

TECHNICAL FIELD

[0001] The present disclosure relates to methods of treating diseases. More particularly, the disclosure relates to methods of treating or preventing colorectal cancer and related diseases and methods related thereto.

BACKGROUND

[0002] Polyposis syndromes are disorders in which patients present with multiple growths or polyps in the gastrointestinal tract. Familial adenomatous polyposis (FAP) is a hereditary syndrome where patients progressively accumulate polyps in their gastrointestinal tract. Over time, the patients accumulate up to thousands of these polyps and eventually some of these growths will become cancerous. Without treatment, 100% of these patients will progress to colorectal cancer. These patients are also at risk for other cancers throughout life even after their bowel has been surgically removed. Attenuated FAP results in fewer polyps but can also lead to colorectal cancer.

[0003] Lynch syndrome, often called hereditary nonpolyposis colorectal cancer (HNPCC), is an inherited disorder that increases the risk of colorectal cancer. Lynch syndrome is not considered a polyposis syndrome.

[0004] There continues to be a need for development of treatments and preventative therapies for colorectal cancer, related diseases, and associated symptoms.

SUMMARY

[0005] Provided herein are methods of treating colorectal cancer in a subject 55 years or older by administering Compound A or a pharmaceutically acceptable salt thereof. Also provided are methods of treating familial adenomatous polyposis (FAP) in a subject 55 years or older by administering a therapeutically effective amount of Compound A or a pharmaceutically acceptable salt thereof. In various cases, Compound A is administered orally once per day at a dose of 4 mg, 8 mg, or 12 mg.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] The embodiments disclosed herein will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings.

[0007] FIG. 1 is a graphical representation of a model of polyp formation with a spheroid assay.

[0008] FIG. 2A is a graphical representation of spheroid growth measured as normalized spheroid area over time post-treatment with various concentrations of Compound A.

[0009] FIG. 2B are images showing spheroids when optimal density was reached (upper panels) and on day five after treatment (lower panels) with Compound A (right panels) or DMSO as a control (left panels).

[0010] FIG. 2C is a graphical representation of viability of spheroids treated with various concentrations of Compound A or other treatments.

[0011] FIG. 2D is a graphical representation of viability of spheroids treated with various concentrations of Compound A or other treatments.

[0012] FIG. 3 is a graphical representation of viability of various colon cells treated with Compound A or DMSO as a control.

[0013] FIG. 4 are graphical representations of viability of various colon cells treated with Compound A or Selumetinib. Colo-205 versus NHCE (upper panel), SW48 versus NHCE (middle panel), and HT-29 versus NHCE (lower panel) are shown.

[0014] FIG. 5A is graphical representation of the effect of Compound A and celecoxib on polyp numbers in a genetic model of familial adenomatous polyposis. Average polyp counts per treatment group are shown.

[0015] FIG. 5B is graphical representation of the effect of Compound A and celecoxib on polyp numbers in a genetic model of familial adenomatous polyposis. The number of polyps per animal is shown.

[0016] FIG. 5C is a graphical representation of the percentage of pre-cancerous polyps in a genetic model of familial adenomatous polyposis following treatment with Compound A or celecoxib.

[0017] FIG. 6 are graphical representations of the activity of Compound A on biomarkers p-ERK (upper left panel), COX2 (right panel), and MCP1 (lower left panel) in histological samples.

[0018] FIG. 7 is a graphical representation of the effect of Compound A treatment on body weight of an animal model.

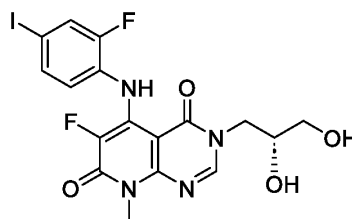
DETAILED DESCRIPTION

[0019] The present disclosure relates to methods of treating cancer, and more specifically colorectal cancer, to a subject that is 55 years or older. In any of the embodiments disclosed herein, the cancer that is treated may be an adenomatous polyposis. In particular, the adenomatous polyposis may be familial adenomatous polyposis (FAP). Alternatively, the adenomatous polyposis may be attenuated familial adenomatous polyposis (AFAP). Additionally, symptoms of FAP or AFAP may be prevented or treated using the methods disclosed herein. In some of the embodiments disclosed herein, the disease treated may be Lynch syndrome and the symptoms thereof may be treated or prevented.

