Protocol Title: A Phase 2, Open-Label, Single-Arm Study of Single-Dose Lead-In and Neoadjuvant Trilaciclib and Chemotherapy in Patients with Early-Stage Triple Negative Breast Cancer (TNBC)

Protocol Number: G1T28-212

Compound: Trilaciclib for Injection, 300 mg/vial

Study Phase: 2

Study Name: G1T28-212

Sponsor Name: G1 Therapeutics, Inc.

Legal Registered Address: G1 Therapeutics, Inc.
700 Park Offices Dr. Suite 200
PO Box 110341
Research Triangle Park, NC 27709

Regulatory Agency Identifier Numbers: IND 145091

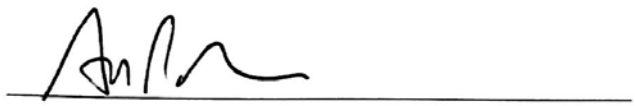
Approval Date: 23 Mar 2022 (Version 2.0)

Previous Version: 30 Jun 2021 (Version 1.0)

PROTOCOL SIGNATURE PAGE

Sponsor's Approval

I have read and understand the contents of this clinical protocol, Version 2.0 for Study G1T28-212 dated 23 Mar 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.



23 MARCH 2022

Andrew Beelen, MD
Vice President, Clinical Development
G1 Therapeutics, Inc.

Date

INVESTIGATOR'S AGREEMENT

Clinical Study Protocol G1T28-212: A Phase 2, Open-Label, Single-Arm Study of Single-Dose Lead-In and Neoadjuvant Trilaciclib and Chemotherapy in Patients with Early-Stage Triple Negative Breast Cancer (TNBC)

Protocol (Version 2.0) Issue Date: 23 Mar 2022

I have read the G1T28-212 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, Inc.		
Name of Investigational Product: Trilaciclib for Injection, 300 mg/vial		
Name of Active Ingredient: trilaciclib dihydrochloride (hereafter referred to as trilaciclib) (G1T28)		
Protocol Number: G1T28-212	**Phase:** 2	**Regions:** United States
Title of Study: A Phase 2, Open-Label, Single-Arm Study of Single-Dose Lead-In and Neoadjuvant Trilaciclib and Chemotherapy in Patients with Early-Stage Triple Negative Breast Cancer (TNBC)		
Study centers: Approximately 10 centers		
Study period (years): Approximately 15 months Date first patient enrolled: Q1 2022 Estimated date last patient enrolled: Q3 2022 Estimated date last patient completed: Q1 2023		
Objectives: Primary objective: To evaluate the immune-based mechanism of action of trilaciclib after a single-dose as measured by the change in cluster of differentiation 8 positive (CD8+) tumor-infiltrating lymphocytes (TILs)/regulatory T cell (Treg) ratio in tumor tissue. Secondary objectives: To assess the pathologic complete response (pCR) rate at the time of definitive surgery. To evaluate the safety and tolerability of trilaciclib in combination with standard neoadjuvant systemic therapies. Exploratory objectives: To characterize the intratumoral immune profile following trilaciclib. To evaluate the kinetics of the peripheral immune response following trilaciclib. To identify molecular and cellular biomarkers in tumor or blood samples that may be indicative of clinical response/resistance, pharmacodynamic activity, and/or the mechanism of action of trilaciclib and other systemic treatments.		
Study Design: This is a Phase 2 multicenter, open-label, single-arm, neoadjuvant study with 4 phases: 1) Screening Phase, 2) Trilaciclib Lead-In Phase, 3) Treatment Phase, and 4) Surgery and Follow-Up Phase. During the Screening Phase, tumor tissue (sample #1) will be obtained at baseline prior to any study drug. This sample may be archival tissue, or if unavailable/insufficient, then a fresh biopsy is required. Patients with adequate archival tissue will also have the option to undergo an additional fresh biopsy at baseline. Patients will receive a single dose of monotherapy trilaciclib 240 mg/m^2 in the Trilaciclib Lead-In Phase, followed by tumor biopsy (sample #2) 7 (±1) days later. Following the biopsy, patients will enter the Treatment Phase in which trilaciclib 240 mg/m^2 on Day 1 of each cycle will be administered along with dose-dense anthracycline/cyclophosphamide followed by weekly taxane chemotherapy (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 [AC] every 2 weeks for 4 cycles [Cycles 1-4], then weekly paclitaxel [T] 80 mg/m^2 weekly for 12 cycles [Cycles 5-16]). If		

pembrolizumab is given (per Investigator discretion), it will start with AC (Cycle 1 of chemotherapy). If carboplatin is given (per Investigator discretion) it will start with paclitaxel (Cycle 5).

Three to 5 weeks after the last dose of chemotherapy, patients will proceed to definitive surgery at which time tumor tissue (sample #3) will be collected if the patient has residual disease. A 30-day Safety Follow-up Visit will occur 30 (+7) days after last dose of trilaciclib and an End of Study Visit will occur within 14 days after surgery. Both the Safety Follow-up Visit and End of Study Visit may be conducted in person or by telephone and may occur at the same visit.

Methodology:

Sample Size Justification:

Sample size justification is based off of previously published data on the CD8+/Treg ratio from early stage TNBC patients who underwent neoadjuvant chemotherapy. Using a 2-sided significance level of 0.05, and anticipating that 10% patients enrolled to this study will not have paired data (resulting in 27 patients with paired data), the power of the study to detect a respective mean of paired difference in CD8+/Tregs ratio using paired Wilcoxon signed-rank tests has been calculated using PASS 2019 (v19.0.3) and is presented in the table below.

Mean Change in CD8+/Tregs	Power
1.8	72%
2.0	81%
2.3	90%
2.5	94%
Based on the assumption of an estimated standard deviation of paired differences of 3.4.	

Number of patients (planned): Approximately 30 patients are planned to be enrolled in this study.

Diagnosis and main eligibility criteria:

Female patients ≥18 years of age at the time of signing the informed consent with treatment-naïve early-stage breast cancer in which neoadjuvant chemotherapy with an anthracycline/taxane combination is deemed to be a suitable therapy and curative surgery is intended. Documented diagnosis of TNBC (defined as <1% estrogen receptor [ER] and progesterone receptor [PR] positive stained cells utilizing an assay consistent with local standards and HER2-negative as per 2018 College of American Pathologists criteria), a primary tumor ≥1.5 cm, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Patients must also have adequate organ function as demonstrated by laboratory values and left ventricular ejection fraction of ≥50% or ≥ institution lower limit of normal (LLN). If the Investigator chooses to add pembrolizumab, there must be no active autoimmune disease that has required systemic treatment in the past 2 years or corticosteroids within the past 7 days. Patients are required to have baseline archival tumor tissue available or a fresh biopsy must be obtained, and patient must agree to an on-treatment research biopsy.

Investigational product, dosage, and mode of administration:

Trilaciclib will be administered as monotherapy as a single dose of 240 mg/m^2 intravenously (IV) over 30 minutes during the Trilaciclib Lead-In Phase of the study, and then at the same dose with each chemotherapy cycle during the Treatment Phase.

Duration of treatment: Study treatment will continue as per protocol to completion or early discontinuation of chemotherapy, until unacceptable toxicity, Investigator's decision to withdraw the patient from study treatment, pregnancy of the patient, noncompliance with study treatment or

procedure requirements, consent withdrawal, becoming lost-to-follow-up, death, or administrative reasons requiring cessation of treatment.

Standard of care chemotherapy backbone, dosage, and mode of administration:

Doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 will be administered IV once every 2 weeks in Cycles 1-4, followed by paclitaxel 80 mg/m^2 administered IV once every week in Cycles 5-16.

Treatment per Investigator discretion, dosage, and mode of administration:

Carboplatin: If carboplatin is administered, the dose amount required will be calculated as area under the curve (AUC) at a dose level of AUC 1.5 weekly during paclitaxel treatment (Cycle 5-16). Carboplatin will be administered IV.

Pembrolizumab: If pembrolizumab is administered, the dose level will be 400 mg every 6 weeks starting with Cycle 1 of AC and continued while receiving chemotherapy. Pembrolizumab will be administered as an IV infusion.

Growth factor support:

Granulocyte-colony stimulating factor (G-CSF) should be administered after each cycle of AC chemotherapy or per Investigator discretion and as clinically indicated for subsequent cycles with paclitaxel. G-CSF 5 μg/kg/day administered subcutaneously (SC) should be initiated at 24 hours post-dose and continued for at least 72 hours. Pegfilgrastim administered SC as either a single dose of 100 μg/kg (individualized) or a single dose of 6 mg (general approach) at 24 hours after chemotherapy is also acceptable.

Criteria for evaluation:

Efficacy: pCR will be evaluated using the definition of ypT0/Tis ypN0 (i.e., no invasive residual in breast or nodes; residual noninvasive in breast allowed) as assessed by the local pathologist at the time of definitive surgery.

Safety: Safety will be evaluated by monitoring adverse events (AEs), clinical laboratory test results (hematology and serum chemistry), and vital sign measurements (blood pressure and pulse rate) as well as electrocardiogram (ECG) and ejection fraction changes if clinically indicated.

Statistical methods:

Summary statistics will be used to summarize data collected from this study. The descriptive summary for the categorical variables will include counts and percentages. The descriptive summary for the continuous variables will include means, standard deviations, medians, 25% and 75% percentiles, and minimum and maximum values. For the immune-response measures and other biomarkers, median will be used to present the central point of the population being studied.

Analysis population

The Full Analysis Set - 1 (FAS1) population includes enrolled patients who have received trilaciclib during the Trilaciclib Lead-in Phase. This dataset will be used to assess immune response in tumor tissue.

The Full Analysis Set – 2 (FAS2) population includes enrolled patients who have received at least one dose of a study drug during the Treatment Phase of the study. This dataset will be used to assess safety and tolerability and the rate of pCR for trilaciclib in combination with neoadjuvant systemic therapies.

Statistical analysis method for primary and secondary efficacy endpoints

Analysis for primary immune-response measure

Summary statistics for primary immune-response measure, the ratio of CD8+ TIL over Forkhead Box P3 (FOXP3)+ regulatory T cells (abbreviated as CD8+/Tregs), will be provided for measures taken at

baseline (pre-trilaciclib), 7 ± 1 days after single dose trilaciclib (post-trilaciclib), and the change scores (post minus pre). Median will be used as the central point with Q1 and Q3 as the measure of spread. The significance of the magnitude of change will be tested by paired Wilcoxon Signed-Rank test. Analyses on the primary endpoint will be based on the FAS1 population.

Analysis for pCR

For the clinical efficacy endpoint, pCR rate will be reported with a 95% confidence interval using the Clopper-Pearson method based on FAS2 population. In addition, patients will be dichotomized by median value of changes in CD8+/Tregs ratio, and pCR rate will be summarized by the subgroup of patients with high or low change scores. The association between pCR status and high/low in ratio change will be tested using a Chi-square test. These analyses will be performed on the FAS2 population.

Analysis for safety endpoints

For trilaciclib alone, safety will be assessed by AE reporting during the Trilaciclib Lead-in Phase based on FAS1 population. For trilaciclib in combination with other systemic therapies, safety and tolerability will be assessed by AEs, dose modification, laboratory tests, vital signs, and ECGs based on FAS2 population. AEs are defined as those events occurring or worsening after treatment has begun on this study. Collected AE terms will be coded to system organ class and preferred term using the latest version of MedDRA. AEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v5.0 by Investigators. AEs overall, AEs related to each study drug, AEs leading to study drug discontinuation or dose modification, and AEs of special interests for trilaciclib will be summarized by system organ class, preferred term and CTCAE grade, as appropriate. In the tabulation of severity and causality for an AE, if the same AE occurs on multiple occasions, the highest grade and strongest relationship to study drug will be used. Concomitant medications will be coded to Anatomical Therapeutic Classification using the World Health Organization-Drug Dictionary and summarized.

For laboratory assessments, vital signs, and ECG parameters, observed values and changes from baseline will be summarized. Chemistry and hematology laboratory parameters will be characterized according to CTCAE toxicity grade from 1 to 5, 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. Safety data collected through scheduled or unscheduled visits will all be included in the safety evaluation.

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

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

3. LIST OF ABBREVIATIONS

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

Table 1: Abbreviations

Abbreviation	Definition
5-FU	5-fluorouracil
AC	anthracycline/cyclophosphamide
AC/T	anthracycline/cyclophosphamide/taxane
AE	adverse event
AESI	adverse event of special interest
AJCC	American Joint Committee of Cancer
ALT	alanine aminotransaminase
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
β-hCG	beta human chorionic gonadotropin
BSA	body surface area
CBC	complete blood count
CD8+	cluster of differentiation 8 positive
CDK	cyclin-dependent kinase
CI	confidence interval
COVID-19	coronavirus disease 19
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	cytotoxic T-lymphocyte associated protein 4
CYP	cytochrome P450
DCIS	ductal carcinoma in situ
DDI	drug-drug interaction
DFS	disease-free survival
DSN	duration of severe neutropenia
ECG	electrocardiogram
ECHO	echocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EFS	event-free survival

Abbreviation	Definition
E/P	etoposide plus carboplatin
E/P/A	etoposide, carboplatin, and atezolizumab
ER	estrogen receptor
ES-SCLC	early-stage small cell lung cancer
FACT-B	Functional Assessment of Cancer Therapy - Breast
FAS	Full Analysis Ste
FDA	Food and Drug Administration
FFPE	formalin-fixed paraffin-embedded
FOXP3	Forkhead Box P3
FSH	follicle stimulating hormone
G_1	gap 1 phase of the cell cycle
GC	gemcitabine and carboplatin
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GnRH	gonadotrophin-releasing hormone
HBV	hepatitis B virus
HCV	hepatitis C virus
HER2	human epidermal growth factor receptor 2
HIV	human immunodeficiency virus
HRT	hormone replacement therapy
HSPC	hematopoietic stem and progenitor cell
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
ICI	immune checkpoint inhibitors
IEC	independent ethics committee
IFN	interferon
IHC	immunohistochemistry
ILD	interstitial lung disease
INR	international normalized ratio
IRB	institutional review board

Abbreviation	Definition
IV	intravenous
LAG-3	lymphocyte activation gene 3
LFT	liver function test
MATE1 or 2-K	multidrug and toxin extrusion 1 or 2-K
MedDRA	Medical Dictionary for Regulatory Activities
mCRC	metastatic colorectal cancer
MHC	major histocompatibility complex
MOA	mechanism of action
mRNA	messenger ribonucleic acid
MRP1 or 2	multidrug resistance protein 1 or 2
MUGA	multigated acquisition
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NFAT	nuclear factor of activated T cell
NSCLC	non-small cell lung cancer
NYHA	New York Heart Association
OAT1 or 3	organic anion transporter 1 or 3
OATP1B1 or 1B3	organic anion transporting polypeptide 1B1 or 1B3
OCT1 or 2	organic cation transporter 1 or 2
ORR	objective response rate
OS	overall survival
PAM50	Prosigna Breast Cancer Prognostic Gene Signature Assay
pCR	pathologic complete response
PCS	potentially clinically significant
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
PFS	progression-free survival
PK	pharmacokinetic(s)
PR	progesterone receptor
PRO	patient-reported outcome
PTFE	polytetrafluorethylene
PVG	pharmacovigilance

Abbreviation	Definition
Q3W	every 3 weeks
RBC	red blood cell
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SCLC	small cell lung cancer
SDV	source data verification
SMC	Safety Monitoring Committee
SN	severe neutropenia
SOC	standard of care
SUSAR	suspected unexpected serious adverse reactions
T	taxane
TEAE	treatment-emergent adverse event
TGIT	T cell Ig and ITIM domain
TIL	tumor-infiltrating lymphocyte
TIM3	T cell immunoglobulin and mucin domain 3
TNBC	triple negative breast cancer
Treg	regulatory T cell
ULN	upper limit of normal
US	United States
WHO-DD	World Health Organization Drug Dictionary

4. INTRODUCTION

4.1. Triple Negative Breast Cancer

Triple negative breast cancer (TNBC) is a heterogeneous disease with distinct pathological, genetic, and clinical features, and is associated with younger age and more advanced tumor stage at diagnosis, African American race/ethnicity, higher tumor grade, poor overall survival (OS), higher risk of disease recurrence, and higher recurrence in visceral organs within 5 years of diagnosis. Treatments which are effective for hormone receptor-positive breast cancer and human epidermal growth factor receptor 2 (HER2)-positive breast cancer, such as endocrine therapy or HER2-targeted therapies (e.g., trastuzumab), respectively, are not effective in TNBC, which lacks these markers. Neoadjuvant chemotherapy (systemic therapy given prior to definitive surgery) with an anthracycline/taxane-based chemotherapy regimen (e.g., AC/T chemotherapy) has been considered an important and standard part of the treatment strategy for patients with early-stage TNBC for both tumor control and improving the curability rate among subtypes (NCCN, 2021; Cardoso, 2019).

