5-(pyrazin-2-yl)pyridin-2-yl)-2-(4-(pyridazin-4-yl)-3-(trifluoromethyl)phenyl)acetamide; tert-butyl 4-(5-(2-(4-(2-methylpyridin-4-yl)phenyl)acetamido)pyridin-2-yl)piperazine-1-carboxylate; N-(5-(3-fluorophenyl)pyridin-2-yl)-2-(4-(pyridazin-4-yl)phenyl)acetamide; N-(2,3'-bipyridin-6'-yl)-2-(4-(2-methylpyridin-4-yl)-3-(trifluoromethyl)phenyl)acetamide; N-(5-(pyridazin-3-yl)pyridin-2-yl)-2-(4-(pyridazin-4-yl)-3-(trifluoromethyl)phenyl)acetamide; N-(2-(3-fluorophenyl)pyrimidin-5-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(2,3'-bipyridin-6'-yl)-2-(2',3'-dimethyl-2,4'-bipyridin-5-yl)acetamide; tert-butyl 4-(6-(2-(4-(2-methylpyridin-4-yl)phenyl)acetamido)pyridin-3-yl)piperazine-1-carboxylate; N-(2,3'-bipyridin-

6'-yl)-2-(2'-methyl-2,4'-bipyridin-5-yl)acetamide; N-(6-(1-acetylpiperidin-4-yl)pyridin-3-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; 2-(2'-methyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; N-(5-(pyrazin-2-yl)pyridin-2-yl)-2-(6-(pyridazin-4-yl)pyridin-3-yl)acetamide; 2-(5-methyl-6-(pyridazin-4-yl)pyridin-3-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; methyl 4-(6-(2-(4-(2-methylpyridin-4-yl)phenyl)acetamido)pyridin-3-yl)piperazine-1-carboxylate; 2-(3-methyl-4-(pyridazin-4-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; 2-(3-methyl-4-(pyridazin-4-yl)phenyl)-N-(5-(pyridazin-4-yl)pyridin-2-yl)acetamide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyridazin-4-yl)pyridin-2-yl)acetamide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(6-(pyridazin-4-yl)pyridin-3-yl)acetamide; 2-(3-methyl-4-(pyridazin-4-yl)phenyl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; tert-butyl 4-(6-(2-(4-(2-methylpyridin-4-yl)phenyl)acetamido)pyridin-3-yl)piperidine-1-carboxylate; 2-(3-methyl-4-(pyridazin-4-yl)phenyl)-N-(6-(pyridazin-4-yl)pyridin-3-yl)acetamide; 2-(6-(4-acetylpiperazin-1-yl)pyridin-3-yl)-N-(5-(3-fluorophenyl)pyridin-2-yl)acetamide; 2-(4-(2-methylpyridin-4-yl)phenyl)-N-(5-(3-oxopiperazin-1-yl)pyridin-2-yl)acetamide; 4-(6-(2-(4-(2-methylpyridin-4-yl)phenyl)acetamido)pyridin-3-yl)piperazine-1-carboxamide; N-(5-(4-methyl-1H-imidazol-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; 2-(4-(4-methyl-1H-imidazol-1-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; 2-(5-methyl-6-(pyridazin-4-yl)pyridin-3-yl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; 2-(5-methyl-6-(pyridazin-4-yl)pyridin-3-yl)-N-(5-(pyridazin-4-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide; N-(5-(3S,5R)-4-acetyl-3,5-dimethylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(4-(1-acetylpiperidin-4-yl)phenyl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(6-(4-(2-hydroxyethyl)piperazin-1-yl)pyridin-3-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(6-(3-fluorophenyl)pyridin-3-yl)-2-(5-methyl-6-(pyridazin-4-yl)pyridin-3-yl)acetamide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(4-(pyrazin-2-yl)phenyl)acetamide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(4-(pyridazin-3-yl)phenyl)acetamide; 2-(2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamido)-5-(pyrazin-2-yl)pyridine 1-oxide; 2',3-dimethyl-5-(2-oxo-2-(5-(pyrazin-2-yl)pyridin-2-ylamino)ethyl)-2,4'-bipyridine 1'-oxide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(6-(pyrazin-2-yl)pyridin-3-yl)acetamide; N-(5-(4-isobutyrylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-methyl-4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(6-(4-acetylpiperazin-1-yl)pyridin-3-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide; (R)-N-(6-(4-acetyl-3-methylpiperazin-1-yl)pyridin-3-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; (S)-N-(6-(4-acetyl-3-methylpiperazin-1-yl)pyridin-3-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; (S)-N-(6-(4-acetyl-3-methylpiperazin-1-yl)pyridin-3-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide; (R)-N-(6-(4-acetyl-3-methylpiperazin-1-yl)pyridin-3-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide; N-(5-(3S,5R)-4-acetyl-3,5-dimethylpiperazin-1-yl)pyridin-2-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide; methyl 4-(6-(2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamido)pyridin-3-yl)piperazine-1-carboxylate; methyl 4-(6-(2-(3-methyl-4-(2-methylpyridin-4-yl)phenyl)acetamido)pyridin-3-yl)piperazine-1-carboxylate; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-fluoro-4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-methyl-2,4'-bipyridin-5-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-chloro-4-(2-methylpyridin-4-yl)phenyl)acetamide; ethyl 4-(6-(2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamido)pyridin-3-yl)piperazine-1-carboxylate; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)-3-

(trifluoromethyl)phenyl)acetamide; 2-(3-cyano-4-(2-methylpyridin-4-yl)phenyl)-N-(6-phenylpyridin-3-yl)acetamide; 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(4-propionylpiperazin-1-yl)pyridin-2-yl)acetamide; N-(5-(4-(cyanomethyl)piperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(5-(4-cyanopiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-chloropyridin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-fluoropyridin-4-yl)phenyl)acetamide; 2-(3-cyano-4-(2-methylpyridin-4-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; 2-(3-cyano-4-(2-methylpyridin-4-yl)phenyl)-N-(6-(pyrazin-2-yl)pyridin-3-yl)acetamide; 2-(3-cyano-4-(2-methylpyridin-4-yl)phenyl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-methoxy-4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-chloro-2'-methyl-2,4'-bipyridin-5-yl)acetamide; (S)-N-(5-(4-acetyl-3-methylpiperazin-1-yl)pyridin-2-yl)-2-(3-cyano-4-(2-methylpyridin-4-yl)phenyl)acetamide; (R)-N-(5-(4-acetyl-3-methylpiperazin-1-yl)pyridin-2-yl)-2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamide; isopropyl 4-(6-(2-(2',3-dimethyl-2,4'-bipyridin-5-yl)acetamido)pyridin-3-yl)piperazine-1-carboxylate; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-cyano-2'-methyl-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-(3-fluoro-2'-methyl-2,4'-bipyridin-5-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2-fluoro-5-methyl-4-(2-methylpyridin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyrimidin-4-yl)-3-(trifluoromethyl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2',3-difluoro-2,4'-bipyridin-5-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-methylpyrimidin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(5-fluoropyrimidin-4-yl)phenyl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-methyl-3-(methylsulfonyl)-2,4'-bipyridin-5-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(6-methylpyrimidin-4-yl)phenyl)acetamide; 2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; 2-(4-(2-fluoropyridin-4-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(2-(difluoromethyl)pyridin-4-yl)phenyl)acetamide; N-(6-(4-acetylpiperazin-1-yl)pyridin-3-yl)-2-(4-(2-(difluoromethyl)pyridin-4-yl)phenyl)acetamide; 2-(4-(2-(difluoromethyl)pyridin-4-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(4-(5-fluoropyrimidin-4-yl)-3-methylphenyl)acetamide; 2-(2',3-difluoro-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; 2-(3-cyano-4-(2-fluoropyridin-4-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-fluoro-4-(2-fluoropyridin-4-yl)phenyl)acetamide; 2-(2'-fluoro-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(2'-fluoro-2,4'-bipyridin-5-yl)acetamide; 2-(2',3-difluoro-2,4'-bipyridin-5-yl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; N-(5-(4-acetylpiperazin-1-yl)pyridin-2-yl)-2-(3-cyano-4-(2-fluoropyridin-4-yl)phenyl)acetamide; 2-(3-fluoro-4-(2-fluoropyridin-4-yl)phenyl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide; 2-(3-fluoro-4-(2-fluoropyridin-4-yl)phenyl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; 2-(3-cyano-4-(2-fluoropyridin-4-yl)phenyl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; 2-(4-(2-fluoropyridin-4-yl)phenyl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide; and 2-(2'-fluoro-3-methyl-2,4'-bipyridin-5-yl)-N-(5-(pyridazin-3-yl)pyridin-2-yl)acetamide, or a physiologically acceptable salt thereof.

50. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 20 or a pharmaceutical combination according to any one of items 21 to 48, wherein the Wnt inhibitor is 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide.

51. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to any one of items 4, 6 to 20, 49 or 50, or a pharmaceutical combination according to any one of items 21, 22, 24 to 29, 31 to 36 or 39 to 50, wherein the BRAF inhibitor is selected from the group consisting of: (S)-methyl-1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate;
methyl N-[(2S)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2S)-1-({4-[3-(2,5-difluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2S)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-ethyl-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2S)-1-({4-[3-(2-fluoro-3-methanesulfonamido-5-methylphenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2S)-1-({4-[3-(2-chloro-3-methanesulfonamido-5-methylphenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2S)-1-({4-[3-(2-chloro-5-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2R)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
methyl N-[(2S)-1-({4-[3-(2,5-dichloro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate; and
vemurafenib.

52. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to any one of items 4, 6 to 20, 49 or 50, or a pharmaceutical combination according to any one of items 21, 22, 24 to 29, 31 to 36 or 39 to 50, wherein the BRAF inhibitor is (S)-methyl-1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate, methyl N-[(2S)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate or vemurafenib.

53. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to any one of items 4, 6 to 20, 49 or 50, or a pharmaceutical combination according to any one of items 21, 22, 24 to 29, 31 to 36 or 39 to 50, wherein the BRAF inhibitor is (S)-methyl-1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate.

54. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 6 to 20, or 49 to 53, or a pharmaceutical combination according to any one of items 22 to 53, wherein the EGFR inhibitor is selected from a group consisting of gefitinib, erlotinib, lapatinib, XL-647, HKI-272 (Neratinib), BIBW2992 (Afatinib), EKB-569 (Pelitinib), AV-412, canertinib, PF00299804, BMS 690514, HM781-36b, WZ4002, AP-26113, cetuximab, panitumumab, matuzumab, trastuzumab, or pertuzumab, (R,E)-N-(7-chloro-1-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

N-(7-chloro-1-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

(R,E)-N-(7-chloro-1-(1-(4-(3-fluoroazetidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

N-(7-chloro-1-(1-(4-(3-fluoroazetidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

(S)-N-(1-(1-(1-acryloylpiperidin-3-yl)-5-methyl-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl) benzamide;

N-(1-(1-(1-acryloylpiperidin-3-yl)-5-methyl-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl) benzamide;

(R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

(R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;

N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;

(R,E)-N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

(R,E)-N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-(trifluoromethyl)isonicotinamide;

N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-(trifluoromethyl)isonicotinamide;

(R)-N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-methoxy-1H-benzo[d]imidazol-2-yl)pyridazine-4-carboxamide;

N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-methoxy-1H-benzo[d]imidazol-2-yl)pyridazine-4-carboxamide;

N-(7-methyl-1-(1-(vinylsulfonyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl)benzamide;

N-(1-(1-(1-acryloylazepan-3-yl)-7-(pyrrolidin-1-ylmethyl)-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl)benzamide;

tert-butyl 4-acryloyl-6-(7-chloro-2-(2-methylisonicotinamido)-1H-benzo[d]imidazol-1-yl)-1,4-diazepane-1-carboxylate;

N-(1-(1-(1-acryloyl-1,4-diazepan-6-yl)-7-chloro-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

N-(1-(1-(1-acetyl-4-acryloyl-1,4-diazepan-6-yl)-7-chloro-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

(R)-N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-(2-(pyrrolidin-1-yl)ethoxy)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;

N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-(2-(pyrrolidin-1-yl)ethoxy)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;

(R,E)-N-(7-chloro-1-(1-(4-(dicyclopropylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

N-(7-chloro-1-(1-(4-(dicyclopropylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;

(R)-1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxylic acid;

1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxylic acid;

(R)-1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxamide;

1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxamide; and

1-(1-(1-acryloylazepan-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-7-carboxylic acid.

55. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 6 to 20, or 49 to 53, or a pharmaceutical combination according to any one of items 22 to 53, wherein the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide or (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide.

56. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 6 to 20, or 49 to 53, or a pharmaceutical combination according to any one of items 22 to 53, wherein the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide.

57. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 6 to 20, or 49 to 53, or a pharmaceutical combination according to any one of items 22 to 53, wherein the EGFR inhibitor is cetuximab.

58. The pharmaceutical combination according to any one of items 21 to 57 in a form of a pharmaceutical composition.

59. A method of treating a patient having colorectal cancer further defined as in any one of items 1 to 20, or 49 to 57, wherein the amount of the inhibitor administered to a patient is therapeutically effective.

60. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of items 1 to 20 or 49 to 57, for use *in vivo*.

“Colorectal cancer” as used herein means a neoplasm arising from the colon and/or rectum, particularly from the epithelium of the colon and/or rectum.

“Mutated” or “mutation” denotes herein an alteration from a normal functional or wild type protein, cDNA, gene or mRNA, which results in changed activity, or loss thereof. It includes changed mutation status like for example frame-shift mutation, deletion, translocation, insertion, duplication, inversion, functional mutation; or combinations thereof. It includes also DNA modification, cDNA modification, mRNA modification, protein modification, DNA function, cDNA function, mRNA function, protein function, DNA mutation, cDNA mutation, mRNA mutation, protein mutation, or combinations thereof; preferably is DNA mutation. DNA modification includes DNA alkylation or acylation. For example, methylation is a biochemical process involving the addition of a methyl group to the cytosine or adenine DNA nucleotides. mRNA modification includes RNA editing, which is a biochemical process involving the change of nucleotides after they have been generated by RNA polymerase to form a sequence. cDNA

modification includes any modification that was made at the mRNA level will be translated into cDNA modification. Protein modification includes a biochemical process involving the change of amino acids after they have been translated. Protein function is understood for proteins to carry out the duties specified by the information encoded in genes, including facilitation of signaling transduction, enzymatic reactions etc.

"Gene expression" or "expression" are both used herein interchangeably and refer to the nucleic acids or amino acids (e.g., peptide or polypeptide) generated when a gene is transcribed and translated. In a particular embodiment "gene expression" or "expression" denote DNA expression, mRNA expression, cDNA expression, transcription, protein transcription or protein expression. Detection of gene expression can be by any appropriate method, including for example, detecting the quantity of mRNA transcribed from the gene or the quantity of cDNA produced from the reverse transcription of the mRNA transcribed from the gene or the quantity of the polypeptide or protein encoded by the gene. Transcription or translation can be determined using known techniques. For example, an amplification method such as PCR may be useful. The same principles apply to "overexpression".

"Control" denotes herein to the sequence, parameter or level measured for comparison in a non-cancerous, healthy, wild-type tissue or cell. For example, the control can be the sequence, parameter or level measured in a normal colonic epithelium. A Wnt pathway in the colorectal cancer can be activated (increased Wnt signaling) or inactivated (decreased Wnt signaling) compared to a control. This refers to the increased or decreased, respectively, expression or functional effect of Wnt pathway genes, corresponding mRNA and equally to transcription or functional effect of proteins encoded by said genes or mRNA.

"Mutated Wnt pathway" or "upstream Wnt pathway mutation" as used herein means any mutation in a gene encoding a protein in the Wnt pathway that increases Wnt signaling in colorectal cancer relative to a control, in a Wnt ligand-dependent manner. The control can be Wnt signaling in normal colonic epithelium. In addition to inactivating mutations in RNF43 and fusions involving RSPO2 or RSPO3, mutations in the following genes may increase Wnt signaling in a Wnt ligand-dependent manner: ZNRF3, RSPO1, RSPO4, LGR4-6, Frizzled1-10, LRP5, LRP6, WISP3, Wise, SFRP1-3, WIF-1, DKK1, DKK4, LKB1/STK11, Keap-1, and NRF2. Mutations in the Wnt pathway or upstream Wnt pathway mutations can be detected by using Next-Generation DNA/RNA sequencing, RT-PCR or Nanostring technology. Additional DNA/RNA platforms could also be employed.

