Official Title:	PRESERVE 1: A Phase 3 Randomized, Double-blind Trial of Trilaciclib versus Placebo in Patients Receiving FOLFOXIRI/Bevacizumab for Metastatic Colorectal Cancer
NCT Number:	NCT04607668
Document Date:	15-Dec-2022

Protocol Title: PRESERVE 1: A Phase 3 Randomized, Double-blind Trial of Trilaciclib versus Placebo in Patients Receiving FOLFOXIRI/Bevacizumab for Metastatic Colorectal Cancer

Protocol Number: G1T28-207

Compound: Trilaciclib for Injection, 300 mg/vial

Study Phase: 3

Short Title: Trilaciclib versus placebo in patients receiving FOLFOXIRI/bevacizumab for metastatic colorectal cancer

Study Name: PRESERVE 1

Sponsor Name: G1 Therapeutics, Inc.

Legal Registered Address: G1 Therapeutics, Inc.
700 Park Offices Dr. Suite 200
Research Triangle Park, NC 27709

Regulatory Agency Identifier Numbers: IND: 119254
EudraCT: 2019-003826-25

Approval Date: 15 December 2022 (Version 6.1)

Replaces Amendment v5.1 (21 November 2022) _ Released to Italy AIFA

Original Protocol: 12 June 2020 (Version 1.0)

PROTOCOL SIGNATURE PAGE

Sponsor's Approval

I have read and understand the contents of this clinical protocol, Version 6.1 for Study G1T28-207 dated 15 December 2022 and I agree to meet all obligations of the Sponsor as detailed in all applicable regulations and guidelines. In addition, I will inform the Principal Investigator and all other investigators of all relevant information that becomes available during the conduct of the study.

PPD MD Executive Director, Clinical Development Physician Sponsor Representative G1 Therapeutics	Date

INVESTIGATOR'S AGREEMENT

Clinical Study Protocol G1T28-207: PRESERVE 1: A Phase 3 Randomized, Double-blind Trial of Trilaciclib versus Placebo in Patients Receiving FOLFOXIRI/Bevacizumab for Metastatic Colorectal Cancer

Version 6.1 Issue Date: 15 December 2022

Version 5.1 Issue Date: 21 November 2022

Version 4.1 Issue Date: 06 September 2022

Version 3.1 Issue Date: 27 July 2021

Version 2.1 Issue Date: 20 January 2021

Original Protocol (Version 1.0) Issue Date: 12 June 2020

I have read the G1T28-207 protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Principal Investigator Signature	Date
Principal Investigator Name	Institution

1. SYNOPSIS

Name of Sponsor/Company: G1 Therapeutics		
Name of Investigational Product: trilaciclib for injection, 300 mg/vial		
Name of Active Ingredient: trilaciclib hydrochloride (hereafter referred to as trilaciclib) (G1T28)		
Protocol Number: G1T28-207	**Phase:** 3	**Regions:** United States, Western Europe, Eastern Europe, China
Title of Study: PRESERVE 1: A Phase 3 Randomized, Double-blind Trial of Trilaciclib versus Placebo in Patients Receiving FOLFOXIRI/Bevacizumab for Metastatic Colorectal Cancer		
Study center(s): Approximately 120 centers		
Studied period (years): Estimated date first patient randomized: Jan 2021 Estimated date last patient completed: Q2 2025		**Phase of development:** 3

Objectives:

Primary:

To assess the effects of trilaciclib on the neutrophil lineage compared with placebo in patients receiving FOLFOXIRI/bevacizumab for proficient mismatch repair/microsatellite stable (pMMR/MSS) metastatic colorectal cancer (mCRC).

Key Secondary:

To assess the effect of trilaciclib on overall survival (OS) compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.

Study Design:

This is 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. Patients 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). There will be three stratification factors for randomization: country, 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). Study drugs administered during Induction are as follows:

- Trilaciclib (240 mg/m^2) or placebo – administered as a 30-minute IV infusion no more than 4 hours prior to chemotherapy on each day chemotherapy is administered. The second dose of trilaciclib/placebo should be administered as a 30-minute infusion on Day 2.
- Irinotecan 165 mg/m^2 –IV, Day 1
- Oxaliplatin 85 mg/m^2 – IV, Day 1
- Leucovorin 400 mg/m^2 – IV, Day 1; LEVOLeucovorin 200 mg/m^2 is an acceptable alternative
- Fluorouracil 2400 – 3200 mg/m^2 – continuous infusion (CI) over 46 – 48 hours beginning on Day 1; this dose range is provided to reflect geographic variations in prescribed

fluorouracil (5FU) dose; however, the same dose should be continued throughout the study for each patient, except where dose modifications are required for toxicity management.

- Bevacizumab 5 mg/kg – IV, Day 1

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. However, if a patient is unable to complete the maximum number of 12 Induction cycles because of toxicity, and the treating physician feels the patient will receive additional clinical benefit by transitioning to Maintenance, the patient will be allowed to discontinue oxaliplatin and irinotecan, and continue on Maintenance therapy as described below.

Following completion of Induction, patients will continue in Maintenance, where they will receive trilaciclib or placebo per randomization allocation at study entry. Trilaciclib/placebo will be administered prior to infusional-5FU/leucovorin/bevacizumab at the same dose and schedule used during Induction. The patient may continue to receive treatment on study until disease progression, unacceptable toxicity, withdrawal of consent, discontinuation by Investigator, or the end of the trial, whichever occurs first. Treatment cycles will occur consecutively without interruption, except when necessary to manage toxicities or for administrative reasons.

Upon discontinuation of study treatment, patients will be followed for survival, ie, 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.

Methodology:

Sample Size Justification:

The primary and key secondary objectives of this study are to evaluate the myelopreservation and anti-tumor efficacy of trilaciclib administered prior to FOLFOXIRI/bevacizumab (referred to as trilaciclib hereafter) compared with placebo administered prior to FOLFOXIRI/bevacizumab (referred to as placebo hereafter). The study has two primary myelosuppression endpoints (duration of severe [Grade 4] neutropenia [DSN] in Cycle 1 – 4 and occurrence of severe neutropenia [SN] during Induction and one key secondary anti-tumor efficacy endpoint, OS. To ensure strong control of family-wise type I error rate at the level of 2-sided 0.05 when performing statistical analyses, the overall 2-sided α of 0.05 will be split between the analyses of the two primary endpoints (using $\alpha_1 = 0.04$) and analyses for OS (using $\alpha_2 = 0.01$).

The sample size is determined to support primary efficacy analysis on the two primary efficacy endpoints. Overall, 282 patients (141 per group) are needed to detect the assumed treatment effect for each of the two co-primary myelosuppression endpoints with 90% power at the 2-sided significance level of 0.04. Assuming 5% of randomized patients will not have any post-baseline data, a total of 296 patients (148 per group) will be required for the study.

Although the number of patients is determined to ensure adequate power for the evaluation of the myelopreservation efficacy of trilaciclib, statistical comparisons for anti-tumor efficacy will also be conducted and the statistical significance is set to be 0.01 initially for testing the treatment effect for OS. A total of 157 deaths are estimated to be observed during 52-month of study duration based on the following assumptions: 18 months of accrual with 34 months follow up after the last patient is randomized, a monthly drop-out rate of 0.0029 (assuming an exponential distribution), and the median OS of 31 months for placebo group (patients receiving FOLFOXIRI/bevacizumab plus placebo) (Loupakis, 2014). It is also assumed that the hazard ratio (trilaciclib vs. placebo) is 0.75.

A total of 44 patients from Ukraine were randomized to the study as of 24 February 2022. To mitigate the potential impact of Russian-Ukraine war on data integrity and ensure the objectives of PRESERVE-1 will not be compromised, patients who were randomized from Ukraine prior to 09

September 2021 will be included in efficacy analyses while all randomized patients from Ukraine will be included in the safety evaluation. Therefore, a total of 30 randomized patients from Ukraine will be excluded from the efficacy evaluation. This will preserve the study power as it was originally designed, as patients randomized prior to 09 September 2021 should have completed or had the opportunity to complete Induction period per protocol (approximately 24 weeks), prior to the start of the war (24 February 2022).
Number of patients (planned): Approximately 326 patients (296 patients in the mITT population) with mCRC are planned in this study. The original study population included 296 patients; 30 additional patients are planned to be enrolled to replace patients in Ukraine for the efficacy analyses.
Diagnosis and main criteria for inclusion: Patients ≥18 years of age at the time of signing the informed consent (patients > 70 years of age must have a G8 Health State Screening Tool [geriatric screening tool] score > 14) with pMMR/MSS, and histologically or cytologically-confirmed adenocarcinoma of the colon or rectum. Patients with any BRAF or KRAS mutation status (wild type or mutant) are eligible. If historical pMMR/MSS and/or BRAF V600E mutational status are not known, a tumor specimen (archival or fresh biopsy) must be sent for testing and results must be available at the time of randomization in interactive web response system (IWRS). If testing cannot be completed using a standard clinical assay performed institutionally/locally, the tumor specimen may be sent to the Sponsor's designated central laboratory for analysis; only historical KRAS mutational status will be collected (ie, no testing required prior to study entry). Patients must have unresectable and evaluable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, and Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1. At the time of providing informed consent, a formalin-fixed paraffin-embedded (FFPE) tumor specimen (from archival or fresh biopsy) with an associated pathology report documenting pMMR/MSS mCRC for retrospective biomarker analysis must be confirmed to be available to send to Sponsor for planned retrospective biomarker analyses.
Investigational product, dosage, and mode of administration: In each 14-day cycle in Induction and Maintenance, a dose of trilaciclib 240 mg/m^2 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 chemotherapy administration. The second dose of trilaciclib should be administered on Day 2.
Duration of treatment: Each patient may receive a maximum of 12 cycles of therapy (trilaciclib or placebo plus FOLFOXIRI/bevacizumab) during Induction. Following completion of Induction, patients will continue on Maintenance therapy. Patients will receive trilaciclib or placebo (per randomization allocation at study entry) administered prior to infusional-5FU/leucovorin/bevacizumab at the same dose and schedule used during Induction. Treatment in both Induction and Maintenance will continue until disease progression, unacceptable toxicity, withdrawal of consent, discontinuation by Investigator, or the end of the trial, whichever occurs first.
Reference therapy, dosage and mode of administration: In each 14-day cycle in Induction and Maintenance, a dose of placebo (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 chemotherapy administration. The second dose of placebo should be administered on Day 2. FOLFOXIRI/bevacizumab will be administered IV in accordance with the prescribing information and according to the study site's standard practice.

Criteria for evaluation:

Efficacy:

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.

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 body temperature), 12-lead safety electrocardiogram (ECG) results, dose modifications, and physical examination findings.

Statistical methods:

Data will be summarized by treatment group. The descriptive summary for the categorical variables will include counts and percentages. The descriptive summary for the continuous variables will include means, medians, standard deviations, 25% and 75% percentiles, and minimum and maximum values.

Stratification factors and factors used in statistical models:

There are three stratification factors for randomization: country, prior therapy in adjuvant/neoadjuvant setting, and BRAF V600E mutational status. A randomization schema will be in place to ensure the best possible balance of treatment assignment within each country, and between patients with history of systemic cytotoxic therapy in the adjuvant/neoadjuvant setting (yes/no) and patients with/without BRAF V600E mutation. The countries will be grouped into four different regions as US, Eastern Europe, Western Europe, and China in the statistical models to account for regional differences in clinical practice. It is anticipated that all three factors (region, prior therapy in the adjuvant/neoadjuvant setting, and BRAF status) would have an impact on patient's anti-tumor efficacy outcome, but only region and prior therapy in adjuvant/neoadjuvant setting could impact the myelosuppression measures. Therefore, region and prior therapy in adjuvant/neoadjuvant setting will be included as the factors in statistical analysis models evaluating trilaciclib's myelopreservation efficacy, while all three factors are included in the statistical analysis models to assess trilaciclib's anti-tumor efficacy. Unless otherwise specified, the strata information as entered in IWRS at the time of randomization except for "region" will be used as the factors for all stratified statistical analyses.

Analysis population

The intent-to-treat (ITT) population includes all randomized patients. Analyses for the ITT population will be conducted based on the randomly assigned treatment regardless of whether the patient received study treatment or was compliant with the protocol.

To account for the data integrity issues potentially resulting from the war in Ukraine, a modified intent-to-treat (mITT) population is the primary population for all efficacy evaluations. The criteria for the mITT population are as follows:

- All patients randomized in countries other than the Ukraine.
- All patients in the Ukraine who were randomized prior to 09 September 2021. This group should have completed, or had the opportunity to complete, Induction period per protocol (approximately 24 weeks) prior to the start of the war (24 February 2022).

As a subset of ITT population, the analyses performed on mITT will be consistent with the ITT principle, that is, data will be analyzed based on the randomly assigned treatment regardless of whether the patient received study treatment or was compliant with the protocol.

A per-protocol (PP) population is a subset of the mITT population that includes only those patients who have no key protocol deviations (eg, a subset of major protocol deviations that might substantially affect the accuracy of the study efficacy data) and who receive at least one dose of the randomly assigned treatment. The PP population will be used to analyze selected efficacy endpoints to evaluate the robustness of the efficacy findings observed in the mITT population. Analyses on the PP population will be based on the randomly assigned treatment.

The response evaluable (RE) population will include patients in the mITT population who have a measurable lesion (target lesions) at the baseline tumor assessment, and either (i) have at least 1 post-dose tumor assessment, or (ii) do not have post-dose tumor assessment but have clinical progression as noted by the Investigator, or (iii) have died due to disease progression before their first post-dose tumor scan. RE population will be the primary analysis set to evaluate tumor response and the data will be analyzed based on the actual treatment received.

The safety population includes all enrolled patients who received at least 1 dose of study drug. Analyses conducted on the safety population will be based on the actual treatment received. All safety analyses will be conducted using the safety population.

The pharmacokinetic (PK) population will include all dosed patients with evaluable PK data.

Unless otherwise specified, myelopreservation efficacy and its effect on PRO endpoints will be evaluated based on the data that are collected during Induction, which is defined as the time period between the date of randomization and the end of the last cycle in Induction (ie, last cycle of placebo or trilaciclib plus FOLFOXIRI/bevacizumab). Treatment effect on OS will be evaluated based on the estimated number of events and will not be limited to where the patient is in the study (eg, Induction, Maintenance or Survival Follow-Up).

Statistical analysis methods for primary and key secondary endpoints

General testing strategy

The treatment effects on the primary myelosuppression endpoints (DSN in Cycle 1 – 4 and occurrence of SN during Induction) will be tested as 2-sided α_1=0.04 level, while the treatment effect on OS will be tested at 2-sided α_2=0.01 level. Both DSN in Cycle 1 – 4 and occurrence of SN in Induction will be tested at the 2-sided α_1=0.04 level simultaneously. If statistical significance is established at the 2-sided 0.04 level for the two primary endpoints, the assigned α_1=0.04 will be recycled to test treatment effect on OS following a fallback procedure (Wiens, 2003). In that occasion, testing OS will be conducted at the level of 2-sided 0.05 (ie, 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 – 4 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 ≥ Grade 4 toxicity (ie, ANC < 0.5 x 10^9/L in SI Unit). For patients with at least one SN event in Cycle 1 – 4, the duration of SN (DSN) is defined as the number of days for the first SN event that occurred in Cycle 1, 2, 3, or 4 for patients who had at least one SN event in the first 4 cycles of Induction. Specifically, it is calculated as the days from the date of the first ANC value of <0.5 × 10^9/L to the date of the first ANC value ≥0.5 × 10^9/L where no additional ANC values <0.5 × 10^9/L are observed for the remainder of that cycle. All observed data from scheduled or non-scheduled visits within Cycle 1 – 4 will be included in the derivation. For those patients without any SN in Cycle 1– 4, DSN will be set to be 0. The treatment group difference in DSN in Cycle 1 – 4 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, Western Europe, and China), 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 – 4 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.

Analysis for OS

At the time when 157 death events are observed or at 52 months after the date of first randomization, whichever comes first, the study will be concluded, and the final study database will be locked to perform the OS analysis. Treatment effect on 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 terms as in the stratified log-rank test will be used to estimate the HR between the 2 treatment groups (trilaciclib vs placebo) for OS along with its (1-α) x100% confidence interval.

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 (eg, 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 anti-cancer 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 (eg, hematology, chemistry) will be characterized according to National Cancer Institute (NCI) -CTCAE v5.0, when possible, and the number and percentage of patients within each CTCAE grade will be summarized for the overall treatment period as well as for each cycle. Scheduled and unscheduled safety data will be included in safety evaluation.

The pharmacokinetics of trilaciclib and any metabolites 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.

2. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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3. LIST OF ABBREVIATIONS

The following abbreviations and specialist terms are used in this study protocol.

Table 1: Abbreviations

Abbreviation	Definition
AC	adriamycin and cyclophosphamide
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ANCOVA	analysis of covariance
aPTT	activated partial thromboplastin time
aRR	adjusted relative risk
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Classification
BCRP	breast cancer resistance protein
BED	biologically effective dose
Bev	bevacizumab
β-hCG	beta human chorionic gonadotropin
BICR	blinded independent central review
BSEP	bile salt export pump
CBC	complete blood count
CD	cluster of differentiation
CDK	cyclin-dependent kinase
CFR	Code of Federal Regulations
CI	continuous infusion
CIM	chemotherapy-induced myelosuppression
CL	clearance
CLIA	Clinical Laboratory Improvement Amendments
CMH	Cochran–Mantel–Haenszel
CNS	central nervous system
CR	complete response

Abbreviation	Definition
CRC	colorectal cancer
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450
D5W	dextrose 5% in water
DCAF	d-type cyclin activating features
DDI	drug-drug interaction
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DOR	duration of objective response
DPD	dipyrimidine dehydrogenase
DSN	duration of severe (Grade 4) neutropenia
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
EdU	5-ethynyl-2′-deoxyuridine
EGFR	epidermal growth factor receptor
E/P	etoposide plus carboplatin
ER	estrogen receptor
ESA	erythropoiesis-stimulating agent
ESMO	European Society of Medical Oncology
EQ-5D-5L	5-level EQ-5D
EQ-VAS	EQ visual analogue scale
FACT-An	Functional Assessment of Cancer Therapy – Anemia
FACT-C	Functional Assessment of Cancer Therapy – Colorectal
FACT-G	Functional Assessment of Cancer Therapy – General
FDA	Food and Drug Administration
FFPE	formalin-fixed paraffin-embedded
FN	febrile neutropenia
FSH	follicle stimulating hormone

Abbreviation	Definition
5FU/FU	fluorouracil
G1	gap 1
G2	gap 2
G8	geriatric 8
G/C	gemcitabine plus carboplatin
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GI	gastrointestinal
HFS	Hand-Foot Syndrome
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HR	hazard ratio
HRT	hormone replacement therapy
HSPCs	hematopoietic stem and progenitor cells
IB	Investigator's Brochure
IC_{50}	half maximal inhibitory concentration
ICF	informed consent form
ICH	International Council for Harmonisation
ID	identification
IEC	Independent Ethics Committee
ILD	interstitial lung disease
INR	international normalized ratio
IP	intraperitoneal
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	intravenous
IWRS	interactive web response system
Ki	inhibitory constant
LCV	leucovorin
M	mitosis

Abbreviation	Definition
mab	monoclonal antibody
MATE	multidrug and toxin extrusion
mCRC	metastatic colorectal cancer
MOA	mechanism of action
mITT	modified intent-to-treat
MRI	magnetic resonance imaging
MRP	multidrug resistance protein
MSI	microsatellite instable
MSS	microsatellite stable
NCI	National Cancer Institute
NMPA	National Medical Products Administration
NYHA	New York Heart Association
OAT1	organic anion transporter 1
OATP1B1	organic anion transporting polypeptide 1B1
OATP1B3	organic anion transporting polypeptide 1B3
OCT2	organic cation transporter 2
ORR	objective response rate
OS	overall survival
PD	progressive disease
PDX	patient derived xenografts
PFS	progression-free survival
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
p-gp	p glycoprotein
PK	pharmacokinetic(s)
pMMR	proficient mismatch repair
PP	Per Protocol
PR	partial response
PRES	Posterior Reversible Encephalopathy Syndrome
PRO	patient reported outcome
PT	preferred term

Abbreviation	Definition
PVG	pharmacovigilance
QTcF	QTc corrected by Fridericia's formula
RB	retinoblastoma
RB1	retinoblastoma gene
RBC	red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	ribonucleic acid
RP2D	recommended phase 2 dose
RPLS	Reversible Posterior Leukoencephalopathy Syndrome
S	synthesis
SAE	serious adverse event
SAP	Statistical Analysis Plan
SCLC	small cell lung cancer
SD	stable disease
SN	severe neutropenia
SUSAR	suspected unexpected serious adverse reactions
$t_{1/2}$	half-life
T_{max}	time to reach the maximum concentration
TNBC	triple negative breast cancer
TTCD-fatigue	time to first confirmed deterioration of fatigue
ULN	upper limit of normal
US	United States
V	volume of distribution
VEGF	vascular endothelial growth factor
WHO	World Health Organization
WHO-DD	World Health Organization-Drug Dictionary
WOCBP	women of childbearing potential

4. INTRODUCTION

DISEASE

Although the solid tumor cancer treatment paradigm has advanced over the last 20 years with the introduction of targeted therapies (e.g., vascular endothelial growth factor [VEGF] inhibitors, epidermal growth factor receptor [EGFR] inhibitors) and immuno-oncology agents (e.g., checkpoint inhibitors), chemotherapy remains the cornerstone of treatment for patients with solid tumor cancers in both the curative and palliative settings. Consequently, chemotherapy-related toxicity, including chemotherapy-induced myelosuppression (CIM), diarrhea, and stomatitis, can influence the risk/benefit ratio of a given chemotherapy treatment.

CIM is a significant issue in cancer treatment; 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 (Caggiano, 2005; Gustinetti, 2016; Bodey, 1966; Li, 2016). Moreover, CIM commonly leads to dose reductions and delays, which limit therapeutic dose intensity and can compromise the anti-tumor efficacy benefits of chemotherapy. 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. Therefore, preserving the bone marrow and immune system from the cytotoxic effects of chemotherapy has the potential to maximize the anti-tumor activity of the chemotherapy while minimizing the consequences to the patient of chemotherapy-induced cellular damage.

CHEMOTHERAPY AND TUMOR TYPE

Globally, 1.85 million people will be diagnosed with colorectal cancer (CRC) each year and approximately 880,000 will die of their disease (WHO International Agency for Cancer Research, 2019). Multi-agent chemotherapy remains the cornerstone of treatment for metastatic disease and almost all patients will receive some combination of fluorouracil (5FU), oxaliplatin, irinotecan, a monoclonal antibody (mab) targeting the VEGF pathway and, for those with KRAS wild type disease, a mab targeting the EGFR pathway. How these drugs are combined with one another and administered to patients depends on a variety of factors including physician preference, patient preference, and patient characteristics. Geographic differences in practice patterns also influence the choice of therapies and how they are administered. In general, until relatively recently, the standard of care focused on the use of either FOLFOX (5FU, leucovorin, oxaliplatin) or FOLFIRI (5FU, leucovorin, 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 switch to FOLFIRI/mab upon progression on FOLFOX/mab. However, recent studies combining all four drugs (FOLFOXIRI) indicate that four drugs are better than three with respect to anti-tumor efficacy in metastatic colorectal cancer (mCRC).

In the TRIBE trial, patients on FOLFOXIRI/bevacizumab had a median progression free survival (PFS) of 12.1 months compared to those receiving FOLFIRI/bevacizumab who had a median PFS of 9.7 months (hazard ratio [HR]: 0.75); and a median overall survival (OS) of 29.8 months compared to 25.8 months in the FOLFIRI/bevacizumab arm (HR: 0.8) (Loupakis, 2014; Cremolini, 2015). 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, 2019). Unfortunately, the improvements in PFS and OS have come at the expense of increased chemotherapy-induced toxicity as would be expected when the chemotherapy regimen is intensified by adding additional cytotoxic agents (Montagnani, 2011). For example, 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 (Loupakis, 2014). Similarly, in VISNU-1, rates of ≥ 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).

One significant challenge when considering the FOLFOXIRI/bevacizumab published literature is that these trials were primarily designed to demonstrate anti-tumor efficacy and not designed to provide detailed assessments of CIM, as demonstrated by limited number of timepoints for blood counts and less frequent testing for blood counts. In addition, application of supportive care measures were left flexible and not regulated during these trials. For these reasons, the published literature likely underestimates the actual degree and rate of myelosuppression of FOLFOXIRI, which is hypothesized to be similar to that seen with the small cell lung cancer (SCLC) chemotherapy regimens studied in the trilaciclib program.

As a result of the increased toxicity associated with FOLFOXIRI, the use of FOLFOXIRI is frequently limited to younger patients with fewer comorbidities and in need of aggressive cytoreduction. In fact, most trials where FOLFOXIRI is being evaluated specifically exclude patients over 70 with an Eastern Cooperative Oncology Group (ECOG) performance status other than 0. In the United States (US), the median age for colon cancer detection is 68 in men and 72 in women suggesting that a substantial portion of those diagnosed are not considered for the FOLFOXIRI/bevacizumab regimen (ACS, 2019). In addition, some members of the oncology community believe that using all of the most active drugs together in the first line setting is inferior to a sequential approach. While recent data show no advantage to a sequential approach and suggest that patients should ideally receive the triple drug combination in the first line setting (Cremolini, 2019), the issue of increased chemotherapy-induced toxicity remains.

Currently, there are no approved treatments to prevent the chemotherapy-induced cellular damage that leads to chemotherapy-induced toxicities like CIM, diarrhea, and stomatitis. Although some therapies may help to address CIM once they have occurred (eg, transfusions, growth factors), there are currently no available options that provide patients with the means to effectively protect hematopoietic stem and progenitor cells (HSPCs). HSPC protection would help to limit reductions in therapeutic dose intensity as well as off-target lymphocyte depletion that decreases host anti-tumor immunity (Lyman, 2006; Smith, 2006). Introducing a therapy that can protect the host from chemotherapy-induced cellular damage has the potential to minimize chemotherapy-induced toxicity while optimizing anti-tumor activity.

CYCLIN-DEPENDEINT KINASES 4/6 PATHWAY AND COLORECTAL CANCER

Cyclin-dependent kinases (CDKs) are a family of enzymes that regulate the cell cycle by binding to cyclins A-E and activating transcription factors that regulate cellular transition from gap 1 (G1) (growth phase) to synthesis (S) (deoxyribonucleic acid [DNA] replication) and gap 2 (G2) (growth phase) to M (mitosis) (Asghar, 2015). Specifically, in G1, association of cyclin D with

CDK4 and/or CDK6 (CDK4/6) forms a complex that results in the activation of CDK4/6. The activated CDK4/6 complex phosphorylates the retinoblastoma (RB) protein leading to transcription of genes required for the G1/S transition and subsequent cell cycle progression. Similarly, other cyclin-CDK complexes (cyclin E-CDK2, cyclin A-CDK2, cyclin A-CDK1 and cyclin B-CDK1) regulate the transition through G1/S and the other phases of the cell cycle (S, G2, M).

While CDK4/6 has been shown to orchestrate a variety of complex biological processes, all are associated with cell division including (1) cellular senescence (Anders, 2011) (2) cell differentiation (He, 2017; Sicinski, 1995; Lazarov, 2002; Shen, 2006; Fujimoto, 2007) (3) metabolism (Sakamaki, 2006; Wang, 2006) and (4) DNA repair (Cook, 2015; Hashizume, 2016). In addition to these normal cellular processes, dysregulation of the CDK4/6 pathway described above leads to uncontrolled tumor proliferation including loss of retinoblastoma gene (RB1) and increased p16INK4a expression (George, 2015; Herschkowitz, 2008; Subhawong, 2009). In addition to cell cycle regulation, CDK4/6 inhibition has been shown to regulate anti-tumor immunity through increased antigen presentation in tumor cells, (Goel, 2017; Schaer, 2018; Teo, 2017; G1 data not shown), reduced intra-tumoral frequency of T regulatory cells, and increased tumor infiltration and activation of cluster of differentiation (CD)4+ and CD8+ T cells (Deng, 2018; Schaer, 2018; Lai, 2018).

While the data evaluating the role of the CDK4/6 pathway in CRC is relatively limited, the available nonclinical (*in vivo* and *in vitro*) and clinical data indicate that CRC is largely insensitive to CDK4/6 inhibitors (including trilaciclib); thereby suggesting that normal cell cycle controls mediated by the CDK4/6 pathway have been dysregulated (Lai, 2018; Sorrentino, 2017; Pek, 2017; Lee, 2016; Shapiro, 2013; O' Hara, 2015). In addition, the observation that CRC is a chemosensitive tumor, and that loss of CDK4/6 pathway function correlates with increased tumor proliferation and improved tumor response to chemotherapy in a variety of cancer types, also indirectly indicates that CRC is generally CDK4/6 independent (Socinski, 2009; Herschkowitz, 2008; Ertel, 2010; Witkiewicz, 2012). Lastly, applying a DNA signature analysis algorithm to the cBioportal dataset to characterize CRC tumors as CDK4/6 dependent, independent or indeterminate, only 7.5% of microsatellite stable (MSS) CRC would be classified as CDK4/6 dependent (Gao, 2013; Cerami, 2012).

