



(51) International Patent Classification:

A61K 36/484 (2006.01) A61K 36/65 (2006.01)
A61K 36/539 (2006.01)

(21) International Application Number:

PCT/US2021/055681

(22) International Filing Date:

19 October 2021 (19.10.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/093,756	19 October 2020 (19.10.2020)	US
63/094,193	20 October 2020 (20.10.2020)	US
63/196,148	02 June 2021 (02.06.2021)	US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW,

(54) Title: IMPROVED FLUOROURACIL-BASED MULTI-AGENT CHEMOTHERAPY FOR TREATMENT OF METASTATIC COLORECTAL CANCER

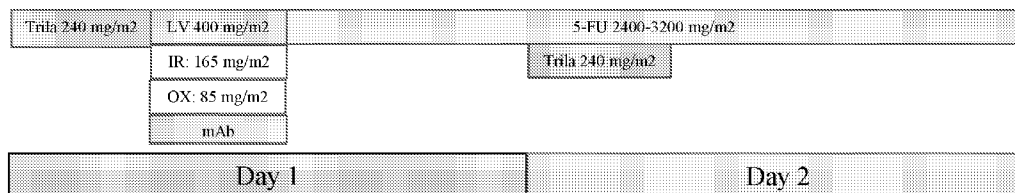
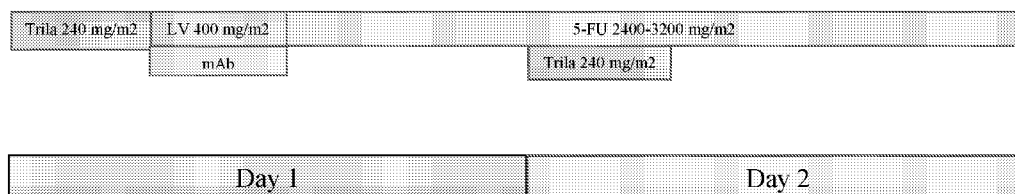
Trilaciclib/FOLFOXIRIInductionMaintenance

Fig.3A

(57) Abstract: This invention is in the area of improved colorectal cancer treatment protocols that provide reduced toxicity compared to currently used regimens, including reduced chemotherapy -induced myelosuppression (CIM), diarrhea, mucositis, and/or stomatitis, while, in some embodiments, also improving anti-cancer efficacy. The improved protocols incorporate a highly selective, transient cyclin dependent kinase (CDK) 4/6 inhibitor into the colorectal cancer treatment protocol, allowing for the expanded use of effective fluorouracil-based multi-agent chemotherapies, for example FOLFOXIRI or FOLFIRINOX, into patient populations previously excluded from these protocols.



SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

- (84) Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*

IMPROVED FLUOROURACIL-BASED MULTI-AGENT CHEMOTHERAPY FOR TREATMENT OF METASTATIC COLORECTAL CANCER

Cross-Reference to Related Applications

5 This application claims the benefit of U.S. Provisional Patent Application No. 63/196,148, filed June 2, 2021, U.S. Provisional Patent Application No. 63/094,193, filed on October 20, 2020, and U.S. Provisional Patent Application No. 63/093,756, filed on October 19, 2020. The entirety of each of these applications is hereby incorporated by reference for all purposes.

10 Field of the Invention

This invention is in the area of improved colorectal cancer treatment protocols that provide reduced toxicity compared to currently used regimens, including reduced chemotherapy-induced myelosuppression (CIM), diarrhea, mucositis, and/or stomatitis, while in some embodiments also improving anti-cancer efficacy. The improved protocols incorporate a highly selective, transient
15 cyclin dependent kinase (CDK) 4/6 inhibitor into the colorectal cancer treatment protocol, allowing for the expanded use of these effective fluorouracil-based multi-agent chemotherapies, for example but not limited to FOLFOXIRI and FOLFIRINOX, into patient populations previously excluded from these protocols.

20 Background of the Invention

1.85 Million people will be diagnosed with colorectal cancer (CRC) each year and approximately 880,000 will die of the disease (WHO International Agency for Cancer Research, 2019). Fluorouracil-based multi-agent chemotherapy remains the cornerstone of treatment for metastatic disease and almost all patients will receive some combination of fluorouracil (5-FU),
25 folinic acid (e.g., leucovorin or levoleucovorin), oxaliplatin, and/or irinotecan, and either a monoclonal antibody (mAb) targeting the VEGF pathway, for example bevacizumab or, for those with KRAS wild type and BRAF wild type disease, or a mAb targeting the EGFR pathway, for example cetuximab or panitumumab.

Until recently, the standard of care focused on the use of either FOLFOX (5-FU, folinic
30 acid (e.g., leucovorin or levoleucovorin), and oxaliplatin) or FOLFIRI (5-FU, folinic acid, and irinotecan) in the first line setting to which a mAb (anti-VEGF or anti-EGFR) would be added.

Upon progression on this therapy, the patient then received the alternative therapy. For example, if a patient started on FOLFOX/mAb, they would be switched to FOLFIRI/mAb upon progression on FOLFOX/mAb, and vice-versa. Recent studies combining all four drugs—termed FOLFOXIRI (5-FU, folinic acid (e.g., leucovorin or, alternatively levoleucovorin), oxaliplatin, and irinotecan; and a further modified protocol termed FOLFIRINOX)—now, indicate that four
5 drugs are better than three with respect to anti-tumor efficacy in metastatic colorectal cancer (mCRC).

For example, in the TRIBE trial, patients treated with FOLFOXIRI/bevacizumab had a median progression free survival (PFS) of 12.1 months compared to those treated with FOLFIRI/bevacizumab who had a median PFS of 9.7 months (hazard ratio [HR]: 0.75); and a
10 median overall survival (OS) of 29.8 months compared to 25.8 months in the FOLFIRI/bevacizumab arm (HR: 0.8) (Loupakis et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med.* 2014; 371(17):1609-18; Cremolini et al. FOLFOXIRI/bevacizumab versus FOLFIRI/bevacizumab as first-line treatment of patients
15 with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol.* 2015; 16: 1306-15). Similarly, in VISNU-1, in a subset of mCRC patients with a poor prognosis being treated in the first line setting, FOLFOXIRI/bevacizumab was statistically significantly better than FOLFOX/bevacizumab at
prolonging median PFS (12.4 months vs 9.3 months) (Sastre et al., Randomized phase III study
20 comparing FOLFOX + bevacizumab versus FOLFOXIRI + bevacizumab (BEV) as 1st line treatment in patients with metastatic colorectal cancer (mCRC) with ≥ 3 baseline circulating tumor cells (bCTCs). *J Clin Oncol.* 2019;37: suppl abstract 3507).

While the FOLFOXIRI protocol has shown significantly better progression free survival (PFS) and overall survival (OS) compared to either FOLFOX or FOLFIRI, such improvements,
25 however, have come at the expense of increased chemotherapy-induced toxicity (see Montagnani et al., A systematic review of FOLFOXIRI chemotherapy for the first-line treatment of metastatic colorectal cancer: improved efficacy at the cost of increased toxicity. *Colorectal Dis.* 2011; 13: 846-52). Although the toxicities associated with FOLFOX and FOLFIRI treatment protocols are significant, the toxicities associated with FOLFOXIRI are significantly more severe. For example,
30 in the TRIBE study, patients receiving FOLFOXIRI/bevacizumab had higher rates of Grade 3 or

4 neutropenia (50% vs 20.5%, febrile neutropenia (8.8% vs 6.3%) diarrhea (18.8% vs 10.6%), and stomatitis (8.8% vs 4.3%) compared to FOLFIRI/bevacizumab (see Loupakis et al., Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med.* 2014; 371(17):1609-18). Similarly, in VISNU-1, rates of $\geq$ Grade 3 neutropenia, febrile neutropenia, and diarrhea were all significantly higher in patients receiving FOLFOXIRI/bevacizumab (35%, 9%, 21%) than in those receiving FOLFOX/bevacizumab (26%, 2%, 6%). Mucositis was also numerically higher in the FOLFOXIRI arm at 9% as compared to 4% in the FOLFOX arm in VISNU-1 (Sastre, 2019).

Although the development of chemotherapy-induced myelosuppression (CIM) is potentially problematic in all fluorouracil-based multi-agent chemotherapy, such CIM is particularly problematic during FOLFOXIRI and FOLFIRINOX treatment, as the additional chemotherapeutic agents add to myelosuppressive toxicity, and patients with myelosuppression are more likely to experience infections, sepsis, bleeding, and fatigue, often leading to the need for hospitalizations, hematopoietic growth factor support, transfusions (red blood cells [RBCs] and/or platelets), and even death (see, e.g., Gustinetti et al., Bloodstream infections in neutropenic cancer patients: A practical update. *Virulence.* 2016; 7(3): 280-97; Li et al., Relationship between severity and duration of chemotherapy-induced neutropenia and risk of infection among patients with nonmyeloid malignancies. *Support Care Cancer* 2016; 24(10): 4377-83; Caggiano et al., Incidence, cost, and mortality of neutropenia hospitalization associated with chemotherapy. *Cancer.* 2005; 103(9): 1916-24). Moreover, CIM commonly leads to dose reductions and delays, which limit therapeutic dose intensity and can compromise the anti-tumor efficacy benefits of fluorouracil-based multi-agent chemotherapy. Attempts at developing and implementing clinical algorithms to guide chemotherapy dose reductions and treatment delays in patients with neutropenia and/or thrombocytopenia during treatments have been examined (see, for example, Clinical Trial of a Novel Dose Adjustment Algorithm for Preventing Cytopenia-Related Delays During FOLFOX Chemotherapy, ClinicalTrials.gov Identifier: NCT04526886). Nonetheless, chemotherapy-induced cellular damage to the immune system may also limit anti-tumor efficacy due to an inability of the host immune system to effectively mount a response against the cancer. Prolonged exposure to myelosuppressive agents can lead to cumulative bone marrow toxicity and myelosuppression that can limit the ability to deliver subsequent lines of therapy at the standard

of care doses and schedule. To date, there is no single regulatory approved treatment available that prevents or mitigates the myelosuppressive effects of the fluorouracil-based multi-agent chemotherapy protocols before they occur. Existing interventions are generally used reactively to treat acute cytopenias and are lineage specific; each of these have their own set of associated risks (see, e.g., Blumberg et al., (2010) Platelet transfusions: trigger, dose, benefits, and risks. *F1000 Med Rep* 2:5. <https://doi.org/10.3410/m2-5>; Bohlius et al., (2019) Management of cancer-associated anemia with erythropoiesis-stimulating agents: ASCO/ASH Clinical Practice Guideline Update. *J Clin Oncol* 37 (15):1336-1351. <https://doi.org/10.1200/jco.18.02142>; Xu et al., (2016) Risk factors for bone pain among patients with cancer receiving myelosuppressive chemotherapy and pegfilgrastim. *Support Care Cancer* 24 (2):723-730. <https://doi.org/10.1007/s00520-015-2834-2>; Corey-Lisle et al., (2014) Transfusions and patient burden in chemotherapy-induced anaemia in France. *Ther Adv Med Oncol* 6 (4):146-153. <https://doi.org/10.1177/1758834014534515>).

The development of chemotherapy-induced diarrhea (CID) can be debilitating and, in some cases, life threatening. Findings in such patients include volume depletion, renal failure, and electrolyte disorders such as metabolic acidosis and depending upon water intake, hyponatremia (increased water intake that cannot be excreted because of the hypovolemic stimulus to the release of antidiuretic hormone) or hypernatremia (insufficient water intake to replace losses) (Maroun et al., (2007) Prevention and management of chemotherapy-induced diarrhea in patients with colorectal cancer: a consensus statement by the Canadian working group on chemotherapy-induced diarrhea. *Curr Oncol* 14: 13–20). CID can interfere with and detract from cancer treatment by causing dosing delays or reductions which may have an impact on survival. Patients experiencing multiple bouts of severe diarrhea (grade 3 or greater) while on fluorouracil-based multi-agent chemotherapy, especially FOLFOXIRI and FOLFIRINOX, may have the treatment permanently discontinued due to the associated risks.

Stomatitis/mucositis is a result of the toxic effect of chemotherapeutics on the rapidly dividing epithelial cells lining the gastro-intestinal tract (which goes from the mouth to the anus), leaving the mucosal tissue open to ulceration and infection. Stomatitis/mucositis generally begins 5-10 days following the initiation of chemotherapy and lasts anywhere from one week to six weeks or more. Many patients with stomatitis/mucositis develop significant nutritional issues due to the

inability to eat because of the associated pain, leading to hypovolemia, electrolyte abnormalities, malnutrition, and even death. Severe stomatitis/mucositis often results in dose reductions of or interruptions to the therapeutic protocol.

Consequently, chemotherapy-related toxicity, including chemotherapy-induced
5 myelosuppression (CIM), diarrhea, stomatitis, and mucositis can influence the risk/benefit ratio of fluorouracil-based multi-agent chemotherapy administration such as FOLFOXIRI and FOLFIRINOX. As a result of the increased toxicity associated with more aggressive protocols such as FOLFOXIRI and FOLFIRINOX, their use is frequently limited to younger patients with fewer comorbidities and in need of aggressive cytoreduction surgery. Most clinical trials
10 evaluating FOLFOXIRI specifically exclude patients over 70 years of age with an Eastern Cooperative Oncology Group (ECOG) performance status other than 0 (fully active, able to carry on all pre-disease performance without restriction). For example, in the United States (US), the median age for colon cancer detection is 68 in men and 72 in women suggesting that a substantially large portion of those diagnosed will not be considered for more aggressive fluorouracil-based
15 multi-agent chemotherapy regimens such as FOLFOXIRI. In addition, prolonged exposure to the cytotoxic chemotherapeutic agents comprising fluorouracil-based multi-agent chemotherapy regimens such as FOLFOXIRI, can lead to cumulative bone marrow toxicity and myelosuppression that can limit the ability to deliver subsequent lines of therapy at the standard of care doses and schedule.

Accordingly, there is a need for improved protocols which increase the safety profile of
20 fluorouracil-based multi-agent chemotherapy regimens, and allow for a broader and more aggressive administration of efficacious protocols such as FOLFOXIRI and FOLFIRINOX to a larger colorectal cancer patient population.

25 **Summary of the Invention**

The present invention provides improved fluorouracil-based multi-agent chemotherapy
protocols for treating colorectal cancer in a human subject by incorporating into the protocol the selective, short-acting, and transient CDK4/6 inhibitor trilaciclib, or a pharmaceutically acceptable
salt thereof, which provides for the reduction of chemotherapy associated side-effects, including
30 chemotherapy-induced myelosuppression (CIM) and gastrointestinal toxicities. Alternatively, the

present invention provides improved anti-cancer efficacy, progression free survival, and/or overall survival.

By incorporating trilaciclib into the fluorouracil-based multi-agent chemotherapy protocols and reducing chemotherapy associated toxicities, the protocol can be more safely administered at the standard of care dose and schedule. Whereas current treatments for CIM such as the administration of an erythropoiesis-stimulating agent (ESA) and/or granulocyte-colony stimulating factor (G-CSF) are hematological lineage specific and used after the damage to hematopoietic stem and progenitor cells (HSPCs) has already occurred—placing patients at risk for the development of additional toxicities associated with the administration of these CIM treatments—incorporation of trilaciclib into the fluorouracil-based multi-agent chemotherapy protocols prevents or limits damage to hematopoietic stem and progenitor cells (HSPCs) and therefore associated CIM, thus resulting in an overall improved safety profile and reduced use of standard hematological lineage specific treatments. Furthermore, by reducing chemotherapy associated toxicities, patient populations previously excluded from treatment protocols such as FOLFOXIRI—e.g., those individuals over 70 years of age with an ECOG performance status other than 0—can receive these protocols safely. This is especially significant in protocols such as FOLFOXIRI and FOLFIRINOX, as FOLFOXIRI and FOLFIRINOX are more efficacious than either FOLFOX or FOLFIRI, but may be less tolerated by older, sicker patients. By including trilaciclib in a protocol such as FOLFOXIRI or FOLFIRINOX, an increased benefit across a larger patient population than is currently being treated may be achieved, leading to superior quality of life and/or outcomes for the colorectal cancer patient population as a whole.

In addition to the improved safety profile with the inclusion of trilaciclib in fluorouracil-based multi-agent chemotherapy, trilaciclib is also capable of enhancing the anti-cancer effect of these protocols in certain patient subgroups having colorectal tumors that may be immunologically susceptible to immune suppression. It has been shown that metastatic colorectal cancer can have variable tumor-immune microenvironment across the patient population, allowing the tumor to be immunologically classified as immunologically hot, altered, and cold based on immune effector cell populations and the presence or absence of certain immunogenic biomarkers and signals, which correlate with prognosis and relapse (see Camus et al., Coordination of intratumoral immune reaction and human colorectal cancer recurrence, Cancer Research 69, 2685-2693 (2009),

incorporated herein by reference). Furthermore, a fluorouracil-based multi-agent chemotherapy protocol employs an agent associated with the induction of immunogenic cell death (ICD), including 5-FU (or a 5-FU derivative, analog, or pro-drug), irinotecan and/or oxaliplatin (see Ruan et al., Immunogenic cell death in colon cancer prevention and therapy. *Molecular Carcinogenesis* 2020; 59:783-793). ICD is a form of regulated cell death apoptosis that induces the release of tumor associated antigens and is capable of triggering an anti-tumor immune response, and involves the release of damage-associated molecular pathways (DAMPs) that alert the host's immune system that the cell is damaged (see Locy et al., immunomodulation of the tumor microenvironment: turn foe into friend: *Frontiers in Immunology*, 2018; 9:2020; Wang et al., Immunogenic effects of chemotherapy induced tumor cell death. *Genes & Diseases* (2018) 5, 194-203).

The addition of trilaciclib to fluorouracil-based multi-agent chemotherapy, which includes one or more ICD-inducing chemotherapeutic agents, in addition to reducing CIM and other toxicities, may enhance quality of life and/or anti-tumor efficacy and increase the progression free survival and/or overall survival in patients having immunologically hot or altered tumors due to trilaciclib's preservation of immune effector cells during chemotherapy and differential G1 arrest of cytotoxic T-cells (CTLs) and regulatory T-cells (Tregs), which allows a faster recovery of cytotoxic T-cells from G1 arrest compared with regulatory T-cells in the tumor microenvironment. This differential alteration of cell-cycle kinetics between CTLs and Tregs results in a higher proportion of CTLs to Tregs, the enhancement of T-cell activation, and a decrease in Treg-mediated immunosuppressive functions. Therefore, the anti-tumor effect of trilaciclib may result from the transient proliferative arrest of T-cells (protecting them from chemotherapy-induced damage), followed by activation of CTLs in the tumor microenvironment in the context of fewer Tregs. In addition, trilaciclib may play an important role in expanding anti-tumor T-cell subsets during treatment, and inducing an immune mediated response.

Importantly, this improved immune mediated response occurs without the addition of an immune checkpoint inhibitor, for example an anti-PD-1, anti-PD-L1, or anti-CTLA4 agent such as an antibody.

Accordingly, the present invention provides a method of treating a human subject having colorectal cancer, including metastatic colorectal cancer, comprising administering to the subject

an effective amount of trilaciclib and fluorouracil-based multi-agent chemotherapy, wherein the trilaciclib is administered prior to receiving a chemotherapeutic agent administered during the protocol.

In one aspect, trilaciclib is added to a FOLFOX-based regimen. The FOLFOX regimen
5 generally includes the administration of 5-FU, folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt), and oxaliplatin. In the FOLFOX regimen, 5-FU is administered either as a continuous infusion, or as both a bolus administration and a continuous infusion, wherein the continuous infusion is administered over, for example 22 hours a day for 2 days, or over between about 44 and 48 hours over 2-days. Examples of FOLFOX regimens used in the treatment of
10 colorectal cancer and suitable for inclusion of trilaciclib include FOLFOX4, FOLFOX6, modified FOLFOX6 (mFOLFOX6), FOLFOX7, and modified FOLFOX7 (mFOLFOX7). The various FOLFOX protocols provide variations in dosing and/or administration timing. An anti-VEGF or anti-EGFR monoclonal antibody or compound may also be administered in the various FOLFOX protocols. In some embodiments, in the FOLFOX protocol and variations thereof, trilaciclib is
15 administered a first time about 4 hours or less prior to the initiation of the FOLFOX protocol, and a second time between 18 and 26 hours after the first administration. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In an alternative aspect, trilaciclib is added to a FOLFIRI-based regimen. The FOLFIRI regimen generally includes the administration of 5-FU, folinic acid (e.g., leucovorin or
20 levoleucovorin, or similar acting folinate salt), and irinotecan, wherein 5-FU is administered as either a continuous infusion, or both a bolus administration and a continuous infusion, wherein the continuous infusion is administered over, for example 22 hours a day for 2 days, or over between about 44 and 48 hours over 2-days. A modification of the FOLFIRI protocol has been administered to colorectal patients providing variations in dosing and/or administration timing. An anti-VEGF
25 or anti-EGFR monoclonal antibodies may also be administered in the various FOLFIRI protocols. In some embodiments, in the FOLFIRI protocol and variations thereof, trilaciclib is administered a first time about 4 hours or less prior to the initiation of the FOLFIRI protocol, and a second time between 18 and 26 hours after the first administration. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In an alternative aspect, trilaciclib is added to a FOLFOXIRI-based regimen. The FOLFOXIRI regimen comprises 5-FU, folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt), oxaliplatin, and irinotecan, wherein 5-FU is administered as a continuous infusion over between about 44 and 48 hours over 2-days. Modification of the FOLFOXIRI protocol have been administered to colorectal patients involving variations to dosing and/or administration timing. An anti-VEGF or anti-EGFR monoclonal antibody or compound may also be administered in the various FOLFOXIRI protocols. In some embodiment, the trilaciclib-FOLFOXIRI-based regimen is administered to a patient previously ineligible for FOLFOXIRI administration, including, but not limited to, a patient over 70 years of age with an ECOG ≥ 1 . In some embodiments, in the FOLFOXIRI protocol and variations thereof, trilaciclib is administered a first time about 4 hours or less prior to the initiation of the FOLFOXIRI protocol, and a second time between 18 and 26 hours after the first administration. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In an alternative aspect, trilaciclib is added to a FOLFIRINOX-based regimen. The FOLFIRINOX regimen comprises 5-FU, folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt), oxaliplatin, and irinotecan, wherein 5-FU is administered as both a bolus administration and a continuous infusion, wherein the continuous infusion is administered over between about 44 and 48 hours over 2-days. Modification of the FOLFIRINOX protocol have been administered to colorectal patients involving variations to dosing and/or administration timing. An anti-VEGF or anti-EGFR monoclonal antibody or compound may also be administered in the various FOLFIRI protocols. In some embodiment, the trilaciclib-FOLFOXIRI-based regimen is administered to a patient previously ineligible for FOLFIRINOX administration, including, but not limited to, a patient over 70 years of age with an ECOG ≥ 1 . In some embodiments, in the FOLFIRINOX protocol and variations thereof, trilaciclib is administered a first time about 4 hours or less prior to the initiation of the FOLFIRINOX protocol, and a second time between 18 and 26 hours after the first administration. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In some embodiments, the colorectal cancer treated is metastatic colorectal cancer (mCRC). In some embodiments, the metastatic colorectal cancer is proficient mismatch repair (pMMR) mCRC. In some embodiments, the metastatic colorectal cancer is microsatellite stable

(MSS) mCRC. In some embodiments, the metastatic colorectal cancer is proficient mismatch repair (pMMR)/microsatellite stable (MSS) mCRC (pMMR/MSS mCRC). In some embodiments, the pMMR/MSS mCRC has not been responsive to a previous treatment with a single agent checkpoint inhibitor. In some embodiments, the mCRC has a BRAF or KRAS mutation. In some
5 embodiments, the BRAF mutation is a V600E substitution mutation. In some embodiments, the colorectal cancer is CDK4/6 replication dependent. In some embodiments, the colorectal cancer is CDK4/6 replication independent. In some embodiments, the colorectal cancer is CDK4/6 replication indeterminate. In some embodiments, the subject being treated has a tumor that exhibits specific characteristics as described herein according to the Ayer's interferon- γ signature,
10 the Ayer's expanded immune signature or the Thorsson et al Six Class Immune Signature. In some embodiments, the tumor has is interferon- γ (IFN- γ) dominant according to Thorsson's Six Class Immune Signature, or a high IFN- γ signature or expanded immune signature according to the Ayer's IFN- γ Signature Score or Expanded Immune Signature Score. In some embodiments, the cancer is PD-L1 positive.

15 In some embodiments, the inclusion of trilaciclib in the protocol results in a reduction and/or prevention in one or more fluorouracil-based multi-agent chemotherapy-associated toxicities, for example, but not limited to, chemotherapy-induced myelosuppression (CIM), chemotherapy-induced diarrhea (CID), stomatitis, and/or mucositis. In some embodiments, the inclusion of trilaciclib results in an improvement in progression free survival (PFS) in comparison
20 to the predicted PFS based on administration of fluorouracil-based multi-agent chemotherapy without the inclusion of trilaciclib.

In some embodiments, the inclusion of trilaciclib results in an improvement in overall survival (OS) in comparison to the predicted OS based on administration of fluorouracil-based multi-agent chemotherapy alone.

25 In some embodiments, the subject has not received a prior chemotherapeutic regimen for the treatment of the colorectal cancer. In some embodiments, the subject has failed prior chemotherapeutic regimen for the treatment of colorectal cancer. In some embodiments, the trilaciclib/fluorouracil-based multi-agent chemotherapy is administered in a cycle every 14 days. In some embodiments, the trilaciclib/fluorouracil-based multi-agent chemotherapy is administered
30 in 3 or more induction cycles, 4 or more induction cycles, 5 or more induction cycles, 6 or more

induction cycles, 7 or more induction cycles, 8 or more induction cycles, 9 or more induction cycles, 10 or more induction cycles, or 11 or more induction cycles. In some embodiments, the trilaciclib/fluorouracil-based multi-agent chemotherapy is administered up to 12 times. In some embodiments, the subject is further administered a maintenance regimen of trilaciclib, 5-FU, and
5 a folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt) following cessation of the trilaciclib/fluorouracil-based multi-agent chemotherapy induction protocol. In some embodiments, the subject maintains an absolute neutrophil count (ANC) of greater than $1.0 \times 10^9/L$ when measured prior to the beginning of each trilaciclib/fluorouracil-based multi-agent chemotherapy treatment cycle. In some embodiments, the subject maintains a platelet count of
10 greater than $75 \times 10^9/L$ when measured prior to the beginning of each trilaciclib/fluorouracil-based multi-agent chemotherapy treatment cycle.

In one aspect, a method of treating a human subject with colorectal cancer is provided comprising:

- i) administering to the subject an effective amount of trilaciclib;
- 15 ii) administering to the subject an effective amount of 5-FU;
- iii) administering to the subject an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt);
- iv) administering to the subject an effective amount of oxaliplatin and/or irinotecan; and,
- 20 v) optionally administering to the subject an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered prior to the initiation of administration of the 5-FU, folinic acid, and oxaliplatin and/or irinotecan. In some embodiments, the trilaciclib is administered about 4 hours or less prior to the administration of the 5-FU, folinic acid, and oxaliplatin and/or
25 irinotecan. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, folinic acid, and oxaliplatin and/or irinotecan, and a second time between 18 and 26 hours after the first administration. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiments, the
30 subject is further administered an anti-VEGF or an anti-EGFR antibody or compound. In some

embodiments, the subject is administered an anti-VEGF antibody selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept. In some embodiments, the subject is administered an anti-EGFR antibody selected from cetuximab or panitumumab. In some embodiments, the subject is administered oxaliplatin. In some embodiments, the subject is administered irinotecan. In some embodiments, the subject is administered both irinotecan and oxaliplatin. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, provided herein is a method of treating a human subject with colorectal cancer wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- ii) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 20 and 24 hours, or
- iii) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt); and,
- iv) administering to the subject on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- v) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and oxaliplatin on day 1 of each 14-day cycle, and about 4 hours or less prior to the initiation of administration of 5-FU and folinic acid on day 2 of each cycle. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered

up to 12 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid on the same schedule above, following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided comprising one or more 14-day cycles comprising:

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- ii) administering to the subject on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus intravenous injection;
- iii) administering to the subject starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iv) administering to the subject on day 1 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt); and,
- v) administering to the subject on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and oxaliplatin on day 1 of each 14-day cycle, and wherein the trilaciclib is administered on day 2 about 18 to 26 hours after its administration on day 1. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiment, trilaciclib is administered on day 2 between about 20 and 24 hours after its administration on day 1. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each

cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered up to 12 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid on the same schedule above following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- ii) administering to the subject starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iii) administering to the subject on day 1 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt); and,
- iv) administering to the subject on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- v) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and oxaliplatin on day 1 of each 14-day cycle, and wherein the trilaciclib is administered on day 2 about 18 to 26 hours after its administration on day 1. In

some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiment, trilaciclib is administered on day 2 between about 20 and 24 hours after its administration on day 1. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5-FU-related myelosuppression is exacerbated rather than mitigated. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered up to 12 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid (e.g., leucovorin or levoleucovorin), on the same schedule as the protocol above following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- ii) administering to the subject on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus intravenous injection;
- iii) administering to the subject starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iv) administering to the subject on day 1 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt); and,

- v) administering to the subject on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- vi) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

5 wherein the trilaciclib is administered about 4 hours or less prior to initiation of administration of 5-FU, leucovorin, and irinotecan on day 1 of each 14-day cycle, and wherein the trilaciclib is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 24 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5-FU is at sufficient concentrations such that 5-FU-related myelosuppression is exacerbated rather than mitigated. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered up to 12 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid on the same schedule as the above protocol following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- ii) administering to the subject starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iii) administering to the subject on day 1 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt); and,
- iv) administering to the subject on day 1 of each 14-day cycle an effective amount of irinotecan; and
- v) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered about 4 hours or less prior to initiation of administration of 5-FU, folinic acid, and irinotecan on day 1 of each 14-day cycle, and wherein the trilaciclib is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 24 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered up to 12 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid, on the same schedule above following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the

folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided, wherein the treatment comprises one or more 14-day cycles comprising

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- ii) administering to the subject starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iii) administering to the subject on day 1 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt);
- iv) administering to the subject on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- v) administering to the subject on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- vi) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered about 4 hours or less prior to administration of 5-FU, folinic acid, oxaliplatin, and irinotecan on day 1 of each 14-day cycle, and wherein the trilaciclib is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 24 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated. In some embodiments, the

subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered up to 12 cycles of the protocol
5 above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid on the same schedule above following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In
10 some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided, wherein the treatment comprises one or more 14-day cycles comprising

- i) administering to the subject on day 1 and day 2 of each 14-day cycle an effective amount of trilaciclib;
- 15 ii) administering to the subject on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;
- iii) administering to the subject starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours beginning on day 1;
- 20 iv) administering to the subject on day 1 of each 14-day cycle an effective amount of folinic acid (e.g., leucovorin levoleucovorin, or similar acting folinate salt);
- v) administering to the subject on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) administering to the subject on day 1 of each 14-day cycle an effective amount of
25 irinotecan; and,
- vii) optionally administering to the subject on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the trilaciclib is administered about 4 hours or less prior to initiation of administration of 5-FU, folinic acid, oxaliplatin, and irinotecan on day 1 of each 14-day cycle, and
30 wherein the trilaciclib is administered on day 2 of each cycle between about 18 and 26 hours after

its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered a first time about 4 hours or less prior to the administration of the 5-FU, and a second time between 18 and 26 hours after the first administration. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 24 hours after its administration on day 1 of each cycle. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5-FU-related myelosuppression is exacerbated rather than mitigated. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1 of each 14-day cycle. In some embodiments, anti-VEGF antibody is selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is cetuximab or panitumumab. In some embodiments, the subject is administered up to 12 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid on the same schedule as the protocol above following cessation of the protocol above. In some embodiments, the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In one aspect, a method of treating a human subject with colorectal cancer is provided, wherein the treatment comprises one or more 56-day cycles comprising

- i) administering to the subject on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of trilaciclib;
- ii) administering to the subject on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;
- iii) administering to the subject on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of folinic acid (e.g., leucovorin or levoleucovorin, or similar acting folinate salt);

iv) administering to the subject on day 1, day 15, and day 29 of each 56-day cycle an effective amount of oxaliplatin; and,

v) optionally administering to the subject on day 1, day 15, and day 29 of each 56-day cycle an anti-VEGF or anti-EGFR monoclonal antibody;

5 wherein the trilaciclib is administered about 4 hours or less prior to initiation of administration of the 5-FU, folinic acid, or oxaliplatin. In some embodiments, the trilaciclib is administered about 4 hours or less prior to the administration of the 5-FU. In some embodiments, the subject is further administered an anti-VEGF or an anti-EGFR antibody or compound on day 1, day 15, and day 29 of each 56-day cycle. In some embodiments, the anti-VEGF antibody is
10 selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept, and the anti-EGFR antibody is selected from cetuximab or panitumumab. In some embodiments, the subject is administered up to 3 cycles of the protocol above. In some embodiments, the subject is further administered one or more maintenance cycles of trilaciclib, 5-FU, and folinic acid on the same schedule as the protocol above following cessation of the protocol above. In some embodiments,
15 the folinic acid is leucovorin. In some embodiments, the folinic acid is levoleucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In some embodiments, the colorectal cancer treated in one of the 5 fluorouracil-based
20 multi-agent protocols provided herein is metastatic colorectal cancer. In alternative embodiments, the cancer is selected from pancreatic cancer, gastric cancer, gastroesophageal junction adenocarcinoma, biliary tract cancer, neuroendocrine carcinoma, peritoneal carcinosis, or liver cancer.

In some embodiments, the treatment results in a reduction and/or prevention in one or more
25 fluorouracil-based multi-agent chemotherapy-associated toxicities, for example, chemotherapy-induced myelosuppression (CIM), chemotherapy-induced diarrhea (CID), stomatitis, and/or mucositis. In some embodiments, the treatment results in an improvement in progression free survival (PFS) in comparison to the predicted PFS based on administration of the fluorouracil-based multi-agent chemotherapy regimen without trilaciclib. In some embodiments, the treatment

results in an improvement in overall survival (OS) in comparison to the predicted OS based on administration of the fluorouracil-based multi-agent chemotherapy regimen without trilaciclib.

In some embodiments, the administration of a protocol described herein provides improved myelopreservation of hematopoietic stem and progenitor cells (HSPCs) and immune effector cells such as lymphocytes including T-lymphocytes. In some embodiments, the administration of a protocol described herein provides enhanced anti-tumor efficacy in subjects compared to those receiving the fluorouracil-based multi-agent chemotherapy without trilaciclib. In some embodiments, the administration of a protocol described herein provides myelopreservation of the neutrophil lineage in subjects compared to those receiving fluorouracil-based multi-agent chemotherapy without trilaciclib. In some embodiments, the administration of a protocol described herein provides a reduction in the duration of severe (Grade 4) neutropenia in subjects compared to those receiving fluorouracil-based multi-agent chemotherapy without trilaciclib. In some embodiments, the administration of a protocol described herein provides a reduction of chemotherapy-induced fatigue (CIF) in subjects compared to those receiving fluorouracil-based multi-agent chemotherapy without trilaciclib. In some embodiments, the reduction of CIF is a reduction in the time to first confirmed deterioration of fatigue (TTCD-fatigue), as measured by the Functional Assessment of Cancer Therapy-Fatigue (FACIT-F). In some embodiments, the administration of a protocol described herein provides improved progression free survival (PFS) and/or overall survival (OS) in subjects compared to those receiving fluorouracil-based multi-agent chemotherapy without trilaciclib. In some embodiments, an improvement in PFS is based on per Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1). In some embodiments, the administration of a protocol described herein provides a reduction in severe neutropenia events, a reduction in granulocyte-colony stimulating factor (G-CSF) treatment, or a reduction in febrile neutropenia (FN) adverse events (AEs). In some embodiments, the administration of a protocol described herein provides a reduction in Grade 3 or 4 decreased hemoglobin laboratory values, red blood cell (RBC) transfusions, or erythropoiesis-stimulating agent (ESA) administration. In some embodiments, the administration of a protocol described herein provides a reduction in Grade 3 or 4 decreased platelet count laboratory values and/or the number of platelet transfusions. In some embodiments, the administration of a protocol described herein provides a reduction in Grade 3 or 4 hematologic laboratory values. In some embodiments, the administration of a protocol described

herein provides a reduction in all-cause dose reductions or cycle delays and relative dose intensity for fluorouracil-based multi-agent chemotherapy. In some embodiments, the administration of a protocol described herein provides a reduction in i) hospitalizations, including but not limited to those due to all causes, febrile neutropenia/neutropenia, anemia/RBC transfusion, thrombocytopenia/bleeding and infections) or ii) antibiotic use, including but not limited to intravenous (IV), oral, and oral and IV administered antibiotics. In some embodiments, the administration of a protocol described herein provides an improvement to one or more of: Functional Assessment of Cancer Therapy-General (FACT-G) domain scores (physical, social/family, emotional, and functional well-being); Functional Assessment of Cancer Therapy-Anemia (FACT-An): Anemia; Functional Assessment of Cancer Therapy-colorectal cancer (FACT-C) : Colorectal Cancer Subscale; 5-level EQ-5D (EQ-5D-5L); Patient Global Impression of Change (PGIC) fatigue item; or Patient Global Impression of Severity (PGIS) fatigue item.

Brief Description of the Figures

Fig. 1A is a schematic of an exemplary FOLFOX4 protocol incorporating trilaciclib. Trila = trilaciclib; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 1B is a schematic of an exemplary FOLFOX6 protocol incorporating trilaciclib. Trila = trilaciclib; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 1C is a schematic of an exemplary mFOLFOX6 protocol incorporating trilaciclib. Trila = trilaciclib; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or

compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 1D is a schematic of an exemplary FOLFOX7 protocol incorporating trilaciclib. Trila = trilaciclib; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 1E is a schematic of an exemplary mFOLFOX7 protocol incorporating trilaciclib. Trila = trilaciclib; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 2A is a schematic of an exemplary FOLFIRI protocol incorporating trilaciclib. Trila = trilaciclib; IR = irinotecan; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 2B is a schematic of an exemplary mFOLFIRI protocol incorporating trilaciclib. Trila = trilaciclib; IR = irinotecan; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half

the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 3A is a schematic of an exemplary FOLFOXIRI protocol incorporating trilaciclib. Trila = trilaciclib; IR = irinotecan; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin.

Fig. 3B is a schematic of an alternative exemplary FOLFOXIRI protocol incorporating trilaciclib. Trila = trilaciclib; IR = irinotecan; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LLV = levoleucovorin.