"Microsatellite-unstable tumor" is a tumor that is hypermutable due to inactivation of one or more mismatch repair system genes, for example MLH1, MSH2, MSH6, and PMS2.

Description of the Figures

Compounds mentioned in the figures:

- Compound A (Wnt inhibitor): 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide

- Compound B (BRAF inhibitor): (S)-methyl 1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate
- Cetuximab

Figure 1. *RNF43* mutations and *RSPO* fusions co-occur with *BRAF*^{V600E} mutations in patient CRC samples. *APC* mutations show a strong tendency to be non-overlapping with *RNF43* mutations and *RSPO* fusions. Quilt plot of mutations and fusions in *BRAF*^{V600E}-mutant CRC samples acquired from Asterand (*n* = 45). Dark gray boxes = mutations/variants that alter the amino acid sequence. Black boxes = fusions.

Figure 2. JHOM-2B cell line is derived from a lower GI adenocarcinoma. (A) Representative images of CK7 staining (Left) or CK20 staining (Right) of a JHOM-2B tumor xenograft. (B) DNA sequence of the fusion breakpoint between exon1 of *PTPRK* and exon 2 of *RSPO3* in JHOM-2B.

Figure 3. JHOM-2B tumor cells are sensitive to COMPOUND A; however, COMPOUND A antagonizes the tumor growth inhibition of COMPOUND B plus cetuximab *in vitro*. (A) In a foci formation assay, decreased JHOM-2B colonies are seen after treatment with COMPOUND A. (B) COMPOUND A inhibits the proliferation of JHOM-2B tumor cells in a 7-day CTG assay, with an EC₅₀ of 0.06 μ M. (C) Addition of COMPOUND A to COMPOUND B+cet has an antagonistic effect on tumor growth inhibition *in vitro*.

Figure 4. JHOM-2B tumor cells exhibit autocrine Wnt signaling. Quantitative RT-PCR of beta-catenin-target genes (*AXIN2*, *LGR5*, *c-MYC*, and *CCND1*) after 72 hours of treatment with 100 nM COMPOUND A. ****P* < 0.001.

Figure 5. Effects of COMPOUND A, COMPOUND B, cetuximab and combination treatments on Wnt and MAPK signaling pathways in JHOM-2B tumor cells. JHOM-2B tumor cells were treated with 100 nM COMPOUND A, 100 nM COMPOUND B, and/or 50 nM cetuximab for 72 hours prior to collection of protein lysates and immunoblotting.

Figure 6. The triple combination of COMPOUND A+COMPOUND B+cet is efficacious in JHOM-2B tumor xenografts *in vivo*. (A) JHOM-2B-tumor-bearing mice were treated with 10 ml/kg vehicle, p.o., BID (filled circles); 5 mg/kg COMPOUND A, p.o. BID (open squares); 20 mg/kg COMPOUND B, p.o., qd (filled stars); 20 mg/kg cetuximab, i.p., 2x/wk (filled upside down triangles); COMPOUND A+COMPOUND B (filled diamonds); COMPOUND A+cet (open circles); COMPOUND B+cet (filled squares); or COMPOUND A+COMPOUND B+cet (open triangles) for 11 days. The tumor volume is plotted as the mean $\pm$ SEM (*n* = 5-6 per treatment group). (B) Scatter plot of individual tumor volumes.

Figure 7. COMPOUND A induces cell cycle arrest in JHOM-2B tumor xenografts, as a single agent and in combination. (A) Representative images of pHH3 staining by immunohistochemistry after 14 days of treatment (the COMPOUND A and COMPOUND A+cet treatment groups were collected after 11 days of treatment). (B) Percentage of pHH3-positive pixels. Graphs represent mean $\pm$ SEM (*n* = 5-6 per treatment group). ***P* < 0.01; ****P* < 0.001.

Figure 8. COMPOUND A induces mucinous differentiation of JHOM-2B tumor xenografts, as a single agent and in combination. (A) Representative images of Alcian blue staining after 14 days of treatment (the COMPOUND A and COMPOUND A+cet treatment groups were collected after 11 days of treatment). (B) Percentage of blue-positive pixels. Graphs represent mean $\pm$ SEM ($n = 5-6$ per treatment group). *** $P < 0.001$.

Figure 9. The triple combination of COMPOUND A+COMPOUND B+cet is efficacious in HCOX1329 patient-derived xenografts *in vivo*. (A) HCOX1329-tumor-bearing mice were treated with 10 ml/kg vehicle, p.o., BID (filled circles); 5 mg/kg COMPOUND A, p.o. BID (open squares); 20 mg/kg COMPOUND B, p.o., qd (filled stars); 20 mg/kg cetuximab, i.p., 2x/wk (filled upside down triangles); COMPOUND A+COMPOUND B (filled diamonds); COMPOUND A+cet (open circles); COMPOUND B+cet (filled squares); or COMPOUND A+COMPOUND B+cet (open triangles) for 10 days. The tumor volume is plotted as the mean $\pm$ SEM ($n = 4-5$ per treatment group). (B) Scatter plot of individual tumor volumes.

Figure 10. COMPOUND A and COMPOUND B modestly induce cell cycle arrest in HCOX1329 patient-derived tumor xenografts; additional cell cycle arrest is seen with combination treatments. (A) Representative images of Ki67 staining by immunohistochemistry after 10 days of treatment. (B) Percentage of Ki67-positive nuclei. Graphs represent mean $\pm$ SEM ($n = 4-5$ per treatment group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 11. The combinations of COMPOUND A+COMPOUND B and COMPOUND A+COMPOUND B+cet induce mucinous differentiation of HCOX1329 tumor xenografts. (A) Representative images of Alcian blue staining after 10 days of treatment. In contrast to JHOM-2B xenografts that accumulate intra- and extracellular mucin upon treatment, treatment of HCOX1329 xenografts results in a selective increase in intracellular mucin (arrows). (B) Percentage of blue-positive pixels. Graphs represent mean $\pm$ SEM ($n = 4-5$ per treatment group). * $P < 0.05$; ** $P < 0.01$.

Figure 12. No statistically significant decrease in tumor volume is seen in T70 patient-derived xenografts treated with COMPOUND A, cetuximab, or COMPOUND A+cetuximab *in vivo*. (A) T70-tumor-bearing mice were treated with 10 ml/kg vehicle, p.o., BID (filled circles); 5 mg/kg COMPOUND A, p.o. BID (open squares); 20 mg/kg cetuximab, i.p., 2x/wk (filled upside down triangles); or COMPOUND A+cetuximab (open circles) for 14 days. The tumor volume is plotted as the mean $\pm$ SEM ($n = 16$ per treatment group). (B) Scatter plot of individual tumor volumes.

Figure 13. COMPOUND A and COMPOUND A+cetuximab induce cell cycle arrest in T70 patient-derived tumor xenografts. (A) Representative images of Ki67 staining by immunohistochemistry after 14 days of treatment. (B) Percentage of Ki67-positive nuclei. Graphs represent mean $\pm$ SEM ($n = 16$ per treatment group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

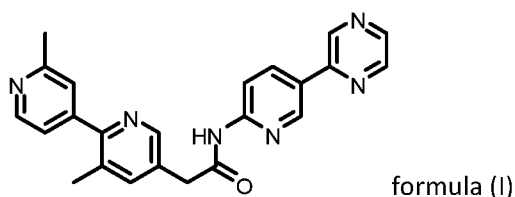
Figure 14. COMPOUND A and COMPOUND A+cetuximab induce mucinous differentiation of T70 tumor xenografts. (A) Representative images of Alcian blue staining after 14 days of treatment. (B) Percentage of blue-positive pixels. Graphs represent mean $\pm$ SEM ($n = 16$ per treatment group). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Detailed Description of the Invention

Surprisingly it has been found that a Wnt inhibitor, or a combination of Wnt inhibitor and either a BRAF inhibitor, or EGFR inhibitor, or both, according to the present disclosure, pharmaceutical compositions or combination product of the present disclosure, are especially useful for treatment of a patient with a *BRAF*^{V600E}-mutant colorectal cancer and microsatellite-unstable, BRAF wild-type (*BRAF*^{WT}), KRAS wild-type (*KRAS*^{WT}) colorectal cancer harboring, particularly the cancer with a mutated Wnt pathway. Wnt/ β -catenin signaling promotes cell survival in various cell types (Orford et al., J Cell Biol, 146:855-868 (1999); Cox et al., Genetics, 155:1725-1740 (2000); Reya et al., Immunity, 13:15-24 (2000); Satoh et al., Nat Genet, 24:245-250 (2000); Shin et al., Journal of Biological Chemistry, 274:2780-2785 (1999); Chen et al., J Cell Biol, 152:87-96 (2001); Loannidis et al., Nat Immunol, 2:691-697 (2001)). Wnt signaling pathway is also thought to be associated with tumor development and/or progression (Polakis et al., Genes Dev, 14:1837-1851 (2000); Cox et al., Genetics, 155). According to the present disclosure, a Wnt inhibitor can be any compound which targets and decreases or inhibits the Wnt pathway.

A Wnt inhibitor can be a compound, protein or antibody that reduces the beta-catenin target gene expression of *AXIN2* by at least 50% with an EC₅₀ of 10 μ M or less, preferably less than 1 μ M, in a tumor cell line with autocrine Wnt signaling (HPAF-II). Gene expression is measured against a control, which can be either gene expression of *AXIN2* in an untreated cell sample, and/or against the gene expression of housekeeping genes in the treated sample, such as gene expression of for example *GAPDH*, *HSP90*, *β -actin*, *ACTB* and *BM*.

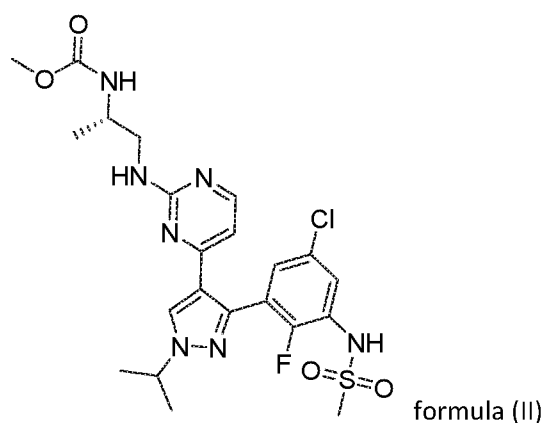
The Wnt inhibitor is particularly a compound, protein or an antibody which inhibit members of the Wnt pathway, for example inhibits or reduces activity of Porcupine, RSPO2, RSPO3, Frizzled, LRP6 or Tankyrase. The inhibitor can be for example XAV939, IWR1, IWP-1, IWP-2, JW74, JW55, Tautomycin, SB239063, SB203580, ADP-HPD, 2-[4-(4-fluorophenyl)piperazin-1-yl]-6-methylpyrimidin-4(3H)-one, PJ34, Niclosamide, Cambinol, Sulindac, 3289-8625, J01-017a, NSC668036, Filipin, IC261 or other Wnt inhibitors disclosed herein. A Wnt inhibitor can especially be a compound that inhibits Frizzled or Porcupine, most preferably Porcupine. In one embodiment, the Wnt inhibitor according to the present disclosure is 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide (**compound A**). The compound A can be prepared as described in WO2010/101849 and has a structure of formula (I):



Mitogen-activated protein kinase (MAPK) hyper-activation is a common property of human cancers and is often due to activating mutations in the *BRAF* and *RAS* genes. *BRAF* kinase domain mutations result in the production of a constitutively activated form of the protein and occur in approximately 8% of human

tumors (Davies et al., 2002; Wan et al., 2004). *BRAF* mutation stimulates extracellular signal-regulated kinase (ERK) signaling, induces proliferation and is capable of promoting transformation. BRAF inhibitors are compounds, especially compounds, proteins or antibodies which target BRAF, particularly mutant BRAF, and its downstream effectors. BRAF inhibitor can be selected from the group consisting of: ((S)-methyl-1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-yl)carbamate;
 methyl N-[(2S)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2S)-1-({4-[3-(2,5-difluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2S)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-ethyl-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2S)-1-({4-[3-(2-fluoro-3-methanesulfonamido-5-methylphenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2S)-1-({4-[3-(2-chloro-3-methanesulfonamido-5-methylphenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2S)-1-({4-[3-(2-chloro-5-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2R)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate;
 methyl N-[(2S)-1-({4-[3-(2,5-dichloro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl]pyrimidin-2-yl}amino)propan-2-yl]carbamate; and
 vemurafenib.

In one embodiment, the BRAF inhibitor is (S)-methyl 1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate of formula (II) (**compound B**).



The BRAF inhibitors can be obtained by the processes disclosed in WO2011/025927.

Where the colorectal cancer is BRAF wild-type, like for example in the microsatellite-unstable BRAF wild-type and KRAS wild-type colorectal cancer, the BRAF inhibitor may not be used in combination with the Wnt inhibitor.

EGFR inhibitor can be any compound that targets, decreases or inhibits the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers) and their mutants. Such compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind to EGF or EGF related ligands, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180); e.g. trastuzumab (HerceptinTM), cetuximab (ErbixTM), Iressa, Tarceva, OSI-774, CI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3, and 7H-pyrrolo-[2,3-d]pyrimidine derivatives which are disclosed in WO 03/013541, gefitinib, erlotinib, lapatinib, XL-647, HKI-272 (Neratinib), BIBW2992 (Afatinib), EKB-569 (Pelitinib), AV-412, canertinib, PF00299804, BMS 690514, HM781-36b, WZ4002, AP-26113, panitumumab, matuzumab, pertuzumab, or (R,E)-N-(7-chloro-1-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 N-(7-chloro-1-(1-(4-(pyrrolidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 (R,E)-N-(7-chloro-1-(1-(4-(3-fluoroazetidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 N-(7-chloro-1-(1-(4-(3-fluoroazetidin-1-yl)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 (S)-N-(1-(1-(1-acryloylpiperidin-3-yl)-5-methyl-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl) benzamide;
 N-(1-(1-(1-acryloylpiperidin-3-yl)-5-methyl-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl) benzamide;
 (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;
 N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;
 (R,E)-N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 (R,E)-N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-(trifluoromethyl)isonicotinamide;

N-(1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-7-methyl-1H-benzo[d]imidazol-2-yl)-2-(trifluoromethyl)isonicotinamide;
 (R)-N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-methoxy-1H-benzo[d]imidazol-2-yl)pyridazine-4-carboxamide;
 N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-methoxy-1H-benzo[d]imidazol-2-yl)pyridazine-4-carboxamide;
 N-(7-methyl-1-(1-(vinylsulfonyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl)benzamide;
 N-(1-(1-(1-acryloylazepan-3-yl)-7-(pyrrolidin-1-ylmethyl)-1H-benzo[d]imidazol-2-yl)-3-(trifluoromethyl)benzamide;
 tert-butyl 4-acryloyl-6-(7-chloro-2-(2-methylisonicotinamido)-1H-benzo[d]imidazol-1-yl)-1,4-diazepane-1-carboxylate;
 N-(1-(1-(1-acryloyl-1,4-diazepan-6-yl)-7-chloro-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 N-(1-(1-(1-acetyl-4-acryloyl-1,4-diazepan-6-yl)-7-chloro-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 (R)-N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-(2-(pyrrolidin-1-yl)ethoxy)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;
 N-(1-(1-(1-acryloylazepan-3-yl)-7-chloro-6-(2-(pyrrolidin-1-yl)ethoxy)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide;
 (R,E)-N-(7-chloro-1-(1-(4-(dicyclopropylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 N-(7-chloro-1-(1-(4-(dicyclopropylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide;
 (R)-1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxylic acid;
 1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxylic acid;
 (R)-1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxamide;
 1-(1-(1-acryloylpiperidin-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-5-carboxamide; and
 1-(1-(1-acryloylazepan-3-yl)-2-(3-(trifluoromethyl)benzamido)-1H-benzo[d]imidazole-7-carboxylic acid,
 which are disclosed in WO2013184757.

In one embodiment the EGFR inhibitor is cetuximab.