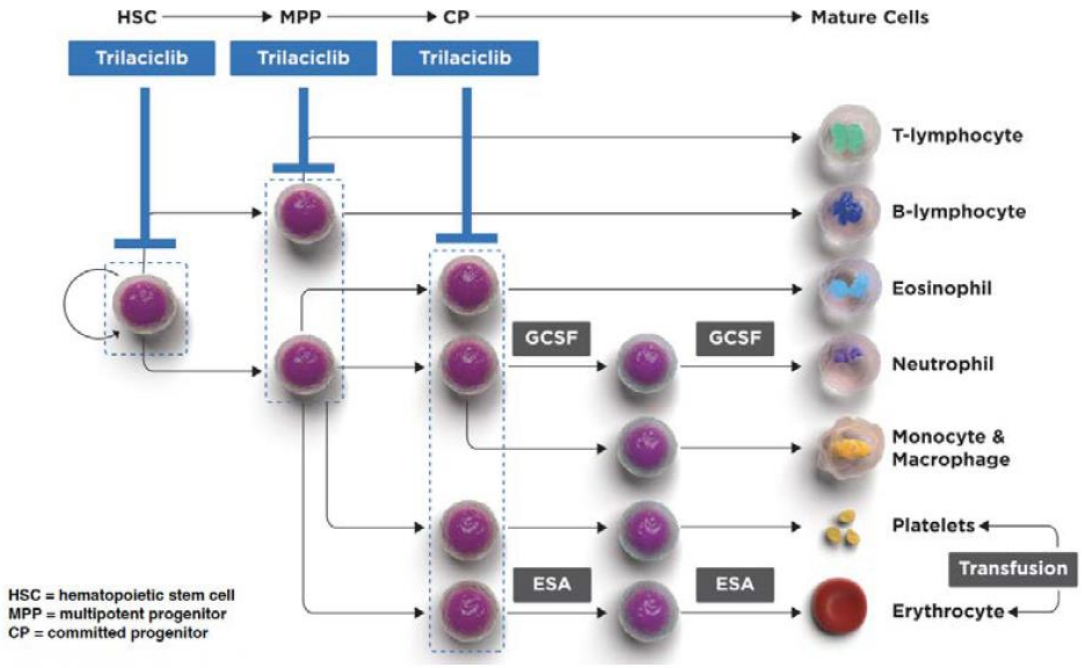
4.1. Study Rationale

Trilaciclib is a highly potent and selective, reversible, CDK4/6 inhibitor that is administered intravenously (IV) prior to chemotherapy and is designed to preserve HSPCs during chemotherapy (myelopreservation) and enhance anti-tumor immunity (anti-tumor efficacy). Both HSPC and lymphocyte populations are dependent on CDK4/6 activity (Kozar, 2004; Malumbres, 2004; Ramsey, 2007; Horsley, 2008) for proliferation and become arrested in the G1 phase of the cell cycle upon exposure to trilaciclib (He, 2017). This transient, drug-induced, cell cycle arrest by trilaciclib has been demonstrated to provide protection from chemotherapy-induced cell damage by preventing HSPCs from proliferating in the presence of cytotoxic chemotherapy and to favorably alter the tumor immune microenvironment through transient T cell inhibition when combined with chemotherapy (He, 2017; Bisi, 2016, Lai, 2020).

Whereas current treatments for CIM are lineage specific, used after the damage to HSPCs has occurred, and place patients at risk for additional toxicities, trilaciclib improves the overall

chemotherapy experience for patients by the prevention of damage to HSPCs and therefore CIM, thus resulting in an overall improved safety profile and reduced utilization of available SOC treatments (Figure 1).

Figure 1: Comparison of Trilaciclib and Current Myelosuppression Therapies



ESA=erythropoiesis stimulating agent; G-CSF=granulocyte colony-stimulating factor.

Three randomized, double-blind Phase 2 clinical trials evaluating trilaciclib/placebo administered prior to chemotherapy in patients with SCLC have demonstrated in a variety of clinical settings (first, second, third line SCLC treated with several different classes of chemotherapy) that trilaciclib can prevent CIM as measured by multiple endpoints (Weiss, 2019; Hart, 2019; Daniel, 2019). Analysis of the exploratory patient reported outcome (PRO) endpoints in these patients suggested that trilaciclib administered prior to chemotherapy may offer potential benefit in multiple aspects of health-related quality of life with a greater magnitude of effect observed for fatigue and anemia symptoms and functional limitations (Weiss, 2019). In an analysis of integrated data from all three studies, the delivery of trilaciclib prior to chemotherapy results in a 4-day reduction in the duration of severe neutropenia in Cycle 1 and an approximately 76% reduction (52.9% placebo to 11.4% trilaciclib) in the occurrence of severe neutropenia during chemotherapy treatment. The occurrence of RBC transfusions on or after 5 weeks on study was reduced by almost half from 26.1% in those treated with placebo to 14.6% in those receiving trilaciclib with an associated 31.9% to 20.3% reduction in the rates of Grade 3 or 4 anemia. Grade 3 or 4 thrombocytopenia was decreased from 36.1% to 19.5% with the addition of trilaciclib and diarrhea was decreased from 17.8% to 13.1%. An analysis of integrated fatigue subscale data from Functional Assessment of Cancer Therapy – Anemia (FACT-An) in the three studies showed that trilaciclib delayed the median time to deterioration by approximately 5 months for fatigue (7.03 months in the trilaciclib group vs 2.33 months in the placebo group, HR: 0.56 [0.37, 0.85]). In addition, these myelopreservation benefits were observed in the setting

of stable PFS and OS as evidenced by hazard ratios < 1.0 in almost all clinical settings for trilaciclib compared to placebo.

In February 2021, the FDA approved trilaciclib (COSELA™) as a treatment to decrease the incidence of chemotherapy-induced myelosuppression in adult patients when administered prior to a platinum/etoposide-containing regimen or topotecan-containing regimen for extensive-stage small cell lung cancer (ES-SCLC).

In contrast, addition of trilaciclib to chemotherapy in patients with triple negative breast cancer (TNBC) in a fourth randomized, Phase 2 trial did not result in the striking statistically significant effects on CIM observed in the SCLC trials but did substantially impact anti-tumor efficacy as measured by OS (median OS in control 12.6 months vs 17.8 months or 20.1 months in the two trilaciclib groups) (Tan, 2019). The differences between the observed results in the SCLC trials and those in the TNBC trial, are hypothesized to result from differences in the key variables between the two clinical situations, ie, chemotherapy type, chemotherapy schedule, trilaciclib schedule, tumor type and host.

Based on the mechanism of action (MOA) of trilaciclib, the effects of trilaciclib on CIM are predicted to be primarily influenced by the host, chemotherapy type and schedule, and trilaciclib schedule; the tumor type should be irrelevant since myelosuppression is not normally influenced by the type of solid tumor. Evaluation of these variables in the two clinical settings highlights an important observation: SCLC chemotherapy is delivered over sequential days (1-3 for etoposide/carboplatin or 1-5 for topotecan) in a cyclical fashion while in the TNBC trial, the chemotherapy (gemcitabine/carboplatin) was delivered intermittently (Days 1 and 8) in a cyclical fashion. This difference in chemotherapy schedule is hypothesized to be the key explanation for the differences in the magnitude of the effects on CIM in these settings. With sequential day chemotherapy, the HSPCs are relatively quiescent in their natural unstimulated state when chemotherapy begins. With the first dose of chemotherapy, the HSPCs and peripheral blood cells are damaged, which is hypothesized to signal the HSCPs to begin proliferating to repair the damage and replenish the peripheral blood counts. If chemotherapy continues to be administered for several days after that first stimulus, the HSPCs are predicted to be damaged further as they increase proliferation (Wang, 2006). In contrast, if proliferation is paused in G1 by trilaciclib, the HSPCs do not begin proliferating while chemotherapy is present and are predicted not to be damaged. Hence, with sequential day chemotherapy (eg, SCLC), trilaciclib prevents CIM. In contrast, with intermittent chemotherapy (e.g., TNBC trial), the chemotherapy is hypothesized to be delivered in a situation where the HSPCs are not fully protected from damage by trilaciclib.

Unlike CIM, the effects of trilaciclib on anti-tumor efficacy are predicted to be primarily driven by the tumor type, chemotherapy type and host, ie, (1) the tumor type must be sufficiently responsive to chemotherapy such that maintenance of chemotherapy dose intensity is beneficial, (2) the tumor must be sufficiently immunogenic and sensitive to the host cytolytic efforts as to see improvement in anti-tumor endpoints like OS, (3) the chemotherapy should promote immune activation, and (4) the host must be able to tolerate the standard of care chemotherapy dose intensity and (5) the host must be able to mount an effective cytolytic response against the tumor. In the SCLC trials, the addition of trilaciclib to the standard of care therapies provided modest improvement, to neutral effects, on measures of anti-tumor efficacy including objective response rate (ORR), PFS and OS. These results are not surprising considering that SCLC is one of the most aggressive solid tumors, relapses quickly after completion of chemotherapy, has been

shown to be relatively insensitive to multiple attempts to intensify the chemotherapy regimen beyond the current SOC, and is not particularly immunogenic or sensitive to immune modulation. In contrast, some TNBC outcomes can be improved with maintenance of dose intensity (improved OS with dose dense adriamycin and cyclophosphamide [AC] compared to traditional schedule AC) (Citron, 2003; Loibl 2011), and TNBC has been shown to be immunogenic and more sensitive to immune modulation compared to SCLC (Semenova, 2015; He, 2017; Carvajal-Hausdorf, 2019, Liu, 2018). Hence, trilaciclib added to standard of care therapy in TNBC appeared to improve anti-tumor efficacy outcomes.

In summary, the magnitude of benefit provided by trilaciclib for the prevention or mitigation of CIM and/or improving anti-tumor efficacy is hypothesized to depend on the chemotherapy type and schedule, host immune system, and tumor type. PRESERVE 1 has been designed to understand how these variables influence the manifestation of trilaciclib benefits (i.e., prevent CIM, improve anti-tumor efficacy or both) by combining trilaciclib with a chemotherapy regimen with a sequential day schedule that is intermediate between that evaluated in the SCLC and TNBC trial (FOLFOXIRI/bevacizumab with 48 hours infusional-5FU). In addition, enrollment of patients with proficient mismatch repair (pMMR)/MSS CRC, which has not been responsive to single agent checkpoint inhibitors in the clinic (Ros, 2018; Hermel, 2019), will explore the extent to which the immune microenvironment of the tumor, alone or in combination with intensification of chemotherapy, plays a role in the ability of trilaciclib to benefit patients with improvement in anti-tumor efficacy.

FOLFOXIRI is a four-drug regimen composed of infusional-5FU, oxaliplatin, irinotecan and leucovorin administered for a maximum of 12 Induction cycles (every 14 days) followed by Maintenance consisting of infusional-5FU and leucovorin administered in 14-day cycles until disease progression, unacceptable toxicity etc. FOLFOXIRI was chosen as the chemotherapy backbone for this trial (compared to FOLFOX or FOLFIRI) for a multitude of reasons including:

- FOLFOXIRI is the most myelosuppressive of the 5FU-based regimens. Patients receiving this regimen will receive the maximal benefit from the ability of trilaciclib to prevent or mitigate CIM.
- FOLFOXIRI is more efficacious than either FOLFOX or FOLFIRI such that if patients could receive this regimen safely at the standard of care dose and schedule, they would derive more benefit.
- Because of the chemotherapy-induced toxicity associated with FOLFOXIRI, its use is limited to younger patients with a poorer prognosis; prevention or mitigation of those toxicities could lead to expansion of its use to more patients who could benefit from its ability to deliver superior outcomes.

pMMR/MSS mCRC was chosen because it is the most common tumor type treated with FOLFOXIRI and it has been shown to be sensitive to chemotherapy intensification, ie, the four drug regimen is more efficacious than the three drug regimens, so there is a potential for patients to benefit from decreased CIM leading to maintenance of standard of care chemotherapy dose and schedule.

This study will enroll patients with unresectable disease. However, patients with metastases confined to the liver have been shown to have higher rates of curative intent surgical resection with FOLFOXIRI/bevacizumab (Gruenberger, 2015). Such patients achieving gross total

resection have longer PFS and OS (Cremolini, 2017). Patients enrolled in this study who may become eligible for and proceed to surgical resection will be appropriately managed both clinically and with respect to analysis of their study data (see Section 12).

While preventing or mitigating CIM is the primary objective of this trial, there are other cells in the host that are dependent on CDK4/6 for proliferation and that suffer from chemotherapy-induced damage similar to HSPCs and lymphocytes. Examples include the stem cells of hair follicles and the stem and progenitor cells of the intestinal crypts (Purba, 2019; Yu, 2008; Wei, 2016; Yang, 2007). In a manner similar to what is seen with protection of HSPCs and lymphocytes, it is possible that protection of these cell populations could lead to improvements in chemotherapy-induced alopecia, stomatitis and diarrhea. This possibility is supported by results from the integrated analysis of the SCLC trials where, although the rates of stomatitis were low in both arms as expected with the chemotherapy regimens utilized, trilaciclib administered prior to chemotherapy resulted in a decreased percentage of patients experiencing diarrhea (13.1% vs 17.8%) and alopecia (13.1% vs 25.4%) compared to placebo.

4.2. Background

4.2.1. Summary of Nonclinical Data

A summary of the trilaciclib nonclinical data is presented in the Trilaciclib Investigator's Brochure (IB).

Nonclinical data related to FOLFOXIRI/bevacizumab are provided in the prescribing information (Section 17.5 and Section 17.6).

4.2.1.1. Pharmacology Studies

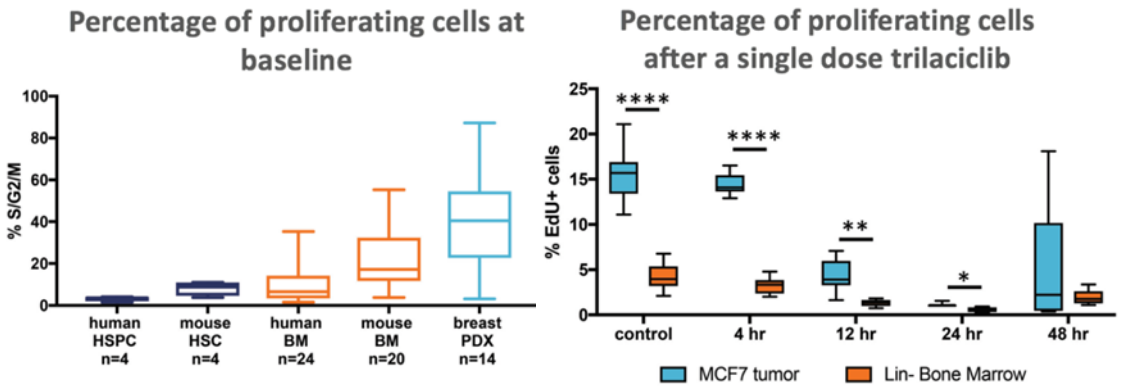
Trilaciclib-induced G1 arrest of HSPCs has been shown to be transient and readily reversible in both in vitro and in vivo models. In mice treated with a single dose of 5FU, the rapid depletion of various cellular lineages (neutrophils, red blood cells, lymphocytes, platelets) is not prevented with the administration of trilaciclib, but animals that received trilaciclib + 5FU demonstrate a faster rate of recovery and improved survival compared with the 5FU alone group. In accordance with the single-dose study, trilaciclib administration with all cycles of 5FU maintained the protective effect against 5FU-induced DNA damage in HSPCs over multiple cycles, leading to an effect that persisted and was greater following multiple cycles of trilaciclib + 5FU compared with 5FU alone. In addition, bone marrow obtained from mice that received trilaciclib in combination with 5FU for a total of 4 cycles was more robust at hematopoietic reconstitution of lethally irradiated mice compared with bone marrow obtained from mice that received 4 cycles of 5FU alone, suggesting that trilaciclib administered with chemotherapy can preserve stem cell function. Additionally, when a separate cohort of 5FU ± trilaciclib-treated mice was analyzed 13 months after the last dose of 5FU (in the absence of bone marrow transplantation), trilaciclib-treated mice demonstrated significantly higher peripheral blood lymphoid-to-myeloid cell ratios compared to 5FU only treated mice, further supporting trilaciclib's long-term protection of hematopoietic stem cell function (He, 2017) following 5FU based chemotherapy.

As a consequence of trilaciclib's MOA, there is a hypothetical risk that administration of trilaciclib prior to chemotherapy could decrease chemotherapy efficacy by pausing CDK4/6-dependent tumor cells in G1 in the presence of chemotherapy; however, nonclinical

data suggest this risk is not clinically significant. To understand why trilaciclib should not antagonize chemotherapy treatment, the baseline proliferation rates of hematopoietic stem and progenitor cells (HSPCs), bone marrow, and patient derived xenografts (PDX) were examined using flow cytometric analysis of the cell cycle. A bar graph depicting mean percentage of cells in S/G2/M demonstrates there are a higher proportion of cells cycling in tumor cells when compared to bone marrow/HSPCs. Specifically, < 4% of human HSPCs are cycling, while in breast tumors an average of ~40% of cells were cycling (Figure 2, left). These results illustrate why tumor cells are more susceptible to chemotherapy-induced damage compared to HSPCs, since chemotherapy treatment is predominantly effective in the S/G2/M phases of the cell cycle.

To evaluate the effects of trilaciclib on HSPCs and CDK4/6 dependent tumor cells, MCF7 tumor-bearing mice were given a single dose of trilaciclib, and bone marrow and MCF7 tumor cell proliferation was evaluated. MCF7 tumors are a well-established estrogen receptor (ER)+ breast cancer model known to be CDK4/6 dependent (Hafner, 2019). After treatment, trilaciclib inhibits proliferation (% 5-ethynyl-2′-deoxyuridine [EdU]+ cells) at a faster rate and more completely in bone marrow than tumor cells (Figure 2, right). The differences in inhibition rates between HSPCs and MCF7 tumors cells after trilaciclib treatment creates a therapeutic window for the selective protection of bone marrow compared to CDK4/6-dependent tumor cells from the cytotoxic effects of chemotherapy.

Figure 2: Proliferation Kinetics Are Different Between Bone Marrow and Tumor Cells

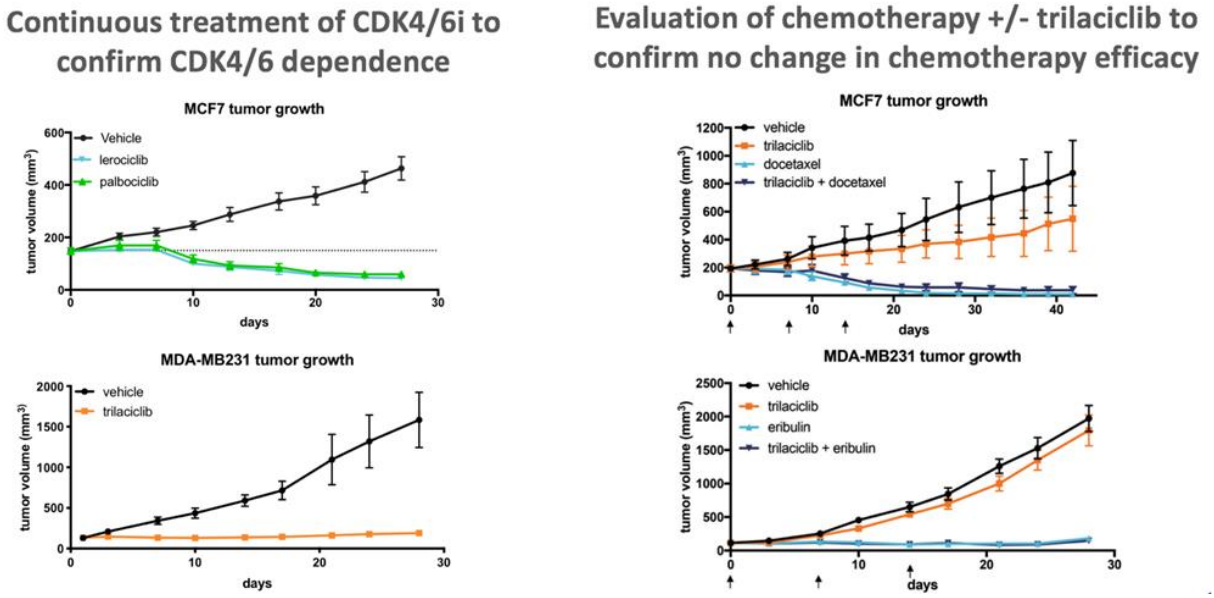


A) Mean differences in baseline proliferation rates of hematopoietic stem cells (HSCs), hematopoietic stem and progenitor cells (HSPCs), bone marrow (BM), and patient derived xenograft (PDX) tumor cells in mice and humans, were examined using flow cytometric analysis of the cell cycle (He, 2017). Error bars represent the minimum and maximum. B) MCF7 tumor-bearing mice were treated with a single dose of trilaciclib (intraperitoneal [IP], 100 mg/kg) or vehicle control. 4, 12, 24, and 48 hours post-treatment. Animals were pulsed with 5 ethynyl-2′ -deoxyuridine (EdU; IP, 200 mg) and tumors and femurs were harvested after 4 hours of EdU dosing. HSPC in bone marrow is defined as cell populations negative for lineage markers Mac-1, Gr-1, Ter119, B220, CD4, and CD8. Proliferations was measured by % EdU incorporated. ****p≤ 0.0001, **p≤ 0.01, *p≤ 0.05

To test whether trilaciclib could decrease chemotherapy efficacy in CDK4/6 dependent tumor models, two different models were treated with chemotherapy and trilaciclib. To confirm CDK4/6 dependence in MCF7 tumors, two alternative CDK4/6 inhibitors (palbociclib and lerociclib) were administered continuously to MCF7 tumor-bearing mice for 28 days, with tumors regressing when on treatment. MCF7 tumor-bearing mice were treated with docetaxel (IV, 5 mg/kg) +/- trilaciclib once weekly for three doses. Although weekly trilaciclib treatment caused some tumor growth inhibition, administering trilaciclib with docetaxel did not alter the

effects of chemotherapy (Figure 3, top right). Next, MDA-MB231 tumor-bearing mice were treated with daily trilaciclib (intraperitoneal [IP], 100 mg/kg) for 28 days to confirm CDK4/6 dependence. Continuous treatment with trilaciclib caused stable disease in MDA-MB231 tumor-bearing mice (Figure 3, bottom left). MDA-MB231 tumor-bearing mice were then treated with eribulin (IV, 0.5 mg/kg) +/- trilaciclib once weekly for three doses. Similar to the MCF7 model, the addition of trilaciclib to eribulin treatment did not impact chemotherapy efficacy (Figure 3, bottom right). These experiments demonstrate that transient CDK4/6 inhibition prior to chemotherapy (eribulin, docetaxel) does not antagonize the intended anti-tumor effects of the chemotherapy in these CDK4/6-dependent tumor models.

Figure 3: CDK4/6 Inhibition Does Not Antagonize Anti-Tumor Efficacy of Chemotherapy in CDK4/6 Dependent Xenograft Models



4.2.1.2. Pharmacokinetic Studies

In vitro analyses of direct and time-dependent inhibition suggest that drug interactions based on inhibition of cytochrome P450 (CYP)1A2-, 2B6-, 2C8-, 2C9-, 2C19-, and 2D6-mediated metabolic pathways are unlikely at clinical doses, however, the studies do suggest that drug interactions via trilaciclib-mediated inhibition of CYP3A4 metabolic pathways are possible. A clinical DDI study using the index CYP3A substrate midazolam indicated that trilaciclib had no impact on CYP3A activity. Two clinical DDI studies using a strong CYP3A inhibitor itraconazole were also conducted. No clinically significant changes in exposure were observed for trilaciclib when co-administered with itraconazole. In vitro induction studies of the 3 major inducible CYP enzymes (CYP1A2, CYP3A4, and CYP2B6) in human hepatocytes suggest that trilaciclib mediated induction is unlikely.

In vitro inhibition studies with membrane transporter model systems also suggest trilaciclib is unlikely to cause a drug-drug interaction (DDI) based on inhibition of breast cancer resistance protein (BCRP)-, bile salt export pump (BSEP)-, organic anion transporter 1 (OAT1)-, organic anion transporter 3 (OAT3)-, organic anion transporting polypeptide 1B1 (OATP1B1),

p glycoprotein (p-gp), multidrug resistance protein (MRP) 1, MRP2, organic cation transporter 1 (OCT1), or organic anion transporting polypeptide 1B3 (OATP1B3) mediated transport. However, in vitro, trilaciclib is a potent inhibitor of multidrug and toxin extrusion 1 (MATE1), multidrug and toxin extrusion 2-K (MATE2-K), organic cation transporter 1 (OCT1), and organic cation transporter 2 (OCT2). Clinical data from the evaluation of trilaciclib administered prior to topotecan (Study G1T28-03) suggests that drugs that are substrates of multidrug and toxin extrusion (MATE)1 and MATE2 do not pose a clinically relevant risk of DDIs when administered concomitantly with trilaciclib. In an additional clinical DDI assessment, trilaciclib increased metformin (MATE1, MATE2-K and OCT2 substrate) exposure by 65%, in healthy subjects, compared to administration of metformin alone. Avoid concomitant use of trilaciclib with certain OCT2, MATE1, and MATE2-K substrates (e.g., dofetilide, dalfampridine) where minimal concentration changes may lead to serious or life-threatening toxicities. Refer to the prescribing information for these concomitant medications for assessing the benefit and risk of concomitant use with trilaciclib.

Study G1T28-11 was a single ascending dose study, with both placebo and positive control groups, with the objective to evaluate potential effects of trilaciclib on ECG parameters, including concentration-QTc (C-QTc) analysis. Trilaciclib transiently increased heart rate by approximately 10 beats per minute. At the clinical dose of 240 mg/m^2, trilaciclib did not have a clinically relevant effecton QTc (i.e., > 10 msec).

Irinotecan is converted by enzymes into its active metabolite SN-38. SN-38 is inactivated by the enzyme UGT1A1 via glucuronidation. The inhibition of UGT1A1 could potentially elevate the SN-38 exposure causing toxicity. The inhibitory effect of trilaciclib on UGT1A1 was tested in vitro using 17ß-Estradiol as a substrate. The estimated half maximal inhibitory concentration (IC_{50}) was 20 µM. The highest concentration of trilaciclib observed in the clinical studies was ~1800 ng/mL (~4 µM) at recommended dose of 240 mg/m^2. The unbound fraction of trilaciclib in human plasma is ~30%. By assuming inhibitory constant (Ki)=IC_{50}/2, the R1 value recommended by the Food and Drug Administration (FDA) is calculated to be 1.12 which is slightly higher than the 1.02 threshold proposed by FDA for clinical assessment. Given trilaciclib showed a three-compartment pharmacokinetic (PK) profile with an alpha phase half-life ($t_{1/2}$) of 0.25 hour, the concentration of trilaciclib will drop below the 1.02 threshold approximately 40 minutes after reaching the maximum concentration (T_{max}). Therefore, the overall impact on the SN-38 exposure via UGT1A1 inhibition by trilaciclib is regarded to be minimal.

Oxaliplatin undergoes nonenzymatic conversion in physiologic solutions to active derivatives via displacement of the labile oxalate ligand. There is no evidence of cytochrome P450-mediated metabolism in vitro. The major route of platinum elimination is renal excretion. In addition, oxaliplatin does not inhibit P450 enzymes *in vitro*. Hence, a PK drug-drug interaction between trilaciclib and oxaliplatin is not expected.

5FU is a prodrug and it has a narrow therapeutic range. In humans, the majority of 5FU dosage is degraded by dihydropyrimidine dehydrogenase. Approximately 60 to 90% of the administered dose is excreted in the urine within 24 hours, primarily as alpha-fluoro-beta-alanine. 5FU inhibits CYP2C9. However, triaciclib is not a substrate of CYP2C9. Therefore, a PK DDI between trilaciclib and 5FU is not expected.

4.2.2. Summary of Clinical Data

4.2.2.1. Trilaciclib

A summary of the clinical study results with trilaciclib is available in the Trilaciclib IB. Results relevant to this clinical study are summarized below.

The safety and efficacy of administering trilaciclib prior to chemotherapy was tested in one completed and one ongoing Phase 1b/2 study (G1T28-02 and G1T28-03) and 2 completed Phase 2 studies (G1T28-05 and G1T28-04) in patients with SCLC or TNBC. The Phase 2 portions of Studies G1T28-02, G1T28-03 and Study G1T28-05 were randomized, double blind and placebo-controlled. Study G1T28-04 was randomized and included a control arm but was not double-blinded.