Fig. 4A is a schematic of an exemplary FOLFIRINOX protocol incorporating trilaciclib. Trila = trilaciclib; IR = irinotecan; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin.

Fig. 4B is a schematic of an exemplary FOLFIRINOX protocol incorporating trilaciclib. Trila = trilaciclib; IR = irinotecan; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LLV = levoleucovorin.

Fig. 5 is a schematic of an exemplary FLOX protocol incorporating trilaciclib. Trila = trilaciclib; OX = oxaliplatin; mAb = anti-VEGF or anti-EGFR monoclonal antibody or compound; 5-FU = fluorouracil; LV = leucovorin. In alternative embodiments, levoleucovorin can be substituted for leucovorin as the folinic acid, wherein levoleucovorin is administered at one-half the dose of leucovorin. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered instead of leucovorin or levoleucovorin.

Fig. 6 is a reproduction of figure 3 found in Galon, J., and Bruni, D., *Approaches to treat immune hot, altered and cold tumours with combination immunotherapies*, Nature Reviews Drug Discovery (18), March 2019, 197-218, incorporated herein in its entirety, describing an immunogram for use as a tool to direct anticancer therapy. Cancers can be classified into four main subtypes (hot, altered- excluded, altered- immunosuppressed and cold) according to their associated T cell (CD3+ and CD8+) presence and distribution. Hot cancers are defined by the simultaneous presence of immune contexture parameters: the cell type (CD3+, CD8+, follicular helper T (TFH), T helper 1 (TH1), memory and exhausted T cells); the location (invasive margin, tumor core and tertiary lymphoid structures); the density (immune density and quantity); and the functional immune orientation (chemokines, cytokines, cytotoxic factors, adhesion, attraction and

TH1). As provided in the immunogram: DORA2A, A2A adenosine receptor; β m, β 2-microglobulin; BET, bromodomain and extra-terminal motif proteins; BTLA, B and T lymphocyte attenuator; CAR T-cell, chimeric antigen receptor T-cell; CCR, CC-chemokine receptor; CIN, chromosomal instability; CSF1R, colony-stimulating factor 1 receptor; CTL A4, cytotoxic T lymphocyte-associated antigen; CXCL, CXC-chemokine ligand; DDR, DNA damage response; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; FDA, US Food and Drug Administration; FGFR3, fibroblast growth factor receptor 3; FOXP3, forkhead box P3; GITR, glucocorticoid-induced TNFR-related protein; GM-CSF, granulocyte–macrophage colony-stimulating factor; HDAC, histone deacetylase; HIF1 α , hypoxia-inducible factor 1- α ; HLA, human leukocyte antigen; HMA, hypomethylating agent; IAP, inhibitors of apoptosis family (also known as XIAP); ICAM1, intercellular adhesion molecule 1; ICD, immunogenic cell death; ICOS, inducible T-cell co-stimulator; ICP, immune checkpoint; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; LAG3, lymphocyte activation gene 3; LIGHT, tumor necrosis factor superfamily member 14; MAdCAM1, mucosal addressin cell adhesion molecule 1; MCL1, induced myeloid leukemia cell differentiation protein Mcl1; MDSCs, myeloid-derived suppressor cells; MEK, mitogen-activated protein kinase kinase; MET, mesenchymal–epithelial transition; MSI, microsatellite instability; NK, natural killer; NOS1, nitric oxide synthase 1; PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand; PI3K γ , phosphoinositide 3-kinase- γ ; PPAR γ , peroxisome proliferator-activated receptor- γ ; SIGLEC9, sialic acid-binding Ig-like lectin 9; STING, stimulator of interferon genes; TDO, tryptophan 2,3-dioxygenase; TGF β , transforming growth factor- β ; TIGIT, T-cell immunoglobulin and ITIM domain; TIM3, T-cell immunoglobulin and mucin domain-containing 3; TKI, tyrosine kinase inhibitor; TLR, Toll-like receptor; Treg cells, regulatory T-cells; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VISTA, V-domain Ig suppressor of T-cell activation; XCL1, lymphotactin; XCR1, chemokine XC receptor 1. Lower case “i” following any acronym or abbreviation indicates inhibitor; lower case “a” following any acronym or abbreviation indicates agonist.

Fig. 7A is a reproduction of Fig. 1A found in Galon, J., and Bruni, D., *Approaches to treat immune hot, altered and cold tumors with combination immunotherapies*, Nature Reviews Drug Discovery (18), March 2019, 197-218, incorporated herein in its entirety, which illustrates examples of hot, altered and cold immune cancers. Dark (3,3'-diaminobenzidine (DAB)) staining

represents CD3+ T cells and lighter (alkaline phosphatase) counterstaining provides homogeneous tissue background staining. The level and spatial distribution of CD3+ and CD8+ T cell infiltration differentiates four distinct solid tumor phenotypes: hot (or inflamed); altered, which can be excluded or immunosuppressed; and cold (or non- inflamed). These tumor phenotypes are characterized by high, intermediate, and low immunoscore, respectively.

Fig. 7B is a reproduction of Fig. 1B found in Galon, J., and Bruni, D., *Approaches to treat immune hot, altered and cold tumors with combination immunotherapies*, Nature Reviews Drug Discovery (18), March 2019, 197-218, incorporated herein in its entirety, which is a schematic representation of the four subtypes of immune tumor. Of note, in altered- excluded tumors, CD3+ and CD8+ T cell infiltrates are low at the tumor center and high at the invasive margin, resulting overall in an intermediate immunoscore. Altered- immunosuppressed tumors display instead a more uniform pattern of (low) CD3+ and CD8+ T cell infiltration. CT, center of tumor; Hi, high; IM, invasive margin; Lo, low.

Fig. 8 is a schematic of a randomized, double-blind, placebo-controlled, global, multicenter, Phase 3 trial evaluating the impact of trilaciclib on myelopreservation and anti-tumor efficacy when administered prior to FOLFOXIRI/bevacizumab in patients with pMMR/MSS mCRC who have not received systemic therapy for metastatic disease.

Detailed Description of the Invention

Provided herein are improved methods and compositions for use in treating colorectal cancer, including metastatic or advanced colorectal cancer. In particular, provided herein are improved fluorouracil-based multi-agent chemotherapy regimens such as FOLFOX, FOLFIRI, FOLFOXIRI, FOLFIRINOX, and FLOX that include the CDK4/6 inhibitor trilaciclib.

Trilaciclib is a highly potent and selective, reversible, CDK4/6 inhibitor that is typically administered intravenously (IV) prior to fluorouracil-based multi-agent chemotherapy administration, including but not limited to FOLFOXIRI or FOLFIRINOX, and is designed to preserve HSPCs during chemotherapy (myelo-preservation) and enhance anti-tumor immunity (anti-tumor efficacy). Both HSPC and lymphocyte populations are dependent on CDK4/6 activity for proliferation (see, e.g., Kozar et al., Mouse development and cell proliferation in the absence of D-cyclins. Cell. 2004;118(4):477–91; Malumbres et al., Mammalian cells cycle without the D-

type cyclin-dependent kinases Cdk4 and Cdk6. Cell. 2004;118(4):493-504.; Ramsey et al., Expression of p16Ink4a Compensates for p16Ink4a Loss in Cyclin-Dependent Kinase 4/6-Dependent Tumors and Tissues. Cancer Res 2007; 67: 4732-41; Horsley et al., NFATc1 balances quiescence and proliferation of skin stem cells. Cell. 2008;132(2):299-310). HSPCs and lymphocytes become arrested in the G1 phase of the cell cycle upon exposure to trilaciclib. This transient, drug-induced, cell cycle arrest by trilaciclib provides protection from chemotherapy-induced cell damage during treatment by preventing HSPCs and immune effector cells from proliferating in the presence of cytotoxic chemotherapy and to favorably alter the tumor immune microenvironment through transient T cell inhibition when combined with ICD-chemotherapy. Upon release of the transient G1 arrest, immune effector cells are positioned to respond to the increased antigenicity induced through the ICD-chemotherapeutic agents contained in these chemotherapeutic protocols. Furthermore, in addition to the myelopreservation protection, trilaciclib may protect other CDK4/6-dependent replicating cells such as the epithelial lining of the gastro-intestinal tract from the harmful and toxic effects associated with these protocols, and reduce side effects such as mucositis/stomatitis.

A safer, less toxic fluorouracil-based multi-agent chemotherapy protocol, for example FOLFOXIRI or FOLFIRINOX, allows for administration to patients previously excluded from these otherwise efficacious treatments, for example patients 70 years older with ECOG scores of 1 or greater.

Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this application belongs. In the specification, singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice and testing of the present application, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference. The references cited herein are not admitted to be prior art to the claimed application. In the case of conflict, the present specification, including definitions, will

control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

Compounds are described using standard nomenclature. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

Compounds are described using standard nomenclature. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

In some embodiments of each compound described herein, the compound may be in the form of a racemate, enantiomer, mixture of enantiomers, diastereomer, mixture of diastereomers, tautomer, N-oxide, or isomer, such as a rotamer, as if each is specifically described unless specifically excluded by context.

The terms “a” and “an” do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced items. The term “or” means “and/or”. Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individual recited herein. The endpoints of all ranges are included within the range and independently combinable. All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of examples, or exemplary language (e.g., “such as”), is intended merely to better illustrate the invention and do not pose a limitation on the scope of the invention unless otherwise claimed.

In some embodiments of each compound described herein, the compound may be in the form of a tautomer, N-oxide, or isomer, such as a rotamer, as if each is specifically described unless specifically excluded by context.

An “effective amount” as used herein, means an amount which provides a therapeutic or prophylactic benefit.

To “treat” a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease, disorder, or side-effect experienced by a subject (i.e. palliative treatment) or to decrease a cause or effect of the disease, disorder (i.e. disease-modifying

treatment), or side effect experienced by a subject as a result of the administration of a therapeutic agent.

Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and should not be construed as a limitation on the scope of the invention. The description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

As used herein, “pharmaceutical compositions” are compositions comprising at least one active agent, and at least one other substance, such as a carrier. “Pharmaceutical combinations” are combinations of at least two active agents which may be combined in a single dosage form or provided together in separate dosage forms with instructions that the active agents are to be used together to treat any disorder described herein.

As used herein, “pharmaceutically acceptable salt” is a derivative of the disclosed compound in which the parent compound is modified by making inorganic and organic, non-toxic, acid or base addition salts thereof. The salts of the present compounds can be synthesized from a parent compound that contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are typical, where practicable. Salts of the present compounds further include solvates of the compounds and of the compound salts.

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 include the conventional non-toxic salts and the quaternary ammonium salts of the parent compound formed, for example,

from non-toxic inorganic or organic acids. For example, conventional non-toxic acid salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, mesylic, esylic, besylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, $\text{HOOC}-(\text{CH}_2)_n\text{-COOH}$ where n is 0-4, and the like, or using a different acid that produces the same counterion. Lists of additional suitable salts may be found, e.g., in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., p. 1418 (1985). Wherein the methods described herein identify the administration of a particular compound, it is understood that administration of the compound's pharmaceutically acceptable salt, if applicable, is encompassed as an embodiment.

As used herein, the term "prodrug" means a compound which when administered to a host in vivo is converted into the parent drug. As used herein, the term "parent drug" means any of the presently described chemical compounds that are useful to treat any of the disorders described herein, or to control or improve the underlying cause or symptoms associated with any physiological or pathological disorder described herein in a host, typically a human. Prodrugs can be used to achieve any desired effect, including to enhance properties of the parent drug or to improve the pharmaceutic or pharmacokinetic properties of the parent. Prodrug strategies exist which provide choices in modulating the conditions for in vivo generation of the parent drug, all of which are deemed included herein. Nonlimiting examples of prodrug strategies include covalent attachment of removable groups, or removable portions of groups, for example, but not limited to acylation, phosphorylation, phosphonylation, phosphoramidate derivatives, amidation, reduction, oxidation, esterification, alkylation, other carboxy derivatives, sulfoxy or sulfone derivatives, carbonylation or anhydride, among others.

The term "carrier" applied to pharmaceutical compositions/combinations of the invention refers to a diluent, excipient, or vehicle with which an active compound is provided.

A "pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition/combination that is generally safe, and neither biologically nor otherwise inappropriate for administration to a host, typically a human. In some embodiments, an excipient is used that is acceptable for veterinary use.

In non-limiting embodiments, trilaciclib can be used in a form that has at least one desired isotopic substitution of an atom, at an amount above the natural abundance of the isotope, i.e., enriched. Isotopes are atoms having the same atomic number but different mass numbers, i.e., the same number of protons but a different number of neutrons.

5 Examples of isotopes that can be incorporated into trilaciclib for use in the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, chlorine and iodine such as 2H , 3H , 11C , 13C , 14C , 15N , 18F , 31P , 32P , 35S , 36Cl , and 125I respectively. In one non-limiting embodiment, isotopically labelled compounds can be used in metabolic studies (with 14C), reaction kinetic studies (with, for example 2H or 3H), detection or imaging techniques, such
10 as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. In particular, an 18F labeled compound may be particularly desirable for PET or SPECT studies. Isotopically labeled compounds of this invention and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the schemes or in the examples and
15 preparations described below by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

By way of general example and without limitation, isotopes of hydrogen, for example, deuterium (2H) and tritium (3H) may be used anywhere in described structures that achieves the desired result. Alternatively, or in addition, isotopes of carbon, e.g., 13C and 14C , may be used.

20 Isotopic substitutions, for example deuterium substitutions, can be partial or complete. Partial deuterium substitution means that at least one hydrogen is substituted with deuterium. In certain embodiments, the isotope is 90, 95 or 99% or more enriched in an isotope at any location of interest. In one non-limiting embodiment, deuterium is 90, 95 or 99% enriched at a desired location.

25 Trilaciclib for use in the present invention may form a solvate with solvents (including water). Therefore, in one non-limiting embodiment, the invention includes a solvated form of trilaciclib. The term "solvate" refers to a molecular complex of trilaciclib (including a salt thereof) with one or more solvent molecules. Non-limiting examples of solvents are water, ethanol, dimethyl sulfoxide, acetone and other common organic solvents. The term "hydrate" refers to a
30 molecular complex comprising a compound of the invention and water. Pharmaceutically

acceptable solvates in accordance with the invention include those wherein the solvent may be isotopically substituted, e.g. D₂O, d₆-acetone, d₆-DMSO. A solvate can be in a liquid or solid form.

The “patient” or “subject” treated is typically a human patient, although it is to be understood the methods described herein are effective with respect to other animals, such as mammals. More particularly, the term patient can include animals used in assays such as those used in preclinical testing including but not limited to mice, rats, monkeys, dogs, pigs and rabbits; as well as domesticated swine (pigs and hogs), ruminants, equine, poultry, felines, bovines, murines, canines, and the like.

As generally contemplated herein, the term “hematopoietic stem and progenitor cells” (HSPCs) includes, but are not limited to, long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), hematopoietic progenitor cells (HPCs), multipotent progenitors (MPPs), oligodendrocyte pre-progenitors (OPPs), monocyte progenitors, granulocyte progenitors, common myeloid progenitors (CMPs), common lymphoid progenitors (CLPs), granulocyte-monocyte progenitors (GMPs), granulocyte progenitors, monocyte progenitors, and megakaryocyte-erythroid progenitors (MEPs), megakaryocyte progenitors, erythroid progenitors, HSC/MPPs (CD45dim/CD34+/CD38-), OPPs (CD45dim/CD34+/CD38+), monocyte progenitors (CD45+/CD14+/CD11b+), granulocyte progenitors (CD45+/CD14-/CD11b+), erythroid progenitors (CD45-/CD71+), and megakaryocyte progenitors (CD45+/CD61+).

The term “immune effector cell” generally refers to an immune cell that performs one or more specific functions. Immune effector cells are known in the art and include for example, but are not limited to, T-cells, including Naïve T-cells, Memory T-cells, Activated T-cells (Thelper (CD4+) and Cytotoxic T cells (CD8+)), TH1 activated T-cells, TH2 activated T-cells, TH17 activated T-cells, Naïve B cells, Memory B cells, plasmablasts, dendritic cells, monocytes, and natural killer (NK) cells.

As used herein, the term “immune checkpoint inhibitor (ICI)” refers to inhibitory therapy targeting immune checkpoints, key regulators of the immune system that when stimulated can dampen the immune response to an immunologic stimulus. Some cancers can protect themselves from attack by stimulating immune checkpoint targets. ICIs block inhibitory checkpoints, restoring immune system function. ICIs include those targeting immune checkpoint proteins such

as programmed cell death-1 protein (PD-1), PD-1 Ligand-1 (PD-L1), PD-1 Ligand-2 (PD-L2), CTLA-4, LAG-3, TIM-3, and V-domain Ig suppressor of T-cell activation (VISTA), B7-H3/CD276, indoleamine 2,3-dioxygenase (IDO), killer immunoglobulin-like receptors (KIRs), carcinoembryonic antigen cell adhesion molecules (CEACAM) such as CEACAM-1, CEACAM-3, and CEACAM-5, sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15), T cell immunoreceptor with Ig and ITIM domains (TIGIT), and B and T lymphocyte attenuator (BTLA) protein. Immune checkpoint inhibitors are known in the art.

Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death. Most cases of CRC are detected in Western countries, with its incidence increasing year by year. The probability of suffering from colorectal cancer is about 4%–5% and the risk for developing CRC is associated with personal features or habits such as age, chronic disease history, and lifestyle. By gender, CRC is the second most common cancer in women (9.2%) and the third in men (10%) (Stewart B., Wild C.P., editors. World Cancer Report 2014. International Agency for Research on Cancer (IARC); Lyon, France: 2014. The incidence of CRC has risen by more than 200,000 new cases per year from 1990 to 2012).

Colorectal cancer starts in the colon or the rectum. These cancers can also be called colon cancer or rectal cancer, depending on where they start. Adenocarcinomas of the colon and rectum make up 95 percent of all colorectal cancer cases. Other types include carcinoid tumors which involve the hormone-producing cells in the intestines, gastrointestinal stromal tumors which are soft tissue sarcomas which start in the blood vessels or connective tissue of the colon, and colorectal lymphoma.

CRC, like numerous other solid tumors, is a heterogeneous disease in which different subtypes may be distinguished by their specific clinical and/or molecular features. Mutations in specific genes can lead to the onset of colorectal cancer, as happens in other types of cancer. Those mutations can appear in oncogenes, tumor suppressor genes and genes related to DNA repair mechanisms. Depending on the origin of the mutation, colorectal carcinomas can be classified as sporadic, inherited and familial.

Point mutations, which appear during life, are not associated with inherited syndromes and only affect individual cells and their descendants. Cancers derived from point mutations are called sporadic cancers, and account for 70% of all colorectal cancers. The molecular pathogenesis of sporadic cancer is heterogeneous as mutations can target different genes (see Marmol et al.,
5 Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. *Int. J. Mol. Sci.* 2017 Jan; 18(1):197). However, approximately 70% of CRC cases follow a specific succession of mutations that is then translated into a specific morphological sequence, starting with the formation of an adenoma and ending in the carcinoma state. The first mutation occurs in adenomatous polyposis coli (APC), a tumor suppressor gene, triggering the formation of non-
10 malignant adenomas, also called polyps. Approximately 15% of those adenomas are expected to be promoted to the carcinoma state within a period of ten years. This APC mutation is followed by mutations in KRAS, TP53 and, finally, DCC (deleted in colorectal cancer) (see Marmol 2017).

Genomic instability is an important feature underlying colorectal cancer. The pathogenic mechanisms leading to this situation can be included in three different pathways, namely
15 chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) (see Marmol 2017).

The CIN pathway, which is also considered to be the classical pathway since it represents the cause of up to 80%–85% of all CRC cases, is characterized by imbalances in the number of chromosomes, thus leading to aneuploidic tumors and loss of heterozygosity (LOH). The
20 mechanisms underlying CIN include alterations in chromosome segregation, telomere dysfunction and DNA damage response, which affect critical genes involved in the maintenance of correct cell function, such as APC, KRAS, PI3K, and TP53 amongst others. APC mutations cause the translocation of β -catenin to the nucleus and drive the transcription of genes implicated in tumourigenesis and invasion, whereas mutations in KRAS and PI3K lead to a constant activation
25 of MAP kinase, thus increasing cell proliferation. Finally, loss-of-function mutations in TP53, which encodes for p53, the main cell-cycle checkpoint, cause an uncontrolled entry in the cell cycle (Pino et al., The chromosomal instability pathway in colon cancer. *Gastroenterology*. 2010 Jun; 138(6):2059-72).

The Microsatellite instability pathway is caused by a hypermutable phenotype due to loss
30 of DNA repair mechanisms. The ability to repair short DNA chains or tandem repeats (two to five

base-pair repeats) is decreased in tumors with microsatellite instability; therefore, mutations tend to accumulate in those regions. These mutations can affect non-coding regions as well as codifying microsatellites, and tumors develop when reading frames of oncogenes or tumor suppressor genes codified in microsatellites are altered (see Marmol 2017). Loss of expression of mismatch repair genes (MMR) can be caused by spontaneous events (promoter hypermethylation) or germinal mutations such as those found in Lynch syndrome. These tumors are mainly diploid and harbor less LOH. Genes mutated in tumors with microsatellite instability include MLH1, MSH2, MSH6, PMS1 and PMS2. In general, MSI tumors have a better prognosis than sporadic tumors (Umar et al., Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004 Feb 18; 96(4):261-8). To access the MSI status of a cancer, a standard panel of five microsatellite markers, including two mononucleotide (BAT26 and BAT25) and three dinucleotide (D2S123, D5S346, and D17S250) repeats, has been recommended according to the Bethesda Guidelines. Tumors are then classified based on the number of microsatellites exhibiting instability. Particularly, tumors are classified as MSI high (MSI-H) when $\geq 30\%$ of the markers exhibit instability; those with $< 30\%$ markers exhibiting instability are defined as MSI low, and those with no apparent instability are microsatellite stable (MSS). It is now accepted that MSI is associated with post-replicative DNA MMR deficiency, primarily involving mutL homolog 1 (MLH1) and mutS homolog 2 (MSH2) (see Nguyen et al., The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy (Review) . Impairment of MMR genes can occur by either mutational inactivation or by epigenetic inactivation through CpG island methylation of the promoter of the genes. Loss or insufficiency of MMR activity leads to replication errors with an increased mutation rate and a higher potential for malignancy.

Epigenetic instability, which is responsible for the CpG island methylator phenotype, is another common feature in CRC. The main characteristic of CIMP tumors is the hypermethylation of oncogene promoters, which leads to genetic silencing and a loss of protein expression. Genetics and epigenetics are not exclusive in colorectal cancer, and both cooperate in its development, with more methylation events than point mutations often being found (Lao et al., Nat Rev Gastroenterol Hepatol. 2011 Oct 18; 8(12):686-700). One example of the combined effect of genetics and epigenetics in the colorectal cancer development process is the presence of BRAF mutations as

well as microsatellite instability in many CIMP tumors (Weisenberger et al., CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat. Genet.* 2006;38:787–793. doi: 10.1038/ng1834).

Methods for detecting MSI, CIN, and CIMP are well known in the art, and can be
5 determined from a tumor sample, the blood, and/or stool. A review of techniques for determining CRC mutational status is provided in Marmol et al., *Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer*. *Int. J. Mol. Sci.* 2017 Jan; 18(1):197, incorporated herein in its entirety by reference.

Colorectal cancers are generally CDK4/6 replication independent, although heterogeneity
10 does exist. CDK4/6-replication independent colorectal cancer refers to a CRC that does not significantly require the activity of CDK4/6 for replication. CDK4/6 replication independent cancers generally have a retinoblastoma gene (Rb1) aberration. The gene product of Rb1—Rb-protein—is a downstream target of CDK4/6. RB1 is commonly dysregulated in cancer cells through deletion, mutation or epigenetic modification resulting in loss of RB expression, as well
15 as by aberrant CDK kinase activity leading to excessive phosphorylation and inactivation of RB function (Chen et al. *Novel RB1-Loss Transcriptomic Signature Is Associated with Poor Clinical Outcomes across Cancer Types*. *Clin Cancer Res.* 2019;25(14); Sherr, C.J., and McCormick, F. *The RB and p53 pathways in cancer*. *Cancer Cell*, 2002;2:103–12.). CCNE1/2 (cyclin E) is part of a parallel pathway that provides functional redundancy with CDK4/6 and helps to transition cells
20 from the G1 to S phase. Overexpression will decrease the reliance on the CDK4/6 pathway leading to CDK4/6 independence (Turner et al., *Cyclin E1 Expression and Palbociclib Efficacy in Previously Treated Hormone Receptor-Positive Metastatic Breast Cancer*. *J Clin Oncol.* 2019;37(14):1169–78.). Therefore, a tumor with either CCNE1/2 amplification or RB loss will generally be considered “CDK4/6 independent”.

25 Due to its heterogeneity, a small portion of colorectal cancers are CDK4/6 replication dependent. Cancers that are CDK4/6 replication dependent require the activity of CDK4/6 for replication or proliferation. CDK 4/6 replication dependent CRCs generally have an intact and functional Rb pathway and/ increased expression of CDK4/6 activators (cyclin D), and/or a d-type cyclin activating features (DCAF) — including CCND1 translocation, CCND1-3 3’UTR loss, and
30 amplification of CCND2 or CCND3 (see Gong et al. *Genomic aberrations that activate D-type*

cyclins are associated with enhanced sensitivity to the CDK4 and CDK5 inhibitor abemaciclib. Cancer Cell. 2017;32(6):761-76). Tumors that are wildtype for RB and CCNE1/2 as well as have one of the DCAF described above are generally classified as “CDK4/6 dependent”.

5 Tumors that cannot be classified as either CDK4/6-replication dependent or CDK4/6-replication independent is generally classified as “CDK4/6 indeterminate” since they cannot be confirmed as CDK4/6 dependent or independent.

10 Methods of determining CDK4/6 genetic signature analysis are known in the art and involve the utilization of tumor tissue collected from a patients’ biopsy (colorectal primary or metastatic site) and are described in Shapiro GI. Genomic biomarkers predicting response to selective CDK4/6 inhibition: Progress in an elusive search. Cancer Cell. 2017;32(6):721-3 and Gong et al. Genomic aberrations that activate D-type cyclins are associated with enhanced sensitivity to the CDK4 and CDK5 inhibitor abemaciclib. Cancer Cell. 2017;32(6):761-76.

15 The use of anti-VEGF or anti-EGFR targeting agents in colorectal cancer is dependent on the mutational status of KRAS (Kirsten rat sarcoma 2 viral oncogene homolog) and BRAF (v-Raf murine sarcoma viral oncogene homolog B) in the tumor. The use of anti-EGFR targeting agents is generally limited to those having wild-type KRAS and BRAF status. Comparatively, the use of anti-VEGF agents is not dependent on KRAS and BRAF status. Methods for the assessment of BRAF and KRAS mutations are generally known in the art. Several methods have been developed and are currently used for the detection of BRAF and KRAS mutations, including High-Resolution
20 Melting curve analysis (HRM)/Sanger sequencing, pyrosequencing, mutation-specific Polymerase Chain Reaction (PCR) and mutation-specific real-time PCR (Cobas), immunohistochemistry (IHC), digital droplet PCR (ddPCR), Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS; Sequenom), and Next-Generation Sequencing (NGS). These methods are well known in the art and are described in Vanni I et al., The Current State of
25 Molecular Testing in the BRAF-Mutated Melanoma Landscape. Front Mol Biosci. 2020;7:113. Published 2020 Jun 30. doi:10.3389/fmolb.2020.00113.

Accordingly, the methods described herein are directed to the treatment of a subject with colorectal cancer. In some embodiments, the colorectal cancer is colorectal adenocarcinoma. In some embodiments, the colorectal cancer is metastatic colorectal cancer (mCRC). In some
30 embodiments, the colorectal cancer is proficient mismatch repair (pMMR) mCRC. In some

embodiments, the CRC is not pMMR. In some embodiments, the colorectal cancer is microsatellite stable (MSS) CRC. In some embodiments, the colorectal cancer is MSI-Hi CRC. In some embodiment, the colorectal cancer is MSI-Lo CRC. In some embodiments, the colorectal cancer is pMMR/MSS mCRC. In some embodiments, the colorectal cancer is pMMR/MSS mCRC and has not been responsive to previous treatment with single agent checkpoint inhibitors. In some embodiments, the colorectal cancer has a mutation, chromosomal change, or translocation which affects one or more of the WNT, MAPK/PI3K, TGF- β , or TP53 pathways. In some embodiments, the colorectal cancer has a mutation in a gene selected from c-MYC, KRAS, NRAS, HRAS, BRAF, PIK3CA, PTEN, SMAD2, or SMAD4. In some embodiments, the mCRC has a BRAF or KRAS mutation. In some embodiments, the BRAF mutation is V600E substitution mutation. In some embodiments, the KRAS mutation is a V9, G12, G13, V14, L19, Q22, D33, A59, G60, Q61 R68, K117, A146, R164, K176, or K180 substitution. In some embodiments, the KRAS mutation is a G12D, G12V, G12C, G12A, or other G12 variant. In some embodiments, the NRAS mutation is selected from G12, G13, G60, Q61, E123, or P185. In some embodiments, the HRAS mutation is G12, G13, Q61, K117, R164, or P167. In some embodiments, the colorectal cancer is KRAS wild-type. In some embodiments, the colorectal cancer is BRAF wild-type.

In alternative embodiments, the cancer to be treated according to the protocols described herein is pancreatic cancer. In some embodiments, the pancreatic cancer is metastatic or advanced pancreatic cancer.

In an alternative embodiment, the cancer to be treated according to the protocols described herein is gastric cancer. In some embodiments, the gastric cancer is metastatic or advanced gastric cancer.

In an alternative embodiment, the cancer to be treated according to the protocols described herein is gastroesophageal junction adenocarcinoma. In some embodiments, the gastroesophageal junction adenocarcinoma is metastatic or advanced gastroesophageal junction adenocarcinoma.

In an alternative embodiment, the cancer to be treated according to the protocols described herein is biliary tract cancer (cholangiocarcinoma), including intrahepatic cholangiocarcinoma, hilar cholangiocarcinoma, and distal cholangiocarcinoma. In some embodiments, the biliary tract cancer is metastatic or advanced biliary tract cancer.

In an alternative embodiment, the cancer to be treated according to the protocols described herein is neuroendocrine carcinoma. In some embodiments, the neuroendocrine carcinoma is metastatic or advanced neuroendocrine carcinoma.

5 In an alternative embodiment, the cancer to be treated according to the protocols described herein is peritoneal carcinosis. In some embodiments, the peritoneal carcinosis is metastatic or advanced peritoneal carcinosis.

10 In an alternative embodiment, the cancer to be treated according to the protocols described herein is liver cancer, including but not limited to hepatocellular carcinoma (HCC). In some embodiments, the liver cancer, including but not limited to hepatocellular carcinoma, is metastatic or advanced.

Immunologically Susceptible Colorectal Cancer

15 In some embodiments, the colorectal cancer to be treated is an immunologically susceptible cancer. In some embodiments, the cancer that is highly immunogenic or “hot,” or alternatively “altered-immunosuppressed.” In some embodiments, the colorectal cancer to be treated has a high-immunoscore. In some embodiments, the subject being treated has a colorectal cancer that exhibits specific characteristics as described herein according to the Ayer’s interferon- γ signature, the Ayer’s expanded immune signature or the Thorsson et al Six Class Immune Signature. In some
20 embodiments, the cancer is interferon- γ (IFN- γ) dominant according to Thorsson’s Six Class Immune Signature, or has a high IFN- γ signature or expanded immune signature according to the Ayer’s IFN- γ Signature Score or Expanded Immune Signature Score. In some embodiments, the cancer is PD-L1 positive.

25 Specifically, progression free survival and/or overall survival can be improved when a cancer that is highly immunogenic, for example, a hot tumor (as defined in Galon, J., and Bruni, D., Approaches to treat immune hot, altered and cold tumours with combination immunotherapies”, Nature Reviews Drug Discovery (18), March 2019, 197-218 incorporated herein by reference and discussed further below), high IFN- γ expression, or other acceptable indicator of immunogenic susceptibility is treated with a chemotherapy that causes an immune-mediated response including, but not limited to, immunogenic cell death and/or regulatory T-cell
30 (Treg cell) suppression, in combination with a short acting CDK4/6 inhibitor administered at least

prior to the administration of the chemotherapy or alternatively, administered both prior to and concurrently with the chemotherapy. When a cancer therapy includes these three components in the appropriate dosage regimen, there is an immuno-oncology effect that promotes progression free survival and/or overall survival by alteration of the milieu of T-cells away from an immunosuppressive environment (i.e., Treg cells) and toward an enhancement of T-cell activity and an increase in cytotoxic T cells (CD8⁺ cells).

As provided herein, hot immune tumors are those that have (i) a high degree of T-cell and cytotoxic T cell infiltration, i.e., a high immunoscore; and (ii) ability for checkpoint activation (programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated antigen 4 (CTLA4), T-cell immunoglobulin mucin receptor 3 (TIM3) and lymphocyte activation gene 3 (LAG3)) or otherwise impaired T-cell functions (for example, extracellular potassium-driven T-cell suppression). In addition to presence of tumor-infiltrating lymphocytes (TILs) and the expression of anti-programmed death-ligand 1 (PD-L1) on tumor-associated immune cells, hot tumors characteristically display possible genomic instability and the presence of a pre-existing antitumor immune response. See, e.g., in Galon, J., and Bruni, D., Approaches to treat immune hot, altered and cold tumors with combination immunotherapies, *Nature Reviews Drug Discovery* (18), March 2019, 197-218, incorporated herein in its entirety. An exemplified resected tumor having the characteristics of a hot immune tumor is illustrated in Fig. 7A.

Altered-immunosuppressed tumors are categorized by (i) poor, albeit not absent, T-cell and cytotoxic T-cell infiltration (intermediate immunoscore), (ii) presence of soluble inhibitory mediators (transforming growth factor- β (TGF β), interleukin 10 (IL-10) and vascular endothelial growth factor (VEGF)), (iii) the presence of immune suppressive cells (myeloid-derived suppressor cells and regulatory T-cells), and (iv) presence of T-cell checkpoints (PD-1, CTLA4, TIM3 and LAG3). Altered-immunosuppressed tumor sites display a low degree of immune infiltration (FIG. 7A), which suggests the absence of physical barriers and the presence of an immuno-suppressive environment that limits further T-cell recruitment and expansion. See, e.g., in Galon, J., and Bruni, D., Approaches to treat immune hot, altered and cold tumors with combination immunotherapies, *Nature Reviews Drug Discovery* (18), March 2019, 197-218, incorporated herein in its entirety. An exemplified resected tumor having the characteristics of an altered-immunosuppressed immune tumor is illustrated in Fig. 7A.

The determination of the immunogenic classification of a tumor can be carried out on resected tumors (primary or metastatic) (see e.g., Fig. 7A). Less invasive diagnostic procedures, such as immuno-positron-emission tomography (PET) imaging detecting intratumoral CD8⁺ T-cells can also be used. For a description of immuno-PET detection of intratumoral CD8⁺ T-cells, see Rooney et al., Molecular and genetic properties of tumors associated with local immune cytolytic activity, *Cell* 160, 48–61 (2015), incorporated herein by reference.

In addition to the above, bulk gene expression profiling can be used to determine the immunogenic classification of a tumor (see Ali et al., Patterns of immune infiltration in breast cancer and their clinical implications: a gene- expression-based retrospective study, *PLOS Med.* 13, e1002194 (2016); Newman et al., Robust enumeration of cell subsets from tissue expression profiles, *Nat. Methods* 12, 453–457 (2015); Rooney et al., Molecular and genetic properties of tumors associated with local immune cytolytic activity, *Cell* 160, 48–61 (2015), Bindea, G. et al., Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 39, 782–795 (2013), each of which is incorporated herein by reference).

Multiple additional tools, such as CIBERSORT (which infers the relative fractions of immune subsets in the total leukocyte population), xCell (which predicts the abundance of immune cells in the overall TME), TIMER (which generates enrichment scores on the basis of proportions among 64 immune and stromal cell types) and integrated immunogenomics methods (using a CIBERSORT- based approach, which, of note, identified six immune subtypes of cancer) can be used to estimate the abundance of intra-tumoral immune infiltrates by using deconvolution of bulk gene expression data (see Newman et al., Robust enumeration of cell subsets from tissue expression profiles, *Nat. Methods* 12, 453–457 (2015); Gentles et al., The prognostic landscape of genes and infiltrating immune cells across human cancers, *Nat. Med.* 21, 938–945 (2015); Aran et al., xCell: digitally portraying the tissue cellular heterogeneity landscape, *Genome Biol.* 18, 220 (2017); Li et al., TIMER: a web server for comprehensive analysis of tumor- infiltrating immune cells, *Cancer Res.* 77, e108–e110 (2017); Thorsson, V. et al., The immune landscape of cancer. *Immunity* 48, 812–830 (2018), each incorporated herein by reference).

In some embodiments, the cancer to be treated has a high immunoscore. Immunoscore is a digital pathology, IHC-based immune assay measuring the densities of CD3⁺ and CD8⁺ T cells at different tumor locations. The Immunoscore scoring has been defined in a large international

SITC-led retrospective validation study conducted on more than 2500 St I-III colon cancer patients (see Pagès et al, International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study, *The Lancet* Volume 391, ISSUE 10135, P2128-2139, May 26, 2018, incorporated herein by reference). Commercial Immunoscore assays are available through, for example, HalioDx, Inc. (Richmond, Va). Briefly, CD3- and CD8-immunostained formalin-fixed, paraffin-embedded (FFPE) slides are scanned and the two corresponding digital images validated by the operator. Image analysis is performed via a dedicated software (Immunoscore Analyzer, HalioDx): automatic detection of the tissue histologic structure is followed by an operator-guided definition of the tumor, healthy tissue (submucosa, muscularis propria, serosa), and the epithelium (mucosa). The operator also excludes all areas of necrosis, abscess, and artifacts (bubbles folds, torn areas, background) to avoid false positives. The IM, spanning 360 μ m into the healthy tissue and 360 μ m into the tumor, is calculated automatically by the software. In the presence of multiple FFPE blocks, the one to select for the Immunoscore evaluation is the one containing the IM.

In some embodiments, the cancer to be treated has a high IFN- γ Signature of Expanded Immune Signature, for example as described in Ayers et al. Ayers M, et al. "IFN- γ -Related MRNA Profile Predicts Clinical Response to PD-1 Blockade." *Journal of Clinical Investigation*, vol. 127, no. 8, 2017, pp. 2930–2940., doi:10.1172/jci91190 (incorporated herein by reference in its entirety), who outline a thorough, iterative approach to building a gene expression signature predictive of response to immune checkpoint inhibitors (e.g., pembrolizumab). Starting in melanoma data, one-sided t-tests were used to detect differentially expressed genes between responders and non-responders to pembrolizumab. Noting that many of these differentially expressed genes were associated with IFN- γ signaling, Ayers et al. developed a preliminary IFN- γ signature for ICI response by averaging expression within the IFN- γ pathway and its correlated genes.