In another embodiment the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide.

In yet another embodiment the EGFR inhibitor is (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2,6-dimethylisonicotinamide.

In one embodiment, the combination partners are 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide, (S)-methyl 1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate and/or cetuximab.

In yet another embodiment the combination partners are 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide, (S)-methyl 1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate and/or (R,E)-N-(7-chloro-1-(1-(4-(dimethylamino)but-2-enoyl)azepan-3-yl)-1H-benzo[d]imidazol-2-yl)-2-methylisonicotinamide.

The present disclosure embodiments also include pharmaceutically acceptable salts of the compounds useful according to the disclosure described herein. As used herein, "pharmaceutically acceptable salt" refers to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and *Journal of Pharmaceutical Science*, 66, 2 (1977), each of which is incorporated herein by reference in its entirety. For example, the salt is sulphate salt, or bisulphate salt.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The term "treating" or "treatment" as used herein comprises a treatment relieving, reducing or alleviating at least one symptom in a subject or effecting a delay of progression of a disease. For example, treatment can be the diminishment of one or several symptoms of a disorder or complete eradication of a disorder, such as cancer. Within the meaning of the present disclosure, the term "treat" also denotes to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease. The term "protect" is used herein to mean prevent delay or treat, or all, as appropriate, development or continuance or aggravation of a disease in a subject, e.g., a mammal or human. The term "prevent", "preventing" or "prevention" as used herein comprises the prevention of at least one symptom associated with or caused by the state, disease or disorder being prevented.

The compounds useful according to the disclosure (= being included in a combination, especially a pharmaceutical combination, according to the disclosure, respectively, or being used according to the

disclosure, optionally also including further co-agents as defined below, that is, all active ingredients), as well as their pharmaceutically acceptable salts, can also be present as tautomers, N-oxides or solvates, e.g. hydrates. All these variants, as well as any single one thereof or combination of two or more to less than all such variants, are encompassed and to be read herein where a compound included in the inventive combination products, e.g. a Wnt inhibitor and/or a BRAF inhibitor, is mentioned.

In one embodiment the present disclosure relates to a pharmaceutical combination, especially a pharmaceutical combination product, comprising the mentioned combination partners and at least one pharmaceutically acceptable carrier.

“Pharmaceutical combination” refers to use, application or formulations of the separate partners with or without, preferably with, instructions for combined use or to combination products. The combination partners may thus administered entirely separately or be entirely separate pharmaceutical dosage forms. The combination partners may be pharmaceutical compositions that are also sold independently of each other and where just instructions for their combined use are provided in the package equipment, e.g. leaflet or the like, or in other information e.g. provided to physicians and medical staff (e.g. oral communications, communications in writing or the like), for simultaneous or sequential use for being jointly active, especially as defined below. It can refer to either a fixed combination in one dosage unit form, or a kit of parts for the combined administration where a Wnt inhibitor and either a BRAF inhibitor or EGFR inhibitor, or both (and optionally yet a further combination partner (e.g. an other drug, also referred to as “co-agent”)) may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative (= joint) effect. In one embodiment the effect is synergistic. The selected combination partner are to be administered to a single subject in need thereof (e.g. a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration and/or at the same time. The term “pharmaceutical combination” as used herein thus means a pharmaceutical product that results from the mixing or combining of more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients (which may also be combined).

The compounds of the present disclosure can be used as a fixed or non-fixed combination. The term “fixed combination” means that the active ingredients, e.g. a Wnt inhibitor and a BRAF inhibitor, are both administered to a patient simultaneously in the form of a single entity or dosage. In other terms: the active ingredients are present in one dosage form, e.g. in one tablet or in one capsule.

The term “non-fixed combination” means that the active ingredients are both administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients. The term “non-fixed combination” thus defines especially administration, use, composition or formulation in the sense that the combination partners (i) Wnt inhibitor and either (ii) BRAF inhibitor or (iii) EGFR inhibitor, or both (and if present further one or more co-agents); for example (i) Wnt inhibitor and EGFR inhibitor alone; as defined herein can be dosed independently of each other or by

use of different fixed combinations with distinguished amounts of the combination partners, i.e. simultaneously or at different time points, where the combination partners may also be used as entirely separate pharmaceutical dosage forms or pharmaceutical formulations that are also sold independently of each other and just instructions of the possibility of their combined use is or are provided in the package equipment, e.g. leaflet or the like, or in other information e.g. provided to physicians and medical staff. The independent formulations or the parts of the formulation, product, or composition, can then, e.g. be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. Particularly, the time intervals are chosen such that the effect on the treated disease in the combined use of the parts is larger than the effect which would be obtained by use of only any one of the combination partners (i), (ii) and (iii), thus being jointly active. The total amounts of the combination partners to be administered in the combined preparation can be varied, e.g. in order to cope with the needs of a patient sub-population to be treated or the needs of the single patient which different needs can be due to age, sex, body weight, etc. of the patients.

The combination partners, a Wnt inhibitor, a BRAF inhibitor and a EGFR inhibitor in any disclosed embodiment are preferably formulated or used to be jointly (prophylactically or especially therapeutically) active. This means in particular that there is at least one beneficial effect, e.g. a mutual enhancing of the effect of the combination partners, in particular a synergism, e.g. a more than additive effect, additional advantageous effects (e.g. a further therapeutic effect not found for any of the single compounds), less side effects, a combined therapeutic effect in a non-effective dosage of one, both or all three combination partners, and very preferably a clear synergism of the combination partners.

For example, the term "jointly (therapeutically) active" may mean that the compounds may be given separately or sequentially (in a chronically staggered manner, especially a sequence-specific manner) in such time intervals that they preferably, in the warm-blooded animal, especially human, to be treated, and still show a (preferably synergistic) interaction (joint therapeutic effect). A joint therapeutic effect can, inter alia, be determined by following the blood levels, showing that both compounds are present in the blood of the human to be treated at least during certain time intervals, but this is not to exclude the case where the compounds are jointly active although they are not present in blood simultaneously.

The present disclosure thus pertains to a combination product for simultaneous or sequential use, such as a combined preparation or a pharmaceutical fixed combination, or a combination of such preparation and combination.

In the combination therapies of the disclosure, the compounds useful according to the disclosure may be manufactured and/or formulated by the same or different manufacturers. Moreover, the combination partners may be brought together into a combination therapy: (a) prior to release of the combination product to physicians (e.g. in the case of a kit comprising the compound of the disclosure and the other therapeutic agent); (b) by the physician themselves (or under the guidance of a physician) shortly before administration; (c) in the patient themselves, e.g. during sequential administration of the compound of the disclosure and the other therapeutic agent.

Also in this case, the combination partners forming a corresponding combination according to the disclosure may be mixed to form a fixed pharmaceutical composition or they may be administered separately or pairwise (i.e. before, simultaneously with or after the other drug substance(s)).

The term "pharmaceutically effective" preferably relates to an amount that is effective against the progression of a disease or disorder as disclosed herein.

A Wnt inhibitor, the combinations according to the present disclosure, pharmaceutical compositions or combination product of the present disclosure is especially suitable for treatment of a patient suffering from a proliferative disorder, in particular a solid tumor, *BRAF*^{V600E}-mutant colorectal cancer, which can be microsatellite-unstable, or microsatellite-unstable *BRAF* wild-type and *KRAS* wild-type colorectal cancer. In one embodiment a Wnt pathway in the *BRAF*^{V600E}-mutant colorectal cancer or microsatellite-unstable *BRAF* wild-type and *KRAS* wild-type colorectal cancer is activated compared to a control. In a further embodiment *AXIN2* expression in the colorectal cancer is higher compared to a control. In yet another embodiment, the cancer comprises mutated *RNF43*, mutated *RSPO2* or mutated *RSPO3*, particularly mutated *RNF43*. Because *RNF43* and *ZNRF3* are two homologous Zinc/RING finger cell surface transmembrane E3 ubiquitin ligases for Wnt receptor Frizzled that inhibit the cell surface levels of Wnt receptor complex composing Frizzled and LRP6, a Wnt inhibitor can also be used in *ZNRF3* mutated colorectal cancer. The cancer can alternatively or in addition comprise *RSPO* fusion.

The term "a therapeutically effective amount" of a compound of the present disclosure refers to an amount of the compound of the present disclosure that will elicit the biological or medical response of a subject, for example, reduction or inhibition of an enzyme or a protein activity, or ameliorate symptoms, alleviate conditions, slow or delay disease progression, or prevent a disease, etc. In one non-limiting embodiment, the term "a therapeutically effective amount" refers to the amount of the compound of the present disclosure that, when administered to a subject, is effective to (1) at least partially alleviating, inhibiting, preventing and/or ameliorating a condition, or a disorder or a disease (i) mediated by a Wnt pathway, or mediated by *BRAF* activity and/or *EGFR* activity, or (ii) characterized by activity (normal or abnormal) of Wnt pathway, *EGFR* and/or *BRAF*; or (2) reducing or inhibiting the activity of Wnt pathway, *EGFR* and/or of *BRAF*.

As used herein, the term "subject" refers to an animal. Typically the animal is a mammal. A subject also refers to for example, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice, fish, birds and the like. In certain embodiments, the subject is a primate. In yet other embodiments, the subject is a human.

Wnt inhibitor can be for example administered in unit dosage of about 1-5000 mg of active ingredient(s) for a subject of about 50-70 kg, or about 1mg – 3g or about 1-250 mg or about 1-150 mg or about 0.5-100 mg, or about 1-50 mg of active ingredients. The therapeutically effective dosage of a compound, the pharmaceutical composition, or the combinations thereof, is dependent on the species of the subject, the body weight, age and individual condition, the disorder or disease or the severity thereof being treated. A physician, clinician or veterinarian of ordinary skill can readily determine the effective amount

of each of the active ingredients necessary to prevent, treat or inhibit the progress of the disorder or disease.

BRAF inhibitor of the present disclosure can be administered in therapeutically effective amounts via any of the usual and acceptable modes known in the art. A therapeutically effective amount may vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compound used and other factors. In general, satisfactory results are indicated to be obtained systemically at daily dosages of from about 0.03 to 30mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5mg to about 2000mg, conveniently administered, e.g. in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from about 1 to 500mg active ingredient.

In general, satisfactory results are indicated to be obtained systemically at daily dosages of an EGFR inhibitor according to the present disclosure from about 0.03 to 2.5 mg/kg per body weight. An indicated daily dosage in the larger mammal, e.g. humans, is in the range from about 0.5 mg to about 100 mg, conveniently administered, e.g. in divided doses up to four times a day or in retard form. Suitable unit dosage forms for oral administration comprise from ca. 1 to 50 mg active ingredient.

In certain embodiments, a therapeutic amount or dose of the compounds of the present disclosure may range from about 0.1 mg/kg to about 500 mg/kg, alternatively from about 1 to about 50 mg/kg. In general, treatment regimens according to the present invention comprise administration to a patient in need of such treatment from about 10 mg to about 1000 mg of the compound(s) of this invention per day in single or multiple doses (such as two, three, or four times daily). Therapeutic amounts or doses will also vary depending on route of administration, as well as the possibility of co-usage with other agents.

In general, the dosage of the active ingredient to be applied to a warm-blooded animal depends upon a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound employed. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

As used herein, the term "carrier" or "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, and the like and combinations thereof, as would be known to those skilled in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289- 1329). Except insofar as any

conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The pharmaceutical combination product according to the disclosure (as fixed combination, or as kit, e.g. as combination of a fixed combination and individual formulations for one or both combination partners or as kit of individual formulations of the combination partners) comprises the combination of the present disclosure and one or more pharmaceutically acceptable carrier materials (carriers, excipients). The pharmaceutical combination or the combination partners constituting it can be formulated for particular routes of administration such as oral administration, parenteral administration, and rectal administration, etc. In addition, the combination products of the present disclosure can be made up in a solid form (including without limitation capsules, tablets, pills, granules, powders or suppositories), or in a liquid form (including without limitation solutions, suspensions or emulsions). The combination products and/or their combination partners can be subjected to conventional pharmaceutical operations such as sterilization and/or can contain conventional inert diluents, lubricating agents, or buffering agents, as well as adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers and buffers, etc.

In one embodiment, the pharmaceutical compositions are tablets or gelatin capsules comprising the active ingredient together with one or more commonly known carriers, e.g. one or more carriers selected from the group consisting of

- a) diluents, *e.g.*, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine;
- b) lubricants, *e.g.*, silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol; for tablets also
- c) binders, *e.g.*, magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; if desired
- d) disintegrants, *e.g.*, starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and
- e) absorbents, colorants, flavors and sweeteners.

Tablets may be either film coated or enteric coated according to methods known in the art.

Suitable compositions for oral administration especially include an effective amount of one or more or in case of fixed combination formulations each of the combination partners (active ingredients) in the form of tablets, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use are prepared according to any method known in the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets may contain the active ingredient(s) in admixture with nontoxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These

excipients are, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets are uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. Formulations for oral use can be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

Parenteral compositions, transdermal, topical compositions and other can be prepared by known methods in the art.

Examples

The following examples serve to illustrate the invention and provide specific embodiments; however, they do not limit the scope of the invention.

Compounds described in the examples:

- Compound A (Wnt inhibitor): 2-(2',3-dimethyl-2,4'-bipyridin-5-yl)-N-(5-(pyrazin-2-yl)pyridin-2-yl)acetamide
- Compound B (BRAF inhibitor): (S)-methyl 1-(4-(3-(5-chloro-2-fluoro-3-(methylsulfonamido)phenyl)-1-isopropyl-1H-pyrazol-4-yl)pyrimidin-2-ylamino)propan-2-ylcarbamate
- Cetuximab

Example 1: Co-occurrence of *BRAF*^{V600E} and upstream Wnt pathway mutations in patient CRC samples

*Molecular characterization of patient *BRAF*^{V600E}-mutant CRC samples*

There are at least two distinct molecular pathways that underlie the pathogenesis of sporadic CRC: the conventional pathway and the serrated pathway. Approximately 70% of CRCs arise via the conventional pathway. These tumors are characterized by mutational activation of the Wnt pathway through inactivating mutations in *APC* or *AXIN2* or mutations in the GSK3-target residues in *beta-catenin* (Jin, L.H., *et al. Int. J. Cancer*. 2003, 107:696-99; Korinek, V., *et al., Science*. 1997, 275:1784-7; Morin, P.J., *et al., Science*. 1997, 275:1787-90; Rubinfeld, B., *et al., Science*. 1997, 275:1790-2; Liu, W., *et al., Nat. Genet.* 2000, 26:146-7). In normal intestinal cells, APC associates with AXIN, GSK-3 β and casein kinase 1 to form the beta-catenin destruction complex. This complex phosphorylates beta-catenin, resulting in its ubiquitylation and subsequent degradation by the proteasome (Polakis, P. *Curr. Biol.* 2002, 12:R499-R501). In contrast, in cells harboring mutations in *APC*, *AXIN2* or *beta-catenin*, beta-catenin accumulates and upon its translocation to the nucleus, interacts with the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors to activate specific Wnt target genes (Tetsu, O., McCormick, F. *Nature*. 1999, 398:422-6; He, T.C., *et al., Science*. 1998, 281:1509-12). Considerable

evidence from human genetic studies and mouse genetic models suggests that mutational activation of the Wnt pathway can initiate colon tumorigenesis. Inherited *APC* mutations cause familial adenomatous polyposis, and acquired *APC* mutations represent the earliest genetic alteration so far detected in sporadic CRC (Powell, S.M., et al., *Nature*. 1992, 359:235-7). Studies in *APC*-mutant CRC tumor xenografts indicate that Wnt signaling is also required for tumor maintenance, as inducible knockdown of *beta-catenin* in established xenografts inhibits tumor growth *in vivo* (Scholer-Dahirel, A., et al., *Proc. Natl. Acad. Sci.* 2011, 108:17135-40).

The role of Wnt pathway activation in CRCs that develop via the serrated pathway is largely unknown. Serrated pathway CRCs contain either *BRAF* or *KRAS* mutations, and lack *APC* mutations (Bettington, M., et al., *Histopathol.* 2013, 62:367-86). In a mouse model of *BRAF*^{V600E}-induced colon tumorigenesis, Wnt pathway activation was identified as an important step in the progression from intestinal hyperplasia to carcinoma (Rad, R., et al., *Cancer Cell*. 2013, 24:15-29). In contrast to conventional CRCs, *BRAF*-mutant CRCs tend to have minimal chromosomal instability and exhibit hypermethylation of CpG islands, which can decrease the expression of DNA repair genes and cell cycle regulators.