- Study G1T28-02 examined once-daily IV administration of either trilaciclib or placebo on Days 1 to 3 of each 21-day etoposide plus carboplatin (E/P) chemotherapy cycle in patients with treatment naïve extensive-stage SCLC.
- Study G1T28-03 examined once-daily IV administration of trilaciclib or placebo on Days 1 to 5 of each 21-day topotecan chemotherapy cycle in patients with previously treated extensive-stage SCLC.
- Study G1T28-05 examined once-daily IV administration of trilaciclib or placebo on Days 1 to 3 for a maximum of four 21-day cycles of E/P and atezolizumab, followed by monotherapy atezolizumab, in patients with treatment naïve extensive-stage SCLC.
- Study G1T28-04 examined once-daily IV administration of trilaciclib prior to gemcitabine and carboplatin in patients with metastatic TNBC. Patients received 1) gemcitabine plus carboplatin (G/C) therapy only on Days 1 and 8 of a 21-day cycle, 2) trilaciclib and G/C once daily on Days 1 and 8 of each 21-day cycle, OR 3) trilaciclib on Days 1, 2, 8 and 9 with G/C on Days 2 and 9 of each 21-day cycle.

At the recommended phase 2 dose (RP2D) of 240mg/m^2, across all three SCLC trials, trilaciclib administered prior to chemotherapy statistically significantly reduced the duration of severe neutropenia [SN] in Cycle 1 and occurrence of SN (primary endpoints) compared with placebo. An integrated data analysis of the three SCLC studies for 8 of the most relevant myelosuppression endpoints (neutrophils, RBCs and platelets) is summarized in Table 2. Based on this pooled SCLC analysis, statistically significant, and clinically meaningful, improvements for trilaciclib over available therapies is demonstrated in 6 of 8 endpoints across multiple lineages. Importantly, these myelopreservation benefits come with an overall improved safety profile compared to available therapy, as evidenced by reduced high grade treatment-emergent AEs across all SCLC studies, and no detriment to anti-tumor efficacy results.

Table 2: Integrated Analysis for Myelopreservation Efficacy in SCLC (Studies G1T28-02, G1T28-03, and G1T28-05)

	Placebo +Chemo	Trilaciclib +Chemo	2-sided p-value
ITT Set	119	123	
Neutrophils			
DSN [days] in Cycle 1 – Mean (SD)[a]	4 (5.1)	0 (1.8)	<0.0001
Occurrence of SN (Yes, %)[b]	64 (52.9)	14 (11.4)	<0.0001
RBCs			
Occurrence of Grade 3/4 hemoglobin decreased (anemia) (Yes, %)[b]	38 (31.9)	25 (20.3)	0.0279
Occurrence of RBC transfusion on/after 5 weeks (Yes, %)[b]	31 (26.1)	18 (14.6)	0.0252
Cumulative incidence of RBC transfusion on/after 5 weeks - Event rate (per 100 weeks) [c]	3.1	1.5	0.0027
Platelets			
Occurrence of Grade 3/4 platelet count decreased (thrombocytopenia) (Yes, %) [b]	43 (36.1)	24 (19.5)	0.0067
Occurrence of platelet transfusion (Yes, %)[b]	11 (9.2)	10 (8.1)	0.9564
Cumulative incidence of platelet transfusion - Event rate (per 100 weeks)[c]	1.7	1.1	0.5169

ANC=absolute neutrophil count; Chemo=chemotherapy; DSN=duration of severe (Grade 4) neutropenia; ECOG=Eastern Cooperative Group; ITT=intent to treat; SN=severe (Grade 4) neutropenia which is defined as ANC < 500/mm3; RBC=red blood cells, SCLC=small cell lung cancer; SD=standard deviation.
Chemo=etoposide + platinum in G1T28-02, etoposide + platinum + atezolizumab in G1T28-05, and topotecan in G1T28-03. Standard supportive care interventions were allowed for all arms.
a. p-value was obtained from a nonparametric analysis of covariance (ANCOVA).
b. p-value was obtained from a modified Poisson model.
c. p-value was obtained from a negative binomial model. All three models contained fixed effect of treatment, ECOG performance status (0/1 vs. 2), Presence of brain metastases (Y/N), and study (G1T28-02, G1T28-03, or G1T28-05). Corresponding baseline assessment was also included as a covariate.

Results from Study G1T28-04 demonstrated that although the addition of trilaciclib to GC did not statistically significantly improve CIM as measured by the neutrophil-based primary endpoints of duration of severe (Grade 4) neutropenia (DSN) in Cycle 1 and occurrence of SN, there were trends toward improvement in RBC and platelet-based measures. In addition, anti-tumor efficacy results demonstrated a clinically meaningful improvement in PFS and most importantly OS with HR: 0.31 (median OS: not reached [NR]; 95% confidence interval [10.2, NR]) and 0.40 ([median OS: 17.8 months; 95% confidence interval [12.9, 32.7]) for the two trilaciclib arms, respectively (see Trilaciclib IB). This meaningful anti-tumor efficacy was observed across multiple subgroups and in both of the trilaciclib groups compared with the control group.

As mentioned in Section 4.2.1.1, there is a hypothetical risk that administration of trilaciclib prior to chemotherapy could decrease chemotherapy efficacy by pausing CDK4/6-dependent

tumor cells in G1 in the presence of chemotherapy. This hypothetical risk is countered by the results observed in Study G1T28-04 which suggested that the addition of trilaciclib improves the anti-tumor efficacy of gemcitabine and carboplatin regardless of the CDK4/6 status of the TNBC tumor. Although TNBC tumors are predominantly classified as CDK4/6 independent (ie, their replication is not sensitive to CDK4/6 inhibition), there is a small subset of patients whose tumors are classified as either CDK4/6 indeterminate or CDK4/6 dependent. When the TNBC population enrolled in Study G1T28-04 is divided into these subsets, evaluation of the anti-tumor efficacy in patients whose tumors are classified as CDK4/6 indeterminate or CDK4/6 dependent suggests that trilaciclib does not antagonize the anti-tumor effects of gemcitabine and carboplatin. Specifically, PFS and OS did not decrease when trilaciclib was added to gemcitabine and carboplatin in the most CDK4/6 dependent population (see Trilaciclib IB).

4.2.2.2. FOLFOXIRI/Bevacizumab

4.2.2.2.1. FOLFOXIRI

FOLFOXIRI is a combination of 5FU, leucovorin, oxaliplatin, and irinotecan commonly used in the treatment of mCRC (NCCN, 2019). Complete prescribing information for each component is provided in Section 17.5.

4.2.2.2.2. Bevacizumab

Bevacizumab has been tested in clinical studies and is currently approved by the US FDA for the treatment of mCRC, in combination with fluorouracil-based chemotherapy for first- or second-line treatment (Avastin®, 2020; Avastin®, 2019). Complete prescribing information is provided in Section 17.6.

4.2.3. Risks

4.2.3.1. Trilaciclib

Reproductive/embryo-fetal effects is an important potential risk of trilaciclib. Both nonclinical toxicology studies with trilaciclib, and clinical studies with other compounds with a similar MOA, report effects on either the reproductive system or embryo/fetus. Since this clinical study will focus on trilaciclib administered prior to cytotoxic chemotherapy (which carries its own risk of reproductive/embryo-fetal toxicity), the risks specific to trilaciclib are consistent with those experienced with chemotherapy. In addition, female patients will be monitored for pregnancy and eligibility criteria describing specific birth control methods are incorporated. Dose discontinuation recommendations for female patients who become pregnant while receiving trilaciclib are also provided in the protocol. Detailed information regarding all important identified and important potential risks of trilaciclib administration can be found in the Trilaciclib IB.

Adverse events of special interest (AESIs) identified for trilaciclib are described below. Rates reported below reflect those observed in the integrated safety summary from the four Phase 2 oncology trials conducted with trilaciclib to date (G1T28-02 [database lock: 05 May 2019], G1T28-03 [data cutoff: 31 May 2019], G1T28-05 [data cutoff: 28 June 2019], and G1T28-04 [data cutoff: 28 June 2019]). Some AESIs have been infrequently reported (or not reported) in the trilaciclib clinical program to date but are considered to be potential class effects of CDK4/6

inhibitors. However, as trilaciclib is given intravenously and only when chemotherapy is administered, the safety profile of trilaciclib appears to be different from that of the oral, chronically-dosed members of its pharmacologic class. All patients will be monitored for these events and dose modification and discontinuation guidelines are provided in Section 9.5.

Trilaciclib AESIs:

1. **Injection Site Reaction/Phlebitis/Thrombophlebitis:** Infusion of trilaciclib may result in local irritation manifesting as erythema, pain, and swelling; and in a subset of patients, the irritation may cause phlebitis/thrombophlebitis. Injection site reactions have been observed in approximately 15 to 20% of patients receiving trilaciclib and the events have been Grade 1 or 2. Phlebitis/thrombophlebitis has been noted in approximately 5 to 10% of patients receiving trilaciclib and the events were primarily Grade 1 or 2. Supportive care interventions such as slowing the infusion rate, flushing the infusion line with a minimum of 20 ml normal saline or 5% dextrose, rotating IV catheter sites or removing the IV (when given peripherally) following each trilaciclib infusion can decrease the frequency and severity of these events. In addition, if symptoms are observed with normal saline as diluent/flush, use of 5% dextrose as an alternative may decrease the symptoms. Central access may also be considered.
2. **Acute Drug Hypersensitivity Reaction:** Trilaciclib administration may cause acute drug hypersensitivity reaction characterized by symptoms like facial edema, pruritis, and urticaria. These events have been reported in approximately 6% of patients receiving trilaciclib compared with approximately 3% in patients receiving placebo, and were classified as Grade 1 and 2. Only one case of grade 2 anaphylaxis was observed, which occurred 4 days after the preceding dose of trilaciclib, resolved on the same day, was not serious, and did not result in discontinuation of treatment.
3. **Pneumonitis/Interstitial Lung Disease (ILD)**: A rare (1 to 3%) but serious class effect (< 1% fatal) associated with CDK4/6 inhibitors. Symptoms include hypoxia, cough, dyspnea, or interstitial infiltrates on radiologic exams in patients in whom infectious, neoplastic or other causes have been excluded. The only case of pneumonitis reported in patients who received trilaciclib was observed in a patient in whom trilaciclib had been discontinued for two months and who had been receiving atezolizumab (known to cause pneumonitis) as monotherapy. The pneumonitis event was Grade 3 and considered by the Investigator to be related to atezolizumab.
4. **Hepatotoxicity:** Both nonclinical toxicology studies with trilaciclib, and clinical studies with other compounds with a similar MOA, report reversible elevations in transaminases with continuous dosing. There has been only 1 instance of Grade 4 alanine aminotransferase (ALT) increase in a patient receiving trilaciclib, no Grade 4 aspartate aminotransferase (AST) increases, and no cases of Hy's law reported in patients receiving trilaciclib. However, generally low grade and transient increases in AST, ALT or bilirubin have been observed in a small number of patients (~ 5%) receiving trilaciclib prior to chemotherapy. Patients with mild hepatic impairment have been treated with trilaciclib without a clinically significant increase in exposure or the frequency/severity of AEs.

5. **Embolic and Thrombotic Events, Venous:** The CDK4/6 inhibitor abemaciclib has been associated with an increased risk for venous thromboembolism when combined with endocrine therapy in patients with breast cancer. This same risk has not been reported for the other approved CDK4/6 inhibitors (ribociclib and palbociclib); therefore, it is not clear if this is a class effect. Approximately 3% of cancer patients that received trilaciclib prior to chemotherapy experienced a venous thromboembolic event and half of those events (3/6) were Grade 3 or 4. No Grade 5 events were reported. Approximately 2% of patients receiving chemotherapy alone or with placebo reported an embolic or thrombotic event, 1 of 3 such events was Grade 3.

4.2.3.2. FOLFOXIRI/Bevacizumab

4.2.3.2.1. FOLFOXIRI

4.2.3.2.1.1. Fluorouracil

Per Warnings and Precautions in the prescribing information for fluorouracil (Section 17.5.1), the following are important risks related to fluorouracil use:

- Increased Risk of Serious or Fatal Adverse Reactions in Patients with Low or Absent dipyrimidine dehydrogenase (DPD) Activity. No fluorouracil dose has been proven safe in patients with absent DPD activity.
- 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).
- Mucositis (severe).
- Increased Risk of Elevated international normalized ratio (INR) with Warfarin: Concurrent administration with warfarin can result in clinically significant increases in coagulation parameters.
- Embryofetal Toxicity: Fluorouracil can cause fetal harm.

Please refer to the full fluorouracil prescribing information (Section 17.5.1) for specific information on adverse reactions and management of toxicity.

4.2.3.2.1.2. Leucovorin

Per Warnings and Precautions in the prescribing information for leucovorin (Section 17.5.2), the following are important risks related to leucovorin use:

- Therapy with leucovorin and 5FU must not be initiated or continued in patients who have symptoms of gastrointestinal toxicity of any severity, until those symptoms have completely resolved.
- Seizures and/or syncope have been reported rarely in cancer patients receiving leucovorin, usually in association with fluoropyrimidine administration, and most commonly in those with central nervous system metastases or other predisposing factors, however, a causal relationship has not been established.
- Leucovorin enhances the toxicity of 5FU.

Please refer to the full leucovorin prescribing information (Section 17.5.2) for specific information on adverse reactions and management of toxicity.

4.2.3.2.1.3. Oxaliplatin

Per Warnings and Precautions in the prescribing information for oxaliplatin (Section 17.5.3), 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 (eg, 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).
- Oxaliplatin has been associated with pulmonary fibrosis (< 1% of study patients), which may be fatal. In case of unexplained respiratory symptoms such as non-productive cough, dyspnea, crackles, or radiological pulmonary infiltrates, ELOXATIN should be discontinued until further pulmonary investigation excludes interstitial lung disease or pulmonary fibrosis.
- Hepatotoxicity, as evidenced in the adjuvant study by increase in transaminases and alkaline phosphatase, was observed more commonly in the oxaliplatin combination arm than in the control arm.

- QT prolongation and ventricular arrhythmias including fatal torsade de pointes have been reported in post marketing experiences following oxaliplatin administration.
- Oxaliplatin may cause fetal harm when administered to a pregnant woman.

Please refer to the full oxaliplatin prescribing information (Section 17.5.3) for specific information on adverse reactions and management of toxicity. Oxaliplatin is also associated with high rates of gastrointestinal toxicity such as nausea/vomiting, diarrhea, abdominal pain and stomatitis. Toxicity should be managed as per package insert and institutional guidelines.

4.2.3.2.1.4. Irinotecan

Per Warnings and Precautions in the prescribing information for irinotecan (Section 17.5.4), 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.
- Hypersensitivity reactions including severe anaphylactic or anaphylactoid reactions have been observed.
- Renal impairment and acute renal failure have been identified, usually in patients who became volume depleted from severe vomiting and/or diarrhea.
- Interstitial Pulmonary Disease-like events, including fatalities, have occurred in patients receiving Irinotecan (in combination and as monotherapy).
- Irinotecan hydrochloride can cause fetal harm when administered to a pregnant woman.
- Hepatic impairment: The use of CAMPTOSAR in patients with significant hepatic impairment has not been established.

Please refer to the full irinotecan prescribing information (Section 17.5.4) for specific information on adverse reactions and management of toxicity.

4.2.3.2.2. Bevacizumab

Per Warnings and Precautions in the prescribing information for bevacizumab (Section 17.6), the following are important risks related to bevacizumab use:

- Serious, and sometimes fatal, gastrointestinal perforation occurred at a higher incidence in patients receiving bevacizumab compared with controls. The majority of cases occurred within the first 50 days of initiation of bevacizumab. Serious and sometimes fatal non-gastrointestinal fistula formation involving tracheo-esophageal, bronchopleural, biliary, vaginal and bladder sites occurs at a higher incidence in bevacizumab -treated patients compared to controls. Most events occurred within the first 6 months of bevacizumab therapy.

- The incidence of wound healing complications, including serious and fatal complications, was 15% in patients with mCRC who underwent surgery while receiving bevacizumab and 4% in patients who did not receive bevacizumab.
- Bevacizumab can result in two distinct patterns of bleeding: minor hemorrhage, which is most commonly Grade 1 epistaxis, and serious hemorrhage, which in some cases has been fatal. Across indications, the incidence of Grade ≥ 3 hemorrhagic events among patients receiving bevacizumab ranged from 1.2 to 4.6%.
- Serious, sometimes fatal, arterial thromboembolic events including cerebral infarction, transient ischemic attacks, myocardial infarction, and angina, occurred at a higher incidence in patients receiving bevacizumab compared with controls.
- Severe hypertension occurred at a higher incidence in patients receiving bevacizumab as compared to controls.
- RPLS has been reported with an incidence of $< 0.1\%$ in clinical studies.
- The incidence and severity of proteinuria was higher in patients receiving bevacizumab as compared with controls.
- Infusion-related reactions reported across clinical studies and post-marketing experience include hypertension, hypertensive crises associated with neurologic signs and symptoms, wheezing, oxygen desaturation, Grade 3 hypersensitivity, chest pain, headaches, rigors, and diaphoresis.
- Bevacizumab may cause fetal harm when administered to pregnant women.

Please refer to the full bevacizumab prescribing information (Section 17.6) for important dose management information specific to adverse reactions.

4.3. Benefit/Risk Assessment

Trilaciclib can provide patient benefit by making chemotherapy safer and allowing patients to receive the full benefit of their chemotherapy treatment, ie, at the standard of care doses and schedule in the setting of an immune system protected from chemotherapy-induced cellular damage. These benefits are provided in the context of minimal toxicity (low grade injection site reactions/phlebitis/thrombophlebitis) being added to their cancer treatment. More specifically, trilaciclib has the potential to make FOLFOXIRI safer and to increase the patient population to which FOLFOXIRI can be administered so that more patients can have access to a chemotherapy regimen with superior outcomes compared to what they would normally be given.

Trilaciclib is not expected to have significant overlapping toxicity with the components of the standard of care regimen of FOLFOXIRI/bevacizumab. Specifically, no overlapping toxicity between 5FU and leucovorin with trilaciclib is anticipated. In fact, trilaciclib is expected to have a favorable impact on specific fluorouracil complications such as myelosuppression and potentially on gastrointestinal (GI) related side effects.

Both trilaciclib and oxaliplatin can be associated with acute hypersensitivity reactions. In addition, oxaliplatin has been associated with pulmonary fibrosis and hepatotoxicity, and chronically dosed oral CDK4/6 inhibitors have been associated with ILD/pneumonitis and hepatotoxicity. Trilaciclib (which is dosed intermittently) has not been associated with an

increased risk of pulmonary toxicity or hepatotoxicity. Patients will be carefully observed during infusions, followed closely for signs of laboratory abnormalities and for signs of pulmonary toxicity. Appropriate actions are described in the dose modifications section (Section 9.5). No overlapping neurologic toxicity is anticipated with trilaciclib, and trilaciclib is expected to decrease the rates of myelosuppression. QT prolongation and ventricular arrhythmias have been reported with Oxaliplatin. No clinically relevant effect on QTc prolongation was observed with trilaciclib at the clinical dose of 240 mg/m^2. As a result, no clinically significant overlapping toxicity is anticipated.

Both trilaciclib and irinotecan can be associated with acute hypersensitivity reactions. In addition, irinotecan and chronically dosed oral CDK4/6 inhibitors have been associated with ILD/pneumonitis. Trilaciclib (which is dosed intermittently) has not been associated with an increased risk of pulmonary toxicity. Patients will be carefully observed during infusions and will be followed for signs of pulmonary toxicity. Appropriate actions are described in the dose modifications section (Section 9.5). Otherwise, overlapping toxicity is not anticipated and trilaciclib is expected to decrease the rates of myelosuppression.

As with multiple drugs in the FOLFOXIRI regimen, bevacizumab has also been associated with GI toxicity including nausea, diarrhea, and stomatitis as well as bone marrow suppression. Overlapping toxicity with trilaciclib is not anticipated. However, bevacizumab is known to have potential impacts on cardiac toxicity, primarily mediated through thromboembolic events. Though this is not an established risk for trilaciclib to date, thromboembolic events are an adverse event of special interest for trilaciclib and will be monitored closely. Management should proceed according to the bevacizumab package insert, institutional guidelines and dose modification tables (Section 9.5).

Trilaciclib has been successfully combined at the RP2D with all the major chemotherapy classes included in the FOLFOXIRI regimen (e.g., alkylating agents, antimetabolites, topoisomerase inhibitors). Based on the PK results described in Section 4.2.1.2, the risk for clinically relevant DDI is low and is not predicted to impact the benefit/risk assessment.

As described in Section 4.2.1.1, there is a hypothetical risk that administration of trilaciclib prior to chemotherapy could decrease chemotherapy efficacy by pausing CDK4/6-dependent tumor cells in G1 in the presence of chemotherapy. However, at this time there is no nonclinical (Section 4.2.1.1) or clinical (Section 4.2.2.1) evidence to support this theoretical risk. In addition, since sensitivity to CDK4/6 inhibitors is a surrogate marker for CDK4/6 dependence, and the oral, chronically-dosed CDK4/6 inhibitors have shown minimal anti-tumor activity in solid tumors other than ER+ breast cancer (Baghdadi, 2019; Goldman, 2014; Goldman, 2018; Gopalan, 2014; Infante, 2016; Karasic, 2018; Rose, 2018), these results suggest that the majority of solid tumor cancers are not CDK4/6 dependent and not susceptible to this hypothetical risk.

Given the potential benefit to patients with mCRC from the addition of trilaciclib to FOLFOXIRI/bevacizumab and the limited potential for significant trilaciclib-related toxicity, the benefit/risk assessment is positive for this combination.

A COVID-19 risk assessment has been performed, documented, and is provided as a separate document (as needed).

5. OBJECTIVES AND ENDPOINTS

The objectives of this study are to assess the myelopreservation and anti-tumor efficacy of trilaciclib administered prior to FOLFOXIRI/bevacizumab in patients receiving treatment for mCRC. Objectives and endpoints are described in Table 3.

Table 3: Objectives and Endpoints

Objectives	Endpoints
Primary Objectives	
• To assess the effects of trilaciclib on the neutrophil lineage compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Duration of severe (Grade 4) neutropenia in Cycle 1 – 4 • Occurrence of severe neutropenia during Induction
Key Secondary Objectives	
• To assess the effect of trilaciclib on OS compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• OS
Secondary Efficacy Objectives: The occurrence and number of events during Induction for each of the following endpoints will be evaluated except as noted.	
• To assess the effects of trilaciclib on additional measures of the neutrophil lineage compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC	• SN (number of events only) • G-CSF administration • FN AEs
• To assess the effects of trilaciclib on the RBC lineage compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Grade 3 or 4 decreased hemoglobin laboratory values • RBC transfusions on or after Week 5 • ESA administration
• To assess the effects of trilaciclib on the platelet lineage compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Grade 3 or 4 decreased platelet count laboratory values • Platelet transfusions
• To assess the effects of trilaciclib on multiple lineages compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Grade 3 or 4 hematologic lab values

Objectives	Endpoints
• To assess the effects of trilaciclib on standard of care dosing compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC	• All-cause dose reductions or cycle delays • Relative dose intensity for FOLFOXIRI/bevacizumab

• To assess the effects of trilaciclib on healthcare utilization compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Hospitalizations
	  • All cause
	  • Febrile neutropenia/ neutropenia
	  • Anemia/RBC transfusions
	  • Thrombocytopenia/bleeding
	  • Infections
	• Antibiotic use
	  • IV
	  • Oral
	  • Oral and IV

• To evaluate the anti-tumor activity of trilaciclib compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• BOR and objective response (CR or PR) per RECIST v1.1 • DOR • PFS (per RECIST 1.1) as assessed by Investigator
• To assess the effects of trilaciclib on chemotherapy-induced fatigue compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• TTCD-fatigue during Induction, as measured by the FACIT-F
Safety Objectives	
• To assess the safety and tolerability of trilaciclib compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Occurrence and severity of AEs by NCI-CTCAE v5 including diarrhoea, stomatitis, and hematologic AEs (neutropenia, anemia, thrombocytopenia) • Changes in laboratory parameters (hematology, chemistry, urinalysis), vital signs and ECG parameters • Grade 3 or 4 abnormalities in chemistry laboratory parameters • Trilaciclib AESIs • Trilaciclib infusion interruptions • Chemotherapy infusion interruptions • Study treatment discontinuation due to AEs

Objectives	Endpoints
Exploratory Objectives	
• To assess the anti-tumor efficacy of trilaciclib in patients with mCRC tumors with different levels of reliance on the CDK4/6 pathway.	ORR (per RECIST v1.1), PFS, and OS in patients with each of the following CDK4/6 dependence signatures: • CDK4/6 dependent • CDK4/6 independent • CDK4/6 indeterminant
• To characterize the population PK of trilaciclib and identify important determinants of variability.	• Exposure-response relationship between trilaciclib as well as any metabolites and response or safety endpoints
• To assess the effects of trilaciclib on CIM -related symptoms and functional limitations compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	Change from baseline and/or time to deterioration in: • FACT-G domain scores (physical, social/family, emotional, and functional well-being) • FACT-An: Anemia • FACT-C (colorectal cancer): Colorectal Cancer Subscale • EQ-5D-5L Percent of patients reporting deterioration and improvement using: • PGIC fatigue item • PGIS fatigue item
• To assess the effects of trilaciclib on the receipt of subsequent anti-cancer therapy(ies) compared with placebo in patients receiving FOLFOXIRI/bevacizumab for pMMR/MSS mCRC.	• Number of patients who receive systemic anti-cancer therapy after discontinuing study drug • Types and number of lines of treatments patients receive after discontinuing study drug

AE=adverse event; BOR=best overall response; CDK=cyclin-dependent kinase; CIM=chemotherapy-induced myelosuppression; CR=complete response; CTCAE=Common Terminology Criteria for Adverse Events; DOR=duration of objective response; ECG=electrocardiogram; ESA=erythropoiesis-stimulating agents; EQ-5D-5L=5-level EQ-5D; FACT-An=Functional Assessment of Cancer Therapy-Anemia; FACT C=Functional Assessment of Cancer Therapy-Colorectal; FACT-G=Functional Assessment of Cancer Therapy-General; G-CSF=granulocyte-colony stimulating factor; pMMR/MSS mCRC=proficient mismatch repair/microsatellite stable metastatic colorectal cancer; NCI=National Cancer Institute; OS=overall survival; PFS=progression free survival; PGIC=Patient Global Impression of Change; PGIS=Patient Global Impression of Severity; PK=pharmacokinetic(s); PR=partial response; RBC=red blood cell; RECIST=Response Evaluation Criteria in Solid Tumors; TTCD-fatigue=time to first confirmed deterioration of fatigue.

5.1. Rationale for Primary and Key Secondary Endpoints

5.1.1. Myelopreservation Efficacy

Patients experiencing CIM often face severe clinical consequences (e.g., febrile neutropenia [FN] predisposes patients to serious infections and even death). For those patients with neutropenia requiring hospitalization, the estimated inpatient mortality rates ranged from 3.4% to 10.5% depending on tumor type in 1 study, with an overall mortality rate of 6.8% (Caggiano, 2005). In another analysis, the overall in hospital rate of death was 9.5%, with rates for solid tumor cancer patients ranging from 3.6% for breast cancer patients to 13.4% for lung cancer patients (Kuderer, 2006). Because both the severity and duration of neutropenia correlate with the risk of FN and infections (Bodey, 1966; Li, 2016; Gustinetti, 2016), a reduction in the occurrence and duration of will decrease the risk of these events and provide an improved patient experience while receiving chemotherapy (Padilla, 2005).

The measurement of DSN is limited to Cycle 1 – 4 because the risk of FN is highest in Cycle 1 – 4 in patients treated with FOLFOXIRI/bevacizumab (Rossini, 2021). Rossini et al describes the occurrence as well as the time course for Grade 3/4, and FN in patients randomized on the TRIBE and TRIBE 2 studies to FOLFOXIRI+B, which is the identical treatment regimen utilized in PRESERVE 1. The TRIBE study randomized 508 patients with mCRC to receive up to 12 cycles of FOLFOXIRI+B or FOLFIRI+B followed by maintenance therapy for both groups. In TRIBE 2, 679 patients were randomized to up to 8 cycles of FOLFOXIRI+B versus mFOLFOX6+B followed by maintenance treatment in both arms. At the time of progression, 8 additional cycles of the randomized regimen could be delivered, again followed by maintenance. The pooled data on these 2 studies (N= 1,155), revealed 51% of patients receiving the FOLFOXIRI+B regimen experienced adverse events of Grade 3/4 neutropenia and 8% experienced FN. In these same patients, the median time to the onset of neutropenia was 0.7 months (i.e., during Cycle 2), suggesting that neutropenic events in this patient population occur later and are not concentrated in Cycle 1. In total, 78.5% of the Grade 3/4 neutropenia events occurred during the first 2 months (or 4 cycles) of treatment. Measuring DSN during the timeframe of occurrence of the majority of SN, i.e., Cycle 1 through 4, will allow an assessment of the risk of FN during the time of greatest clinical risk to patients.

5.1.2. Anti-tumor Efficacy

Measurement of OS is standard in oncology solid tumor trials to measure effects of study treatment on the underlying malignant disease.

5.1.3. Safety

Assessment of AEs, changes in laboratory parameters, vital signs, and ECOG status are all standard assessments used in oncology trials to measure patient safety.

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This is 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. Inclusion/exclusion criteria are outlined in Section 7.1 and Section 7.2.