Alternative neoadjuvant chemotherapy regimens such as those including carboplatin are available to patients with early-stage TNBC, however definite superiority of one regimen has not been demonstrated. The addition of carboplatin to paclitaxel in the neoadjuvant setting increased pathologic complete response (pCR) rates (von Minckwitz, 2014; Sikov, 2015); however, the disease-free survival (DFS) and OS effect with this treatment has not been confirmed and the addition of the platinum-based chemotherapy comes with additional toxicities. More recently, agents that target immune checkpoint pathways, such as the programmed cell death protein 1 (PD-1)/programmed death ligand-1 (PD-L1) pathway, are in clinical development for TNBC, including in the neoadjuvant setting. Pembrolizumab is an anti-PD-1 monoclonal antibody, that has been approved for use in patients with metastatic TNBC with PD-L1 positive disease and recently for high-risk, early-stage TNBC in combination with chemotherapy as neoadjuvant treatment, and then continued as a single agent as adjuvant treatment after surgery (Keytruda® Prescribing Information, 2021). Results from the Phase 3 KEYNOTE-522 study demonstrated that the combination of pembrolizumab to standard neoadjuvant chemotherapy, including carboplatin, led to improved pCR rates and a statistically significant event-free survival (EFS) compared with chemotherapy alone in high-risk, early-stage TNBC patients regardless of PD-L1 status (Schmid, 2020a; Merck Press Release, 2021). Ultimately, the neoadjuvant regimen selected for the individual patient will depend on patient characteristics and disease-specific factors.

In summary, chemotherapy (with or without immunotherapy) remains the cornerstone in treating TNBC. However, toxicity and eventual treatment resistance are significant impediments to its success. Novel therapeutic options that can offer better anti-tumor efficacy without the associated high-grade toxicities are clearly needed for this patient population with high unmet medical need.

4.1.1. T Cell Activity and the Tumor Microenvironment

The importance of the types and crosstalk of immune cell populations within the tumor microenvironment and their impact on cancer progression has been clearly established. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in

tumor tissue and favorable clinical outcomes in various malignancies. In particular, the presence of cytotoxic cluster of differentiation 8 positive (CD8+) T cells and the ratio of cytotoxic CD8+ T cells to Forkhead Box P3 $(FOXP3)^{+}$ regulatory T cells (Treg) correlates with improved prognosis and long-term survival in solid malignancies (Vasievich, 2011; Gonzalez, 2018). Another important factor in discerning the anti-tumor efficacy of the T cells within the tumor microenvironment is their activation and functional potential based on their exhaustion status. T cell exhaustion is a stepwise loss in function that develops in the setting of chronic stimulation and in the presence of an immunosuppressive environment. Exhausted T cells upregulate expression of inhibitory receptors including but not limited to PD-1, cytotoxic T lymphocyte associated protein 4 (CTLA-4), T cell immunoglobulin and mucin domain 3 (TIM3), T cell Ig and ITIM domain (TIGIT), and lymphocyte activation gene 3 (LAG-3), and through interaction with its ligand inhibits T cell activation. Thus, the development of therapeutic approaches that shift the tumor microenvironment to a more immune active environment is an active area of investigation, including in breast cancer.

Relative to estrogen receptor (ER)-positive/HER2-negative breast cancer, TNBC tends to have higher mutational burden and increased potential neo-antigenicity which results in immune stimulation. The immune microenvironment in breast carcinoma has been largely defined by the presence and composition of TILs, which are most pronounced in TNBC and HER2-positive carcinomas (Cimino-Mathews, 2015). TILs are emerging as promising prognostic and predictive biomarkers in breast cancer as demonstrated by several studies in the neoadjuvant setting (Denkert, 2010; Ono, 2012; Yamaguchi, 2012). Analysis of pre-treatment and post-treatment tumor specimens from breast cancer patients showed a correlation between the development of TILs after neoadjuvant paclitaxel chemotherapy and clinical response (Demaria, 2001). As noted for other tumor types, among TIL subsets in breast cancer, the presence of cytotoxic CD8+ T cells is favorable, whereas the presence of the immunosuppressive suppressor FOXP3+ Tregs is unfavorable. There is evidence that a high CD8+/Treg ratio after neoadjuvant chemotherapy predicts OS and is associated with pCR (Ladoire, 2011; Park, 2020). Collectively, these data suggest that immunomodulatory therapies could be added to neoadjuvant chemotherapy to improve current treatment strategies for TNBC.

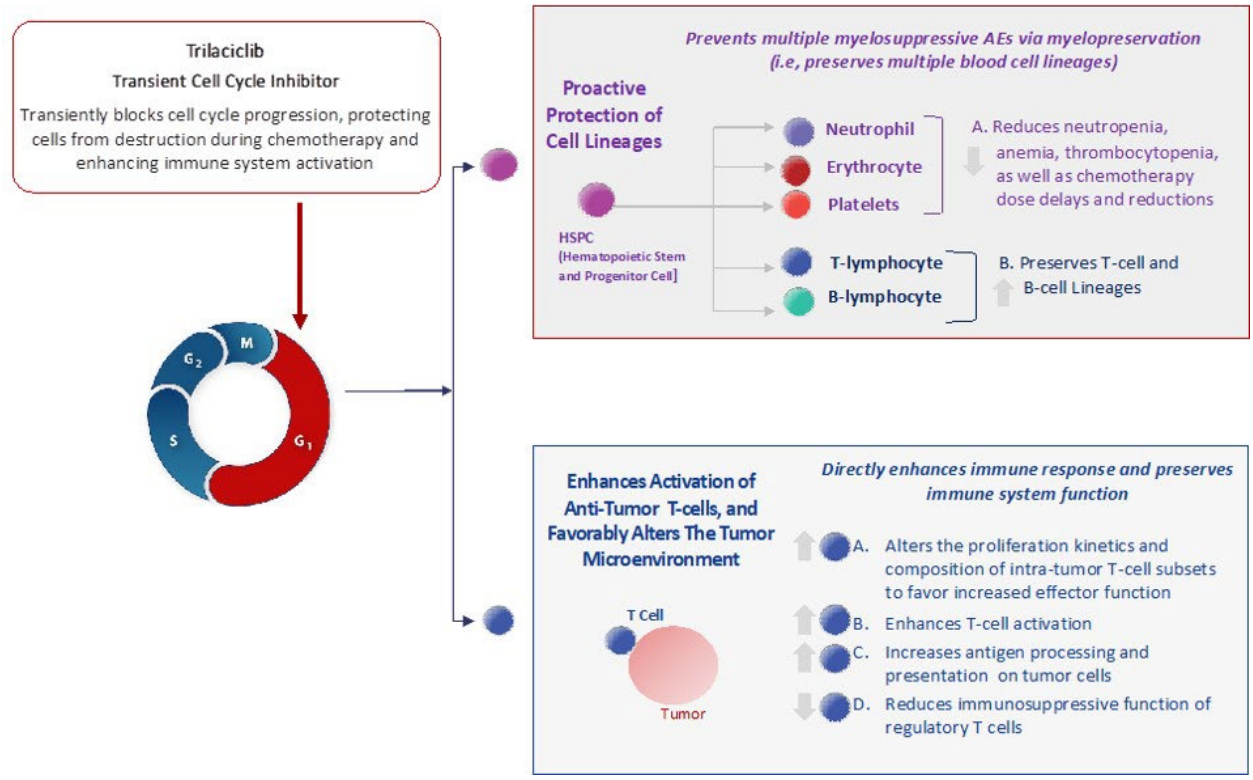
Chemotherapy-induced immunosuppression and depletion of immune cells may also affect anti-tumor efficacy due to an inability of the host immune system to effectively mount a response against the cancer. Therefore, preserving the bone marrow and immune system from the cytotoxic effects of chemotherapy has the potential to maximize the anti-tumor activity of the chemotherapy while minimizing myelotoxicity.

4.2. Trilaciclib

Trilaciclib is a highly potent and selective, reversible, cyclin-dependent kinase (CDK) 4/6 inhibitor that preserves hematopoietic stem and progenitor cells (HSPCs) as well as immune system function during chemotherapy (myeloprotection) in addition to directly enhancing anti-tumor immunity (anti-cancer efficacy) (Figure 1). In February 2021, the United States (US) Food and Drug Administration (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).

Both HSPC and lymphocyte proliferation are dependent on CDK4/6 activity (Kozar, 2004; Malumbres, 2004; Ramsey, 2007; Horsley, 2008) and become arrested in the gap 1 (G_1) phase of the cell cycle upon exposure to trilaciclib (He, 2017a). This trilaciclib-induced transient cell cycle arrest has been demonstrated to provide resistance to chemotherapy-induced cell damage by preventing HSPCs from proliferating in the presence of cytotoxic chemotherapy and favorably altering the tumor immune microenvironment through transient T cell inhibition when combined with chemotherapy (He, 2017b; Bisi, 2016; Lai, 2018; Sorrentino, 2017).

Figure 1: Trilaciclib Transiently Arrests Normal Cells to Prevent Chemotherapy Induced Myelosuppression and Improve Anti-Tumor Efficacy



G_1=Gap 1; G_2=Gap 2; HSPC=hematopoietic stem and progenitor cell; M=mitosis; S=synthesis

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.

Trilaciclib and other CDK4/6 inhibitors have been shown to augment anti-tumor responses in nonclinical settings (Klein, 2018) by enhancing T cell activation through the repression of nuclear factor of activated T cell (NFAT) phosphorylation (Deng, 2018), as well as increasing antigen presentation through upregulation of major histocompatibility complex (MHC) class I and II in CDK4/6-sensitive tumors and myeloid cells (Goel, 2017; Schaer, 2018). Additionally,

CDK4/6 inhibition can upregulate and stabilize the protein expression of PD-L1 on tumor cells (Zhang, 2018). Recently, nonclinical studies investigating CDK4/6 inhibitors in the differentiation of cytotoxic CD8+ T cells revealed a preferential skewing, of CDK4/6 inhibitor exposed cytotoxic CD8+ T cells, into memory precursor cells (Heckler, 2021). When these cells were transferred into mice, the CDK4/6 inhibitor exposed cytotoxic CD8+ T cells demonstrated durability and persistence. Importantly, mice administered with the CDK4/6 inhibitor-exposed T cells demonstrated protection following tumor challenge. Collectively, there is increasing pre-clinical evidence of a direct effect of CDK4/6 inhibition on immune cell activation and differentiation, thereby supporting the critical need to better understand the immunological effects of trilaciclib on the generation of an anti-tumor responses.

4.2.1. Clinical Data with Trilaciclib in TNBC

The use of trilaciclib for patients with TNBC was evaluated in Study G1T28-04, a global, multicenter, randomized, open-label, Phase 2 clinical study to evaluate the safety, efficacy, and pharmacokinetics (PK) of trilaciclib administered prior to gemcitabine/carboplatin (GC) therapy in patients with locally recurrent/metastatic TNBC who had previously been treated with 0 to 2 lines of therapy in the metastatic setting. Patients were randomized 1:1:1 to one of two different trilaciclib + GC treatment regimens or GC alone. Based on its mechanism of action (MOA), it was hypothesized that trilaciclib administered before chemotherapy could protect the bone marrow from the cytotoxic effects of chemotherapy, while also enhancing immune activity in patients with TNBC, thus potentially improving both safety and anti-tumor activity. Additionally, TNBC is considered genomically unstable and the tumor microenvironment is moderately immunogenic (Park, 2018); a state that could be potentially enhanced by trilaciclib in combination with chemotherapy, leading to improved anti-tumor activity.

The G1T28-04 study was the first evaluation of trilaciclib in a tumor type other than small cell lung cancer (SCLC), where trilaciclib development has focused on myeloprotection benefits (Section 4.4.2.1.1). In contrast to previously observed SCLC results, the addition of trilaciclib to chemotherapy for patients with TNBC in G1T28-04 did not result in statistically significant improvements in myeloprotection endpoints, but instead resulted in a substantial improvement in anti-tumor efficacy as measured by multiple anti-tumor efficacy endpoints including OS (median OS duration in control: 12.6 months vs. not evaluable or 17.8 months in the two trilaciclib groups; described in Section 4.4.2.1.1). Efficacy was demonstrated across patient subgroups, including patients with PD-L1 positive and negative tumors and those with different CDK4/6 dependence signatures using two established assays (Prosigna Breast Cancer Prognostic Gene Signature Assay [PAM50] and Lehmann triple-negative breast cancer type) (Prat, 2014; Lehmann, 2016; Asghar, 2017). These data suggest trilaciclib does not antagonize chemotherapy efficacy irrespective of CDK4/6 status, TNBC is predominantly CDK4/6 independent, and therefore not sensitive to CDK4/6 inhibition (Tan, 2019; data on file).

Evaluation of patient-reported outcomes (PROs) by the Functional Assessment of Cancer Therapy – Anemia and Functional Assessment of Cancer Therapy - Breast (FACT-B) indicated that patients treated with trilaciclib were less likely to have a deterioration in their symptoms, and in some cases had improvement in their symptoms, through the first 6 cycles of therapy; and that trilaciclib delayed deterioration of patient functioning and symptoms measures over time compared with GC alone.