Curiously, while the mutant *BRAF* inhibitor vemurafenib achieves a response rate of approximately 80% in *BRAF*^{V600E}-mutant melanoma, the response rate in *BRAF*^{V600E}-mutant CRC is approximately 5% (Kopetz, S. et al. *J. Clin. Oncol. abstr.* 2010, 28:3534; Corcoran, R.B., et al., *J. Clin. Oncol. Suppl. abstr.* 2012, 3528).

Prahallad and co-workers demonstrated in CRC cell line and xenograft models that the unresponsiveness of *BRAF*^{V600E}-mutant CRC to mutant *BRAF* inhibitors is due to feedback activation of EGFR (Prahallad, A. et al., *Nature*. 2012, 483:100-3). Treating *BRAF*^{V600E}-mutant CRC cells with a combination of a mutant *BRAF* inhibitor and an EGFR inhibitor synergistically inhibited tumor growth *in vitro* and *in vivo*. This data was the impetus to initiate clinical trials with combinations of mutant *BRAF* inhibitors and EGFR inhibitors in *BRAF*^{V600E}-mutant CRC. If in addition to the MAPK and PI3K pathways, the Wnt pathway also contributes to the maintenance of *BRAF*^{V600E}-mutant CRC, then patients may derive additional benefit from a combination therapy that also includes a Wnt pathway inhibitor.

Recently, the secreted glycoproteins R-spondins 1-4 (RSPO1-4) have emerged as important activators of the Wnt pathway. RSPOs bind to LGR4-6 receptors and the transmembrane E3 ubiquitin ligases RNF43 and ZNRF3, forming a ternary complex (Chen, P-H., et al., *Genes Dev.* 2013, 27:1345-50; Xie, Y., et al., *EMBO Rep.* 2013, 14:1120-6; Zebisch, M., et al., *Nature Com.* 2013, 4:1-12). RNF43/ZNRF3 antagonize Wnt signaling by promoting the turnover of the Wnt receptors, Frizzled and LRP6 (Hao, H.X., et al., *Nature*. 2012, 485:195-200; Koo, B.K., et al., *Nature*. 2012, 488:665-9). Binding of RSPO induces the endocytosis of RNF43/ZNRF3, thereby increasing levels of membrane-bound Frizzled and LRP6 and enhancing Wnt signaling. RSPO fusions that elevate expression of *RSPO2* or *RSPO3* have been identified in colon cancer (Seshagiri, S., et al., *Nature*. 2012, 488:660-4), and inactivating mutations in *RNF43* have been reported in mucinous tumors of the pancreas, mucinous ovarian carcinoma, and cholangiocarcinoma (Wu, J., et al., *Proc. Natl. Acad. Sci.* 2011, 108:21188-93; Ryland, G.L., et al., *J. Pathol.* 2013, 229:469-76; Ong, C.K., et al., *Nature Genet.* 2012, 44:690-3). As *RSPO* fusions and *RNF43* mutations enhance Wnt ligand-dependent signaling, we hypothesize that Wnt pathway inhibitors, such

as a porcupine inhibitor that exerts its effects upstream in the Wnt pathway, will be efficacious in tumors harboring these genetic alterations.

Forty-five *BRAF*^{V600E}-mutant CRC resection specimens were purchased from Asterand, PLC. The FFPE blocks were sent to Foundation Medicine, Inc. for genetic analysis using the T5b NGS panel. Complete characterization was received, and *BRAF*/Wnt pathway genetic mutation annotation was performed manually. Tabularization and graphical representation was performed using EXCEL.

Presence or absence of *RSPO* fusions in the 45 patient *BRAF*^{V600E}-mutant CRC samples obtained from Asterand, PLC was determined by qPCR assays with either primer or probe spanning published junctions (Seshagiri, S., *et al.*, *Nature*. 2012, 488:660-4). Assays were ordered and synthesized by IDT with FAM labeled probes. The four targeted assays were: EIF3Ee1-*RSPO2*e2 Forward (TTGGATCGGCATCTAGTCTTTC) Probe (TCTGTAAAGGAGGTTCTGTGGCGG) reverse (TCAGTTCAGCGCGATCAG), EIF3Ee1-*RSPO2*e3 Forward (TTCTCTCTGTAAAGGAGCTAGTTATG) Probe (TCCCATTTGCAAGGGTTGTTTGTCTTG) reverse (ACAATGGGTGTAGCCGATG), PTPRKe1-*RSPO3*e2 Forward (GCTTTTGTGGCGCTCTTG) Probe (AGTTCTCCGAGTGCATCCTAACG) reverse (GGGCTTACATGACAAACATCC), and PTPRKe7-*RSPO3*e2del Forward (TTACAAGACCTGGTGAAGGTG) Probe (CTAATCACCAGAACAAAATGTGCAGTGCATCCTAACGTTAGTC) reverse (AGTCTGGGCTTACATGACAAA). RNA was extracted from macrodissected FFPE tissue using Qiagen's RNeasy FFPE kit (Cat# 73504) and subsequently quantified using Nanodrop. 500ngs of total RNA was used for cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat# 4368814) and cDNA was diluted five-fold for a final concentration of 10 ngs/μl. 2 μl of diluted cDNA (total input of 20ngs) in a 10 μl reaction was run in triplicate for each sample for all fusion assays using an ABI7900 in a 384 well format, according to Life Technologies protocol. Threshold setting was automatically set by ABI7900 software (SDS2.4), and all samples were normalized to a *UBC* control gene. Samples with expression higher than 30 Ct's in the fusion assays are considered positive for the corresponding *RSPO* fusion.

Results

A total of 45 patient *BRAF*^{V600E}-mutant CRC samples were analyzed for Wnt pathway mutations (Fig.1). Mutations were screened for by Foundation Medicine, Inc., using their T5b NGS panel, and *RSPO* fusions were separately detected using qPCR methods. We found a striking co-occurrence of *RNF43* mutations with the *BRAF*^{V600E} mutation (64.4% of *BRAF*^{V600E}-mutant CRCs contained *RNF43* mutations). This compares to a *RNF43* mutation frequency of 13.1% when 430 unselected CRC samples in the TCGA database are analyzed with a trial version of OmicSoft. The frequency of *RSPO* fusions may also be enriched in the *BRAF*^{V600E}-mutant CRCs, with a frequency of *RSPO2* plus *RSPO3* fusions of 13.3% in *BRAF*^{V600E}-mutant CRC samples versus 2% when 430 unselected CRC samples in the TCGA database are analyzed with a trial version of OmicSoft. This molecular epidemiology data strongly suggests that Wnt pathway activation, via genetic alterations in upstream Wnt pathway regulators, is important for *BRAF*^{V600E}-mutant CRC tumorigenesis.

Example 2: Efficacy of a Wnt pathway inhibitor and combinations comprising (i) a Wnt pathway inhibitor, (ii) a mutant BRAF inhibitor, and/or (iii) an EGFR inhibitor in a *BRAF*^{V600E};*RSPO3* fusion⁺ lower GI adenocarcinoma cell line model and a patient-derived *BRAF*^{V600E};*RNF43*-mutant CRC xenograft model

DNA sequencing of RSPO3 fusion

Genomic DNA of JHOM-2B cells was isolated. The DNA fragment containing *PTPRK-RSPO3* fusion was amplified by PCR using HH3 and HH4 primers, and analyzed by electrophoresis. DNA sequence of PCR fragment was determined by Sanger sequencing. Sequence of HH3 and HH4 primers are following. HH3: AAAC TCGGCATGGATACGAC. HH4: GCTTCATGCCAATTCTTTCC.

Cell culture

JHOM-2B cells were cultured in DMEM:F-12 (ATCC, Cat# 30-2006), supplemented with 10% fetal bovine serum (5% CO₂ at 37°C).

Foci formation assay

For foci formation assay, approximately 12,000 JHOM-2B cells were seeded in a 6-well tissue culture plate with 2 mL of growth media. After overnight culture for cell attachment, media was replaced with fresh growth media containing 1 µM of COMPOUND A and/or DMSO. When cell colonies reached a desirable size (24 days), cells were fixed with 10% (vol/vol) buffered formalin and stained with crystal violet solution. After several washes, the plate was dried and imaged.

Growth assay

Cell proliferation and EC₅₀ values were determined through the use of CellTiter-Glo® (CTG), a method based on luminescent detection of mitochondrial ATP (Promega, Cat# G7573). JHOM-2B cells were plated in 96 well-clear bottom culture plates (Corning, Cat# 3904) at approximately 9500 cells per well, with 2 identical plates created for a 'time=0' read and 'day 7' read. On day 0, COMPOUND A was added to one plate in the following concentrations (µM); DMSO (vehicle), 0.0015, 0.0046, 0.014, 0.041, 0.123, 0.37, 1.11, 3.33, and 10. The duplicate plate was used for a time=0 CTG read. CTG was determined at day 0 and day 7 of treatment as follows: 100 µl of the CTG reagent was added to each well of the 96 well plates, and placed on a shaker for 30-45 minutes at room temperature. Luminescence was measured using the Perkin Elmer-Victor™ X4. Raw luminescent readings were entered into an Excel spread sheet that was formulated to determine the EC₅₀ value for each PDAC cell lines (day 7 – day 0).

Combination Assay

The combination assay was set-up in the same manner as the growth assay, with several exceptions. Compound was added in an 8 x 8 dose-matrix fashion, with COMPOUND A dilutions added to one axis, and COMPOUND B added to the other axis. The dilution series used for both COMPOUND A and COMPOUND B was as follows (µM): 0.0015, 0.0046, 0.0137, 0.0412, 0.1235, 0.3704, and 1.11.

Cetuximab was added to all of these wells in a single dose of 50 nM, and 16 wells per plate were reserved for the DMSO control. The experiment was run in triplicate, as well as an extra plate for a time=0 CTG read. A CTG assay was performed on the plates after 7 days of compound treatment. Raw luminescent readings were entered into an Excel spread sheet that was formulated to determine inhibition values, which were then entered into an Excel spread sheet that was used uploaded to the Chalice Analyzer server. The final inhibition grid was visualized using the Chalice Analyzer Client program.

Quantitative real-time polymerase chain reaction

JHOM-2B cells were plated onto 10 cm tissue culture dishes at approximately 1.5 e+6 cells per dish, and treated with COMPOUND A (100 nM) or DMSO for 72 hours. Total RNA from COMPOUND A treated and untreated JHOM-2B cells was extracted using the RNeasy Plus Mini Kit (Qiagen, Cat# 74134) and reverse transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat# 4368814), according to the manufacture's protocol. Gene transcript expression levels were determined using the Applied Biosystems 7900HT Fast Real-time PCR System. Quantitative real-time polymerase chain reactions were performed in 12 µl reactions, consisting of 0.6 µl of 20x TaqMan® Gene Expression Assay, 6 µl TaqMan® 2x Universal PCR Master Mix (Applied Biosystems, Cat# 4304437), 0.4 µl DNase/RNase free distilled water and 5 µl cDNA template (1 ng/µl). Thermocycling conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. All experimental reactions were carried out in quadruplicate. Gene expression was quantified using the Δ^2 cycle threshold method, with *18s* serving as an internal control. Applied Biosystems TaqMan® Gene Expression Assay reagents used were as follows: *AXIN2* (Hs00610344_m1), *CCND1* (*CyclinD1*) (Hs0076553_m1), *LGR5* (Hs00969418_m1), *MYC* (Hs00153408_m1) and *18s* (4319413E). Statistical analysis was performed using a Student's t-test.

Immunoblots

JHOM-2B cells were plated onto 8-10 cm tissue culture dishes at approximately 1.5 e+6 cells per dish, and treated for 72 hours under the following conditions: DMSO; COMPOUND A (100 nM); NVP-COMPOUND B-NX-12 (COMPOUND B) (100 nM); cetuximab (50 nM); COMPOUND A (100 nM) + COMPOUND B (100 nM); COMPOUND A (100 nM) + cetuximab (50 nM); COMPOUND B (100 nM) + cetuximab (50 nM); and COMPOUND A (100 nM) + COMPOUND B (100 nM) + cetuximab (50 nM). Western blots were performed as follows: Cell lysates were prepared using NP40 Cell Lysis Buffer (Invitrogen, Cat# FNN0021), supplemented with 1x Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, Cat# 1861281). Thirty micrograms of total cell lysates were separated by SDS-PAGE and electrotransferred to nitrocellulose membrane (Invitrogen, Cat# LC2007). The following primary antibodies and blocking reagents were used: Phospho-LRP6 (Ser1490) (Cell Signaling Technology (CST), Cat# 2568, 2% milk), LRP6 (CST, Cat# 3395, 2 % milk), Phospho-EGFR (Tyr1068) (CST, Cat#3777 , 5 % BSA), EGFR (CST, Cat#2232 , 5 % BSA), Phospho-p44/42 MAPK (Erk1/2)(Thr202/Tyr204) (CST, Cat# 4376, 5 % BSA), p44/42 MAPK (Erk1/2) (CST, Cat# 4695, 5 % BSA), Cleaved Caspase-8 (CST, Cat# 9496, 5 %

BSA), c-Myc (Abcam, Cat# ab32072, 2% milk), p21/Waf1/Cip1 (CST, Cat# 2947, 5% milk), and β -Tubulin (CST, Cat# 2146, 5 % BSA). Blocking reagents were prepared fresh in phosphate buffered saline 0.1% (vol/vol) Tween-20 (PBS-T), and membranes were blocked for 1 hr on a shaker at room temperature. Primary antibodies were added at 1:1000 dilutions (p44/42 MAPK (Erk1/2) 1:5000, and β -Tubulin 1:10,000), and incubated overnight with gentle rocking at 4° C. Immunoblots were washed 3 times, 5 min each with PBS-T, and secondary antibody was added at 1:10,000 dilution into PBS-T milk for 1 hour on a shaker at room temperature. After several washes, enhanced chemiluminescence (ECL) reactions were performed according to manufacture recommendations (SuperSignal® West Dura Extended Duration Substrate; Thermo Scientific, Cat# 34076).

Immunohistochemistry

IHC was performed on the Ventana DISCOVERY XT automated IHC platform (Tuscon, AZ), using optimized protocols described below. FFPE slides were cut at 3.5 μ m thickness, attached to charged slides (Thermo-Scientific Colormark Plus Cat # CM-5951PLUS BLUE), baked at 60°C for 1 hour and loaded on the DISCOVERY XT Staining System. Slides were deparaffinized using Ventana EZ Prep (Cat # 950-100). Antigen retrieval was performed using Ventana Cell Conditioning #1 reagent (Cat # 950-124). Primary antibody diluted in DAKO Cytomation Antibody Diluent (Cat # S0809) or DAKO Cytomation Antibody Diluent with background reducing components (Cat # S3022), was manually applied during the antibody titration step in 100 μ l volume. Ventana OmniMap HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was used at the provided pre-dilute concentrations. Ventana ChromoMap DAB detection kit (Cat # 760-159) was used for chromogenic detection. Slides were counterstained for 4 minutes with Ventana Hematoxylin (Cat # 760-2021), followed by Ventana Bluing Reagent (Cat # 760-2037) for 4 minutes. Coverslipped slides were evaluated by light microscopy. Slides were scanned by Aperio ScanScope slide scanner (Vista, CA). Digital images were then viewed by Aperio ImageScope (Vista, CA) and snapshots were taken by TechSmith Snagit (Okemos, MI).

A rabbit monoclonal antibody produced by immunizing animals with a synthetic peptide corresponding to residues surrounding C-terminus of human keratin 7 protein (Catalog # M3522, Lot# 121022C) was obtained from Spring Bioscience (Pleasanton, CA.). The antibody was stored at 4°C until use. The optimal IHC protocol included Heat and Ventana "Mild- Cell Conditioning 1" setting for antigen retrieval. Primary antibody was diluted at a concentration of 1:100 in DAKO Cytomation Antibody Diluent (Cat # S0809), manually applied during the antibody titration step in 100 μ l volume, and incubated for 60 minutes at 37°C. Subsequently, incubation with Ventana OmniMap prediluted HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was performed for 4 minutes.