The robustness of the preliminary signatures of response was assessed in additional melanomas, HNSCCs and gastric cancers. Preliminary signatures displayed significant association with BOR and PFS, but not OS. To improve prediction in non-melanoma cancers, the immune signatures were trimmed and expanded by assessing univariate associations between individual genes and BOR and PFS within the expanded cohort. Genes with statistically

insignificant associations were pruned from the signature and genes with significant associations were added to form intermediate IFN-gamma signatures.

Citing the success of the preliminary and intermediate IFN-gamma signatures in distinguishing responders and non-responders to anti-PD1 therapies as proof-of-concept for the extension of such predictive signatures to multiple cancer types, a final signature for multiple cancer types was developed using a wide array of samples from KEYNOTE-012 (ClinicalTrials.gov identifier: NCT01848834) and KEYNOTE-028 trials (ClinicalTrials.gov identifier: NCT02054806). Penalized logistic regression on BOR was used to further limit the intermediate signatures to a final set of 18 PD-1/PD-L1-response related genes.

The IFN- γ Signature analysis consists of determining the expression profile of six genes: IDO1, CXCL10, CXCL9, HLA-DRA; STAT1, and IFN- γ . The Expanded Immune Signature analysis consists of determining the expression profile of 18 genes: CCL5, CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DRB1, HLA-DQA1, HLA-E, IDO1, LAG3, NKG7, PDCD1LG2, PSMB10, STAT1, and TIGIT.

Ayers et al. performed sequencing quantitation using a 680 gene panel on the Nanostring platform. To compute a sample's score for either multi-gene signature (IFN- γ Signature or Expanded Immune Signature), quantile normalization is performed prior to a log10 transformation and subsequent averaging across the gene-set. Calculation of the area under the ROC curve was used as a measure of discriminatory ability for the signature scores. The Youden index, a summary measure of the ROC curve (see Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32–35, incorporated herein by reference), was used as an agnostic method for choosing an “optimal” cutoff, that is “high”/“low” on the signature scores to illustrate potential clinical usefulness. For example, a “high” IFN- γ Signature or Expanded Immune Signature can be determined based on comparison to scores of known immunogenic samples. In some embodiments, a “high” IFN- γ Signature or Expanded Immune Signature score is one that is scored greater than at least 2.25, 2.5, or 2.75. In some embodiments, a “high” IFN- γ Signature or Expanded Immune Signature score is one that is scored greater than at least 2.5.

In some embodiments, the cancer to be treated is IFN- γ Dominant in a Six Class Immune Signature category as described in as described by Thorsson et al., “The Immune Landscape of Cancer.” Immunity, vol 51, no. 2, 2018, pp. 812-830 (incorporated herein by reference in its

entirety). Thorsson et al. performed an extensive literature search for expression signatures which characterized various facets of the immune response. This search resulted in 160 signatures for examination. Weighted Gene Correlation Network Analysis (WGCNA) over the entire TCGA (The Cancer Genome Atlas) dataset was used to cluster these 160 signatures into 9 distinguishable signature-modules, or collections of signatures purported to measure consistent immune phenomena. Candidate signatures were then restricted to the signatures which most closely represented the “average profile” from each of the 9 modules.

Further investigation identified that 4 of these 9 representative signatures were not robust classifiers for the TCGA data and resulted in highly variable classifications dependent upon which samples were utilized for model training. This left 5 immune signatures for use in sample classifications, which are identified in Table 1 below.

Table 1: Thorsson et al. Signature Label

Signature Label	Signature Label
CSF1_Response	Representative of activation of macrophages and monocytes
LIexpression_Score	Representative of overall lymphocyte infiltration
TGF- β	Representative of TGF- β response
Module3_IFN_Score	Representative of IFN- γ response
CHANG_CORE_SERUM_RESPONSE_UP	Representative of wound healing (i.e. angiogenesis, etc)

Scores for each of these five signatures were computed across the TCGA dataset using single sample Gene Set Enrichment Analysis (ssGSEA). These data were then clustered using an unsupervised, consensus clustering approach resulting in six identified immune response subtypes, as described in Table 2.

Table 2: Thorsson et al. Six Class Immune Signature

Group	Description
C1	Wound Healing: High proliferation rate, high angiogenesis gene expression, TH2 bias
C2	IFN- γ Dominant : High M1/M2 polarization, Strong CD8 and high TCR diversity
C3	Inflammatory:

	Elevated Th17 and Th1 genes, low to moderate proliferation, low aneuploidy and overall somatic copy number alterations.
C4	Lymphocyte Depleted: Prominent macrophage signature with Th1 suppression and high M2 response
C5	Immunologically Quiet: Low lymphocyte response, High macrophage response dominated by M2.
C6	TGF- β Dominant: Mixed tumour subgroup with high TGF- β and lymphocytic infiltration

In some embodiments, the cancer to be treated is programmed death-1 ligand (PD-L1) positive. PD-L1 is a transmembrane protein that down-regulates immune responses through binding to its two inhibitory receptors, programmed death-1 (PD-1) and B7.1. PD-1 is an inhibitory receptor expressed on T cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer (Blank, C and Mackensen, A, Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. *Cancer Immunol Immunother*, 2007. 56(5): p. 739-745). Binding of PD-L1 with PD-1 inhibits T cell proliferation, cytokine production and cytolytic activity, leading to the functional inactivation or exhaustion of T cells. B7.1 is a molecule expressed on antigen presenting cells and activated T cells. PD-L1 binding to B7.1 on T cells and antigen presenting cells can mediate down-regulation of immune responses, including inhibition of T-cell activation and cytokine production (see Butte MJ, Keir ME, Phamduy TB, et al. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity*. 2007;27(1):111-122). PD-L1 expression has been observed in immune cells and tumor cells. See Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med*. 1999;5(12):1365-1369; Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515(7528):563-567. Aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion. Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T cell immunity suppressed by the expression of PD-L1 in the tumor microenvironment. In cancer, the upregulation of PD-L1 may allow cancers to evade the host immune system.

PD-L1 expression can be determined by methods known in the art. For example, PD-L1 expression can be detected using PD-L1 IHC 22C3 pharmDx, the FDA-approved *in vitro* diagnostic immunohistochemistry (IHC) test developed by Dako and Bristol-Meyers Squibb as a companion test for treatment with pembrolizumab. This is qualitative assay using Monoclonal
 5 Mouse Anti-PD-L1, Clone 22C3 PD-L1 and EnVision FLEX visualization system on Autostainer Lin 48 to detect PD-L1 in formalin-fixed, paraffin-embedded (FFPE) human non-small cell lung cancer tissue. Expression levels can be measured using the tumor proportion score (TPS), which measures the percentage of viable tumor cells showing partial or complete membrane staining. Staining can show PD-L1 expression from 1% to 100%.

10 PD-L1 expression can also be detected using PD-L1 IHC 28-8 pharmDx, the FDA-approved *in vitro* diagnostic immunohistochemistry (IHC) test developed by Dako and Merck as a companion test for treatment with nivolumab. This qualitative assay uses the Monoclonal rabbit anti-PD-L1, Clone 28-8 and EnVision FLEX visualization system on Autostainer Lin 48 to detect PD-L1 in formalin-fixed, paraffin-embedded (FFPE) human non-small cell lung cancer tissue.

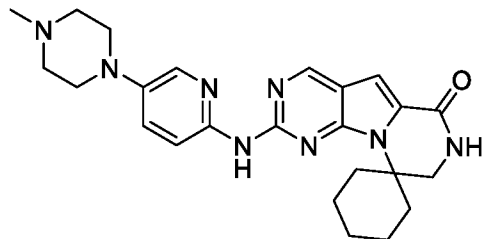
15 Other commercially available tests for PD-L1 detection include the Ventana SP263 assay (developed by Ventana in collaboration with AstraZeneca) that utilizes monoclonal rabbit anti-PD-L1, Clone SP263 and the Ventana SP142 Assay (developed by Ventana in collaboration with Genentech/Roche) that uses rabbit monoclonal anti-PD-L1 clone SP142. Determination of PD-L1 status is indication-specific, and evaluation is based on either the proportion of tumor area
 20 occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity or the percentage of PD-L1 expressing tumor cells (% TC) of any intensity. For example, PD-L1 expression in $\geq 5\%$ IC determined by, for example, the Ventana PD-L1 (SP142) Assay in urothelial carcinoma tissue, whereas a PD-L1 positive status in TNBC is considered $\geq 1\%$ IC and NSCLC is considered $\geq 50\%$ TC or $\geq 10\%$ IC.

25 In some embodiments, the colorectal cancer to be treated is a PD-L1 positive colorectal cancer having PD-L1 expression $\geq 1\%$ IC. In some embodiments, the colorectal cancer to be treated is a PD-L1 positive colorectal cancer having PD-L1 expression $\geq 5\%$ IC

Components of Fluorouracil-Based Multi-Agent Chemotherapy Regimens

30 *Trilaciclib*

Trilaciclib (2'-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)-7',8'-dihydro-6'H-spiro(cyclohexane-1,9'-pyrazino(1',2':1,5)pyrrolo(2,3-d)pyrimidin)-6'-one) is a highly selective CDK4/6 inhibitor having the structure:



5 As provided herein, trilaciclib or its pharmaceutically acceptable salt, composition, isotopic analog, or prodrug thereof is administered in a suitable carrier. Trilaciclib has been approved by the U.S. FDA for the treatment of extensive-stage small cell lung cancer to decrease the incidence of chemotherapy-induced myelosuppression in patients when administered prior to a platinum/etoposide-containing regimen or topotecan-containing regimen, and marketed as under
10 the brand name Cosela® by G1 Therapeutics, Inc. In some embodiments, trilaciclib is provided as a hydrochloride salt, for example, a dihydrochloride salt. In some embodiments, the trilaciclib is reconstituted form the dihydrochloride dihydrate, which may be in lyophilized form.

Trilaciclib is described in US 2013-0237544, incorporated herein by reference in its entirety. Trilaciclib can be synthesized as described in or US 2019-0135820, incorporated herein
15 by reference in its entirety. Trilaciclib can be administered in any manner that achieves the desired outcome, including systemically, parenterally, intravenously, intramuscularly, subcutaneously, or intradermally. For injection, trilaciclib may be provided, in some embodiments, for example, as a 300 mg/vial as a sterile, lyophilized, yellow cake providing 300 mg of trilaciclib (equivalent to 349 mg of trilaciclib dihydrochloride, dihydrate). The product, for example, may be supplied in
20 single-use 20-mL clear glass vials which does not contain a preservative. For example, prior to administration, trilaciclib for injection, 300 mg/vial may be reconstituted with 19.5 ml of 0.9% sodium chloride injection or 5% dextrose injection. This reconstituted solution has a trilaciclib concentration of 15 mg/mL and would typically be subsequently diluted prior to intravenous administration. Trilaciclib can be administered intravenously as described herein. In some
25 embodiments, trilaciclib is administered at between about 180 mg/m² and 300 mg/m². In some embodiments, trilaciclib is administered at about 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 285, or about 300 mg/m². In

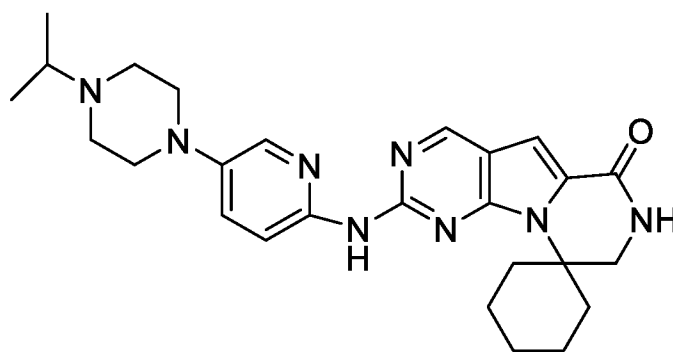
some embodiments, trilaciclib is administered at about 240 mg/m², prior to administration of the other fluorouracil-based multi-agent chemotherapeutic agents, for example prior to about 4 hours or less, for example about 4 hours or less, 3 hours or less, 2 hours or less, about 1 hour or less, or about 30 minutes prior to administration of any of the other fluorouracil-based multi-agent chemotherapeutic agents. In some embodiments, trilaciclib is administered intravenously over a period of about 30 minutes.

As provided herein, trilaciclib is administered on day 1 of each 14-day cycle during the induction and maintenance phase, or as otherwise provided herein. Trilaciclib is administered prior to the initiation of administration of the other chemotherapeutic agents, generally via intravenous injection/infusion over about 30 minutes about 4 hours or less, for example about 3 hours or less, 2 hours or less, 1 hour or less, or about 30 minutes prior to the initiation of the administration of the other agents in the protocol. In some embodiments, the trilaciclib is completely administered on day 1 prior to initiation of the administration any of the other fluorouracil-based multi-agent chemotherapy agents.

Trilaciclib is also administered on day 2 of each 14-day cycle during the induction and optionally during the maintenance phase, if 5-FU is administered over a two-day period as a continuous infusion, or as otherwise provided herein. Trilaciclib can be administered on day 2 between about 16 and 26 hours after its administration on day 1. In a particular embodiment, trilaciclib is administered on day 2 within about 24 hours after administration of trilaciclib on day 1. In some embodiments, trilaciclib is administered on day 2 about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 hours after its administration on day 2. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5-FU is at sufficient concentrations such that 5-FU-related myelosuppression is exacerbated rather than mitigated.

In an alternative embodiment, a different CDK4/6 inhibitor is administered. For example, in alternative embodiments, the CDK4/6 inhibitor used instead of trilaciclib in the protocols described herein is ribociclib (Novartis), palbociclib (Pfizer), or abemaciclib (Eli Lilly), or

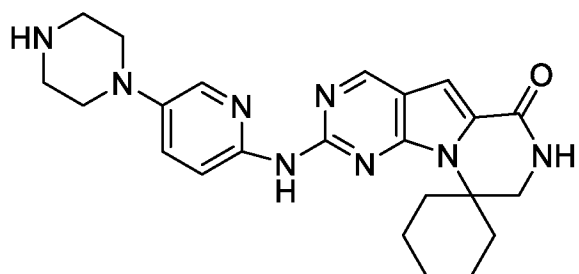
pharmaceutically acceptable salts thereof. In an additional alternative embodiment, the CDK4/6 inhibitor is lerociclib, which has the structure:



, or

or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof, which is described in US 2013-0237544, incorporated herein by reference in its entirety, and can be synthesized as described in US 2019-0135820, incorporated herein by reference in its entirety.

10 In an additional alternative embodiment, the CDK4/6 inhibitor is lerociclib, which has the structure:

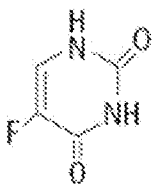


,

or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof, which is described in US 2013-0237544, incorporated herein by reference in its entirety, and can be synthesized as described in US 2019-0135820, incorporated herein by reference in its entirety.

Fluorouracil (5-FU or 5FU)

Fluorouracil, also known as FU, 5FU, or 5-FU, is one of the most commonly used drugs to treat colorectal cancer, and forms the basis of many of the multi-agent chemotherapies used in colorectal treatment. Fluorouracil is an antimetabolite fluoropyrimidine analog of the nucleoside pyrimidine with antineoplastic activity. Fluorouracil and its metabolites possess a number of different mechanisms of action. In *vivo*, fluorouracil is converted to the active metabolite 5-fluoroxymidine monophosphate (F-UMP); replacing uracil, F-UMP incorporates into RNA and inhibits RNA processing, thereby inhibiting cell growth. Another active metabolite, 5-fluoro-2'-deoxyuridine-5'-O-monophosphate (F-dUMP), inhibits thymidylate synthase, resulting in the depletion of thymidine triphosphate (TTP), one of the four nucleotide triphosphates used in the *in vivo* synthesis of DNA. Other fluorouracil metabolites incorporate into both RNA and DNA; incorporation into RNA results in major effects on both RNA processing and functions. Fluorouracil is sold under such brand names as Adrucil among others. Fluorouracil is a fluorinated pyrimidine and has the chemical designation 5-fluoro-2,4 (1H,3H)-pyrimidinedione. The structure of fluorouracil is:



Fluorouracil is contra-indicated for patients with low or absent dihydropyrimidine dehydrogenase (DPD) activity and is associated with a host of toxicities, including:

- Cardiotoxicity: Fluorouracil can cause cardiotoxicity, including angina, myocardial infarction/ischemia, arrhythmia, and heart failure;
- Hyperammonemic Encephalopathy: Altered mental status, confusion, disorientation, coma, or ataxia with elevated serum ammonia level can occur within 72 hours of initiation of fluorouracil;
- Neurologic Toxicity: Fluorouracil can cause acute cerebellar syndrome, confusion, disorientation, ataxia, or visual disturbances.;
- Diarrhea (severe);

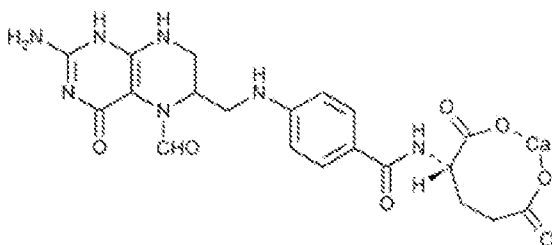
- Palmar-Plantar Erythrodysesthesia (Hand-Foot Syndrome; HFS): Symptoms of HFS include a tingling sensation, pain, swelling, and erythema with tenderness, and desquamation;
- Myelosuppression (severe and potentially fatal); and,
- Mucositis (severe).

In some embodiments, the use of 5-FU in the described embodiments is substituted with an alternative fluoropyrimidine. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU. In some alternative embodiments, a 5-FU prodrug is selected from mono, di, or tri-phosphate 5-FU prodrug, capecitabine, tegafur, tegafur-uracil, tegafur/gimeracil/oteracil, carmofur, doxifluridine, or 5-fluoro-2'-deoxyuridine. In some alternative embodiments, a 5-FU prodrug is an FUDR-phosphoramidate. In some alternative embodiments, the use of 5-FU in the described embodiments is substituted with capecitabine. In some alternative embodiments, the use of 5-FU in the described embodiments is substituted with tegafur. In some embodiments, the use of 5-FU in the described embodiments is substituted with tegafur-uracil (UFT). In some embodiments, the use of 5-FU in the described embodiments is substituted with S-1 (tegafur, gimeracil, and oteracil). In some alternative embodiments, the use of 5-FU in the described embodiments is substituted with carmofur (HCFU). In some alternative embodiments, the use of 5-FU in the described embodiments is substituted with doxifluridine. In some alternative embodiments, the use of 5-FU in the described embodiments is substituted with 5-fluoro-2'-deoxyuridine (5FUdR).

Folinic Acid

The fluorouracil-based multi-agent chemotherapies provided herein generally include the use of a folinic acid. Folinic acid is a 5-FU modulating agent which inhibits thymidylate synthase.

Leucovorin, also known as folinic acid, citrovorum factor, calcium folinate, or 5-formyl-5,6,7,8-tetrahydrofolic acid, or leucovorin calcium, has the chemical designation of Calcium N-[p-[[[(6RS)-2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridiny]methyl]amino]benzoyl]-L-glutamate (1:1). Leucovorin is sold under such brand names as Wellcovorin among others. The structural formula of leucovorin calcium is:



Leucovorin is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid (THF). The biologically active compound of the mixture is the (-)-l-isomer, known as Citrovorum factor or (-)-folinic acid. Leucovorin potentiates the cytotoxic effects of 5-FU in the cell (de Gramont et al., Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J. Clin. Oncol.* 2000 Aug;18(16):2938-47). In the cell, 5-FU converts to fluoro-deoxy uridylic acid, a molecule that functions to inhibit thymidylate synthase. Thymidylate synthase is an enzyme that is important in DNA repair and replication. The functional derivative of folinic acid, 5,10 methylenetetrahydrofolate stabilizes the bound fluoro-deoxy uridylic acid to thymidylate synthase. This interaction yields a ternary complex known as the thymidylate synthase 5-fluorodeoxyuridine monophosphate-methylenetetrahydrofolate complex, which ultimately functions to inhibit thymidylate synthase. Increased cellular amounts of functional folinic acid derivatives lead to increased stability of the aforementioned inhibitory complex, which leads to a depletion of thymidylate synthesis and disrupts DNA synthesis and repair (Priest et al., Pharmacokinetics of leucovorin metabolites in human plasma as a function of dose administered orally and intravenously. *J. Natl. Cancer Inst.* 1991 Dec 18;83(24):1806-120).

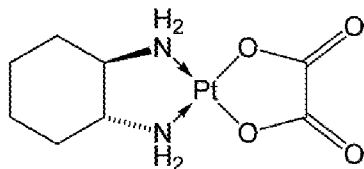
Leucovorin can also be administered as the disodium salt sodium leucovorin.

Levoleucovorin is the levo isomeric form of racemic d,l-leucovorin. Levoleucovorin is the pharmacologically active isomer of leucovorin [(6-S)-leucovorin]. Levoleucovorin is sold under such brand names as Fusilev and Kapzory. Levoleucovorin, when substituted in place of leucovorin, is generally dosed at one-half the usual dose of leucovorin. Levoleucovorin can be administered as a calcium or disodium salt.

In alternative embodiments, a folinate salt with a similar mechanism of action to the folinic acid compounds above are administered.

Oxaliplatin

Oxaliplatin, also sold under various brand names such as Eloxatin, is an antineoplastic agent with the molecular formula $C_8H_{14}N_2O_4Pt$ and the chemical name of cis-[(1 R,2 R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum. Oxaliplatin is an organoplatinum complex in which the platinum atom is complexed with 1,2-diaminocyclohexane (DACH) and with an oxalate ligand as a leaving group, and has the chemical structure:



Oxaliplatin undergoes nonenzymatic conversion in physiologic solutions to active derivatives via displacement of the labile oxalate ligand. Several transient reactive species are formed, including mono-aquo and di-aquo DACH platinum, which covalently bind with macromolecules. Both inter-and intra-strand Pt-DNA crosslinks are formed. Crosslinks are formed between the N7 positions of two adjacent guanines (GG), adjacent adenine-guanines (AG), and guanines separated by an intervening nucleotide (GNG). These crosslinks inhibit DNA replication and transcription. Cytotoxicity is cell-cycle nonspecific.

Oxaliplatin is associated with high rates of gastrointestinal toxicity such as nausea/vomiting, diarrhea, abdominal pain and stomatitis. Additionally, the following are important risks related to oxaliplatin use:

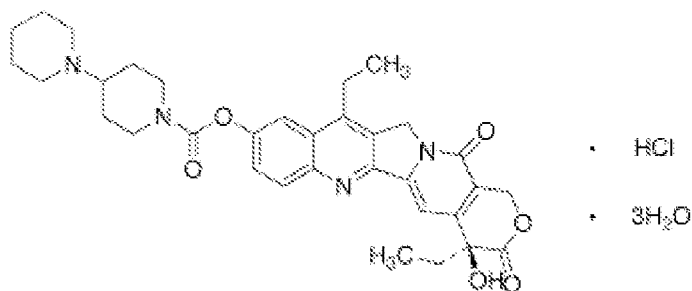
- Anaphylactic reactions to oxaliplatin have been reported, can be fatal, and may occur within minutes of oxaliplatin administration.
- Oxaliplatin is associated with two types of neuropathy:
 - An acute, reversible, primarily peripheral, sensory neuropathy that is of early onset, occurring within hours or one to two days of dosing, that resolves within 14 days, and that frequently recurs with further dosing.
 - A persistent (> 14 days), primarily peripheral, sensory neuropathy that is usually characterized by paresthesias, dysesthesias, hypoesthesias, but may also include deficits in proprioception that can interfere with daily activities

(e.g., writing, buttoning, swallowing, and difficulty walking from impaired proprioception).

- Reversible Posterior Leukoencephalopathy Syndrome (RPLS, also known as PRES, Posterior Reversible Encephalopathy Syndrome) has been observed in clinical trials (< 0.1%) and post-marketing experience.
- Severe neutropenia (Grade 3 or 4).

Irinotecan

Irinotecan, also sold under various brand names such as Campostar, is an antineoplastic agent of the topoisomerase I inhibitor class. The chemical name is (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate, and has the structure:



Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I, which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent re-ligation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

The following are important risks related to irinotecan use:

- Early and late forms of diarrhea can occur. Early diarrhea may be accompanied by cholinergic symptoms. Late diarrhea can be life threatening.
- Severe myelosuppression may occur.

- Individuals who are homozygous for the UGT1A1*28 allele (UGT1A1 7/7 genotype) are at increased risk for neutropenia.
- Renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhea.

5 In some alternative embodiments, irinotecan is administered as a liposomal dispersion (irinotecan liposomal; Onivyde).

Anti-VEGF Compounds

10 The combination of fluorouracil-based multi-agent chemotherapies with an anti-VEGF compound has become a common standard of care for a number of cancers, including colorectal cancer. Anti-VEGF monoclonal antibodies include bevacizumab (Avastin, Genentech) or bevacizumab-awwb (Mvasi, Amgen). Bevacizumab is vascular endothelial growth factor directed antibody. Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that contains human framework regions and murine complementarity-determining regions (CDRs).

15 Bevacizumab binds VEGF and prevents the interaction of VEGF to its receptors (Flt-1 and KDR) on the surface of endothelial cells. The interaction of VEGF with its receptors leads to endothelial cell proliferation and new blood vessel formation in in vitro models of angiogenesis. Administration of bevacizumab causes reduction of microvascular growth and inhibition of metastatic disease progression.

20 Bevacizumab-awwb has a similar mechanism of action and is a biosimilar of bevacizumab.

In fluorouracil-based multi-agent chemotherapy protocols, bevacizumab is generally administered on day 1 of each 14-day cycle of the induction phase and maintenance phase, or once every 2 weeks, as an intravenous injection at a dose of between about 4 and 12 mg/kg, with dosing adjustments as required. In some embodiments, bevacizumab is administered at about 5 mg/kg.

25 In some embodiments, bevacizumab is administered at about 10 mg/kg.

Bevacizumab has been associated with GI toxicity including nausea, diarrhea, and stomatitis as well as bone marrow suppression. The following are important risks related to bevacizumab use:

- Serious, and sometimes fatal, gastrointestinal perforation.

- Serious and sometimes fatal non-gastrointestinal fistula formation involving tracheo-esophageal, bronchopleural, biliary, vaginal and bladder sites.
- Serious, sometimes fatal, arterial thromboembolic events including cerebral infarction, transient ischemic attacks, myocardial infarction, and angina.

5 In an alternative embodiment, the anti-VEGF compound is QL1101A (produced by Qilu Pharmaceutical Co., Ltd.), a biosimilar of bevacizumab.

The anti-VEGF compound ziv-aflibercept (Ziltrap; Sanofi/Genzyme) is a vascular endothelial growth factor inhibitor. It is a recombinant fusion protein consisting of Vascular Endothelial Growth Factor (VEGF)-binding portions from the extracellular domains of human
10 VEGF Receptors 1 and 2 fused to the Fc portion of the human IgG1. Ziv-aflibercept is produced by recombinant DNA technology in a Chinese hamster ovary (CHO) K-1 mammalian expression system. Ziv-aflibercept is a dimeric glycoprotein with a protein molecular weight of 97 kilodaltons (kDa) and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. ZALTRAP (ziv-aflibercept) injection is a sterile,
15 clear, colorless to pale-yellow, non-pyrogenic, preservative-free, solution for intravenous use. ZALTRAP is supplied in single-dose vials of 100 mg/4 mL and 200 mg/8 mL formulated as 25 mg/mL ziv-aflibercept in polysorbate 20 (1 mg/mL), sodium chloride (5.84 mg/mL), sodium citrate (1.45 mg/mL), sodium phosphate (0.8 mg/mL), and sucrose (200 mg/mL), in Water for Injection, USP, at a pH of 6.2.

20 When used in fluorouracil-based multi-agent chemotherapies, ziv-aflibercept is generally administered on day 1 of each 14-day cycle of the induction and maintenance phase, or once every two weeks, as an intravenous infusion at a dose of between about 2 and 10 mg/kg, with dosing adjustments as required. In some embodiments, ziv-aflibercept is administered as an intravenous infusion at about 4 mg/kg over 1 hour.

25 Ziv-aflibercept acts as a soluble receptor that binds to human VEGF-A (equilibrium dissociation constant K_D of 0.5 pM for VEGF-A₁₆₅ and 0.36 pM for VEGF-A₁₂₁), to human VEGF-B (K_D of 1.92 pM), and to human PlGF (K_D of 39 pM for PlGF-2). By binding to these endogenous ligands, ziv-aflibercept can inhibit the binding and activation of their cognate receptors. This inhibition can result in decreased neovascularization and decreased vascular permeability.

Ziv-aflibercept has been associated with GI toxicity including perforation, fistula formation, and diarrhea, neutropenia and neutropenic complications.

Alternatively, aflibercept is administered as the anti-VEGF compound.

Ramucirumab (Cyramza; Eli Lilly) is a human vascular endothelial growth factor receptor 2 (VEGFR2) antagonist that specifically binds VEGFR2 and blocks binding of VEGFR ligands, VEGF-A, VEGF-C, and VEGF-D. As a result, ramucirumab inhibits ligand-stimulated activation of VEGFR2, thereby inhibiting ligand-induced proliferation, and migration of human endothelial cells. Ramucirumab is a recombinant human IgG1 monoclonal antibody. Ramucirumab has an approximate molecular weight of 147 kDa. Ramucirumab is produced in genetically engineered mammalian NS0 cells.

Ramucirumab injection for intravenous use is a sterile, preservative-free, clear to slightly opalescent and colorless to slightly yellow solution. Ramucirumab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-dose vials. Ramucirumab is formulated in glycine (9.98 mg/mL), histidine (0.65 mg/mL), histidine monohydrochloride (1.22 mg/mL), polysorbate 80 (0.1 mg/mL), sodium chloride (4.383 mg/mL), and Water for Injection, USP, pH 6.0.

When used in fluorouracil-based multi-agent chemotherapies, ramucirumab is generally administered on day 1 of each 14-day cycle of the induction and maintenance phase, or once every two weeks, as an intravenous infusion at a dose of between about 5 and 15 mg/kg, with dosing adjustments as required. In some embodiments, ramucirumab is administered at about 8 mg/kg as an intravenous infusion over 1 hour.

In alternative embodiments, the anti-VEGF compound is apatinib. In some embodiments, the apatinib is administered at a dose of between about 100 and 400 mg QD on days 1-14. In some embodiments, the apatinib is administered at a dose of about 250 mg QD on days 1-14.

In an alternative embodiment, the anti-VEGF compound is selected from axitinib (Inlyta), dovitinib, fruquintinib (Elunate), pazopanib (Votrient), sunitinib (Sutent), sorafenib (Nexavar), regorafenib (Stivarga), cabozantinib (Cabometyx), lenvatinib (Lenvima), ponatinib (Iclusig), cabozatinib (Cometriq), tivozanib (Fotivda), or vandetanib (Caperlsa).

Anti-EGFR Monoclonal Antibodies

An anti-EGFR monoclonal antibody or compound is also routinely incorporated in fluorouracil-based multi-agent chemotherapy protocols in patients having colorectal cancer with RAS (including KRAS and NRAS) and BRAF wild-type tumors. Commonly used anti-EGFR monoclonal antibodies include cetuximab (Erbix; Eli Lilly) and panitumumab (Vectibix; Amgen).

EGFR is overexpressed in certain human cancers, including colon and rectum cancers. Interaction of EGFR with its normal ligands (e.g., EGF, transforming growth factor- α) leads to phosphorylation and activation of a series of intracellular proteins, which in turn regulate transcription of genes involved with cellular growth and survival, motility, and proliferation. KRAS (Kirsten rat sarcoma 2 viral oncogene homologue) and NRAS (Neuroblastoma RAS viral oncogene homologue) are highly related members of the RAS oncogene family. Signal transduction through the EGFR can result in activation of the wild-type KRAS and NRAS proteins; however, in cells with activating RAS somatic mutations, the RAS-mutant proteins are continuously active and appear independent of EGFR regulation. Panitumumab binds specifically to EGFR on both normal and tumor cells, and competitively inhibits the binding of ligands for EGFR. binding of the anti-EGFR antibody to the EGFR prevents ligand-induced receptor autophosphorylation and activation of receptor-associated kinases, resulting in inhibition of cell growth, induction of apoptosis, decreased proinflammatory cytokine and vascular growth factor production, and internalization of the EGFR.

Panitumumab is a human IgG2 kappa monoclonal antibody with an approximate molecular weight of 147 kDa that is produced in genetically engineered mammalian (Chinese hamster ovary) cells. In fluorouracil-based multi-agent chemotherapy protocols, panitumumab is generally administered on day 1 of each 14-day cycle of the induction phase and maintenance, or once every two weeks, as an intravenous injection at a dose of between about 2 and 10 mg/kg, with dosing adjustments as required. In some embodiments, panitumumab is administered at about 5 mg/kg.

Cetuximab is a recombinant, human/mouse chimeric monoclonal antibody that binds specifically to the extracellular domain of the human epidermal growth factor receptor (EGFR). Cetuximab is composed of the Fv regions of a murine anti-EGFR antibody with human IgG1 heavy and kappa light chain constant regions and has an approximate molecular weight of 152 kDa. Cetuximab is produced in mammalian (murine myeloma) cell culture. In the fluorouracil-based

multi-agent chemotherapy protocol described herein cetuximab is generally administered on day 1 of each 14-day cycle of the induction and maintenance phase, or once every two weeks, as an intravenous injection at a dose of between about 200 mg/m² and 1000 mg/m² with dosing adjustments as required. In some embodiments, cetuximab is administered at about 500 mg/m².

5 The use of anti-EGFR monoclonal antibodies has been associated with several risks and toxicities, including diarrhea and stomatitis.

In alternative embodiments, the anti-EGFR compound is nimotuzumab. In alternative embodiments, the anti-EGFR compound is necitumumab. In alternative embodiments, the anti-EGFR compound is Mab A13, AMG595, depatuxizumab mafodotin, duligotuzumab
 10 (MEHD7945A, RG7597), futuximab (Sym004), GC1118, imgatuzumab (GA201), matuzumab (EMD 72000), anitumumab (Vectibix, ABX-EGF), zalutumumab, humMR1, tomuzotuximab, erlotinib (Tarceva), gefitinib (Iressa), afatinib (Gilotrif), rociletinib (CO-1686), osimertinib (Tagrisso), olmutinib (Olita), naquotinib (ASP8273), nazartinib (EGF816), PF-06747775 (Pfizer), icotinib (BPI-2009), neratinib (HKI-272; PB272), avitinib (AC0010), EAI045, tarloxotinib (TH-
 15 4000; PR-610), PF-06459988 (Pfizer), tesevatinib (XL647; EXEL-7647; KD-019), transtinib, WZ-3146, WZ8040, CNX-2006, lapatinib (Tykerb; GlaxoSmithKline), brigatinib (Alunbrig; Ariad Pharmaceuticals), sapitinib, CUDC-101, PD153035, pelitinib, AEE788 (NVP-AEE788), AST-1306, AZ5104, lifirafenib (BGB-283), canertinib, CL-387785 (EKI-785), norcantharadin, vandetanib (Caprelsa), aumolertinib, or dacomitinib (PF-00299804; Pfizer)..

20

Colorectal Treatment Using Trilaciclib + FOLFOX

In some aspects, provided herein are improved FOLFOX treatment protocols that include the administration of CDK4/6 inhibitor trilaciclib. Trilaciclib is a highly potent and selective, reversible, CDK4/6 inhibitor that is administered intravenously (IV) on day 1 at between about
 25 200 mg/m² and 280 mg/m², and preferably at about 240 mg/m² no more than about 4 hours prior to the initiation of FOLFOX administration, and administered intravenously (IV) on day 2 at the same dose either about 18 to 26 hours or about 22 to 24 hours or about 20 to 22 hours after its administration on day 1 if fluorouracil is continuously infused over the two days, or on day 2 no more than about 4 hours prior to the initiation of 5-fluorouracil and, if applicable, folinic acid (e.g.,
 30 leucovorin or levoleucovorin) administration if fluorouracil is administered as an infusion on day

one (for example, over about 22 hours) and another infusion on day 2 (for example, over about 22 hours). In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated. This administration cycle for trilaciclib is designed to preserve HSPCs during chemotherapy (myelo-preservation), enhance anti-tumor immunity (anti-tumor efficacy), and reduce other toxicities associated with administration of the protocol such as stomatitis and mucositis.

FOLFOX is a three-drug regimen composed of fluorouracil (5-FU), folinic acid (e.g., leucovorin or levoleucovorin), and oxaliplatin commonly used in the treatment of mCRC (National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology Colon Cancer. Version 2. 2019. NCCN, Fort Washington, PA). Versions of FOLFOX include FOLFOX4, FOLFOX6, modified FOLFOX6 (mFOLFOX6), FOLFOX7, and modified FOLFOX7 (mFOLFOX7).

FOLFOX is administered for a maximum of 12 induction cycles (every 14 days) followed by maintenance consisting of intravenous-5-FU and folinic acid administered in 14-day cycles until disease progression, unacceptable toxicity, etc. FOLFOX is commonly administered in further combination with an anti-VEGF antibody, for example bevacizumab (Avastin, Genentech) or bevacizumab-awwb (Mvasi, Amgen), ramucirumab (Cyramza, Eli Lilly), or ziv-aflibercept (Zaltrap, Sanofi/Regeneron), or an anti-EGFR monoclonal antibody, for example panitumumab (Vectibix, Amgen) (for RAS wild-type (both KRAS and NRAS) CRC) or cetuximab (Erbix, Lilly) (RAS wild-type (both KRAS and NRAS) CRC).

In the FOLFOX-based protocols, oxaliplatin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 85 mg/m² and 130 mg/m², with dosing adjustments as required. In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2 hours at about 85 mg/m², for example FOLFOX4, mFOLFOX6, and mFOLFOX7. In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2 hours at about 100 mg/m² (e.g., FOLFOX6 and mFOLFOX6). In some embodiments, oxaliplatin

is administered as an intravenous infusion over about, for example, 2 hours at about 130 mg/m² (e.g., FOLFOX7). In some embodiments, oxaliplatin is administered simultaneously with folinic acid (e.g., leucovorin or levoleucovorin). In some embodiments oxaliplatin is administered prior to 5FU administration.