Approximately 326 patients will be randomly assigned (1:1) to receive placebo or trilaciclib on Days 1 and 2 administered IV prior to FOLFOXIRI/bevacizumab in 14-day cycles for up to 12 cycles (Induction). There will be three stratification factors for randomization: country, 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 the presence of a BRAF V600E mutation (yes/no). Study drugs administered during Induction are as follows:

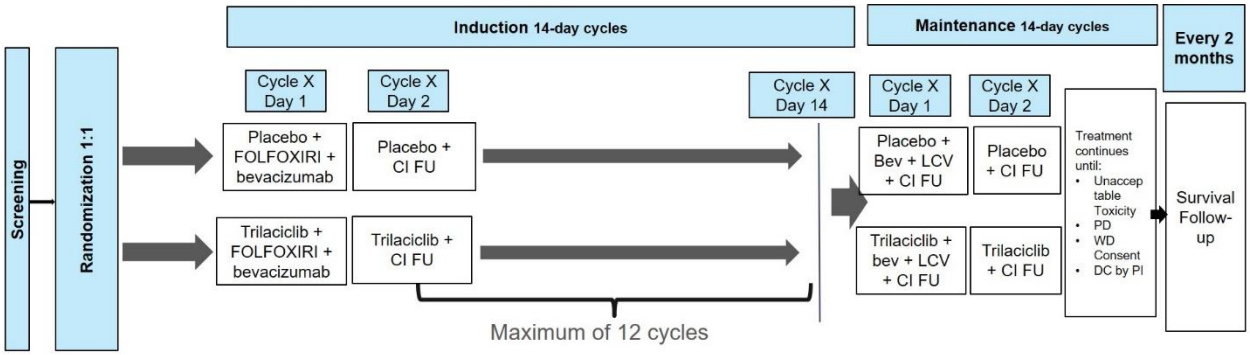
- Trilaciclib (240 mg/m^2) or placebo– administered as a 30-minute IV infusion no more than 4 hours prior to chemotherapy on each day chemotherapy is administered. The second dose of trilaciclib/placebo should be administered as a 30-minute infusion on Day 2.
- Irinotecan 165 mg/m^2 – IV, Day 1
- Oxaliplatin 85 mg/m^2 – IV, Day 1
- Leucovorin 400 mg/m^2 – IV, Day 1; LEVOleucovorin 200 mg/m^2 is an acceptable alternative
- Fluorouracil 2400 – 3200 mg/m^2 – continuous infusion (CI) over 46 – 48 hours beginning on Day 1; this dose range is provided to reflect geographic variations in prescribed 5FU dose, however, the same dose should be continued throughout the study for each patient, except where dose modifications are required for toxicity management.
- Bevacizumab 5 mg/kg – IV, Day 1

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. However, if a patient is unable to complete the maximum number of 12 Induction cycles because of toxicity, and the treating physician feels the patient will receive additional clinical benefit by transitioning to Maintenance, the patient will be allowed to discontinue oxaliplatin and irinotecan, and continue on Maintenance therapy as described below.

Following completion of Induction, patients will continue in Maintenance, where they will continue to receive trilaciclib or placebo per randomization allocation at study entry. Trilaciclib/placebo will be administered prior to infusional-5FU/leucovorin/bevacizumab at the same dose and schedule used during Induction. The patient may continue to receive treatment on study until disease progression, unacceptable toxicity, withdrawal of consent, discontinuation by

Investigator, or the end of the trial, whichever occurs first. Treatment cycles will occur consecutively without interruption, except when necessary to manage toxicities or for administrative reasons.

Figure 4: Study Schema



CI=continuous infusion; DC=discontinued; FU=fluorouracil; LCV= leucovorin; Bev= bevacizumab; PD=progressive disease; PI=Principal Investigator; WD=withdrawal.

Upon discontinuation of study treatment, patients will be followed for survival, ie, 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. The study will be completed when the criteria outlined in Section 10.4 have been met, or upon Sponsor termination of the study.

6.2. Rationale for Dose and Schedule of Study Treatment

6.2.1. Trilaciclib

The RP2D of trilaciclib is 240mg/m^2. This dose was chosen based on (1) a mechanistic PK/pharmacodynamic model informed by nonclinical and human PK and pharmacodynamics and (2) PK and myelopreservation efficacy results from cancer patient studies evaluating trilaciclib administered prior to chemotherapy. In the FIH study (Study G1T28-1-01), assessment of bone marrow aspirates 24 hours after administration of a single dose of trilaciclib at the biologically effective dose (BED) of 192 mg/m^2 revealed a significant decrease in the number of HSPCs in the S/G2/M phases of the cell cycle (ie, an increase in the proportion of cells in G1 arrest). Recovery of the total bone marrow with resumption of proliferation of the bone marrow progenitor subsets was observed by 32 hours post dose, thereby indicating that the arrest was transient and reversible (He, 2017).

When trilaciclib was administered prior to chemotherapy to cancer patients, doses of 200 (rounded up from 192), 240, and 280 mg/m^2 were evaluated. Trilaciclib exposures in cancer patients were slightly lower compared to healthy subjects, such that the dose of 240 mg/m^2 (rather than 200 mg/m^2) more closely matched the BED dose of 192 mg/m^2. In addition, the dose of 240 mg/m^2 demonstrated maximal myelopreservation efficacy benefits (compared to 200 and 280 mg/m^2) as measured by a variety of myelosuppression endpoints. The myelopreservation effect at 240 mg/m^2 was further evaluated and confirmed in three randomized controlled phase 2 trials in SCLC patients. See the Trilaciclib IB for details.

In all trials enrolling cancer patients, trilaciclib is always administered prior to systemic chemotherapy on each day of chemotherapy administration. Since the infusional-5FU component of FOLFOXIRI/bevacizumab is administered over 46 – 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.

Although the Maintenance regimen of infusional-5FU/leucovorin/bevacizumab is not particularly myelosuppressive, 5FU does cause chemotherapy-induced damage to the host leading to other side effects like diarrhea. In a manner similar to the protection of HSPCs, trilaciclib may protect other cell populations that are dependent on CDK4/6 for proliferation. In the integrated data analysis from the 3 randomized SCLC trials, trilaciclib administered prior to chemotherapy resulted in a decreased percentage of patients experiencing diarrhea (13.1% vs 17.8%) compared to placebo. In addition, prolonged exposure to any cytotoxic chemotherapy 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. Therefore, continuation of trilaciclib has the potential to prevent this damage leading to decreases in GI side effects and facilitating maintenance of dose intensity in subsequent lines of therapy; both of which could lead to improved patient outcomes.

6.2.2. FOLFOXIRI/bevacizumab

The doses of FOLFOXIRI/bevacizumab are described in Section 6.1. They are consistent with standard of care as established in multiple clinical trials (Loupakis, 2014; Cremolini, 2019; Sastre, 2019) and the approved labeling for those products. The range of allowable infusional-5FU doses (2400-3200 mg/m^2) reflects differences in practice patterns between different geographic regions and physician preference. The variability in this dose range is not expected to significantly influence the magnitude of CIM or anti-tumor efficacy observed as enrollment will be stratified by country.

Trilaciclib has been successfully combined at the RP2D with all the major chemotherapy classes included in the FOLFOXIRI regimen (e.g., alkylating agents, antimetabolites, topoisomerase inhibitors) in 4 completed or ongoing clinical trials in cancer patients. Integrated safety data from those 4 trials (338 patients) demonstrates that the addition of trilaciclib actually decreases the rate of high-grade AEs associated with the underlying chemotherapy regimen. There is no evidence to suggest that a different result would be expected from the combination of trilaciclib with FOLFOXIRI/bevacizumab. In addition, trilaciclib has a limited toxicity profile and minimal overlapping toxicities are expected with FOLFOXIRI/bevacizumab (Section 4.2.3). The primary AEs anticipated with trilaciclib are low grade injection site reactions including phlebitis/thrombophlebitis. These infusion reactions have generally been mild and occur in association with drug delivery through peripheral access. Central access is likely to reduce/eliminate these issues. The remainder of the trilaciclib AESIs to date have been uncommon and for most, the percentage of patients experiencing these AESIs is not clearly increased in patients receiving trilaciclib compared to placebo. Based on these results a dose-finding study is not needed.

6.3. Rationale for Supportive Care Interventions (Growth Factors and Transfusions)

Primary prophylactic G-CSF will be prohibited in Cycle 1; however therapeutic G-CSF (administered in response to a febrile neutropenia event) in Cycle 1 and secondary prophylactic G-CSF beginning in Cycle 2 and for all subsequent cycles (i.e., after a precipitating event in a prior cycle of therapy) will be allowed per growth factor/neutropenia management guidelines and physician discretion (Aapro, 2010; Smith, 2015). In the clinical trials evaluating FOLFOXIRI, primary prophylactic G-CSF (ie, in Cycle 1) was specifically not recommended per protocol; and in this setting the rates of febrile neutropenia ranged from 3 to 10% and the rates of SN AEs (Grade 4) were approximately 15 to 20%. Based on these data, FOLFOXIRI would be predicted to have an intermediate FN risk of between 10 and 20% which would not require primary prophylactic G-CSF in Cycle 1 per growth factor/neutropenia management guidelines (Cremolini, 2018; Hashimoto, 2016; Loupakis, 2014; Fornaro, 2013; Falcone, 2007; Souglakis, 2006).

Prohibiting primary prophylactic G-CSF in Cycle 1 in order to facilitate an unbiased evaluation of trilaciclib activity in Cycle 1 is permissible as long as the risk to placebo patients is minimized by (1) allowing therapeutic use of G-CSF in Cycle 1, and (2) allowing Investigators to only enroll those patients whose safety (as assessed by the treating physician) is not substantially compromised by this approach. In Cycle 2 and beyond, secondary prophylaxis is permitted in order to reduce the risk of subsequent neutropenia events in patients who have already demonstrated an increased propensity for such events, particularly for the patients enrolled in the placebo arm. However, evaluation of DSN in Cycle 2 – 4 and occurrence of SN in Cycle 2 – 12 could be confounded by the potential use of secondary G-CSF prophylaxis.

In the pooled TRIBE data, only 6% of patients in the FOLFOXIRI+B group experienced a second episode of Grade 3/4 neutropenia while receiving G-CSF as secondary prophylaxis. This finding suggests that the majority of the 78.5% of SN episodes that were reported by Rossini 2021 et al in the first 4 cycles were first occurrences of the event, not recurrent events, and therefore would not be impacted by secondary G-CSF prophylaxis. Further, in the integrated efficacy analysis for the 3 trilaciclib SCLC Phase 2 studies, a consistent treatment effect for trilaciclib compared to placebo in DSN and occurrence of SN was observed in patients who received G-CSF and in those that did not, at each Cycle for Cycle 1 to 4. For example, in Cycle 1, where G-CSF could only be utilized therapeutically in response to an episode of FN, the large reduction in occurrence of SN seen with trilaciclib (5.6% occurrence) compared to placebo (41.3% occurrence) in those that did not receive G-CSF was also observed in those that were treated with G-CSF (trilaciclib 16.7% occurrence vs placebo 80% occurrence). Similarly, in Cycle 2-4 where G-CSF could be utilized prophylactically as well as therapeutically, the benefit of trilaciclib, albeit smaller, persisted. In Cycle 2, an absolute 14% decrease in the occurrence of SN was seen with trilaciclib (compared to placebo) for patients who received G-CSF and an identical absolute decrease was seen with trilaciclib compared to placebo in patients where G-CSF was not utilized. In Cycle 3, the absolute decrease was 10-12% with or without G-CSF and in Cycle 4, approximately 8%. Treatment-by-subgroup interaction was tested for occurrence of SN at Cycle 1 and Cycle 2, respectively, and both showed no statistically significant interaction between treatment effect and the usage of G-CSF. Statistical testing for treatment-by-subgroup interaction was not conducted for Cycle 3 or Cycle 4 given the small total event numbers at

Cycle 3 or 4. A decrease in DSN was also observed with trilaciclib relative to placebo in Cycle 1 in patients receiving G-CSF (7 day decrease for the trilaciclib group); and in patients not receiving G-CSF (4 day decrease for the trilaciclib group). A smaller, but persistent benefit was observed in Cycle 2 – 4, where patients who received G-CSF, and those who did not, experienced a 1 – 2-day improvement in DSN with trilaciclib relative to placebo, respectively. These analytic results and consistent numerical reduction of DSN and the proportion of patients with SN in cycles where G-CSF could be used both prophylactically and therapeutically (Cycle 2 – 4) demonstrate that even in the context of G-CSF use, the beneficial effects of trilaciclib for CIN reduction can still be observed.

Erythropoiesis-stimulating agent (ESA) administration and RBC or platelet transfusions will be allowed per Investigator discretion based on guidelines detailed in Section 9.6.2 and Section 9.6.3. While these interventions may confound analysis of the supportive secondary myelopreservation efficacy endpoints, allowing physicians to provide appropriate supportive care to patients will facilitate patient safety.

6.4. Rationale for Stratification Factors

As outlined in Section 6.1, randomization will be stratified by country, 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 the presence of a BRAF V600E mutation (yes/no). These factors were chosen because they are predicted to have an impact on the primary myelopreservation efficacy endpoints or the key secondary anti-tumor efficacy endpoint such that if there is an imbalance in these factors, interpretation of the results of the study could be compromised.

Country was chosen as a stratification factor to account for the geographic differences in patient populations, treatment patterns including use of different doses of infusional-5FU within FOLFOXIRI and access to subsequent anti-cancer therapy, practice patterns including use of G-CSF and ESAs, and criteria for RBC and platelet transfusions; all of which could influence the primary and key secondary endpoint outcomes.

Prior therapy in adjuvant/neoadjuvant setting was chosen because results observed in the Phase 2 SCLC trials indicate that exposure to prior therapy, and presumably chemotherapy-induced damage to the HSPCs, can influence the magnitude of the effect size observed for the primary endpoints of DSN in Cycle 1 and occurrence of SN. For example, in Study G1T28-05 (1st line SCLC), the occurrence of SN for the placebo group was 49.1% compared with 1.9% for trilaciclib; in Study G1T28-03 (2nd/3rd line SCLC), the occurrence of SN for the placebo group was 75.9% compared with 40.6% for the trilaciclib group. While the chemotherapy backbones on these two trials were different (as reflected by the occurrence of SN rates in the placebo groups), the results suggest that exposure to prior therapy in adjuvant/neoadjuvant setting impacts the ability of trilaciclib to prevent or mitigate SN.

And finally, BRAF mutation status was chosen because patients with BRAF V600E mutations have a significantly worse prognosis than patients without this mutation, and the frequency of this mutation in patients with CRC is low. In one analysis of 2282 patients with CRC, the median OS for BRAF mutation-positive patients was 22.5 months, which was significantly worse than that for patients with wild-type (wt) KRAS and BRAF (49.2 months) (Wang, 2018). While

patients with KRAS mutant CRC also have a poor prognosis compared with patients with wt KRAS tumors, the prognosis is not as poor as BRAF mutant patients (median OS: 36.2 months) and the frequency of KRAS mutations in CRC is much higher than that observed for BRAF mutant patients (KRAS = 47.3%; BRAF = 7.0%) (Wang, 2018). The higher frequency of KRAS mutations means that the two treatment groups are more likely to be balanced for this prognostic factor.

7. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

7.1. Inclusion Criteria

Patients are eligible to be included in the study only if all of the following criteria apply:

1. Age ≥ 18 years of age at the time of signing the informed consent. Patients > 70 years of age must have a G8 Health State Screening Tool (geriatric screening tool) score > 14.
2. Proficient mismatch repair/microsatellite stable (pMMR/MSS), histologically or cytologically-confirmed adenocarcinoma of the colon or rectum. Patients with any BRAF or KRAS mutation status (wild type or mutant) are eligible. If historical pMMR/MSS and/or BRAF V600E mutational status are not known, a tumor specimen (archival or fresh biopsy) must be sent for testing and results must be available at the time of randomization in interactive web response system (IWRS). If testing cannot be completed using a standard clinical assay performed institutionally/locally, the tumor specimen may be sent to the Sponsor's designated central laboratory for analysis; only historical KRAS mutational status will be collected (ie, no testing required prior to study entry). *Note: Any sample sent for MSS/BRAF analysis will be in addition to that required per Inclusion Criterion 5.*
3. Unresectable and measurable or evaluable metastatic colorectal cancer per RECIST v1.1
4. ECOG performance status of 0 to 1
5. A formalin-fixed paraffin-embedded (FFPE) tumor specimen (from archival or fresh biopsy) with an associated pathology report documenting pMMR/MSS mCRC must be confirmed to be available to send to the Sponsor for planned retrospective biomarker analyses (tissue requirements are provided in the associated laboratory manual).
6. Hemoglobin ≥ 9.0 g/dL in the absence of RBC transfusion or ESA administration within 14 days prior to first dose of trilaciclib/placebo
7. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L
8. Platelet count ≥ 100×10^9/L
9. Estimated glomerular filtration rate (eGFR) ≥ 30 mL/minute/1.73m^2
10. Total bilirubin ≤ 1.5 × upper limit of normal (ULN)
11. AST, ALT, and alkaline phosphatase ≤ 3 × ULN for patients without liver or bone metastases; AST, ALT and alkaline phosphatase ≤ 5 × ULN in the presence of liver metastases; AST and ALT ≤ 3 x ULN and alkaline phosphatase ≤ 5 × ULN in the presence of bone metastases
12. Resolution of nonhematologic toxicities from prior therapy or surgical procedures to ≤ Grade 1 or baseline (except alopecia)
13. Urine dipstick protein < 2+. If ≥ 2+ at Screening, then a 24-hour urine collection must be done to demonstrate ≤ 1 g of protein/24 hours

14. Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies. Please see Section 17.4 for detailed instructions on methods of contraception requirements.
15. Capable of giving signed informed consent which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

7.2. Exclusion Criteria

Patients are excluded from the study if any of the following criteria apply:

1. Prior systemic therapy for mCRC. Patients who received adjuvant/neoadjuvant therapy (ie, treatment with curative intent) for colorectal cancer are eligible if it has been ≥ 6 months between the last dose of systemic chemotherapy and the date of informed consent.
2. Any radiotherapy, chemotherapy, immunotherapy, biologic, investigational, or hormonal therapy for cancer treatment (except for adjuvant hormonal therapy for breast cancer or prostate cancer defined as M0 disease or PSA persistence/recurrence without metastatic disease) within 3 weeks prior to the first dose of trilaciclib/placebo.
3. Receipt of any low-dose systemic chemotherapeutic agent (e.g., low-dose methotrexate for rheumatoid arthritis) administered for a nononcologic purpose within 3 weeks prior to the first dose of trilaciclib/placebo.
4. Presence of central nervous system (CNS) metastases/leptomeningeal disease requiring immediate treatment with radiation therapy or steroids (i.e., patient must be off steroids administered for brain metastases for at least 14 days prior to the first dose of trilaciclib/placebo).
5. QTcF interval > 450 msec (males) or > 470 msec (females) at screening (confirmed on repeat). For patients with ventricular pacemakers, QTcF > 500 msec.
6. Personal or family history of long QT syndrome
7. Symptomatic peripheral neuropathy
8. History of interstitial lung disease (ILD)
9. Uncontrolled hypertension (blood pressure ≥ 150/90mm Hg)
10. Clinically significant (i.e., active) cardiovascular disease at the time of signing the informed consent; for example cerebrovascular accidents (≤ 6 months before the first dose of trilaciclib/placebo), myocardial infarction (≤ 6 months before the first dose of trilaciclib/placebo), unstable angina, serious cardiac arrythmia requiring medication, or uncontrolled symptomatic congestive heart failure [Class II or higher as defined by the New York Heart Association [NYHA] functional classification system])
11. Serious, non-healing wound, ulcer, or bone fracture
12. Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to study treatment start, or anticipation of the need for major surgical procedure during the course of the study.

13. Known serious active infection (e.g., human immunodeficiency virus [HIV], hepatitis B or C, tuberculosis, etc.)
14. Known Gilbert's Syndrome or homozygous for the UGT1A1*28 allele. UGT1A1 genotyping is not required for this study.
15. Patients with complete absence of dihydropyrimidine dehydrogenase (DPD) activity as evidenced by the presence of certain genetic mutations or polymorphisms in the DYPD gene or blood uracil level ≥150ng/mL (see Section 17.2 for assay requirements).
16. Chronic inflammatory bowel disease and/or active intestinal obstruction. Patients should not be treated until the intestinal obstruction has resolved.
17. Previous history of significant/severe hemorrhage, within 1 month before randomization. History of previous abdominal fistula or gastrointestinal perforation within 6 months before randomization
18. Known history of bleeding diathesis or coagulopathy
19. INR > 1.5 within 14 days prior to starting study treatment. EXEMPTION: patients on full anticoagulation must have an in-range INR (usually between 2 to 3) if INR is used for monitoring. Any anticoagulation therapy must be at stable dosing prior to enrollment
20. Ongoing or anticipated treatment with potent cytochrome inhibitors CYP450 3A4 (such as ketoconazole) or inducers (such as rifampicin, carbamazepine, phenobarbital, phenytoin or St. John's wort). Irinotecan should not be delivered concurrently.
21. Patients with ongoing or anticipated treatment with sorivudine or its chemically related analogues, such as brivudine.
22. Chronic, daily treatment with high-dose aspirin (> 325 mg/day)
23. Prior allogeneic or autologous hematopoietic stem cell or bone marrow transplantation
24. Receipt of any live attenuated vaccines within 4 weeks prior to first dose of study treatment
25. Known hypersensitivity to any of the drugs used in this study
26. Pregnant or lactating women
27. Legal incapacity or limited legal capacity
28. Other uncontrolled serious chronic disease or psychiatric condition that in the Investigator's opinion could affect patient safety, compliance, or follow-up in the protocol
29. Any contraindications to the administration of FOLFOXIRI and bevacizumab at the discretion of the investigator.

8. SCHEDULE OF ASSESSMENTS

The procedures and assessments to be performed during the study are outlined in Table 4 (Screening and Cycle 1) and Table 5 (Cycle 2 through End of Study). The timing and number of samples collected for PK and biomarker testing may be altered based on emerging data without requiring an amendment if the blood volume per day or overall does not increase and the patient is not required to have additional clinic visits or prolongation of a clinic visit, i.e., the risk-benefit profile for the patient does not worsen.

Study visits are outlined in the Schedule of Assessments. Unless otherwise specified, assessments are to be completed within ± 1 day of the scheduled visit date. When there are multiple procedures at the same or overlapping time points, order of events should be: patient reported outcome measures, ECGs, vitals, blood draw. Unscheduled assessments and visits to manage patient safety may occur at the Investigator's discretion. Study procedures performed at unscheduled visits should be recorded in the appropriate electronic case report form (eCRF).

Table 4: Schedule of Assessments: Screening and Induction Cycle 1

Assessment	**Screening**	**Rand**	**Induction Cycle 1 (14-day cycle)**						
Day	**-28 to -1**	**-3 to -1[a]**	**1**	**2**	**4 (±1 day)**	**6 (±1 day)**	**8 (±1 day)**	**10 (±1 day)**	**12 (±1 day)**
Informed Consent	X								
Demographics	X								
Medical History and CRC History[b]	X								
MMR/MSS and BRAF V600E status[c]	X								
Eligibility Evaluation and Randomization	X	X							
G8 Health Status Screening Tool (Patients > 70yrs only)	X								
Archived tumor sample	X								
ECOG Performance Status	X		X						
Physical Exam[d]	X		X						
Height, Weight	X[e]		X						
Vital Signs	X		X[f]						
12-lead Electrocardiogram	X[g]		X[g]	X[g]					
Clinical Chemistry	X		X[h]						
DPD testing[i]	X								
Hematology	X		X[j]	X	X	X	X	X	X
INR/aPTT	X								
Urinalysis (dipstick)	X		X						
Pregnancy test (WOCBP)[k]		X							
Tumor Assessment by RECIST v1.1[l]	X								
Trilaciclib or Placebo[m]			X	X					

Assessment	Screening	Rand	Induction Cycle 1 (14-day cycle)						
Day	**-28 to -1**	**-3 to -1[a]**	**1**	**2**	**4 (±1 day)**	**6 (±1 day)**	**8 (±1 day)**	**10 (±1 day)**	**12 (±1 day)**
Irinotecan			X						
Oxaliplatin			X						
Leucovorin			X						
Fluorouracil			X	X					
Bevacizumab			X						
FACT-An (includes FACIT-F), FACT-C, PGIS, and PGIC[n]			X				X		
EQ-5D-5L			X				X		
Blood sample for PK[o]			X	X					
Blood sample for Immunologic and Hematologic Markers			X						
AEs			X						
Concomitant Medications	X[b]	X[b]	X						

AE=adverse event; aPTT=activated partial thromboplastin time; β-hCG=beta human chorionic gonadotropin; CRC=colorectal cancer; CT=computed tomography; ECOG=Eastern Cooperative Oncology Group; EQ-5D-5L=5-level EQ-5D; FACT-An=Functional Assessment of Cancer Therapy – Anemia; FACIT-F=Functional Assessment of Chronic Illness Therapy – Fatigue; FACT-C=Functional Assessment of Cancer Therapy – Colorectal; FACT-G=Functional Assessment of Cancer Therapy – General; INR=international normalized ratio; IWRS=interactive web response system; MMR=mismatch repair; MRI=magnetic resonance imagining; MSI=microsatellite instable; PE=physical exam; PGIC=Patient Global Impression of Change; PGIS=Patient Global Impression of Severity; PK=pharmacokinetic; pMMR/MSS=proficient mismatch repair/microsatellite stable; RECIST=Response Evaluation Criteria in Solid Tumors; yrs=years; WOCBP=women of childbearing potential.

[a] Patients can be randomized and receive Cycle 1 Day 1 on the same day. When required by institutional procedures, randomization will be allowed up to 7 days prior to dosing without a protocol deviation. Screening procedures must be completed prior to randomization and the screening window remains 28 days.

[b] Including medical and surgical history, documentation of CRC diagnosis and prior CRC therapy, and prior medications (current and those taken within 14 days of informed consent).

[c] Documentation of BRAF V600E and pMMR/MSS is required; patients with deficient MMR/MSI-high or MSI-low CRC are not eligible for this study. If historical pMMR/MSS and/or BRAF V600E mutational status are not known, a tumor specimen (archival or fresh biopsy) must be sent for testing and results must be available at the time of randomization in IWRS. If testing cannot be completed using a standard clinical assay performed institutionally/locally, the tumor specimen may be sent to the Sponsor's designated central laboratory for analysis. If institutional/local testing is unavailable, please contact your CRA.

[d] Full PE at Screening, brief PE at all other timepoints.

[e] Height measured only at Screening; weight measured at Screening and Day 1 of each cycle in Induction and Maintenance.

[f] Vitals (BP, HR, temperature) should be taken 15 minutes (± 10 minutes) before and after the trilaciclib/placebo infusion.

[g] Perform triplicate ECGs at Screening and on Day 1 of Cycle 1 at the following timepoints: predose (trilaciclib/placebo) at any time prior to dosing, within 30 minutes following the end of the trilaciclib/placebo infusion, and at 3 hours after the start of trilaciclib/placebo infusion (± 30 minutes). Triplicate ECGs should also be performed on Day 2 of Cycle 1 at the following timepoints: predose (trilaciclib/placebo) at any time prior to dosing and within 30 minutes following the end of the trilaciclib/placebo infusion. Additional ECGs during the study may be performed as clinically indicated. Any ECG with a QTc value of > 500 or any other clinically significant abnormal finding should be repeated every 5 minutes for a total of three ECGs to confirm this finding, if not already recorded in triplicate.

[h] Clinical chemistry and urinalysis may be obtained up to 3 calendar days prior to dosing.

[i] Patients with complete absence of dihydropyrimidine dehydrogenase (DPD) activity as evidenced by the presence of certain genetic mutations or polymorphisms in the DYPD gene or blood uracil level ≥150ng/mL must be excluded. Fluorouracil dose modification should be considered for those with partial DPD deficiency as evidenced by certain genetic mutations/polymorphisms in the DYPD gene or blood uracil values >16 but <150 ng/mL (Section 9.5).

[j] Hematology may be obtained up to 1 calendar day prior to dosing.

[k] Female patients of childbearing potential: serum β-hCG at enrolment (eg, signed consent and during window allowing randomization in IWRS; results should be available prior to randomization).

[l] Initial staging should include, at a minimum, CT or MRI of chest, abdomen and pelvis. IV contrast should be used unless contraindicated. Oral contrast can be used at the Investigator's discretion.

[m] Trilaciclib or placebo will be administered as a 30-minute IV infusion prior to FOLFOXIRI/bevacizumab chemotherapy on Day 1 and on Day 2 of every cycle. Chemotherapy cannot be administered until after completion of the trilaciclib or placebo infusion on Day 1. The interval between the dose of trilaciclib or placebo and the first dose of chemotherapy on Day 1 should not be greater than 4 hours. The second dose of trilaciclib/placebo should be administered on Day 2. Trilaciclib or placebo will only be administered if chemotherapy is also to be administered on that day.