A rabbit monoclonal antibody produced by immunizing animals with a synthetic peptide corresponding to residues surrounding C-terminus of human keratin 20 protein (Catalog # M3332, Lot# 121218CD) was obtained from Spring Bioscience (Pleasanton, CA.). The antibody was stored at 4°C until use. The optimal IHC protocol included Ventana "Mild- Cell Conditioning 1" setting for antigen retrieval. Primary antibody was diluted at a concentration of 1:100 in DAKO Cytomation Antibody Diluent with background reducing

components (Cat # S3022), manually applied during the antibody titration step in 100 µl volume, and incubated for 60 minutes at RT. Subsequently, incubation with Ventana OmniMap prediluted HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was performed for 4 minutes.

A rabbit polyclonal antibody produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser10 of phospho-Histone H3 (Catalog # 9701, Lot# 13) was obtained from Cell Signaling Technology (Danvers, MA). The antibody was stored at -20°C until use. The optimal IHC protocol included Heat and Ventana "Standard- Cell Conditioning 1" setting for antigen retrieval. Primary antibody was diluted at a concentration of 1:100 in DAKO Cytomation Antibody Diluent (Cat # S0809), manually applied during the antibody titration step in 100 µl volume, and incubated for 60 minutes at 37°C. Subsequently, incubation with Ventana OmniMap prediluted HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was performed for 4 minutes.

A rabbit polyclonal antibody produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ki67 (Catalog # VP-RM04, Lot# 20425) was obtained from Cell Signaling Technology (Burlingame, Ca.). The antibody was stored at -20°C until use. The optimal IHC protocol included Ventana "Standard- Cell Conditioning 1" setting for antigen retrieval. Primary antibody was diluted at a concentration of 1:100 in DAKO Cytomation Antibody Diluent (Cat # S0809), manually applied during the antibody titration step in 100 µl volume, and incubated for 60 minutes at RT. Subsequently, incubation with Ventana OmniMap prediluted HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was performed for 4 minutes.

Alcian blue staining

FFPE slides were cut at 3.5µm thickness, attached to charged slides (Thermo-Scientific Colormark Plus Cat # CM-5951PLUS BLUE), baked at 60°C for 1 hour. Slides were then placed in a rack and deparaffinized with 3 changes of xylene 5 minutes each, 2 changes of 100% alcohol 1 minute each and 2 changes of 95% alcohol 1 minute each. Then slides were rinsed thoroughly in distilled water to let stand until staining procedure is ready to be performed. Alcian Blue pH2.5 staining kit (Cat# k066) was obtained from Poly Scientific R&D Corp (Bayshore, NY). Alcian Blue pH 1.0 (Cat# s2412) was obtained and used in place of Alcian Blue pH2.5. Slides were transferred from distilled water and placed in 3% aqueous acidic acid for 3 minutes RT. Slides were then directly placed in Alcian Blue pH1.0 for 30 minutes. Then slides are washed in running tap water for 10 minutes, rinsed in distilled water, and placed in 0.1% Nuclear Fast Red Kernechtrot for 5 minutes. Slides were removed and then washed in running water for 1 minute. After wash slides are dehydrated in 95% alcohol, 100% alcohol, and cleared in xylene, 2 changes each. Slides were then mounted in xylene based mounting media.

Image analysis

Images were captured using the Aperio Scanscope AT Turbo scanner (Leica Microsystems Inc, Buffalo Grove, IL). Whole tumor sections were analyzed using Visiopharm Integrator System (V.4.5.6.580; Visiopharm, Hørsholm, Denmark). Necrotic regions were manually excluded from Alcian blue staining image analysis using the drawing tools provided by the analysis software. Necrotic regions and stroma

were excluded from JHOM-2B xenograft pHH3 image analysis by applying tumor masks generated from CK20-stained adjacent tissue sections using the Tissue Align module. Tumor tissues were segmented using the VisiomorphDP module. Necrotic regions and stroma were manually excluded from HCOX1329 xenograft Ki67 image analysis, and the tumor tissues were segmented using TissueMorphDP module. Staining was quantified as percent blue pixels for Alcian blue, percent positive pixels for pHH3, or percent positive nuclei for Ki67. Unpaired *t* tests were used to determine statistical significance. Symbols used: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *ns*, not significant.

In vivo efficacy studies

JHOM-2B human lower GI tumor cells were obtained from RIKEN (Japan) and a working stock was frozen at passage 14. The line was shown to be free of Mycoplasma sp. and murine viruses in the IMPACT VIII PCR assay panel (RADIL, University of Missouri, Columbia, MO). These cells were maintained in DMEM:HAM's F12 1:1 (ATCC #30-2006) plus 10% FBS (Omega Scientific Inc. #FB-09) at 37°C in a humidified atmosphere containing 5% carbon dioxide.

JHOM-2B cells were harvested at 95-100% confluency, washed, and resuspended in a 1:1 mixture of cold DPBS and Matrigel™ (Becton-Dickinson #354234) at a concentration of 1×10^8 cells/1.5 ml. Finally, 1×10^7 cells in a total volume of 150 μ L were implanted subcutaneously into the right axillary region of female nu/nu mice.

HCOX1329 is a human primary xenograft model derived from a poorly differentiated colonic adenocarcinoma, which was implanted and passaged. HCOX1329 contains a *BRAF*^{V600E} mutation and several mutations/variants in *RNF43* (*P192fs*, *G659fs*, and *C272R*) (Korn, J., *et al.*, *Manuscript in preparation*).

Female nu/nu mice were implanted subcutaneously with 3x3x3mm tumor fragment containing 50% phenol red-free matrigel (BD Biosciences) in DMEM. For tumor implantation, mice were anesthetized with continuous flow of 1.5-3.5% isoflurane/oxygen mixture using the integrated multi chambers anesthesia center (IMCAC). Use a 12G trocar to take one fragment with 0.1 ml of the diluted matrigel and implant subcutaneously into the right axillary region of nude mice.

Mice with average tumor volume of 200 mm³ were randomized into 8 experimental groups on day 21 (JHOM-2B) or day 17 (HCOX1329) post implantation. Tumors were measured with digital calipers twice a week during active dosing period. Tumor volumes were calculated using the ellipsoid formula: (length x width²)/2. Body weights were recorded twice a week at the time of tumor measurement. After 11 days (Groups 2 and 6) or 14 days of treatment, the dosing of mice harboring JHOM-2B xenografts was terminated, and animals euthanized. After 10 days of treatment, the dosing of mice harboring HCOX1329 xenografts was terminated, and animals euthanized. Plasma and tumors were collected at 7hr post last dose for pharmacokinetic (PK) and pharmacodynamic (PD) analysis.

All data were expressed as mean $\pm$ standard error of the mean (SEM). Delta tumor volume and body weight were used for statistical analysis. Between group comparisons were carried out using the Kruskal-Wallis ANOVA followed by a post hoc Dunn's test. For all statistical evaluations, the level of significance was set at $p < 0.05$. Significance compared to the vehicle control group is reported unless otherwise stated. The standard protocols used in pharmacology studies are not pre-powered to demonstrate statistically significant superiority of a combination over the respective single agent treatment. The statistical power is often limited by potent single agent response and/or model variability.

Results

Two pre-clinical CRC models with the appropriate genetic background (JHOM-2B and HCOX1329) were identified for testing the porcupine inhibitor COMPOUND A as a single agent and in combination with the mutant BRAF inhibitor COMPOUND B and the EGFR inhibitor cetuximab.

JHOM-2B is a human cancer cell line that is reported to be derived from a mucinous ovarian carcinoma (Riken Bioresource Center). However, it can be extremely challenging to clinically differentiate a mucinous tumor of the ovary from a colorectal or appendiceal tumor, because all of these tumor types disseminate widely throughout the abdomen. Recently, it has become clear that the expression pattern of CK7 and CK20 is very useful in determining the site of origin of such tumors (Vang, R., *USCAP presentation*. 2013). JHOM-2B xenografts have rare cells that stain positively for CK7, whereas CK20 is diffusely positive (Fig. 2A). This expression pattern is consistent with lower GI, not ovarian, origin. Furthermore, JHOM-2B contains a *BRAF*^{V600E} mutation (Barretina, J., *et al.*, *Nature*. 2012, 492:603-7), which has not been reported in appendiceal cancer (Shen, J.P., *et al.*, *J. Clin. Oncol.* 2012, suppl 34; abstr 397), strongly suggesting the JHOM-2B is of colorectal origin. A survey for genetic alterations in upstream Wnt pathway regulators revealed that JHOM-2B also harbors a fusion between exon1 of *PTPRK* and exon2 of *RSPO3* (Fig. 2B).

HCOX1329 is a human primary xenograft model derived from a poorly differentiated colonic adenocarcinoma. HCOX1329 contains a *BRAF*^{V600E} mutation and several mutations/variants in *RNF43* (*P192fs*, *G659fs*, and *C272R*) (Korn, J., *et al.*, *Manuscript in preparation*).

JHOM-2B tumor cells are sensitive to treatment with COMPOUND A *in vitro*, as assessed by both a foci formation assay (Fig. 3A) and a 7-day CTG assay (Fig. 3B), with an EC₅₀ of approximately 0.06 μ M. Treatment of JHOM-2B tumor cells with COMPOUND A results in a robust decrease in beta-catenin-target genes (*AXIN2*, *LGR5*, *c-MYC* and *CCND1*), indicating that JHOM-2B cells exhibit autocrine Wnt signaling (Fig. 4). *In vitro* by 7-day CTG assay, the addition of COMPOUND A to COMPOUND B plus cetuximab leads to antagonism of the growth inhibitory effects of COMPOUND B+cet (Fig. 3C). This is likely because the cell cycle arrest induced by COMPOUND A protects JHOM-2B cells from apoptosis (Fig. 5, cleaved CASP8, an apoptotic marker, decreases upon COMPOUND A treatment). Immunoblotting of JHOM-2B tumor cells confirms that i) COMPOUND A inhibits phosphorylation of the Wnt co-receptor LRP6 and induces cell cycle arrest (increased c-MYC and decreased p21); and ii) inhibition of both mutant BRAF and EGFR is required to inhibit the MAPK pathway (decreased pERK) (Fig.5).

In vivo treatment of JHOM-2B xenografts with 5 mg/kg COMPOUND A BID resulted in modest but significant tumor growth inhibition (T/C 48%) (Fig. 6). Treatment with the triple combination of COMPOUND A+COMPOUND B+cet caused regression (T/T0 -26%), which although statistically not superior to COMPOUND B+cet, showed a trend toward greater anti-tumor activity (Fig. 6). Treatment with COMPOUND A as a single agent or in combination decreased cell proliferation (Fig. 7) and induced mucinous differentiation of JHOM-2B tumor cells (Fig. 8). The accumulation of mucin in xenografts treated with COMPOUND A (as a single agent or in combination) may affect tumor volume measurements and lead to an underestimate of anti-tumor activity.

Treatment of HCOX1329 xenografts with 5 mg/kg COMPOUND A BID did not statistically inhibit tumor growth *in vivo* (Fig. 9). Strong trends toward combination activity were observed, resulting in tumor stasis in the COMPOUND A+COMPOUND B treatment group (T/T0 -12%) and tumor regression in the COMPOUND A+COMPOUND B+cet treatment group (T/T0 -36%) (Fig. 9). Anti-tumor combination activity correlated well with decreased tumor cell proliferation (Fig. 10). Accumulation of intracellular mucin, consistent with differentiation, was seen in the COMPOUND A+COMPOUND B and COMPOUND A+COMPOUND B+cet treatment groups (Fig.11).

Review of the genetic profile of HCOX1329 revealed that it is also microsatellite unstable (IHC of the patient's tumor sample at the time of diagnosis was reported as MLH1, negative; PMS2, negative; MSH2, positive; MSH6, positive) and harbors two mutations/variants in *ZNRF3* (*H537R* and *E383fs*).

Example 3: Phase 1, open-label, dose escalation and dose expansion study, to evaluate the safety, pharmacokinetics, and activity of the porcupine inhibitor COMPOUND A in patients with *BRAF*^{V600E}-mutant CRC harboring upstream Wnt pathway mutations

Inclusion criteria:

1. Male or female patients ≥ 18 years of age
2. WHO performance status (PS) of 0-2
3. Histologically confirmed diagnosis of colorectal adenocarcinoma that has progressed despite standard therapy or for which no effective standard therapy exists
4. Molecular confirmation that the tumor contains *BRAF*^{V600E} mutation
 - Dose escalation cohorts: No additional molecular pre-selection
 - Dose expansion cohort: Additional molecular pre-selection for *RNF43* mutations or *RSPO* fusions

Example 4: Phase 1b, open label, multicenter, dose escalation and expansion study, to evaluate the safety, pharmacokinetics, and activity of the porcupine inhibitor COMPOUND A in combination with the *BRAF* inhibitor COMPOUND B and the EGFR inhibitor cetuximab in patients with *BRAF*^{V600E}-mutant CRC harboring upstream Wnt pathway mutations

Inclusion criteria:

1. Male or female patients ≥ 18 years of age
2. WHO performance status (PS) of 0-2
3. Histologically confirmed diagnosis of colorectal adenocarcinoma that has progressed despite standard therapy or for which no effective standard therapy exists
4. Molecular confirmation that the tumor contains $BRAF^{V600E}$ mutation
 - Dose escalation cohorts: No additional molecular pre-selection
 - Dose expansion cohort: Additional molecular pre-selection for $RNF43$ mutations or $RSPO$ fusions
5. Failed prior therapy for unresectable or metastatic $BRAF^{V600E}$ -mutant CRC
 - Dose escalation cohorts: Prior treatment with chemotherapy permitted; prior treatment with RAF inhibitors and EGFR monoclonal antibodies permitted.
 - Dose expansion cohort: Prior treatment with chemotherapy permitted; prior treatment with RAF inhibitors and EGFR monoclonal antibodies not allowed.

Example 5: Efficacy of a Wnt pathway inhibitor and combinations comprising (i) a Wnt pathway inhibitor, (ii) a BRAF inhibitor, and/or (iii) an EGFR inhibitor in additional patient-derived, microsatellite-unstable, $BRAF^{V600E}$, $RNF43$ -mutant CRC xenograft models

In vivo studies in two additional patient-derived, microsatellite-unstable, $BRAF^{V600E}$ CRC xenograft models [HCOX1167 ($RNF43^{G659fs}$; $ZNRF3^{R146H, R246H, R393fs}$) and HCOX2239 ($RNF43^{A169T, G659fs}$, $ZNRF3^{L780M}$)] were performed. In both models statistically significant anti-tumor activity versus vehicle was observed upon treatment with COMPOUND A, COMPOUND A+COMPOUND B, AND COMPOUND A+COMPOUND B+cetuximab. An *in vivo* study in a third additional patient-derived, microsatellite-unstable, $BRAF^{V600E}$ CRC xenograft model [HCOX2761 ($RNF43^{G659fs}$)] showed no statistically significant decrease in tumor volume with any of the treatments.

Example 6: Decreased cell proliferation and mucinous differentiation induced by a Wnt pathway inhibitor and a combination comprising (i) a Wnt pathway inhibitor and (ii) an EGFR inhibitor in a patient-derived microsatellite-unstable, $RNF43$ and $ZNRF3$ -mutant CRC xenograft model that is wild type for $BRAF$ and $KRAS$

In vivo efficacy study

T70 is a human primary xenograft model derived from a poorly differentiated colonic adenocarcinoma, which was implanted and passaged in nu/nu mice. T70 is microsatellite unstable, $BRAF^{WT}$, $KRAS^{WT}$, and harbors mutations/variants in $RNF43$ ($W200L$, $E278delE$) and $ZNRF3$ ($Q226^*$, $C230fs$) (as determined by Haloplex custom cancer panel).

Female nu/nu mice were implanted subcutaneously with 100,000 tumor cells containing 50% phenol red-free matrigel (BD Biosciences) in DMEM. For tumor implantation, mice were anesthetized with continuous flow of 1.5-3.5% isoflurane/oxygen mixture using the integrated multi chambers anesthesia center (IMCAC). A 12G trocar was used to take tumor cells with 0.1 ml of the diluted matrigel and implant them subcutaneously into the right axillary region of nude mice, and this was repeated in the left axillary region.