[0020] In any of the embodiments disclosed herein, the methods may include treating or preventing polyps in the gastrointestinal tract or symptoms related to the polyps, including FAP or AFAP polyps. A size, a number, or both, of the polyps may be reduced by the methods disclosed herein.

[0021] The treatment as disclosed herein comprises administering a therapeutically effective amount of Compound A or a pharmaceutically acceptable salt thereof to a subject in need of treatment.

[0022] Compound A has an IUPAC name of 3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-(2-fluoro-4-iodoanilino)-8-methylpyrido[2,3-d]pyrimidine-4,7-dione. The CAS number for Compound A is 1035555-63-5. Compound A is



also known as TAK-733. Compound A has a structure of

[0023] Compound A can be present as a pharmaceutically acceptable salt. Such salts are meant to comprise the therapeutically active non-toxic acid addition salt forms which the compounds disclosed herein are able to form. The salts can conveniently be obtained by treating the base form with such appropriate acids as inorganic acids, for example, hydrohalic acids, e.g. hydrochloric, hydrobromic and the like; sulfuric acid; nitric acid; phosphoric acid and the like; or organic acids, for example, acetic, propanoic, hydroxy-acetic, 2-hydroxypropanoic, 2-oxopropanoic, oxalic, malonic, succinic, maleic, fumaric, malic, tartaric, 2-hydroxy-1,2,3-propanetricarboxylic, methanesulfonic, ethanesulfonic, benzenesulfonic, 4-methylbenzenesulfonic, cyclohexanesulfamic, 2-hydroxybenzoic, 4-amino-2-hydroxybenzoic and the like acids. Conversely the salt form can be converted by treatment with alkali into the free base form. The compounds disclosed herein containing acidic protons may be converted into their therapeutically active non-toxic metal or amine addition salt forms by treatment with appropriate organic and inorganic bases. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. primary, secondary and tertiary aliphatic and aromatic amines such as methylamine, ethylamine, propylamine, isopropylamine, the four butylamine isomers, dimethylamine, diethylamine, diethanolamine, dipropylamine, diisopropylamine, di-n-butylamine, pyrrolidine, piperidine, morpholine, trimethylamine, triethylamine, tripropylamine, quinuclidine, pyridine, quinoline and isoquinoline, the benzathine, N-methyl-D-glucamine, 2-amino-2-(hydroxymethyl)-1,3-propanedi-ol, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like. Conversely the salt form can be converted by treatment with acid into the free acid form. Compound A may be administered as a salt of Compound A such as a hydrochloride or fumarate salt.

[0024] A compound as disclosed herein can be present with enriched active isotopes, such as deuterium or tritium for hydrogen, or ^{13}C or ^{14}C for carbon, or ^{123}I , ^{125}I , or ^{131}I for iodine, or ^{18}F for fluorine.

[0025] As used herein, "administering" encompasses either administering a compound, or a pharmaceutical compositions comprising the compound, directly to isolated cells or to an animal, or administering to cells or an animal another agent to cause the presence or formation of the compound inside the cells or the animal. Accordingly, the "another agent" may administered in a sufficient amount to achieve a therapeutically effective amount of the compound inside the cells or the animal.

[0026] In any of the embodiments disclosed herein, administering a therapeutically effective amount of

Compound A or a pharmaceutically acceptable salt thereof may include administering a composition or formulation consisting essentially of Compound A, or a pharmaceutically acceptable salt thereof.

[0027] In any of the embodiments disclosed herein, the administering may include orally administering. Parenteral routes of administration are also contemplated, such as intracerebroventricular, intravenous, intramuscular, subcutaneous, and transdermal.