4.2.2. T Cell Activity with Trilaciclib

Nonclinical data demonstrate that trilaciclib enhances immune activation and promotes anti-tumor immunity by differentially arresting cytotoxic and regulatory T cell subsets followed by a faster recovery of cytotoxic T lymphocytes than Tregs in tumor (Section 4.4.1.1). To evaluate the effect of trilaciclib on T cell expansion in G1T28-04, T cell receptor was longitudinally sequenced from the peripheral blood. Simpson clonality significantly decreased over time in patients that received trilaciclib in addition to GC when compared to GC alone ($P_{interaction}$=0.012) meaning that patients receiving trilaciclib and GC demonstrated T cell diversity based on increased numbers of T cell clones. Furthermore, when patients were stratified above or below median Simpson clonality at baseline, there was a trend for improved OS among patients with decreased peripheral clonality and a statistically significant improvement in OS among patients receiving trilaciclib (P=0.02) (O'Shaughnessy, 2020). In addition to a decrease in Simpson clonality, responders receiving trilaciclib prior to GC had more newly detected expanded clones compared with responders receiving GC alone (P=0.09; data on file). These data are consistent with the analyses of patient peripheral T cells from another clinical study (G1T28-02; NCT02499770) demonstrating that transient exposure of trilaciclib in patients with SCLC during chemotherapy treatment both preserved and increased peripheral lymphocyte counts and enhanced T cell activation (Lai, 2020). Other immune activating effects of CDK4/6 inhibition have been demonstrated, including increasing antigen presentation through MHC class I, decreasing T cell exhaustion markers (e.g., PD-1, CTLA-4, and TIM3), stabilizing expression of PD-L1 on tumor cells, and increasing T cell effector function through high interferon (IFN)γ production. Collectively, these data provide insight into the variety of mechanisms by which trilaciclib enhances T cell immunity leading to an anti-tumor efficacy. In addition, nonclinical studies have suggested there is synergy between inhibition of CDK4/6 and anti-PD-1 blocking antibodies in multiple tumor models (Chaikovsky and Sage, 2018; Deng, 2018; Goel, 2017; Schaer, 2018; Lai, 2020; Bonelli, 2019; Teh and Aplin, 2019). Clinical data in SCLC demonstrated that the addition of trilaciclib prior to chemotherapy and the PD-L1 antibody, atezolizumab, generated more newly expanded T cell clones with significantly greater expansion among patients with clinical response vs placebo (Daniel, 2020). Treatment with CDK4/6 inhibitors may increase the effectiveness of immune checkpoint blockade therapies or provide benefit in patients after progressing on immune checkpoint inhibitor (ICI) treatment.

4.3. Study Rationale

This study is designed to elucidate the immune-based MOA of trilaciclib independent of other treatments in a neoadjuvant setting of TNBC. Nonclinical and recent clinical studies with trilaciclib demonstrate enhanced immune activation and anti-tumor immunity through differentially arresting cytotoxic and regulatory T cell subsets followed by a faster recovery of cytotoxic T lymphocytes than Tregs in tumors. This differential alteration of cell cycle kinetics between cytotoxic T lymphocytes and Tregs results in a higher proportion of cytotoxic T lymphocytes than Tregs, enhancement of T cell activation, and a decrease in Treg-mediated immunosuppressive functions. Based on these anti-neoplastic driven effects on the immune response, the study is designed to define quantitative and qualitative changes in immune cell phenotype and function following neoadjuvant trilaciclib treatment. These include a measurement of cytotoxic CD8+ T cell infiltration into the tumor microenvironment following a single dose of trilaciclib and changes in ratio of cytotoxic CD8+ T cells to Tregs. Together, these

events promote the cytotoxic T lymphocyte-mediated clearance of tumor cells. Moreover, the anti-tumor effects of trilaciclib might result from the transient proliferative arrest of T cells (protecting them from chemotherapy-induced damage), followed by activation of cytotoxic T lymphocytes in the context of fewer Tregs.

To evaluate the impact of trilaciclib on changes of the tumor-associated immune response in TNBC treated with standard of care (SOC) neoadjuvant chemotherapy (plus carboplatin and/or pembrolizumab per Investigator discretion), immunophenotypic changes will be compared in tumor biopsies at baseline, after a single dose of monotherapy trilaciclib, and at definitive surgery (if available). Serial peripheral blood collection will also be obtained in parallel with tumor sample collection to identify immune correlates associated with trilaciclib treatment. For peripheral blood, an extra timepoint will be collected prior to Cycle 2 Day 1 (C2D1) to define the kinetics of the immune response following trilaciclib plus chemotherapy. Longitudinal analysis after trilaciclib treatment will enable comprehensive assessment of the immune and anti-neoplastic MOA of trilaciclib with potential to identify biomarkers for patient selection in future studies and understand mechanisms of resistance.

Finally, flexibility was built into the protocol to allow the Investigator to tailor neoadjuvant therapy to each patient. As the treatment paradigm for early-stage TNBC continues to evolve, platinum chemotherapy and immunotherapy may be added per Investigator discretion to the standard anthracycline/taxane-backbone. This inclusion will also provide additional safety and efficacy data for a novel combination.

4.4. Background

4.4.1. Summary of Nonclinical Data

A brief summary of the trilaciclib nonclinical data is provided in the following sections. Detailed information is presented in the Trilaciclib Investigator's Brochure (IB).

Nonclinical data related to chemotherapy agents (doxorubicin, cyclophosphamide, paclitaxel, and carboplatin) and immunotherapy (pembrolizumab) are provided in the US prescribing information for each agent.

4.4.1.1. Pharmacology Studies

Through a structure-based design approach to optimize potency, selectivity, and drug metabolism and PK properties, G1 Therapeutics, Inc. identified trilaciclib as a highly potent inhibitor of CDK4 and CDK6 (half maximal inhibitory concentration values of 1 nM and 4 nM, respectively) that is highly selective for CDK4/Cyclin D1 versus CDK2/Cyclin E (>2500-fold selectivity). Trilaciclib also demonstrated reversible inhibition of CDK4/Cyclin D1, with an inhibition constant value of 0.78 nM.

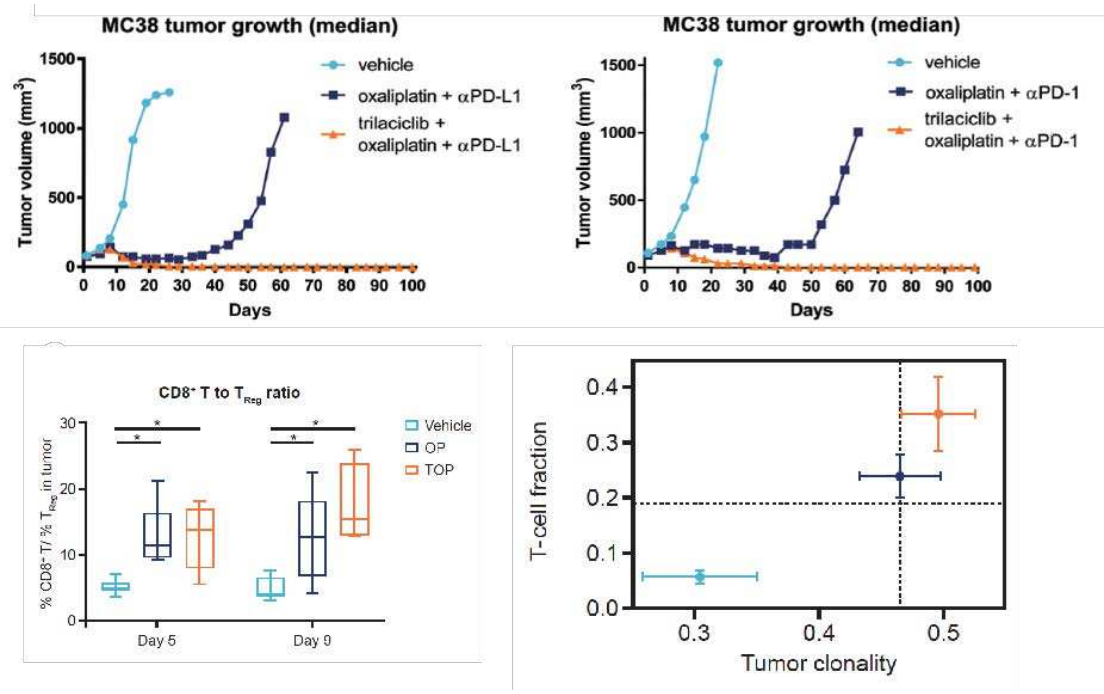
The trilaciclib-induced G_1 arrest of HSPCs has been shown to be transient and readily reversible in both in vitro and in vivo models. In vivo analysis has demonstrated that trilaciclib administered prior to myelosuppressive chemotherapy leads to improved complete blood count (CBC) recovery of all blood lineages and increased survival. Specifically, in a model using the highly myelosuppressive chemotherapy 5-fluorouracil (5-FU), while the extent and duration of nadir in CBCs worsened after each cycle of 5-FU administered alone, trilaciclib administered prior to 5-FU ameliorated this worsening effect and the animals that received trilaciclib + 5-FU

demonstrated a faster rate of recovery of CBCs compared with the 5-FU alone group following Cycle 4 (He, 2017a).

Nonclinical data have shown trilaciclib enhances immune activation and promotes anti-tumor immunity by differentially arresting cytotoxic and regulatory T cell subsets followed by a faster recovery of cytotoxic T lymphocytes than Tregs in tumors. Specifically, the addition of trilaciclib to various chemotherapy/ICI treatment combinations resulted in enhanced tumor growth delay and durability of the anti-tumor response. Trilaciclib favorably modulated the proliferation of T cell subsets in the tumor microenvironment, consistent with an enhanced cytotoxic T cell response (Figure 2; Lai, 2020). This differential alteration of cell cycle kinetics between cytotoxic T lymphocytes and Tregs results in a higher proportion of cytotoxic T lymphocytes than Tregs, enhancement of T cell activation, and a decrease in Treg-mediated immunosuppressive functions (Chaikovsky and Sage, 2018; Deng, 2018; Goel, 2017; Schaer, 2018). Together, these events promote the cytotoxic T lymphocyte-mediated clearance of tumor cells. Therefore, these data support the hypothesis that trilaciclib-mediated transient proliferative arrest of T cells (protecting them from chemotherapy-induced damage), followed by activation of cytotoxic T lymphocytes in the context of fewer Tregs led to the anti-tumor response observed.

Nonclinical data have shown that in an extremely CDK4/6 dependent estrogen receptor positive breast cancer model, intermittent trilaciclib dosing during the time of chemotherapy treatment did not negatively affect chemotherapy treatment. The lack of antagonism when trilaciclib was added to chemotherapy treatment was observed in multiple CDK4/6 dependent models (Sorrentino, 2018).

Figure 2: The Addition of Trilaciclib to Chemotherapy/Checkpoint Inhibitor Treatment Enhances Efficacy Through T Cell Activation



CD=cluster of differentiation; PD-1=programmed cell death protein 1; PD-L1=programmed death-ligand 1; Treg=regulatory T cell.

4.4.1.2. Pharmacokinetic Studies

Pharmacokinetic studies in rats and dogs showed that the relationship between dose level and plasma exposure to trilaciclib was generally similar between males and females and did not change with repeated daily dosing. Exposure to trilaciclib increased with dose level, but not always proportionally.

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 based on trilaciclib-mediated inhibition of CYP3A4-mediated metabolic pathways are possible, but clinical studies have confirmed no clinically significant drug-drug interaction (DDI) based on CYP3A (see Section 4.4.2.1.2 for additional details). Additionally, 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 DDI based on inhibition of breast cancer resistance protein-, bile salt export pump-, organic anion transporter 1 (OAT1)-, organic anion transporter 3 (OAT3)-, organic anion transporting polypeptide 1B1 (OATP1B1)-, p-glycoprotein-, multidrug resistance protein 1 (MRP1)-, multidrug resistance protein 2 (MRP2)-, 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) (see Section 4.4.2.1.2 for additional details).

4.4.2. Summary of Clinical Data

A brief summary of the trilaciclib clinical data is provided in the following sections. Detailed information is presented in the trilaciclib IB.

4.4.2.1. Clinical Trials

The safety and efficacy of administering trilaciclib prior to chemotherapy has been evaluated in one completed and one ongoing Phase 1b/2 study (G1T28-02 and G1T28-03) and two 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.

Additional studies that are ongoing include a Phase 3 study in metastatic colorectal cancer (mCRC; G1T28-207), a Phase 3 study in TNBC (G1T28-208); a Phase 2 study in urothelial carcinoma (G1T28-209), and a Phase 2 study in TNBC (trilaciclib + sacituzumab govitecan; G1T28-213).

- Study G1T28-02 evaluated 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 ES-SCLC.

- Study G1T28-03 evaluated administration of trilaciclib or placebo on Days 1 to 5 of each 21-day topotecan chemotherapy cycle in patients with previously treated ES-SCLC.
- Study G1T28-05 evaluated administration of trilaciclib or placebo on Days 1 to 3 for a maximum of four 21-day cycles of Etoposide, carboplatin, and atezolizumab (E/P/A), followed by monotherapy atezolizumab, in patients with treatment naïve ES-SCLC.
- Study G1T28-04 evaluated administration of trilaciclib prior to GC in patients with metastatic TNBC who had received 0 to 2 lines of previous therapy in the metastatic setting. Patients received 1) GC therapy only on Days 1 and 8 of a 21-day cycle, 2) trilaciclib and GC once daily on Days 1 and 8 of each 21-day cycle, OR 3) trilaciclib on Days 1, 2, 8 and 9 with GC on Days 2 and 9 of each 21-day cycle (further noted as Group 1, 2, or 3, respectively).
- Study G1T28-207 (PRESERVE 1) is evaluating administration of either trilaciclib or placebo on Days 1 and 2 of each 14-day FOLFOXIRI/bevacizumab cycle in patients with proficient mismatch repair/microsatellite stable mCRC who have not received systemic therapy for metastatic disease.
- Study G1T28-208 (PRESERVE 2) is evaluating administration of either trilaciclib or placebo on Day 1 and Day 8 of each 21-day GC cycle in patients receiving first line treatment for locally advanced unresectable/metastatic TNBC.
- Study G1T28-209 (PRESERVE 3) is evaluating administration of trilaciclib on Day 1 of each 21-day platinum-based chemotherapy cycle (4-6 cycles) followed by Day 1 of each 14-day avelumab maintenance cycle in patients receiving first-line treatment for advanced/metastatic urothelial carcinoma.
- Study G1T28-213 is evaluating the administration of trilaciclib prior to sacituzumab govitecan-hziy on Day 1 and Day 8 of each 21-day cycle in patients with unresectable locally advanced or metastatic TNBC who received at least 2 prior treatments, at least 1 in the metastatic setting.

Based on data from the aforementioned SCLC trials, the US 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 ES-SCLC in February 2021.

At the approved dose of 240 mg/m^2, across all three SCLC studies, trilaciclib administered prior to chemotherapy statistically significantly reduced the duration of severe neutropenia (DSN) in Cycle 1 and occurrence of severe neutropenia (SN) (primary endpoints) compared with placebo. An integrated data analysis of the three SCLC studies (G1T28-02, G1T28-03, and G1T28-05) for 8 of the most relevant myelosuppression endpoints (e.g., neutrophils, red blood cells [RBCs] and platelets) demonstrated statistically significant, and clinically meaningful, improvements for trilaciclib over available therapies in 6 of 8 endpoints across multiple lineages. Importantly, these myeloprotective benefits come with an overall improved safety profile compared with available therapy, as evidenced by reduced high grade treatment-emergent adverse events (AEs) across all

SCLC studies, and no detriment to anti-tumor efficacy results. For a more detailed description of the results, refer to the trilaciclib IB.