[n] FACT-An, FACT-C, PGIS, and EQ-5D-5L will be administered on Days 1 and 8 of Cycle 1. PGIC will only be administered on Day 8 of Cycle 1. FACIT-F is embedded within the FACT-An. Patient-reported outcome questionnaires may be administered up to 1 calendar day prior to the first dose of each cycle; questionnaires administered on Day 8 may be obtained ±1 day of scheduled visit; **NOTE**: questionnaires should be completed on the same day blood sample is collected for hematology assessment. Questionnaires must be completed prior to the administration of any study drug and should be administered prior to the conduct of any study procedures at visit.

[o] Four blood samples for PK analysis of trilaciclib and any metabolites will be collected: on Cycle 1 Day 1 at the end of trilaciclib/placebo infusion (within 5 minutes prior to the end of the infusion), and at 25 to 45 minutes and 4-6 hours post the end of the trilaciclib/placebo infusion, as well as predose (trilaciclib/placebo) on Cycle 1 Day 2. Three samples will be collected for PK analysis of irinotecan and its metabolite (SN-38): on Cycle 1 Day 1 at the end of irinotecan infusion (within 5 minutes prior to the end of the infusion) and at 4-6 hours post the end of the irinotecan infusion as well as predose (trilaciclib/placebo) on Cycle 1 Day 2.

Table 5: Schedule of Assessments: Induction (Cycle 2-12), Maintenance, and Survival Follow-up

Assessment	**Induction (14-day cycles) [Maximum 12 cycles]**			**Last Induction Cycle only if no Maintenance**	**Maintenance (14-day cycles)**		**Post Treatment Visit**	**Survival Follow Up**
Day	**1**	**2**	**8 (±1 day)**	**15 (±1 day)**	**1**	**2**	**30 ±7d post last dose**	
ECOG Performance Status	X				X		X	
Physical Exam [Odd cycles only in both Induction and Maintenance, eg, C1, 3, 5, etc)	X				X		X	
Weight	X				X		X	
Vital Signs[a]	X				X		X	
12-lead Electrocardiogram	X[b] [C2 only]	X[b] [C2 only]						
Clinical Chemistry[c]	X				X		X	
Hematology[d]	X		X [C2-4 only]	X	X		X	
INR/aPTT (as clinically indicated during the study)								
Urinalysis[c] (dipstick)	X				X			
Pregnancy test (WOCBP, q month)[e]	X				X		X	
Tumor Assessment by RECIST v1.1	X[f] (q8 weeks regardless of dosing)				X[f] (q12 weeks)		X[g]	X[g]
Trilaciclib or placebo[h]	X	X			X	X		
Irinotecan	X							

Assessment	**Induction (14-day cycles) [Maximum 12 cycles]**			**Last Induction Cycle only if no Maintenance**	**Maintenance (14-day cycles)**		**Post Treatment Visit**	**Survival Follow Up**
Day	**1**	**2**	**8 (±1 day)**	**15 (±1 day)**	**1**	**2**	**30 ±7d post last dose**	
Oxaliplatin	X							
Leucovorin	X				X			
Fluorouracil	X	X			X	X		
Bevacizumab	X				X			
FACT-An (includes FACIT-F), FACT-C, PGIS, PGIC[i]	X		X [C2-4 only]	X[j]	X		X	
EQ-5D-5L	X		X [C2-4 only]	X[j]	X		X	
Blood sample for Immunologic and Hematologic Markers	X [C2, C5, only]				X [C1 only]			
AEs	X							
Concomitant Medications	X							
Survival Follow-up Contact (every other month)								X[k]

AE=adverse event; aPTT=activated partial thromboplastin time; β-hCG=beta human chorionic gonadotropin; C=cycle; ECOG=Eastern Cooperative Oncology Group; EQ-5D-5L=5-level EQ-5D; FACT-An=Functional Assessment of Cancer Therapy – Anemia; FACIT-F=Functional Assessment of Chronic Illness Therapy-Fatigue; FACT-C=Functional Assessment of Cancer Therapy – Colorectal; FACT-G=Functional Assessment of Cancer Therapy – General; INR=international normalized ratio; IWRS=interactive web response system; PE=physical exam; PGIC=Patient Global Impression of Change; PGIS=Patient Global Impression of Severity; RECIST=Response Evaluation Criteria in Solid Tumors; WOCBP=women of childbearing potential.

[a] Vitals (blood pressure, heart rate, temperature) should be taken 15 minutes (± 10 minutes) before and after the trilaciclib/placebo infusion.

[b] Perform triplicate ECGs on Day 1 of Cycle 2 at the following timepoints: predose (trilaciclib/placebo) at any time prior to dosing, within 30 minutes following the end of the trilaciclib/placebo infusion, and 3 hours after the start of trilaciclib/placebo infusion (± 30 minutes). Triplicate ECGs should also be performed on Day 2 of Cycle 2 at the following timepoints: predose (trilaciclib/placebo) at any time prior to dosing and within 30 minutes of the end of the trilaciclib/placebo

infusion. Additional ECGs during the study may be performed as clinically indicated. Any ECG with a QTc value of > 500 or any other clinically significant abnormal finding should be repeated every 5 minutes for a total of three ECGs to confirm this finding, if not already recorded in triplicate .

[c] Clinical chemistry and urinalysis may be obtained up to 3 calendar days prior to dosing.

[d] Hematology may be obtained up to 1 calendar day prior to dosing. If the initiation of the next cycle is delayed due to an AE, the patient should have (at least) weekly visits, including CBCs if the AE is hematologic, to follow the AE. Clinical laboratory assessments should be completed on the scheduled Day 1 as well as on the actual first dosing day of that delayed cycle.

[e] Female patients of childbearing potential: serum or urine pregnancy testing every 2nd cycle (eg, every month) during treatment (Induction and Maintenance) and at the post-treatment visit.

[f] Tumor assessments of chest, abdomen, and pelvis using the same imaging modality as at baseline should be performed every 8 weeks ± 7 days during Induction and every 12 weeks ± 14 days during Maintenance. IV contrast should be used unless contraindicated. Oral contrast can be used at the Investigator's discretion. Timing of scans should be regardless of dosing (e.g. from date of first dose). Additional scans may be performed during the study if clinically indicated.

[g] Perform tumor assessments at the Post Treatment Visit (30 +7d after last dose of study drug) only for patients who have not progressed at the time of study drug discontinuation. For those patients being followed for survival who have not progressed at the time of study drug discontinuation, tumor assessments will be repeated every 12 weeks ± 14 days until the occurrence of disease progression, withdrawal of consent, initiation of subsequent anti-cancer therapy, or study completion. Tumor assessments of chest, abdomen, and pelvis using the same imaging modality as at baseline should be performed. IV contrast should be used unless contraindicated; oral contrast can be used at the Investigator's discretion.

[h] Trilaciclib or placebo will be administered as a 30-minute IV infusion prior to chemotherapy on Day 1 and on Day 2 of every cycle during Induction and Maintenance. Chemotherapy cannot be administered until after completion of the trilaciclib or placebo infusion on Day 1. The interval between the dose of trilaciclib or placebo and the first dose of chemotherapy on Day 1 should not be greater than 4 hours. The second dose of trilaciclib/placebo should be administered on Day 2. Trilaciclib or placebo will only be administered if chemotherapy is also to be administered on that day.

[i] FACIT-F is embedded in the FACT-An. Patient reported outcome questionnaires should be completed at the following timepoints: Induction Cycles 2 to 4 (Days 1 and 8), Induction Cycle 5-12 (Day 1), Day 1 of each cycle in Maintenance, and at the post-treatment visit. If a cycle is delayed, the patient should still complete the questionnaires on the scheduled Day 1 of that cycle, as well as the actual first dosing day of that cycle. PRO questionnaires may be administered up to 1 calendar day prior to the first dose of each cycle; questionnaires administered on Day 8 may be obtained ±1 day of scheduled visit. **NOTE**: questionnaires should be completed on the same day blood sample is collected for hematology assessment. Questionnaires must be completed prior to the administration of any study drug and should be administered prior to conduct of any study procedure at that visit.

[j] Administer PRO questionnaires on Day 15 of last induction cycle only if patient does not continue with Maintenance therapy. When patient does not continue in Maintenance, questionnaires do not need to be repeated at both Post Treatment Visit and Day 15 of last induction cycle if < 3 weeks between these 2 timepoints.

[k] If a patient withdraws consent for further study treatment and/or procedures, the site should clarify if the patient remains open to survival contact and associated data collection. See Section 11.12 for details of information to be collected during Survival Follow-up.

9. STUDY TREATMENT

9.1. Study Drugs Administered

Study drugs are defined as any investigational agent(s), marketed product(s), or placebo intended to be administered to a study patient according to the study protocol. Study drugs used in this protocol are described in Table 6.

Table 6: Study Drugs

Name	Trilaciclib	Placebo	FOLFOXIRI	Bevacizumab
Type	Active Drug	Placebo	SOC Therapy	SOC Therapy
Dose Formulation	Single-use, sterile powder to be reconstituted with 250 mL of dextrose 5% in water or normal saline (sodium chloride solution 0.9%) per the Pharmacy Manual	250 mL of dextrose 5% in water or normal saline (sodium chloride solution 0.9%)	Descriptions of the formulations are provided in the current prescribing information (Section 17.5)	Description of the formulation is provided in the current prescribing information (Section 17.6)
Unit Dose Strength(s)	300 mg/20 mL	N/A	See Section 17.5	See Section 17.6
Dosage Level(s)	240 mg/m^2 administered on Days 1 and 2 over 30 minutes	N/A – administered on Days 1 and 2 to match trilaciclib	Irinotecan 165 mg/m^2 (Day 1) Oxaliplatin 85 mg/m^2 (Day 1) Leucovorin 400 mg/m^2 (Day 1); LEVOleucovorin 200 mg/m^2 is an acceptable alternative Fluorouracil 2400 – 3200 mg/m^2 (continuous infusion over 48 hours; Days 1 and 2); actual dose to be determined by the Investigator	Bevacizumab 5 mg/kg (Day 1)
Route of Administration	IV	IV	IV	IV
Use	Experimental	Placebo	SOC	SOC
Sourcing	Provided by the Sponsor	Prepared locally at the study site	Commercially available; may be supplied by study site or Sponsor	Commercially available; may be supplied by study site or Sponsor
Packaging and Labeling	Study Intervention will be provided in flint glass vials. Each vial will be labeled as required per country requirements.	Placebo may be provided by the study site.	SOC may be supplied by the study site in the commercially available packaging or by the Sponsor.	SOC may be supplied by the study site in the commercially available packaging or by the Sponsor.

IV=intravenous; N/A=not applicable; SOC=standard of care; USP=United States Pharmacopeia.

9.1.1. Dose, Dosing Regimen, and Route

9.1.1.1. Trilaciclib

Trilaciclib for injection, (300 mg/vial, also referred to as "Trilaciclib Sterile Powder" for concentrate for solution for IV infusion, 300 mg/vial) 240 mg/m^2 diluted in 250 mL of dextrose 5% in water (D5W) or sodium chloride solution 0.9% is to be administered by IV infusion over 30 (±10) minutes by peripheral or central access. During sequential drug administration (e.g., Day 1 infusions), trilaciclib can be delivered through the same access point through which chemotherapy/bevacizumab will be delivered. However, when trilaciclib is given concurrently with another infusion (e.g., during Day 2 5FU infusion), trilaciclib must be delivered through either a separate peripheral line or through central access that is designed for delivery of incompatible medications. Following trilaciclib administration in either scenario, flushing the infusion line with a minimum of 20 ml of 5% dextrose or normal saline is recommended. In addition, for peripheral access delivery, rotating the IV catheter sites and starting a new IV with each day of trilaciclib infusion can decrease the frequency and severity of local reactions. Administration of trilaciclib infusion by central access may be considered to limit these local reactions.

Actual body weight (not ideal body weight) should be utilized for dose calculations. At a minimum, if there is a change in body weight of > 10% relative to the weight at the time of the last dose calculation, doses should be recalculated. Recalculation of the dose more frequently per local institutional guidelines is permitted. Dose recalculation to adjust for changes in body weight will not be considered a dose reduction.

If there is any study drug remaining in the infusion bag at the end of the 30 minutes, the infusion should be continued at the same rate until the entire contents of the bag have been administered to ensure patients receive the full dose. Details regarding the reconstitution and dilution of trilaciclib vials is detailed in the Pharmacy Manual.

The infusion rate may be decreased to manage an infusion-related AE; for example, if a patient experiences a burning sensation during infusion, the infusion may be increased to 45 minutes (or longer if clinically indicated) to alleviate the symptoms.

9.1.1.2. Placebo

The placebo formulation of 250 mL of D5W or sodium chloride solution 0.9% will be administered by IV infusion over 30 (±10) minutes by peripheral or central access. If there is any volume remaining in the infusion bag at the end of the 30 minutes, the infusion should be continued at the same rate until the entire contents of the bag have been administered to ensure patients receive the full dose.

9.1.1.3. FOLFOXIRI/bevacizumab

FOLFOXIRI/bevacizumab will be administered IV in accordance with the prescribing information (see Section 17.5 and Section 17.6) and according to the study site's standard practice. Fluorouracil 2400 – 3200 mg/m^2 (continuous infusion over 48 hours; Days 1 and 2) will be administered, with the actual dose to be determined by the Investigator at the beginning of Induction (e.g., Cycle 1 Day 1). The same dose of 5FU must be administered to a given patient

throughout the study (Induction and Maintenance) except when dose modification for toxicity is necessary. If a bevacizumab biosimilar is used, the biosimilar must be approved for use by the FDA, European Commission, or the China National Medical Products Administration (NMPA).

At a minimum, if there is a change in body weight of > 10% relative to the weight at the time of the last dose calculation, doses should be recalculated. Recalculation of the dose more frequently per local institutional guidelines is permitted. Dose recalculation to adjust for changes in body weight will not be considered a dose reduction.

9.1.2. Preparation, Handling, Storage, and Accountability

The Investigator or designee, institution, or the head of the medical institution (where applicable) is responsible for study medication accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). Further guidance and information are provided in the Pharmacy Manual.

9.1.3. Treatment Compliance

The Investigator or designee will dispense the study drugs, via an unblinded Pharmacist/Designee, only for use by patients enrolled in the study as described in this protocol. The study drugs are not to be used for reasons other than those described in this protocol. The clinical study site will maintain records of study drugs' receipt, preparation, and dispensing, including the applicable lot numbers; patient's height, body weight, and body surface area; date and time of the start and end of each trilaciclib or placebo, FOLFOXIRI, and bevacizumab infusion; and total drug administered in milligrams. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy (e.g., interruption of infusion without restarting) will be recorded on eCRF and in the source documents.

9.2. Induction

During Induction, all patients will receive trilaciclib 240 mg/m^2 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 outlined in Section 9.4 before initiation of Cycle 2 and each subsequent Induction cycle. Criteria for study drug discontinuation are outlined in Section 10.1.

Since individual drugs may be discontinued for toxicity, Induction refers to any cycles of therapy where 5FU and either oxaliplatin or irinotecan is also being administered, e.g., 5FU + oxaliplatin + bevacizumab; 5FU+ irinotecan + bevacizumab; 5FU + oxaliplatin; or 5FU + irinotecan.

9.3. Maintenance

Patients who complete Induction without radiographic or clinical disease progression and remain eligible to receive 5FU/leucovorin/bevacizumab will continue with Maintenance therapy. 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 additional clinical benefit by transitioning to Maintenance. Patients will receive trilaciclib or placebo (per same randomization allocation at study entry) prior to infusional-5FU/leucovorin/bevacizumab at the same dose and schedule in each 14-day cycle during Maintenance. Infusional-5FU/leucovorin/bevacizumab should not be administered on Day 1 until after the completion of administration of the trilaciclib or placebo infusion. Patients should meet the requirements outlined in Section 9.4 before initiation of each Maintenance cycle. Criteria for study drug discontinuation are outlined in Section 10.1.

Since individual drugs may be discontinued for toxicity, Maintenance refers to any cycles of therapy where at least 5FU is administered.

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

Following completion of Cycle 1, patients must meet all the following minimum 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,
- Platelet count $\geq 75 \times 10^9$/L, and
- Nonhematologic drug-related toxicities must be $\leq$ Grade 1. However, Grade 2 toxicities not specifically noted in Table 9 as requiring a dose delay until toxicity resolves to < G1 (ex. neuropathy, mucositis, palmar-plantar erythrodysesthesia) can be managed according to local standard of care in consultation with the Medical Monitor.

If the initiation of the next cycle is delayed due to an AE, the patient should have (at least) weekly visits, including CBCs if the AE is hematologic, to monitor the toxicity until treatment criteria described above are met or until they discontinue treatment (e.g., if more than 4 weeks elapse from the last chemotherapy dose). The patient should still complete the clinical laboratory assessments and the PRO questionnaires on the scheduled Day 1 (entered as Unscheduled assessments in EDC) as well as on the actual first dosing day of that cycle.

There should be no more than 4 weeks (for toxicity and/or administrative reasons) between doses of chemotherapy; however dosing delays > 4 weeks may be permitted on a case by-case basis with the documented approval of the Investigator and Medical Monitor.

Local treatment of symptomatic lesion(s) with palliative therapy (ex. surgery, radiotherapy, radiofrequency ablation, embolization) is permitted at the Investigator's discretion to control disease symptoms. However, use of techniques that incorporate systemic therapy, including local administration, is not permitted (see Section 9.7). Patients requiring palliative therapy may continue receiving study drug until documented disease progression (radiographic or clinical) if, in the Investigator's opinion, the patient is continuing to receive clinical benefit and they meet the requirements described above.

Patients with metastases confined to the liver who experience a robust response to chemotherapy may also proceed with curative intent surgical resection at the Investigator's discretion. In that

event, study treatment in this trial will be considered complete and patients will enter into Survival Follow-up. Tumor assessments will continue as directed for survival follow-up (Table 5, footnote g). For the time-to-event endpoints, except OS, these patients are considered to be censored at the date of surgery. Any anti-cancer therapy for colorectal cancer that is initiated post-surgical resection is at the discretion of the Investigator and will be recorded as subsequent anti-cancer therapy in the EDC.

9.5. Toxicity Management and Dose Modifications

All dose reductions for hematologic or non-hematologic toxicity management will only be made on Day 1 of a cycle. All dose reductions for an individual patient are permanent and no dose increases will occur following a dose reduction. Recommended dose levels for reduction of irinotecan, oxaliplatin and 5FU are described in Table 7.

Table 7: Recommended Chemotherapy Dose Modifications

	IRIN	OXALI	5FU	LCV
Starting Dose	165 mg/m^2	85 mg/m^2	2400-3200 mg/m^2 (actual dose to be determined by Investigator)	400 mg/m^2; LEVOleucovorin at 200 mg/m^2 is an accepted alternative
Dose Level -1	125 mg/m^2 (~75% of starting dose)	65 mg/m^2 (~75% of starting dose)	1800-2400 mg/m^2 (75% of starting dose)	No dose adjustment (per package insert)
Dose Level -2	80 mg/m^2 (~50% of starting dose)	40 mg/m^2 (~50% of starting dose)	1200-1600 mg/m^2 (50% of starting dose)	

5FU=fluorouracil; IRIN=irinotecan; OXALI=oxaliplatin; LCV=leucovorin.
No dose modification is permitted for trilaciclib.
*In case of partial DPD deficiency as evidenced by certain genetic mutations/polymorphisms in the DYPD gene or blood uracil levels <150 ng/mL but >16 ng/mL, consideration should be given to modifying the starting dose of 5FU, taking into account the specific mutation/polymorphism or blood uracil level as well as the individual patient risk factors for bone marrow toxicity. Depending on treatment-related toxicity, a therapeutic dose adjustment may be required in Cycle 2.

The recommended dose modification procedures for hematologic toxicities are described in Table 8, non-hematologic toxicities are described in Table 9 and those for bevacizumab toxicities are described in Table 10. Recommendations for hepatobiliary toxicity management are in Section 9.5.2. All recommendations are intended as a minimum guideline for toxicity management and are not a replacement for independent medical judgement tailored to specific circumstances with an individual patient. Please also refer to the individual package inserts (Section 17.5) for additional information and recommendations.

Note that dose modifications (specifically dose reductions/discontinuations) are recommended for those events considered related to drug (i.e., toxicities) such that decreasing the dose or stopping the drug will lead to improved patient safety. If an event is not thought to be related to study drug by the Investigator, dose reduction/discontinuation is not required since decreasing the dose or stopping the drug would not be expected to alter the risk of the event occurring again;

rather the Investigator should consider if treatment should be delayed until the event recovers to ≤ Grade 1 or baseline before resumption of treatment. Decreased neutrophil, platelet counts or hemoglobin values that occur in isolation (e.g., at nadir) that do not result in a dosing delay, febrile neutropenia, serious adverse event (SAE) or other consequence, do not require dose modification.

Dose modifications for drug-related toxicity should be done according to the organ system showing the greatest degree of drug-related toxicity (e.g., modify dose of the suspect drug) and may be independent of other drugs in the regimen. If drug-related toxicity requires discontinuation of both oxaliplatin and irinotecan, Induction will be considered complete and Maintenance therapy with 5FU, leucovorin and bevacizumab may continue per the clinical judgment of the Investigator.

If fluorouracil is discontinued during Induction or Maintenance, then the patient must permanently discontinue all study drugs (e.g., End of Treatment) and should complete the Post Treatment Visit and enter Survival Follow-up.

9.5.1. Trilaciclib and FOLFOXIRI

The dose of trilaciclib will not be modified and will remain at 240 mg/m^2 throughout the study. The interval between the dose of trilaciclib or placebo and the first dose of chemotherapy on Day 1 should not be greater than 4 hours. The second dose of trilaciclib/placebo should be administered on Day 2.

Trilaciclib or placebo will only be administered with FOLFOXIRI/bevacizumab therapy. If administration of chemotherapy/bevacizumab is held or discontinued, trilaciclib or placebo will also be held or discontinued. Conversely, if trilaciclib or placebo is held on Day 1 of any given cycle, chemotherapy/bevacizumab will not be administered until trilaciclib/placebo can be given on the same day.

Table 8: Dose Modifications for Drug-related Hematologic Toxicity

		Action Taken					
Event, CTCAE Grade or Lab Value	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Hematologic toxicity on scheduled Day 1 in Cycle 2 and all subsequent cycles resulting in delay of treatment							
ANC $< 1.0 \times 10^9$/L ($\geq$ G3)	1st episode	Hold drug until ANC criteria for dosing reached. No change in dose.	Hold drug until ANC criteria for dosing reached. No change in dose.			Hold drug until ANC criteria for dosing reached. No change in dose.	Administer prophylactic G-CSF with each subsequent cycle of chemotherapy.
	2nd, 3rd and 4th episode	Hold drug until ANC criteria for dosing reached. No change in dose.	Hold drug until ANC criteria for dosing reached. Reduce dose of one drug by one dose level and resume dosing; Investigator discretion as to which drug is reduced.			Hold drug until ANC criteria for dosing reached. No change in dose.	Continue prophylactic G-CSF with each subsequent cycle of chemotherapy. Prolonged severe myelosuppression should prompt evaluation for dihydropyrimidine dehydrogenase deficiency.
	5th episode	Permanently discontinue					
Platelets $< 75 \times 10^9$/L ($\geq$ G2)	1st, 2nd and 3rd episode	Hold drug until platelet criteria for dosing reached. No change in dose.	Hold drug until platelet criteria for dosing reached. Reduce dose of one drug by one dose level and resume dosing; Investigator discretion as to which drug is reduced.			Hold drug until platelet criteria for dosing reached. No change in dose.	Monitor for signs and symptoms of bleeding. Consider treatment as clinically indicated.
	4th episode	Permanently discontinue.					

		Action Taken					
Event, CTCAE Grade or Lab Value	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Hematologic toxicity at any point during study treatment							
Febrile neutropenia (G3 or 4) **OR** G4 neutropenia lasting > 5 days	1st episode	Hold drug until ANC criteria for dosing reached. No change in dose.	Hold drug until ANC criteria for dosing reached. Consider reducing one drug by one dose level.			Hold drug until ANC criteria for dosing reached. No change in dose.	Administer prophylactic G-CSF with each subsequent cycle of chemotherapy. Prolonged severe myelosuppression should prompt evaluation for dihydropyrimidine dehydrogenase deficiency
	2nd episode	Hold drug until ANC criteria for dosing reached. No change in dose.	Hold drug until ANC criteria for dosing reached. Reduce dose of one drug by two dose levels **OR** the dose of two drugs by one dose level each and resume dosing; Investigator discretion as to which option is selected.			Hold drug until ANC criteria for dosing reached. No change in dose.	Continue prophylactic G-CSF with each subsequent cycle of chemotherapy. Prolonged severe myelosuppression should prompt evaluation for dihydropyrimidine dehydrogenase deficiency
	3rd episode	Permanently discontinue.					

		Action Taken					
Event, CTCAE Grade or Lab Value	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Symptomatic thrombocytopenia (≥ G3 with bleeding)	1st and 2nd episode	Hold drug until ANC criteria for dosing reached. No change in dose.	Hold drug until ANC criteria for dosing reached. Reduce dose of one drug by one dose level and resume dosing; Investigator discretion as to which drug is reduced.			Hold drug until ANC criteria for dosing reached. No change in dose.	Consider treatment as clinically indicated.
	3rd episode	Permanently discontinue.					

ANC=absolute neutrophil count; CTCAE=Common Terminology Criteria for Adverse Events; G=Grade; G-CSF=granulocyte colony-stimulating factor;5FU= fluorouracil; IRIN=irinotecan; OXALI=oxaliplatin; LCV=leucovorin.

Table 9: Dose Modification for Drug-related Non-hematologic Toxicity

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Acute drug hypersensitivity reaction G1 (defined as mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated).	Any occurrence.	No change in dose.	No change in dose.			No change in dose.	Initiate appropriate medical therapy and monitor as clinically indicated.
Acute drug hypersensitivity reaction G2 (defined as moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL).	1st episode	Stop infusion and hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Stop infusion and hold drug until toxicity resolves to ≤G1 or baseline. No change in dose.			Stop infusion and hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	
Acute drug hypersensitivity reaction G3-4 (defined as severe or life-threatening consequences)	2nd episode G2 or any occurrence G3-4	Permanently discontinue					
Diarrhea G1	Any occurrence.	No change in dose.	No change in dose; continue dosing and consider starting anti-diarrheal therapy.			No change in dose.	Manage with appropriate antidiarrheal therapy per

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Diarrhea G2	Any occurrence.	No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Resume dosing at the same dose and consider starting anti-diarrheal therapy.			No change in dose.	institutional standards. Encourage patients to take plenty of oral fluids.
Diarrhea G3	1st and 2nd episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose of one drug by one dose level and resume dosing; Investigator discretion as to which drug is reduced.			Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Severe diarrhea should prompt evaluation for dihydropyrimidine dehydrogenase deficiency. Consider referral to dietician for dietary management. Consider antidiarrheal therapy such as loperamide with titration to effect. Consider work-up for infectious cause if clinical signs of infection or unresponsive to conservative management.
	3rd episode	Permanently discontinue.					
Diarrhea G4	1st episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose of one drug by two dose levels **OR** the dose of two drugs by one dose level each and resume dosing: Investigator discretion as to which option is selected.			Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	
	2nd episode	Permanently discontinue.					
Hepatobiliary toxicity	See Section 9.5.2						
	Hy's Law [ALT and/or AST ≥3×ULN, ALP ≤2×ULN, and total bilirubin ≥2×ULN]	Permanently discontinue.					See Section 9.5.2

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Injection site reactions including phlebitis and thrombophlebitis G1 (defined as tenderness with or without symptoms [e.g. warmth, erythema, itching])	Any occurrence	Pause or slow the infusion.	No change in dose.			No change in dose.	Initiate supportive care interventions. Upon completion of infusion, flush line/cannula with at least 20 ml sterile 5% dextrose or normal saline. If normal saline is being used as a diluent/flush, consider changing to 5% dextrose as appropriate for subsequent infusions. Central access may also be considered.
Injection site reactions including phlebitis and thrombophlebitis G2 (defined as pain; lipodystrophy; edema; phlebitis)	Any occurrence	Pause infusion. If pain not severe, follow instructions for G1. Otherwise, stop infusion in extremity and rotate site of infusion to site in alternative extremity.	No change in dose.			No change in dose.	

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Injection site reactions including phlebitis and thrombophlebitis G3-4 (G3 defined as ulceration or necrosis; severe tissue damage; operative intervention required; G4 defined as life-threatening consequences including need for urgent intervention)	Any occurrence	Permanently discontinue.					
Interstitial lung disease /pneumonitis G1 (asymptomatic)	Any occurrence	No change in dose.	No change in dose.			No change in dose.	Initiate appropriate medical therapy and monitor as clinically indicated.
Interstitial lung disease /pneumonitis G2 (symptomatic)	1st episode	Hold drug until toxicity resolves to ≤G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.			Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	
	2nd episode	Permanently discontinue.					
Interstitial lung disease /pneumonitis G3-4	Any occurrence	Permanently discontinue.					