[0028] In any of the embodiments disclosed herein, a second therapeutic agent may be administered. The agent may include NSAIDs, COX-2 inhibitors, EGFR inhibitors, and MEK inhibitors. The agent may be, but not limited to, Sulindac, Celecoxib, Erlotinib, Selumetinib, Cobimetinib, Binimetinib, or Trametinib.

[0029] In any of the embodiments disclosed herein, the administering may be long term or short term. In some cases, the administering is for at least 6 weeks. In some cases, the administering is for not more than 18 months.

[0030] In any of the embodiments disclosed herein, the methods may increase the probability that the subject can avoid surgery, delay surgery, or both. Accordingly, methods of delaying or preventing surgery are contemplated herein, the methods comprising administering a therapeutically effective amount of Compound A, or a pharmaceutically acceptable salt thereof to a subject in need of treatment.

[0031] In particular embodiments, the subject treated has a high frequency of deoxyribonucleic acid (DNA) somatic copy number alterations (SCNA), increased Wnt pathway activation, and increased MYC gene activation or is otherwise classified as Consensus Molecular Subtypes (CMS) Consortium subtype 2 (CMS2). For example, adenomatous polyposis coli (APC) gene mutations are significantly enriched in CMS2.

[0032] Another aspect of the disclosure relates to methods of treating or preventing diseases or symptoms associated with mutation in an adenomatous polyposis coli (APC) gene of a subject, the method comprising administering a therapeutically effective amount of Compound A or a pharmaceutically acceptable salt thereof to a subject in need of treatment. For example, the subject may be a mammal and the disease treated or prevented may be colorectal cancer or desmoid tumor.

[0033] Another aspect of the disclosure relates to methods of treating cells, such as the cells of a mammal, with a mutation in an *APC* gene, the method comprising administering an effective amount of Compound A or a pharmaceutically acceptable salt thereof. When the cells are in or from a mammal, the methods may further include identifying the mammal as having a mutation in the *APC* gene. Identifying the mammal as having cells polyps may involve endoscopic evaluation. In any relevant embodiments disclosed herein, the mutation in the *APC* gene may result in loss of function of the *APC* gene, generation of truncated *APC* gene products, or both.

EXAMPLES

[0034] To further illustrate these embodiments, the following examples are provided. These examples are not intended to limit the scope of the claimed invention, which should be determined solely on the basis of the attached claims.

Example 1 — Compound A treatment in cells and spheroids

[0035] Human colon cancer cell line HT-29 (APC^{mut}) was seeded in 384-well round bottom plates at 4,000 cells/well and incubated for five days at 37°C in 5% CO₂. Spheres began forming over the first day and reached optimal density within 18 hours. Growth of the spheres was evident over the next four days and consistent across multiple plates, with respect to total spheroid area (FIG. 1). Wells were imaged every four hours using the IncuCyte® Live Cell Analysis System, and HT-29 spheroid size was calculated using a custom built Matlab script. Data represents the mean spheroid area $\pm$ SEM (N = 10).

[0036] Compound A reduced 3D spheroid growth of HT-29 cells in a dose-dependent manner (FIG. 2A). Compound treatment occurred on day one of five. Wells were imaged every four hours using the IncuCyte® Live Cell Analysis System, and HT-29 spheroid size was calculated using the custom built Matlab script. Data represents the mean spheroid area (a.u.) $\pm$ SEM (N = 4/concentration). Study was repeated four times on separate days. Images show spheroid growth after optimal density had been reached until day five when the assay was completed (FIG. 2B). Compound treatment occurred on day one of five. Representative images of Compound A, at 3.33 μ M showed significant reductions in size and apparent viability of the spheroid, compared to DMSO controls (FIG. 2B). Concentration-response of Compound A (with pathway controls; ERK inhibitor, U0126 and clinical stage MEK inhibitor, selumetinib) reduced spheroid growth over five days (FIG. 2C). Spheroid viability was measured using CellTiter-Glo™ cell viability assay. Data represent normalized mean luminescence $\pm$ SEM, n = 6 per concentration group. Compound treatment occurred on day one of five.