5 In the FOLFOX protocol, leucovorin is generally administered on day 1 of each 14-day cycle of the induction and maintenance phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 50 mg/m² to about 400 mg/m², with dosing adjustments as required. In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours on day 1 at about 400 mg/m² (e.g., FOLFOX6, mFOLFOX6, FOLFOX7). In
10 some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 200 mg/m² on both day 1 and day 2 (e.g., FOLFOX 4). In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours on day 1 at about 200 mg/m² (e.g., mFOLFOX7). In some embodiments, leucovorin is administered simultaneously with oxaliplatin. In some embodiments leucovorin is administered prior to 5FU.
15 Where levoleucovorin is used, it is generally dosed at one-half the racemic d,l-leucovorin, for example, in the FOLFOX protocol, on day 1 of each 14-day cycle of the induction and maintenance phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 25 mg/m² to about 200 mg/m², with dosing adjustments as required. In some embodiments, levoleucovorin is administered as an intravenous infusion over about, for example, 2 hours on day
20 1 at about 200 mg/m² (e.g., FOLFOX6, mFOLFOX6, FOLFOX7). In some embodiments, levoleucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 100 mg/m² on both day 1 and day 2 (e.g., FOLFOX 4). In some embodiments, levoleucovorin is administered as an intravenous infusion over about, for example, 2 hours on day 1 at about 100 mg/m² (e.g., mFOLFOX7). In some embodiments, levoleucovorin is administered simultaneously
25 with oxaliplatin. In some embodiments levoleucovorin is administered prior to 5FU. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered in place of leucovorin or levoleucovorin.

In the FOLFOX protocol, fluorouracil is administered initially as a bolus intravenous injection of 400 mg/m² on day 1 followed by administration as a continuous infusion (CI) over
30 about 22 hours on Day 1 and a separate continuous infusion over about 22 hours on day 2 (e.g.,

FOLFOX4), or a single continuous infusion over about 46-48 hours beginning on Day 1 (e.g., FOLFOX6, mFOLFOX6, and FOLFOX7) of each 14-day cycle in the induction and maintenance phase. In some embodiments, fluorouracil is administered initially as a bolus intravenous injection of about 400 mg/m² on day 1 followed by administration as a continuous infusion (CI) over about 22 hours on Day 1 and a separate continuous infusion over about 22 hours on day 2 (e.g., FOLFOX4), or a single continuous infusion over about, for example, 46 hours beginning on Day 1 (e.g., FOLFOX6, mFOLFOX6, and FOLFOX7) of each 14-day cycle in the induction and maintenance phase. The bolus dose of fluorouracil can vary, from about 240 mg/m² to about 400 mg/m², with dosing adjustments as required. The continuous infusion dose of fluorouracil can vary, from about 600 mg/m² to about 2400 mg/m², with dosing adjustments as required. In some embodiments, the fluorouracil is administered only as a continuous infusion (CI) of about 2400 mg/m² over 46 hours with dosing adjustments as required (e.g., mFOLFOX7). In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In some embodiments, an anti-VEGF or anti-EGFR monoclonal antibody or compound as described herein is administered on day 1. In some embodiments, the anti-VEGF or anti-EGFR monoclonal antibody or compound is administered at a dose according to the approved label.

Non-limiting representative examples of the administration of trilaciclib and FOLFOX4 protocol is provided in Table 3 and Figure 1A.

Table 3 – Trilaciclib + FOLFOX4 Protocol

Exemplary FOLFOX4 Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFOX4 protocol Day 2 - 240 mg/m ² 4 hours or less prior to administration of leucovorin/fluorouracil
Oxaliplatin	Day 1 – 85 mg/m ²
Leucovorin	Day 1 – 200 mg/m ² (or, alternatively, 100 mg/m ² of levoleucovorin)

	Day 2 – 200 mg/m ² (or, alternatively, 100 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 600 mg/m ² continuous infusion over 22 hours Day 2 - 600 mg/m ² continuous infusion over 22 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² 4 hours or less prior to administration of leucovorin/fluorouracil
Leucovorin	Day 1 – 200 mg/m ² (or, alternatively, 100 mg/m ² of levoleucovorin) Day 2 – 200 mg/m ² (or, alternatively, 100 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 600 mg/m ² continuous infusion over 22 hours Day 2 - 600 mg/m ² continuous infusion over 22 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)

Non-limiting representative examples of the administration of trilaciclib and FOLFOX6 protocol is provided in Table 4 and Figure 1B.

Table 4 – Trilaciclib + FOLFOX6 Protocol

Exemplary FOLFOX6 Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFOX6 protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Oxaliplatin	Day 1 – 100 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400-3000 mg/m ² continuous infusion over about 46 - 48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400-3000 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)

Non-limiting representative examples of the administration of trilaciclib and mFOLFOX6 protocol is provided in Table 5 and Figure 1C.

Table 5 – Trilaciclib + mFOLFOX6 Protocol

Exemplary mFOLFOX6 Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of mFOLFOX6 protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Oxaliplatin	Day 1 – 85 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours

Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 –dosing according to approved label (e.g., bevacizumab 5 mg/kg)
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Non-limiting representative examples of the administration of trilaciclib and FOLFOX7 protocol is provided in Table 6 and Figure 1D.

5 **Table 6 – Trilaciclib + FOLFOX7 Protocol**

Exemplary FOLFOX7 Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFOX7 protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Oxaliplatin	Day 1 – 130 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 –dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)

Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 –2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 –dosing according to approved label (e.g., bevacizumab 5 mg/kg)

Non-limiting representative examples of the administration of trilaciclib and mFOLFOX7 protocol is provided in Table 7 and Figure 1E.

5 **Table 7 – Trilaciclib + mFOLFOX7 Protocol**

Exemplary mFOLFOX7 Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of mFOLFOX7 protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Oxaliplatin	Day 1 – 85 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 –2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody	Day 1 –dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil

	Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (or 200 mg/m ² of levoleucovorin)
Fluorouracil	Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)

Colorectal Treatment Using Trilaciclib + FOLFIRI

In some aspects, provided herein are improved FOLFIRI treatment protocols that include administering the CDK4/6 inhibitor trilaciclib. As provided herein, trilaciclib is administered intravenously (IV) on day 1 at between about 200 mg/m² and 280 mg/m², and preferably at about 240 mg/m² no more than about 4 hours prior to the initiation of FOLFIRI administration, for example about 1 hour or less prior to the initiation of FOLFIRI, and administered intravenously (IV) on day 2 about 18 to 26 hours or about 22 to 24 hours after its administration on day 1. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5-FU is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated. Trilaciclib can be administered at a dose of between about 200 mg/m² to about 280 mg/m², but preferably at about 240 mg/m² as an intravenous injection or infusion over about 30 minutes. Trilaciclib can be administered at a dose of between about 200 mg/m² to about 280 mg/m², but preferably at about 240 mg/m². This administration cycle for trilaciclib can be incorporated into any of the various FOLFIRI protocols, and is designed to preserve HSPCs during chemotherapy (myelo-preservation), enhance anti-tumor immunity (anti-tumor efficacy), and reduce other toxicities associated with administration of the protocol such as stomatitis and mucositis.

FOLFIRI is a three-drug regimen composed of fluorouracil (5-FU), folinic acid (e.g., leucovorin or levoleucovorin), and irinotecan commonly used in the treatment of mCRC (National

Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology Colon Cancer. Version 2. 2019. NCCN, Fort Washington, PA). FOLFIRI is administered for a maximum of 12 induction cycles (every 14 days) followed by maintenance consisting of 5-FU and folinic acid administered in 14-day cycles until disease progression, unacceptable toxicity, etc. FOLFIRI is commonly administered in further combination with an anti-VEGF antibody, for example bevacizumab (Avastin, Genentech) or bevacizumab-awwb (Mvasi, Amgen), ramucirumab (Cyramza, Eli Lilly), or ziv-aflibercept (Zaltrap, Sanofi/Regeneron) or an anti-EGFR monoclonal antibody, for example panitumumab (Vectibix, Amgen) (for RAS wild-type (both KRAS and NRAS) CRC) or cetuximab (Erbix, Lily) (RAS wild-type (both KRAS and NRAS) CRC).

In the FOLFIRI protocol, irinotecan is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 90 minutes at a dose of between about 120 mg/m² and 180 mg/m², with dosing adjustments as required. In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 90 minutes at about 180 mg/m². In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 90 minutes at about 150 mg/m². In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 90 minutes at about 120 mg/m². In some embodiments, irinotecan is administered simultaneously with leucovorin. In some embodiments, irinotecan is administered prior to 5FU.

In the FOLFIRI protocol, leucovorin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 200 mg/m² and 400 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 200 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 400 mg/m². In some embodiments, leucovorin is administered simultaneously as irinotecan. In some embodiments, leucovorin is administered prior to 5FU. If levoleucovorin is used, it is generally dosed at one-half the dose of racemic *d,l*-leucovorin. For example, in the FOLFIRI protocol, levoleucovorin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 100 mg/m² and 200 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 100 mg/m². In some embodiments,

leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 200 mg/m². In some embodiments, leucovorin is administered simultaneously as irinotecan. In some embodiments, leucovorin is administered prior to 5-FU. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered in place of leucovorin or levoleucovorin.

In the FOLFIRI protocol, fluorouracil is administered either as a bolus intravenous injection, e.g., about 400 mg/m², on day 1 followed by administration as a continuous infusion (CI) over about 46 hours beginning on Day 1 of each 14-day cycle in the induction and maintenance phase (e.g., FOLFIRI), or only as a continuous infusion (CI) over about 46-48 hours beginning on Day 1 of each 14-day cycle in the induction and maintenance phase (e.g., mFOLFIRI). The bolus dose of fluorouracil can vary, from about 240 mg/m² to about 400 mg/m², with dosing adjustments as required. In some embodiments, the bolus dose is about 400 mg/m². In some embodiments, the bolus dose is about 320 mg/m². The continuous infusion dose of fluorouracil can vary, from about 1600 mg/m² to about 2800 mg/m² over about 46-48 hours, with dosing adjustments as required. In some embodiments, the continuous infusion dose is about 2800 mg/m². In some embodiments, the continuous infusion dose is about 2400 mg/m². In some embodiments, the continuous infusion dose is about 2000 mg/m². In some embodiments, the continuous infusion dose is about 1600 mg/m². In some embodiments, the fluorouracil is administered as a bolus intravenous injection of about 400 mg/m² on day 1 followed by administration as a continuous infusion (CI) of about 2400 mg/m² over about 46-48 hours. In some embodiments, the fluorouracil is administered as a bolus intravenous injection of about 320 mg/m² on day 1 followed by administration as a continuous infusion (CI) of about 2000 mg/m² over about 46-48 hours. In some embodiments, the fluorouracil is administered as a bolus intravenous injection of about 240 mg/m² on day 1 followed by administration as a continuous infusion (CI) of about 1600 mg/m² over about 46 hours. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In some embodiments, an anti-VEGF or anti-EGFR monoclonal antibody or compound as described herein is administered on day 1. In some embodiments, the anti-VEGF or anti-EGFR monoclonal antibody or compound is administered at a dose according to the approved label.

Non-limiting representative examples of the administration of trilaciclib and FOLFIRI protocol is provided in Table 8 and Figure 2A.

Table 8 – Trilaciclib + FOLFIRI Protocol

Exemplary FOLFIRI Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFIRI protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Irinotecan	Day 1 – 180 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or, alternatively, 200mg/m ² is levoleucovorin)
Fluorouracil	Day 1 – bolus dose of 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (or, alternatively, 200mg/m ² is levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46 hours

Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 –dosing according to approved label (e.g., bevacizumab 5 mg/kg)
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Non-limiting representative examples of the administration of trilaciclib and mFOLFIRI protocol is provided in Table 9 and Figure 2B.

5 **Table 9 – Trilaciclib + mFOLFIRI Protocol**

Exemplary mFOLFIRI Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of mFOLFIRI protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Irinotecan	Day 1 – 180 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or, alternatively, 200mg/m ² is levoleucovorin)
Fluorouracil	Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1

Leucovorin	Day 1 – 400 mg/m ² (or, alternatively, 200mg/m ² is levoleucovorin)
Fluorouracil	Day 1 –2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 –dosing according to approved label (e.g., bevacizumab 5 mg/kg)

Colorectal Treatment Using Trilaciclib + FOLFOXIRI

In particular, provided herein is an improved FOLFOXIRI treatment protocol that includes administration of the CDK4/6 inhibitor trilaciclib. As provided herein, trilaciclib is administered intravenously (IV) on day 1 no more than about 4 hours prior to the initiation of FOLFOXIRI administration, for example about 1 hour or less prior to the initiation of FOLFOXIRI, and administered intravenously (IV) on day 2 about 18 to 26 hours or about 22 to 24 hours after its administration on day 1. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5-FU is at sufficient concentrations such that 5-FU-related myelosuppression is exacerbated rather than mitigated. Trilaciclib can be administered at a dose of between about 200 mg/m² to about 280 mg/m², but preferably at about 240 mg/m² as an intravenous injection or infusion over about 30 minutes. This administration cycle for trilaciclib can be incorporated into any variation of the FOLFOXIRI protocol, and is designed to preserve HSPCs during chemotherapy (myelo-preservation), enhance anti-tumor immunity (anti-tumor efficacy), reduce other toxicities associated with administration of the protocol such as stomatitis and mucositis, and, importantly, extend the availability of FOLFOXIRI treatment to previously excluded patients. In some embodiments, the trilaciclib-FOLFOXIRI protocol described herein is administered to a patient greater than 70 years of age with an ECOG of ≥ 1 .

FOLFOXIRI is a four-drug regimen composed of infusional fluorouracil (5-FU), folinic acid (e.g., leucovorin or levoleucovorin), oxaliplatin, and irinotecan commonly used in the

treatment of mCRC (National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology Colon Cancer. Version 2. 2019. NCCN, Fort Washington, PA). FOLFOXIRI is administered for a maximum of 12 induction cycles (every 14 days) followed by maintenance cycles consisting of infusional-5-FU and folinic acid (leucovorin or levoleucovorin) administered in 14-day cycles until disease progression, unacceptable toxicity, etc. FOLFOXIRI is commonly administered in further combination with an anti-VEGF antibody, for example bevacizumab (Avastin, Genentech) or bevacizumab-awwb (Mvasi, Amgen), ramucirumab (Cyramza, Eli Lilly), or ziv-aflibercept (Zaltrap, Sanofi/Regeneron) or an anti-EGFR monoclonal antibody, for example panitumumab (Vectibix, Amgen) (for RAS wild-type (both KRAS and NRAS) CRC) or cetuximab (Erbix, Lilly) (RAS wild-type (both KRAS and NRAS) CRC).

In the FOLFOXIRI protocol, irinotecan is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 125 mg/m² and 200 mg/m², with dosing adjustments as required. In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 2 hours at about 165 mg/m². In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 2 hours at about 175 mg/m². In some embodiments, irinotecan is administered prior to leucovorin and oxaliplatin administration.

In the FOLFOXIRI protocol, leucovorin is generally administered on day 1 of each 14-day cycle of the induction and maintenance phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 140 mg/m² to about 400 mg/m², with dosing adjustments as required. In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 200 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 400 mg/m². In some embodiments, leucovorin is administered simultaneously with oxaliplatin. In some embodiments, leucovorin is administered after irinotecan. In some embodiments, leucovorin is administered prior to fluorouracil. If levoleucovorin is used, it is generally dosed at one-half the dose of racemic d,l-leucovorin, for example in FOLFOXIRI, , levoleucovorin is generally administered on day 1 of each 14-day cycle of the induction and maintenance phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 70 mg/m² to about 200 mg/m², with dosing adjustments as required. In some embodiments, levoleucovorin is administered as an intravenous

infusion over about, for example, 2 hours at about 100 mg/m². In some embodiments, levoleucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 200 mg/m². In some embodiments, levoleucovorin is administered simultaneously with oxaliplatin. In some embodiments, levoleucovorin is administered after irinotecan. In some
5 embodiments, levoleucovorin is administered prior to fluorouracil. In alternative embodiments, a folinate salt with a similar mechanism of action as leucovorin or levoleucovorin is administered in place of leucovorin or levoleucovorin.

In the FOLFOXIRI protocol, oxaliplatin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose
10 of between about 50 mg/m² and 100 mg/m², with dosing adjustments as required. In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2 hours at about 85 mg/m². In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2 hours at about 100 mg/m². In some embodiments, oxaliplatin is administered simultaneously as leucovorin. In some embodiments, oxaliplatin is administered
15 after irinotecan. In some embodiments, oxaliplatin is administered prior to fluorouracil.

In the FOLFOXIRI protocol, fluorouracil is administered as a continuous infusion (CI) over 48 hours beginning on Day 1 on each 14-day cycle in the Induction and Maintenance phase. The dose of fluorouracil can vary, from about 1200 mg/m² to about 4000 mg/m², with dosing adjustments as required. In some embodiments, the fluorouracil is administered as a continuous
20 infusion over about, for example, 46-48 hours at a dose of between about 2400 mg/m² to about 3200 mg/m², with dosing adjustments as required. In some embodiments, the fluorouracil is administered as a continuous infusion over about, for example, 46-48 hours at a dose of 3200 mg/m². In some embodiments, the fluorouracil is administered as a continuous infusion over about, for example, 46-48 hours at a dose of 3800 mg/m². In some alternative embodiments, a 5-FU
25 prodrug is administered instead of 5-FU.

In some embodiments, an anti-VEGF or anti-EGFR monoclonal antibody or compound as described herein is administered on day 1. In some embodiments, the anti-VEGF or anti-EGFR monoclonal antibody or compound is administered at a dose according to the approved label.

Non-limiting representative examples of the administration of trilaciclib and FOLFOXIRI
30 protocol is provided in Table 10 and Figure 3A.

Table 10 – Trilaciclib + FOLFOXIRI Protocol

Exemplary FOLFOXIRI Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFOXIRI protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Irinotecan	Day 1 – 165 mg/m ²
Oxaliplatin	Day 1 – 85 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (or, alternatively, 200 mg/m ² if levoleucovorin)
Fluorouracil	Day 1 – 2400-3200 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (or, alternatively, 200 mg/m ² if levoleucovorin)
Fluorouracil	Day 1 – 2400-3200 mg/m ² continuous infusion over about 46-48 hours
Optionally:	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)

Anti-EGFR or Anti-VEGF antibody or compound	
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Non-limiting representative examples of the administration of trilaciclib and an alternative FOLFOXIRI protocol is provided in Table 11 and Figure 3B.

In some embodiments of the above protocol, the 5-FU is administered at a dose of about 75% of the above, that is about 1800-2400 mg/m². In some embodiments of the above protocol, the 5-FU is administered at a dose of about 50% of the above, that is about 1200-1600 mg/m².

In some embodiments of the above protocol, the irinotecan is administered at a dose of about 75% of the above, that is about 125 mg/m². In some embodiments of the above protocol, the irinotecan is administered at a dose of about 50% of the above, that is about 80 mg/m².

In some embodiments of the above protocol, the oxaliplatin is administered at a dose of about 75% of the above, that is about 65 mg/m². In some embodiments of the above protocol, the oxaliplatin is administered at a dose of about 50% of the above, that is about 40 mg/m².

Table 11 – Trilaciclib + FOLFOXIRI Protocol

Exemplary FOLFOXIRI Protocol	
Induction (up to 12 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFOXIRI protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Irinotecan	Day 1 – 165 mg/m ²
Oxaliplatin	Day 1 – 85 mg/m ²
Leucovorin	Day 1 – 200 mg/m ² (or, alternatively, 100 mg/m ² if levoleucovorin)
Fluorouracil	Day 1 – 2400-3200 mg/m ² continuous infusion over about 46-48 hours

Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 200 mg/m ² (or, alternatively, 100 mg/m ² if levoleucovorin)
Fluorouracil	Day 1 – 2400-3200 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)

In some embodiments of the above protocol, the 5-FU is administered at a dose of about 75% of the above, that is about 1800-2400 mg/m². In some embodiments of the above protocol, the 5-FU is administered at a dose of about 50% of the above, that is about 1200-1600 mg/m².

5 In some embodiments of the above protocol, the irinotecan is administered at a dose of about 75% of the above, that is about 125 mg/m². In some embodiments of the above protocol, the irinotecan is administered at a dose of about 50% of the above, that is about 80 mg/m².

In some embodiments of the above protocol, the oxaliplatin is administered at a dose of about 75% of the above, that is about 65 mg/m². In some embodiments of the above protocol, the
10 oxaliplatin is administered at a dose of about 50% of the above, that is about 40 mg/m².

Colorectal Treatment Using Trilaciclib + FOLFIRINOX

In particular, provided herein is an improved FOLFIRINOX treatment protocol that includes administration of the CDK4/6 inhibitor trilaciclib. As provided herein, trilaciclib is

administered intravenously (IV) on day 1 no more than about 4 hours prior to the initiation of FOLFIRINOX administration, for example about 1 hour or less, and administered intravenously (IV) on day 2 about 18 to 26 hours or about 22 to 24 hours after its administration on day 1. In some embodiments, the trilaciclib is administered on day 2 of each cycle between about 20 and 22 hours after its administration on day 1 of each cycle. The day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of trilaciclib) does not occur while 5FU is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated. This administration cycle for trilaciclib can be incorporated into any variation of the FOLFIRINOX protocol, and is designed to preserve HSPCs during chemotherapy (myelo-preservation), enhance anti-tumor immunity (anti-tumor efficacy), reduce other toxicities associated with administration of the protocol such as stomatitis and mucositis, and, importantly, extend the availability of FOLFIRINOX treatment to previously excluded patients.

FOLFIRINOX is a four-drug regimen composed of bolus + intravenous fluorouracil (5-FU), intravenous folinic acid (e.g., leucovorin or levoleucovorin), intravenous oxaliplatin, and intravenous irinotecan commonly used in the treatment of mCRC (National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology Colon Cancer. Version 2. 2019. NCCN, Fort Washington, PA). FOLFIRINOX is administered for a maximum of 12 induction cycles (every 14 days) followed by maintenance cycles consisting of intravenous-5-FU and leucovorin (folinic acid) administered in 14-day cycles until disease progression, unacceptable toxicity, etc. FOLFIRINOX is commonly administered in further combination with an anti-VEGF antibody, for example bevacizumab (Avastin, Genentech), bevacizumab-awwb (Mvasi, Amgen), ramucirumab (Cyramza, Eli Lilly), or ziv-aflibercept (Zaltrap, Sanofi/Regeneron) or an anti-EGFR monoclonal antibody, for example panitumumab (Vectibix, Amgen) (for RAS wild-type (both KRAS and NRAS) CRC) or cetuximab (Erbix, Lilly) (RAS wild-type (both KRAS and NRAS) CRC).

In the FOLFIRINOX protocol, oxaliplatin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 60 mg/m² and 85 mg/m², with dosing adjustments as required. In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2

hours at about 85 mg/m². In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2 hours at about 60 mg/m². In some embodiments, oxaliplatin is administered prior to irinotecan and leucovorin.

5 In the FOLFIRINOX protocol, irinotecan is generally administered on day 1 of each 14-day cycle of the Induction phase as an intravenous infusion over about, for example, 90 minutes at a dose of between about 120 mg/m² and 180 mg/m², with dosing adjustments as required. In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 90 minutes at about 180 mg/m². In some embodiments, irinotecan is administered as an intravenous infusion over about, for example, 90 minutes at about 150 mg/m². In some
10 embodiments, irinotecan is administered as an intravenous infusion over about, for example, 90 minutes at about 120 mg/m². In some embodiments, irinotecan is administered simultaneously as leucovorin. In some embodiments, irinotecan is administered after oxaliplatin. In some embodiments, irinotecan is administered prior to 5FU.

15 In the FOLFIRINOX protocol, leucovorin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 200 mg/m² and 400 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 200 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 400 mg/m². In some embodiments, leucovorin is administered simultaneously
20 with irinotecan. In some embodiments, leucovorin is administered after oxaliplatin. In some embodiments, leucovorin is administered prior to 5FU. If levoleucovorin is administered, it is generally administered at one-half the dose of d,l-leucovorin. For example, in the FOLFIRINOX protocol, levoleucovorin is generally administered on day 1 of each 14-day cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 100
25 mg/m² and 200 mg/m². In some embodiments, levoleucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 100 mg/m². In some embodiments, levoleucovorin is administered as an intravenous infusion over about, for example, 2 hours at about 100 mg/m². In some embodiments, levoleucovorin is administered simultaneously with irinotecan. In some embodiments, levoleucovorin is administered after oxaliplatin. In some embodiments,
30 levoleucovorin is administered prior to 5FU. In alternative embodiments, a folinate salt with a

similar mechanism of action as leucovorin or levoleucovorin is administered in place of leucovorin or levoleucovorin.

In the FOLFIRINOX protocol, fluorouracil is administered initially as a bolus intravenous injection of about 400 mg/m² on day 1 followed by administration as a continuous infusion (CI) over about 46 hours beginning on Day 1 of each 14-day cycle in the induction and maintenance cycles. The bolus dose of fluorouracil can vary, from about 200 mg/m² to about 400 mg/m², with dosing adjustments as required. In some embodiments, the bolus dose is about 400 mg/m². In some embodiments, the bolus dose is about 300 mg/m². In some embodiments, the bolus dose is about 200 mg/m². The continuous infusion dose of fluorouracil can vary, from about 1200 mg/m² to about 2400 mg/m² over about 46-48 hours, with dosing adjustments as required. In some embodiments, the continuous infusion dose is about 2400 mg/m². In some embodiments, the continuous infusion dose is about 1800 mg/m². In some embodiments, the continuous infusion dose is about 1200 mg/m². In some embodiments, the fluorouracil is administered as a bolus intravenous injection of about 400 mg/m² on day 1 followed by administration as a continuous infusion (CI) of about 2400 mg/m² over about 46-48 hours. In some embodiments, the fluorouracil is administered as a bolus intravenous injection of about 300 mg/m² on day 1 followed by administration as a continuous infusion (CI) of about 1800 mg/m² over about 46-48 hours. In some embodiments, the fluorouracil is administered as a bolus intravenous injection of about 200 mg/m² on day 1 followed by administration as a continuous infusion (CI) of about 1200 mg/m² over about 46-48 hours. In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In some embodiments, an anti-VEGF or anti-EGFR monoclonal antibody or compound as described herein is administered on day 1. In some embodiments, the anti-VEGF or anti-EGFR monoclonal antibody or compound is administered at a dose according to the approved label.

Non-limiting representative examples of the administration of trilaciclib and FOLFIRINOX protocol is provided in Table 12 and Figure 4.

Table 12 – Trilaciclib + FOLFIRINOX Protocol

Exemplary FOLFIRINOX Protocol	
Induction (up to 12 cycles)	

Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of FOLFIRINOX protocol Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Irinotecan	Day 1 – 165 mg/m ²
Oxaliplatin	Day 1 – 85 mg/m ²
Leucovorin	Day 1 – 400 mg/m ² (200 mg/m ² if levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label (e.g., bevacizumab 5 mg/kg)
Maintenance (as long as tolerated)	
Drug	Schedule and Dose
Trilaciclib	Day 1 – 240 mg/m ² 4 hours or less prior to initial administration of leucovorin/fluorouracil Day 2 - 240 mg/m ² between about 18-24 hours after administration of trilaciclib on day 1
Leucovorin	Day 1 – 400 mg/m ² (200 mg/m ² if levoleucovorin)
Fluorouracil	Day 1 – Bolus dose 400 mg/m ² Day 1 – 2400 mg/m ² continuous infusion over about 46-48 hours
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1 – dosing according to approved label

Colorectal Treatment Using Trilaciclib + FLOX

In some alternative aspects, provided herein are improved FLOX treatment protocols that include the administration of CDK4/6 inhibitor trilaciclib. Trilaciclib is a highly potent and selective, reversible, CDK4/6 inhibitor that is administered intravenously (IV) on day 1, day 8, day 15, day 22, day 29, and day 36 of each 8-week cycle (56 days) at between about 200 mg/m² and 280 mg/m², and preferably at about 240 mg/m² no more than about 4 hours prior to the initiation of FLOX administration. This administration cycle for trilaciclib can be incorporated into any of the various FLOX protocols, and is designed to preserve HSPCs during chemotherapy (myelo-preservation), enhance anti-tumor immunity (anti-tumor efficacy), and reduce other toxicities associated with administration of the protocol such as stomatitis and mucositis.

FLOX is a three-drug regimen composed of fluorouracil (5-FU), folinic acid (leucovorin or levoleucovorin), and oxaliplatin commonly used in the treatment of CRC (National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology Colon Cancer. Version 2. 2019. NCCN, Fort Washington, PA).

FLOX is administered for a maximum of 3 induction cycles (every 8 weeks). FLOX can be administered in further combination with an anti-VEGF antibody, for example bevacizumab (Avastin, Genentech) or bevacizumab-awwb (Mvasi, Amgen), ramucirumab (Cyramza, Eli Lilly), or ziv-aflibercept (Zaltrap, Sanofi/Regeneron), or an anti-EGFR monoclonal antibody, for example panitumumab (Vectibix, Amgen) (for RAS wild-type (both KRAS and NRAS) CRC) or cetuximab (Erbix, Lily) (RAS wild-type (both KRAS and NRAS) CRC).

In the FLOX-based protocols, oxaliplatin is generally administered on day 1, day 15, and day 29 of an 8-week cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 65 mg/m² and 105 mg/m², with dosing adjustments as required. In some embodiments, oxaliplatin is administered as an intravenous infusion over about, for example, 2 hours at about 85 mg/m². In some embodiments, oxaliplatin is administered simultaneously with leucovorin. In some embodiments oxaliplatin is administered prior to leucovorin and 5FU administration.

In the FLOX protocol, leucovorin is generally administered on day 1, day 8, day 15, day 22, day 29, and day 36 of each 8-week cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 25 mg/m² to about 600 mg/m², with dosing adjustments as required. In some embodiments, leucovorin is administered as an intravenous

infusion over about, for example, 2 hours on day 1 at about 500 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours on day 1 at about 50 mg/m². In some embodiments, leucovorin is administered simultaneously with oxaliplatin. In some embodiments leucovorin is administered after 5-FU is administered. If
 5 leucovorin is administered, it is generally administered at one-half the dose of d,l-leucovorin. For example, in the FLOX protocol, leucovorin is generally administered on day 1, day 8, day 15, day 22, day 29, and day 36 of each 8-week cycle of the induction phase as an intravenous infusion over about, for example, 2 hours at a dose of between about 12.5 mg/m² to about 300 mg/m², with dosing adjustments as required. In some embodiments, leucovorin is
 10 administered as an intravenous infusion over about, for example, 2 hours on day 1 at about 250 mg/m². In some embodiments, leucovorin is administered as an intravenous infusion over about, for example, 2 hours on day 1 at about 25 mg/m². In some embodiments, leucovorin is administered simultaneously with oxaliplatin. In some embodiments leucovorin is administered after 5-FU is administered. In alternative embodiments, a folinate salt with a similar
 15 mechanism of action as leucovorin or leucovorin is administered in place of leucovorin or leucovorin.

In the FLOX protocol, fluorouracil is administered as a bolus intravenous injection on day 1, day 8, day 15, day 22, day 29, and day 36 of each 8-week cycle. The bolus dose of fluorouracil can vary, from about 200 mg/m² to about 1000 mg/m², with dosing adjustments as required. In
 20 some embodiments, the fluorouracil is administered at about 500 mg/m². In some alternative embodiments, a 5-FU prodrug is administered instead of 5-FU.

In some embodiments, an anti-VEGF or anti-EGFR monoclonal antibody or compound as described herein is administered on day 1, day 15, and day 29 of each 8-week cycle. In some
 25 embodiments, an anti-VEGF or anti-EGFR monoclonal antibody or compound as described herein is administered at a dose according to the approved label.

Non-limiting representative examples of the administration of trilaciclib and FLOX protocol is provided in Table 13 and Figure 5.

Table 13 – Trilaciclib + FLOX Protocol

Exemplary FLOX Protocol	
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Induction (up to 3 cycles)	
Drug	Schedule and Dose
Trilaciclib	Day 1, day 8, day 15, day 22, day 29, and day 36 – 240 mg/m ² 4 hours or less prior to initial administration of FLOX protocol
Oxaliplatin	Day 1, day 15, day 29 – 85 mg/m ²
Leucovorin	Day 1, day 8, day 15, day 22, day 29, day 36 – 500 mg/m ² (or, alternatively, 250 mg/m ² if levoleucovorin)
Fluorouracil	Day 1, day 8, day 15, day 22, day 29, and day 36 – Bolus dose 500 mg/m ²
Optionally: Anti-EGFR or Anti-VEGF antibody or compound	Day 1, day 15, day 29 – dosing according to approved label

Anti-tumor Efficacy Assessment

In some embodiments, the inclusion of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein provides for an increased anti-tumor efficacy compared to those subjects receiving a fluorouracil-based multi-agent chemotherapy protocols without trilaciclib. Methods of accessing tumor response are well known in the art and include, for example RECIST v1.1 (Eisenhauer et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45: 228-247). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the inclusion of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein provides for an increased or extended progression free survival (PFS) compared to those subjects not receiving trilaciclib. PFS is generally defined as the time (number of months) from date of protocol administration until the date of documented radiologic disease progression or death from any cause. Methods of accessing increased PFS are well known in the art and include, for example RECIST v1.1 (Eisenhauer et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;

45: 228-247). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the inclusion of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein provides for an increased or extended overall survival (OS) compared to those subjects not receiving trilaciclib. OS is generally calculated as the time (months) from the date of the onset of protocol administration to the date of death due to any cause.

In some embodiments, the inclusion of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein provides for an improved objective response rate (ORR) compared to those subjects not receiving trilaciclib. ORR is generally defined as the proportion of patients with tumor size reduction of a predefined amount and for a minimum time period. Objective response (OR) includes a complete response (CR), which is the disappearance of all signs of the tumor in response to treatment and a partial response (PR), which is a decrease in the size of a tumor in response to treatment. In some embodiments, the objective response (OR) is a complete response (CR). In some embodiments, the objective response (OR) is a partial response (PR). The ORR is an important parameter to demonstrate the efficacy of a treatment and it serves as a primary or secondary end-point in clinical trials. Methods of assessing ORR are well known in the art and include, for example RECIST v1.1 (Eisenhauer et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009; 45: 228-247) and World Health Organization (WHO) (World Health Organization. WHO Handbook for Reporting Results of Cancer Treatment. World Health Organization Offset Publication No. 48; Geneva (Switzerland), 1979). Statistical methods of measuring objective response rate are well known in the art and include, for example, the Clopper-Pearson Method (Clopper, C.; Pearson, E. S. (1934). "The use of confidence or fiducial limits illustrated in the case of the binomial". *Biometrika*. 26 (4): 404–413. doi:10.1093/biomet/26.4.404). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the inclusion of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein provides for an improved Duration of Objective Response (DOR) compared to those subjects not receiving trilaciclib. DOR is generally defined as the time between first objective response of CR or PR and the first date that progressive disease is

objectively documented or death, whichever comes first. Methods of assessing improved DOR are well known in the art and include, for example RECIST v1.1 (Eisenhauer et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45: 228-247). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

Reduction of Toxicity

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in improved myelopreservation of hematopoietic stem and progenitor cells (HSPCs) and immune effector cells such as lymphocytes including T-lymphocytes, as well as enhanced anti-tumor efficacy in subjects compared to those receiving a fluorouracil-based multi-agent chemotherapy protocol without trilaciclib. Improvements in myelopreservation of hematopoietic stem and progenitor cells (HSPCs) and immune effector cells such as lymphocytes including T-lymphocytes are measured with increases in hematology assessments (complete blood count (CBC), red blood cell count (RBC), platelet count, white blood cell count (WBC) and absolute neutrophil count (ANC)), reduction in severe adverse events (AEs), reduction in supportive care interventions (including transfusions and G-CSF administration), reduction in dose modifications, and improved patient recorded outcomes (PROs). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in myelopreservation of the neutrophil lineage in subjects compared to those receiving a fluorouracil-based multi-agent chemotherapy protocol without trilaciclib. Endpoints to measure myelopreservation of the neutrophil lineage include a reduction in duration of severe neutropenia, for example after induction cycle 1, and the reduction in the occurrence of severe neutropenia. Neutropenia is generally defined as a condition that results when the body does not have enough neutrophils, an important white blood cell that fights infections. The lower the neutrophil count, the more vulnerable one is to infectious diseases. Neutropenia is generally quantified as an absolute neutrophil count (ANC) of less than 1500 per microliter (1500/ μ L). Severe neutropenia is generally quantified as an absolute neutrophil count

(ANC) of less than 500 per microliter (500/ μ L). Methods of calculating absolute neutrophil count (ANC) are well known in the art and include multiplying the WBC count times the percent of neutrophils in the differential WBC count. The percent of neutrophils consists of the segmented (fully mature) neutrophils) + the bands (almost mature neutrophils). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in the duration of severe (Grade 4) neutropenia (DSN) in subjects compared to those receiving a fluorouracil-based multi-agent chemotherapy protocol without trilaciclib. The duration of SN (DSN) is generally defined as the number of days from the date of the first ANC value of $<0.5 \times 10^9/L$ to the date of the first ANC value $\geq 0.5 \times 10^9/L$ where no additional ANC values $<0.5 \times 10^9/L$ are observed for the remainder of that cycle. Severe neutropenia is generally quantified as an absolute neutrophil count (ANC) of less than 500 per microliter (500/microL). Methods of calculating absolute neutrophil count (ANC) are well known in the art and include multiplying the WBC count times the percent of neutrophils in the differential WBC count. The percent of neutrophils consists of the segmented (fully mature) neutrophils) + the bands (almost mature neutrophils). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction of chemotherapy-induced fatigue (CIF) in subjects compared to those receiving a fluorouracil-based multi-agent chemotherapy protocol without trilaciclib. In some embodiments, the reduction of CIF is a reduction in the time to first confirmed deterioration of fatigue (TTCD-fatigue), as measured by the Functional Assessment of Cancer Therapy-Fatigue (FACIT-F). FACIT-F is a 13-item subscale that measures fatigue severity and the impact of fatigue on functioning and is described in Yellen et al., Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. J Pain Symptom Manage. 1997; 13: 63-74. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in severe neutropenia events, a reduction in granulocyte-colony stimulating factor (G-CSF) treatment, or a reduction in febrile neutropenia (FN) adverse events (AEs). G-CSF treatment will be utilized according to the treatment guidelines outlined in Aapro et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer. 2011; 47:8-32. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in Grade 3 or 4 decreased hemoglobin laboratory values, red blood cell (RBC) transfusions, or erythropoiesis-stimulating agent (ESA) administration. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in Grade 3 or 4 decreased platelet count laboratory values and/or the number of platelet transfusions. As described in (Kaufman, 2015; Schiffer, 2017), platelets are generally transfused at a threshold of $\leq 10,000/\mu\text{L}$. Platelets are also generally transfused in any patient who is bleeding with a platelet count $< 50,000/\mu\text{L}$ ($100,000/\mu\text{L}$ for central nervous system or ocular bleeding). In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in Grade 3 or 4 hematologic laboratory values. In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in all-cause dose reductions or cycle delays and relative dose intensity or a fluorouracil-based multi-agent chemotherapy protocol described herein. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in i) hospitalizations, including but not limited to those due to all causes, febrile neutropenia/neutropenia, anemia/RBC transfusion, thrombocytopenia/bleeding and infections) or ii) antibiotic use, including but not limited to
5 intravenous (IV), oral, and oral and IV administered antibiotics. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in an improvement to one or more of: Functional
10 Assessment of Cancer Therapy-General (FACT-G) domain scores (physical, social/family, emotional, and functional well-being); Functional Assessment of Cancer Therapy-Anemia (FACT-An): Anemia; Functional Assessment of Cancer Therapy-colorectal cancer (FACT-C) : Colorectal Cancer Subscale; 5-level EQ-5D (EQ-5D-5L); Patient Global Impression of Change (PGIC) fatigue item; or Patient Global Impression of Severity (PGIS) fatigue item. In some
15 embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in the number of severe diarrhea episodes (Grade 3 or greater) experienced by a subject compared to those a fluorouracil-based
20 multi-agent chemotherapy protocol described herein without trilaciclib. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent chemotherapy protocol described herein results in a reduction in the development of, severity of,
25 or episodes of mucositis experienced by a subject compared to those receiving a fluorouracil-based multi-agent chemotherapy protocol described herein without trilaciclib. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

In some embodiments, the use of trilaciclib in a fluorouracil-based multi-agent
30 chemotherapy protocol described herein results in a reduction in the development of, severity of,

or episodes of stomatitis experienced by a subject compared to those receiving a fluorouracil-based multi-agent chemotherapy protocol described herein without trilaciclib. In some embodiments, the fluorouracil-based multi-agent chemotherapy protocol is FOLFOXIRI or FOLFOXIRI + an anti-VEGF or anti-EGFR antibody or compound.