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Mucositis G1-2	Any occurrence	No change in dose	No change in dose			No change in dose	Manage with appropriate oral care per institutional standards.
Mucositis G3	1st and 2nd episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose by one level and resume dosing.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	In addition to oral care, consider use of topical or systemic analgesia Severe mucositis should prompt evaluation of dihydropyrimidine dehydrogenase deficiency.
	3rd episode	Permanently discontinue This toxicity is associated with 5FU infusion such that per protocol, if 5FU is permanently discontinued, then all study drugs should also be discontinued.					
Mucositis G4	1st episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose by two dose levels and resume dosing.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	
	2nd episode	Permanently discontinue This toxicity is associated with 5FU infusion such that per protocol, if 5FU is permanently discontinued, then all study drugs should also be discontinued.					

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Myocardial ischemia, OR hyperammonemic encephalopathy, OR CNS toxicity including acute cerebellar syndrome, confusion, disorientation, ataxia, or visual disturbances	Any occurrence	Permanently discontinue. These toxicities are associated with 5FU infusion such that if they are experienced by a patient and considered related to 5FU, 5FU should be discontinued. Per protocol, if 5FU is permanently discontinued, then all study drugs should also be discontinued.					Manage per institutional standards
Palmar-plantar erythrodysesthesia (hand-foot syndrome) G1-2	Any occurrence	No change in dose.	No change in dose.			No change in dose.	Manage with appropriate supportive care per institutional standards
Palmar-plantar erythrodysesthesia (hand-foot syndrome) G3	1st episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose by two dose levels and resume dosing.	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Symptoms may be managed using topical wound care, cold compresses, topical corticosteroids and or topical or systemic analgesics.
	2nd episode	Permanently discontinue This toxicity is associated with 5FU infusion such that per protocol, if 5FU is permanently discontinued, then all study drugs should also be discontinued.					
Peripheral sensory neuropathy G1-2	Any occurrence	No change in dose.	No change in dose.			No change in dose.	Manage with appropriate supportive care per institutional standards

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Peripheral sensory neuropathy G2 lasitng > 14 days	Any occurrence	No change in dose	No change in dose	Consider reducing dose by one dose level and resume dosing	No change in dose	No change in dose	Consider use of systemic analgesics, particularly those utilized for neuropathic pain.
Peripheral sensory neuropathy **OR** G3 lasting < 14 days	1st episode	Hold drug until toxicity resolves to ≤ G2 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G2 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G2 or baseline. Reduce dose by two dose levels and resume dosing.	Hold drug until toxicity resolves to ≤ G2 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G2 or baseline. No change in dose.	
Peripheral sensory neuropathy	2nd G3 <14 days **OR** any occurrence of G3 lasting ≥ 14 days **OR** any occurrence of G4	No change in dose.	No change in dose.	Permanently discontinue.	No change in dose.	No change in dose.	
Any other G1-2 non-hematologic toxicity	Any occurrence	No change in dose.	No change in dose.			No change in dose.	Supportive care as appropriate.

		Action Taken					
Event, CTCAE Grade	**Frequency**	**Trilaciclib**	**IRIN**	**OXAL**	**5FU**	**LCV**	**Toxicity Management**
Any other G3 non-hematologic toxicity	1st and 2nd episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose of one drug by one dose level and resume dosing; Investigator discretion as to which drug is reduced.			Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	
	3rd episode	Permanently discontinue.					
Any other G4 non-hematologic toxicity	1st episode	Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	Hold drug until toxicity resolves to ≤ G1 or baseline. Reduce dose of one drug by two dose levels or the dose of two drugs by one dose level and resume dosing: Investigator discretion as to which drug is reduced.			Hold drug until toxicity resolves to ≤ G1 or baseline. No change in dose.	
	2nd episode	Permanently discontinue.					

CNS=central nervous system; CTCAE=Common Terminology Criteria for Adverse Events; G=Grade; G-CSF=granulocyte colony-stimulating factor; 5FU=fluorouracil; IRIN=irinotecan; OXALI=oxaliplatin; LCV=leucovorin.

9.5.2. Hepatobiliary Toxicity Management

Abnormal values in AST and/or ALT concurrent with abnormal elevations in total bilirubin that meet the criteria outlined below in the absence of other causes of liver injury are considered potential cases of drug-induced liver injury (potential Hy's Law cases) and should always be considered important medical events.

The threshold of laboratory abnormalities for a potential case of drug-induced liver injury depends on the patient's individual baseline values and underlying conditions. Patients who present with the following laboratory abnormalities should be evaluated further to definitively determine the etiology of the abnormal laboratory values:

- Patients with AST or ALT and total bilirubin baseline values within the normal range who subsequently present with AST or ALT ≥3 times the upper limit of normal (X ULN) concurrent with a total bilirubin ≥2 X ULN with no evidence of hemolysis and an alkaline phosphatase ≤2 X ULN or not available.
- For patients with preexisting ALT OR AST OR total bilirubin values above the upper limit of normal, the following threshold values should be used in the definition mentioned above:
  - For patients with pre-existing AST or ALT baseline values above the normal range: AST or ALT ≥2 times the baseline values and ≥3 X ULN, or ≥8 X ULN (whichever is smaller).

Concurrent with

  - For patients with pre-existing values of total bilirubin above the normal range: Total bilirubin increased by two times the baseline value OR ≥3 times the upper limit of normal (whichever is smaller).

The patient should return to the investigational site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history and physical assessment. In addition to repeating AST and ALT, laboratory tests should include albumin, creatine kinase, total bilirubin, direct and indirect bilirubin, gamma-glutamyl transferase, activated partial thromboplastin time (aPTT)/prothrombin time (PT)/International Normalized Ratio (INR), and alkaline phosphatase. A detailed history, including relevant information, such as review of ethanol, acetaminophen, recreational drug and supplement consumption, family history, occupational exposure, sexual history, travel history, history of contact with a jaundiced patient, surgery, blood transfusion, history of liver or allergic disease, and work exposure, should be collected. Further testing for acute hepatitis A, B, or C infection and liver imaging (eg, biliary tract) may be warranted. All cases confirmed on repeat testing as meeting the laboratory criteria defined above, with no other cause for liver function test (LFT) abnormalities identified at the time should be considered potential Hy's Law cases irrespective of availability of all the results of the investigations performed to determine etiology of the abnormal LFTs. Such potential Hy's Law cases should be reported as SAEs.

9.5.3. Bevacizumab

There are no recommended dose reductions for bevacizumab, per package insert. In cases where bevacizumab is permanently discontinued, continuation of chemotherapy may be considered. If administration of chemotherapy is held or discontinued, bevacizumab will also be held or discontinued (e.g., bevacizumab should not be administered in isolation).

Table 10: Dose Modification for Bevacizumab-related Toxicity Management at Any Point During the Study

Adverse Event	CTCAE Grade	Bevacizumab Dose Modification	Toxicity Management
Hypertension	3	Hold or Discontinue	Discontinue if not controlled with triple-drug medication.
	4	Discontinue	
Hemorrhage	≥ 2 [pulmonary or CNS]	Discontinue	
	3 [non-pulmonary and non-CNS]	Hold	Hold bevacizumab until all of the following criteria are met, and then resume dosing: • Bleeding has resolved and hemoglobin is stable. • There is no bleeding diathesis that would increase the risk of therapy. • There is no anatomic or pathologic condition that significantly increases the risk of hemorrhage recurrence.
	4	Discontinue	
Venous thrombosis	3 or 4	Discontinue	
Arterial thromboembolic event	Any Grade	Discontinue	
Congestive heart failure	3	Hold	Hold bevacizumab until ≤ Grade 2; resume dosing.
	4	Discontinue	
Proteinuria	≥ 2+ [urine dipstick]	Hold	Assess with 24-hour urine collection. Hold bevacizumab for ≥ 2 g/24-hour urine collection; resume dosing when proteinuria is < 2 g/24 hours.
	nephrotic syndrome	Discontinue	
GI perforation	-	Discontinue	
Non-GI fistulas	-	Discontinue	

Adverse Event	CTCAE Grade	Bevacizumab Dose Modification	Toxicity Management
Bowel obstruction	1	Continue dosing	Patients who experience partial obstruction not requiring medical intervention may continue on bevacizumab.
	2	Hold	Hold bevacizumab in patients who experience partial obstruction requiring medical intervention. Resume dosing upon complete resolution of event.
	3 or 4	Discontinue	
Infusion reactions (ex. hypertension, hypertensive crises associated with neurologic signs and symptoms, wheezing, oxygen desaturation, Grade 3 hypersensitivity, chest pain, headaches, rigors, and diaphoresis)	3 or 4	Discontinue	Administer appropriate medical therapy.
Reversible posterior leukoencephalopathy syndrome (RPLS)	-	Discontinue	
Wound dehiscence	-	Discontinue	Bevacizumab should be discontinued 28 days prior to elective surgery.
Other unspecified bevacizumab-related adverse events	3	Hold	Hold bevacizumab until ≤ Grade 1; resume dosing.
	4	Discontinue	

CNS=central nervous system; CTCAE=Common Terminology Criteria for Adverse Events; GI=gastrointestinal.

9.6. Supportive Care Interventions

9.6.1. Colony Stimulating Factor Usage

Use of primary **prophylactic** colony stimulating factors (e.g., G-CSF; granulocyte-macrophage colony-stimulating factor [GM-CSF]) during Cycle 1 is not allowed; therapeutic use of G-CSF/GM-CSF is permitted in any cycle, including Cycle 1, as described below. In subsequent cycles (Cycle 2 and beyond), secondary prophylactic colony stimulating factors are allowed as outlined in regional guidelines or consensus (e.g., American Society of Clinical Oncology [ASCO], European Society for Medical Oncology [ESMO], Chinese Anti-Cancer Association) and package inserts. G-CSF products (i.e., Neupogen or biosimilars) may be administered starting 24 to 48 hours after chemotherapy is complete on Day 2 and must be stopped 48 hours prior to study drug administration in the next cycle. Similarly, Neulasta (or biosimilars) or other long half-life GCSF (ex. macapegfilgrastim) may be administered up to 48 hours after

chemotherapy but due to its prolonged half-life should be administered as soon as clinically acceptable and should not be repeated within that cycle.

If in any cycle (including Cycle 1), a patient experiences febrile neutropenia and is at high risk for infection-associated complications OR has prognostic factors that are predictive of poor clinical outcomes (Table 11), G-CSF/GM-CSF may be used to treat the febrile neutropenia event per regional guidelines and package insert (i.e., **therapeutic** use of G-CSF).

Table 11: Patient Risk Factors for Poor Clinical Outcomes Resulting from Febrile Neutropenia or Infection

Risk Factor
Sepsis syndrome
Age > 65 years
Profound neutropenia (absolute neutrophil count < 0.1×10^9/L)
Neutropenia expected to last > 10 days
Pneumonia
Invasive fungal infection
Other clinically documented infections
Hospitalization at time of fever
Prior episode of febrile neutropenia

Source: Table recreated from Table 2 of the ASCO guidelines (Smith, 2015; Smith, 2006).

9.6.2. Erythropoiesis-Stimulating Agent Usage

If a patient experiences a hemoglobin level < 10.0 g/dL or symptomatic anemia during study treatment, ESAs may be used per the current prescribing information (Bohlius, 2019) (Procrit®, 2017; Aranesp®, 2011).

9.6.3. Transfusions

Red Blood Cells

Based on the NCCN Clinical Practice Guidelines in Oncology for Hematopoietic Growth Factors Version 2.2020 and the AABB Clinical Practice Guidelines, the following RBC transfusion thresholds are recommended (Carson, 2016; Goel, 2017); however, the patient's clinical situation should always be the primary guiding factor when deciding to transfuse.

- Transfusion is not indicated until the hemoglobin level is ≤ 7 g/dL for hospitalized adult patients who are hemodynamically stable.
- An RBC transfusion threshold of ≤ 8 g/dL is recommended for patients undergoing orthopedic surgery, cardiac surgery, and those with preexisting cardiovascular disease.
- Patients with symptomatic anemia should be transfused per the Investigator discretion regardless of hemoglobin levels.

PLATELETS

Platelets should be transfused at a threshold of ≤ 10,000/µL. Platelets should also be transfused in any patient who is bleeding with a platelet count < 50,000/µL (100,000/µL for central nervous system or ocular bleeding) (Kaufman, 2015; Schiffer, 2017).

9.7. Prior/Concomitant Medications

Any medication (including over-the-counter or prescription medicines, vitamins, herbal supplements, growth factors, blood products, and/or parenteral nutrition) that the patient has received within 14 days prior to date of informed consent or receives during the study and through 30 days after the last dose of study drug must be recorded in the eCRF. During the study, prescription and commercially available products, including traditional Chinese medications, with known or suspected immunomodulatory properties should be used with caution or avoided entirely.

Supportive care as described in Section 9.6, including the administration of colony stimulating factors or ESA and RBC or platelet transfusions, must be recorded in the eCRF.

Trilaciclib is a time-dependent inhibitor of CYP3A4 and is a substrate for CYP3A4. No clinically meaningful change in trilaciclib exposure was observed when co-administered with a CYP3A4 inhibitor or inducer. Trilaciclib is not expected to affect the exposure of CYP3A substrates following coadministration.

Avoid concomitant use of trilaciclib with certain OCT2, MATE1, and MATE2-K substrates (e.g., dofetilide, dalfampridine) where minimal concentration changes may lead to serious or life-threatening toxicities. Refer to the prescribing information for these concomitant medications for assessing the benefit and risk of concomitant use of trilaciclib (Section 4.2.1.2).

Refer to the current, regional package insert of fluorouracil for recommendations on monitoring for INR or prothrombin time in patients receiving concomitant coumarin-derivative anticoagulants such as warfarin (e.g. low dose as prophylaxis for port) in order to adjust the anticoagulant dose accordingly. Administration of aspirin doses > 325 mg/day is not permitted.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Administration of other systemic concomitant non-protocol anti-cancer therapies prior to progression is not permitted while on this study with the exception of adjuvant endocrine therapy for breast cancer or prostate cancer defined as M0 disease or PSA persistence/recurrence without metastatic disease.

Local treatment of symptomatic lesion(s) with palliative therapy (ex. surgery, radiotherapy, radiofrequency ablation, embolization) is permitted at the Investigator's discretion to control disease symptoms. However, use of techniques that incorporate systemic therapy, including local administration, is not permitted. If the lesion(s) treated is being followed for evaluation by RECIST (target or nontarget lesion), then "not evaluable" should be reported in EDC for this lesion at subsequent disease assessments following palliative treatment. Patients requiring palliative therapy may continue receiving study drug until documented disease progression (radiographic or clinical) if, in the Investigator's opinion, the patient is continuing to receive clinical benefit and they meet the requirements described in Section 9.4. However, for patients

who have not had disease progression at the time of the need for palliative treatment, the requirement for intervention will be regarded as disease progression in the study's analyses and will be entered as such in the EDC.

Patients with metastases confined to the liver who experience a robust response to chemotherapy may also proceed with curative intent surgical resection at the Investigator's discretion. In that event, study treatment in this trial will be considered complete and patients will enter into Survival Follow-up. Tumor assessments will continue as directed for survival follow-up. Any anti-cancer therapy for colorectal cancer that is initiated post-surgical resection is at the discretion of the Investigator, and will be recorded as subsequent anti-cancer therapy in the EDC.

Administration of other concomitant investigational agents for any indication or any live attenuated vaccines is not permitted while on this study. Necessary supportive care (antiemetics, antidiarrheals, etc.) per the standard of care at the study center will be permitted.

9.8. Measures to Minimize Bias: Randomization and Blinding

The study will be randomized and double-blind. Patients meeting all inclusion and exclusion criteria will be randomized 1:1 by an IWRS according to a randomization schedule generated by an unblinded external statistician. Each patient will be assigned a unique randomization number, which will not be reused. There will be three stratification factors for randomization: country, prior therapy in adjuvant/neoadjuvant setting, and presence of BRAF V600E mutation. Within each country, patients will be stratified by systemic cytotoxic therapy in the adjuvant/neoadjuvant setting (yes/no) and BRAF V600E mutation (yes/no).

Each site will have an unblinded Pharmacist/Designee, who will have access to the treatment assignment. Because the active drug product trilaciclib has a faint yellow color when reconstituted and diluted, additional procedures will be implemented to ensure the blind is maintained for the patient, Investigator, Research Nurse, Study Coordinator, and other blinded site personnel. Details regarding the preparation and administration of trilaciclib/placebo in a manner to maintain the blinding will be included in the Pharmacy Manual.

The patients, Investigators, other site staff involved in the clinical care of the patients, and the Sponsor or Designees involved in the conduct of the study will not be aware of the treatment group to which a particular patient has been randomized. If an Investigator determines that a patient's assignment should be unblinded for reasons of safety, this should be discussed with the study Medical Monitor prior to unblinding, unless an urgent and immediate intervention is required that precludes this discussion. If unblinding of the treatment assignment is necessary, the Investigator or Designee will obtain the treatment assignment details from the IWRS. Unblinding of personnel should be limited to the minimum needed for mitigation of safety concern. Following unblinding, patient continuation on study will be determined on a case by case basis. Any unplanned unblinding must be communicated to the Project Manager, Study Statistician, and Sponsor for documentation in the study files and the clinical study report.

9.9. Intervention after End of Study Treatment

Following completion of study treatment on the study, patients will receive standard of care treatment as determined by their healthcare provider. During Survival Follow-up, patients and their caregivers will be contacted to record their status (alive or dead) as well as details of any subsequent systemic anti-cancer therapy initiated (see Section 11.12).

10. DISCONTINUATION OF STUDY INTERVENTION AND PATIENT DISCONTINUATION/WITHDRAWAL

10.1. Discontinuation of Study Treatment

Study drug will be discontinued if any of the following events occur during the study:

- A patient suffers an AE that, in the judgment of the Investigator, Sponsor, or Medical Monitor, presents an unacceptable risk to the patient
- General or specific changes in the patient's condition (e.g., a significant intercurrent illness or complication) that, in the judgment of the Investigator, are unacceptable for further administration of study drug
- Occurrence of pregnancy during the study
- Significant noncompliance with protocol requirements
- The Sponsor or legal representative of the Sponsor requests the patient to withdraw
- Patient has documented disease progression (radiographic or clinical progression). See Section 9.7 for details regarding palliative therapy and curative intent surgical resection.
- Where permanent discontinuation of all study drugs is indicated in the toxicity management recommendations

At the time of study drug discontinuation, a Post-treatment Visit should be completed with assessments performed as shown in the Schedule of Assessments (Table 5). The Investigator or Designee will document the reason for study drug discontinuation on the applicable eCRF. When discontinuation is due to a SAE or a Grade 3 or 4 toxicity considered to be related to study medication, the Investigator should follow the event until resolution, return to baseline, or it is deemed that further recovery is unlikely. Data on these events should be collected on the AE eCRF. In the event a patient discontinues due to pregnancy, the Investigator or designee should notify the Medical Monitor by telephone within 24 hours of pregnancy confirmation (see Section 17.4).

10.2. Discontinuation/Withdrawal from the Study

A patient may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. At the time of discontinuing from the study, if patient has not already discontinued study intervention, a Post-treatment Visit should be completed with assessments performed as shown in the Schedule of Assessments (Table 5).

If the patient withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a patient withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator or designee must document this in the site study records.

If a patient withdraws consent for further study procedures, the site should clarify if the patient remains open to survival contact and associated data collection.

10.3. Lost to Follow Up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions should be taken if a patient fails to return to the clinic for a required study visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible and counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- Before a patient is deemed lost to follow up, the Investigator or Designee should make every effort to regain contact with the patient per institutional practice. These contact attempts should be documented in the patient's medical record.
- Should the patient continue to be unreachable, he/she will be considered to have withdrawn from the study.

10.4. Study and Site Start and Closure

The study start date is the date on which the first site is open for recruitment of patients.

The Sponsor's designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected, a study-site closure visit has been performed, and the site has closed all regulatory activities with the Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

The Investigator may be requested to initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Should this occur with patients receiving study drug, the patients will transition to receive standard of care treatment by their healthcare provider outside of this study.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or Good Clinical Practice (GCP) guidelines
- Inadequate recruitment of patients by the Investigator
- Discontinuation of further development of trilaciclib

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the patient and should assure appropriate patient therapy and/or follow-up.

11. STUDY ASSESSMENTS

Study procedures and their timing are summarized in the Schedule of Assessments (Table 4 and Table 5). Adherence to the study design requirements, including those specified in the Schedule of Assessments, is essential and required for study conduct. Immediate safety concerns should be discussed with the study Medical Monitor upon occurrence or awareness to determine if the patient should continue or discontinue study intervention.

The Investigator or Designee will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable. The following information for screening failures should be recorded into appropriate eCRFs: patient identification (ID), demographic data, and reason for failing the screening process. Patients may only be rescreened one time at the discretion of the Investigator. For abnormal laboratory values, a second test to confirm the first is permitted. If a patient is rescreened, they should receive a new patient ID. Procedures conducted as part of the patient's routine clinical management (e.g., hematology, clinical chemistry) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and are performed within 28 days prior to Cycle 1 Day 1.

For the patients who undergo curative intent surgical resection, no additional myelosuppression, or PRO data will be collected following surgery. However, tumor assessments will continue as directed in survival follow-up.

11.1. Randomization

Eligibility should be determined prior to randomization and the start of study treatment. Eligible patients will be instructed on all protocol requirements, including any restrictions on concomitant medication usage.

Randomization will be performed via IWRS within 3 days prior to the first dose of trilaciclib or placebo + FOLFOXIRI/bevacizumab therapy, following confirmation that the patient is eligible for the study. Patients can be randomized and receive Cycle 1 Day 1 on the same day.

11.2. Myelopreservation Efficacy Assessments

Myelosuppression will be assessed based on hematology assessments, severe AEs, supportive care interventions (including transfusions), dose modifications, and PROs.

11.2.1. Hematology Sampling Schedule

Trilaciclib has been shown in SCLC patients to prevent the myelosuppressive effects of chemotherapy across multiple cellular lineages, including neutrophils, RBCs, and platelets, by protecting HSPCs. In order to adequately characterize the myelopreservation benefit of trilaciclib, more frequent hematology lab assessments will be done during Cycle 1, as outlined in the Schedule of Assessments (Table 4 and Table 5). The frequent assessments will only be done during Cycle 1 because the data collected in Cycle 1 won't be subject to bias due to primary prophylactic G-CSF administration, and more frequent monitoring in subsequent cycles would be an unreasonable burden on the patient.

11.3. Anti-tumor Efficacy Assessment

Tumor response criteria will be based on RECIST v1.1 (Eisenhauer, 2009).

Initial staging studies should include computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, pelvis at a minimum. IV contrast should be used unless contraindicated. Oral contrast can be used at the Investigator's discretion. Post-baseline tumor assessment using the same imaging modality and region(s) as at baseline should be performed as outlined in the Schedule of Assessments (Table 4 and Table 5) until the occurrence of disease progression, withdrawal of consent, the initiation of subsequent anti-cancer therapy, or study completion. These assessments should be done regardless of the timing of study drug administration, e.g. if cycles are delayed.

Any CT, MRI, or other staging study obtained as standard of care prior to signing the informed consent will not need to be repeated if performed within 28 days prior to Cycle 1 Day 1 as long as it meets the requirements above. Additional scans may be obtained at the discretion of the Investigator during the course of the study, if clinically indicated.

For those patients who have not progressed at the time of study drug discontinuation and enter into Survival Follow-up, radiological tumor assessments should continue, utilizing the same imaging modality as used at screening as outlined in the Schedule of Assessments (Table 4 and Table 5) until progression, withdrawal of consent, initiation of subsequent anti-cancer therapy, or study completion.

For definitions of tumor response per RECIST v1.1 see Section 17.7.

During the course of this trial, scans may be collected and sent to Sponsor or Designee for storage. Centralized storage is intended for possible blinded independent central review (BICR) of disease assessments. At the discretion of the Sponsor, BICR of all scans by RECIST v1.1 may be conducted retrospectively. The blinded review will include all patients with available images. However, the raw images for patients enrolled in China cannot be stored outside of China and therefore these images will be stored in the server in China for central imaging review if approved by the related authority. If needed, guidelines for imaging collection and storage will be provided in a separate document. The clinical management of patients will be based solely upon the results of the assessment conducted by the Investigator based on RECIST v1.1 per protocol.

11.4. Safety Assessments

11.4.1. Demographics

Age, gender, race and ethnicity will be collected during the Screening period.

11.4.2. Medical History and Colorectal Cancer Disease History

Medical and surgical history, including past and current conditions, will be collected. Concomitant medications taken within 14 days of signing the informed consent will be recorded.

Documentation of CRC history, including date of diagnosis as well as BRAF V600E (required) and KRAS (as available) mutational status, will be collected. Patients will be stratified based on a locally available BRAF mutation test result that has been performed in a Clinical Laboratory

Improvement Amendments (CLIA)-certified laboratory (US sites) or an accredited laboratory (sites outside of the US). A liquid biopsy is acceptable to confirm BRAF V600E mutational status if tissue cannot be utilized. If historical BRAF V600E mutational status is not known, a tumor specimen (archival, fresh, or biopsy) must be sent for testing. If appropriate testing cannot be performed at the local institution, a tumor specimen (archival or fresh tissue only) may be sent to the Sponsor's designated central laboratory for analysis. Results must be available at the time of randomization in IWRS. Any tumor sample sent for analysis of BRAF will be in addition to that required for the retrospective biomarker analysis described in Section 11.8.1.

Documentation of pMMR/MSS is also required; patients with deficient MMR/microsatellite instable (MSI)-high or MSI-low CRC are not eligible for this study. Patients with MSI-high tumors have a more favorable prognosis than those with MSS or MSI-low tumors (Malesci, 2007; Hutchins, 2011; Sinicrope, 2013; Zaanan, 2018) and have been shown to have longer progression-free survival with the addition of immunotherapy to common colorectal chemotherapy regimens (Helwick, 2020). The clinical behavior of MSI-low tumors is incompletely characterized but has been associated with worse outcomes relative to MSI-high and MSS tumors (Kohonen-Corish, 2005; Wright, 2005; Mojarad, 2016). Because MSI-low tumors make up only 3-10% of an unselected colorectal cancer population (de la Chapelle, 2010) and could have a negative impact on prognosis, stratification should be considered if these subjects are included. At the planned sample size for this study, an additional stratification factor is not optimal and therefore, only patients with MSS disease will be eligible. Patients will be eligible based on a locally available pMMR/MSS test result that has been performed in a CLIA-certified laboratory (US sites) or an accredited laboratory (sites outside of the US). If historical pMMR/MSS status is not known, a tumor specimen (archival or fresh biopsy) must be sent for testing. If appropriate testing cannot be performed at the local institution, the tumor specimen may be sent to the Sponsor's designated central laboratory for analysis. Results must be available at the time of randomization in IWRS. Any tumor sample sent for analysis of pMMR/MSS will be in addition to that required for the retrospective biomarker analysis described in Section 11.8.1.

Prior radiation, surgery, and systemic chemotherapy for CRC will also be recorded.

11.4.3. Vital Signs

The following will be collected per the Schedule of Assessments (Table 4 and Table 5):

- Body temperature, pulse rate, blood pressure (diastolic and systolic)
  - Blood pressure and pulse measurements should be assessed in the supine position.
  - Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the patient in a quiet setting without distractions (e.g., television, cell phones).
- Height in centimeters (Screening visit only) and body weight in kilograms

11.4.4. Physical Examination

Full physical examination evaluations at screening should include general appearance, skin, neck, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and neurological examinations. Subsequent physical exams should include body systems as appropriate (e.g., brief physical exam).

Information about the physical examination must be present in the source documentation at the study site. Clinically relevant findings observed **prior** to the start of study drug, should be recorded as medical history. Clinically relevant findings observed **after** the start of study drug, which meet the definition of an AE, must be recorded on the AE eCRF.

11.4.5. Electrocardiogram

Triplicate 12-lead single electrocardiograms (ECGs) will be performed as outlined in the Schedule of Assessments (Table 4 and Table 5). Patients should rest for 5 minutes prior to the ECG assessment. Additional ECGs during the study may be performed as clinically indicated.

Any ECG with a QTc value of > 500 or any other clinically significant abnormal finding should be repeated every 5 minutes for a total of three ECGs to confirm this finding, if not already recorded in triplicate. The QTc value should also be confirmed via manual read.

The Investigator or qualified designee should review the ECGs for any abnormalities as compared with the baseline ECG.

11.4.6. Clinical Safety Laboratory Assessments

Hematology, clinical chemistry, coagulation (INR, activated partial thromboplastin time [aPTT]), and urinalysis will be performed at the site's local certified laboratory per the schedule outlined in the Schedule of Assessments (Table 4 and Table 5). Clinical laboratory samples may be collected from patients at a different location that the Investigator's clinic following approval by the Medical Monitor. A list of clinical laboratory tests to be performed is provided in Section 17.2. Clinical chemistry and hematology results should be reviewed before dosing. Laboratory toxicities will be assessed using the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE), v5.0.

An abnormal laboratory value is not an AE unless it is considered to be clinically significant. Laboratory parameters for which clinically significant values are noted will be re-measured on the appropriate clinical follow-up arranged by the Investigator. Any laboratory value that remains abnormal at the end of the study and that is considered clinically significant should be followed according to accepted medical standards for up to 30 days or until the values return to normal or baseline or are no longer considered clinically significant by the Investigator. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Medical Monitor notified.