Mice with average tumor volume of 200 mm³ were randomized into 4 experimental groups on day 21 post implantation. Tumors were measured with digital calipers twice a week during active dosing period. Tumor volumes were calculated using the ellipsoid formula: (length x width²)/2. Body weights were recorded twice a week at the time of tumor measurement. After 14 days the dosing of mice harboring T70 xenografts was terminated, and animals euthanized. Plasma and tumors were collected at 7hr post last dose for pharmacokinetic (PK) and pharmacodynamic (PD) analysis.

All data were expressed as mean $\pm$ standard error of the mean (SEM). Delta tumor volume and body weight were used for statistical analysis. Between group comparisons were carried out using the Kruskal-Wallis ANOVA followed by a post hoc Dunn's test. For all statistical evaluations, the level of significance was set at $p < 0.05$. Significance compared to the vehicle control group is reported unless otherwise stated. The standard protocols used in pharmacology studies are not pre-powered to demonstrate statistically significant superiority of a combination over the respective single agent treatment. The statistical power is often limited by potent single agent response and/or model variability.

Immunohistochemistry and Alcian blue staining

IHC and Alcian blue staining was performed as described above.

Image analysis

Image analysis was performed as described above.

Results

Treatment of T70 xenografts with 5 mg/kg COMPOUND A BID, 20 mg/kg cetuximab, i.p., 2x/wk, or COMPOUND A+cetuximab did not statistically decrease tumor volume *in vivo* (Fig. 12). The lack of a statistically significant decrease in tumor volume was due to extensive variability in the growth of this patient-derived xenograft model. However, a marked decrease in cell proliferation was observed upon treatment with COMPOUND A or COMPOUND A+cetuximab (Fig. 13). The decrease in cell proliferation was accompanied by mucinous differentiation (Fig. 14). These findings show that COMPOUND A or COMPOUND A+cetuximab can have anti-tumor activity in CRCs with this genetic background.

Example 7: Phase 1, open-label, dose escalation and dose expansion study, to evaluate the safety, pharmacokinetics, and activity of the porcupine inhibitor COMPOUND A in patients with microsatellite-unstable CRC harboring upstream Wnt pathway mutations

Inclusion criteria:

1. Male or female patients ≥ 18 years of age
2. WHO performance status (PS) of 0-2
3. Histologically confirmed diagnosis of colorectal adenocarcinoma that has progressed despite standard therapy or for which no effective standard therapy exists
4. Molecular and/or immunohistochemical confirmation that the tumor is microsatellite unstable
 - Dose escalation cohorts: No additional molecular pre-selection
 - Dose expansion cohort: Additional molecular pre-selection for *RNF43* mutations, *RSPO* fusions, or other upstream Wnt pathway mutations

Example 8: Phase 1b, open label, multicenter, dose escalation and expansion study, to evaluate the safety, pharmacokinetics, and activity of the porcupine inhibitor COMPOUND A in combination with the EGFR inhibitor cetuximab in patients with microsatellite-unstable, *BRAF*^{WT}, *KRAS*^{WT} CRC harboring upstream Wnt pathway mutations

Inclusion criteria:

1. Male or female patients ≥ 18 years of age
2. WHO performance status (PS) of 0-2
3. Histologically confirmed diagnosis of colorectal adenocarcinoma that has progressed despite standard therapy or for which no effective standard therapy exists
 - Dose escalation cohorts: Prior treatment with chemotherapy permitted; prior treatment with EGFR monoclonal antibodies permitted
 - Dose expansion cohort: Prior treatment with chemotherapy permitted; prior treatment with EGFR monoclonal antibodies not allowed
4. Molecular and/or immunohistochemical confirmation that the tumor is microsatellite unstable; and molecular confirmation that the tumor is wild type for *BRAF* and *KRAS*
 - Dose escalation cohorts: No additional molecular pre-selection
 - Dose expansion cohort: Additional molecular pre-selection for *RNF43* mutations, *RSPO* fusions, or other upstream Wnt pathway mutations

What is claimed is:

1. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer.
2. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to claim 1, wherein the cancer is microsatellite-unstable.
3. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to claim 1 or 2, wherein the cancer is *KRAS* wild-type.
4. A Wnt inhibitor for use in the treatment of *BRAF*^{V600E}-mutant colorectal cancer according to any one of claims 1 to 3 in combination with a BRAF inhibitor.
5. A Wnt inhibitor for use in the treatment of microsatellite-unstable, BRAF wild-type and KRAS wild-type colorectal cancer.
6. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 5 in combination with an EGFR inhibitor.
7. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 6, wherein a Wnt pathway in the colorectal cancer is activated compared to a control.
8. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 7, wherein *AXIN2* expression in the colorectal cancer is higher compared to a control.
9. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 8, wherein the colorectal cancer comprises mutated *RNF43*, mutated *RSPO2* or mutated *RSPO3*.
10. A Wnt inhibitor for use in the treatment of colorectal cancer according to claim 9, wherein the colorectal cancer comprises mutated *RNF43*.
11. A Wnt inhibitor for use in the treatment of colorectal cancer according to claim 9, wherein the colorectal cancer comprises *RSPO* fusion.
12. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 8, wherein the colorectal cancer comprises mutated ZNRF3, RSPO1, RSPO4, LGR4-6, Frizzled1-10, LRP5, LRP6, WISP3, Wise, SFRP1-3, WIF-1, DKK1, DKK4, LKB1/STK11, Keap-1 and/or NRF2.
13. A Wnt inhibitor for use in the treatment of microsatellite-unstable colorectal cancer according to any one of claims 2 to 12, wherein at least one mismatch repair system gene is inactivated in the colorectal cancer compared to a control.
14. A Wnt inhibitor for use in the treatment of microsatellite-unstable colorectal cancer according to claim 13, wherein the mismatch repair system gene is MLH1, MSH2, MSH6 or PMS2.
15. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of previous claims, wherein the Wnt inhibitor is the inhibitor of Porcupine or Frizzled.

16. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of previous claims, wherein the Wnt inhibitor is a Porcupine inhibitor.
17. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 4 to 16, wherein the Wnt inhibitor and the inhibitor in the combination are to be administered simultaneously or sequentially.
18. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 4 to 17, wherein the Wnt inhibitor and the inhibitor in the combination are used in the form of a fixed combination.
19. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 18, further comprising at least one pharmaceutically acceptable carrier.
20. A Wnt inhibitor for use in the treatment of colorectal cancer according to any one of claims 1 to 19 in a form of a pharmaceutical composition.
21. A pharmaceutical combination comprising (i) a Wnt inhibitor and (ii) a BRAF inhibitor.
22. The pharmaceutical combination according to claim 21 further comprising an EGFR inhibitor.
23. A pharmaceutical combination comprising a Wnt inhibitor and an EGFR inhibitor.
24. The pharmaceutical combination according to any one of claims 21 to 23, wherein the pharmaceutical combination comprises the inhibitors separately or together.
25. The pharmaceutical combination according to any one of claims 21 to 24 for simultaneous or sequential use of the inhibitors.
26. The pharmaceutical combination according to any one of claims 21 to 25, further comprising at least one pharmaceutically acceptable carrier.
27. The pharmaceutical combination according to any one of claims 21 to 26 in the form of a fixed combination.
28. The pharmaceutical combination according to any one of claims 21, 22, or 24 to 27, in the form or a kit of parts for the combined administration, wherein the Wnt inhibitor and the BRAF inhibitor are administered independently at the same time or separately within time intervals, and the time intervals allow the Wnt inhibitor and the BRAF inhibitor to be jointly active.
29. The pharmaceutical combination according to any one of claims 21 to 27 in the form or a kit of parts for the combined administration, wherein the Wnt inhibitor and the BRAF inhibitor and/or EGFR inhibitor are administered independently at the same time or separately within time intervals, and the time intervals allow the Wnt inhibitor and the BRAF inhibitor and/or EGFR inhibitor to be jointly active.
30. The pharmaceutical combination according to any one of claims 23 to 27 in the form or a kit of parts for the combined administration, wherein the Wnt inhibitor and the EGFR inhibitor are administered

independently at the same time or separately within time intervals, and the time intervals allow the Wnt inhibitor and the EGFR inhibitor to be jointly active.

31. The pharmaceutical combination according to any one of claims 21 to 30, wherein the inhibitors are in a quantity which is jointly therapeutically effective for the treatment of cancer.

32. The pharmaceutical combination according to any one of claims 21 to 30 for use in the treatment of cancer.

33. The pharmaceutical combination according to claims 31 or 32, wherein the cancer is colorectal cancer.

34. The pharmaceutical combination according to any one of claims 31 to 33, wherein the cancer is microsatellite-unstable.

35. The pharmaceutical combination according to any one of claims 33 or 34, wherein the cancer is *KRAS* wild-type.

36. The pharmaceutical combination according to any one of claims 31 or 35, wherein the cancer is *BRAF*^{V600E}-mutant colorectal cancer.

37. The pharmaceutical combination according to any one of claims 33 or 35, wherein the cancer is *BRAF* wild-type.

38. The pharmaceutical combination according to any one of claims 31 to 35 or 37, wherein the cancer is microsatellite-unstable, *BRAF* wild-type and *KRAS* wild-type colorectal cancer.

39. The pharmaceutical combination according to any one of claims 33 to 38, wherein a Wnt pathway in the colorectal cancer is activated compared to a control.

40. The pharmaceutical combination according to any one of claims 33 to 39, wherein *AXIN2* expression in the colorectal cancer is higher compared to a control.

41. The pharmaceutical combination according to any one of claims 33 to 40, wherein the colorectal cancer comprises mutated *RNF43*, mutated *RSPO2* or mutated *RSPO3*.

42. The pharmaceutical combination according to any one of claims 33 to 41, wherein the colorectal cancer comprises mutated *RNF43*.

43. The pharmaceutical combination according to any one of claims 33 to 41, wherein the colorectal cancer comprises *RSPO* fusion.

44. The pharmaceutical combination according to any one of claims 33 to 43, wherein the colorectal cancer comprises mutated *ZNRF3*, *RSPO1*, *RSPO4*, *LGR4-6*, *Frizzled1-10*, *LRP5*, *LRP6*, *WISP3*, *Wise*, *SFRP1-3*, *WIF-1*, *DKK1*, *DKK4*, *LKB1/STK11*, *Keap-1* and/or *NRF2*.

45. The pharmaceutical combination for use in the treatment of microsatellite-unstable colorectal cancer according to any one of claims 34 to 44, wherein at least one mismatch repair system gene is inactivated in the colorectal cancer compared to a control.

46. The pharmaceutical combination for use in the treatment of microsatellite-unstable colorectal cancer according to claim 45, wherein the mismatch repair system gene is MLH1, MSH2, MSH6 or PMS2.

47. The pharmaceutical combination according to any one of claims 21 to 46, wherein the Wnt inhibitor is an inhibitor of Porcupine or Frizzled.

48. The pharmaceutical combination according to any one of claims 21 to 47, wherein the Wnt inhibitor is the inhibitor of Porcupine.

Figure 1

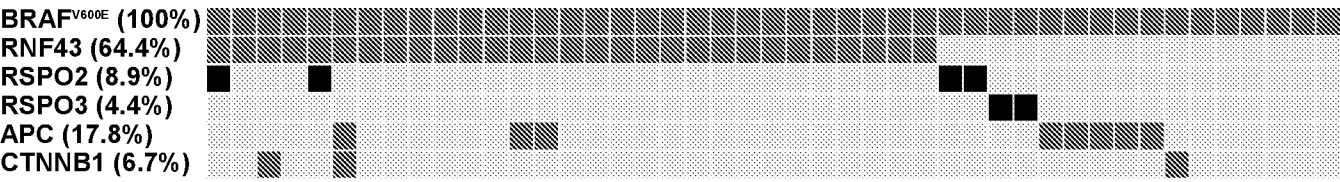


Figure 2

Figure 2A

JHOM-2B

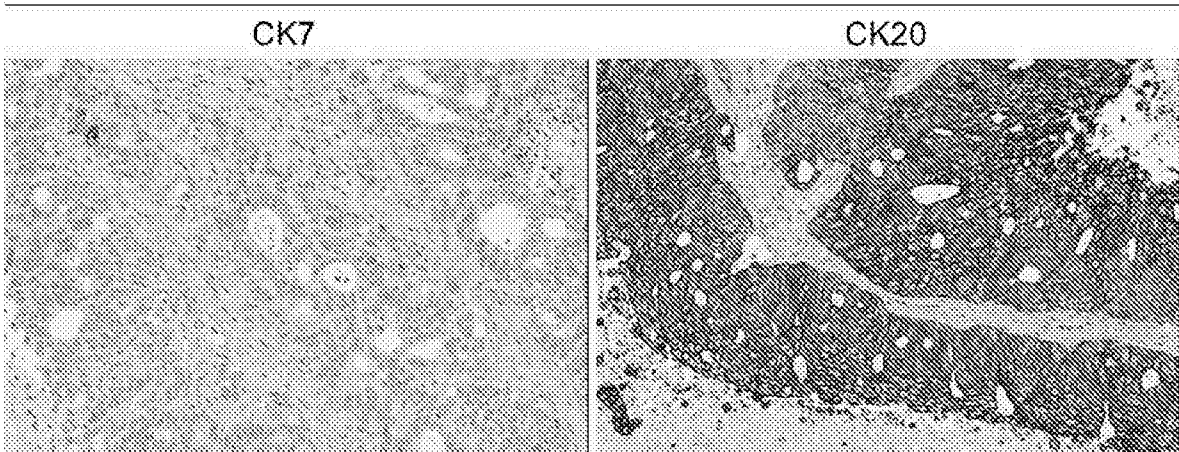


Figure 2B

PTPRK exon1								RSPO3 exon2							
Arg	Pro	Lys	Ala	Ser	Ser	Pro	Gln	Cys	Ile	Leu	Thr	Leu	Val	Lys	Ala
CGGCC	CAAGGCCAGT	TCTCCGCACT	GCATCCTAAC	GTTAGTCAAG	GCT										
GCCGG	GTTCCGGTCA	AGAGGCGTCA	CGTAGGATTG	CAATCAGTTC	CGA										