[0037] In a separate experiment, other MAPK pathways were investigated. Compound A was compared against other MEK inhibitors (selumetinib), ERK (ERK5-in-1), and p38 MAPK (Arry-614) (FIG. 2D). Compound treatment occurred on day one of five. Compound A was superior at halting the growth of the spheroid, which was measured by using CellTiter-Glo™ cell viability assay. Data represent normalized mean luminescence $\pm$ SEM; n = 6 per concentration group.

[0038] Compound A efficacy across multiple colorectal cancer (CRC) lines was investigated in a 2-dimensional (2D) monolayer experiment. Normal human colon epithelial (NHCE) and HCT-116 both wild-type for APC was compared against three separate APC mutant CRC lines (FIG. 3). Compound A showed preferential cell killing of APC mutant lines vs wild-type. Viability was measured using CellTiter-Glo™ cell viability assay and data represent the normalized mean luminescence $\pm$ SEM; n = 6 per concentration group. Compound A showed selective potency against APC mutant cells in 2D and 3D assays (Table 1).

Table 1. Potency against APC mutant cells in 2D and 3D assays.

Name	Target	NHCE	HCT-116	SW48 ¹	HT-29	Colo-205	HT-29
EC50 (nM) - 2D							EC50 (nM) - Spheroid

Compound A	MEK1/2 inhibitor	> 2,000 ‡	1,222 ± 323 ‡	1.0 ± 0.5 *	1.1 ± 0.6 *	0.6 ± 0.1 *	3.4 ± 0.7 *
U0126²	<i>MEK1/2 inhibitor</i>	> 10,000 ‡	> 10,000 ‡	211.0 ± 21.2 †	> 10,000 ‡	381.0 ± 93.3 †	> 10,000 ‡
Selumetinib	<i>MEK inhibitor</i>	> 2,000 ‡	38.0 ± 13 †	685.2 ± 10.8 †	130.5 ± 83.8 †	26.3 ± 3.5 †	ETA (9/10)

¹ – Nonsynonymous single nucleotide variant for APC

² – POC compound (MEK/ERK as target)

* - potent; † - active; ‡ - inactive

[0039] Compound A impact on 2D viability was compared to clinical stage MEK inhibitor, selumetinib across the three APC mutant CRC lines vs. NHCE (APC wild-type normal colon epithelium) (FIG. 4). Results showed preferential cell killing of APC mutant vs wild-type. Viability was measured using CellTiter-Glo™ cell viability assay. Data represent the normalized mean luminescence ± SEM and n = 6 per concentration group.

Example 2 — Compound A treatment in mice

[0040] The C57BL/6J-ApcMin/J (APC^{min}) strain is highly susceptible to spontaneous intestinal adenoma formation. Dosing with Compound A (PO administration) and/or vehicle (0.5% methylcellulose, PO) commenced when mice were 10 weeks of mean age and continued for up to 8 weeks. Positive control, celecoxib was added to the drinking water for the entire duration of the study. At termination of the study, intestine and plasma samples were collected from all mice. The complete intestinal tract from the small bowel to large bowel was removed and flushed with cold PBS to remove lumen content. Following flushing, polyps were counted manually in both the small and large intestine. The results showed that a statistically significant inhibitory effect on polyp formation was observed for all treatment groups when compared with the vehicle group. Total polyps were counted from all animals in the study at either the end, or when animals were sacrificed due to clinical signs detailed in the IACUC euthanasia criteria (FIG. 5A), and FIG. 5B shows the total polyps from all animals completing the study. Percent pre-cancerous polyps (adenomas) were counted and normalized to total number of polyps (FIG. 5C). Results show a significant reduction in pre-cancerous polyps in Compound A treatment groups compared to vehicle treated controls. The average number of polyps between groups were compared by one-way ANOVA and the Dunnett's multiple comparison test. Significance was set at a threshold of greater than 0.5. Data represent the mean ± SEM (n = 15 per experimental group).