If a subsequent cycle is delayed for toxicity, the patient should still complete the clinical laboratory assessments on the scheduled Day 1 (entered as an Unscheduled assessment in EDC) as well as on the actual first dosing day of that cycle. If the delay is secondary to hematologic toxicity, weekly repeat hematology assessments should continue until the finding has returned to baseline or is deemed not clinically significant by the Investigator.

If laboratory values from non-protocol specified laboratory assessments are performed at the institution's local laboratory as part of the monitoring of an SAE, then the results should be provided to the Pharmacovigilance group as part of the safety reporting.

11.5. Geriatric 8 Health Status Screening Tool

In prior studies, the administration of FOLFOXIRI has generally been limited to patients < 75 years of age because of concerns regarding the potential risks of chemotherapy-induced toxicities like CIM. However, the evolving field of geriatric oncology has demonstrated that chronologic age is not necessarily the best measure of a patient's physiologic age and the ability to tolerate more intensive chemotherapy regimens (Soto Perez de Celis, 2018). Therefore, rather than implementing an arbitrary chronologic age cutoff for enrollment in this trial, the G8 (geriatric 8) Health Status screening tool will be used to evaluate a prospective patient's fitness for treatment. This tool has been shown to predict outcomes and AEs during the management of the elderly with cancer (Bellera, 2012; Kenis, 2014). The G8 Health Status screening tool will be completed by the Investigator or designee.

The G8 consists of 8 items concerning nutritional status, weight loss, body mass index, motor skills, psychological status, number of medications, self-perception of health, and age (< 80, 80 to 85, > 85). Scores range from 17 (not impaired) to 0 (heavily impaired). A score of > 14 is suggestive of a geriatric cancer patient able to tolerate standard treatment without major modification; low G8 scores have been associated with functional decline and poorer survival (Kenis, 2014). The G8 Health Status screening tool will be completed by the Investigator or designee once during Screening only for patients > 70 years old.

11.6. Patient Reported Outcomes

11.6.1. Functional Assessment of Cancer Scales

The Functional Assessment of Cancer Therapy – Colorectal (FACT-C) and FACT-An are validated instruments that will be administered to patients by a designated trained study site staff member. The instruments will be administered on the days outlined in the Schedule of Assessments (Table 4 and Table 5). If the cycle is delayed, the patient should still complete the PRO on the scheduled Day 1 (entered as an Unscheduled assessment), as well as on the actual first dosing day of that cycle. The PROs will be administered electronically during a protocol-specified clinic visit; if a hematology assessment is performed at the visit then the questionnaires should be completed on the same day a blood sample is collected for that assessment. Questionnaires must be administered prior to administration of any study drug and should be administered prior to conduct of any study procedure at that visit.

A core general questionnaire (FACT-G) that measures physical, social/family, emotional, and functional well-being is embedded in both the FACT-C and FACT-An instruments. The FACT-C has an additional subscale to measure aspects of health-related quality of life that are specific to colorectal cancer patients. The FACT-An includes the FACIT-F, a 13-item subscale that measures fatigue severity and the impact of fatigue on functioning (Yellen, 1997). The core general questionnaire (FACT-G) is the same for both instruments; as such, at each assessment, the core questionnaire will be administered only once along with the condition-specific colorectal and anemia subscales, including the FACIT-F.

11.6.2. Patient Global Impression of Change and Patient Global Impression of Severity

The Patient Global Impression of Change (PGIC) and Patient Global Impression of Severity (PGIS) items will be administered to patients by a designated trained study site staff member. The instruments will be administered on the days outlined in the Schedule of Assessments (Table 4 and Table 5). The PGIC item will ask the patient to rate the change in their fatigue since taking study medication on a 7-point scale ranging from -3 (very much worse) to 3 (very much better). The PGIS item, will ask the patient to rate the overall severity of their fatigue over the past week on a 5-point scale ranging from 0 (none) to 4 (severe).

PGIC and PGIS will be administered electronically during a protocol-specified clinic visit; if a hematology assessment is performed at the visit then the questionnaires should be completed on the same day a blood sample is collected for that assessment. The questionnaires must be administered prior to administration of any study drug and should be administered prior to conduct of any study procedure at that visit.

11.6.3. 5-Level EQ-5D

The 5-level EQ-5D (EQ-5D-5L) is a standardized instrument by which treatment effects can be assessed by measuring health status at different points in time, e.g., before and after treatment, and then investigating the gains (or losses) in reported health status. The EQ-5D-5L consists of the EQ-5D descriptive system and the EQ visual analogue scale (EQ-VAS). For the EQ-5D, respondents will rate their health on each of 5 dimensions: mobility, self-care, usual activities, pain/discomfort, anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The patient will be asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the five dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the five dimensions can be combined into a 5-digit number that describes the patient's health state. The EQ-VAS records the patient's self-rated health on a vertical visual analogue scale, where the endpoints are labelled "The best health you can imagine" and "The worst health you can imagine". The VAS can be used as a quantitative measure of health outcome that reflect the patient's own judgement.

The EQ-5D-5Lwill be administered to patients by a designated trained study site staff member on the days outlined in the Schedule of Assessments (Table 4 and Table 5). It will be administered electronically during a protocol-specified clinic visit; if a hematology assessment is performed at the visit then the EQ-5D-5L should be completed on the same day a blood sample is collected for that assessment. The questionnaire must be administered prior to administration of any study drug and should be administered prior to conduct of any study procedure at that visit.

11.7. Pharmacokinetics

Blood samples for PK analysis will be collected at the time points indicated in the Schedule of Assessments (Table 4 and Table 5) from all patients. Four blood samples for PK analysis will be collected for trilaciclib and any metabolites and three samples will be collected for PK analysis of irinotecan and its metabolite (SN-38. The actual date and time of each blood sample collection, the scheduled timepoint of collection, and the time of dose administration prior to the

pharmacokinetic sample will be recorded. Details of PK blood sample collection including volume to be collected, timing of samples, processing, storage, and shipping procedures are provided in the study procedure manual.

11.8. Biomarkers (CDK4/6 Signature)

11.8.1. Rationale for Archival Tumor Collection

Because trilaciclib maintains G1 arrest in CDK4/6 dependent cells, there is a hypothetical risk that trilaciclib could antagonize the anti-tumor efficacy of cytotoxic chemotherapy by maintaining a G1 cell cycle arrest of CDK4/6 dependent tumor cells during chemotherapy. While the currently available nonclinical and clinical data indicate that this risk remains hypothetical and does not influence the overall benefit/risk assessment for combining trilaciclib with chemotherapy, this study will evaluate in a retrospective fashion the relationship between CDK4/6 status and measures of anti-tumor efficacy; the planned statistical analyses are described in Section 12.4.5.1.

To support these analyses, prior to signing the informed consent, a FFPE archival tumor specimen with an associated pathology report documenting pMMR/MSS CRC for each patient must be confirmed to be available to send to the Sponsor for planned retrospective biomarker analyses. Tumor tissue must consist of 20 (15 minimum) freshly cut unstained slides, 4-6 microns in thickness from a serial section close to the section of the block taken for local H&E assessment. If sectioning is not feasible, an FFPE tumor block, 100 microns preferred (or 75 microns at the minimum) is an acceptable alternative. If archival tissue is not available, a fresh biopsy must be obtained. Acceptable samples include core needle biopsies, tumor tissue - excisional or incisional, punch, or forceps biopsies for cutaneous, subcutaneous, or mucosal lesions. Fine-needle aspiration, brushing, cell pellet from pleural effusion, bone metastases, and lavage samples are not acceptable. Additional guidance is provided in the laboratory manual. Any remaining tumor tissue following the described analyses will be banked and may be analyzed for relevant DNA, ribonucleic acid (RNA), or protein biomarkers and their role in cancer, including to better understand immune-related mechanisms. Note: No tumor tissue will be archived for study centers in China.

11.8.2. Description of CDK4/6 Signature

In order to characterize this hypothetical risk in a tumor type without a clear CDK4/6 response phenotype, archival tumor tissue will be collected from patients' most recent biopsy (colorectal primary or metastatic site) and subjected to a genetic signature analysis based on published literature in an effort to characterize patient tumors into one of the following three groups (Shapiro, 2017; Gong, 2017):

- CDK4/6 independent: As the canonical downstream target of CDK4/6, the RB1 gene and expression of its gene product (RB protein) has been well studied as a marker of resistance and sensitivity to CDK4/6 inhibitors. RB1 is commonly dysregulated in cancer cells through deletion, mutation or epigenetic modification resulting in loss of RB expression, as well as by aberrant CDK kinase activity leading to excessive phosphorylation and inactivation of RB function (Chen, 2019; Sherr, 2002). CCNE1/2 (cyclin E) is part of a parallel pathway that provides functional redundancy

with CDK4/6 and helps to transition cells from the G1 to S phase. Overexpression will decrease the reliance on the CDK4/6 pathway leading to CDK4/6 independence (Turner, 2019). Therefore, a tumor with either CCNE1/2 amplification or RB loss will be classified as "CDK4/6 independent".

- CDK4/6 dependent: Attempts to identify markers of CDK4/6 dependence have evaluated increased expression of CDK4/6 activators (cyclin D). In PALOMA-1, when the Cyclin D1 gene (CCND1) amplification and loss of p16IN4a were evaluated in patients receiving palbociclib plus letrozole, anti-tumor efficacy was improved regardless of CCND1 or p16INK4a status (Finn, 2019); thereby showing the futility of this approach. One explanation of this result is that assessment of cyclin D1 alone is insufficient to predict CDK4/6 sensitivity. Lending support to this hypothesis is a nonclinical study evaluating a broader set of d-type cyclin activating features (DCAF) — including CCND1 translocation, CCND1-3 3'UTR loss, and amplification of CCND2 or CCND3 — which were shown to predict in vitro sensitivity to abemaciclib treatment (Gong, 2017). Therefore, after excluding tumors that are "CDK4/6 independent", any remaining tumors that are WT for RB and CCNE1/2 as well as have one of the DCAF described above will be classified as "CDK4/6 dependent".
- CDK4/6 indeterminate: Any remaining tumor will be classified as "CDK4/6 indeterminate" since they cannot be confirmed as CDK4/6 dependent or independent.

The evaluation of the relationship between CDK4/6 status and anti-tumor outcomes will be performed when the OS analysis is performed, that is, when the predicted death events are observed. The use of mature OS data, in addition to the ORR and PFS data, will allow for a more robust interpretation of the relationship between CDK4/6 status and anti-tumor outcomes without the complication of trying to draw conclusions with inadequate information (ie, immature anti-tumor efficacy data).

This timing is considered to be appropriate since all currently available data indicate that if there is a risk, it is not clinically relevant; therefore, waiting until mature OS data does not pose a significant hazard to the enrolled patients, particularly since the percentage of patients at risk for a potential negative outcome (i.e., who have CDK4/6 dependent tumors) is predicted to be < 10% (Section 4).

In addition to this prespecified retrospective analysis of CDK4/6 status and response when OS data are mature, during the study an unblinded DMC (Section 11.11) will also review the response rate of the overall population (eg, without subsets of CDK4/6 status) to monitor any potential detriment in chemotherapy efficacy in those patients receiving trilaciclib versus placebo. Sponsor, Investigators, and patients will remain blinded. Timing of this review and details of the associated analysis plan will be included in the DMC Charter.

11.9. Immunologic and Hematologic Markers

Chemotherapeutic agents may elicit part of their anti-tumor efficacy by modulating the immune system to enhance antigen presentation, uptake, and processing; prime the immune response through immunodepletion; inhibit regulatory cells; and stimulate immune effector cells (Zitvogel, 2008; McDonnell, 2011; Bracci, 2014). Conversely, immunosuppression from direct

cytotoxicity to the bone marrow and immune system over repeated cycles of chemotherapy may counterbalance the positive immunostimulatory effects of chemotherapy. Therefore, therapeutic approaches to maintain bone marrow health and immune system function should enhance the immune-mediated anti-tumor activity.

To evaluate the impact of trilaciclib administration on chemotherapy-induced changes of the immune and hematopoietic system, immunophenotypic and hematologic changes will be compared (as data warrant) between patients receiving FOLFOXIRI/bevacizumab therapy with either placebo or trilaciclib. To assess these changes, peripheral blood will be collected per the timepoints in the Schedule of Assessments (Table 4 and Table 5). At each time point (Cycle 1 Day 1, Cycle 2 Day 1, Cycle 5 Day 1, and Maintenance Cycle 1 Day 1), collection should occur prior to trilaciclib dosing. These blood samples will be banked and may be analyzed for relevant DNA, RNA, or protein biomarkers and their role in cancer. Note: No blood will be archived for study centers in China.

11.10. Adverse and Serious Adverse Events

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up, as applicable, AEs that are serious, considered related to the study drugs or study procedures, or that caused the patient to discontinue the study or study drugs (see Section 10.1). Patients should be encouraged to report AEs spontaneously or in response to general, nondirected questioning. Adverse events should be reported on the appropriate page of the eCRF.

11.10.1. Time Period and Frequency for Collecting Adverse and Serious Adverse Event Information

All AEs and SAEs will be collected from the day of first dose of any study drug until 30 days after the last dose of study drug. Any SAE occurring between the date the patient signs informed consent and the first dose of any study drug, and which the investigator feels is related to a study specific procedure (i.e. would not have occurred unless the patient was on the study), should also be reported.

All SAEs will be recorded and reported to G1 Therapeutics pharmacovigilance (PVG) or designee immediately and under no circumstance should this exceed 24 hours after becoming aware of the event, as indicated in Section 17.3. The Investigator or designee will submit any updated SAE data to G1 Therapeutics PVG within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after 30 days following the last dose of study drugs on this study. However, if the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the Investigator or designee must promptly notify G1 Therapeutics PVG or designee.

11.10.2. Method of Detecting Adverse and Serious Adverse Events

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Section 17.3.

Care should be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the patient is the preferred method to inquire about AE occurrences.

11.10.3. Follow-up of Adverse and Serious Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each patient at subsequent visits/contacts. All AEs (both serious and nonserious) will be followed in accordance with good medical practice until resolution, return to baseline, or it is deemed that further recovery is unlikely. All measures required for AE management and the ultimate outcome of the AE will be recorded in the source document and reported to G1 Therapeutics PVG or designee.

All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the patient is lost to follow-up (as defined in Section 10.3). Further information on follow-up procedures is provided in Section 17.3.

11.10.4. Regulatory Reporting Requirements for Serious Adverse Events

Prompt notification of G1 Therapeutics PVG or designee by the Investigator (or designee) of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of patients and the safety of a study intervention under clinical investigation are met.

G1 Therapeutics has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. G1 Therapeutics will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.

For all studies, except those utilizing medical devices, Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and G1 Therapeutics policy and forwarded to Investigators as necessary.

An Investigator who receives an Investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from G1 Therapeutics or designee will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

11.10.5. Pregnancy

Details of all pregnancies in female patients and female partners of male patients will be collected after the start of study intervention and until 3 weeks and 3 months respectively, after the last dose of study drug.

If a pregnancy is reported, the Investigator or designee should inform G1 Therapeutics PVG or designee within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 17.4.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

11.10.6. Adverse Events of Special Interest

All Grade 3 or Grade 4 trilaciclib AESIs should be reported as SAEs using the procedures outlined in Section 11.10.1 to Section 11.10.4. For a list of trilaciclib AESIs, please see Section 4.2.3.1.

11.11. Data Monitoring Committee

An unblinded, independent data monitoring committee (DMC) will monitor accumulating safety and disposition data with the first meeting planned for when approximately 30 patients have enrolled and completed at least 2 cycles of study treatment. The meetings will continue approximately every 4 months while patients are on study treatment depending upon the enrollment rate. Additional reviews may occur based on DMC requests.

A DMC charter will define the roles and responsibilities of the DMC and its members. Additional details regarding the committee's composition, scope, objectives, procedures and policies, including data to be reviewed and strategy for maintaining study blind for the Sponsor, Investigators and patients, are described in the DMC charter. The DMC will monitor accumulating safety and disposition data entirely independent of the conduct of the study.

In addition to its periodic review of all available safety data, the DMC will also review at one meeting the response data of the overall population to monitor any potential detriment in chemotherapy efficacy in those patients receiving trilaciclib. Sponsor, Investigators, and patients will remain blinded. Timing of this review and details of the associated analysis plan will be included in the DMC Charter.

11.12. Survival Follow-up

When a patient permanently discontinues study treatment (End of Treatment [EOT]), the patient should complete a Post-treatment follow-up visit as outlined in Table 5. The patient will then continue in the study in Survival Follow-up. If a patient withdraws consent for further study treatment and/or procedures, the site should clarify if the patient remains open to survival contact and associated data collection.

During Survival Follow-up, 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). In addition, details of any subsequent systemic anti-cancer therapy initiated, including name(s) of agent(s), dates (start/stop) administered, best response to the treatment, and date of progression should also be reported in EDC. As available, results of CBC performed prior to the start of each regimen of subsequent systemic anti-cancer therapy should also be reported in the EDC. Provided that the patient has not withdrawn consent for follow up contact, information from medical records may be substituted for phone or other contact, provided that records are available as source documentation. Public records may be used to verify survival status if permitted by institutional or country guidance.

For those patients who have not progressed at the time of study drug discontinuation and enter into Survival Follow-up, radiological tumor assessments should continue as outlined in Table 5 until progression, withdrawal of consent, initiation of the first subsequent anti-cancer therapy, or

study completion (see Section 11.3). Results of these scans should be assessed by RECIST 1.1 and data entered in EDC in the corresponding tumor assessment forms.

For those patients who undergo curative intent surgical resection during this trial, the patient will discontinue study treatment at the time of resection and continue in Survival Follow-up. Tumor assessments will continue as directed for survival follow-up. Any anti-cancer therapy for colorectal cancer that is initiated post-surgical resection is at the discretion of the Investigator. Details of subsequent anti-cancer therapy received post-surgical resection (even if a continuation of the study regimen), including name(s) of agent(s), dates (start/stop) administered, best response to the treatment, and date of progression should also be reported in EDC. Subsequent anti-cancer therapy data following curative intent surgical resection will be summarized separately from subsequent anti-cancer therapy data reported following disease progression.

12. STATISTICAL CONSIDERATIONS

Full details on the statistical analyses to be performed will be provided in a separate statistical analysis plan (SAP).

12.1. Sample Size Determination

The primary and key secondary objectives of this study are to evaluate the myelopreservation and anti-tumor efficacy of trilaciclib administered prior to FOLFOXIRI/bevacizumab (referred to as trilaciclib hereafter) compared with placebo administered prior to FOLFOXIRI/bevacizumab (referred to as placebo hereafter). To ensure strong control of family-wise type I error rate at the level of 2-sided 0.05 when performing statistical analyses for the two primary myelosuppression endpoints, and key secondary anti-tumor efficacy endpoint, the overall 2-sided α of 0.05 will be split between the analyses of the primary endpoints (using $\alpha_1 = 0.04$) and analysis for OS (using $\alpha_2 = 0.01$).

The sample size is determined to support primary efficacy analysis on the two primary efficacy endpoints.

To detect the assumed treatment effect of an absolute reduction of 15% on the proportion of patients who have SN in Induction at the significance level of 2-sided 0.04 with 90% power, assuming the event rate for placebo is 25%, a sample size of 282 (141 per group) is needed using a Chi-square test without continuity correction.

The sample size needed to detect a treatment effect on DSN in Cycle 1 – 4 (refer to Section 12.4.4.1 for definition) is estimated through simulations to detect a treatment effect of 2.4 days reduction for DSN. In derivation of this endpoint, duration of 0 will be assigned to patients who do not experience any SN in Cycle 1 – 4 (see details in Section 12.4.4.1). With a large proportion of 0 values, the variable of DSN in Cycle 1– 4 does not follow a normal distribution but approximately a Poisson distribution with the mean parameter as -log (proportion of patients without any SN at Cycle 1– 4). Assuming approximately 80% of SN that occurs during Induction will take place in the first 4 cycles of Induction as reported in (Rossini, 2021) and assuming that trilaciclib will have an effect of 15% absolute reduction on SN in the first 4 cycles, , it is estimated that the proportion of patients with SN in Cycle 1 – 4 is 20% for placebo and 5% for the trilaciclib group, respectively. Hence, the Poisson means are estimated to be 0.2231 and 0.0513 for placebo and trilaciclib groups, respectively. 10,000 trials were simulated based on the Poisson distributions to obtain DSN in Cycle 1– 4. Treatment group difference is evaluated using the Mann-Whitney-Wilcoxon test, and the power is estimated by the proportion of the trials that have achieved a 2-sided p-value ≤ 0.04 over 10,000 simulated trials. From the simulation results, a sample size of 200 patients (100 per group) can achieve an empirical power of 91%.

Overall, 282 patients (141 per group) are needed to detect the assumed treatment effect for each of the two co-primary myelosuppression endpoints with 90% power at the 2-sided significance level of 0.04. Assuming 5% of randomized patients will not have any post-baseline data, a total of 296 patients (148 per group) will be required for the study.

Although the number of patients is determined to ensure adequate power for the evaluation of the myelopreservation efficacy of trilaciclib, statistical comparisons for anti-tumor efficacy will also be conducted and the statistical significance is set to be 0.01 for testing the treatment effect on

OS. A total of 157 deaths are estimated to be observed during 52-month of study duration based on the following assumptions: 18 months of accrual with 34 months follow up after the last patient is randomized, a monthly drop-out rate of 0.0029 (assuming an exponential distribution), and the median OS of 31months for placebo group (patients receiving FOLFOXIRI/bevacizumab plus placebo) (Loupakis, 2014). It is also assumed that the hazard ratio (trilaciclib vs. placebo) is 0.75.

A total of 44 patients from Ukraine were randomized to the study as of 24 February 2022. To mitigate the potential impact of Russian-Ukraine war on data integrity and ensure the objectives of PRESERVE-1 will not be compromised, patients who were randomized from Ukraine prior to 09 September 2021 will be included in efficacy analyses while all randomized patients from Ukraine will be included in the safety evaluation. Therefore, a total of 30 randomized patients from Ukraine will be excluded from efficacy evaluation. To preserve the study power as it was originally designed, additional 30 patients will be randomized in countries other than Ukraine. As such, the total sample size for this study will be 326, with 296 patients included in the mITT population for the primary efficacy analyses. The reason that 09 September 2021 was chosen is because patients randomized prior to this date should have completed or had the opportunity to complete Induction period per protocol (approximately 24 weeks), prior to the start of the war (24 February 2022).

12.2. Analysis Population

The intent-to-treat (ITT) population includes all randomized patients. Analyses for the ITT population will be conducted based on the randomly assigned treatment regardless of whether the patient received study treatment or was compliant with the protocol.

To account for the data integrity issues resulting from the war in Ukraine, a modified intent-to-treat (mITT) population will be utilized as the primary analysis population for all efficacy evaluations. The criteria for the mITT population are as follows:

- All patients randomized in countries other than the Ukraine.
- All patients in the Ukraine who were randomized prior to 09 September 2021. This group should have completed, or had the opportunity to complete, Induction period per protocol (approximately 24 weeks) prior to the start of the war (24 February 2022).

As a subset of ITT population, the analyses performed on mITT will be consistent with the ITT principle, that is, data will be analyzed based on the randomly assigned treatment regardless of whether the patient received study treatment or was compliant with the protocol.

A per-protocol (PP) population is the subset of the mITT population that includes only those patients who have no key protocol deviations (e.g., a subset of major protocol deviations that might significantly affect the accuracy of the study efficacy data) and who receive at least one dose of the randomly assigned study treatment. The PP population will be used to analyze selected endpoints to evaluate the robustness of the efficacy findings observed in the mITT population. Analyses on the PP population will be based on the randomly assigned treatment. The criteria for patients to be included in the PP population will be fully defined and documented

prior to database lock, unblinding and performing the analysis of myelopreservation efficacy endpoints.

The response evaluable (RE) population will include patients in the mITT population who have a measurable lesion (target) at the baseline tumor assessment, and either (i) have at least 1 post-dose tumor assessment, or (ii) do not have post-dose tumor assessment but have clinical progression as noted by the Investigator, or (iii) have died due to disease progression before their first post-dose tumor scan. RE population will be the primary analysis set to evaluate tumor response and the data will be analyzed based on real received treatment.

The safety population includes all enrolled patients who received at least 1 dose of study drug. Analyses conducted on the safety population will be based on the actual treatment received. All safety analyses will be conducted using the safety population.

The PK population will include all dosed patients with evaluable PK data.

12.3. Timing of Planned Analyses

12.3.1. First Planned Analysis – Evaluation of Myelopreservation Efficacy

The first planned analysis will be conducted at the time that all randomized patients have finished 12 cycles of Induction treatment or discontinued during Induction. Trilaciclib's effects on myelopreservation will be evaluated in this analysis. The clinical trial database will be locked to support this first planned analysis.

This analysis is estimated to occur approximately 24 months from the date of the first patient randomized in the study. At the time of performing this analysis, the Sponsor will be unblinded; however, Investigators and patients will remain blinded.

Analysis for tumor response data will also be performed at this time.

12.3.2. Second Planned Analysis - Analysis for Progression Free Survival

The planned second analysis of the study is to perform the PFS analysis around the time that is 35 months post first randomization.

12.3.3. Third Analysis – Analysis for Overall Survival

At the time when 157 death events are observed or at 52 months after the date of first randomization, whichever comes first, the study will be concluded, and the final study database will be locked to perform the OS analysis.

PFS analysis will also be performed at this time along with other endpoints that will be analyzed based on the final study database lock, such as safety endpoints.

12.4. Statistical Analysis Methods

An SAP will be developed and finalized prior to the first planned database lock and will include more details related to the statistical analysis of this study's data. This section is a summary of the key aspects of the planned statistical analyses.

12.4.1. General Considerations

All statistical analyses will be performed using SAS® v9.4 or higher.

Data will be summarized descriptively by treatment group. The descriptive summary for the categorical variables will include counts and percentages. The descriptive summary for the continuous variables will include means, medians, standard deviations, 25% and 75% percentiles, and minimum and maximum values.

There are three stratification factors for randomization: country, prior therapy in adjuvant/neoadjuvant setting, and BRAF V600E mutational status. Within each country, patient randomization will be stratified by history of prior therapy in the adjuvant/neoadjuvant setting (yes/no) and BRAF V600E mutational status ("BRAF status"; yes/no). The countries will be grouped into the factor of "region" with four different entries of US, Eastern Europe, Western Europe, and China. The factor "region" will be used instead of "country" in the statistical analysis models to account for regional differences in clinical practice. It is anticipated that all three factors (region, prior therapy in adjuvant/neoadjuvant setting and BRAF status) would have an impact on patients' anti-tumor efficacy outcome, but only region and prior therapy in adjuvant/neoadjuvant setting would have an impact on myelosuppression measures. Therefore, region and prior therapy in adjuvant/neoadjuvant setting will be included as the factors in statistical analysis models evaluating trilaciclib myelopreservation efficacy, while all three factors are included in the statistical analysis models to assess trilaciclib's anti-tumor efficacy. Unless otherwise specified, the strata information as entered in IWRS at the time of randomization except for "region" will be used as the factors for all stratified statistical analyses.

For the patients who undergo curative intent surgical resection, myelosuppression and tumor assessments collected prior to the date of surgery will be included in the efficacy analyses, while all safety data will be included in the safety evaluation.

12.4.2. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for the ITT population descriptively by treatment and overall.

12.4.3. Prior and Subsequent Anti-cancer Therapies

Prior and subsequent anti-cancer therapy verbatim terms will be coded to Anatomical Therapeutic Classification (ATC) and preferred term (PT) using the most recent World Health Organization-Drug Dictionary (WHO-DD). For the ITT population, summary statistics will be provided for prior or subsequent systemic anti-cancer therapies by treatment group and overall. For the subsequent systemic anti-cancer therapies, the lines of therapy, response to each treatment regimen, and disease progression status will also be summarized by treatment group and overall.

For patients who undergo surgical resection with curative intent, surgery will be recorded as a subsequent surgery/procedure. The number and percentage of these patients will be summarized by treatment group and overall. Any post-surgical resection systemic anti-tumor therapy will be recorded on the subsequent anti-cancer therapy eCRF. Subsequent anti-cancer therapy data following curative intent surgical resection will be summarized separately from subsequent anti-cancer therapy reported following disease progression.

12.4.4. Efficacy Analyses

Unless otherwise specified, all efficacy analyses will be performed on the mITT population. Unless otherwise specified, myelopreservation efficacy will be evaluated based on the data that are collected during Induction as of the data cutoff date of the first planned analysis. Treatment effects on PFS and OS will be evaluated based on the estimated number of events and will not be limited to where the patient is in the study (e.g., Induction, Maintenance or Survival Follow-Up).

To ensure strong control of family-wise Type I error rate at the level of 2-sided 0.05 when performing statistical analyses for the two primary myelosuppression endpoints (DSN in Cycle 1 – 4 and occurrence of SN during Induction) and OS, the overall 2-sided α of 0.05 will be split between two groups of analysis: the analyses of the primary endpoints (using $\alpha = 0.04$), and the analysis for OS (using $\alpha = 0.01$). Testing strategies within each group and the recycling algorithm from the first group to the next are described in Section 12.4.4.2 and Section 12.4.4.3.1, respectively.