5

Combination with an Immune-Checkpoint Inhibitor

In some alternative embodiments, the fluorouracil-based multi-agent chemotherapy protocols for administration to a subject with colorectal cancer, or other cancer described herein, further includes the administration of an immune-checkpoint inhibitor or immune modulating agent. In some embodiments, the immune checkpoint inhibitor or immune modulating agent is administered on day 1 of each 14-day cycle, or every two weeks. Example of immune checkpoint inhibitors and immune modulating agents include, but are not limited to, a PD-1 inhibitor, PD-L1 inhibitor, PD-L2 inhibitor, CTLA-4 inhibitor, LAG-3 inhibitor, TIM-3 inhibitor, V-domain Ig suppressor of T-cell activation (VISTA) inhibitors, TIGIT inhibitor, Siglec-15 inhibitor, B7-H3 (CD272) inhibitor, BTLA inhibitor (CD272), small molecule, peptide, nucleotide, or other inhibitor. In certain aspects, the immune modulator is an antibody, such as a monoclonal antibody.

In some alternative embodiments, the immune checkpoint inhibitor is a PD-1 inhibitor that blocks the interaction of PD-1 and PD-L1 by binding to the PD-1 receptor, and in turn inhibits immune suppression. In some alternative embodiments, the immune checkpoint inhibitor is a PD-1 immune checkpoint inhibitor selected from, but not limited to, nivolumab (Opdivo®), pembrolizumab (Keytruda®), pidilizumab (Medivation), AMP-224 (Amplimmune); sasanlimab (PF-06801591; Pfizer), spartalizumab (PDR001; Novartis), cemiplimab (Libtayo®; REGN2810; Regeneron), retifanlimab (MGA012; MacroGenics), tislelizumab (BGB-A317; BeiGene), camrelizumab (SHR-1210; Jiangsu Hengrui Medicine Company and Incyte Corporation), and dostarlimab (TSR-042; Tesaro).

In some alternative embodiments, the immune checkpoint inhibitor is a PD-L1 inhibitor that blocks the interaction of PD-1 and PD-L1 by binding to the PD-L1 receptor, and in turn inhibits immune suppression. PD-L1 inhibitors include, but are not limited to, atezolizumab (Tecentriq®, Genentech), durvalumab (Imfinzi®, AstraZeneca); avelumab (Bavencio®; Merck), envafolimab (KN035; Alphamab), BMS-936559 (Bristol-Myers Squibb), lodapolimab

(LY3300054; Eli Lilly), cosibelimab (CK-301; Checkpoint Therapeutics), sugemalimab (CS-1001; Cstone Pharmaceuticals), adebrelimab (SHR-1316; Jiangsu HengRui Medicine), CBT-502 (CBT Pharma), and BGB-A333 (BeiGene).

5 In some alternative embodiments, the immune checkpoint inhibitor is a PD-L1/VISTA inhibitor. PD-L1-VISTA inhibitors include, but are not limited to, CA-170 (Curis Inc.). In some alternative embodiments, the immune checkpoint inhibitor is a VISTA immune checkpoint inhibitor. VISTA inhibitors include, but are not limited to, JNJ-61610588 (Johnson & Johnson).

10 In one aspect of this embodiment, the immune checkpoint inhibitor is a CTLA-4 immune checkpoint inhibitor that binds to CTLA-4 and inhibits immune suppression. CTLA-4 inhibitors include, but are not limited to, ipilimumab (Yervoy®, Bristol Myers Squibb); tremelimumab (AstraZeneca/MedImmune), zalifrelimab (AGEN1884; Agenus) and AGEN2041 (Agenus).

15 In another embodiment, the immune checkpoint inhibitor is a LAG-3 immune checkpoint inhibitor. Examples of LAG-3 immune checkpoint inhibitors include, but are not limited to, relatlimab (BMS-986016; Bristol-Myers Squibb), GSK2831781 (GlaxoSmithKline), eftilagimod alpha (IMP321; Prima BioMed), leramilimab (LAG525; Novartis), MK-4280 (Merck), REGN3767 (Regeneron), TSR-033 (Tesar), BI754111 (Bohringer Ingelheim), Sym022 (Symphogen), the dual PD-1 and LAG-3 inhibitor tebotelimab (MGD013; MacroGenics), and the dual PD-L1 and LAG-3 inhibitor FS118 (F-Star).

20 In yet another aspect of this embodiment, the immune checkpoint inhibitor is a TIM-3 immune checkpoint inhibitor. TIM-3 inhibitors include, but are not limited to, TSR-022 (Tesar), MBG453 (Novartis), Sym023 (Symphogen), INCAGN2390 (Incyte), LY3321367 (Eli Lilly and Company), BMS-986258 (BMS), SHR-1702 (Jiangsu HengRui), and RO7121661 (Roche).

25 In yet another aspect of this embodiment, the immune checkpoint inhibitor is a TIGIT (T cell immunoreceptor with Ig and ITIM domains) immune checkpoint inhibitor. TIGIT immune checkpoint inhibitors include, but are not limited to, MK-7684 (Merck), Etigilimab /OMP-313 M32 (OncoMed), Tiragolumab/MTIG7192A/RG-6058 (Genentech), BMS-986207 (BMS), AB-154 (Arcus Biosciences), and ASP-8374 (Potenza).

30 Other immune checkpoint inhibitors for use in the invention described herein include, but are not limited to, B7-H3/CD276 immune checkpoint inhibitors such as enoblituzumab (MGA217, MacroGenics) MGD009 (MacroGenics), 131I-8H9/omburtamab (Y-mabs), and I-

8H9/omburtamab (Y-mabs), indoleamine 2,3-dioxygenase (IDO) immune checkpoint inhibitors such as Indoximod and INCB024360, killer immunoglobulin-like receptors (KIRs) immune checkpoint inhibitors such as Lirilumab (BMS-986015), carcinoembryonic antigen cell adhesion molecule (CEACAM) inhibitors (e.g., CEACAM-1, -3 and/or -5). Exemplary anti-CEACAM-1
5 antibodies are described in WO 2010/125571, WO 2013/082366 and WO 2014/022332, e.g., a monoclonal antibody 34B1, 26H7, and 5F4; or a recombinant form thereof, as described in, e.g., US 2004/0047858, U.S. Pat. No. 7,132,255 and WO 99/052552. In other embodiments, the anti-CEACAM antibody binds to CEACAM-5 as described in, e.g., Zheng et al. PLoS One. 2010 September 2; 5(9). pii: e12529 (DOI:10.1371/journal.pone.0021146), or cross-reacts with
10 CEACAM-1 and CEACAM-5 as described in, e.g., WO 2013/054331 and US 2014/0271618.

Still other checkpoint inhibitors can be molecules directed to B and T lymphocyte attenuator molecule (BTLA), for example as described in Zhang et al., Monoclonal antibodies to B and T lymphocyte attenuator (BTLA) have no effect on in vitro B cell proliferation and act to inhibit in vitro T cell proliferation when presented in a cis, but not trans, format relative to the
15 activating stimulus, Clin Exp Immunol. 2011 Jan; 163(1): 77–87, and TAB004/JS004 (Junshi Biosciences).

In another embodiment, the immune checkpoint inhibitor is a sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) inhibitor, including, but not limited to, NC318 (an anti-Siglec-15 mAb).

20 In an alternative embodiment, the fluorouracil-based multi-agent chemotherapy protocol administered to the subject does not include an immune checkpoint inhibitor or immune modulatory agent other than trilaciclib.

Pharmaceutical Compositions

25 The selected compounds of the protocols described herein or their pharmaceutically acceptable salts can be administered as the neat chemical, but is more typically administered as a pharmaceutical composition, that includes an effective amount for a patient, typically a human, in need of such treatment in a pharmaceutically acceptable carrier. The pharmaceutical composition may contain a compound or salt thereof as the only active agent, or, in an alternative embodiment,
30 the compound or its salt and at least one additional active agent for the disease to be treated.

The pharmaceutical compositions may be administered in a therapeutically effective amount by any desired mode of administration, but is typically administered as an intravenous injection or infusion. In alternative embodiments, the compounds or pharmaceutically acceptable salts are delivered in an effective amount with a pharmaceutically acceptable carrier for oral
5 delivery. As more general non-limiting examples, the pharmaceutical composition one suitable for oral (including buccal and sub-lingual), rectal, nasal, topical, transdermal, pulmonary, vaginal or parenteral (including intramuscular, intra-arterial, intrathecal, subcutaneous and intravenous), injections, inhalation or spray, intra-aortal, intracranial, subdermal, intraperitoneal, subcutaneous, or by other means of administration containing conventional pharmaceutically acceptable carriers.

10 Suitable dosage ranges depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and the preferences and experience of the medical practitioner involved. One of ordinary skill in the art of treating such diseases will be able, without undue experimentation and in reliance upon personal knowledge and the disclosure of this application,
15 to ascertain a therapeutically effective amount of the compositions of the disclosure for a given disease.

In certain embodiments the pharmaceutical composition is in a dosage form that contains from about 0.01 mg to about 1000 mg, from about 0.1 mg to about 750 mg, from about 1 mg to about 500 mg, or from about 5, 10, 15, or 20 mg to about 250 mg of the active compound or its
20 pharmaceutically acceptable salt. Examples are dosage forms are those delivering at least 0.01, 0.05, 0.1, 1, 5, 10, 25, 50, 100, 200, 250, 300, 400, 500, 600, 700, or 750 mg of active compound, or its salt. When the weight is used herein, it can refer to either the compound alone or the compound in combination with its pharmaceutially acceptable salt.

An effective amount of the disclosed compound or its salt may be administered based on
25 the weight, size or age of the patient. For example, a therapeutic amount may for example be in the range of about 0.01 mg/kg to about 250 mg/kg body weight, or about 0.1 mg/kg to about 10 mg/kg, in at least one dose. The patient can be administered as many doses as are required to reduce and/or alleviate and/or cure the disorder in question. When desired, formulations can be prepared with enteric coatings adapted for sustained or controlled release administration of the
30 active ingredient.

In certain embodiments the dose ranges from about 0.01-100 mg/kg of patient bodyweight, for example about 0.01 mg/kg, about 0.05 mg/kg, about 0.1 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 55 mg/kg, about 60 mg/kg, about 65 mg/kg, about 70 mg/kg, about 75 mg/kg, about 80 mg/kg, about 85 mg/kg, about 90 mg/kg, about 95 mg/kg, or about 100 mg/kg.

The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component.

The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packed tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

In certain embodiments the compound is administered as a pharmaceutically acceptable salt. Non-limiting examples of pharmaceutically acceptable salts include: acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, and valerate salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, and ethylamine.

Depending on the intended mode of administration, the pharmaceutical compositions can be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, syrup, suspensions, creams, ointments, lotions,

paste, gel, spray, aerosol, foam, or oil, injection or infusion solution, a transdermal patch, a subcutaneous patch, an inhalation formulation, in a medical device, suppository, buccal, or sublingual formulation, parenteral formulation, or an ophthalmic solution, or the like, preferably in unit dosage form suitable for single administration of a precise dosage.

5 Some dosage forms, such as tablets and capsules, are subdivided into suitably sized unit doses containing appropriate quantities of the active components, e.g., an effective amount to achieve the desired purpose. The compositions will include an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, can include other pharmaceutical agents, adjuvants, diluents, buffers, and the like.

10 Carriers include excipients and diluents and should be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the patient being treated. The carrier can be inert or it can possess pharmaceutical benefits of its own. The amount of carrier employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound.

15 Classes of carriers include, but are not limited to adjuvants, binders, buffering agents, coloring agents, diluents, disintegrants, excipients, emulsifiers, flavorants, gels, glidants, lubricants, preservatives, stabilizers, surfactants, solubilizer, tableting agents, wetting agents or solidifying material.

20 Some carriers may be listed in more than one class, for example vegetable oil may be used as a lubricant in some formulations and a diluent in others.

25 Exemplary pharmaceutically acceptable carriers include sugars, starches, celluloses, powdered tragacanth, malt, gelatin; talc, petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers and vegetable oils. Optional active agents may be included in a pharmaceutical composition, which do not substantially interfere with the activity of the compound of the present invention.

30 Some excipients include, but are not limited, to liquids such as water, saline, glycerol, polyethylene glycol, hyaluronic acid, ethanol, and the like. The compound can be provided, for example, in the form of a solid, a liquid, spray dried material, a microparticle, nanoparticle, controlled release system, etc., as desired according to the goal of the therapy. Suitable excipients for non-liquid formulations are also known to those of skill in the art. A thorough discussion of

pharmaceutically acceptable excipients and salts is available in Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990).

Additionally, auxiliary substances, such as wetting or emulsifying agents, biological buffering substances, surfactants, and the like, can be present in such vehicles. A biological buffer
5 can be any solution which is pharmacologically acceptable, and which provides the formulation with the desired pH, i.e., a pH in the physiologically acceptable range. Examples of buffer solutions include saline, phosphate buffered saline, Tris buffered saline, Hank's buffered saline, and the like.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc,
10 cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, and the like, an active compound as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered can
15 also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and the like. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, referenced above.

20 In yet another embodiment provided is the use of permeation enhancer excipients including polymers such as: polycations (chitosan and its quaternary ammonium derivatives, poly-L-arginine, aminated gelatin); polyanions (N-carboxymethyl chitosan, poly-acrylic acid); and, thiolated polymers (carboxymethyl cellulose-cysteine, polycarbophil-cysteine, chitosan-thiobutylamidine, chitosan-thioglycolic acid, chitosan-glutathione conjugates).

25 In certain embodiments the excipient is selected from butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol,
30 polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac,

silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

The pharmaceutical compositions/combinations can be formulated for oral administration.

5 For oral administration, the composition may take the form of a tablet, capsule, a softgel capsule or can be an aqueous or nonaqueous solution, suspension or syrup. Tablets and capsules are typical oral administration forms. Tablets and capsules for oral use can include one or more commonly used carriers such as lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. Typically, the compositions of the disclosure can be combined with an oral,
10 non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and
15 synthetic gums such as acacia, tragacanth, or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, and the like.

20 When liquid suspensions are used, the active agent can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like and with emulsifying and suspending agents. If desired, flavoring, coloring and/or sweetening agents can be added as well. Other optional components for incorporation into an oral formulation herein include, but are not limited to, preservatives, suspending agents, thickening agents, and the like.

25 For ocular delivery, the compound can be administered, as desired, for example, via intravitreal, intrastromal, intracameral, sub-tenon, sub-retinal, retro-bulbar, peribulbar, suprachoroidal, conjunctival, subconjunctival, episcleral, periocular, transscleral, retrobulbar, posterior juxtасleral, circumcorneal, or tear duct injections, or through a mucus, mucin, or a mucosal barrier, in an immediate or controlled release fashion or via an ocular device.

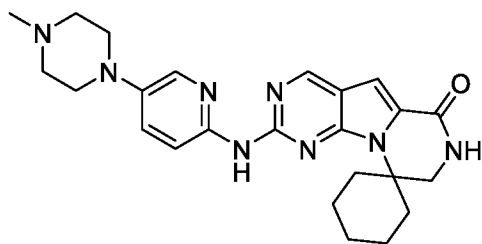
Parenteral formulations can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solubilization or suspension in liquid prior to injection, or as emulsions. Typically, sterile injectable suspensions are formulated according to techniques known in the art using suitable carriers, dispersing or wetting agents and suspending agents. The sterile injectable formulation can also be a sterile injectable solution or a suspension in a acceptably nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils, fatty esters or polyols are conventionally employed as solvents or suspending media. In addition, parenteral administration can involve the use of a slow release or sustained release system such that a constant level of dosage is maintained.

Parenteral administration includes intraarticular, intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, and include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Administration via certain parenteral routes can involve introducing the formulations of the disclosure into the body of a patient through a needle or a catheter, propelled by a sterile syringe or some other mechanical device such as a continuous infusion system. A formulation provided by the disclosure can be administered using a syringe, injector, pump, or any other device recognized in the art for parenteral administration.

Embodiments

Provided herein are at least the following embodiments:

1. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:

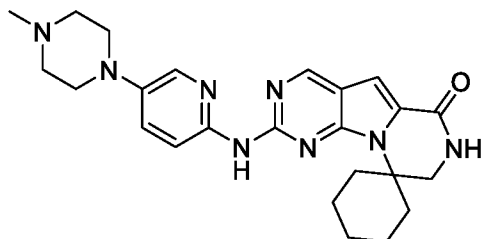


, or a pharmaceutically acceptable salt thereof,

wherein the CDK4/6 inhibitor is administered in a FOLFOXIRI chemotherapy protocol,

and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFOXIRI chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

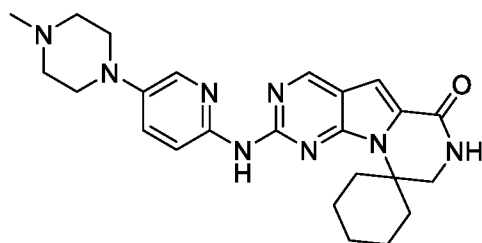
2. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFOXIRI chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and

wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFOXIRI chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

3. A method of treating colorectal cancer in a human comprising:
- vi) administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



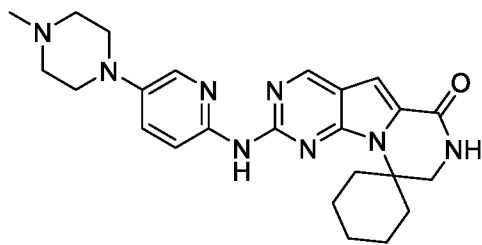
, or a pharmaceutically acceptable salt

thereof;

- vii) administering to the human an effective amount of 5-FU;
- viii) administering to the human an effective amount of folinic acid;
- ix) administering to the human an effective amount of oxaliplatin;
- x) administering to the human an effective amount of irinotecan; and,
- xi) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan, and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

4. The method of any of embodiments 1-3, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.
5. The method of any of embodiments 1-4, wherein the CDK4/6 inhibitor is administered a second time between 20 and 24 hours after the first administration.
6. The method of any of embodiments 1-4, wherein the CDK4/6 inhibitor is administered a second time between 20 and 22 hours after the first administration.
7. The method of any of embodiments 1-6, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
8. The method of any of embodiments 1-7, wherein the human is administered an anti-VEGF antibody selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
9. The method of embodiment 8, wherein the human is administered bevacizumab, or a biosimilar thereof.
10. The method of any of embodiments 1-7, wherein the human is administered an anti-EGFR antibody selected from cetuximab or panitumumab.
11. The method of embodiment 10, wherein the human is administered cetuximab, or a biosimilar thereof.
12. The method of any of embodiments 1-11, wherein the folinic acid administered is leucovorin.
13. The method of any of embodiments 1-11, wherein the folinic acid administered is levoleucovorin.
14. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising
 - i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor of structure:



, or a pharmaceutically acceptable salt thereof,

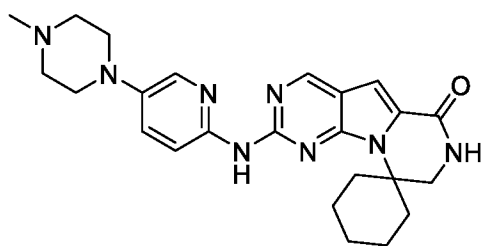
- ii) administering to the human starting on day 1 of each 14-day cycle an effective amount of fluorouracil (5-FU), wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- 5 iii) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid;
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- v) administering to the human on day 1 of each 14-day cycle an effective amount of
- 10 irinotecan; and,
- vi) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle.

- 15 15. The method of any of embodiments 14, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.
16. The method of embodiments 14 or 15, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 24 hours after its administration on day 1.
- 20 17. The method of embodiments 14 or 15, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 22 hours after its administration on day 1.
18. The method of any of embodiments 14 to 17, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
- 25 19. The method of embodiment 18, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.

20. The method of embodiment 19, wherein the human is administered bevacizumab, or a biosimilar thereof.
21. The method of any of embodiments 14-17, wherein the human is administered an anti-EGFR antibody selected from cetuximab or panitumumab.
- 5 22. The method of embodiment 21, wherein the human is administered cetuximab, or a biosimilar thereof.
23. The method of any of embodiments 14 to 17, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
- 10 24. The method of embodiment 23, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
25. The method of any of embodiments 14 to 24, wherein the 5-FU is administered as a continuous infusion between about 2200 mg/m² and 3800 mg/m² over between about 44 hours and 48 hours.
- 15 26. The method of embodiment 25, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² and 3200 mg/m² over about 46-48 hours.
27. The method of embodiment 26, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² over about 46-48 hours.
28. The method of any of embodiments 14 to 27, wherein the human is administered a bolus dose of 5-FU of between about 200 and 600 mg/m² on day 1.
- 20 29. The method of embodiment 28, wherein the human is administered a bolus dose of 5-FU of between about 400 mg/m² on day 1.
30. The method of any of embodiments 14-29, wherein the human is administered a dose of irinotecan of between about 150 and 200 mg/m².
- 25 31. The method of embodiment 30, wherein the human is administered a dose of irinotecan of about 165 mg/m².
32. The method of embodiment 30, wherein the human is administered a dose of irinotecan of about 180 mg/m².
33. The method of any of embodiments 14-32, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².

34. The method of embodiment 33, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
35. The method of embodiment 33, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
- 5 36. The method of embodiment 33, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
37. The method of any of embodiments 14-36, wherein the human is administered a dose of leucovorin on day 1 of between about 100 mg/m² and 500 mg/m².
- 10 38. The method of embodiment 37, wherein the human is administered a dose of leucovorin on day 1 of about 400 mg/m².
39. The method of embodiment 37, wherein the human is administered a dose of leucovorin on day 1 of about 200 mg/m².
40. The method of any of embodiments 14-36, wherein the human is administered a dose of leucovorin on day 1 of between about 50 mg/m² and 250 mg/m².
- 15 41. The method of embodiment 40, wherein the human is administered a dose of levoleucovorin on day 1 of about 200 mg/m².
42. The method of embodiment 40, wherein the human is administered a dose of leucovorin on day 1 of about 100 mg/m².
43. The method of any of embodiments 14-42, wherein the human is administered up to 12 14-day cycles.
- 20 44. The method of any of embodiments 1-43, wherein the human is further administered a maintenance regimen comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of embodiments 14-43.
45. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:
- 25

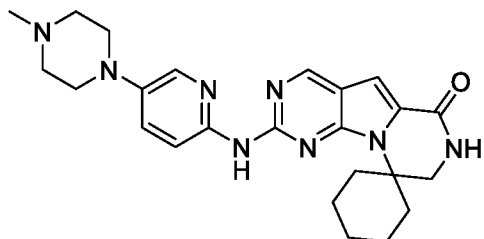


, or a pharmaceutically acceptable salt thereof,

wherein the CDK4/6 inhibitor is administered in a FOLFIRINOX chemotherapy protocol,

and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFIRINOX chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

46. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFIRINOX chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:

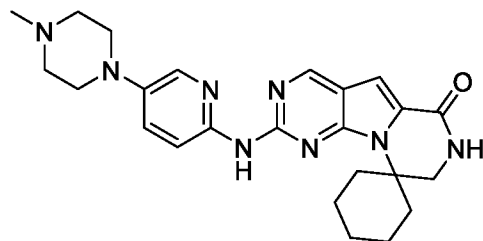


, or a pharmaceutically acceptable salt thereof, and

wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFIRINOX chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

47. A method of treating colorectal cancer in a human comprising:

- i) administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof;

- ii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;
- iii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI);
- iv) administering to the human an effective amount of folinic acid;
- v) administering to the human an effective amount of oxaliplatin; and,
- vi) administering to the human an effective amount of irinotecan; and,

vii) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is first administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan, and wherein the CDK4/6 inhibitor is administered a second time between about 18 and 26 hours after its first administration.

48. The method of any of embodiments 45-47, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.

49. The method of any of embodiments 45-48, wherein the CDK4/6 inhibitor is administered a second time between 20 and 24 hours after the first administration.

50. The method of any of embodiments 45-48, wherein the CDK4/6 inhibitor is administered a second time between 20 and 22 hours after the first administration

51. The method of any of embodiments 45-50, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.

52. The method of any of embodiments 45-51, wherein the human is administered an anti-VEGF antibody selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.

53. The method of embodiment 52, wherein the human is administered bevacizumab, or a biosimilar thereof.

54. The method of any of embodiments 45-51, wherein the human is administered an anti-EGFR antibody selected from cetuximab or panitumumab.

55. The method of embodiment 54, wherein the human is administered cetuximab, or a biosimilar thereof.

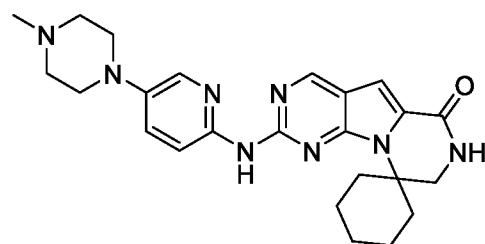
56. The method of any of embodiments 45-54, wherein the folinic acid administered is leucovorin.

57. The method of any of embodiments 45-54, wherein the folinic acid administered is levoleucovorin.

58. The method of any of embodiments 45-54, wherein the human is further administered a maintenance regimen comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of embodiments 45-55.

59. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

- ii) administering to the human on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;
- iii) administering to the human starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours beginning on day 1;
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid;
- v) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) administering to the human on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- vii) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle.

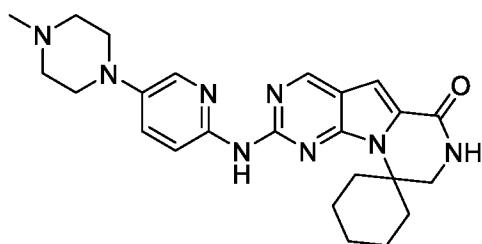
60. The method of embodiment 59, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.

61. The method of embodiment 60, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 24 hours after its administration on day 1.
62. The method of embodiment 60, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 22 hours after its administration on day 1.
- 5 63. The method of any of embodiments 60-62, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
64. The method of embodiment 63, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
- 10 65. The method of embodiment 63, wherein the human is administered bevacizumab or a biosimilar thereof.
66. The method of embodiment 63, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
67. The method of any of embodiments 60-66, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
- 15 68. The method of embodiment 67, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
69. The method of any of embodiments 60-68, wherein the 5-FU administered as a bolus injection is administered between about 100 mg/m² and 500 mg/m².
- 20 70. The method of embodiment 69, wherein the 5-FU bolus injection is administered between about 400 mg/m².
71. The method of embodiment 69, wherein the 5-FU bolus injection is administered between about 200 mg/m².
72. The method of any of embodiments 60-71, wherein the 5-FU continuous infusion is administered between about 2200 mg/m² and 3800 mg/m² over between about 44 hours and 48 hours.
- 25 73. The method of embodiment 72, wherein the 5-FU administered as a continuous infusion is administered between about 2400 mg/m² and 3200 mg/m² over about 46-48 hours.
74. The method of embodiment 72, wherein the 5-FU administered as a continuous infusion is administered at about 2400 mg/m² over about 46-48 hours.
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75. The method of any of embodiments 60-74, wherein the human is administered a dose of irinotecan of between about 150 and 200 mg/m².
76. The method of embodiment 75, wherein the human is administered a dose of irinotecan of about 165 mg/m².
- 5 77. The method of embodiment 75, wherein the human is administered a dose of irinotecan of about 180 mg/m².
78. The method of any of embodiments 60-77, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².
79. The method of embodiment 78, wherein the human is administered a dose of oxaliplatin of
10 about 85 mg/m².
80. The method of embodiment 78, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
81. The method of embodiment 78, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
- 15 82. The method of embodiments 60-81, wherein the human is administered a dose of leucovorin on day 1 of between about 100 mg/m² and 500 mg/m².
83. The method of embodiment 82, wherein the human is administered a dose of leucovorin on day 1 of about 400 mg/m².
84. The method of embodiment 82, wherein the human is administered a dose of leucovorin
20 on day 1 of about 200 mg/m².
85. The method of embodiments 60-81, wherein the human is administered a dose of levoleucovorin on day 1 of between about 50 mg/m² and 250 mg/m².
86. The method of embodiment 85, wherein the human is administered a dose of leucovorin on day 1 of about 200 mg/m².
- 25 87. The method of embodiment 85, wherein the human is administered a dose of leucovorin on day 1 of about 100 mg/m².
88. The method of any of embodiments 60-87, wherein the human is administered up to 12 14-day cycles.

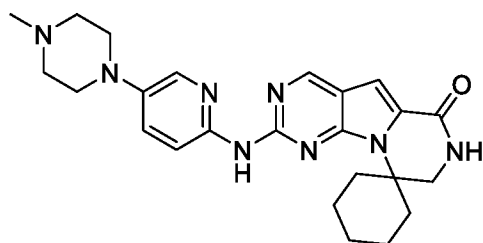
89. The method of any of embodiments 60-88, wherein the human is further administered a maintenance regimen comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of embodiments 60-88.

90. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, wherein the CDK4/6 inhibitor is administered in a FOLFOX-based chemotherapy protocol, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of the FOLFOX-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

91. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFOX-based chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFOX-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

92. The method of embodiments 90-91, wherein the FOLFOX-based chemotherapy protocol is FOLFOX4.

93. The method of embodiments 90-91, wherein the FOLFOX-based chemotherapy protocol is FOLFOX6.

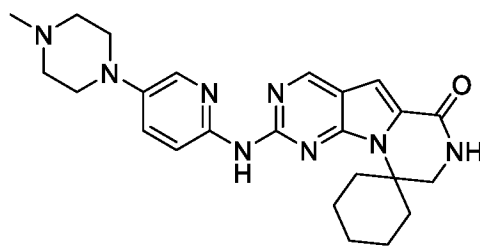
94. The method of embodiments 90-91, wherein the FOLFOX-based chemotherapy protocol is modified FOLFOX6 (mFOLFOX6).

95. The method of embodiments 90-91, wherein the FOLFOX-based chemotherapy protocol is FOLFOX7.

96. The method of embodiments 90-91, wherein the FOLFOX-based chemotherapy protocol is modified FOLFOX7 (mFOLFOX7).

97. A method of treating colorectal cancer in a human comprising:

- i) administering to the human an effective amount of a CDK4/6 inhibitor having the structure;



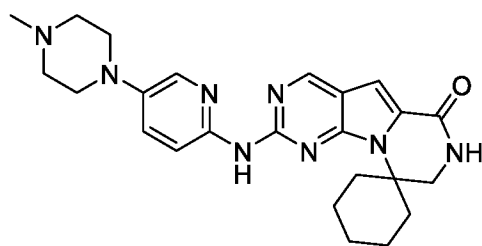
, or a pharmaceutically acceptable salt thereof,

- ii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI);
- iii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a bolus dose;
- iv) administering to the human an effective amount of a folinic acid; and,
- v) administering to the human an effective amount of oxaliplatin; and,
- vi) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is first administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, or oxaliplatin, and wherein the CDK4/6 inhibitor is administered a second time between about 18 and 26 hours after its first administration.

98. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure;



, or a pharmaceutically acceptable salt

thereof,

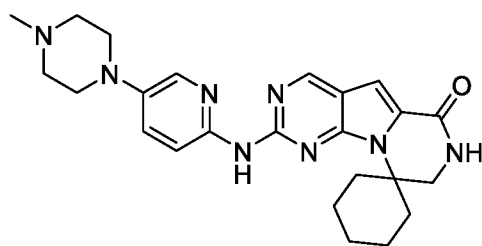
- ii) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 20 and 24 hours;
- iii) administering on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus dose;
- iv) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a folinic acid; and,
- v) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and oxaliplatin on day 1 of each 14-day cycle, and

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to the initiation of administration of 5-FU and folinic acid on day 2 of each cycle.

99. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

- ii) administering to the human on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus intravenous injection;
- 5 iii) administering to the human starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid; and,
- 10 v) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, or oxaliplatin on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle.

100. The method of any of embodiments 90-99, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of either the bolus 5-FU or intravenous 5-FU.

101. The method of embodiments 90-95, 97, and 99-100, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 24 hours after its administration on day 1.

102. The method of embodiments 90-95, 97, and 99-100, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 22 hours after its administration on day 1.

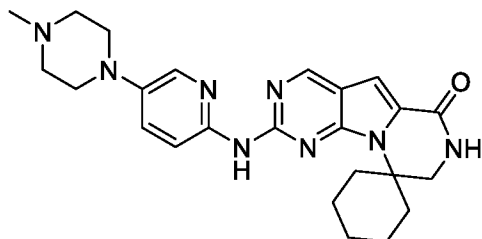
25 103. The method of any of embodiments 90-103, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.

104. The method of embodiment 103, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
105. The method of embodiment 103, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
106. The method of any of embodiments 90-105, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
107. The method of embodiment 106, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
108. The method of any of embodiments 96, 98, and 100-107, wherein the 5-FU is administered as a continuous infusion between about 400 mg/m² and 800 mg/m² over about 22 hours on day 1 and day 2.
109. The method of embodiment 108, wherein the 5-FU is administered as a continuous infusion of about 600 mg/m² over about 22 hours on day 1 and day 2.
110. The method of any of embodiments 90-95, 97, and 99-107, wherein the 5-FU is administered as a continuous infusion between about 2000 mg/m² and 3200 mg/m² over about 46-48 hours on day 1 and day 2.
111. The method of embodiment 110, wherein the 5-FU is administered as a continuous infusion between about 2400 mg/m² and 3000 mg/m² over about 46-48 hours on day 1 and day 2.
112. The method of embodiment 110, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² over about 46-48 hours on day 1 and day 2.
113. The method of any of embodiments 90-112, wherein the human is administered a bolus dose of 5-FU of between about 200 and 600 mg/m² on day 1.
114. The method of embodiment 113, wherein the human is administered a bolus dose of 5-FU of about 200 mg/m².
115. The method of embodiment 113, wherein the human is administered a bolus dose of 5-FU of between about 400 mg/m² on day 1.
116. The method of any of embodiments 90-114, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².

117. The method of embodiment 115, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
118. The method of embodiment 115, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
- 5 119. The method of embodiment 115, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
120. The method of any of embodiments 90-118, wherein the folinic acid is leucovorin.
121. The method of embodiment 96, 98, 100-109, 113-119 wherein the human is administered a dose of leucovorin on day 1 and day 2 of between about 100 mg/m² and
10 500 mg/m².
122. The method of embodiment 121, wherein the human is administered a dose of leucovorin on day 1 and day 2 of about 200 mg/m².
123. The method of embodiment 90-95, 97, 99-107, 110-120, wherein the human is administered a dose of leucovorin on day 1 of between about 100 mg/m² and 500 mg/m².
- 15 124. The method of embodiment 123, wherein the human is administered a dose of leucovorin on day 1 of about 400 mg/m².
125. The method of any of embodiments 90-118, wherein the folinic acid is levoleucovorin.
126. The method of embodiment 96, 98, 100-109, 113-119 wherein the human is administered a dose of levoleucovorin on day 1 and day 2 of between about 50 mg/m² and
20 250 mg/m².
127. The method of embodiment 126, wherein the human is administered a dose of levoleucovorin on day 1 and day 2 of about 100 mg/m².
128. The method of embodiment 90-95, 97, 99-107, 110-120, wherein the human is administered a dose of levoleucovorin on day 1 of between about 50 mg/m² and 250 mg/m².
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129. The method of embodiment 128, wherein the human is administered a dose of levoleucovorin on day 1 of about 200 mg/m².
130. The method of any of embodiments 90-129, wherein the human is administered up to 12 14-day cycles.

131. The method of any of embodiments 90-130, wherein the human is further administered a maintenance regimen of comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of embodiments 90-130.