[0041] The data suggests that patients classified under the Consensus Molecular Subtypes (CMS) Consortium as having CMS2 (a high frequency of deoxyribonucleic acid (DNA) somatic copy number alterations (SCNA), increased Wnt pathway activation, increased MYC gene activation, and enriched in adenomatous polyposis coli (APC) gene mutations) may benefit from treatment with Compound A.

[0042] Immunohistochemical (IHC) staining for pERK, CCL2(MCP1+), and COX2 was performed on the

intestine samples collected from the APC^{min} *in vivo* study (FIG. 6). The IHC score for pERK and CCL2 was presented as the ratio of the pERK positive cells area against the total cells area of whole section; whereas for COX2 the score was measured based on the number of positive cells vs total number of cells. Differences between groups were compared by one-way ANOVA and the Dunnett's multiple comparison test. Significance was set at a threshold of greater than 0.5. Data represent the mean $\pm$ SEM (n = 15 per experimental group). Compound A may work through a mechanism independent of COX-2 inhibition.

[0043] Mice were weighed daily following commencement of dosing with compounds (FIG. 7). Mean body weights $\pm$ SEM were captured. Results showed no significant decreases in body weights among the doses of Compound A administered and the vehicle control or positive control, celecoxib.

[0044] It will be apparent to those having skill in the art that many changes may be made to the details of the above-described embodiments without departing from the underlying principles of the invention.

We Claim:

1. A method of treating or preventing a colorectal cancer related disease, or a symptom thereof in a subject who is 55 years or older, the method comprising administering to the subject a therapeutically effective amount of 3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-(2-fluoro-4-iodoanilino)-8-methylpyrido[2,3-d]pyrimidine-4,7-dione ("Compound A") or a pharmaceutically acceptable salt thereof.
2. The method of claim 1, wherein the administering comprises administering a composition consisting essentially of Compound A or salt thereof.
3. The method of claim 1 or claim 2, wherein administering comprises orally administering Compound A.
4. The method of claim 3, wherein Compound A is administered at a dose of 4 mg.
5. The method of claim 3, wherein Compound A is administered at a dose of 8 mg.
6. The method of claim 3, wherein Compound A is administered at a dose of 12 mg.
7. The method of any one of claims 1, 2, and 4 to 6, wherein administering comprises parenterally administering Compound A.
8. The method of any one of claims 1 to 7, wherein Compound A is administered once daily.
9. The method of claim 8, wherein Compound A is orally administered once daily at a daily dose of 4 mg.
10. The method of claim 8, wherein Compound A is orally administered once daily at a daily dose of 8 mg.
11. The method of claim 8, wherein Compound A is orally administered once daily at a daily dose of 12 mg.
12. The method of any one of claims 1 to 11, wherein the subject suffers from familial adenomatous polyposis (FAP), and the method treats FAP or symptoms thereof.
13. The method of any one of claims 1 to 11, wherein the subject suffers from polyps in the gastrointestinal tract, and the method treats the polyps in the gastrointestinal tract or symptoms thereof.
14. The method of any one of claims 1 to 11, wherein the subject suffers from Lynch syndrome, and the method treats Lynch syndrome or symptoms thereof.
15. The method of any one of claims 1 to 14, further comprising administering a second therapeutic agent to the subject.
16. The method of claim 15, wherein the second therapeutic agent is selected from a group consisting of a NSAID, a COX-2 inhibitor, an EGFR inhibitor, and a MEK inhibitor.