12.4.4.1. Analyses of Primary Myelosuppression Endpoints: DSN in Cycle 1 – 4 and Occurrence of SN during Induction

Severe neutropenia (SN) is defined as an ANC of $< 0.5 \times 10^9$/L (Grade 4). Hematology laboratory assessments obtained at scheduled and non-scheduled visits will all be included to identify SN events and in the DSN derivation.

The co-primary endpoint DSN in Cycle 1 – 4, is defined as the number of days of the first SN event that occurred in the first 4 cycles of Induction. Specifically, for patients with at least 1 SN event in Induction in Cycle 1, 2, 3 or 4, DSN is calculated for the first occurrence of the event following the rules described below.

- For patients whose SN is resolved (defined as an ANC $\geq 0.5 \times 10^9$/L at a date after the initial SN occurrence and maintained until the end of the cycle), DSN will be derived as the number of days from the date of the first SN occurrence to the date of SN resolution.
- For patients who withdraw from the study with unresolved neutropenia, DSN will be derived as the number of days from the date of the first SN occurrence to the date of withdrawal (i.e., the last date in the study).

For patients without any SN in Cycle 1– 4 of Induction, or those who randomized but who did not receive any study drug, DSN will be set to 0.

DSN is a continuous random variable but is not normally distributed given that a big proportion of the values are set as 0 per the definition described above.

Treatment effect on DSN in Cycle 1 – 4 will be evaluated using 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, Western Europe, and China), and prior therapy in the adjuvant/neoadjuvant setting (Yes or No) with the rank-transformed baseline ANC (within each stratum) as a covariate. In addition, the mean difference (trilaciclib – placebo), the standard error and the 96% confidence interval for the difference 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 thus is a binary variable The treatment effect for occurrence of SN will be evaluated using modified Poisson regression (Zou, 2004). The model will include the treatment, region, and prior therapy in the adjuvant/neoadjuvant setting (yes/no) as the fixed effect with baseline ANC value as a covariate. The log-transformed number of cycles will be used as the offset in the model to account for the variable duration during Induction for each patient. The 2-sided p-value, adjusted relative risk (aRR) (trilaciclib vs placebo) and its 96% confidence interval will be calculated.

12.4.4.2. Multiplicity Control for the Primary Myelosuppression Endpoints

For each of the primary endpoints, the treatment effect will be tested at 2-sided $\alpha_1 = 0.04$ level. That is, the statistically significant treatment effect for DSN in Cycle $1-4$ will be established if the 2-sided p-value from non-parametric ANCOVA model (Section 12.4.4.1) is ≤ 0.04, and the statistically significant treatment effect for SN during Induction will be established if the 2-sided p-value from the modified Poisson model (Section 12.4.4.1) is ≤ 0.04. When the statistically significant treatment effects are established for both primary endpoints, the primary objective of the study is met.

12.4.4.3. Analysis of Key Secondary Anti-Tumor Efficacy Endpoint: Overall Survival

OS will be calculated as the time (months) from the date of randomization to the date of death due to any cause. Patients who do not die during the study will be censored at the date last known to be alive. Patients lacking data beyond the date of randomization will have their survival time censored at the date of randomization. OS will not be censored if a patient receives subsequent anti-cancer treatments after discontinuation of the study drugs.

12.4.4.3.1. Statistical Significance Level and Testing Strategy for Testing Treatment Effect on Overall Survival

As described in Section 12.4.4, a Type I error rate of 2-sided $\alpha_2 = 0.01$ is originally assigned to the analysis for OS with $\alpha_1 = 0.04$ being used for testing treatment effect on the primary endpoints (DSN in Cycle $1-4$ and occurrence of SN in Induction). If the statistically significant treatment effect are established at 0.04 level for the two primary endpoints, then $\alpha_1 = 0.04$ will be added onto to $\alpha_2 = 0.01$ to allow a full α of 2-sided 0.05 to test the treatment effects on OS following the fallback procedure (Wiens, 2003). That is, there are two scenarios for statistical significance level in testing OS:

- **Scenario 1:** If one of the primary endpoints fails to establish the statistical significance, OS will be tested at 2-sided 0.01 level.
- **Scenario 2:** If the statistical significance for DSN in Cycle $1-4$, and occurrence of SN are established at the level of 2-sided 0.04, OS will be tested at 2-sided 0.05 level.

12.4.4.3.2. Statistical Analysis Models

The Kaplan-Meier plot will be produced and the within-group median, 25% and 75% percentile of time-to-event will be estimated using the Kaplan-Meier method with their corresponding 95% confidence interval, which are calculated based on the method by Brookmeyer and Crowley

(1982). Additionally, Kaplan-Meier estimates will be provided for the survival probability along with their 95% confidence intervals (Kalbfleisch, 1980) at selected landmarks, for example, at 12, 24, and 36 months.

The treatment effect will be evaluated using a stratified log-rank test accounting for factors of region, prior therapy in the adjuvant/neoadjuvant setting, and BRAF status. The HR between the 2 treatment groups (trilaciclib vs placebo), together with its (1-α) x100% confidence intervals, will be calculated from a Cox proportional hazard model with the same factors as included in the stratified log-rank test.

12.4.4.4. Analysis for Secondary Myelosuppression Endpoints

The detailed derivation for these endpoints along with their analysis methods will be included in the SAP. The myelosuppression endpoints will be used to evaluate the potential of trilaciclib to prevent or mitigate CIM by assessing effects on multiple lineages (e.g., neutrophils, RBCs, platelets) and current standard of care interventions to treat CIM (e.g., G-CSF administration, ESA administration, RBC or platelet transfusion, and dose reductions).

In general, a binary response variable, eg, occurrence of FN adverse events or Grade 3 or 4 decreased hemoglobin laboratory values (measured by hemoglobin laboratory value < 8.0 g/dL) in Induction, will be analyzed using the modified Poisson regression model (Zou, 2004) as outlined in Section 12.4.4.1 with the corresponding baseline laboratory value as a covariate. A counting variable, e.g., number of RBC transfusions on/after Week 5, will be analyzed using a negative binomial regression model. The model contains the same terms as included in the modified Poisson model. In both the modified Poisson and negative binomial models, the variable duration of Induction for each patient will be adjusted in assessing treatment effect by using the log-transformed cycles (or weeks) as the offset variable in the model. In addition, baseline variation of the corresponding laboratory values will also be adjusted by including baseline value as a covariate in the model. For both models, the 2-sided p-value, aRR (trilaciclib vs placebo), and 95% confidence interval will be generated and presented.

12.4.4.5. Analysis for Secondary Anti-tumor Efficacy Endpoints

Secondary anti-tumor endpoints will include the following:

- Objective response (complete response [CR] or partial response [PR])
- Best overall response (BOR)
- Duration of objective response (DOR)

At each tumor assessment visit, an overall time point response by RECIST v1.1 will be determined programmatically using the measurements provided by the Investigator for target lesions, non-target lesions, and new lesions collected in the eCRF. Best overall response (BOR) will be determined using all visit responses prior to or on the date of (i) radiographic disease progression; (ii) withdrawal of consent to obtain scans; (iii) death; (iv) lost to follow-up; or (v) initiation of subsequent anti-cancer therapy other than the study drugs, whichever is earlier.

Objective response rate (ORR) along with its exact 95% two-sided confidence interval using the Clopper-Pearson method will be computed for each treatment group. The treatment effect on ORR will be evaluated using a stratified Cochran–Mantel–Haenszel (CMH) method to account

for the factors of region, prior therapy in the adjuvant/neoadjuvant setting, and BRAF status. The adjusted proportion difference (trilaciclib vs placebo) and its 95% confidence intervals will be calculated using Cochran–Mantel–Haenszel (CMH) weight (as described in Kim, 2013).

DOR is 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. Patients who do not experience objective CR or PR will not be included in the analysis. For patients with objective response of CR or PR but (i) withdrew consent to obtain scans; (ii) is considered lost to follow up; (iii) initiated subsequent anti-cancer therapy other than the study drugs, or (iv) died, the DOR of this patient will be censored at the earliest date among these four.

The Kaplan-Meier method will be used to estimate the median, 25% and 75% percentile of DOR for each treatment group, along with the 95% confidence interval which is calculated using the method by Brookmeyer and Crowley (1982).

The analysis for objective response, BOR, and DOR will be based on response evaluable population, unless otherwise specified.

12.4.4.6. Analysis of Secondary Endpoint – Progression Free Survival

PFS is defined as the time (number of months) from date of randomization until date of documented radiologic disease progression per RECIST v1.1 or death due to any cause, whichever comes first. More specifically, PFS will be determined using all data until the last evaluable visit prior to or on the date of (i) radiographic disease progression per RECIST v1.1; (ii) withdrawal of consent to obtain additional scans on study; or (iii) initiation of subsequent anti-cancer therapy other than the study drugs whichever is earlier. Death is always categorized as a confirmed progressive disease (PD) event. Censoring rules for patients who do not experience PD or death will be described in the study SAP.

PFS analysis will be performed twice in this study. The first time is around the time point of 35 months post first randomization and the second time at the end of the study.

The Kaplan-Meier plots will be produced and the within-group median, 25% and 75% percentile of time-to-event will be estimated using the Kaplan-Meier method with their corresponding 95% confidence interval, which are calculated based on the method by Brookmeyer and Crowley (1982).

The treatment effect will be evaluated using a stratified log-rank test accounting for factors of region, prior therapy in the adjuvant/neoadjuvant setting, and BRAF status. The HR between the 2 treatment groups (trilaciclib vs placebo), together with its (1-α) x100% confidence intervals, will be calculated from a Cox proportional hazard model with the same factors as included in the stratified log-rank test.

12.4.4.7. Analysis of Secondary Endpoint - Time to Confirmed Deterioration of Fatigue

The secondary endpoint, TTCD-fatigue, is calculated from the patient reported outcome FACIT-F. Time (months) to the first deterioration of fatigue (confirmed at the next visit) is calculated as the duration of time from date of randomization to the date of first deterioration which is

confirmed at the next visit. 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).

For time to first deterioration of fatigue, the Kaplan-Meier plots will be produced and the median, 25% and 75% percentile of TTCD-fatigue will be estimated using Kaplan-Meier method with their corresponding 95% confidence interval, which are calculated based on the method by Brookmeyer and Crowley (1982). Treatment group difference in TTCD-fatigue will be evaluated using a stratified log-rank test to account for the factors of region and prior therapy in the adjuvant/neoadjuvant setting. The HR between the 2 treatment groups (trilaciclib vs placebo), together with its 95% confidence intervals, will be calculated from a Cox proportional hazard model with the same terms as used in the stratified log-rank test.

12.4.4.8. Analysis of Exploratory Endpoints

The FACT (FACT-An and FACT-C), EQ-5D-5L, and PGIC/PGIS instruments will be used to assess the impact of trilaciclib on various quality of life measures. The analysis will be based on the mITT population.

12.4.5. Safety Analyses

Summaries of safety data will be performed using the safety population. Adverse event data will be coded to system organ class and preferred term using MedDRA (Version 23 or later). 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, chemotherapy, 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. AESIs for trilaciclib, AEs leading to study drug discontinuation, dose reductions, cycle delays, and use of concomitant medications to treat AEs will be tabulated separately.

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 (e.g., hematology, chemistry) will be characterized according to NCI-CTCAE, Version 5.0, 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 the safety evaluation. Graphical presentations of safety data will be presented as is deemed appropriate.

Relative dose intensity of chemotherapy (fluorouracil, leucovorin, irinotecan, oxaliplatin, bevacizumab), dose modifications, dose reduction, dose interruptions, chemotherapy delay, compliance, and patient exposure will be summarized for each study therapy component, where appropriate.

12.4.5.1. Analysis of Anti-tumor Endpoints by CDK4/6 Biomarker Signature

In addition to summarizing the distribution of CDK4/6 signature by treatment groups as described in Section 11.8.2, selected anti-tumor endpoints (ORR, PFS, and OS) will be evaluated by subgroup of patients with different CDK 4/6 signature status (i.e., CDK4/6 independent, CDK4/6 dependent, and CDK4/6 indeterminate). Within each type of signature, the analysis methods for PFS and OS will follow the approaches as outlined in Section 12.4.4.3.2, and method for ORR will follow the approach as outlined in Section 12.4.4.5.

The planned timing of the analysis is detailed in Section 12.3. The evaluation of CDK4/6 status will be performed retrospectively when final OS results are available.

12.4.6. Pharmacokinetic Analyses

The pharmacokinetics of trilaciclib and any metabolites will be determined using a non-linear mixed effects modeling approach. Population pharmacokinetic parameters including CL, V, and other parameters will be estimated as data permit. Alternatively, if population pharmacokinetic models already exist, the predictability of the model to describe trilaciclib (and any metabolites) concentration obtained in this study will be evaluated. Population pharmacokinetic modeling will be performed using the non-linear mixed effects software such as NONMEM (ICON Development Solutions, Ellicott City, MD) or other similar software. Further details of population pharmacokinetic analyses will be described in the population pharmacokinetic/pharmacodynamic analysis plan.

12.4.7. Pharmacokinetic/Pharmacodynamic Analyses

If data warrant, exploratory analyses may be performed to examine the relationship(s) between exposure to trilaciclib (and any metabolites) and pharmacodynamic endpoints or other clinical and safety endpoints. Further details of population PK/pharmacodynamic analyses will be described in a separate population PK/pharmacodynamic analysis plan.

13. ETHICS

13.1. Ethics Review

The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), International Council for Harmonisation (ICH) guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

13.2. Ethical Conduct of the Study

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
- Applicable ICH Good GCP Guidelines
- Applicable laws and regulations

13.3. Written Informed Consent

The Principal Investigator(s) at each center will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Principal Investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the patient.

Recommendations for the informed consent process are provided in Section 17.1.

14. DATA HANDLING AND RECORDKEEPING

14.1. Data Protection

Patients will be assigned a unique identifier by the Sponsor. Any patient records or datasets that are transferred to the Sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred.

The patient must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient who will be required to give consent for their data to be used as described in the informed consent.

The patient must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

14.2. Data Quality Assurance

- All patient data relating to the study will be recorded on eCRF unless transmitted to the Sponsor or Designee electronically. The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or Designee is responsible for the data management of this study including quality checking of the data.
- Study Monitors will perform ongoing source data verification at the frequencies and SDV extent as outlined in the Monitoring Plan to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study are required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and

FDA is notified. However, if required by local regulation that these documents should be retained for a longer period, then Retention of Documentation will abide by local regulation, no records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained.

A study-specific COVID-19 Assessment Plan will be utilized for any necessary modifications and/or mitigation to the data collection, monitoring or other associated activities during this study due to the COVID-19 pandemic.

14.3. Source Documents

Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

14.4. Audits and Inspections

Authorized representatives of G1 Therapeutics, a regulatory authority, an IEC or IRB may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. The Investigator should contact G1 Therapeutics immediately if contacted by a regulatory agency about an inspection.

15. PUBLICATION POLICY

By signing the study protocol, the Investigator and his or her institution agree that the results of the study may be used by G1 Therapeutics for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement.

Initial publication of the results of this study will be of a cooperative nature that may include authors representing the Sponsor, Investigator(s), and collaborating scientists. Independent publications by involved individuals may follow. Investigators and their institutions agree not to publish or publicly present any interim results of studies without the prior written consent of G1 Therapeutics. G1 Therapeutics reserves the right to request modification of any publication, presentation or use by the Investigator if such activity may jeopardize a patent application, an existing patent, or other proprietary rights. G1 Therapeutics shall determine order of authorship of any publication combining all clinical results of this trial.

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17. APPENDICES

17.1. Informed Consent Process Recommendations

- The Investigator or his/her representative will explain the nature of the study to the patient and answer all questions regarding the study.
- Patients must be informed that their participation is voluntary. Patients or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before any study related procedures in the study were performed and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Patients must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the patient or the patient's legally authorized representative.

17.2. Clinical Laboratory Tests

- The timing and laboratory tests detailed in Schedule of Assessments (Table 4 and Table 5) will be performed by a local laboratory.
- Protocol-specific requirements for inclusion or exclusion of patients are detailed in Section 7 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations

Table 12: Protocol-specified Safety Laboratory Assessments

Laboratory Assessment	Parameters			
Hematology	Platelet Count	Hemoglobin		
	Complete Blood Count (CBC) with Differential (% and/or absolute values): neutrophils, monocytes, lymphocytes			
Clinical Chemistry	Blood Urea Nitrogen (BUN) or Urea	Serum Creatinine	Lactate Dehydrogenase	Albumin
	Aspartate Aminotransferase (AST)	Alanine Aminotransferase (ALT)	Alkaline Phosphatase	Total Bilirubin
	Potassium	Sodium	Calcium	Chloride
	Inorganic Phosphorous or Phosphate	Total Protein	Glucose (non-fasting)	
Coagulation	International Normalized Ratio (INR)		Activated Partial Thromboplastin Time (aPTT)	
Urinalysis	Semiquantitative dipstick: specific gravity, pH, evaluation of glucose, protein, bilirubin, ketones, leukocytes, and hemoglobin			
	Microscopic examination (including RBC, WBC, and casts) will be performed, if clinically warranted			
Other Tests	Serum or urine human chorionic gonadotropin (hCG) pregnancy test (for women of childbearing potential only)			
	serum FSH for postmenopausal females (Screening only)			
	DPD testing – genetic testing or blood uracil level (Screening only)			

To guarantee the reliability of blood uracil results, the following conditions are recommended:

- use of tubes without separating gel and with anticoagulant to carry out the sampling (restriction of sampling times not mandatory);
- time between sampling and centrifugation of 1h30 if the sample is stored at room temperature and 4h if it is placed at + 4°C;
- centrifugation preferably at + 4°C then immediate freezing of the obtained plasma;
- transport must respect the cold chain.

Failure to comply with these conditions must lead to a report of non-compliance brought to the attention of the clinician as soon as possible so that a new sample can be sent to the laboratory quickly.

17.3. Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

17.3.1. Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study patient, temporally associated with the use of study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.
Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant and require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) in the medical and scientific judgment of the Investigator (i.e., not related to progression of underlying disease) unless they are associated with an already reported clinical event, e.g. elevated liver enzymes in a patient with jaundice. • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the patient's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

17.3.2. Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
1. Results in death
2. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
4. Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
5. Is a congenital anomaly/birth defect
6. Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. • Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

17.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the eCRF.
- It is not acceptable for the Investigator to send photocopies of the patient's medical records to G1 Therapeutics (or designee) in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by G1 Therapeutics (or designee). In this case, all patient identifiers, with the exception of the patient number, will be redacted on the copies of the medical records before submission to G1 Therapeutics (or designee).
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- All SAEs should be reported to G1 Therapeutics PVG (or designee) within 24 hours of notification on a SAE Form in the eCRF. Any relevant source data related to the SAE that cannot be entered in the EDC should be emailed or faxed to G1 Therapeutics PVG (or designee):

  G1 Therapeutics Pharmacovigilance

  Email: safetyreporting@g1therapeutics.com

  Fax: +1-984-285-7131

Assessment of Intensity

Intensity will be assessed using NCI-CTCAE v5.0 criteria, as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

AE and SAE Recording

Assessment of Causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The Investigator will also consult the IB and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to G1 Therapeutics PVG (or designee). However, it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to G1 Therapeutics PVG (or designee).
- The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by G1 Therapeutics PVG (or designee) to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any new or updated SAE data to G1 Therapeutics PVG (or designee) within 24 hours of receipt of the information:

  G1 Therapeutics Pharmacovigilance

  Email: safetyreporting@g1therapeutics.com

  Fax: +1-984-285-7131

17.3.4. Reporting of SAEs

SAE Reporting to G1 Therapeutics (or designee) via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE to G1 Therapeutics (or designee) will be the electronic data collection tool (EDC). • If the electronic system is unavailable, then the site will use the paper SAE Report Form in order to report the event within 24 hours via email or fax (see below for SAE reporting contact information). • The site will enter the SAE data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study patient or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site should report this information on a paper SAE Report form or notify the Medical Monitor by telephone. • Contact for SAE reporting: **G1 Therapeutics Pharmacovigilance** **Email: safetyreporting@g1therapeutics.com** **Fax: +1-984-285-7131**

17.4. Contraceptive Guidance and Collection of Pregnancy Information

WOCBP Definition

Woman of Childbearing Potential

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (e.g., amenorrhea in adolescents or athletes), additional evaluation should be considered.

Women in the following categories are not considered Woman of Childbearing Potential

1. Premenarchal

   Note: Documentation can come from the site personnel's review of the patient's medical records, medical examination, or medical history interview.

2. Premenopausal female with 1 of the following acceptable surgical sterilization techniques: complete or partial hysterectomy, bilateral tubal ligation or occlusion with surgery at least 6 months prior to dosing, or bilateral oophorectomy with surgery at least 2 months prior to dosing.

3. Postmenopausal female: defined as spontaneous amenorrhea for > 12 months prior to screening without alternative cause (e.g., implantable contraceptive, side effect of medication, etc.) and a serum follicle stimulating hormone (FSH) within the laboratory's reference range for postmenopausal females.

   - Women taking hormone replacement therapy (HRT) must discontinue HRT at least 2-4 weeks prior to screening for accurate assessment of FSH (though exact interval will depend on the type and dosage of HRT and should be determined by the principal Investigator).

Contraception Guidance

Contraceptive use by men or women should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

- Male patients: Males must be surgically sterile prior to screening with appropriate documentation (absence of sperm in ejaculate 6 months after procedure) or have a female partner(s) who is either postmenopausal, surgically sterile, or using 2 forms of concurrent contraception as defined below. In addition, males must also refrain from sperm donation during the study and utilize a barrier method with intercourse during and for 6 months following discontinuation of chemotherapy.
- Female patients: All females of childbearing potential must have a negative serum beta human chorionic gonadotropin (β-hCG) test result at screening, and negative serum or urine pregnancy test results approximately every month at the start of a cycle during Induction and Maintenance and at the post-treatment visit.

- Females must be either postmenopausal, surgically sterile, or agree to use 2 concurrent forms of contraception during the study and for 6 months following last dose of chemotherapy. Acceptable forms of contraception include:
  - Established use of oral, injected or implanted hormonal methods of contraception (stable dose at least 3 months prior to dosing)
  - Intrauterine device or intrauterine system
  - Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository. Barrier methods alone (without spermicide) are not acceptable methods. Likewise, spermicide alone is not an acceptable method
  - Male sterilization prior to screening with the appropriate post-vasectomy documentation (absence of sperm in the ejaculate 6 months after procedure). For female patients on the study, the vasectomized male partner should be the sole partner for that patient
  - True abstinence when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception
- For patients who are exclusively in same-sex relationships, contraceptive requirements do not apply. If a patient who is in a same-sex relationship at the time of signing the ICF becomes engaged in a heterosexual relationship, they must agree to use contraception as described previously. If a patient who is abstinent at the time of signing the ICF becomes sexually active, they must agree to use contraception as described above.

Collection of Pregnancy Information

Male participants with partners who become pregnant

- The Investigator or designee will attempt to collect pregnancy information on any male patient's female partner who becomes pregnant while the male patient is in this study. This applies only to males who receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator or designee will record pregnancy information on the Pregnancy Reporting and Outcome Form and submitted to G1 Therapeutics PVG or designee within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to G1 Therapeutics PVG or designee. Generally, the follow-up will be no longer than 12 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Patients who become pregnant

- The Investigator or designee will collect pregnancy information on any female patient who becomes pregnant while participating in this study. The initial Information will be recorded on the Pregnancy Reporting and Outcome Form and submitted to G1 PVG or designee within 24 hours of learning of a patient's pregnancy within 24 hours of learning of a patient's pregnancy.
- The patient will be followed to determine the outcome of the pregnancy. The Investigator or designee will collect follow-up information on the patient and the neonate and the information will be forwarded to G1Therapeutics PVG or designee. Generally, follow-up will not be required for longer than 12 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE. A spontaneous abortion (occurring at < 22 weeks gestational age) or still birth (occurring at > 22 weeks gestational age) is always considered to be an SAE and will be reported as such. Any post-study pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to G1 Therapeutics PVG or designee. While the Investigator is not obligated to actively seek this information in former study patients, he or she may learn of an SAE through spontaneous reporting.
- Any female patient who becomes pregnant while participating in the study will discontinue study intervention.

17.5. FOLFOXIRI Components Package Inserts

Links to US package inserts in this section are provided for reference only. Please refer to the inserts with the product(s) available at your individual center for the most current and region-specific information.

17.5.1. Fluorouracil

US package insert:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/012209s040lbl.pdf

17.5.2. Leucovorin

US package insert:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/040347s010lbl.pdf

17.5.3. Oxaliplatin

US package insert:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021492s011,021759s009lbl.pdf

17.5.4. Irinotecan

US package insert:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/020571s048lbl.pdf

17.6. Bevacizumab Package Insert

The following link to the US package insert is provided for reference only. Please refer to the insert with the product available at your individual center for the most current and region-specific information.

US package insert:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125085s319lbl.pdf

17.7. 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 (Eisenhauer, 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, non-cystic 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.

Timepoint Response

Patients with Measurable Disease (i.e., Target ± Non-Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	NE	No	PR
PR	Non-CR/Non-PD/NE	No	PR
SD	Non-CR/Non-PD/NE	No	SD
NE	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD[a]	Yes or No	PD
Any	Any	Yes	PD

CR=complete response; NE=not evaluable; PD: progressive disease; PR=partial response; SD=stable disease.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.
[a] Unequivocal progression in non-target lesions may be accepted as disease progression.

Patients with Evaluable or Non-Measurable Disease Only (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[a]
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR=complete response; NE=not evaluable; PD: progressive disease; SD=stable disease.

[a] Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Clinical Study Protocol G1T28-207_PRESERVE 1
A Phase 3 Randomized, Double-blind Trial of Trilaciclib versus Placebo in Patients Receiving FOLFOXIRI/Bevacizumab for Metastatic Colorectal Cancer

Summary of Changes

Amendment Version 6.1	Date: 15 December 2022
Rationale of Changes:	Herein is a summary of the changes made to Amendment Version 5.1 of the study dated 21 November 2022 and reflected in amendment version 6.1 dated 15 December 2022. Changes are clearly identified in the track changes version of the amendment. Minor administrative changes are not described below. This amendment is considered a non-substantial amendment. Changes to key secondary objective and endpoint: 1. Synopsis: The key secondary objective of progression free survival (PFS) was deleted because this objective is no longer considered a key secondary objective for this study. 2. Section 5: PFS was removed as a key secondary objective/endpoint and was added as a secondary objective and endpoint to evaluate anti-tumor activity. 3. Section 5.1: The rationale for the PFS as an anti-tumor efficacy endpoint in the study was deleted since PFS is no longer a key secondary endpoint in the study. 4. Section 12: PFS was deleted in every mention as an objective and endpoint since this is no longer considered a key secondary endpoint and it was clarified that OS is the only key secondary endpoint in the study. Changes to the sample size determination and timing of planned analyses: 5. Synopsis: PFS was deleted from the sample size justification as it is no longer a key secondary objective. 6. Synopsis: Assumptions for the number of PFS events were deleted as PFS is no longer a key secondary endpoint. The timing of the final database lock for OS analysis was added to the Synopsis. 7. Section 12.1: Assumptions around the accrual of PFS events were deleted as PFS is no longer a key secondary endpoint. Wording around the assumptions for OS events were updated to reflect that OS is the only key secondary endpoint. 8. Section 12.3.2: OS was removed as a potential analysis to occur at the time of the second planned analysis. The second analysis will now be for PFS and occur around the time that is 35 months post first randomization. 9. Section 12.3.3: The third analysis was updated to remove "optional," and text was added to specify that the final database lock for the OS analysis will occur "at the time when 157 death events are observed or at 52 months after the date of first randomization, whichever comes first." PFS analysis at the time of the final study database lock was added. Changes to the statistical analysis method for primary and secondary endpoints: 10. Synopsis: PFS was deleted from the testing strategy and analysis sections since it is no longer a key secondary endpoint. Instead, OS will be the only key secondary endpoint. 11. Synopsis: The analysis section was updated with the addition of the following text to reflect the timing of the analysis as follows: "At the time when 157 death events are observed or at 52 months after the date of first randomization, whichever comes first, the study will be concluded, and the final study database will be locked to perform the OS analysis." 12. Section 12.4.4.3: PFS, the definition of PFS and the method of determining PFS were deleted as PFS was removed as a key secondary endpoint. 13. Section 12.4.4.3.1: This entire section, 'Timing for PFS and OS analysis' was deleted, as the timing of the final OS analysis is described in other sections. 14. Section 12.4.4.3.2.: Language regarding the hierarchical testing strategy for PFS and OS was deleted as it is no longer applicable with the removal of PFS as a key secondary endpoint and OS as the only key secondary endpoint.

	15. Section 12.4.4.6: Section was added to reflect PFS as a secondary endpoint. Added to this section were the definition of PFS, the timing of the two PFS analyses, description of the Kaplan-Meier plots and associated calculations, and evaluation of the treatment effect.