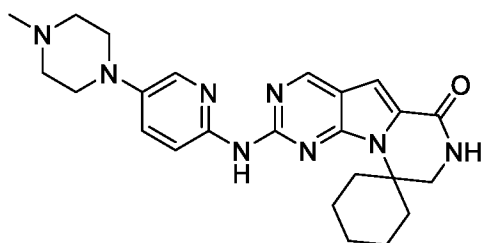
132. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof,

wherein the CDK4/6 inhibitor is administered in a FOLFIRI-based chemotherapy protocol, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of the FOLFIRI-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

133. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFIRI-based chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and

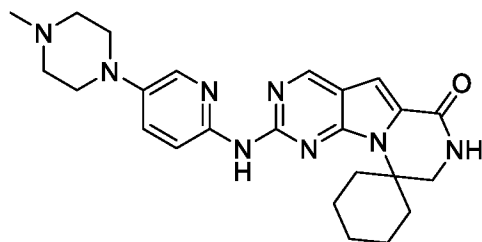
wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFIRI-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

134. The method of embodiments 90-91, wherein the FOLFIRI-based chemotherapy protocol is FOLFIRI.

135. The method of embodiments 90-91, wherein the FOLFOX-based chemotherapy protocol is modified FOLFIRI (mFOLFIRI).

136. A method of treating colorectal cancer in a human comprising:

- i) administering to the human an effective amount of a CDK4/6 inhibitor having the structure;



, or a pharmaceutically acceptable salt

thereof,

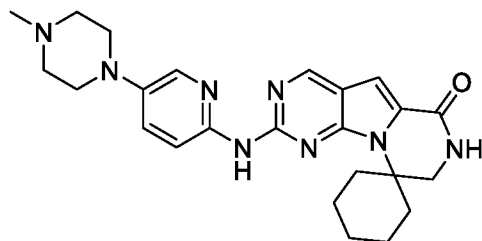
- ii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI);
 iii) administering to the human an effective amount of a folinic acid; and,
 iv) administering to the human an effective amount of irinotecan; and,
 v) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is first administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, or irinotecan, and wherein the CDK4/6 inhibitor is administered a second time between about 18 and 26 hours after its first administration.

137. The method of embodiment 136, wherein the human is further administered 5-FU in a bolus dose.

138. A method of treating colorectal cancer in a human wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

- ii) administering to the human starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iii) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid; and,
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- v) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and irinotecan on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 about 18 to 26 hours after its administration on day 1.

139. The method of embodiment 138, further comprising administering to the human on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection.

140. The method of any of embodiments 132-139, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of either the bolus 5-FU or intravenous 5-FU.

141. The method of embodiments 132-140, wherein the CDK4/6 inhibitor is administered the second time between about 20 and 24 hours after its first administration.

142. The method of embodiments 132-140, wherein the CDK4/6 inhibitor is administered the second time between about 20 and 24 hours after its first administration.

143. The method of any of embodiments 132-142, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.

144. The method of embodiment 143, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.

145. The method of embodiment 143, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.

146. The method of any of embodiments 132-145, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
147. The method of embodiment 146, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
- 5 148. The method of any of embodiments 132-147, wherein the 5-FU is administered as a continuous infusion between about 2200 mg/m² and 3800 mg/m² over between about 44 hours and 48 hours.
149. The method of embodiment 148, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² and 3000 mg/m² over about 46-48 hours.
- 10 150. The method of embodiment 148, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² over about 46-48 hours.
151. The method of any of embodiments 134, 137, and 139-150, wherein the human is administered a bolus dose of 5-FU of between about 200 and 600 mg/m² on day 1.
152. The method of embodiment 151, wherein the human is administered a bolus dose
15 of 5-FU of between about 400 mg/m² on day 1.
153. The method of any of embodiments 132-152, wherein the human is administered a dose of irinotecan of between about 150 and 200 mg/m².
154. The method of embodiment 153, wherein the human is administered a dose of irinotecan of about 165 mg/m².
- 20 155. The method of embodiment 153, wherein the human is administered a dose of irinotecan of about 180 mg/m².
156. The method of any of embodiments 132-155, wherein the folinic acid is leucovorin.
157. The method of embodiment 156, wherein the human is administered a dose of leucovorin of between about 100 mg/m² and 500 mg/m².
- 25 158. The method of embodiment 157, wherein the human is administered a dose of leucovorin of about 400 mg/m².
159. The method of any of embodiments 132-155, wherein the folinic acid is levoleucovorin.
160. The method of embodiment 159 wherein the human is administered a dose of
30 levoleucovorin of between about 50 mg/m² and 250 mg/m².

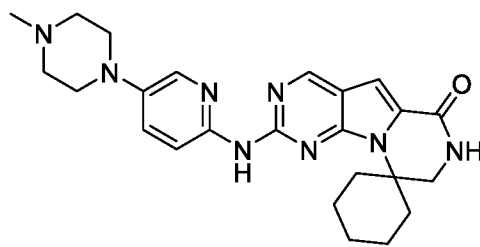
161. The method of embodiment 160, wherein the human is administered a dose of levoleucovorin of about 200 mg/m².

162. The method of any of embodiments 132-161, wherein the human is administered up to 12 14-day cycles.

5 163. The method of any of embodiments 132-162, wherein the human is further administered a maintenance regimen of CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of embodiments 132-162.

164. A method of treating a human with colorectal cancer, wherein the treatment comprises one or more 56-day cycles comprising

10 i) administering to the human on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof;

15 ii) administering to the human on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;

iii) administering to the human on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of folinic acid;

20 iv) administering to the human on day 1, day 15, and day 29 of each 56-day cycle an effective amount of oxaliplatin; and,

v) optionally administering to the human on day 1, day 15, and day 29 of each 56-day cycle an anti-VEGF or anti-EGFR monoclonal antibody;

25 wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to initiation of administration of the 5-FU, folinic acid, or oxaliplatin.

165. The method of embodiment 166, wherein CDK4/6 inhibitor is administered about 4 hour or less prior to the administration of the 5-FU.

166. The method of any of embodiments 164-165, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
167. The method of embodiment 166, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
168. The method of embodiment 166, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
169. The method of any of embodiments 164-168, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
170. The method of embodiment 169, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
171. The method of any of embodiments 164-170, wherein the 5-FU is administered as a bolus dose of between about 400 mg/m² and 600 mg/m².
172. The method of embodiment 171, wherein the 5-FU is administered as a bolus dose of about 500 mg/m².
173. The method of any of embodiments 164-172, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².
174. The method of embodiment 173, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
175. The method of embodiment 173, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
176. The method of embodiment 173, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
177. The method of any of embodiments 164-176, wherein the folinic acid is leucovorin.
178. The method of embodiment 177, wherein the human is administered a dose of leucovorin of between about 100 mg/m² and 600 mg/m².
179. The method of embodiment 178, wherein the human is administered a dose of leucovorin of about 500 mg/m².
180. The method of any of embodiments 164-176, wherein the folinic acid is levoleucovorin.

181. The method of embodiment 180, wherein the human is administered a dose of levoleucovorin of between about 50 mg/m² and 300 mg/m².
182. The method of embodiment 181, wherein the human is administered a dose of leucovorin of about 250 mg/m².
- 5 183. The method of any of embodiments 164-182, wherein the human is administered up to 3 56-day cycles.
184. The method of any of embodiments 164-183, wherein the human is further administered a maintenance regimen of CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of embodiment 164-183.
- 10 185. The method of any of embodiments 1-184, wherein the treatment results in a reduction and/or prevention in one or more toxicities selected from chemotherapy-induced myelosuppression (CIM), chemotherapy-induced diarrhea (CID), stomatitis, and mucositis.
186. The method of any of embodiments 1-185, wherein the treatment results in an
15 improvement in progression free survival (PFS) in comparison to the predicted PFS based on administration of the chemotherapy protocol without the CDK4/6 inhibitor.
187. The method of any of embodiments 1-186, wherein the treatment results in an improvement in overall survival (OS) in comparison to the predicted OS based on administration of chemotherapy protocol without the CDK4/6 inhibitor.
- 20 188. The method of any of embodiments 1-187, wherein the treatment results in myelopreservation of hematopoietic stem and progenitor cells (HSPCs).
189. The method of any of embodiments 1-188, wherein the treatment results in protection of immune effector cells.
190. The method of any of embodiments 1-189, wherein the treatment results in
25 enhanced anti-tumor efficacy in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.
191. The method of any of embodiments 1-190, wherein the treatment results in myelopreservation of the neutrophil lineage in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.

192. The method of any of embodiments 1-191, wherein the treatment results in a reduction in the duration of severe (Grade 4) neutropenia in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.
193. The method of any of embodiments 1-192, wherein the treatment results in a reduction of chemotherapy-induced fatigue (CIF) in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.
194. The method of any of embodiments 1-193, wherein the treatment results in reduction of CIF is a reduction in the time to first confirmed deterioration of fatigue (TTCD-fatigue),
195. The method of any of embodiments 1-194, wherein the treatment results in an improvement in PFS is based on per Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1).
196. The method of any of embodiments 1-195, wherein the treatment results in a reduction in severe neutropenia events, a reduction in granulocyte-colony stimulating factor (G-CSF) treatment, or a reduction in febrile neutropenia (FN) adverse events (AEs).
197. The method of any of embodiments 1-196, wherein the treatment results in a reduction in Grade 3 or 4 decreased hemoglobin laboratory values, red blood cell (RBC) transfusions, or erythropoiesis-stimulating agent (ESA) administration.
198. The method of any of embodiments 1-197, wherein the treatment results in a reduction in Grade 3 or 4 decreased platelet count laboratory values and/or the number of platelet transfusions.
199. The method of any of embodiments 1-198, wherein the treatment results in a reduction in Grade 3 or 4 hematologic laboratory values.
200. The method of any of embodiments 1-199, wherein the treatment results in a reduction in i) hospitalizations due to all causes, febrile neutropenia/neutropenia, anemia/RBC transfusion, thrombocytopenia/bleeding, or infections or ii) antibiotic use.
201. The method of any of embodiments 1-200, wherein the treatment results in an improvement to one or more of: Functional Assessment of Cancer Therapy-General (FACT-G) domain scores (physical, social/family, emotional, and functional well-being); Functional Assessment of Cancer Therapy-Anemia (FACT-An): Anemia; Functional

Assessment of Cancer Therapy-colorectal cancer (FACT-C) : Colorectal Cancer Subscale; 5-level EQ-5D (EQ-5D-5L); Patient Global Impression of Change (PGIC) fatigue item; or Patient Global Impression of Severity (PGIS) fatigue item.

202. The method of any of embodiments 1-201, wherein the colorectal cancer treated is metastatic colorectal cancer (mCRC).

203. The method of any of embodiments 1-202, wherein the colorectal cancer is proficient mismatch repair (pMMR) mCRC.

204. The method of any of embodiments 1-203, wherein the colorectal cancer is microsatellite stable (MSS) mCRC.

205. The method of any of embodiments 1-204, wherein the colorectal cancer is proficient mismatch repair (pMMR)/microsatellite stable (MSS) mCRC (pMMR/MSS mCRC).

206. The method of any of embodiments 1-205, wherein the colorectal cancer has not been responsive to previous treatment with single agent checkpoint inhibitors. I

207. The method of any of embodiments 1-206, wherein the colorectal cancer has a BRAF or KRAS mutation.

208. The method of any of embodiments 1-207, wherein the colorectal cancer has a BRAF V600E substitution mutation.

209. The method of any of embodiments 1-208, wherein the colorectal cancer is CDK4/6 replication dependent.

210. The method of any of embodiments 1-208, wherein the colorectal cancer is CDK4/6 replication independent.

211. The method of any of embodiments 1-208, wherein the colorectal cancer is CDK4/6 replication indeterminate.

212. The method of any of embodiments 1-211, wherein the colorectal cancer is interferon- γ (IFN- γ) dominant according to Thorsson's Six Class Immune Signature.

213. The method of any of embodiments 1-212, wherein the colorectal cancer has a high IFN- γ signature or expanded immune signature according to the Ayer's IFN- γ Signature Score or Expanded Immune Signature Score.

214. The method of any of embodiments 1-213, wherein the colorectal cancer is PD-L1 positive.
215. The method of any of embodiments 1-214, wherein the colorectal cancer has a KRAS mutation selected from a V9, G12, G13, V14, L19, Q22, D33, A59, G60, Q61 R68, K117, A146, R164, K176, or K180 substitution.
216. The method of any of embodiments 1-214, wherein the colorectal cancer has a KRAS mutation selected from G12D, G12V, G12C, G12A, or other G12 variant.
217. The method of any of embodiments 1-214, wherein the colorectal cancer has a NRAS mutation selected from G12, G13, G60, Q61, E123, or P185.
218. The method of any of embodiments 1-194, wherein the colorectal cancer has an HRAS mutation selected from G12, G13, Q61, K117, R164, or P167.
219. The method of any of embodiments 1-214, wherein the colorectal cancer is KRAS wild-type.
220. The method of any of embodiments 1-214, wherein the colorectal cancer is BRAF wild-type.
221. The method of any of embodiments 1-220, wherein the human has not received a prior chemotherapeutic regimen for the treatment of the colorectal cancer.
222. The method of any of embodiments 1-221, wherein the human has failed prior chemotherapeutic regimen for the treatment of colorectal cancer.
223. The method of any of embodiments 1-222, wherein the human maintains an absolute neutrophil count (ANC) of greater than $1.0 \times 10^9/\text{L}$ when measured prior to the beginning of each chemotherapy treatment cycle.
224. The method of any of embodiments 1-223, wherein the human maintains a platelet count of greater than $75 \times 10^9/\text{L}$ when measured prior to the beginning of each chemotherapy treatment cycle.
225. The method of any of embodiments 1-224, wherein the human has proficient mismatch repair (pMMR)/microsatellite stable (MSS) metastatic colorectal cancer (pMMR/MSS mCRC) and has not received systemic therapy for metastatic disease.
226. The method of any of embodiments 1-225, wherein the chemotherapy protocol does not include the administration of an immune checkpoint inhibitor.

227. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic pancreatic cancer.

228. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic gastric cancer.

5 229. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic gastroesophageal junction adenocarcinoma.

230. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic biliary tract cancer.

10 231. The method of embodiment 230, wherein the advanced or metastatic biliary tract cancer is intrahepatic cholangiocarcinoma.

232. The method of embodiment 230, wherein the advanced or metastatic biliary tract cancer is hilar cholangiocarcinoma.

15 233. The method of embodiment 230, wherein the advanced or metastatic biliary tract cancer is distal cholangiocarcinoma.

234. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic neuroendocrine carcinoma.

20 235. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic peritoneal carcinosis.

236. In alternative embodiments of the method of any of embodiments 1-225, the cancer treated is not colorectal cancer but instead advanced or metastatic liver cancer.

25 237. In alternative embodiments of the method of any of embodiments 1-236, the use of 5-FU in the described embodiments is substituted with an alternative fluoropyrimidine, wherein the alternative fluoropyrimidine is selected from capecitabine, tegafur, carmofur (HCFU), or doxifluridine.

238. The method of any of embodiments 1-237, wherein the treatment results in a reduction and/or prevention in chemotherapy-induced myelosuppression (CIM).

30 239. The method of any of embodiments 1-238, wherein the treatment results in a reduction and/or prevention in chemotherapy-induced diarrhea (CID).

240. The method of any of embodiments 1-239, wherein the treatment results in a reduction and/or prevention in stomatitis.

241. The method of any of embodiments 1-240, wherein the treatment results in a reduction and/or prevention in mucositis.

5 242. The method of any of embodiments 1-241, wherein a 5-FU prodrug is administered instead of 5-FU.

Examples:

10 **Example 1 - A Phase 3 Randomized, Double-blind Trial of Trilaciclib versus Placebo in Patients Receiving FOLFOXIRI/Bevacizumab for Metastatic Colorectal Cancer**

A randomized, double-blind, placebo-controlled, global, multicenter, Phase 3 trial evaluating the impact of trilaciclib on myelopreservation and anti-tumor efficacy when administered prior to FOLFOXIRI/bevacizumab in patients with pMMR/MSS mCRC who have not received systemic therapy for metastatic disease is underway. A general schematic describing the clinical trial is provided in Fig. 8.

15 Approximately 296 patients with metastatic colorectal cancer (mCRC) will be randomly assigned (1:1) to receive placebo or trilaciclib on Days 1 and 2 administered intravenously (IV) prior to FOLFOXIRI/bevacizumab in 14-day cycles for up to 12 cycles (Induction Period). There are three stratification factors for randomization: country of treatment, prior therapy in adjuvant/neoadjuvant setting, and presence of BRAF V600E mutation. Within each country, patient randomization will be stratified by history of systemic cytotoxic therapy in the adjuvant/neoadjuvant setting (yes/no) and BRAF V600E mutational status (yes/no).

25 Induction

During the induction, all patients will receive trilaciclib 240 mg/m² or placebo IV on Day 1 and Day 2 of each 14-day Induction cycle (up to 12 cycles in total). On Day 1, trilaciclib/placebo will be administered no more than 4 hours prior to the start of chemotherapy administration; the second dose of trilaciclib/placebo should be administered on Day 2. FOLFOXIRI/bevacizumab should not be administered until after the completion of the trilaciclib or placebo infusion on Day

1. Patients should meet the requirements to proceed before initiation of Cycle 2 and each subsequent induction cycle. Since individual drugs may be discontinued for toxicity, induction refers to any cycles of therapy where 5-FU and either oxaliplatin or irinotecan is also being administered, e.g., 5-FU + oxaliplatin + bevacizumab; 5-FU+ irinotecan + bevacizumab; 5-FU +
 5 oxaliplatin; or 5-FU + irinotecan. Though there is no requirement for a minimum number of chemotherapy cycles during induction, patients should continue with Induction therapy (in the absence of disease progression) as long as it is tolerated.

Study drugs administered during Induction are as follows:

- Trilaciclib (240 mg/m²) or placebo: In each 14-day cycle in Induction and
 10 Maintenance, a dose of trilaciclib 240 mg/m² diluted in 250 mL of dextrose 5% in water or sodium chloride solution 0.9% will be administered by IV infusion over approximately 30 (±10) minutes no more than 4 hours prior to each Day 1 FOLFOXIRI/bevacizumab administration. The second dose of trilaciclib should be administered on Day 2.
- FOLFOXIRI/bevacizumab – In each 14-day cycle in Induction the following agents
 15 will be administered according to standard labeling instructions:
 - Irinotecan 165 mg/m² –IV, Day 1.
 - Oxaliplatin 85 mg/m² – IV, Day 1.
 - Folinic acid (leucovorin 400 mg/m² or levoleucovorin 200 mg/m²)-IV, Day 1.
 - 20 ○ 5-FU (Fluorouracil) 2400 – 3200 mg/m² – continuous infusion (CI) over 48 hours beginning on Day 1; this dose range is provided to reflect geographic variations in prescribed fluorouracil (5-FU) dose; however, the same dose should be continued throughout the study for each patient, except where dose modifications are required for toxicity management.
 - 25 ○ Bevacizumab 5 mg/kg – IV, Day 1.

Since the infusional-5FU component of FOLFOXIRI/bevacizumab is administered over 48 hours, trilaciclib is planned to be administered on both Day 1 and Day 2 of each 14-day cycle. The Day 2 infusion of trilaciclib is being administered to ensure that synchronization and release of the HSPC G1 arrest (~24-32 hours after a single dose of Trilaciclib) does not occur while 5FU

is at sufficient concentrations such that 5FU-related myelosuppression is exacerbated rather than mitigated.

Maintenance

5 Patients who complete induction without radiographic or clinical disease progression and remain eligible to receive 5-FU/folinic acid (leucovorin or levoleucovorin)/bevacizumab will continue with maintenance. These patients include those who were able to complete the maximum of 12 induction cycles as well as those who were unable to complete 12 cycles due to toxicity (without documented disease progression) when the treating physician feels the patient will receive
10 additional clinical benefit by transitioning to maintenance therapy. Patients will receive trilaciclib or placebo (per same randomization allocation at study entry) prior to infusional-5-FU/folinic acid (leucovorin or levoleucovorin)/bevacizumab at the same dose and schedule in each 14-day cycle during maintenance. Infusional 5-FU/folinic acid (leucovorin or levoleucovorin)/bevacizumab should not be administered on Day 1 until after the completion of administration of the trilaciclib
15 or placebo infusion. Patients should meet the requirements before initiation of each maintenance cycle. Since individual drugs may be discontinued for toxicity, maintenance refers to any cycles of therapy where only 5FU/folinic acid (leucovorin or levoleucovorin) or 5FU/folinic acid (leucovorin or levoleucovorin) + bevacizumab is administered. Treatment cycles will occur consecutively without interruption, except when necessary to manage toxicities or for
20 administrative reasons. Upon discontinuation of study treatment, patients will be followed for survival, i.e., patients or their caregivers will be contacted approximately every 2 months until the end of the trial (or death) to record their status (alive or dead) as well as details of any subsequent systemic anti-cancer therapy initiated.

25 Criteria for Starting Cycle 2 and Each Subsequent Cycle in Induction or Maintenance

Following completion of Cycle 1, patients must meet all the following criteria to receive the Day 1 dose of study treatment for Cycle 2 and each subsequent cycle during induction or maintenance:

- $ANC \geq 1.0 \times 10^9 /L$,
- 30 • Platelet count $\geq 75 \times 10^9 /L$, and

- Nonhematologic drug-related toxicities must be $\leq$ Grade 1 or have returned to baseline.

Criteria for evaluation:

Efficacy:

5 Myelopreservation efficacy will be assessed based on reported hematology assessments, severe adverse event (AE) details, and supportive care interventions (including transfusions). Fatigue will be assessed with the Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F). Tumor response criteria including PFS, OS, duration of objective response (DOR) and best overall response (BOR) will be based on RECIST v1.1.

10

Safety:

Safety will be evaluated by monitoring AEs, clinical laboratory test results (hematology, clinical chemistry, coagulation [international normalized ratio, activated partial thromboplastin time], and urinalysis), vital sign measurements (blood pressure, heart rate [HR], and oral body
15 temperature), 12 lead safety electrocardiogram (ECG) results, dose modifications, and physical examination findings.

Statistical analysis methods for primary and key secondary endpoints

General testing strategy

20 The treatment effects on the primary myelosuppression endpoints (DSN in Cycle 1 and occurrence of SN during Induction) and the key secondary PRO endpoint (TTCD-fatigue) will be tested as 2-sided $\alpha_1=0.04$ level, while the treatment effects on PFS and OS will be tested at 2-sided $\alpha_2=0.01$ level. A fixed-sequence procedure will be used for assessing treatment effects on the two primary endpoints and then the key PRO secondary endpoint, while a hierarchical testing strategy
25 from PFS to OS is assumed. If the analyses on DSN in Cycle 1 and occurrence of SN have demonstrated statistically significant results at the 2-sided 0.04 level, the analysis for TTCD-fatigue will then be performed. If statistical significance can be established at the 2-sided 0.04 level for TTCD-Fatigue, the assigned $\alpha_1=0.04$ will be recycled to test PFS and OS following a fallback procedure (Wiens, 2003). In that occasion, testing PFS and OS will be conducted at the
30 level of 2-sided 0.05 (i.e., at the level of $\alpha_1+\alpha_2$).

Analysis for primary and key secondary efficacy endpoints

The primary myelosuppression endpoints are DSN in Cycle 1 and occurrence of SN during Induction. Severe neutropenia (SN) is defined as the absolute neutrophil count (ANC) laboratory value that meets the common terminology criteria for adverse events (CTCAE) criteria for $\geq$ Grade 4 toxicity (i.e., $ANC < 0.5 \times 10^9/L$ in SI Unit). For patients with at least one SN event in Cycle 1, the duration of SN (DSN) is defined as the number of days from the date of the first ANC value of $< 0.5 \times 10^9/L$ to the date of the first ANC value $\geq 0.5 \times 10^9/L$ where no additional ANC values $< 0.5 \times 10^9/L$ are observed for the remainder of that cycle. All observed data from scheduled or non-scheduled visits within Cycle 1 will be included in the derivation. For those patients without any SN in Cycle 1, DSN will be set to be 0. The treatment group difference in DSN in Cycle 1 will be evaluated using a nonparametric analysis of covariance (ANCOVA) (Quade, 1967). In this analysis, the rank transformed (within each stratum) DSN values are analyzed by an ANCOVA model with the terms of treatment, region (US, Eastern Europe, or Western Europe), and history of systemic cytotoxic therapy in the adjuvant/neoadjuvant setting (Yes or No). The rank-transformed baseline ANC (within each stratum) will be included as a covariate in the model. In addition, the mean difference (trilaciclib – placebo), the standard error and the 96% confidence interval generated from a Satterthwaite t test will be presented.

The occurrence of SN during Induction is defined as having SN in at least one cycle in Induction and is thus a binary variable (yes vs no). This co-primary endpoint will be analyzed using a modified Poisson regression model with the same terms as used in the non-parametric ANCOVA model for DSN in Cycle 1 with baseline ANC value as a covariate. The log-transformed number of cycles will be used as the offset in the model. The adjusted relative risk (aRR) (trilaciclib vs placebo) and its 96% confidence interval will be calculated and reported along with the 2-sided p-value.

Once the treatment effects for both primary endpoints are established at the 2-sided significance level of 0.04, the analysis for the key secondary PRO efficacy endpoint of TTCD-fatigue, as measured by the FACIT-F, will be performed. Time (months) to first deterioration of fatigue (confirmed at two consecutive visits) is calculated as the duration of time from date of randomization to the date of first confirmed deterioration. Patients who do not experience

confirmed deterioration will be censored at the date of the last instrument completion (i.e., date of the last non-missing value). Patients with no baseline assessment will be censored at the date of the baseline assessment. For time to first deterioration of fatigue, the Kaplan Meier method will be used to estimate the median, 25% and 75% percentile duration of TTCD-fatigue with their corresponding 96% confidence interval. Treatment group difference in TTCD-fatigue will be evaluated using a stratified log-rank test with the same terms as used in the non-parametric ANCOVA model for DSN in Cycle 1. The hazard ratio (HR) between the 2 treatment groups (trilaciclib vs placebo), together with its 96% confidence intervals, will be calculated from a Cox proportional hazard model using the same terms as included in the stratified log-rank test.

Analysis for PFS and OS

Treatment effects on PFS and OS will be evaluated using the stratified log-rank test controlling for three factors of region, history of systemic cytotoxic therapy in the adjuvant/neoadjuvant setting, and presence of BRAF V600E mutation (yes, or no). In addition, a Cox proportional hazard model with the same covariate terms as in the stratified log-rank test will be used to estimate the HR between the 2 treatment groups (trilaciclib vs placebo) for PFS and OS along with its $(1-\alpha) \times 100\%$ confidence interval, respectively.

Analysis for safety endpoints

AEs are defined as those events occurring or worsening after treatment has begun on this study. AE data will be coded to system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). The number and percentage of patients experiencing any AE overall, and by system organ class and preferred term will be tabulated for each treatment group. Adverse events considered by the investigator to be related to treatment will also be summarized by the treatment to which it is attributed (e.g., trilaciclib/placebo, fluorouracil, leucovorin, irinotecan, oxaliplatin, bevacizumab) for each treatment group. Severity of AEs will be tabulated based on greatest severity observed for each patient. In the tabulation of grade and causality, if the same AE occurs on multiple occasions, the highest grade and strongest relationship to study drug will be used in a summary. Adverse event of special interests (AESI) for trilaciclib, AEs leading to study drug discontinuation, and use of concomitant medications to treat AEs will

be tabulated separately. Concomitant medications, as well as prior and subsequent anticancer therapies, will be coded to Anatomical Therapeutic Classification (ATC) using the World Health Organization-Drug Dictionary (WHO-DD)

Observed values and change (including maximum and minimum values) from baseline to each visit in vital signs, ECG intervals, and laboratory assessments of hematology, clinical chemistry, urinalysis, and liver function parameters will be tabulated, as appropriate. Toxicity grades for clinical lab parameters (ego, hematology, chemistry) will be characterized according to National Cancer Institute (NCI) CTCAE v5.0, when possible. Shifts in toxicity grades from baseline to each visit, and from baseline to the worst grade during the study will be summarized. Both scheduled and unscheduled data will be included in safety evaluation. Graphical presentations of safety data will be presented as is deemed appropriate.

The pharmacokinetics of trilaciclib will be determined using a non-linear mixed effects modeling approach. Population pharmacokinetic parameters including clearance (CL), volume of distribution (V), and other parameters will be estimated as data permit.

Definitions of Tumor Response and Disease Progression (per RECIST v1.1)

The determination of tumor response and progression will be based on the RECIST v1.1 criteria (**Error! Reference source not found.**, 2009). Tumor lesions will be categorized as follows:

Measurable lesions: tumor lesions with a longest diameter (measured in at least 1 dimension) with a minimum size as follows:

- 10 mm by CT or MRI (with a scan slice thickness of no greater than 5 mm)
- Measurable lymph nodes must be ≥ 15 mm on the short axis by CT or MRI (with a scan slice thickness of no greater than 5 mm); only the short axis is to be measured at baseline and follow-up.
- Lytic bone lesions or mixed lytic-blastic lesions with a soft tissue component meeting the definition of measurability above can be considered measurable lesions.

- Cystic lesions representing cystic metastases that meet the definition of measurability described above can be considered measurable lesions. If present, noncystic lesions should be selected as target lesions for this study.
- A tumor lesion that has been previously irradiated may be considered measurable if unequivocal growth of the lesion has been demonstrated.

Target lesions: At baseline, up to 5 measurable tumor lesions/lymph nodes (with a maximum of 2 lesions per organ) should be identified as target lesions. Lesions with the longest diameter, that are representative of all involved organs, and for which reproducible repeated measurements can be obtained should be selected as the target lesions. Malignant lymph node is considered an organ in this study, therefore only 2 malignant lymph nodes (regardless of location) may be selected as target lesions and all others should be entered as nontarget lesions.

Non-measurable Lesions: tumor lesions with a longest diameter < 10 mm, lymph nodes with ≥ 10 to < 15 mm short axis, or non-measurable lesions such as leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, or abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by CT scan or MRI.

Nontarget lesions: All other lesions (or sites of disease) identified at baseline should be identified as nontarget lesions and recorded in the eCRF. Measurements of these lesions are not required, but the presence, absence, or unequivocal progression of each nontarget lesion should be recorded in the eCRF at each follow up time point. Multiple nontarget lesions in the same organ may be noted as a single item on the eCRF.

Evaluation of Target Lesions

The definitions for tumor response for the target lesion per RECIST v1.1 are as follows:

- Complete Response: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm (< 1 cm).

- Partial Response: At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease: At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progression).
- Stable Disease: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Evaluation of Non-Target Lesions

- Complete Response: Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm [< 1 cm] short axis).
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- Progressive Disease: Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. ‘Unequivocal progression’ represents a substantial increase in overall tumor burden such that treatment should be discontinued even in the setting of stable disease (SD) or PR in the target disease. Although a clear progression of “non-target” lesions only is rare, the opinion of the treating physician should prevail in such circumstances.

Appearance of New Lesions

The appearance of new lesion(s) is considered PD according to RECIST v1.1.

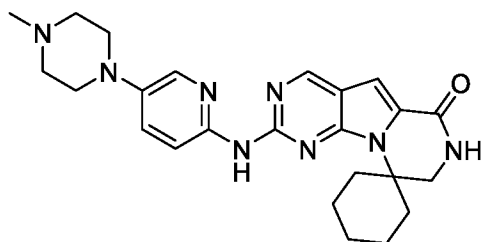
This specification has been described with reference to embodiments of the invention. However, one of ordinary skill in the art appreciates that various modification and changes can be made without departing from the scope of the invention as set forth in the claims below.

Accordingly, the specification is to be regarded in an illustrative rather than a restrictive sense, and all such modifications are intended to be included within the scope of the invention.

Claims of the Invention

We claim:

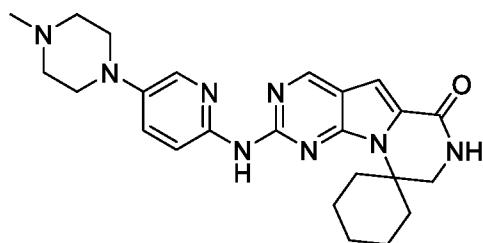
1. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof,

wherein the CDK4/6 inhibitor is administered in a FOLFOXIRI chemotherapy protocol, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFOXIRI chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

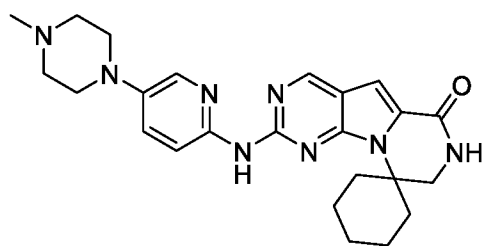
2. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFOXIRI chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and

wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFOXIRI chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

3. A method of treating colorectal cancer in a human comprising:
 - i) administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

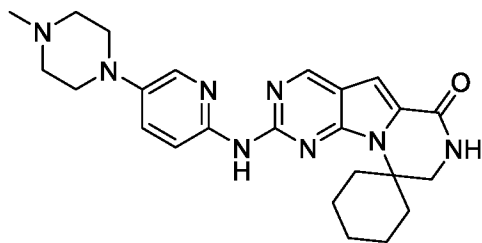
- ii) administering to the human an effective amount of 5-FU;
- iii) administering to the human an effective amount of folinic acid;
- iv) administering to the human an effective amount of oxaliplatin;
- v) administering to the human an effective amount of irinotecan; and,
- vi) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan, and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

4. The method of any of claims 1-3, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.
5. The method of any of claims 1-4, wherein the CDK4/6 inhibitor is administered a second time between 20 and 24 hours after the first administration.
6. The method of any of claims 1-4, wherein the CDK4/6 inhibitor is administered a second time between 20 and 22 hours after the first administration
7. The method of any of claims 1-6, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
8. The method of any of claims 1-7, wherein the human is administered an anti-VEGF antibody selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
9. The method of claim 8, wherein the human is administered bevacizumab, or a biosimilar thereof.
10. The method of any of claims 1-7, wherein the human is administered an anti-EGFR antibody selected from cetuximab or panitumumab.

11. The method of claim 10, wherein the human is administered cetuximab, or a biosimilar thereof.
12. The method of any of claims 1-11, wherein the folinic acid administered is leucovorin.
13. The method of any of claims 1-11, wherein the folinic acid administered is levoleucovorin.
14. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor of structure:



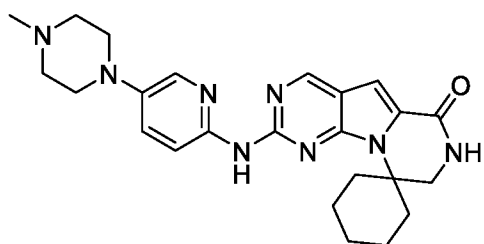
, or a pharmaceutically acceptable salt thereof,

- ii) administering to the human starting on day 1 of each 14-day cycle an effective amount of fluorouracil (5-FU), wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iii) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid;
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- v) administering to the human on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- vi) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;
- wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle.
15. The method of any of claims 14, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.

16. The method of claims 14 or 15, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 24 hours after its administration on day 1.
17. The method of claims 14 or 15, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 22 hours after its administration on day 1.
18. The method of any of claims 14 to 17, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
19. The method of claim 18, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
20. The method of claim 19, wherein the human is administered bevacizumab, or a biosimilar thereof.
21. The method of any of claims 14-17, wherein the human is administered an anti-EGFR antibody selected from cetuximab or panitumumab.
22. The method of claim 21, wherein the human is administered cetuximab, or a biosimilar thereof.
23. The method of any of claims 14 to 17, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
24. The method of claim 23, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
25. The method of any of claims 14 to 24, wherein the 5-FU is administered as a continuous infusion between about 2200 mg/m² and 3800 mg/m² over between about 44 hours and 48 hours.
26. The method of claim 25, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² and 3200 mg/m² over about 46-48 hours.
27. The method of claim 26, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² over about 46-48 hours.
28. The method of any of claims 14 to 27, wherein the human is administered a bolus dose of 5-FU of between about 200 and 600 mg/m² on day 1.
29. The method of claim 28, wherein the human is administered a bolus dose of 5-FU of between about 400 mg/m² on day 1.

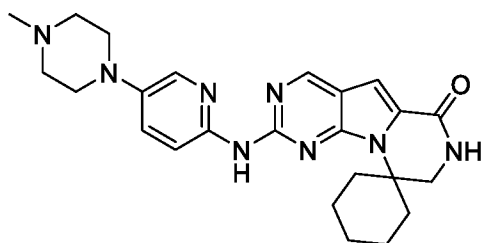
30. The method of any of claims 14-29, wherein the human is administered a dose of irinotecan of between about 150 and 200 mg/m².
31. The method of claim 30, wherein the human is administered a dose of irinotecan of about 165 mg/m².
32. The method of claim 30, wherein the human is administered a dose of irinotecan of about 180 mg/m².
33. The method of any of claims 14-32, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².
34. The method of claim 33, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
35. The method of claim 33, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
36. The method of claim 33, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
37. The method of any of claims 14-36, wherein the human is administered a dose of leucovorin on day 1 of between about 100 mg/m² and 500 mg/m².
38. The method of claim 37, wherein the human is administered a dose of leucovorin on day 1 of about 400 mg/m².
39. The method of claim 37, wherein the human is administered a dose of leucovorin on day 1 of about 200 mg/m².
40. The method of any of claims 14-36, wherein the human is administered a dose of leucovorin on day 1 of between about 50 mg/m² and 250 mg/m².
41. The method of claim 40, wherein the human is administered a dose of levoleucovorin on day 1 of about 200 mg/m².
42. The method of claim 40, wherein the human is administered a dose of leucovorin on day 1 of about 100 mg/m².
43. The method of any of claims 14-42, wherein the human is administered up to 12 14-day cycles.

44. The method of any of claims 1-43, wherein the human is further administered a maintenance regimen comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of claims 14-43.
45. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



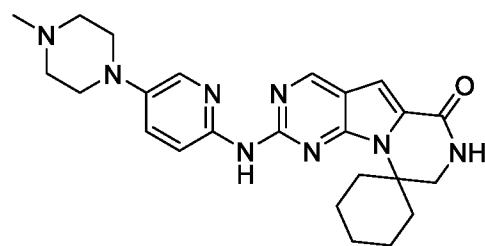
, or a pharmaceutically acceptable salt thereof,

- wherein the CDK4/6 inhibitor is administered in a FOLFIRINOX chemotherapy protocol, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFIRINOX chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.
46. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFIRINOX chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and

- wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFIRINOX chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.
47. A method of treating colorectal cancer in a human comprising:
- administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

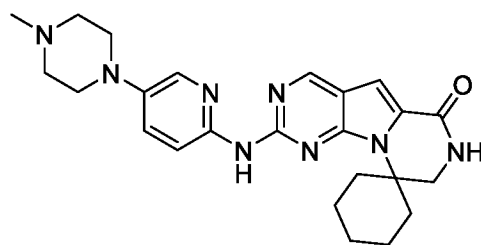
thereof;

- ii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;
- iii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI);
- iv) administering to the human an effective amount of folinic acid;
- v) administering to the human an effective amount of oxaliplatin; and,
- vi) administering to the human an effective amount of irinotecan; and,
- vii) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is first administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan, and wherein the CDK4/6 inhibitor is administered a second time between about 18 and 26 hours after its first administration.