Figure 3

Figure 3A

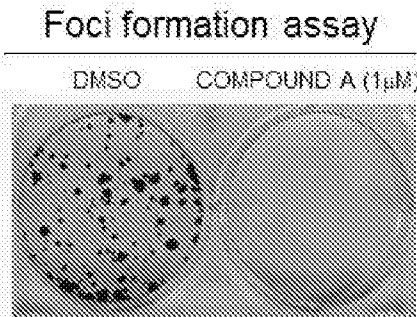


Figure 3B

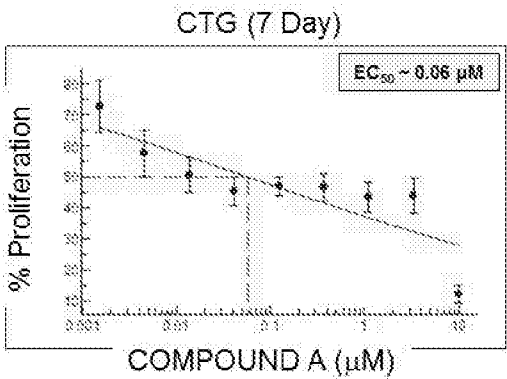


Figure 3C

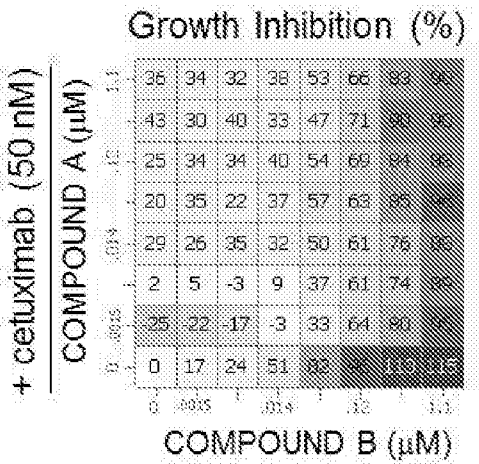


Figure 4

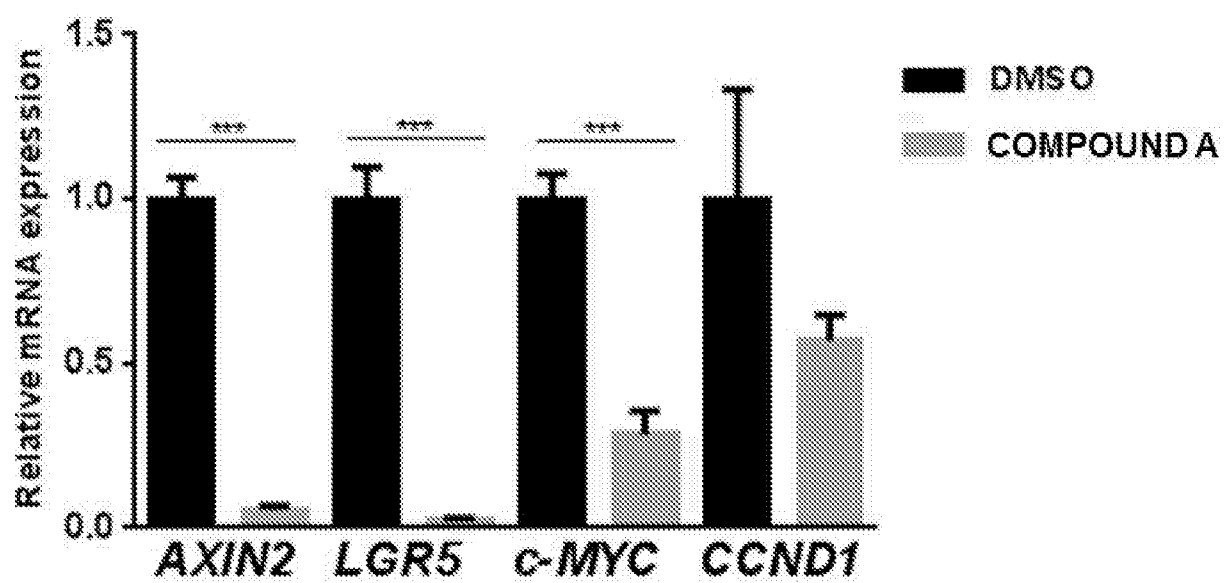


Figure 5

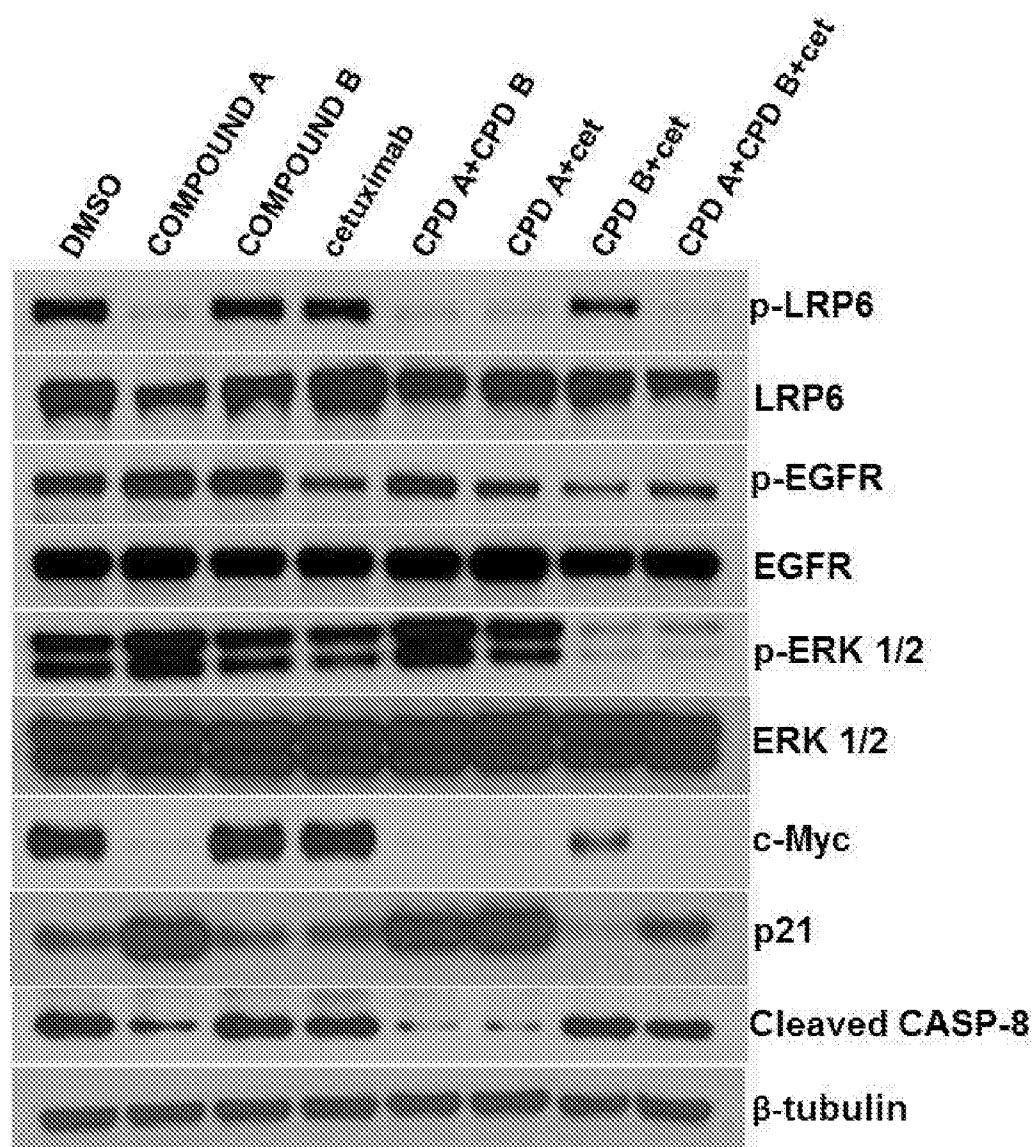


Figure 6

Figure 6A

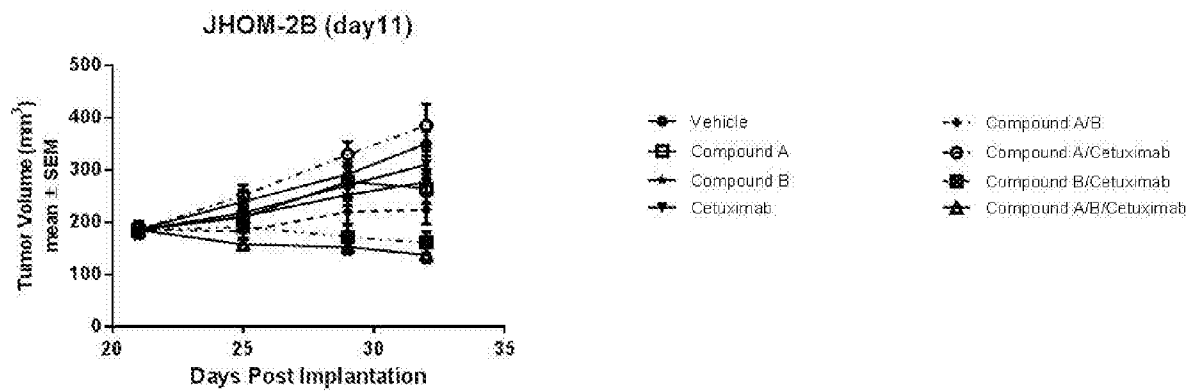


Figure 6B

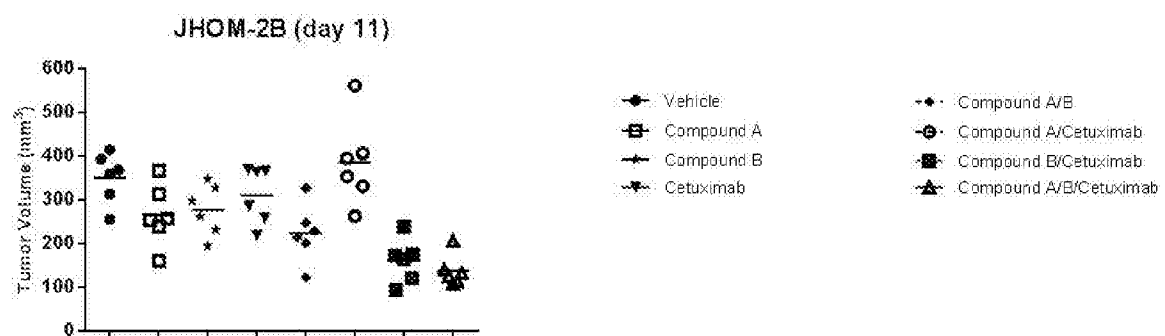


Figure 7

Figure 7A

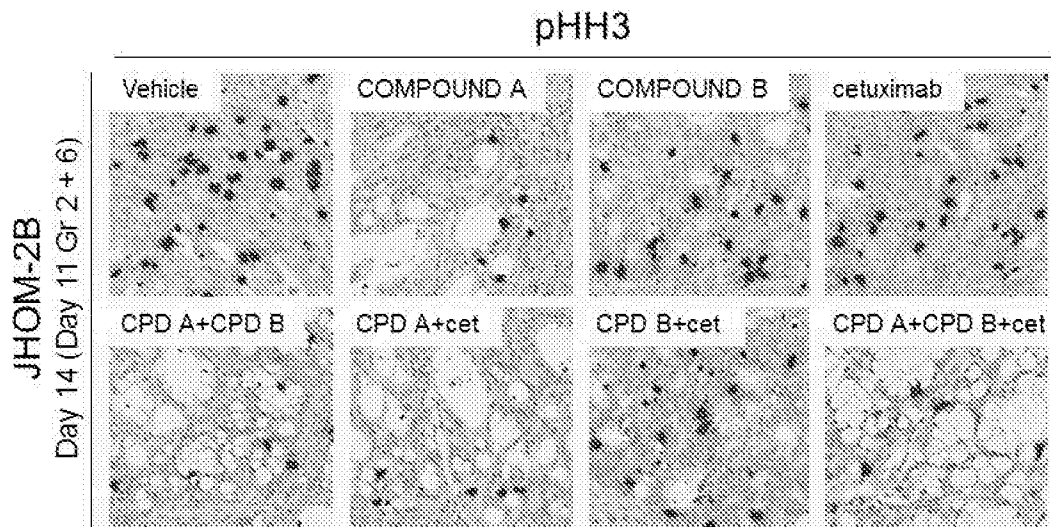


Figure 7B

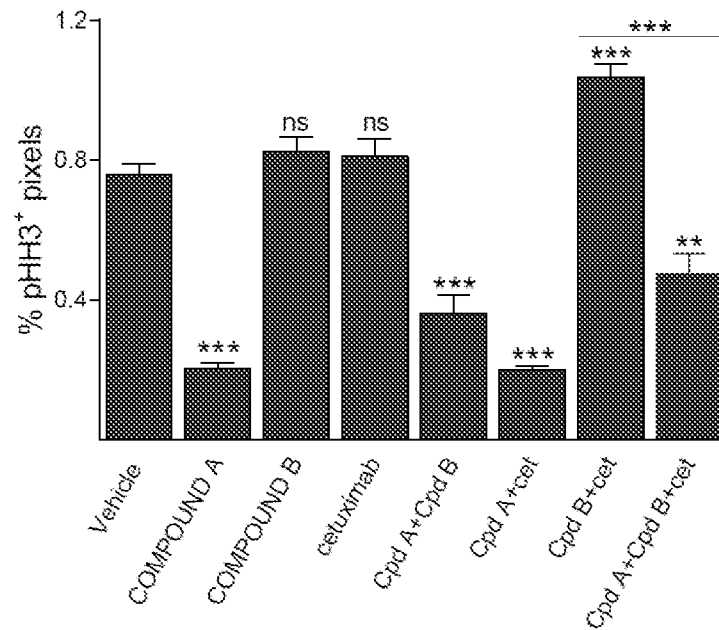


Figure 8

Figure 8A

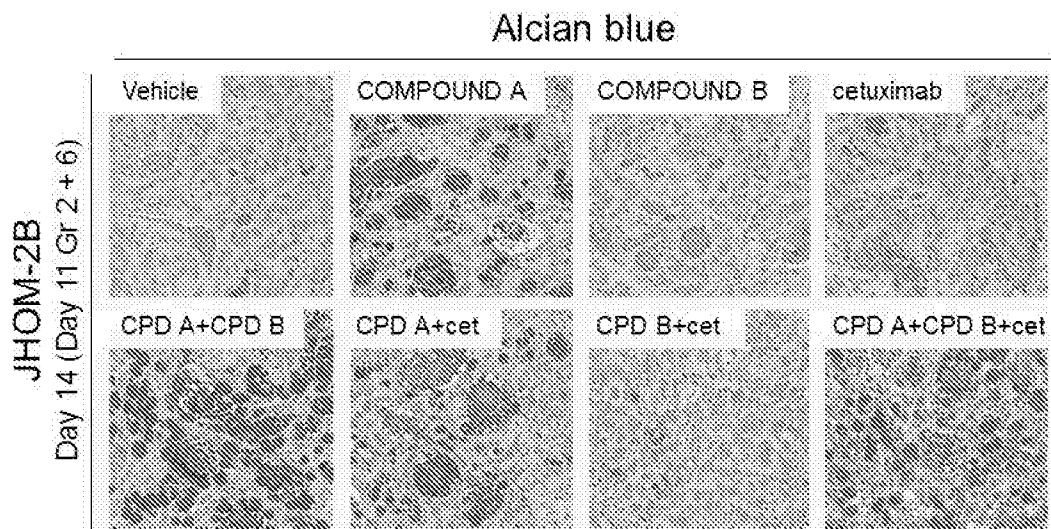


Figure 8B

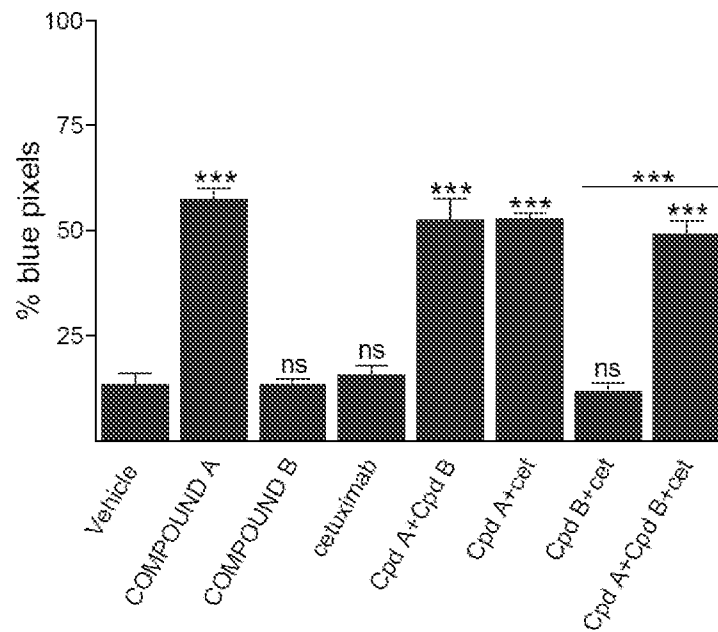


Figure 9

Figure 9A