4.4.2.1.1. Clinical Experience in TNBC

Study G1T28-04 in patients with advanced TNBC did not meet its primary endpoint of myeloprotection (DSN in Cycle 1 and occurrence of SN), though a clinically meaningful benefit in OS was observed when trilaciclib was added to GC chemotherapy. The combination of trilaciclib and GC demonstrated a substantial improvement in anti-tumor efficacy as measured by OS (median OS duration [95% confidence interval (CI)] in control: 12.6 months [6.3, 15.6] vs. not evaluable [10.2, not evaluable] or 17.8 months [12.9, 32.7] in the two trilaciclib groups, respectively) and by progression-free survival (PFS) (median PFS [95% CI] in control 5.7 months [3.3, 9.9] vs. 9.4 months [6.1, 11.9] or 7.3 months [6.2, 13.9] in the two trilaciclib groups, respectively) (Tan, 2019; O'Shaughnessy, 2020). The clinically meaningful anti-tumor efficacy results observed in G1T28-04 were noted across both of the trilaciclib treatment groups and in patients with both PD-L1 positive and negative tumors, and these benefits were observed for multiple anti-tumor efficacy endpoints, with objective response rate (ORR), PFS, and OS endpoints all showing numerical improvement with the addition of trilaciclib to GC compared with GC alone. These results were observed in the context of a control group that is reflective of published literature for this patient population. Furthermore, there were trends toward improvement in RBC and platelet-based measures as well as benefit in PRO endpoints. Given the encouraging evidence of improved anti-tumor activity as well as positive PROs observed in Study G1T28-04, a Phase 3 clinical trial designed to confirm the efficacy of trilaciclib administration prior to treatment with GC in TNBC patients receiving first- or second-line treatment for locally advanced unresectable/metastatic disease is currently ongoing (G1T28-208, PRESERVE 2).

4.4.2.1.2. Pharmacokinetics

The PK and clinical pharmacology of trilaciclib have been assessed in studies with healthy subjects, patients receiving chemotherapy (studies noted above), specific populations, and in DDI studies (see trilaciclib IB for details).

Trilaciclib has a terminal half-life of approximately 14 hours and undergoes extensive metabolism. Mild hepatic impairment and mild and moderate renal impairment had no effect on trilaciclib exposure. Population PK analysis indicated that age, gender, weight and race had no clinically relevant effect on the systemic exposure of trilaciclib. For more information about PK profile of trilaciclib, please refer to the trilaciclib IB.

A clinical DDI study in healthy subjects using the index CYP3A substrate midazolam indicated that trilaciclib had no impact on CYP3A activity and that the strong CYP3A inducer rifampin had no clinically meaningful effect on trilaciclib PK. 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 an additional clinical DDI assessment in healthy subjects, concomitant use of trilaciclib increased metformin (OCT2, MATE1, and MATE-2K substrate) area under the plasma concentration-time curve from time zero to infinity (AUC_{inf}) and maximum concentration by approximately 65% and 81%, respectively. Renal clearance of metformin was decreased by 37%.

There were no clinically significant differences in topotecan (MATE1 and MATE-2K substrate) pharmacokinetics when used concomitantly with trilaciclib.

Trilaciclib is an inhibitor of OCT2, MATE1, and MATE-2K. Co-administration of trilaciclib may increase the concentration or net accumulation of OCT2, MATE1, and MATE-2K substrates in the kidney (e.g., dofetilide, dalfampridine, and cisplatin). Refer to the prescribing information for these concomitant medications for assessing the benefit and risk of concomitant use of trilaciclib.

Table 2: Potentially Significant Drug Interactions with Trilaciclib

Drugs	Recommendations	Comments
Dofetilide	The potential benefits of taking trilaciclib concurrently with dofetilide should be considered against the risk of QT interval prolongation.	Increased dofetilide blood levels may occur in patients who are also receiving trilaciclib. Increased plasma concentrations of dofetilide may cause serious ventricular arrhythmias associated with QT interval prolongation, including torsade de pointes.
Dalfampridine	The potential benefits of taking trilaciclib concurrently with dalfampridine should be considered against the risk of seizures in these patients.	Increased dalfampridine blood levels may occur in patients who are also receiving trilaciclib. Elevated levels of dalfampridine increase the risk of seizure.
Cisplatin	Closely monitor for nephrotoxicity.	Concurrent treatment with trilaciclib may increase the exposure and alter the net accumulation of cisplatin in the kidney, which may associate with dose-related nephrotoxicity.

Based on both nonclinical and clinical data, trilaciclib does not inhibit or induce any of the CYP enzymes. Therefore, the risk of a DDI with the concurrent anticancer medications used in this study (doxorubicin, cyclophosphamide, paclitaxel, carboplatin, and pembrolizumab) based on a CYP mechanism is minimal to nil. Trilaciclib is a known inhibitor of the drug transport proteins OCT2, MATE1, and MATE-2K; however, none of the concurrent anticancer medications used in this study are known substrates of these transporters. Therefore, the risk of a drug transporter based DDI is minimal to nil.

4.4.3. Risks

4.4.3.1. Trilaciclib

Reproductive/embryo-fetal effects are 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 required to have a negative pregnancy test prior to the first dose of study drug and will be monitored while on study. Eligibility criteria describing specific birth control methods are incorporated in the protocol and

the informed consent form (ICF). Dose discontinuation recommendations for female patients who become pregnant while receiving trilaciclib are also provided in the protocol (Section 11.3.7.5). Detailed information regarding all important identified and important potential risks of trilaciclib administration can be found in the trilaciclib IB.

At the clinical dose of 240 mg/m^2 being used in this study, trilaciclib did not have a clinically relevant effect on QTc (i.e., >10 msec).

In an integrated safety analysis from the four Phase 2 oncology studies conducted with trilaciclib to date (G1T28-02 [complete], G1T28-03 [data cutoff: 31 May 2019], G1T28-05 [data cutoff: 28 Jun 2019], and G1T28-04 [complete]), the most common treatment-emergent adverse events (TEAEs) (≥10%) that occurred more frequently in patients receiving trilaciclib compared with placebo were nausea, fatigue, headache, dyspnea, cough, hypokalemia, and infusion related reaction. Trilaciclib-related TEAEs occurring in ≥5% of patients with at least a ≥2% higher incidence in trilaciclib compared with placebo were nausea, fatigue, anemia, headache, infusion related reaction, neutrophil count decreased, decreased appetite, vomiting, and constipation.

Adverse events of special interest (AESIs) identified for trilaciclib in the integrated safety summary are described below. 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 (IV) 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 9.3.

Trilaciclib AESIs:

1. **Injection Site Reaction/Phlebitis/Thrombophlebitis:** Infusion of trilaciclib can cause injection-site reactions including phlebitis and thrombophlebitis. Injection-site reactions including phlebitis and thrombophlebitis occurred in 56 (21%) of 272 patients receiving trilaciclib in clinical trials, including Grade 2 (10%) and Grade 3 (0.4%) AEs. The median time to onset from start of trilaciclib was 15 days (range 1 to 542) and from the preceding dose of trilaciclib was 1 day (1 to 15). The median duration was 1 day (range 1 to 151 for the resolved cases). Injection-site reactions including phlebitis and thrombophlebitis resolved in 49 (88%) of the 56 patients and led to discontinuation of treatment in 3 (1%) of the 272 patients.
2. **Acute Drug Hypersensitivity Reaction:** Trilaciclib can cause acute drug hypersensitivity reactions, including facial edema and urticaria. Acute drug hypersensitivity reactions occurred in 16 (6%) of 272 patients receiving trilaciclib in clinical trials, including Grade 2 reactions (2%). One patient experienced a Grade 2 anaphylactic reaction 4 days after receiving trilaciclib, which resolved with epinephrine, and treatment with trilaciclib was continued. The median time to onset from start of trilaciclib was 77 days (range 2 to 256) and from the preceding dose of trilaciclib was 1 day (range 1 to 28). The median duration was 6 days (range 1 to 69 for the resolved cases). Acute drug hypersensitivity reactions resolved in 12 (75%) of the 16 patients.
3. **Pneumonitis/Interstitial Lung Disease (ILD)**: Severe, life-threatening, or fatal ILD and/or pneumonitis can occur in patients treated with CDK4/6 inhibitors, the same drug class as trilaciclib. ILD/pneumonitis occurred in 1 (0.4%) of 272 patients receiving

trilaciclib in clinical trials. The event was Grade 3 and reported 2 months after discontinuing trilaciclib, in a patient receiving a confounding medication. The event did not resolve.

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 oral 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.

4.4.3.2. Doxorubicin, Cyclophosphamide, and Paclitaxel

4.4.3.2.1. Doxorubicin

Per the boxed warning in the prescribing information for doxorubicin, the following are important risks related to doxorubicin use:

- Cardiomyopathy: Myocardial damage can occur with doxorubicin hydrochloride with incidences from 1% – 20% for cumulative doses from 300 mg/m^2 to 500 mg/m^2 when doxorubicin hydrochloride is administered every 3 weeks. The risk of cardiomyopathy is further increased with concomitant cardiotoxic therapy.
- Secondary Malignancies: Secondary acute myelogenous leukemia and myelodysplastic syndrome occur at a higher incidence in patients treated with anthracyclines, including doxorubicin hydrochloride.
- Extravasation and Tissue Necrosis: Extravasation of doxorubicin hydrochloride can result in severe local tissue injury and necrosis requiring wide excision and skin grafting.
- Severe myelosuppression resulting in serious infection, septic shock, requirement for transfusions, hospitalization, and death may occur.

Per the Warnings and Precautions in the prescribing information for doxorubicin, the following are important risks related to doxorubicin use:

- Radiation-Induced Toxicity: Can be increased by the administration of Doxorubicin Hydrochloride Injection. Radiation recall can occur in patients who receive Doxorubicin Hydrochloride Injection after prior radiation therapy.
- Embryo-Fetal Toxicity: Can cause fetal harm.

In addition, the most common (>10%) adverse reactions observed following doxorubicin treatment are alopecia, nausea, and vomiting.

4.4.3.2.2. Cyclophosphamide

Per the Warnings and Precautions in the prescribing information for cyclophosphamide, the following are important risks related to cyclophosphamide use:

- Myelosuppression, Immunosuppression, Bone Marrow Failure, and Infections: Severe immunosuppression may lead to serious and sometimes fatal infections.
- Urinary Tract and Renal Toxicity: Hemorrhagic cystitis, pyelitis, ureteritis, and hematuria can occur.
- Cardiotoxicity: Myocarditis, myopericarditis, pericardial effusion, arrhythmias, and congestive heart failure, which may be fatal, have been reported.
- Pulmonary Toxicity: Pneumonitis, pulmonary fibrosis and pulmonary veno-occlusive disease leading to respiratory failure may occur.
- Secondary malignancies
- Veno-occlusive Liver Disease: Fatal outcome can occur.
- Alcohol Content: The alcohol content in a dose of Cyclophosphamide Injection may affect the central nervous system. This may include impairment of a patient's ability to drive or use machines immediately after infusion.
- Embryo-Fetal Toxicity: Can cause fetal harm.

In addition, adverse reactions reported most often include neutropenia, febrile neutropenia, fever, alopecia, nausea, vomiting, and diarrhea.

4.4.3.2.3. Paclitaxel

Per the boxed warning in the prescribing information for paclitaxel, the following are important risks related to paclitaxel use:

- Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2 to 4% of patients receiving paclitaxel in clinical studies. Fatal reactions have occurred in patients despite premedication.
- Bone marrow suppression (primarily neutropenia) is dose-dependent and is the dose-limiting toxicity.

Per the Warnings and Precautions in the prescribing information for paclitaxel, the following are also important risks related to paclitaxel use:

- Severe conduction abnormalities have been documented in <1% of patients during paclitaxel therapy and in some cases requiring pacemaker placement.
- Embryo-Fetal Toxicity: Can cause fetal harm.

In addition, adverse reactions reported most often include neutropenia, thrombocytopenia, anemia, nausea, vomiting, diarrhea, infections, and mucositis.

4.4.3.3. Treatment per Investigator Discretion: Carboplatin and Pembrolizumab

4.4.3.3.1. Carboplatin

Per the boxed warning in the prescribing information for carboplatin, the following are important risks related to carboplatin use:

- Bone marrow suppression is dose related and may be severe, resulting in infection and/or bleeding. Anemia may be cumulative and may require transfusion support.
- Vomiting is another frequent drug-related side effect.
- Anaphylactic-like reactions to carboplatin injection have been reported and may occur within minutes of carboplatin injection administration.

Per Warnings and Precautions in the prescribing information for carboplatin, the following are additional important risks related to carboplatin use:

- Nephrotoxic potential: concomitant treatment with aminoglycosides has resulted in increased renal and/or audiologic toxicity
- Peripheral neurotoxicity: observed infrequently, but its incidence is increased in patients older than 65 years and in patients previously treated with cisplatin
- Loss of vision
- Abnormal liver function tests (LFTs)
- Fetal harm

The most common adverse reactions with carboplatin in combination are thrombocytopenia, neutropenia, leukopenia, anemia, nausea, vomiting, other gastrointestinal effects, central neurotoxicity, serum glutamic oxaloacetic transaminase elevations, alkaline phosphatase elevations, hypomagnesemia, pain, asthenia, and alopecia.

4.4.3.3.2. Pembrolizumab

Per Warnings and Precautions in the prescribing information for pembrolizumab, the following are important risks related to pembrolizumab use:

- Immune-mediated adverse reactions
- Infusion-related reactions
- Complications of allogeneic hematopoietic stem cell transplantation
- Fetal harm

The most common adverse reactions with pembrolizumab in combination with chemotherapy are fatigue/asthenia, nausea, constipation, diarrhea, decreased appetite, rash, vomiting, cough, dyspnea, pyrexia, alopecia, peripheral neuropathy, mucosal inflammation, stomatitis, headache, and weight loss.

4.4.3.4. Tumor Biopsy Collection

Risks associated with tumor biopsy may include: stinging pain from injection of local aesthetic; pain or discomfort from the biopsy procedure; bleeding, swelling, scarring, soreness, or bruising; infection of wound; and contamination of cancer cells to unaffected tissue when removing biopsy needle.

4.5. Benefit/Risk Assessment

Clinical trials are designed to provide information about the safety and effectiveness of an investigational medicine which may not always provide direct benefit to the patients participating in the trial. In this study, it is unknown whether patients will receive additional benefit above that of SOC with the addition of trilaciclib. Potential benefits of trilaciclib are addressed in Section 4.4.2. Completed studies with trilaciclib have demonstrated a manageable safety profile in combination with several different chemotherapy regimens. Though trilaciclib has not been fully evaluated with an anthracycline/taxane chemotherapy regimen to date, the risks of AC/T (with and without carboplatin) are well documented. In particular, trilaciclib is not associated with significant cardiac risks, unlike anthracyclines which carry a risk of cardiomyopathy. There are no known risks of DDI with trilaciclib and these chemotherapy agents nor pembrolizumab. In Study G1T28-04, the rates of overall toxicity were comparable in patients who received trilaciclib with GC versus GC alone despite the 2-fold increase in the median number of cycles and 50% increase in the cumulative dose of both gemcitabine and carboplatin in patients who received trilaciclib. Rates of discontinuation due to an AE were not different between the trilaciclib groups and the GC alone group. PRO data also suggested that patients on trilaciclib (compared with GC alone) had a better experience receiving chemotherapy. Finally, efficacy in Study G1T28-04 demonstrated a clinically meaningful benefit in OS and PFS when trilaciclib was added to GC. A coronavirus disease (COVID)-19 risk assessment has been performed, documented, and will be provided as a separate document. Additional details regarding specific benefits and risks for patients participating in this clinical trial may be found in the trilaciclib IB and informed consent documents.