- 48. The method of any of claims 45-47, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.
- 49. The method of any of claims 45-48, wherein the CDK4/6 inhibitor is administered a second time between 20 and 24 hours after the first administration.
- 50. The method of any of claims 45-48, wherein the CDK4/6 inhibitor is administered a second time between 20 and 22 hours after the first administration
- 51. The method of any of claims 45-50, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
- 52. The method of any of claims 45-51, wherein the human is administered an anti-VEGF antibody selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.

53. The method of claim 52, wherein the human is administered bevacizumab, or a biosimilar thereof.
54. The method of any of claims 45-51, wherein the human is administered an anti-EGFR antibody selected from cetuximab or panitumumab.
55. The method of claim 54, wherein the human is administered cetuximab, or a biosimilar thereof.
56. The method of any of claims 45-54, wherein the folinic acid administered is leucovorin.
57. The method of any of claims 45-54, wherein the folinic acid administered is levoleucovorin.
58. The method of any of claims 45-54, wherein the human is further administered a maintenance regimen comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of claims 45-55.
59. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising
- administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

- administering to the human on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;
- administering to the human starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours beginning on day 1;
- administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid;

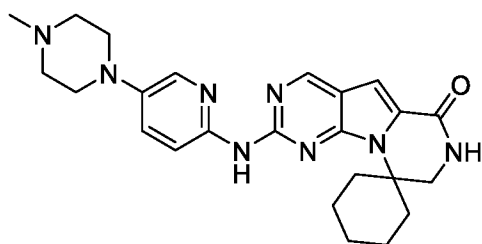
- v) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) administering to the human on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- vii) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, oxaliplatin, or irinotecan on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle.

- 60. The method of claim 59, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of 5-FU.
- 61. The method of claim 60, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 24 hours after its administration on day 1.
- 62. The method of claim 60, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 22 hours after its administration on day 1.
- 63. The method of any of claims 60-62, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
- 64. The method of claim 63, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
- 65. The method of claim 63, wherein the human is administered bevacizumab or a biosimilar thereof.
- 66. The method of claim 63, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
- 67. The method of any of claims 60-66, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
- 68. The method of claim 67, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
- 69. The method of any of claims 60-68, wherein the 5-FU administered as a bolus injection is administered between about 100 mg/m² and 500 mg/m².

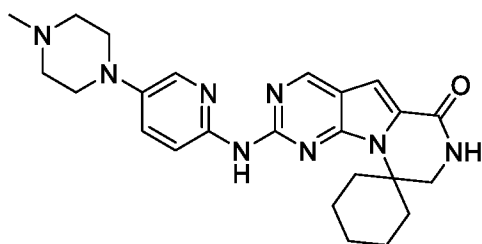
70. The method of claim 69, wherein the 5-FU bolus injection is administered between about 400 mg/m².
71. The method of claim 69, wherein the 5-FU bolus injection is administered between about 200 mg/m².
72. The method of any of claims 60-71, wherein the 5-FU continuous infusion is administered between about 2200 mg/m² and 3800 mg/m² over between about 44 hours and 48 hours.
73. The method of claim 72, wherein the 5-FU administered as a continuous infusion is administered between about 2400 mg/m² and 3200 mg/m² over about 46-48 hours.
74. The method of claim 72, wherein the 5-FU administered as a continuous infusion is administered at about 2400 mg/m² over about 46-48 hours.
75. The method of any of claims 60-74, wherein the human is administered a dose of irinotecan of between about 150 and 200 mg/m².
76. The method of claim 75, wherein the human is administered a dose of irinotecan of about 165 mg/m².
77. The method of claim 75, wherein the human is administered a dose of irinotecan of about 180 mg/m².
78. The method of any of claims 60-77, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².
79. The method of claim 78, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
80. The method of claim 78, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
81. The method of claim 78, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
82. The method of claims 60-81, wherein the human is administered a dose of leucovorin on day 1 of between about 100 mg/m² and 500 mg/m².
83. The method of claim 82, wherein the human is administered a dose of leucovorin on day 1 of about 400 mg/m².
84. The method of claim 82, wherein the human is administered a dose of leucovorin on day 1 of about 200 mg/m².

85. The method of claims 60-81, wherein the human is administered a dose of levoleucovorin on day 1 of between about 50 mg/m² and 250 mg/m².
86. The method of claim 85, wherein the human is administered a dose of leucovorin on day 1 of about 200 mg/m².
87. The method of claim 85, wherein the human is administered a dose of leucovorin on day 1 of about 100 mg/m².
88. The method of any of claims 60-87, wherein the human is administered up to 12 14-day cycles.
89. The method of any of claims 60-88, wherein the human is further administered a maintenance regimen comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of claims 60-88.
90. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof,

- wherein the CDK4/6 inhibitor is administered in a FOLFOX-based chemotherapy protocol, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of the FOLFOX-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.
91. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFOX-based chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and

- wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the

initiation of FOLFOX-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

92. The method of claims 90-91, wherein the FOLFOX-based chemotherapy protocol is FOLFOX4.

93. The method of claims 90-91, wherein the FOLFOX-based chemotherapy protocol is FOLFOX6.

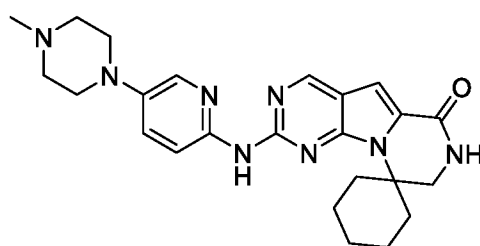
94. The method of claims 90-91, wherein the FOLFOX-based chemotherapy protocol is modified FOLFOX6 (mFOLFOX6).

95. The method of claims 90-91, wherein the FOLFOX-based chemotherapy protocol is FOLFOX7.

96. The method of claims 90-91, wherein the FOLFOX-based chemotherapy protocol is modified FOLFOX7 (mFOLFOX7).

97. A method of treating colorectal cancer in a human comprising:

- i) administering to the human an effective amount of a CDK4/6 inhibitor having the structure;



, or a pharmaceutically acceptable salt thereof,

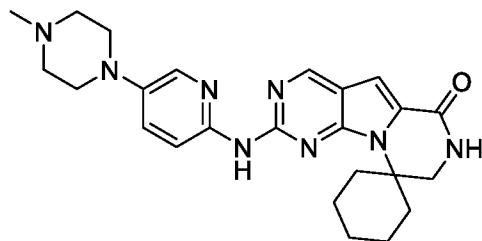
- ii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI);
- iii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a bolus dose;
- iv) administering to the human an effective amount of a folinic acid; and,
- v) administering to the human an effective amount of oxaliplatin; and,
- vi) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is first administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, or oxaliplatin, and

wherein the CDK4/6 inhibitor is administered a second time between about 18 and 26 hours after its first administration.

98. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure;



, or a pharmaceutically acceptable salt

thereof,

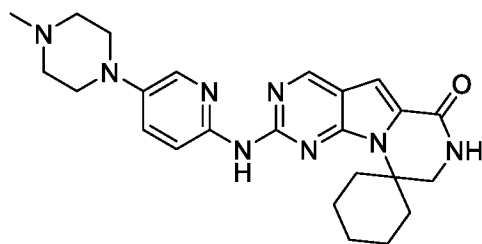
- ii) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 20 and 24 hours;
- iii) administering on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus dose;
- iv) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a folinic acid; and,
- v) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and oxaliplatin on day 1 of each 14-day cycle, and

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to the initiation of administration of 5-FU and folinic acid on day 2 of each cycle.

99. A method of treating colorectal cancer in a human, wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

- ii) administering to the human on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus intravenous injection;
- iii) administering to the human starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid; and,
- v) administering to the human on day 1 of each 14-day cycle an effective amount of oxaliplatin; and,
- vi) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, or oxaliplatin on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 of each cycle between about 18 and 26 hours after its administration on day 1 of each cycle.

100. The method of any of claims 90-99, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of either the bolus 5-FU or intravenous 5-FU.

101. The method of claims 90-95, 97, and 99-100, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 24 hours after its administration on day 1.

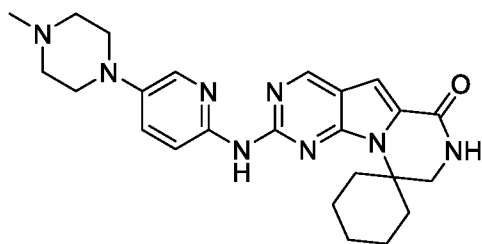
102. The method of claims 90-95, 97, and 99-100, wherein the CDK4/6 inhibitor is administered on day 2 between about 20 and 22 hours after its administration on day 1.

103. The method of any of claims 90-103, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.

104. The method of claim 103, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
105. The method of claim 103, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
106. The method of any of claims 90-105, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
107. The method of claim 106, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
108. The method of any of claims 96, 98, and 100-107, wherein the 5-FU is administered as a continuous infusion between about 400 mg/m² and 800 mg/m² over about 22 hours on day 1 and day 2.
109. The method of claim 108, wherein the 5-FU is administered as a continuous infusion of about 600 mg/m² over about 22 hours on day 1 and day 2.
110. The method of any of claims 90-95, 97, and 99-107, wherein the 5-FU is administered as a continuous infusion between about 2000 mg/m² and 3200 mg/m² over about 46-48 hours on day 1 and day 2.
111. The method of claim 110, wherein the 5-FU is administered as a continuous infusion between about 2400 mg/m² and 3000 mg/m² over about 46-48 hours on day 1 and day 2.
112. The method of claim 110, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² over about 46-48 hours on day 1 and day 2.
113. The method of any of claims 90-112, wherein the human is administered a bolus dose of 5-FU of between about 200 and 600 mg/m² on day 1.
114. The method of claim 113, wherein the human is administered a bolus dose of 5-FU of about 200 mg/m²
115. The method of claim 113, wherein the human is administered a bolus dose of 5-FU of between about 400 mg/m² on day 1.
116. The method of any of claims 90-114, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².

117. The method of claim 115, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
118. The method of claim 115, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
119. The method of claim 115, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
120. The method of any of claims 90-118, wherein the folinic acid is leucovorin.
121. The method of claim 96, 98, 100-109, 113-119 wherein the human is administered a dose of leucovorin on day 1 and day 2 of between about 100 mg/m² and 500 mg/m².
122. The method of claim 121, wherein the human is administered a dose of leucovorin on day 1 and day 2 of about 200 mg/m².
123. The method of claim 90-95, 97, 99-107, 110-120, wherein the human is administered a dose of leucovorin on day 1 of between about 100 mg/m² and 500 mg/m².
124. The method of claim 123, wherein the human is administered a dose of leucovorin on day 1 of about 400 mg/m².
125. The method of any of claims 90-118, wherein the folinic acid is levoleucovorin.
126. The method of claim 96, 98, 100-109, 113-119 wherein the human is administered a dose of levoleucovorin on day 1 and day 2 of between about 50 mg/m² and 250 mg/m².
127. The method of claim 126, wherein the human is administered a dose of levoleucovorin on day 1 and day 2 of about 100 mg/m².
128. The method of claim 90-95, 97, 99-107, 110-120, wherein the human is administered a dose of levoleucovorin on day 1 of between about 50 mg/m² and 250 mg/m².
129. The method of claim 128, wherein the human is administered a dose of levoleucovorin on day 1 of about 200 mg/m².
130. The method of any of claims 90-129, wherein the human is administered up to 12 14-day cycles.
131. The method of any of claims 90-130, wherein the human is further administered a maintenance regimen of comprising administration of the CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of claims 90-130.

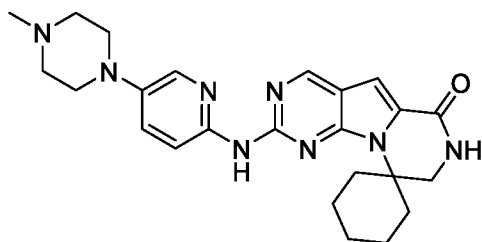
132. A method of treating a human having colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof,

wherein the CDK4/6 inhibitor is administered in a FOLFIRI-based chemotherapy protocol, and wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of the FOLFIRI-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

133. A method of reducing chemotherapy induced myelosuppression in a human undergoing a FOLFIRI-based chemotherapy protocol for the treatment of colorectal cancer comprising administering to the human an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof, and

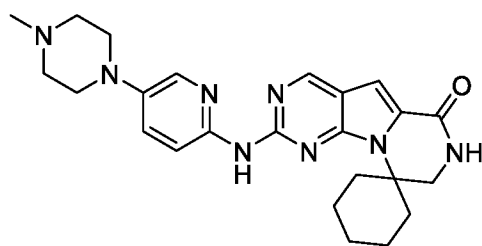
wherein the CDK4/6 inhibitor is administered i) a first time 4 hours or less prior to the initiation of FOLFIRI-based chemotherapy administration and ii) a second time between 18 hours and 26 hours after the first CDK4/6 inhibitor administration.

134. The method of claims 90-91, wherein the FOLFIRI-based chemotherapy protocol is FOLFIRI.

135. The method of claims 90-91, wherein the FOLFOX-based chemotherapy protocol is modified FOLFIRI (mFOLFIRI).

136. A method of treating colorectal cancer in a human comprising:

- i) administering to the human an effective amount of a CDK4/6 inhibitor having the structure;



, or a pharmaceutically acceptable salt

thereof,

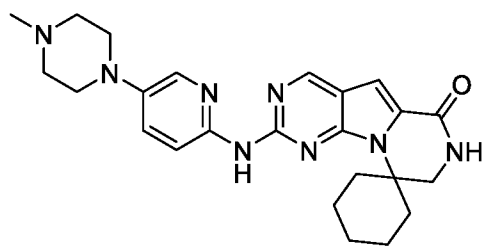
- ii) administering to the human an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI);
- iii) administering to the human an effective amount of a folinic acid; and,
- iv) administering to the human an effective amount of irinotecan; and,
- v) optionally administering to the human an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is first administered about 4 hours or less prior to administration of the first to be administered of 5-FU, folinic acid, or irinotecan, and wherein the CDK4/6 inhibitor is administered a second time between about 18 and 26 hours after its first administration.

137. The method of claim 136, wherein the human is further administered 5-FU in a bolus dose.

138. A method of treating colorectal cancer in a human wherein the treatment comprises one or more 14-day cycles comprising:

- i) administering to the human on day 1 and day 2 of each 14-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt

thereof;

- ii) administering to the human starting on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a continuous infusion (CI) over a period of between about 44 and 48 hours;

- iii) administering to the human on day 1 of each 14-day cycle an effective amount of folinic acid; and,
- iv) administering to the human on day 1 of each 14-day cycle an effective amount of irinotecan; and,
- v) optionally administering to the human on day 1 of each 14-day cycle an anti-VEGF or anti-EGFR monoclonal antibody or compound;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to the initiation of administration of 5-FU, folinic acid, and irinotecan on day 1 of each 14-day cycle, and wherein the CDK4/6 inhibitor is administered on day 2 about 18 to 26 hours after its administration on day 1.

139. The method of claim 138, further comprising administering to the human on day 1 of each 14-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection.
140. The method of any of claims 132-139, wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to administration of either the bolus 5-FU or intravenous 5-FU.
141. The method of claims 132-140, wherein the CDK4/6 inhibitor is administered the second time between about 20 and 24 hours after its first administration.
142. The method of claims 132-140, wherein the CDK4/6 inhibitor is administered the second time between about 20 and 24 hours after its first administration.
143. The method of any of claims 132-142, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.
144. The method of claim 143, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
145. The method of claim 143, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
146. The method of any of claims 132-145, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².

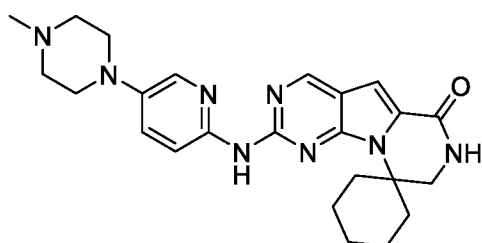
147. The method of claim 146, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
148. The method of any of claims 132-147, wherein the 5-FU is administered as a continuous infusion between about 2200 mg/m² and 3800 mg/m² over between about 44 hours and 48 hours.
149. The method of claim 148, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² and 3000 mg/m² over about 46-48 hours.
150. The method of claim 148, wherein the 5-FU is administered as a continuous infusion of about 2400 mg/m² over about 46-48 hours.
151. The method of any of claims 134, 137, and 139-150, wherein the human is administered a bolus dose of 5-FU of between about 200 and 600 mg/m² on day 1.
152. The method of claim 151, wherein the human is administered a bolus dose of 5-FU of between about 400 mg/m² on day 1.
153. The method of any of claims 132-152, wherein the human is administered a dose of irinotecan of between about 150 and 200 mg/m².
154. The method of claim 153, wherein the human is administered a dose of irinotecan of about 165 mg/m².
155. The method of claim 153, wherein the human is administered a dose of irinotecan of about 180 mg/m².
156. The method of any of claims 132-155, wherein the folinic acid is leucovorin.
157. The method of claim 156, wherein the human is administered a dose of leucovorin of between about 100 mg/m² and 500 mg/m².
158. The method of claim 157, wherein the human is administered a dose of leucovorin of about 400 mg/m².
159. The method of any of claims 132-155, wherein the folinic acid is levoleucovorin.
160. The method of claim 159 wherein the human is administered a dose of levoleucovorin of between about 50 mg/m² and 250 mg/m².
161. The method of claim 160, wherein the human is administered a dose of levoleucovorin of about 200 mg/m².

162. The method of any of claims 132-161, wherein the human is administered up to 12 14-day cycles.

163. The method of any of claims 132-162, wherein the human is further administered a maintenance regimen of CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of claims 132-162.

164. A method of treating a human with colorectal cancer, wherein the treatment comprises one or more 56-day cycles comprising

i) administering to the human on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of a CDK4/6 inhibitor having the structure:



, or a pharmaceutically acceptable salt thereof;

ii) administering to the human on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of 5-FU, wherein the 5-FU is administered as a bolus injection;

iii) administering to the human on day 1, day 8, day 15, day 22, day 29, and day 36 of each 56-day cycle an effective amount of folinic acid;

iv) administering to the human on day 1, day 15, and day 29 of each 56-day cycle an effective amount of oxaliplatin; and,

v) optionally administering to the human on day 1, day 15, and day 29 of each 56-day cycle an anti-VEGF or anti-EGFR monoclonal antibody;

wherein the CDK4/6 inhibitor is administered about 4 hours or less prior to initiation of administration of the 5-FU, folinic acid, or oxaliplatin.

165. The method of claim 166, wherein CDK4/6 inhibitor is administered about 4 hour or less prior to the administration of the 5-FU.

166. The method of any of claims 164-165, wherein the human is further administered an anti-VEGF or an anti-EGFR antibody or compound.

167. The method of claim 166, wherein the human is administered an anti-VEGF antibody or compound selected from bevacizumab, bevacizumab-awwb, ramucirumab, or ziv-aflibercept.
168. The method of claim 166, wherein the human is administered an anti-EGFR antibody or compound selected from cetuximab or panitumumab.
169. The method of any of claims 164-168, wherein the CDK4/6 inhibitor is administered at between about 190 mg/m² and 300 mg/m².
170. The method of claim 169, wherein the CDK4/6 inhibitor is administered at about 240 mg/m².
171. The method of any of claims 164-170, wherein the 5-FU is administered as a bolus dose of between about 400 mg/m² and 600 mg/m².
172. The method of claim 171, wherein the 5-FU is administered as a bolus dose of about 500 mg/m².
173. The method of any of claims 164-172, wherein the human is administered a dose of oxaliplatin of between about 50 and 150 mg/m².
174. The method of claim 173, wherein the human is administered a dose of oxaliplatin of about 85 mg/m².
175. The method of claim 173, wherein the human is administered a dose of oxaliplatin of about 100 mg/m².
176. The method of claim 173, wherein the human is administered a dose of oxaliplatin of about 130 mg/m².
177. The method of any of claims 164-176, wherein the folinic acid is leucovorin.
178. The method of claim 177, wherein the human is administered a dose of leucovorin of between about 100 mg/m² and 600 mg/m².
179. The method of claim 178, wherein the human is administered a dose of leucovorin of about 500 mg/m².
180. The method of any of claims 164-176, wherein the folinic acid is levoleucovorin.
181. The method of claim 180, wherein the human is administered a dose of levoleucovorin of between about 50 mg/m² and 300 mg/m².

182. The method of claim 181, wherein the human is administered a dose of leucovorin of about 250 mg/m².
183. The method of any of claims 164-182, wherein the human is administered up to 3 56-day cycles.
184. The method of any of claims 164-183, wherein the human is further administered a maintenance regimen of CDK4/6 inhibitor, 5-FU, and folinic acid, following cessation of the method of claim 164-183.
185. The method of any of claims 1-184, wherein the treatment results in a reduction and/or prevention in one or more toxicities selected from chemotherapy-induced myelosuppression (CIM), chemotherapy-induced diarrhea (CID), stomatitis, and mucositis.
186. The method of any of claims 1-185, wherein the treatment results in an improvement in progression free survival (PFS) in comparison to the predicted PFS based on administration of the chemotherapy protocol without the CDK4/6 inhibitor.
187. The method of any of claims 1-186, wherein the treatment results in an improvement in overall survival (OS) in comparison to the predicted OS based on administration of chemotherapy protocol without the CDK4/6 inhibitor.
188. The method of any of claims 1-187, wherein the treatment results in myelopreservation of hematopoietic stem and progenitor cells (HSPCs).
189. The method of any of claims 1-188, wherein the treatment results in protection of immune effector cells.
190. The method of any of claims 1-189, wherein the treatment results in enhanced anti-tumor efficacy in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.
191. The method of any of claims 1-190, wherein the treatment results in myelopreservation of the neutrophil lineage in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.
192. The method of any of claims 1-191, wherein the treatment results in a reduction in the duration of severe (Grade 4) neutropenia in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.

193. The method of any of claims 1-192, wherein the treatment results in a reduction of chemotherapy-induced fatigue (CIF) in humans compared to those receiving the chemotherapy protocol without the CDK4/6 inhibitor.
194. The method of any of claims 1-193, wherein the treatment results in reduction of CIF is a reduction in the time to first confirmed deterioration of fatigue (TTCD-fatigue),
195. The method of any of claims 1-194, wherein the treatment results in an improvement in PFS is based on per Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1).
196. The method of any of claims 1-195, wherein the treatment results in a reduction in severe neutropenia events, a reduction in granulocyte-colony stimulating factor (G-CSF) treatment, or a reduction in febrile neutropenia (FN) adverse events (AEs).
197. The method of any of claims 1-196, wherein the treatment results in a reduction in Grade 3 or 4 decreased hemoglobin laboratory values, red blood cell (RBC) transfusions, or erythropoiesis-stimulating agent (ESA) administration.
198. The method of any of claims 1-197, wherein the treatment results in a reduction in Grade 3 or 4 decreased platelet count laboratory values and/or the number of platelet transfusions.
199. The method of any of claims 1-198, wherein the treatment results in a reduction in Grade 3 or 4 hematologic laboratory values.
200. The method of any of claims 1-199, wherein the treatment results in a reduction in i) hospitalizations due to all causes, febrile neutropenia/neutropenia, anemia/RBC transfusion, thrombocytopenia/bleeding, or infections or ii) antibiotic use.
201. The method of any of claims 1-200, wherein the treatment results in an improvement to one or more of: Functional Assessment of Cancer Therapy-General (FACT-G) domain scores (physical, social/family, emotional, and functional well-being); Functional Assessment of Cancer Therapy-Anemia (FACT-An): Anemia; Functional Assessment of Cancer Therapy-colorectal cancer (FACT-C) : Colorectal Cancer Subscale; 5-level EQ-5D (EQ-5D-5L); Patient Global Impression of Change (PGIC) fatigue item; or Patient Global Impression of Severity (PGIS) fatigue item.

202. The method of any of claims 1-201, wherein the colorectal cancer treated is metastatic colorectal cancer (mCRC).
203. The method of any of claims 1-202, wherein the colorectal cancer is proficient mismatch repair (pMMR) mCRC.
204. The method of any of claims 1-203, wherein the colorectal cancer is microsatellite stable (MSS) mCRC.
205. The method of any of claims 1-204, wherein the colorectal cancer is proficient mismatch repair (pMMR)/microsatellite stable (MSS) mCRC (pMMR/MSS mCRC).
206. The method of any of claims 1-205, wherein the colorectal cancer has not been responsive to previous treatment with single agent checkpoint inhibitors. I
207. The method of any of claims 1-206, wherein the colorectal cancer has a BRAF or KRAS mutation.
208. The method of any of claims 1-207, wherein the colorectal cancer has a BRAF V600E substitution mutation.
209. The method of any of claims 1-208, wherein the colorectal cancer is CDK4/6 replication dependent.
210. The method of any of claims 1-208, wherein the colorectal cancer is CDK4/6 replication independent.
211. The method of any of claims 1-208, wherein the colorectal cancer is CDK4/6 replication indeterminate.
212. The method of any of claims 1-211, wherein the colorectal cancer is interferon- γ (IFN- γ) dominant according to Thorsson's Six Class Immune Signature.
213. The method of any of claims 1-212, wherein the colorectal cancer has a high IFN- γ signature or expanded immune signature according to the Ayer's IFN- γ Signature Score or Expanded Immune Signature Score.
214. The method of any of claims 1-213, wherein the colorectal cancer is PD-L1 positive.
215. The method of any of claims 1-214, wherein the colorectal cancer has a KRAS mutation selected from a V9, G12, G13, V14, L19, Q22, D33, A59, G60, Q61 R68, K117, A146, R164, K176, or K180 substitution.

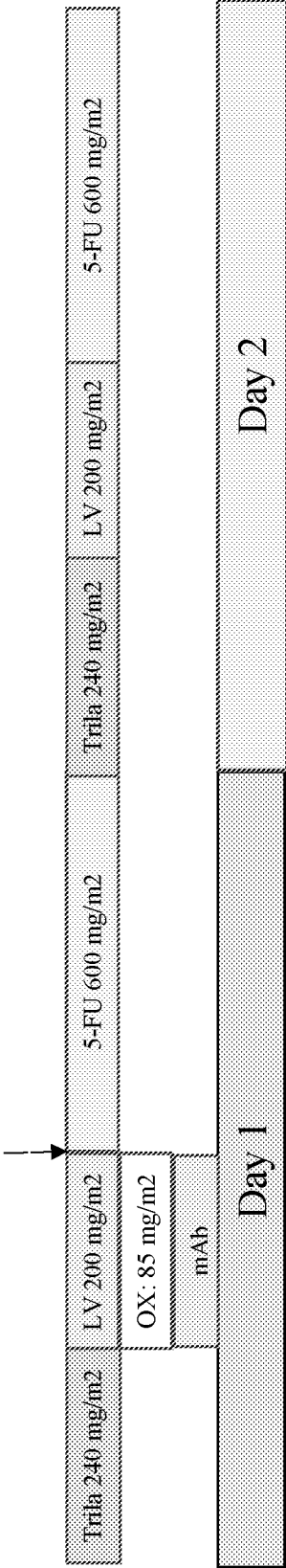
216. The method of any of claims 1-214, wherein the colorectal cancer has a KRAS mutation selected from G12D, G12V, G12C, G12A, or other G12 variant.
217. The method of any of claims 1-214, wherein the colorectal cancer has a NRAS mutation selected from G12, G13, G60, Q61, E123, or P185.
218. The method of any of claims 1-194, wherein the colorectal cancer has an HRAS mutation selected from G12, G13, Q61, K117, R164, or P167.
219. The method of any of claims 1-214, wherein the colorectal cancer is KRAS wild-type.
220. The method of any of claims 1-214, wherein the colorectal cancer is BRAF wild-type.
221. The method of any of claims 1-220, wherein the human has not received a prior chemotherapeutic regimen for the treatment of the colorectal cancer.
222. The method of any of claims 1-221, wherein the human has failed prior chemotherapeutic regimen for the treatment of colorectal cancer.
223. The method of any of claims 1-222, wherein the human maintains an absolute neutrophil count (ANC) of greater than $1.0 \times 10^9/\text{L}$ when measured prior to the beginning of each chemotherapy treatment cycle.
224. The method of any of claims 1-223, wherein the human maintains a platelet count of greater than $75 \times 10^9/\text{L}$ when measured prior to the beginning of each chemotherapy treatment cycle.
225. The method of any of claims 1-224, wherein the chemotherapy protocol does not include the administration of an immune checkpoint inhibitor.
226. The method of any of claims 1-224, wherein the human has proficient mismatch repair (pMMR)/microsatellite stable (MSS) metastatic colorectal cancer (pMMR/MSS mCRC) and has not received systemic therapy for metastatic disease.
227. The method of any of claims 1-225, wherein the chemotherapy protocol does not include the administration of an immune checkpoint inhibitor.
228. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic pancreatic cancer.

229. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic gastric cancer.
230. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic gastroesophageal junction adenocarcinoma.
231. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic biliary tract cancer.
232. The method of claim 230, wherein the advanced or metastatic biliary tract cancer is intrahepatic cholangiocarcinoma.
233. The method of claim 230, wherein the advanced or metastatic biliary tract cancer is hilar cholangiocarcinoma.
234. The method of claim 230, wherein the advanced or metastatic biliary tract cancer is distal cholangiocarcinoma.
235. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic neuroendocrine carcinoma.
236. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic peritoneal carcinosis.
237. The method of any of claims 1-225, wherein the cancer treated is not colorectal cancer but instead advanced or metastatic liver cancer.
238. The method of any of claims 1-237, wherein the treatment results in a reduction and/or prevention of chemotherapy-induced myelosuppression (CIM).
239. The method of any of claims 1-238, wherein the treatment results in a reduction and/or prevention of chemotherapy-induced diarrhea (CID).
240. The method of any of claims 1-239, wherein the treatment results in a reduction and/or prevention of stomatitis.
241. The method of any of claims 1-239, wherein the treatment results in a reduction and/or prevention in mucositis.
242. The method of any of claims 1-241, wherein a 5-FU prodrug is administered instead of 5-FU.

Trilaciclib/FOLFOX4

Induction

5-FU Bolus 400 mg/m²



Maintenance

5-FU Bolus 400 mg/m²

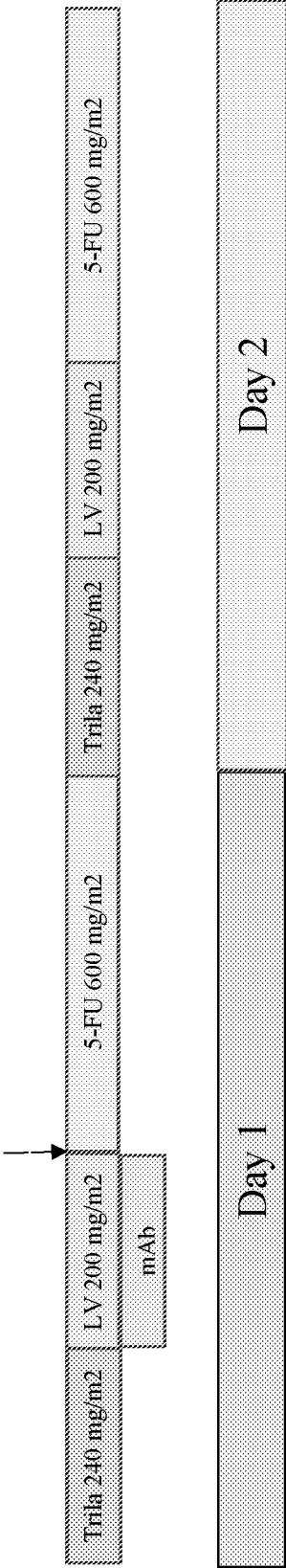


Fig. 1A

Trilaciclib/FOLFOX6

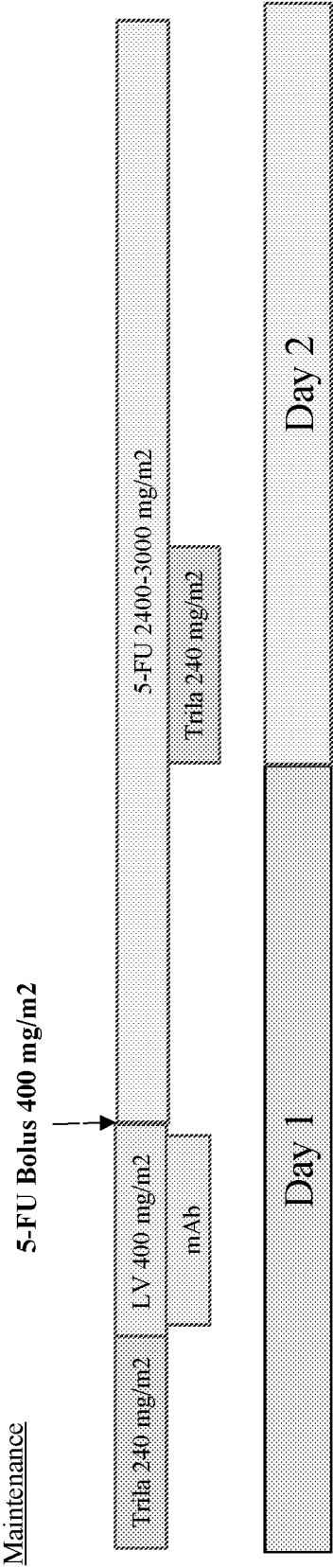
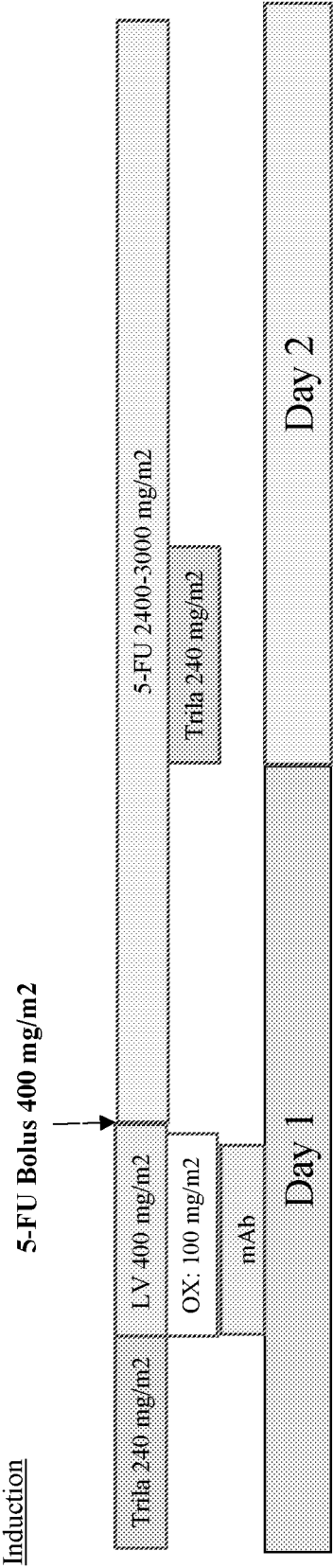


Fig. 1B

Trilaciclib/mFOLFOX6

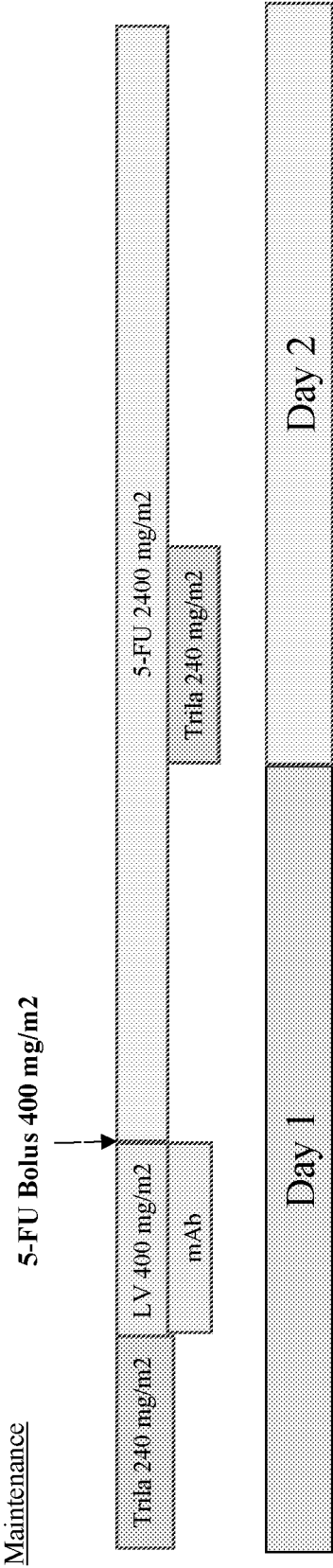
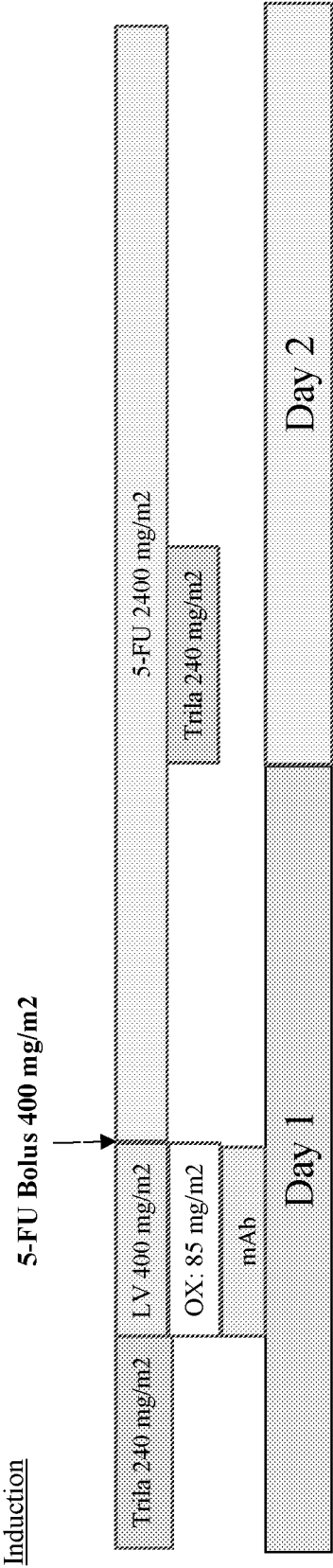