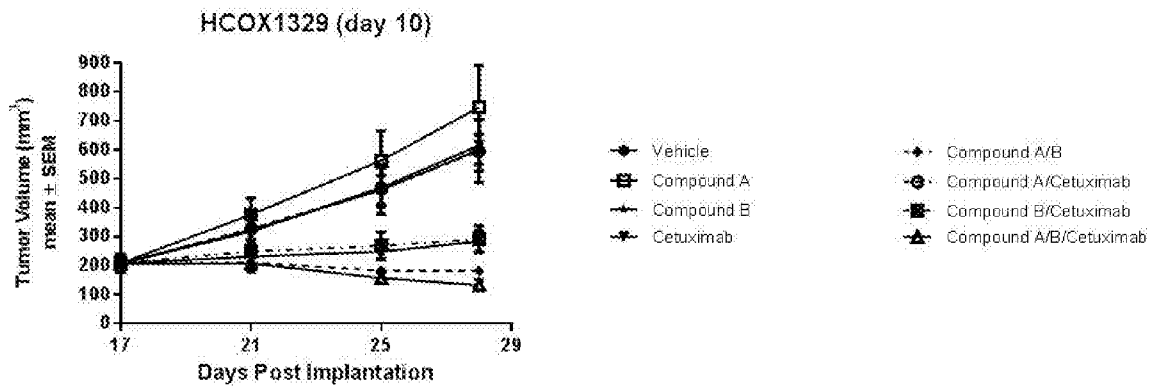


Figure 9B

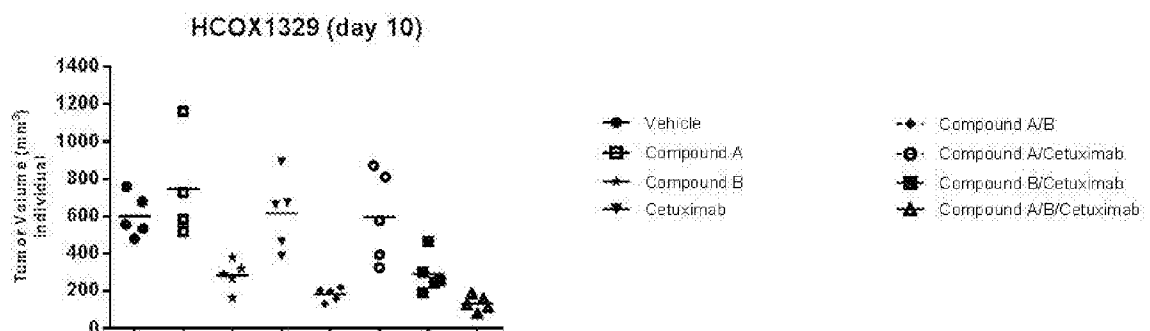


Figure 10

Figure 10A

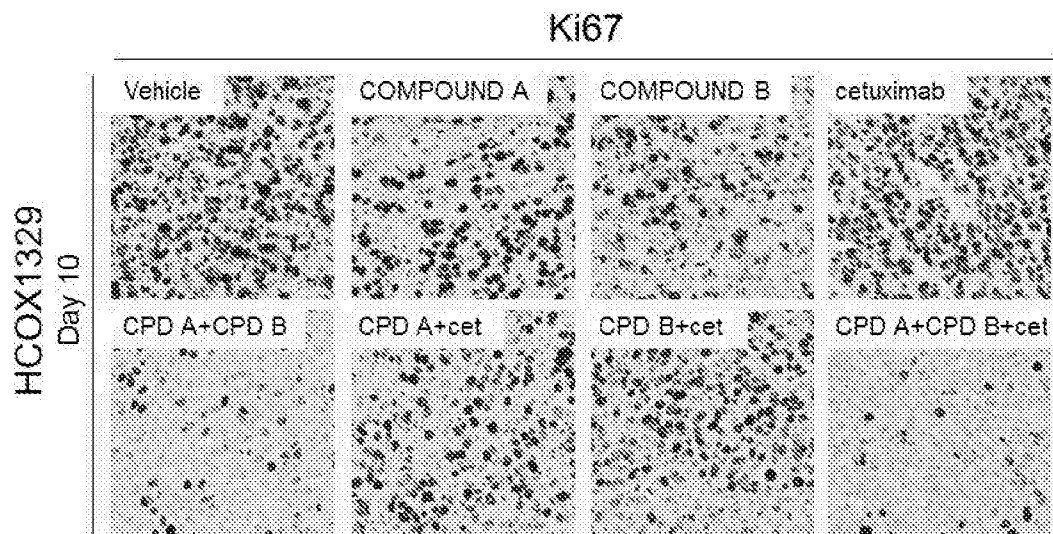


Figure 10B

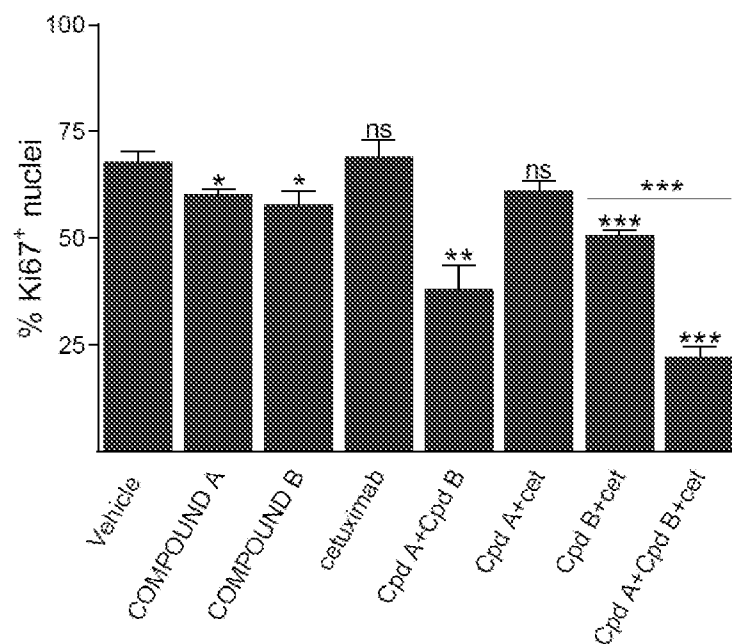


Figure 11

Figure 11A

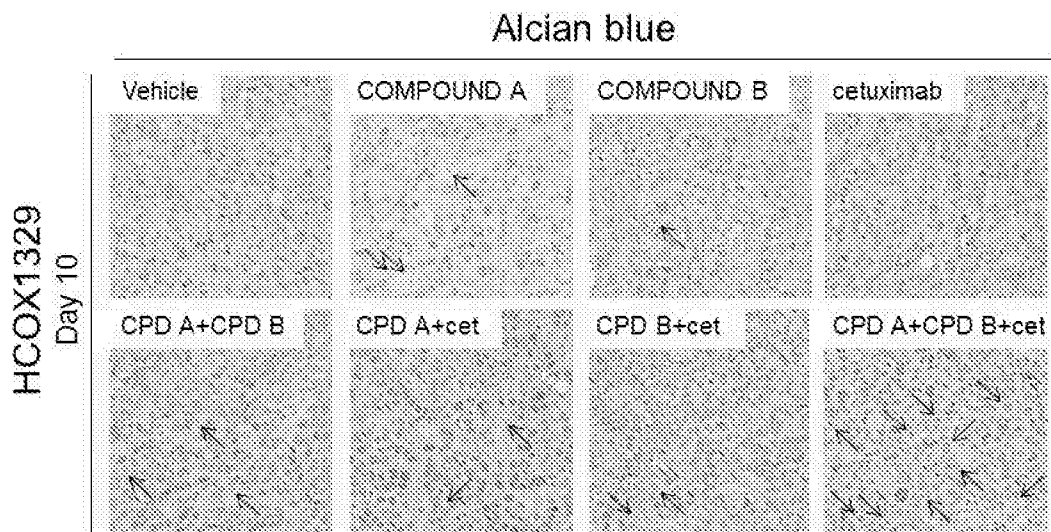


Figure 11B

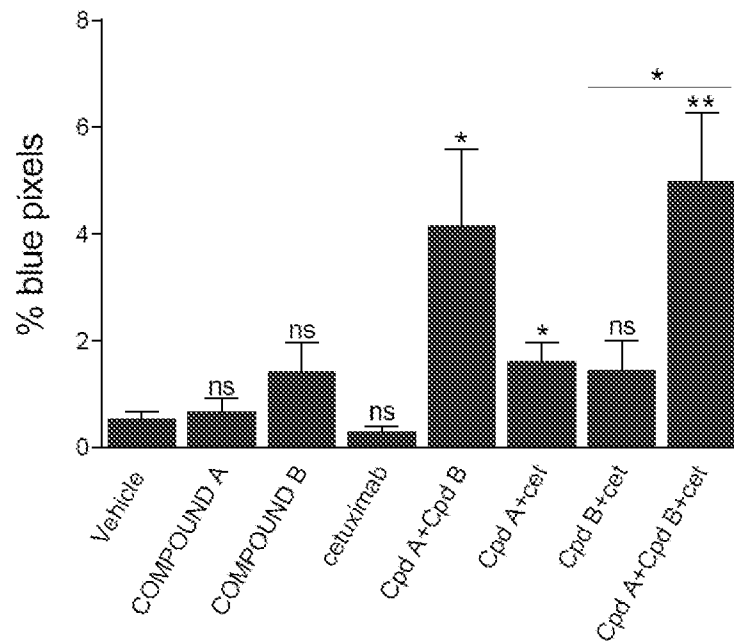


Figure 12

Figure 12A

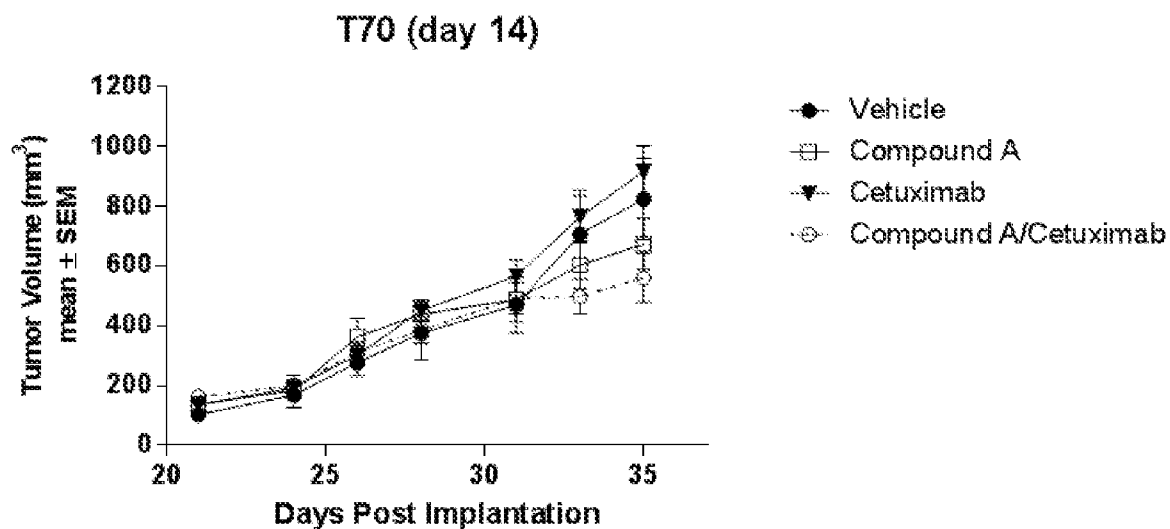


Figure 12 B

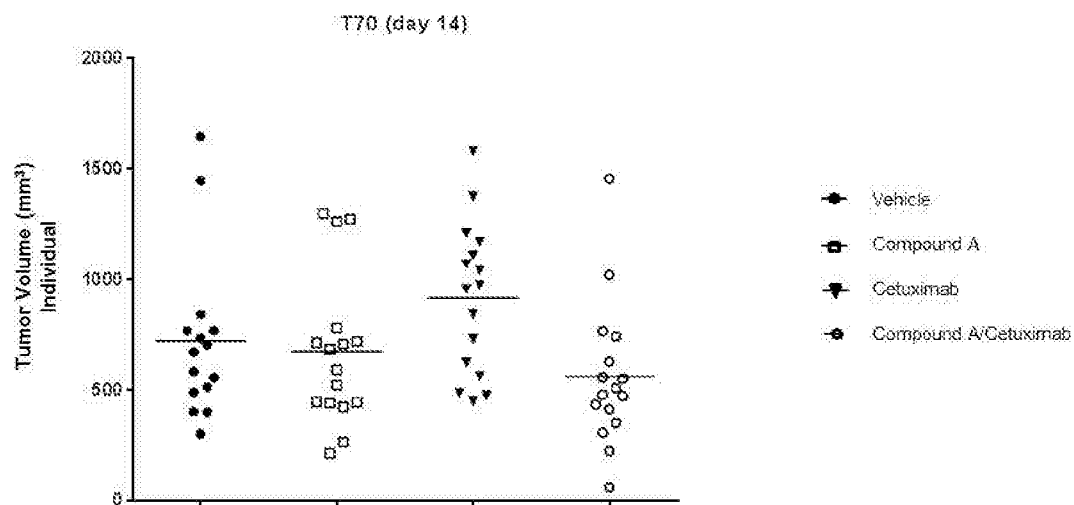


Figure 13

Figure 13A

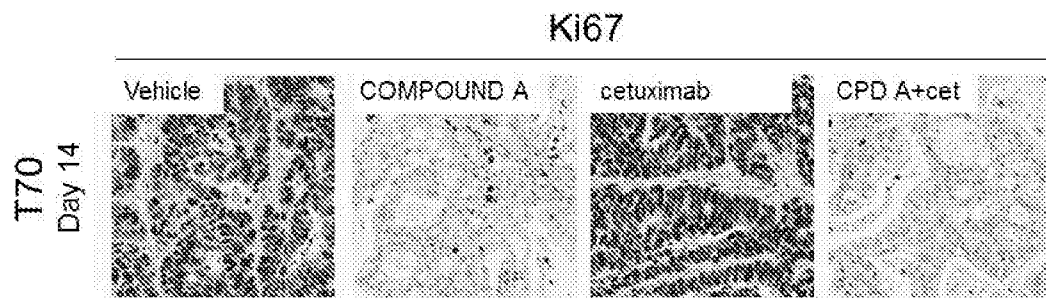


Figure 13B

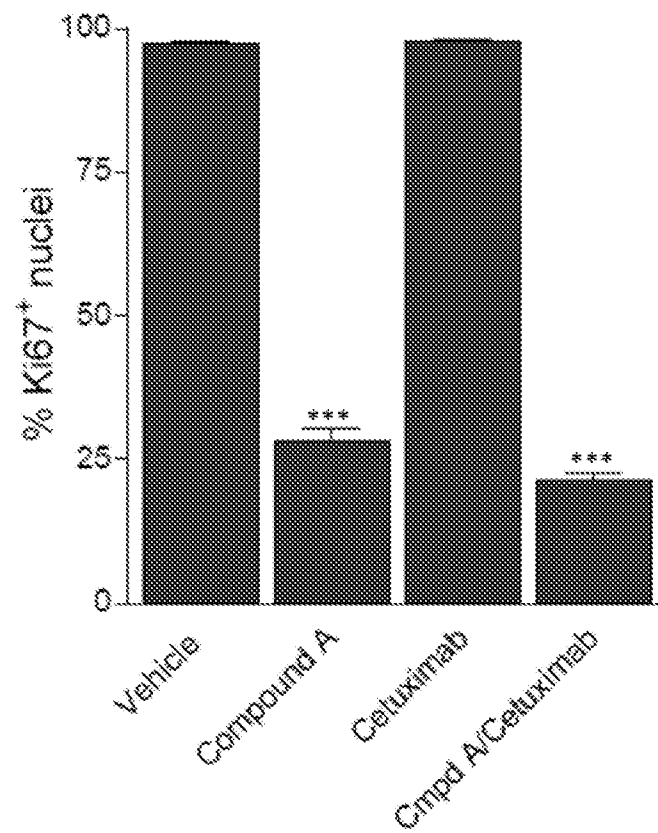


Figure 14

Figure 14A

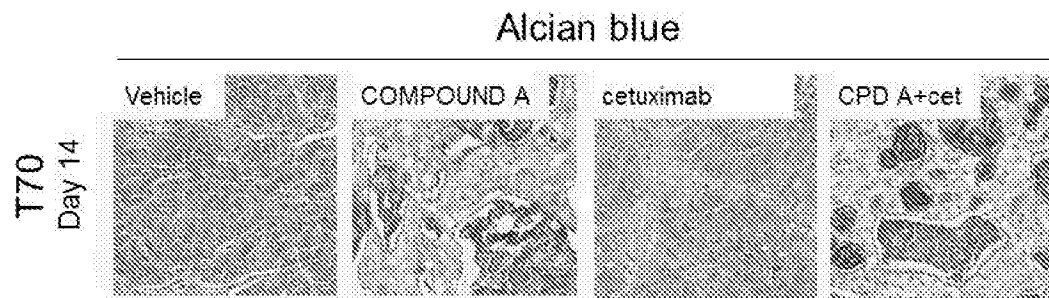


Figure 14B