5. OBJECTIVES AND ENDPOINTS

The primary, secondary, and exploratory objectives of this study and their associated endpoints, for patients with TNBC, are presented in Table 3.

Table 3: Objectives and Endpoints

Objectives	Endpoints
Primary Objective	
• To evaluate the immune-based mechanism of action of trilaciclib after a single-dose as measured by the change in CD8+ TILs/Treg ratio in tumor tissue	• Change in CD8+ TILs/Treg ratio in tumor tissue from baseline to 7 days after single-dose monotherapy trilaciclib administration
Secondary Objectives	
• To assess the pCR rate at the time of definitive surgery	• Rate of pCR using the definition of ypT0/Tis ypN0 (i.e., no invasive residual tumor in breast or nodes; noninvasive breast residuals allowed) as assessed by the local pathologist
• To evaluate the safety and tolerability of trilaciclib in combination with standard neoadjuvant systemic therapies	• Safety/tolerability as per CTCAE version 5.0
Exploratory Objectives	
• To characterize the intratumoral immune profile following trilaciclib	• Immune-specific RNA profiling of tumor tissue at baseline, 7 days after single-dose monotherapy trilaciclib administration, and at surgery as quantified by CD8+ T cell and Treg infiltration
• To evaluate the kinetics of the peripheral immune response following trilaciclib	• Longitudinal immune changes in peripheral blood, measured by frequency of immune subsets and profiling of activation, maturation, and exhaustion status • Ex-vivo measurement of cytokine production to determine T cell function and polyfunctionality
• To identify molecular and cellular biomarkers in tumor or blood samples that may be indicative of clinical response/resistance, pharmacodynamic activity, and/or the mechanism of action of trilaciclib and other systemic treatments	• pCR in patients by subgroups (e.g., CDK4/6 dependence signatures: CDK4/6 dependent, CDK4/6 independent, CDK4/6 indeterminate; PD-L1 status as measured by IHC: positive, negative) • pCR in patients by gene signatures identified in the tumor at baseline • pCR in patients by frequency of immune subsets, immunological markers, and cytokines

CD8+=cluster of differentiation 8 positive; CDK=cyclin dependent kinase; CTCAE=Common Terminology Criteria for Adverse Events; IHC=immunohistochemistry; pCR=pathologic complete response; PD-L1=programmed death-ligand 1; RNA=ribonucleic acid; TIL=tumor-infiltrating lymphocyte; Treg=regulatory T cells

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This is a Phase 2, multicenter, open-label, single-arm, neoadjuvant study with 4 phases: 1) Screening Phase, 2) Trilaciclib Lead-In Phase, 3) Treatment Phase, and 4) Surgery and Follow-Up Phase. A study schema is provided in Figure 3. A total of approximately 30 patients will be enrolled in the study.

During the Screening Phase, tumor tissue (sample #1) will be obtained at baseline prior to any study drug. This sample may be archival tissue, or if unavailable/insufficient, then a fresh biopsy is required. Patients with adequate archival tissue will also have the option to provide a fresh biopsy at baseline. Patients will receive a single dose of monotherapy trilaciclib 240 mg/m^2 in the Trilaciclib Lead-In Phase, followed by tumor biopsy (sample #2) 7 (±1) days later. Following the biopsy, patients will enter the Treatment Phase in which trilaciclib 240 mg/m^2 on Day 1 of each cycle will be administered along with dose-dense anthracycline/cyclophosphamide followed by weekly taxane chemotherapy (doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 [AC] every 2 weeks for 4 cycles [Cycles 1-4], then weekly paclitaxel [T] 80 mg/m^2 weekly for 12 cycles [Cycles 5-16]). If pembrolizumab is given (per Investigator discretion), it will start with AC (Cycle 1 of chemotherapy). If carboplatin is given (per Investigator discretion) it will start with paclitaxel (Cycle 5).

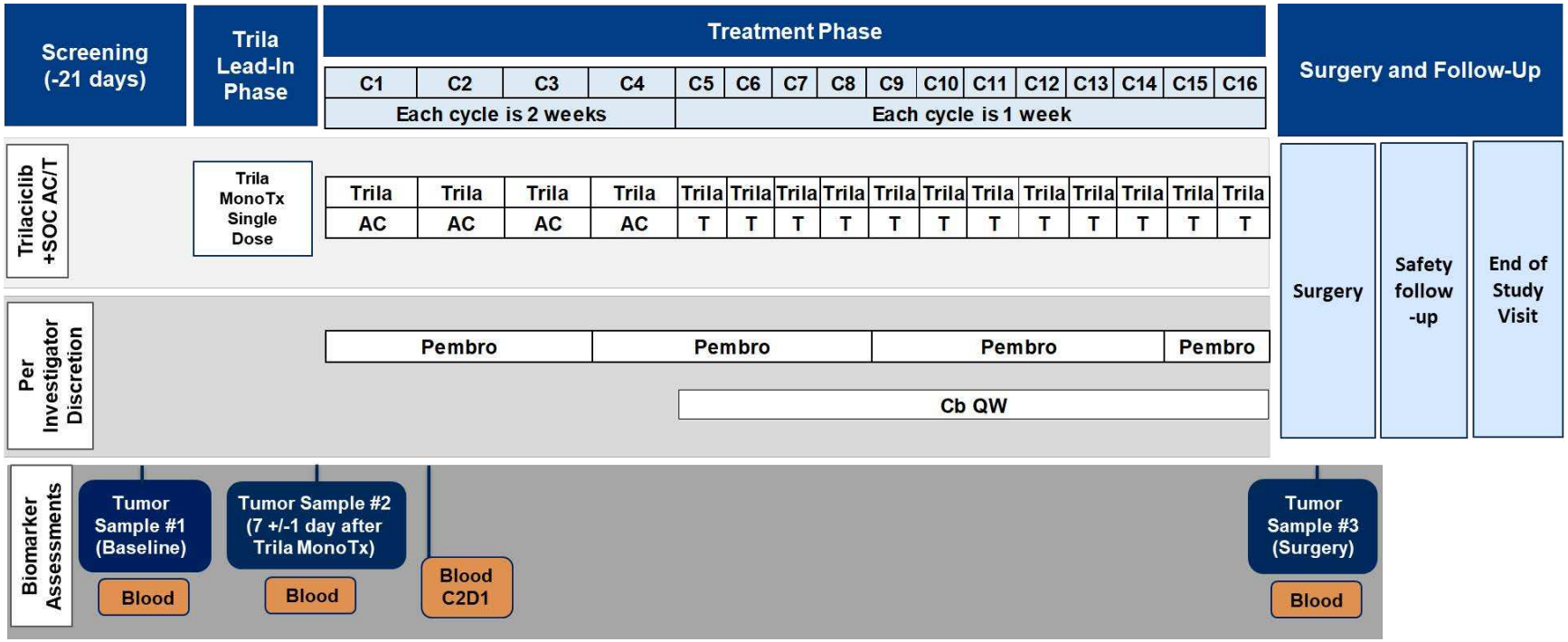
Growth factors (e.g., granulocyte colony stimulating factor [G-CSF]) should be administered after each cycle of AC chemotherapy and/or per Investigator discretion. Premedications will be given in accordance with local institutional guidelines.

Treatment cycles will occur consecutively without interruption, except when necessary to manage toxicities or for administrative reasons. There should be no more than 4 weeks between doses of chemotherapy. Dosing delays >4 weeks may be permitted on a case-by-case basis with the approval of the Investigator and Medical Monitor. Criteria which patients must meet in order to receive study drug on Day 1 are provided in Section 9.2.

Three to 5 weeks after the last dose of chemotherapy, patients will proceed to definitive surgery at which time tumor tissue (sample #3) will be collected if the patient has residual disease. A 30-day Safety Follow-up Visit will occur 30 (+7) days after last dose of trilaciclib and an End of Study Visit will occur within 14 days after surgery. Both the Safety Follow-up Visit and End of Study Visit may be conducted in person or by telephone and may occur at the same visit.

Tumor and blood collection is detailed in Section 11.2. A Safety Monitoring Committee (SMC) will monitor accumulating data throughout the study on a regular basis, as described in Section 11.4. The study will be completed when the criteria outlined in Section 10.4 have been met, or upon Sponsor termination of the study.

Figure 3: Study Schema



AC=doxorubicin/cyclophosphamide; C=cycle; Cb=carboplatin; D=day; Mono=monotherapy; Pembro=pembrolizumab; Q3W=every 3 weeks; QW=every week; SOC=standard of care; T=paclitaxel; TNBC=triple-negative breast cancer; Trila=trilaciclib; Tx=treatment

6.2. Rationale for the Primary Endpoint

Clinically meaningful anti-tumor efficacy results were observed in G1T28-04 in patients with advanced TNBC and were noted across multiple endpoints, including OS, PFS, and ORR. These results are currently being evaluated further in a Phase 3 study in advanced TNBC patients (G1T28-208; PRESERVE 2). However, the MOA of trilaciclib in TNBC is not completely understood and much remains to be learned regarding how best to leverage trilaciclib in treating patients. Earlier pre-clinical and translational data from SCLC studies demonstrate an enhancement of both CD4+ and CD8+ T cell proliferation following trilaciclib treatment (Lai, 2020). Specifically, trilaciclib-exposed T cells were able to withstand Treg suppression and proliferate, and longitudinal assessment of splenic T cell proliferation in trilaciclib treated mice, demonstrated a peak response of 4 days for CD8+ T cells and 7 days for CD4+ T cells. Additionally, in SCLC patients treated with trilaciclib, the ratio of CD8+/Tregs increased over time suggesting an increase in CD8+ T cell proliferation and/or depletion of Tregs in circulation. Based on these changes in the immune response during the course of trilaciclib treatment, the endpoints for this study are to measure cytotoxic CD8+ T cell infiltration in the tumor microenvironment and to quantitate the changes in cytotoxic CD8+ T cell/Tregs 7 days post single dose monotherapy trilaciclib (and prior to any chemotherapy/immunotherapy). Seven days was selected as the optimal timepoint for evaluating the immune MOA of trilaciclib based on parallel assessment of TILs in checkpoint blockade therapies (Kim, 2019; Choe, 2019). Collectively, the primary endpoint for this study will provide insight into the trilaciclib-specific effect on the generation of an anti-tumor T cell response.

6.3. Rationale for Tumor Biopsy Collection

Nonclinical data and data from samples of patients treated with trilaciclib suggest that the MOA of trilaciclib involves enhancement of immune activation and promotes anti-tumor immunity by differentially arresting cytotoxic and regulatory T cell subsets followed by a faster recovery of cytotoxic T lymphocytes than Tregs in tumor (See Section 4.1.1 and Section 4.4.1.1). The immune system is a network of dynamic players that are interdependent and is therefore difficult to capture in a single snapshot. Access to tumor tissue before, during, and after trilaciclib will be critical in understanding the immune response of trilaciclib and the complex interactions within the tumor microenvironment. This study will enable comprehensive analysis of biomarker endpoints and correlation with clinical activity, thus providing important insights into the immune, anti-neoplastic MOA of trilaciclib, identifying biomarkers for patient selection in future studies, and elucidating mechanisms of resistance to trilaciclib treatment.

Longitudinal tissue-based studies will be evaluated at 3 time points. Baseline tumor samples will provide initial immune features including PD-L1 expression and quantitation of cytotoxic CD8+ T cells and Tregs in untreated tumors which may be predictive of treatment response. Subsequent tumor samples will be analyzed similarly to discern dynamic changes during the course of therapy. Several studies have demonstrated that monitoring on-treatment tumor immune response via paired biopsies is more predictive of treatment outcome than immune features observed in only baseline samples (Lesterhuis, 2017; Park, 2020). The 7-day post trilaciclib timepoint is supported by studies with anti-PD-1 therapy showing peak cytotoxic CD8+ T cell proliferation in blood samples approximately 7 days post initial treatment and are

predictive of response (Huang, 2019; Kim, 2019). Overall, assessment of tumor biopsies will provide an immune analysis that is more reflective of the localized tumor immune environment.

6.4. Rationale for Peripheral Blood Collection

Longitudinal blood-based studies will provide a correlative assessment of the immune response during the course of the study. Unlike biopsy collection, blood samples are less invasive to obtain, can be collected more frequently, and provide a significant number of cells for multi-analysis. Blood will be collected at the same time points as tumor biopsy collection plus an additional time point at C2D1 of trilaciclib plus chemotherapy. All blood samples will be analyzed via mass spectrometry for immune profiling and flow cytometry for functional assessment via the measurement of cytokine production.

6.5. Rationale for Dose and Schedule of Trilaciclib

Previous studies demonstrated the recommended phase 2 dose of trilaciclib was 240 mg/m^2, and this dose is now approved by the US FDA for patients with SCLC. When trilaciclib was administered prior to chemotherapy to cancer patients, doses of 200 mg/m^2 (rounded up from 192 mg/m^2), 240 mg/m^2, and 280 mg/m^2 were evaluated. Trilaciclib exposures in cancer patients were slightly lower compared with healthy subjects, such that the dose of 240 mg/m^2 (rather than 200 mg/m^2) more closely matched the biologically effective dose of 192 mg/m^2. In addition, the dose of 240 mg/m^2 demonstrated maximal myeloprotection efficacy benefits (compared with 200 mg/m^2 and 280 mg/m^2) as measured by a variety of myeloprotection endpoints. The myeloprotective effect at 240 mg/m^2 was further evaluated and confirmed in three randomized controlled Phase 2 studies in SCLC patients. See the trilaciclib IB for details.

In all studies enrolling cancer patients, trilaciclib is always administered prior to systemic chemotherapy on each day of chemotherapy administration. Trilaciclib shall not be administered as monotherapy (i.e., on days that chemotherapy will be delayed) except for a single dose during the Trilaciclib Lead-In Phase, which will be used to evaluate changes in the tumor microenvironment prior to beginning the chemotherapy regimen.

6.6. Rationale for Patient Population

Among all breast cancer subtypes, TNBC has the worst prognosis and limited treatment options. Due to lack of specific molecular targets, treatment of TNBC has been relying on chemotherapy, in particular, anthracycline and taxane combinations. In light of the positive impact on OS duration observed in Study G1T28-04, as well as trilaciclib's manageable safety profile and improvement in PRO and myeloprotection endpoints, trilaciclib has the potential to add to the armamentarium of treating TNBC, an area of high unmet medical need. As such, better understanding of the immune mechanism of trilaciclib is greatly warranted.

6.7. Rationale for Neoadjuvant Therapy in TNBC and Secondary pCR Endpoint

Neoadjuvant therapy is an important and standard treatment strategy for patients with locally advanced TNBC as it increases the likelihood of tumor resectability and breast conservation and has a positive and significant correlation of pCR with long-term clinical benefit and OS (Liedtke, 2008). Early prospective observational studies evaluating outcome of neoadjuvant chemotherapy in different breast cancer subtypes revealed that TNBC was more chemosensitive compared with non-TNBC subtypes such as ER-positive, HER2-negative (luminal), with substantially increased pCR rate and clinical response rate. However, as a group, patients with TNBC had a poorer prognosis with significantly higher disease recurrence rate and lower survival rate. The poor long-term outcome in TNBC was found to be driven by those who did not achieve pCR after neoadjuvant chemotherapy (FDA, 2014; EMA, 2014). Patients who achieved pCR demonstrated sustained clinical benefit regardless of breast cancer subtypes (Carey, 2007; Liedtke, 2008).A large, pooled analysis demonstrated patients who attain pCR, when defined as no tumor in both breast and lymph nodes (ypT0 ypN0 or ypT0/is yp N0), have improved survival, with the strongest association observed in patients with TNBC (Cortazar, 2014).