17. The method of claim 15, wherein the second therapeutic agent is Sulindac, Celecoxib, Erlotinib, Selumetinib, Cobimetinib, Binimetinib, or Trametinib, or any combination thereof.
18. The method of any one of claims 1 to 17, wherein administering Compound A comprises administering Compound A to the subject for at least 6 weeks.
19. The method of any one of claims 1 to 18, wherein the subject has one or more of a high frequency of deoxyribonucleic acid (DNA) somatic copy number alterations (SCNA), increased Wnt pathway activation, increased MYC gene activation, and enriched adenomatous polyposis coli (APC) gene mutations.
20. A method of treating or preventing a disease associated with mutation in an adenomatous polyposis coli (APC) gene or a symptom thereof, the method comprising administering to a subject at least 55 years old suffering therefrom a therapeutically effective amount of 3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-(2-fluoro-4-iodoanilino)-8-methylpyrido[2,3-d]pyrimidine-4,7-dione ("Compound A") or a pharmaceutically acceptable salt thereof.
21. The method of claim 20, wherein the subject suffers from familial adenomatous polyposis (FAP), and the method treats or prevents FAP or a symptom thereof.
22. The method of claim 20, wherein the subject suffers from attenuated familial adenomatous polyposis (AFAP), and the method treats or prevents AFAP, or a symptom thereof.
23. The method of any one of claims 20 to 22, wherein the subject suffers from colorectal cancer, and the method treats or prevents colorectal cancer or treats a symptom thereof.
24. The method of any one of claims 20 to 23, wherein the subject suffers from desmoid tumors, and the method treats or prevents desmoid tumors or a symptom thereof.
25. The method of any one of claims 20 to 24, wherein the mutation in the *APC* gene results in loss of function of the *APC* gene, generation of truncated *APC* gene products, or both.
26. The method of any one of claims 20 to 25, wherein the subject has one or more of a high frequency of deoxy ribonucleic acid (DNA) somatic copy number alterations (SCNA), increased Wnt pathway activation, and increased MYC gene activation.
27. The method of any one of claims 20 to 26, further comprising administering a second therapeutic agent to the subject.
28. The method of claim 27, wherein the second therapeutic agent is selected from a group consisting of a NSAID, a COX-2 inhibitor, an EGFR inhibitor, and a MEK inhibitor.
29. The method of claim 27, wherein the second therapeutic agent is Sulindac, Celecoxib, Erlotinib, Selumetinib, Cobimetinib, Binimetinib, or Trametinib, or any combination thereof.
30. The method of any one of claims 20 to 29, wherein a size, a number, or both, of the polyps is reduced.

31. The method of any one of claims 20 to 30, wherein administration of Compound A or salt thereof increases the probability that the subject can avoid surgery, delay surgery, or both.
32. The method of any one of claims 1 to 31, wherein the subject is a mammal.
33. The method of claim 32, wherein the subject is human.
34. A method of treating cells with an *APC* gene mutation, the method comprising administering an effective amount of 3-[(2R)-2,3-dihydroxypropyl]-6-fluoro-5-(2-fluoro-4-iodoanilino)-8-methylpyrido[2,3-d]pyrimidine-4,7-dione ("Compound A or a pharmaceutically acceptable salt thereof).
35. The method of claim 34, wherein the cells are in a mammal.
36. The method of claim 35, further comprising identifying the mammal as having a mutation in the *APC* gene.
37. The method of any one of claims 34 to 36, wherein administering an effective amount of Compound A or salt thereof comprises administering a composition or formulation consisting essentially of Compound A or salt thereof.
38. The method of any one of claims 34 to 37, wherein administering comprises orally administering Compound A or salt thereof.
39. The method of any one of claims 34 to 37, wherein administering comprises parenterally administering Compound A or salt thereof.
40. The method of any one of claims 34 to 39, further comprising administering a second therapeutic agent to the cells.
41. The method of claim 40, wherein the second therapeutic agent is selected from a group consisting of a NSAID, a COX-2 inhibitor, an EGFR inhibitor, and a MEK inhibitor.
42. The method of claim 40, wherein the second therapeutic agent is Sulindac, Celecoxib, Erlotinib, Selumetinib, Cobimetinib, Binimetinib, or Trametinib, or any combination thereof.