Fig. 1C

Trilaciclib/FOLFOX7

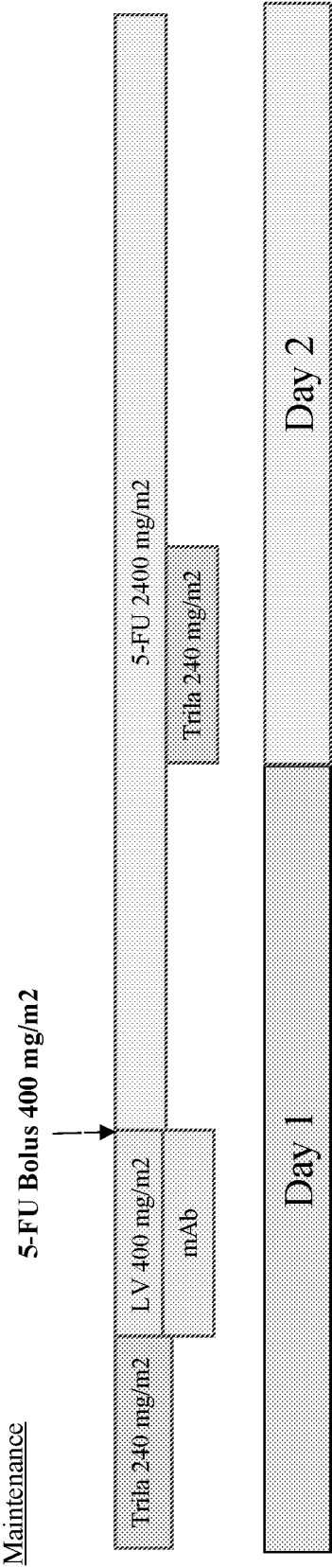
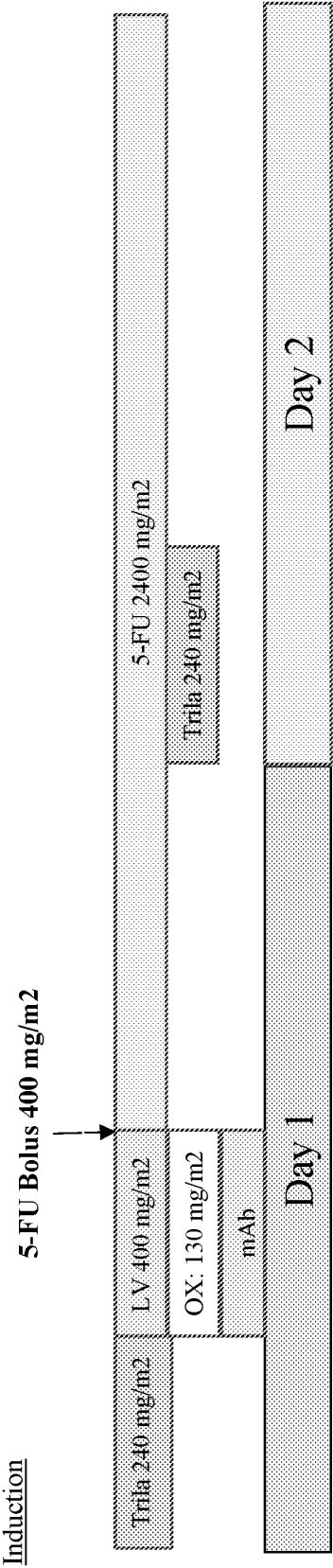
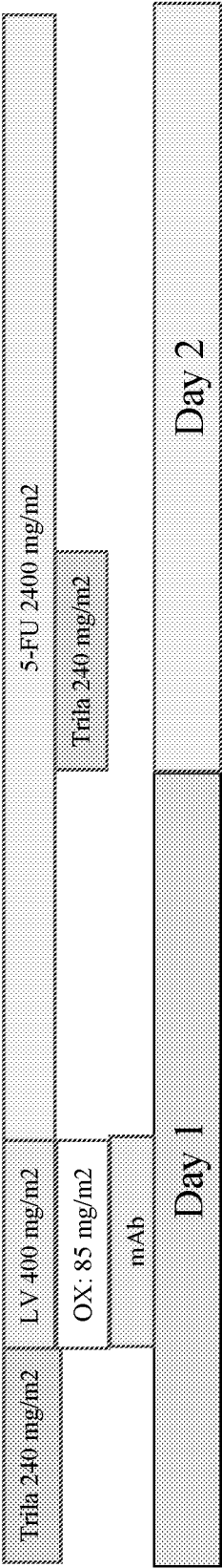


Fig. 1D

Trilaciclib/mFOLFOX7

Induction



Maintenance

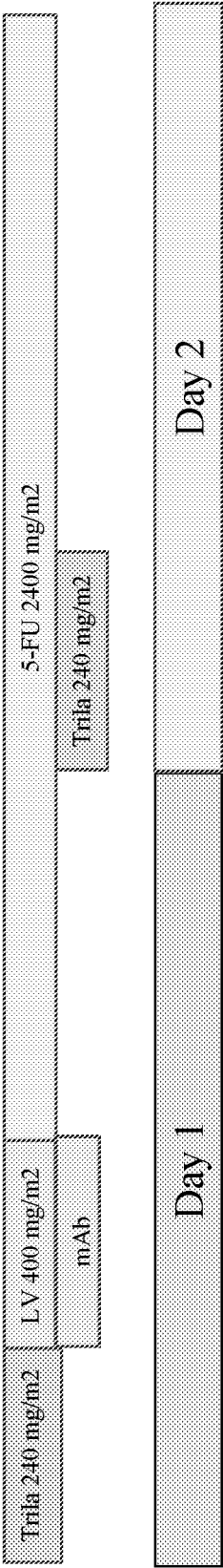
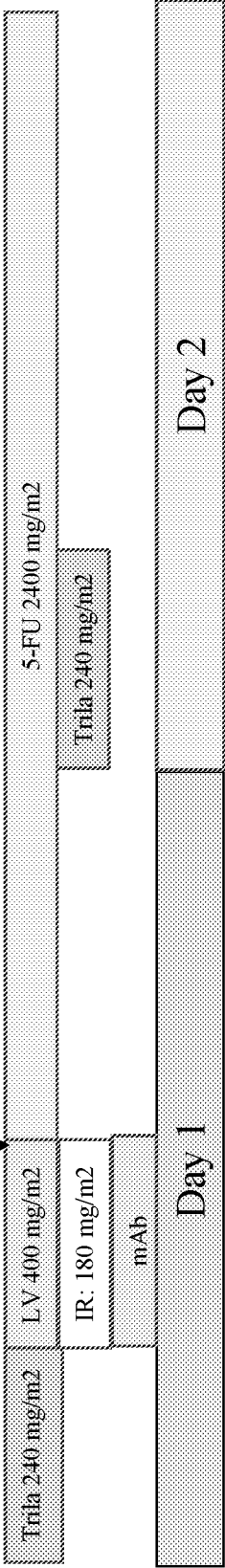


Fig. 1E

Trilaciclib/FOLFIRI

Induction

5-FU Bolus 400 mg/m²



Maintenance

5-FU Bolus 400 mg/m²

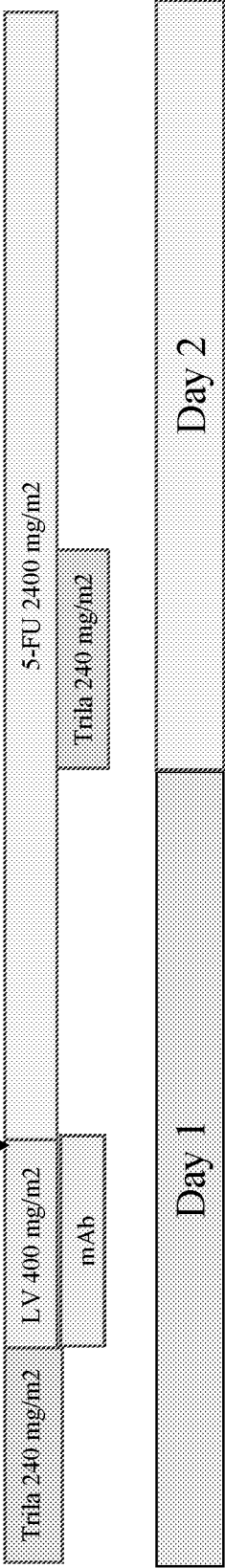
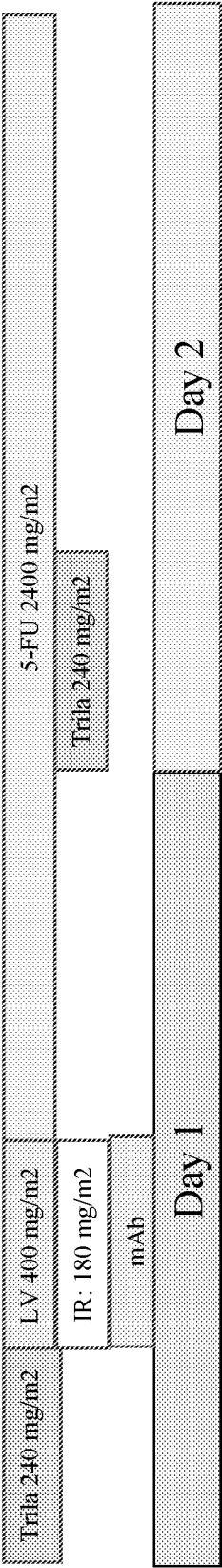


Fig. 2A

Trilaciclib/mFOLFIRI

Induction



Maintenance

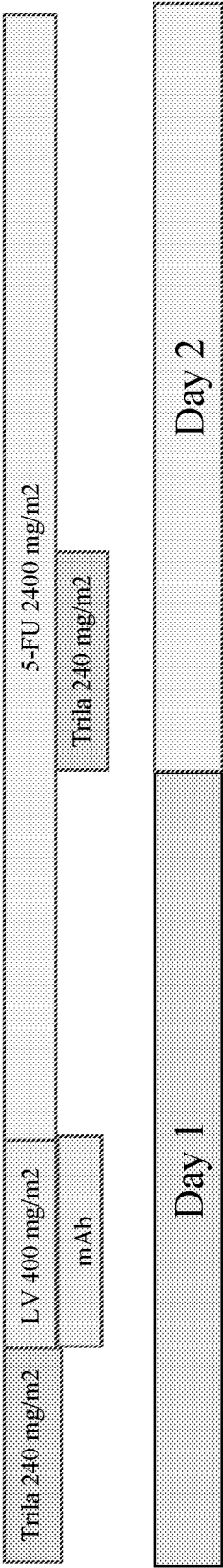
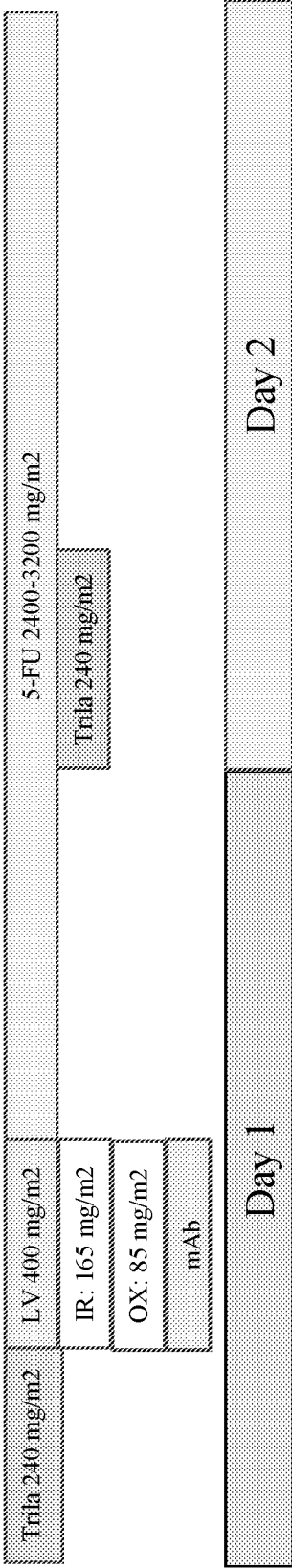


Fig. 2B

Trilaciclib/FOLFOXIRI

Induction



Maintenance

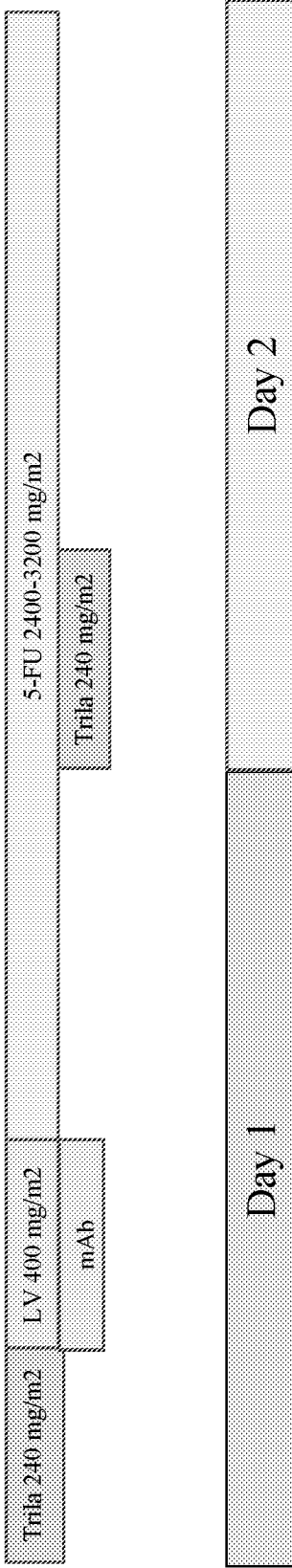
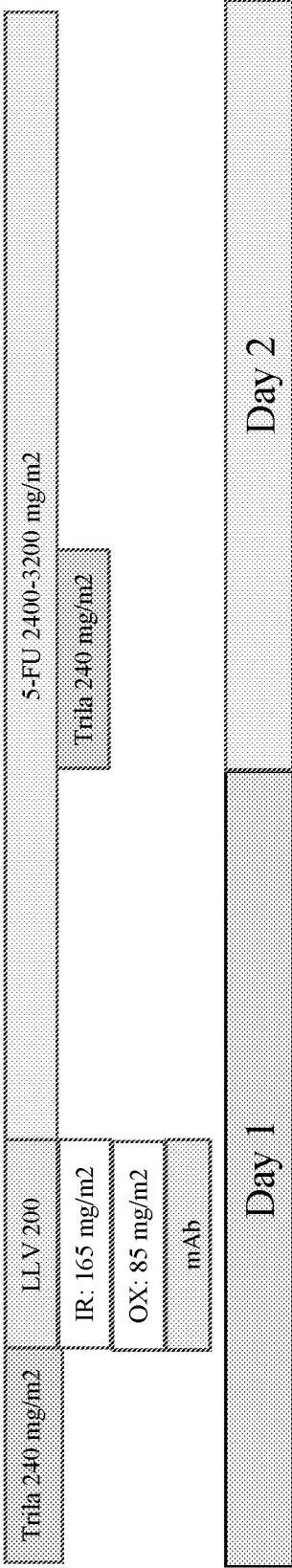


Fig.3A

Trilaciclib/FOLFOXIRI

Induction



Maintenance

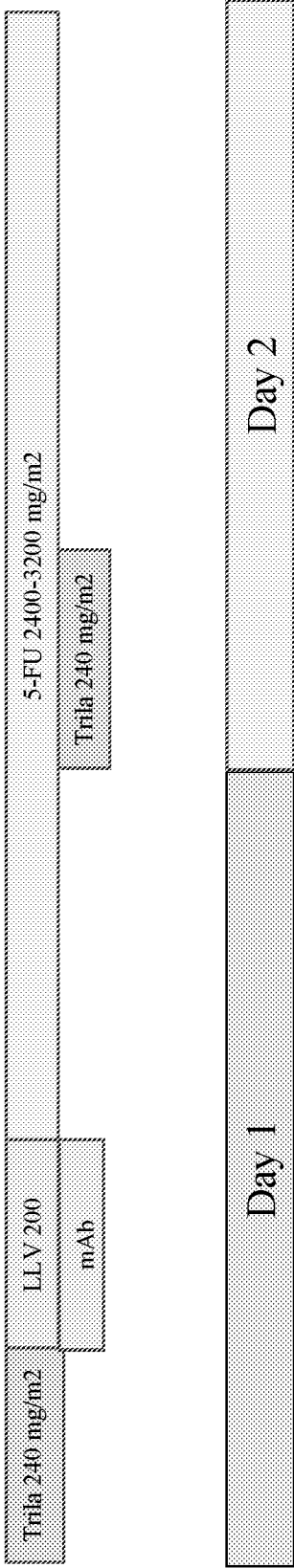


Fig.3B

Trilaciclib/FOLFIRINOX

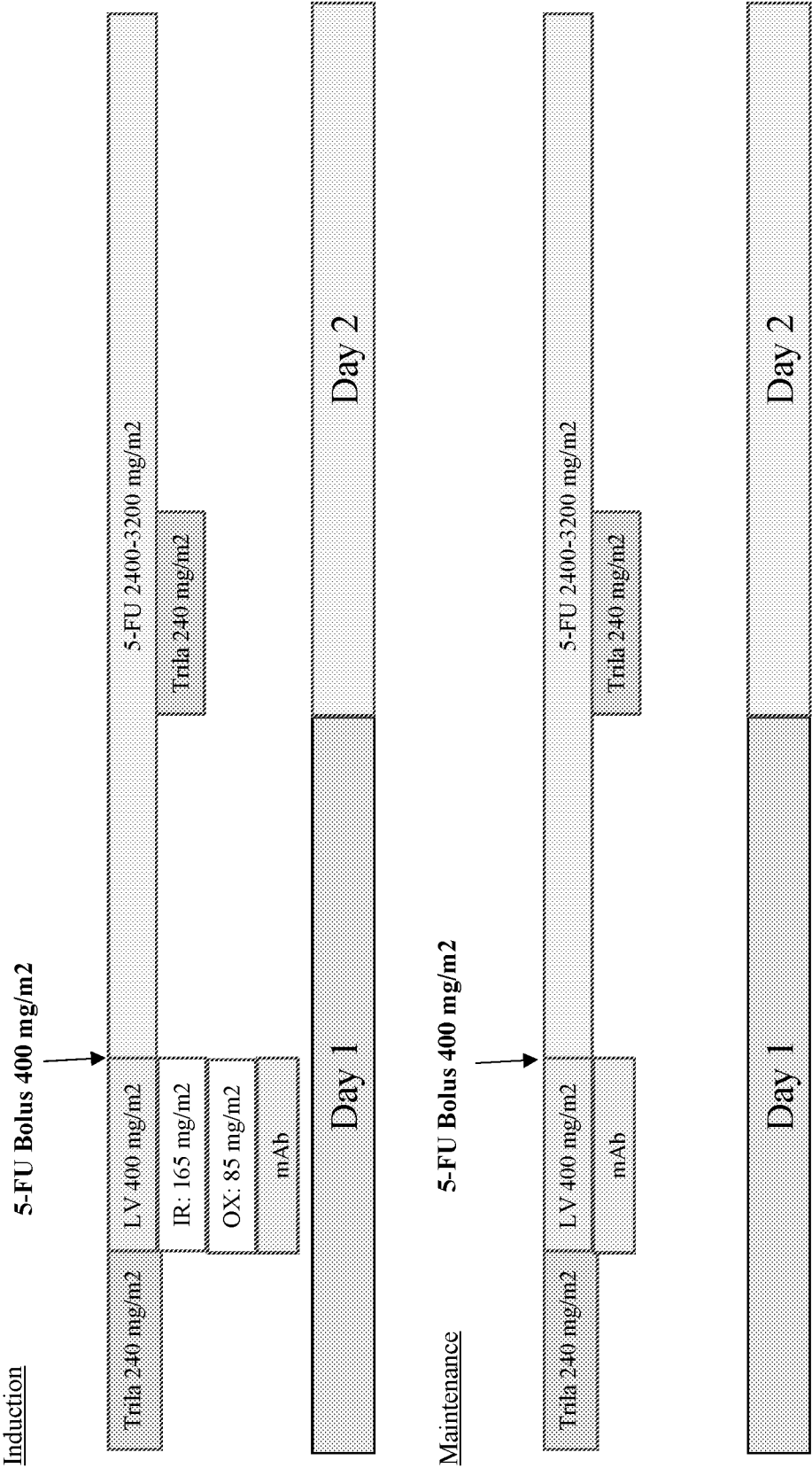
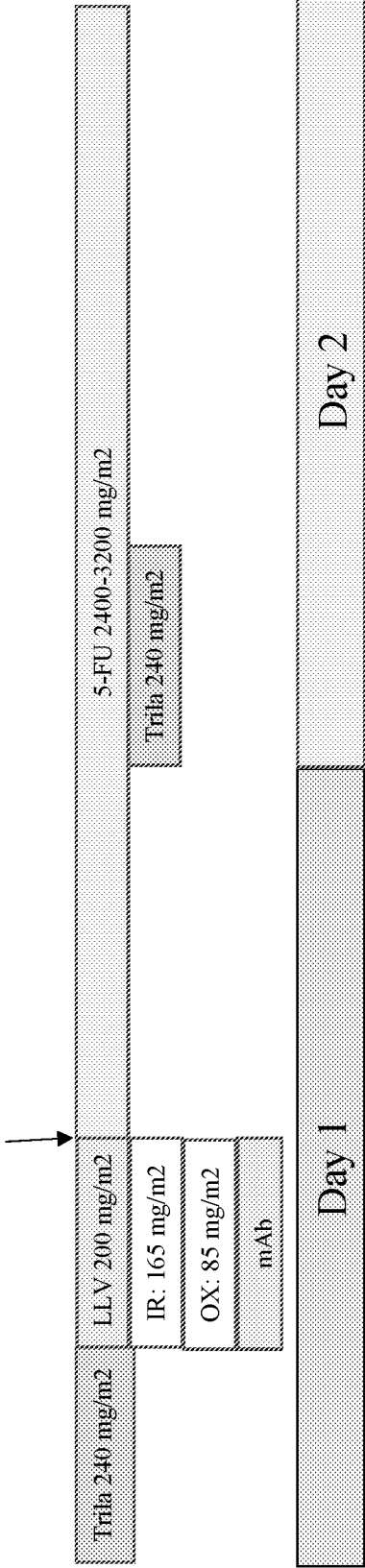


Fig.4A

Trilaciclib/FOLFIRINOX

Induction

5-FU Bolus 400 mg/m²



Maintenance

5-FU Bolus 400 mg/m²

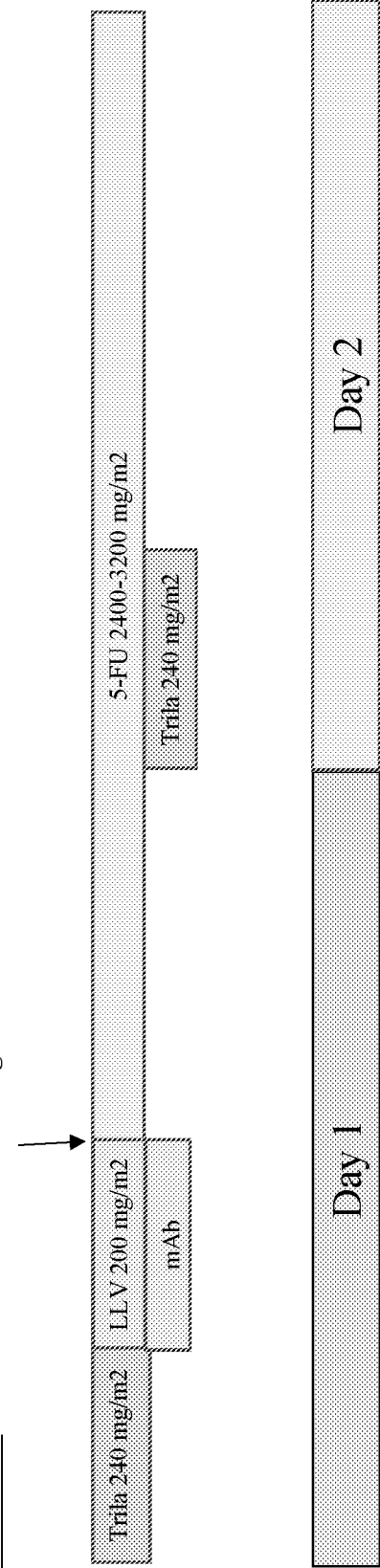


Fig.4B

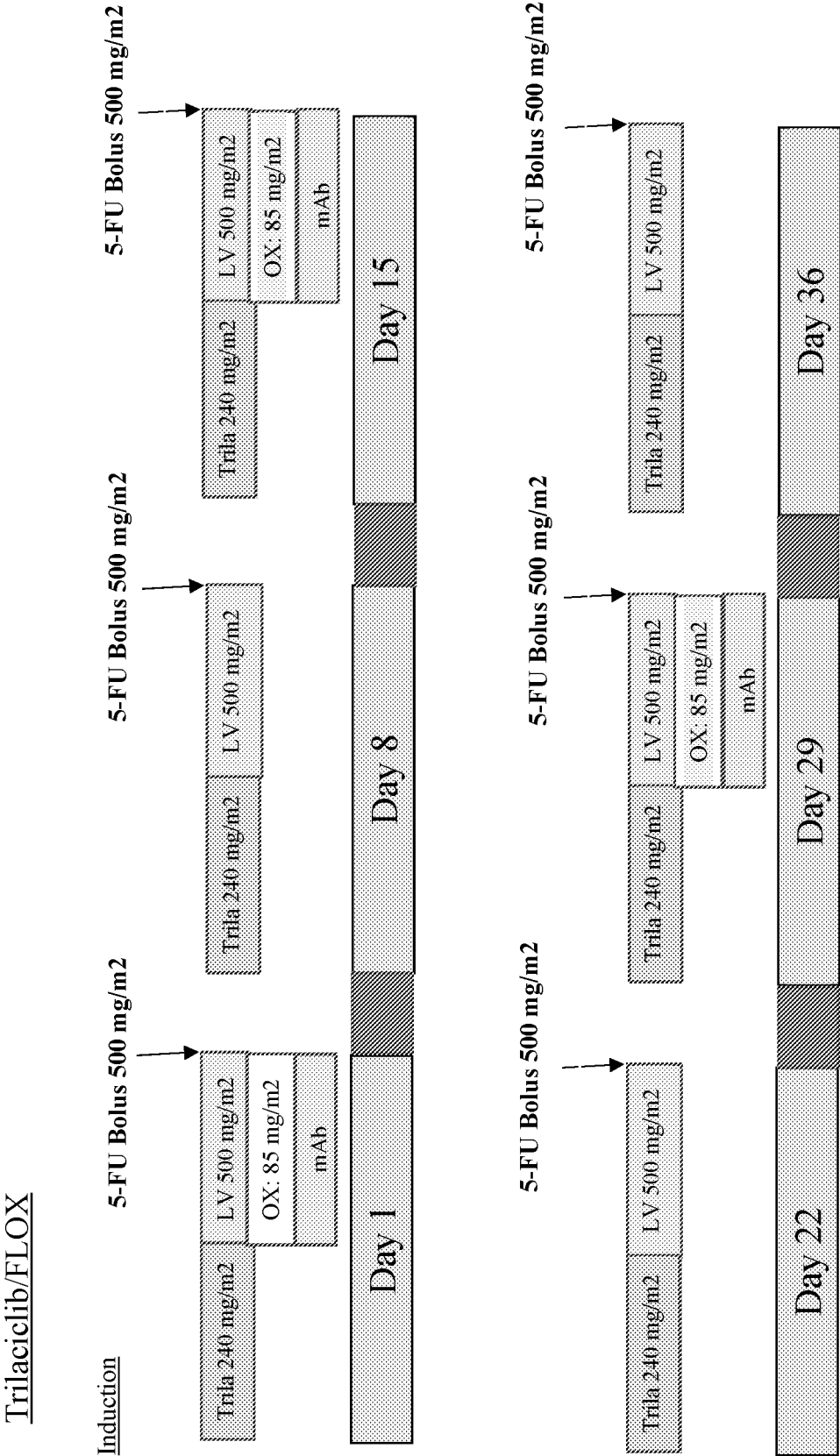


Fig. 5

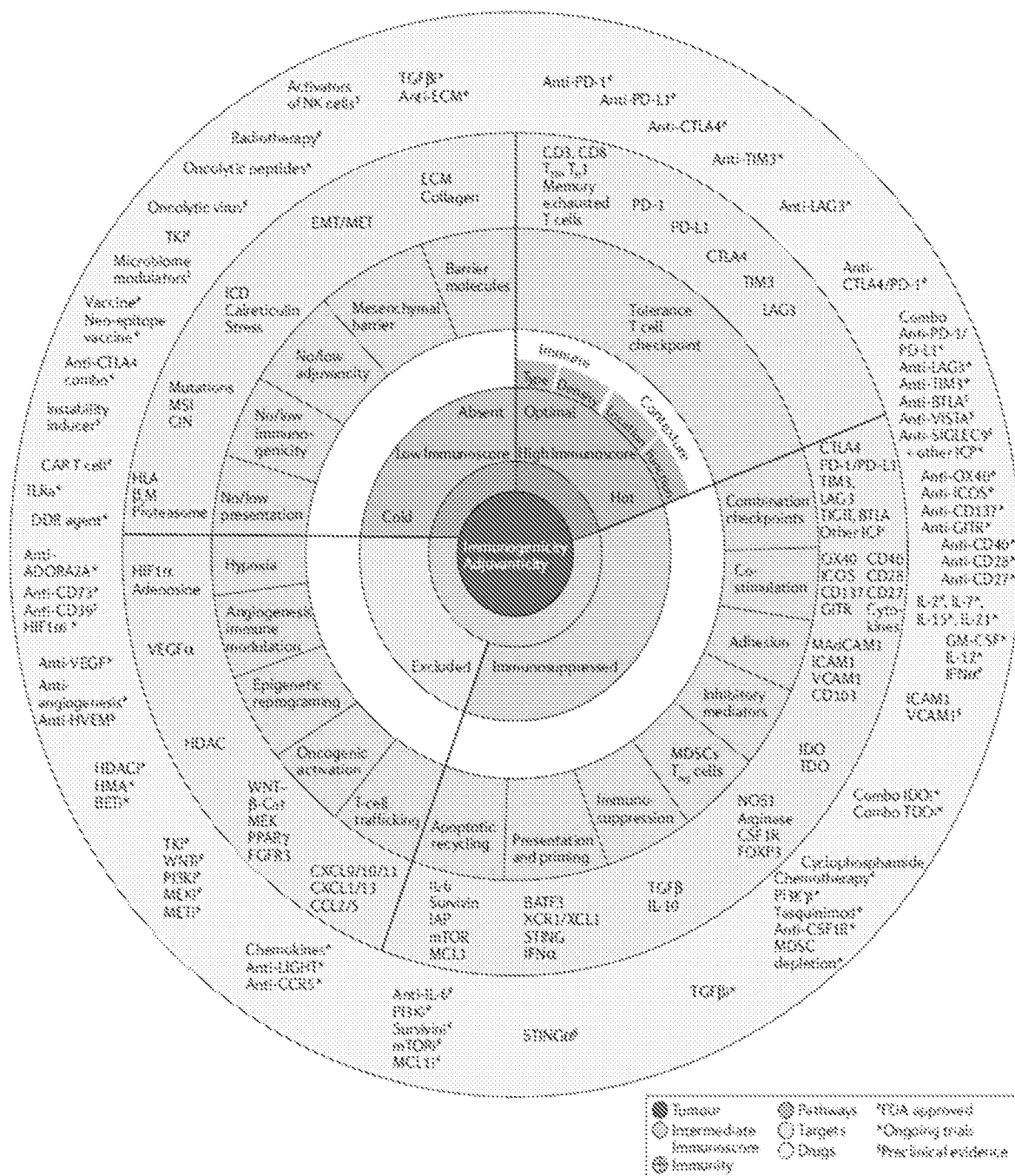


Fig. 6

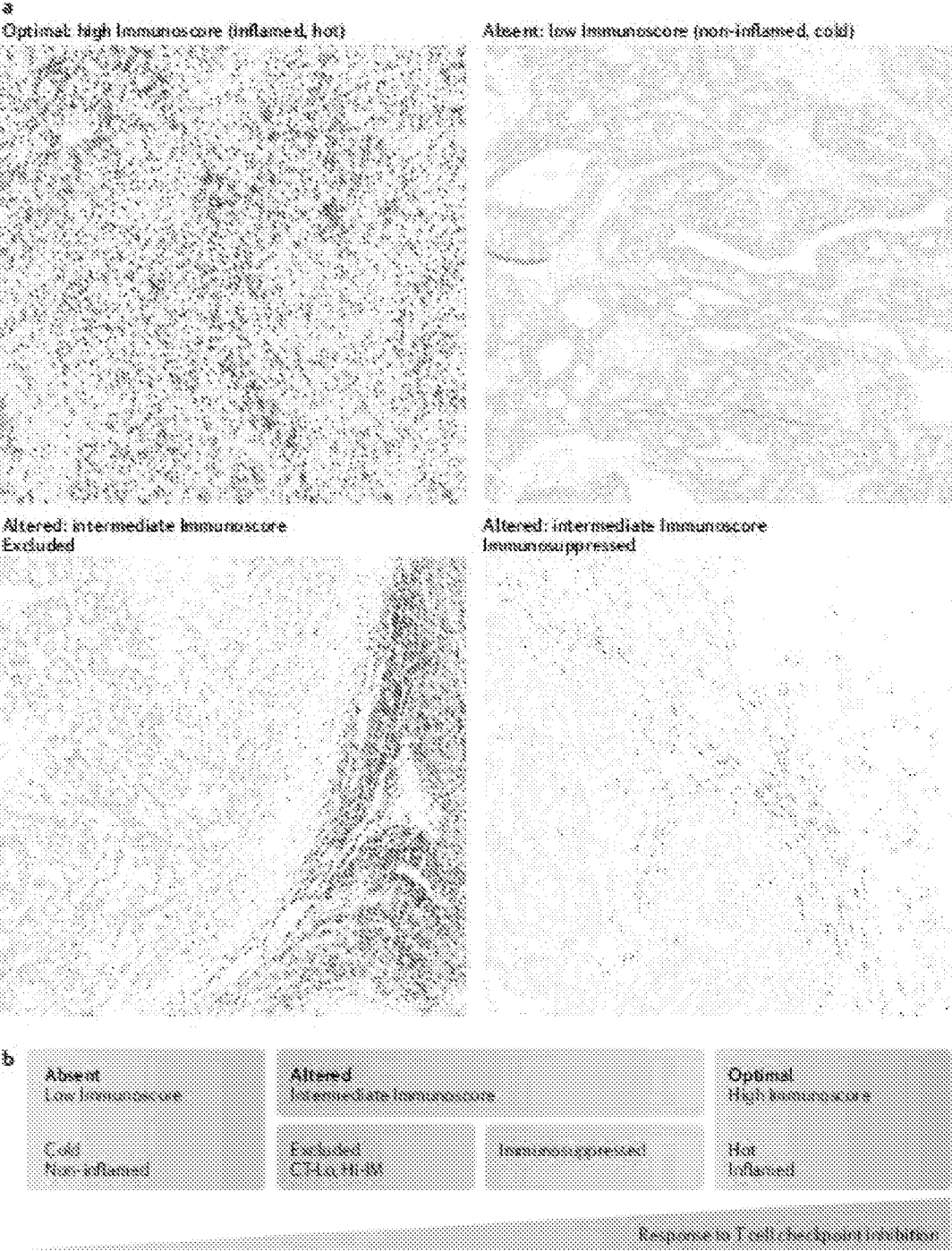


Fig. 7

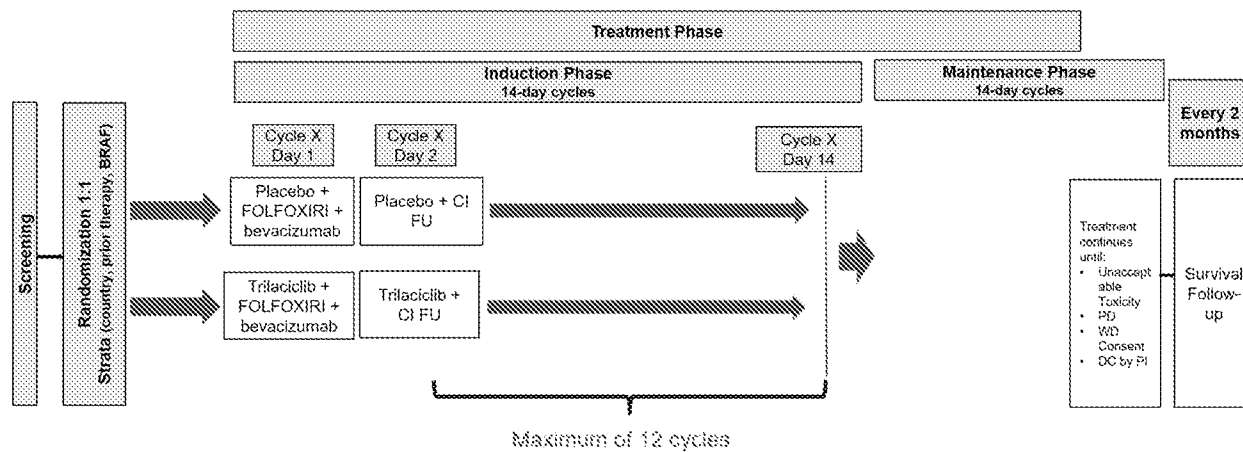


Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/55681

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 36/484; A61K 36/539; A61K 36/65 (2021.01)

CPC - A61K 36/484; A61K 36/53; A61K 36/539

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/106729 A1 (G1 Therapeutics, Inc.) 14 June 2018 (14.06.2018); p5 p10 p13 p18 p50 p51	1-4, 14-17, 45-48, 59-66, 90-91, 97-99, 132-133, 136-139, 164-168
Y	US 2018/0133338 A1 (Amal Therapeutics SA) 17 May 2018 (17.05.2018); para[0602]	1-4, 14-17, 45-48, 59-66, 90-91, 97-99, 132-133, 136-139, 164-168
A	WO 2020/097625 A1 (G1 Therapeutics, Inc.) 14 May 2020 (14.05.2020); entire document	1-4, 14-17, 45-48, 59-66, 90-91, 97-99, 132-133, 136-139, 164-168
A	WO 2019/222521 A1 (G1 Therapeutics, Inc.) 21 November 2019 (21.11.2019); entire document	1-4, 14-17, 45-48, 59-66, 90-91, 97-99, 132-133, 136-139, 164-168
A	US 2016/0258024 A1 (Georgetown University) 08 September 2016 (08.09.2016); entire document	1-4, 14-17, 45-48, 59-66, 90-91, 97-99, 132-133, 136-139, 164-168

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 December 2021

Date of mailing of the international search report

JAN 27 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/55681

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-13, 18-44, 49-58, 67-89, 92-96, 100-131, 134-135, 140-163, 169-242
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.