6.8. Rationale for Anthracycline/Taxane-Based Chemotherapy

Per current National Comprehensive Cancer Network (NCCN) guidelines, dose-dense AC (doxorubicin/cyclophosphamide) followed by paclitaxel is a preferred regimen for neoadjuvant therapy for HER2-negative breast cancer patients. Addition of a taxane to an anthracycline-based regimen as adjuvant chemotherapy has been shown to improve both DFS and OS in locally-advanced breast cancer (De Laurentiis, 2008). The pCR rate for paclitaxel followed by an anthracycline and cyclophosphamide is about 30% (von Minckwitz, 2011).

The optimal schedule of paclitaxel was evaluated in the Eastern Cooperative Oncology Group (ECOG) E1199 trial in which neoadjuvant paclitaxel every 3 weeks for 4 cycles was compared to weekly for 12 cycles (Sparano, 2015). Weekly paclitaxel improved DFS and OS in the TNBC subset compared with the every 3 week paclitaxel arm. Weekly paclitaxel was also used as part of the chemotherapy backbone in the CALGB40603 and GeparSixto trials. As such, dose-dense AC followed by weekly paclitaxel was chosen as the treatment regimen in this neoadjuvant study.

To date, trilaciclib has demonstrated a manageable safety profile in combination with various chemotherapy agents, including gemcitabine, carboplatin, etoposide, and topotecan. Furthermore, the Phase 2 adaptive I-SPY2 study (NCT01042379) has included a study arm to evaluate the safety and efficacy of trilaciclib in combination with neoadjuvant paclitaxel (80 mg/m^2 weekly) followed by doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) every 2 or 3 weeks in locally advanced breast cancer. Data from this study are pending.

6.9. Rationale for Pembrolizumab (per Investigator Discretion)

Pembrolizumab functions as an immune checkpoint blockade by targeting PD-1, which helps to restore endogenous anti-cancer immunity. Pembrolizumab has shown significant clinical anti-cancer activity across multiple tumor types including melanoma, NSCLC, head and neck

cancer, bladder cancer and has gained FDA approval for treating early-stage and advanced TNBC, melanoma, head and neck cancer, and NSCLC.

Support for the use of PD-1/PD-L1 inhibition is provided by a recent meta-analysis which found that the addition of PD-1/PD-L1 blockade to neoadjuvant chemotherapy significantly improves pCR rates in TNBC patients (Tarantino, 2021). In an earlier Phase 1b study, pembrolizumab in combination with different chemotherapy regimens as neoadjuvant treatment for high-risk, early-stage TNBC resulted in pCR rates of 60% across all groups (Schmid, 2020b). These data are further supported by the ongoing Phase 3 study, KEYNOTE-522, which showed a higher pCR rate and improvement in EFS when pembrolizumab was added to standard neoadjuvant chemotherapy, regardless of disease PD-L1 status (Schmid, 2020a; Merck Press Release, 2021). Longer follow-up of the ongoing clinical trials is needed to confirm whether the increased in pCR rate will translate into a meaningful survival benefit.

Based on nonclinical studies, CDK4/6 inhibitors have been shown to enhance anti-tumor T cell activity through stabilization of PD-L1 expression on tumor cells, resulting in increased sensitivity to ICI, such as the PD-1 or PD-L1 monoclonal antibodies (Lai 2020; Deng 2018). Further, the addition of trilaciclib to chemotherapy/ICI combination has been shown to enhance the anti-tumor response (Lai 2020). Collectively, these nonclinical data support the combination of CDK4/6 inhibitors like trilaciclib with ICI. In the clinical setting, trilaciclib was well tolerated when given prior to induction treatment with the PD-L1 antibody, atezolizumab and etoposide/carboplatin (E/P/A) in patients with SCLC (Daniel, 2020). Compared with placebo, fewer patients treated with trilaciclib in combination with E/P/A experienced serious AEs and Grade 3 or Grade 4 AEs, mainly due to fewer hematologic events.

In this study, pembrolizumab can be given per Investigator discretion if deemed the best option for the patient at the dose level of 400 mg every 6 weeks. Though the dose level in KEYNOTE-522 was 300 mg every 3 weeks (Q3W), the FDA granted accelerated approval to the 400 mg every 6 week regimen based on PK modeling, safety, and exposure-response analyses in the Study KEYNOTE-555. In light of these data and convenience with the dose-dense AC and weekly paclitaxel schedule, the every 6 week dosing schedule for pembrolizumab was chosen for this study (Keytruda® Prescribing Information, 2021).

6.10. Rationale for Carboplatin (per Investigator Discretion)

Neoadjuvant carboplatin in combination with weekly paclitaxel at 80 mg/m^2 versus paclitaxel alone followed by the standard anthracycline/cyclophosphamide combination has shown increased pCR rates in patients with TNBC via 2 randomized trials using either weekly carboplatin at AUC 2 (the Phase II GeparSixto trial; von Minckwitz, 2014), or carboplatin at AUC 6 Q3W (the Phase III CALGB 40603 trial; Sikov, 2015). Due to toxicity, in the GeparSixto trial, the dose of carboplatin was reduced to AUC 1.5. A meta-analysis of 1,598 TNBC patients treated with platinum-based neoadjuvant chemotherapy demonstrated a pCR of 45%, with a significantly increased rate of pCR in those treated with platinum therapy vs. nonplatinum agents (risk ratio 1.45 (95% CI, 1.25 -1.68, p<0.0001; Petrelli, 2014). It is worth noting that in the CALGB 40603 study the carboplatin AUC6 Q3W plus weekly paclitaxel 80 mg/m^2 arm showed statistically significant increase in Grade 3/4 neutropenia (56% vs. 22%) and Grade 3/4 thrombocytopenia (20% vs. 4%) compared to paclitaxel alone arm. Taken together, the use of carboplatin in the neoadjuvant setting may provide benefit to some patients (as evidenced by

pCR); however, the survival effect has not been confirmed and platinum-based therapies come with additional hematologic and neurologic toxicities to consider.

Carboplatin (with gemcitabine) has been studied in combination with trilaciclib in the Phase 2 study G1T28-04 in locally recurrent/metastatic TNBC patients (see Section 4.4.2). A total of 68 patients were exposed to both carboplatin and trilaciclib in this study. Despite a longer duration of chemotherapy in the trilaciclib groups, Grade 3 and 4 hematologic toxicities associated with chemotherapy were reported at a similar frequency in patients with and without trilaciclib (Tan, 2019). As such, it is possible that the myeloprotective effects of trilaciclib may lessen the hematologic toxicities well documented with carboplatin.

In this study, carboplatin may be administered per Investigator discretion. The decision to add carboplatin should be made by the first dose of paclitaxel. The option to add pembrolizumab and carboplatin are exclusive of one another.

7. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as a protocol waiver or exemption, is not permitted.

7.1. Inclusion Criteria

Patient eligibility should be reviewed and documented by an appropriately qualified member of the Investigator study team before patients are included in the study. Patients must meet *all* of the following inclusion criteria to be eligible for enrollment into the study:

1. Treatment-naïve early-stage breast cancer in which neoadjuvant chemotherapy with an anthracycline/taxane combination is deemed to be a suitable therapy and patient intends to undergo curative surgery
2. Females ≥ 18 years of age
3. Documented diagnosis of estrogen receptor (ER)-negative and progesterone receptor (PR)-negative tumor, defined as <1% positive stained cells utilizing an assay consistent with local standards. Documented HER2-negative tumor as per 2018 College of American Pathologists criteria
4. Primary tumor ≥ 1.5 cm with any nodal status based on radiological or clinical assessment

   Note: bilateral tumors (i.e., synchronous cancers in both breasts) and/or multi-focal (ie, 2 separate lesions in the same quadrant)/multi-centric (i.e., 2 separate lesions in different quadrants) tumors are allowed as long as TNBC is confirmed for at least one focus. Inflammatory breast cancer is also permitted.
5. Provide archival tissue for the baseline tissue sample, consisting of representative formalin-fixed paraffin embedded (FFPE) tumor specimens in paraffin blocks (at least 75-micron). Unstained slides are not permitted. If archival tissue is insufficient, fresh tissue biopsy is required.

   Note: Sponsor agreement is required for FFPE tumor tissues samples that were obtained greater than 30 days prior to the date that the informed consent was signed.
6. ECOG performance status of 0 or 1
7. Adequate organ function as demonstrated by the following laboratory values:
   a. Hemoglobin ≥ 9 g/dL without RBC transfusion within 2 weeks prior to first dose of study drug
   b. Absolute neutrophil count ≥ 1.5×10^9/L without G-CSF support within 2 weeks prior to first dose of study treatment
   c. Platelet count ≥ 100×10^9/L without transfusion within 2 weeks prior to first dose of study drug
   d. Estimated glomerular filtration rate ≥ 50 mL/minute/1.73 m^2
   e. Total bilirubin ≤ 1.2 × the upper limit of normal (ULN)
   f. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 × ULN

8. Prothrombin time and/or international normalized ratio (INR) must be within normal limits of the local laboratory ranges
9. Patient must agree to research tumor biopsies including at least one on-treatment biopsy (and additional biopsy at baseline, if required)
10. Left ventricular ejection fraction of ≥50% or ≥ institution lower limit of normal as assessed by echocardiogram (ECHO) or multigated acquisition (MUGA) scan performed at screening
11. Contraceptive use by women or male partners should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies. Please see Section 17.3 for detailed instructions on methods of contraception requirements
12. Capable of giving signed informed consent, which includes compliance with the requirements and restrictions listed in the informed consent form and in this protocol

7.2. Exclusion Criteria

A patient will not be eligible for participation in this study if *any* of the following criteria apply:

1. Prior systemic therapies or radiation for current breast cancer
2. History of invasive malignancy ≤3 years prior to signing informed consent except for adequately treated basal cell or squamous cell skin cancer or in situ cervical cancer
3. History of breast cancer including ipsilateral ductal carcinoma in situ (DCIS) treated with radiotherapy at any time
4. History of hypersensitivity to the active ingredient or any component of study drugs to be administered to patient (i.e., trilaciclib, doxorubicin, cyclophosphamide, paclitaxel, carboplatin)
5. Previous exposure to doxorubicin of more than 200 mg/m^2 (as lifetime exposure to doxorubicin is not to exceed 450 mg/m^2)
6. For patients who will receive pembrolizumab:
    a. History of active autoimmune disease that has required systemic treatment in past 2 years (i.e., with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment
    b. Diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study drugs
    c. History of (non-infectious) pneumonitis that required steroids or current pneumonitis
    d. Known history of active tuberculosis (Bacillus Tuberculosis)
    e. History of hypersensitivity to any anti-PD-1, anti-PD-L1, or anti-PD-L2 agent or with an agent directed to another co-inhibitory T cell receptor (e.g., CTLA-4, OX-40, CD137)

7. Receipt of a live, attenuated vaccine within 30 days prior to the first dose of study drugs or anticipation that such a live, attenuated vaccine will be required during the study treatment period. Other vaccine types such as inactivated or nucleic acid vaccines (e.g., those given for COVID-19 or influenza) are permitted if given > 21 days prior to first dose of study drug. In case of COVID-19 vaccinations requiring two doses, the second dose must be given >21 days prior to first dose of study drug
8. History of severe hepatic impairment (defined as Child Pugh Class C or serum bilirubin level greater than 5 mg/dL)
9. Uncontrolled ischemic heart disease or uncontrolled symptomatic congestive heart failure (Class II-IV as defined by the New York Heart Association [NYHA] functional classification system)
10. Known history of stroke, cerebrovascular accident, severe/unstable angina, myocardial infarction, or coronary angioplasty/stenting/bypass grafting within 6 months prior to enrollment
11. QTcF interval >480 msec at Screening. For patients with ventricular pacemakers, QTcF >500 msec
12. Known serious active infection (e.g., human immunodeficiency virus [HIV], hepatitis B or C, tuberculosis). HIV testing is not required. Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as having a negative HBsAg test and a positive antibody to hepatitis B core antigen [anti-HBc] antibody test) are eligible. Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction is negative for HCV ribonucleic acid (RNA).
13. Women who are pregnant or breastfeeding
14. Participation in other studies involving active treatment with investigational drug(s) in Phases 1 to 4 within 30 days prior to the first dose of study drug
15. Prior hematopoietic stem cell or bone marrow transplantation
16. 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
17. Major surgical procedure, open biopsy, or significant traumatic injury within 30 days prior to first dose of study drug

8. SCHEDULE OF ASSESSMENTS

The procedures and assessments to be performed during the study are outlined in Table 4. The timing and number of samples collected for 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.

Unless otherwise specified, assessments are to be completed within ±1 day of the scheduled visit date. Unscheduled assessments and visits to manage patient safety may occur at the Investigator's discretion.

Table 4: Schedule of Assessments

Protocol Activity	Screening	Trilaciclib Lead-In Phase		Treatment Phase					Definitive Surgery	Safety Follow-up Visit	End of Study Visit	See Protocol Section for Additional Details
				Doxorubicin/ Cyclophosphamide +/- pembrolizumab *Each cycle = 2 weeks*				Paclitaxel +/- carboplatin [b] +/- pembrolizumab [b] *Each cycle = 1 week*				
		Single dose mono. trila.	On-tx biopsy	C1 D1[a]	C2 D1	C3 D1	C4 D1	C5 through C16 D1				
Visit Window	**Up to 21 days prior to first dose**	**N/A**	**7d ±1d after trila**	**N/A**	**±2d**	**±2d**	**±2d**	**±1d**	**3-5 weeks following tx.**	**30d (+7) after last trila dose**	**Within 14d after surgery**	
Informed consent	X											Section 13.3
ER-/PR-/HER2- status	X											Section 11.1.1
Inclusion/ exclusion criteria	X											Section 7
Baseline characteristics and demographics	X											Section 11.1.2
Medical and surgical history	X											Section 11.1.3
Concomitant medications and procedures	X	X		X	X	X	X	X (C5, 8, 11, 16 only)	X	X	X	Section 9.5
Complete physical exam	X											Section 11.3.2
Directed physical exam		X		X	X	X	X	X (C5, 8, 11, 16 only)				Section 11.3.2
Vitals	X	X		X	X	X	X	X				Section 11.3.1
ECOG performance status	X	X		X				X (C5 and 16 only)			X	Section 11.3.3
Adverse event reporting	X	X		X	X	X	X	X	X	X	X	Section 11.3.7
Hematology	X	X		X	X	X	X	X				Section 11.3.4
Serum chemistry	X	X		X	X	X	X	X				Section 11.3.4
Coagulation (PT or INR)	X											Section 11.3.4
12-lead ECG	X											Section 11.3.5
Echocardiogram or MUGA for LVEF assessment	X											Section 11.3.6
Urinalysis	X											Section 11.3.4
Urine/serum HCG (WOCBP only)	X			X				X (C5, 11, 16 only)				Section 11.3.4

Protocol Activity	Screening	Trilaciclib Lead-In Phase		Treatment Phase					Definitive Surgery	Safety Follow-up Visit	End of Study Visit	See Protocol Section for Additional Details
				Doxorubicin/ Cyclophosphamide +/- pembrolizumab *Each cycle = 2 weeks*				Paclitaxel +/- carboplatin [b] +/- pembrolizumab [b] *Each cycle = 1 week*				
		Single dose mono. trila.	On-tx biopsy	C1 D1[a]	C2 D1	C3 D1	C4 D1	C5 through C16 D1				
Visit Window	**Up to 21 days prior to first dose**	**N/A**	**7d ±1d after trila**	**N/A**	**±2d**	**±2d**	**±2d**	**±1d**	**3-5 weeks following tx.**	**30d (+7) after last trila dose**	**Within 14d after surgery**	
Biomarker and Efficacy Assessments												
Blood samples for biomarkers		X (pre-dose)	X (pre-biopsy)		X (pre-dose)				X (-3 day)			Section 11.2.2
Tumor sample #1 (Baseline)	X											Section 11.2.1
Tumor sample #2 (On treatment)			X									Section 11.2.1
Tumor sample #3 (Surgery)									X			Section 11.2.1
pCR assessment									X			
Treatment												
Trilaciclib		X		X	X	X	X	X				Section 9.1
Doxorubicin/ cyclophosphamide				X	X	X	X					Section 9.1
Paclitaxel								X				Section 9.1
Carboplatin AUC 1.5 (Investigator discretion)								X				Section 9.1
Pembrolizumab (Investigator discretion)				X			X	X (C9 and C15 only)				Section 9.1
Definitive surgery									X			Section 11.3.8

AUC=area under the curve; C=cycle; chemo=chemotherapy; d=day; ECG=electrocardiogram; ECOG=Eastern Cooperative Oncology Group; ER=estrogen receptor; HCG=human chorionic gonadotropin; HER2=human epidermal growth factor receptor 2; INR=international normalized ratio; LVEF=left ventricular ejection fraction; mono=monotherapy; MUGA=multiple-gated acquisition; N/A=not applicable; pCR=pathologic complete response; PR=progesterone receptor; PT=prothrombin time; q2w=every 2 weeks; trila=trilaciclib; tx=treatment; WOCBP=women of childbearing potential

[a] C1D1 may be the same day as the on-treatment biopsy, as long as the biopsy is done prior to initiation of chemotherapy.

[b] Per Investigator discretion.

9. STUDY TREATMENT

9.1. Study Drugs Administered

Study drugs are defined as any investigational product, chemotherapy, immunotherapy, or growth factor support intended to be administered to a study patient according to the study protocol. Study drugs used in this protocol are described in Table 5.

Table 5: Study Drugs

Name	**Trilaciclib**	**Doxorubicin**	**Cyclophosphamide**	**Paclitaxel**	**Carboplatin**	**Pembrolizumab**	**G-CSF (Filgrastim)**	**G-CSF (Pegfilgastim)**
Type	Investigational Product	Chemotherapy	Chemotherapy	Chemotherapy	Chemotherapy per Investigator discretion	Immunotherapy per Investigator discretion	Growth factor support	Growth factor support
Dose Formulation	Single-use, sterile powder to be reconstituted and further diluted with 250 mL of 5% dextrose in water (D5W) or 0.9% sodium chloride in water (normal saline) per the Pharmacy Manual	See US prescribing information						
Unit Dose Strength(s)	300 mg/20 mL							
Dosage Level(s)	240 mg/m^2 administered as single-dose monotherapy in Trilaciclib Lead-In Phase, then on Day 1 of each chemotherapy cycle	60 mg/m^2 every 2 weeks for 4 cycles (Cycles 1-4)	600 mg/m^2 every 2 weeks for 4 cycles (Cycles 1-4)	80 mg/m^2 every week for 12 cycles (Cycles 5-16)	AUC 1.5 QW starting on Day 1 of paclitaxel chemotherapy (Cycles 5-16)	400 mg fixed dose Q6W starting on Day 1 of AC (Cycle 1) and continued through Treatment Phase	5 µg/kg/day starting approximately 24 hrs after each cycle of AC and continued for at least 72 hours	100 µg/kg or 6 mg administered approximately 24 hrs after each cycle of AC
Route of Administration	IV	IV bolus	IV	IV	IV	IV	SC	SC
Infusion Time	30 minutes	N/A	30 to 60 minutes or per institutional standard	3 hours or per institutional standard	15 to 60 minutes or per institutional standard	30 minutes or per institutional standard	N/A	N/A

AC=doxorubicin/cyclophosphamide; G-CSF=granulocyte colony stimulating factor; IV=intravenous; N/A=not applicable; Q6W=every 6 weeks; QW=every week; SC=subcutaneous; US=United States

9.1.1. Dose, Dosing Regimen, and Route

9.1.1.1. Trilaciclib

Trilaciclib for injection, 300 mg/vial is supplied as a sterile, preservative-free, yellow, lyophilized cake in a single-dose vial (300 mg/20 mL). Trilaciclib 240 mg/m^2 will be administered as an IV infusion as monotherapy during the Trilaciclib Lead-In Phase and just prior to chemotherapy during the Treatment Phase.

Trilaciclib must be reconstituted and further diluted prior to IV infusion as outlined in the Pharmacy Manual. Aseptic technique must be used for reconstitution and dilution. Following reconstitution, the solution must then be diluted to the calculated dose based on the body surface area (BSA) of the patient. Actual body weight should be utilized for dose calculations. If there is a change in body weight ≥10% relative to the weight at the time of the last dose calculation, dose should be recalculated. Recalculation of 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 and will be made at the discretion of the Investigator. No trilaciclib dose reductions for toxicity management will be allowed during the study.

9.1.1.1.1. Administration of Trilaciclib

- Administer diluted trilaciclib solution as a 30-minute IV infusion completed within 4 hours prior to the start of chemotherapy.
- When administered with chemotherapy, trilaciclib is always administered first. Results from hematology labs should be reviewed prior to administration of trilaciclib. If administration of chemotherapy is delayed or discontinued, trilaciclib will also be delayed or discontinued.
- Diluted trilaciclib solution must be administered with an infusion set, including an in-line filter (0.2 or 0.22 micron). Compatible in-line filters include polyether sulfone, polyvinylidene fluoride, and cellulose acetate.
- Do not administer diluted trilaciclib solution with a polytetrafluorethylene (PTFE) in-line filter. PTFE in-line filters are not compatible with diluted trilaciclib solution.
- Do not co-administer other drugs through the same infusion line.
- Do not co-administer other drugs through a central access device unless the device supports co-administration of incompatible drugs.

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. Upon completion of infusion of diluted trilaciclib solution, the infusion line/cannula must be flushed with at least 20 mL sterile 5% dextrose in water or sterile 0.9% sodium chloride in water (normal saline).

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

9.1.1.2. Chemotherapy Backbone: Doxorubicin, Cyclophosphamide, and Paclitaxel

Descriptions of the formulations of commercially available doxorubicin, cyclophosphamide, and paclitaxel can be found in the respective current prescribing information. The dose amount required for chemotherapy will be calculated based on milligrams per square meter of BSA (mg/m^2). Premedications should be administered as per prescribing information or per institutional standard.

Doxorubicin at a dose level of 60 mg/m^2 will be administered by IV on Day 1 of Cycles 1 through 4 as instructed per prescribing information or per institutional standard.

Cyclophosphamide at a dose level of 600 mg/m^2 will be administered by IV as instructed per prescribing information or per institutional standard on Day 1 of Cycles 1 through 4.

Paclitaxel at a dose level of 80 mg/m^2 will be administered by IV per prescribing information or per institutional standard on Day 1 of Cycles 5 through 16.

Actual body weight should be utilized for dose calculations. If there is a change in body weight ≥10% relative to the weight at the time of the last dose calculation, dose should be recalculated. Recalculation of 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 and will be made at the discretion of the Investigator.

9.1.1.3. Treatment per Investigator Discretion: Carboplatin and Pembrolizumab

Descriptions of the formulations of commercially available carboplatin and pembrolizumab can be found in the respective current prescribing information. If carboplatin is administered, the dose amount required will be calculated as AUC at a dose level of 1.5 weekly during paclitaxel treatment. If pembrolizumab is administered, the dose level will be 400 mg every 6 weeks starting with Cycle 1 of AC and continued while receiving chemotherapy. Premedications should be administered as per prescribing information or per institutional standard.

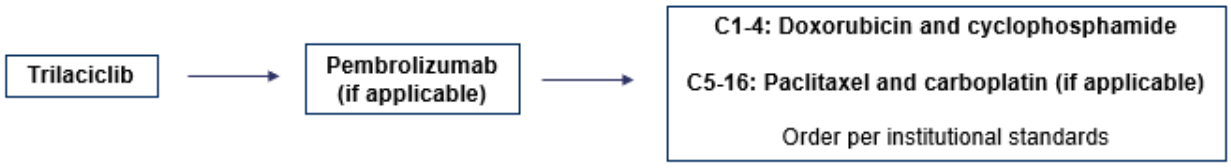
9.1.1.3.1. Sequence of Administration of Study Drugs

The sequence of administration on treatment days as outlined in Table 4 is as follows (Figure 4):

- Trilaciclib is always administered first and infusion must be complete before administrating other agents.
- If pembrolizumab is given, it will be administered after trilaciclib and before chemotherapy. Pembrolizumab may be administered immediately following trilaciclib, but not until the completion of the trilaciclib infusion.
- SOC chemotherapy (doxorubicin/cyclophosphamide or paclitaxel) is always administered after trilaciclib (and pembrolizumab if given). Trilaciclib (and pembrolizumab) infusions must be complete before initiating chemotherapy. The interval between completion of the trilaciclib infusion and the initiation of the first chemotherapy drug should not be greater than 4 hours.
- The order of administration of doxorubicin and cyclophosphamide is per institutional standard.

- If carboplatin is given, the order of administration of paclitaxel and carboplatin is per institutional standard.

Figure 4: Sequence of Administration of IV Study Drugs



9.1.2. Preparation, Handling, Storage, and Accountability

The Investigator or institution is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, dispensing, and final disposition records). The Investigator/institution may assign some or all of the Investigator's/institution's duties for investigational product(s) accountability to an appropriate pharmacist or another appropriate individual who is under the supervision of the Investigator/institution.

Further guidance and information are provided in the Pharmacy Manual.

9.1.3. Treatment Compliance

The Investigator or designee will dispense the study drug, via a Pharmacist/designee, only for use by patients enrolled in the study as described in this protocol (Section 9.1). The study drug shall not be used for reasons other than those described in this protocol. The clinical study site will maintain records of study drug receipt, preparation, and dispensing, including the applicable lot and vial numbers; patient's height, body weight, and BSA; date and time of the start of each infusion for trilaciclib, doxorubicin, cyclophosphamide, paclitaxel (and carboplatin and pembrolizumab if appliable); and planned and actual infusion volumes.

9.2. Criteria for Starting Study Treatment and Each Subsequent Cycle

Patients must meet specific hematologic criteria for each dosing day per Table 6 and Table 7 and any nonhematologic toxicities (except alopecia) must be ≤ Grade 2 or have returned to baseline. Study drug administration will continue as per protocol to completion or early discontinuation of chemotherapy, until unacceptable toxicity, Investigator's decision to withdraw the patient from study treatment, pregnancy of the patient, noncompliance with study treatment or procedure requirements, consent withdrawal, becoming lost-to-follow-up, death, or administrative reasons require cessation of treatment.

Note: C1D1 of the Treatment Phase may be the same day as the on-treatment biopsy (7 days [±1 day] after trilaciclib monotherapy), as long as the biopsy is done prior to initiation of chemotherapy.

9.3. Toxicity Management and Dose Modifications

Toxicities will be graded according to the National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0. For dose delays due to toxicity, the patient should be followed (at least) weekly to monitor the toxicity until treatment criteria are met or until they discontinue treatment. No more than 2 dose reductions in total are allowed per

study drug (expect trilaciclib for which no dose reductions are allowed). Toxicity that requires dose reductions more than twice for one study drug will lead to discontinuation of that study drug. If a dose delay does *not* require discontinuation of chemotherapy, patients may resume treatment when dosing criteria are met and continue on treatment to complete the full number of cycles per protocol.

All dose reductions are permanent, and no dose increases will occur following a dose reduction. Likewise, discontinuation from treatment is permanent and once a patient has had a component or entire treatment discontinued, it may not be restarted.

9.3.1. Dose Modifications for Trilaciclib

Trilaciclib dosing is not to be modified and will remain at 240 mg/m^2 throughout the study. Trilaciclib will be administered only on days that chemotherapy is administered except in the Trilaciclib Lead-In Phase when monotherapy trilaciclib will be given. Recommendations for management of trilaciclib AESIs are provided in Table 9.

9.3.2. Dose Modifications for Chemotherapy

Doxorubicin, cyclophosphamide, paclitaxel, and carboplatin dose reductions for hematologic toxicities are based on values obtained within 24 hours of chemotherapy on Day 1 of a cycle and at any time for non-hematologic toxicities. Dose modifications are permitted for chemotherapy according to the organ system showing the greatest degree of drug-related toxicity and are to be managed per Investigator discretion and institutional guidelines; recommendations are provided in Table 6 and Table 7.

Table 6: Dose Modification Recommendations for Doxorubicin and Cyclophosphamide (AC)

Toxicities	Grade or Actual Value	Action
Hematologic		
Neutropenia	≥1000/mm^3 Grade 1/Grade 2	No change to AC
	<1000/mm^3 Grade 3/Grade 4	Hold AC until ANC≥1000/mm^3. Resume based on timing of recovery: • ≤1 week: No change to AC • ≥1 but <3 weeks: Reduce AC by 20% for all subsequent cycles • ≥3 weeks: Permanently discontinue AC (see Section 9.3.4 below)
Febrile neutropenia	ANC ≤1 000/mm^3, fever ≥38.5°C Grade 3 and Grade 4	Hold AC until resolved (ANC >1000/mm^3, fever <38.5°C, and resolution of any signs of infection) Resume according to number of episodes: • First episode: no change to AC • Second episode: Reduce AC by 20% for all subsequent cycles • Third episode: Permanently discontinue AC (see Section 9.3.4 below)

Toxicities	Grade or Actual Value	Action
Thrombocytopenia	75-<100,000/mm^3 Grade l	Hold AC until ≥100,000/mm^3, resume AC based ontiming of recovery: • ≤1week: No change to AC • ≥1 but <3 weeks: Reduce AC by 20% for all subsequent cycles. • ≥3 weeks: Permanently discontinue AC (see Section 9.3.4 below)
	<75,000/mm^3 ≥ Grade 2	Hold AC until ≥100,000/mm^3 • Reduce AC by 20% for all subsequent cycles • Permanently discontinue AC if held for ≥3 weeks in a row (see Section 9.3.4 below)
Anemia	All grades	No change to AC • Iron studies should be done and iron should be replaced as indicated. • Red blood cell transfusions can be given at the Investigator's discretion.
Non-Hematologic		
Nausea/Vomiting	Grade l or 2	No change to AC
	≥Grade 3	Hold AC until resolved to ≤Grade 1. • Resume AC at same dose with modification of premedication • Second episode ≥Grade 3 despite maximum supportive care, reduce AC by 20% for all subsequent cycles
Mucositis/Stomatitis	Grade l or 2	No change to AC
	≥Grade 3	Hold until resolved to ≤Grade 1. • Resume AC at same dose with modification of premedication • Second episode ≥Grade 3 despite maximum supportive care, reduce AC by 20% for all subsequent cycles
Hepatic (e.g., AST, ALT, and/or bilirubin)	Grade 1	No change to AC
	≥ Grade 2 or 3	• Hold AC until resolved to Grade 1 and resume same dose • Permanently discontinue AC if held for ≥ 3 weeks in a row (see Section 9.3.4 below)
	Grade 4	• Discontinue AC (see Section 9.3.4 below) Note all concurrent ALT/AST >3xULN and total bilirubin >2xULN should be evaluated for potential Hy's law (see Section 9.3.6)
Cardiac toxicity	Grade 1 or 2	No change to AC
	≥ Grade 3	Permanently discontinue doxorubicin (see Section 9.3.4 below)

Toxicities	Grade or Actual Value	Action
Anaphylaxis/ hypersensitivity	Mild	Complete infusion, observe until symptom resolved
	Moderate	• Stop infusion and treat per standard practice • Resume infusion at half of the infusion speed if symptoms resolve • Stop if symptoms recur
	Severe	Stop infusion immediately and permanently discontinue treatment (seeSection 9.3.4 below)
Other significant toxicities excluding fatigue, alopecia and leukopenia per Investigator discretion	Grade 2	• Hold AC until resolve to ≤ Grade 1 • Resume at same dose and increase supportive care measures, if available
	≥ Grade 3	• Hold AC and discuss with Medical Monitorfor further instructions • If ≥ Grade 3 toxicity recurs upon rechallenge, Permanently discontinue (seeSection 9.3.4 below)

AC=doxorubicin/cyclophosphamide; ANC=absolute neutrophil count; ALT=alanine aminotransferase; AST=aspartate aminotransferase; ULN=upper limit of normal

Table 7: Dose Modification Recommendations for Paclitaxel Alone or With Carboplatin

Toxicities	Grade or Actual Value	Action
Hematologic		
Neutropenia	≥1000/mm^3 Grade 1/Grade 2	No change to paclitaxel or carboplatin • For ANC ≤1500/mm^3, prophylactic G-CSF (filgrastim) use at the discretion of the Investigator [a]
	<1000/mm^3 Grade 3/Grade 4	Hold paclitaxel and/or carboplatin until ANC ≥1000/mm^3. G-CSF per Investigator discretion [a]. Resume paclitaxel and/or carboplatin based on timing of recovery: • ≤1 week: No change to paclitaxel and carboplatin • >1 but <3 weeks: Reduce paclitaxel dose by 70 mg/m^{2}and/or carboplatin to AUC 1.1 for all subsequent cycles • ≥3 weeks: Permanently discontinue paclitaxel and/or carboplatin (see Section 9.3.4 below)

Toxicities	Grade or Actual Value	Action
Febrile neutropenia	ANC ≤1000/mm^3 and fever ≥38.5°C Grade 3/Grade 4	Hold paclitaxel and/or carboplatin until resolved (ANC >1000/mm^3, fever <38.5°C, and resolution of any signs of infection). G-CSF (filgrastim) use at the discretion of the Investigator [a] Resume paclitaxel and/or carboplatin according to number of episodes: • First episode: no change to paclitaxel or carboplatin. Recommend adding prophylactic G-CSF for subsequent cycles • Second episode: Reduce paclitaxel dose to 70 mg/m^2 and/or carboplatin to AUC 1.1 for all subsequent cycles • Third episode: Permanently discontinue paclitaxel and/or carboplatin (see Section 9.3.4 below)
Thrombocytopenia	75-<100,000/mm^3 Grade 1	Hold paclitaxel and/or carboplatin until ≥100,000/mm^3, resume treatment based on timing of recovery: • ≤1 week - no change to paclitaxel or carboplatin • >1 but <3 weeks - Reduce paclitaxel dose to 70 mg/m^2 and/ or carboplatin to AUC 1.1) for all subsequent doses • ≥3 weeks: Permanently discontinue paclitaxel and/or carboplatin (see Section 9.3.4 below)
	<75,000/mm^3 ≥Grade 2	Hold paclitaxel and/or carboplatin until ≥100,000/mm^3. • Reduce paclitaxel dose to 70 mg/m^2 and/or carboplatin to AUC 1.1 for all subsequent doses. • Permanently discontinue paclitaxel and/or carboplatin if held for ≥ 3 weeks in a row (see Section 9.3.4 below)
Anemia	All grades	No change to paclitaxel or carboplatin • Iron studies should be done and iron should be replaced as indicated • Red blood cell transfusions can be given at the Investigator discretion
Non-Hematologic		
Nausea/Vomiting	Grade 1 or 2	No change to paclitaxel or carboplatin
	≥ Grade 3	Hold paclitaxel and/or carboplatin until ≤ Grade 1. • Resume paclitaxel and/or carboplatin at same dose with modification of premedication • Second episode ≥ Grade 3 despite with maximum supportive care, reduce paclitaxel to 70 mg/m^2 and/or carboplatin to AUC 1.1 for all subsequent doses
Mucositis/Stomatitis	Grade 1 or 2	No change to paclitaxel or carboplatin
	≥ Grade 3	Hold paclitaxel until ≤Grade 1. • Resume paclitaxel at same dose with modification of premedication • Second episode ≥ Grade 3 despite maximum supportive care, reduce paclitaxel to 70 mg/m^2, and/or carboplatin to AUC 1.1 for all subsequent doses

Toxicities	Grade or Actual Value	Action
Neurotoxicity	Grade 1 or 2	No change to paclitaxel or carboplatin
	Grade 3	Hold paclitaxel and/or carboplatin until ≤Grade 2. • Reduce paclitaxel dose to 70 mg/m^2, and/ or carboplatin to AUC 1.1 for all subsequent doses • Permanently discontinue paclitaxel and/or carboplatin if held for ≥ 3 weeks in a row (see Section 9.3.4 below)
	Grade 4	• Permanently discontinue paclitaxel and/or carboplatin if held for ≥ 3 weeks in a row (see Section 9.3.4 below)
Hepatic (e.g., AST, ALT, and/or bilirubin)	Grade 1	No change to paclitaxel or carboplatin
	Grade 2 or 3	• Bilirubin fractionation should be performed if total bilirubin > 1.5xULN; if mostly indirect, continue dose and evaluate for alternate causes such as Gilbert's syndrome • Hold paclitaxel and/or carboplatin until ≤ Grade 1 and resume at same dose level • Permanently discontinue paclitaxel and/or carboplatin if held for ≥ 3 weeks in a row (see Section 9.3.4 below)
	Grade 4	• Permanently discontinue paclitaxel and/or carboplatin (see Section 9.3.4 below) Note all concurrent ALT/AST >3xULN and total bilirubin > 2xULN should be evaluated for potential Hy's law (see Section 9.3.6)
Anaphylaxis /hypersensitivity	Mild	• Complete paclitaxel or carboplatin infusion, observe until symptoms resolve
	Moderate	• Stop infusion and treat per standard practice • Resume infusion at half of the infusion speed if symptoms resolve • Stop if symptoms recur
	Severe	• Stop infusion immediately and permanently discontinue paclitaxel and/or carboplatin (see Section 9.3.4 below)
Other significant toxicities excluding fatigue, alopecia, and leukopenia per Investigator discretion	Grade 2	• Hold paclitaxel and/or carboplatin until ≤ Grade 1 • Resume at the same dose level and increase supportive care measures, if available
	≥ Grade 3	• Hold paclitaxel and/or carboplatin, and discuss with Medical Monitor for further instructions • If ≥ Grade 3 toxicity recurs upon rechallenge, permanently discontinue paclitaxel and/or carboplatin (see Section 9.3.4 below)

ALT=alanine aminotransferase; ANC=absolute neutrophil count; AST=aspartate aminotransferase; AUC=area under the curve; G-CSF=granulocyte colony stimulating factor; ULN=upper limit of normal

[a] Filgrastim should not be given on the same day as chemotherapy. Pegfilgrastim may not be used with paclitaxel due to its weekly dosing schedule.

9.3.3. Dose Modifications for Pembrolizumab

Pembrolizumab dosing is not to be modified and will remain at 400 mg fixed dose throughout the study. Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Recommendations for withholding or discontinuing pembrolizumab are provided in Table 8 below. Importantly, permanently discontinue for any severe or Grade 3 (Grade 2 for recurrent pneumonitis) drug-related AE that recurs or any life-threatening event.

Table 8: Dose Modification Recommendations for Pembrolizumab

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Diarrhea/Colitis	Grade 2 or 3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks
	Grade 4	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab
AST, ALT, or Increased Bilirubin	Grade 2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose
	Grade 3 or 4	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab
Type 1 diabetes mellitus (T1DM) if newonset including diabetic ketoacidosis or Hyperglycemia (≥ Grade 3) ifassociated withketosis (ketonuria) or metabolic acidosis	See toxicity	Hold pembrolizumab fornew onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patient is clinically and metabolically stable
Hypophysitis	Grade 2 to 4	Toxicity resolves toGrade 0-1. Therapy with pembrolizumab can be continued while endocrine replacement therapy is instituted	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks
Hyperthyroidism	Grade 3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks
	Grade 4	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Treatment Discontinuation
Hypothyroidism	N/A	Therapy with pembrolizumab can becontinued while thyroid replacement therapy is instituted	Therapy with pembrolizumab can be continued while thyroid replacement therapy is instituted
Infusion Reaction	Grade 3 or 4	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab
Pneumonitis	Grade 2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks
	Grade 3-4 or recurrent Grade 2	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab
Renal Failure or Nephritis	Grade 2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within6 weeks
	Grade 3-4	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab
All Other Drug-Related Toxicity[a]	Grade 3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks
	Grade 4	Permanently discontinue pembrolizumab	Permanently discontinue pembrolizumab

AE=adverse event; ALT=alanine aminotransferase; AST=aspartate aminotransferase
[a] Patients with intolerable or persistent Grade 2 drug-related AEs may hold study drug per Investigator discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 6 weeks of the last dose.

9.3.4. Discontinuation of a Component or Entire Regimen

During the study, if one or more than one component of the regimen must be discontinued due to toxicity, the Investigator should follow these recommendations:

- If all chemotherapy drugs are discontinued, then discontinue trilaciclib and proceed with surgery.
- If only trilaciclib is discontinued, then continue chemotherapy ± pembrolizumab for the remaining cycles as planned per protocol, followed by surgery.

- If doxorubicin and/or cyclophosphamide are discontinued, then proceed with trilaciclib and paclitaxel ± carboplatin ± pembrolizumab as planned per protocol, followed by surgery.
- If only paclitaxel is discontinued, then continue with trilaciclib ± carboplatin ± pembrolizumab, if applicable OR if only receiving paclitaxel with trilaciclib, then proceed to surgery as per protocol.
- If only carboplatin is discontinued, then continue with paclitaxel, trilaciclib, ± pembrolizumab for the remaining cycles as planned per protocol, then proceed to surgery.
- If only pembrolizumab is discontinued, then continue with trilaciclib and chemotherapy as planned per protocol, then proceed to surgery.

Please note that with the exception of the trilaciclib monotherapy Lead In Phase, trilaciclib monotherapy is prohibited.

9.3.5. Recommended Actions with Trilaciclib for Adverse Events of Special Interest

Suggested actions to be taken with trilaciclib following AESI are provided in Table 9.

Table 9: Recommended Actions with Trilaciclib Following AESIs

AESI	Severity	Recommended Action
Injection site reactions including phlebitis and thrombophlebitis	Grade 1: Tenderness with or without symptoms (e.g., warmth, erythema, itching)	Interrupt or slow infusion of trilaciclib. If 0.9% normal saline is being used as a diluent/flush, consider changing to 5% dextrose as appropriate for subsequent infusions.
	Grade 2: Pain; lipodystrophy; edema; phlebitis	Interrupt infusion of trilaciclib. If pain not severe, follow instructions for Grade 1. Otherwise, stop infusion in extremity and rotate site of infusion to site in alternative extremity. If 0.9% 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.
	Grade 3: Ulceration or necrosis; severe tissue damage; operative intervention indicated. Grade 4: Life threatening consequences; urgent interventions indicated.	Stop infusion and permanently discontinue trilaciclib.

AESI	Severity	Recommended Action
Acute drug hypersensitivity reactions	Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)	Stop infusion and hold trilaciclib until recovery to Grade ≤1 or baseline, then consider resuming trilaciclib. If Grade 2 recurs, permanently discontinue trilaciclib.
	Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. OR Grade 4: Life-threatening consequences; urgent intervention indicated.	Permanently discontinue trilaciclib.
ILD/pneumonitis	Grade 2 (symptomatic)	Hold trilaciclib until recovery to Grade ≤1 or baseline, then consider resuming trilaciclib. If Grade 2 recurs, permanently discontinue trilaciclib.
	Grade 3: Severe symptoms; limiting self-care ADL; oxygen indicated. OR Grade 4: Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Permanently discontinue trilaciclib.
Other toxicities	Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.	Hold trilaciclib until recovery to Grade ≤1 or baseline, then consider resuming trilaciclib. If Grade 3 recurs, permanently discontinue trilaciclib.
	Grade 4: Life-threatening consequences; urgent intervention indicated.	Permanently discontinue trilaciclib.

AESI=adverse event of special interest; ADL=activities of daily living; ILD=interstitial lung disease

9.3.6. Hy's Law 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.

Patients who present with the following laboratory abnormalities should be evaluated further to determine the etiology of the abnormal laboratory values:

- Baseline AST or ALT and total bilirubin values are within the normal range and the patient subsequently presents with AST or ALT ≥3 × ULN concurrent with a total bilirubin ≥2 × ULN with no evidence of hemolysis and an alkaline phosphatase ≤2 × ULN or not available.
- Pre-existing baseline ALT, AST, OR total bilirubin values are above the ULN, and the patient subsequently presents with:
  - AST or ALT ≥2 × baseline value AND ≥3 × ULN, or ≥8 × ULN (whichever is smaller)
  - **concurrent** with total bilirubin ≥2 × baseline value OR ≥3 × ULN (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, prothrombin time, 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 (e.g., biliary tract) may be warranted. All cases confirmed on repeat testing as meeting the laboratory criteria defined above, with no other cause for 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 serious adverse events (SAEs).

9.3.7. Management of Nausea and Vomiting

Necessary supportive care (e.g., antiemetics, antidiarrheals) administered per standard of care at the study center will be permitted.

9.4. Supportive Care Interventions

9.4.1. Granulocyte-Colony Stimulating Factor Usage

Granulocyte-colony stimulating factor (G-CSF) should be administered after each cycle of AC chemotherapy or per Investigator discretion. G-CSF 5 μg/kg/day administered subcutaneously

(SC) should be initiated at 24 hours post-dose and continued for at least 72 hours. Pegfilgrastim administered SC as either a single dose of 100 µg/kg (individualized) or a single dose of 6 mg (general approach) at 24 hours after chemotherapy is also acceptable.

Prophylactic G-CSF use during treatment with paclitaxel (with or without carboplatin) is allowed per growth factor/neutropenia management guidelines (e.g., American Society of Clinical Oncology [ASCO]), package inserts, and Investigator discretion (Aapro, 2011; Smith, 2015). If in any cycle a patient experiences febrile neutropenia and/or is at high risk for infection-associated complications, G-CSF/granulocyte-macrophage colony-stimulating factor (GM-CSF) should be used to treat the febrile neutropenia event per ASCO guidelines and package inserts.

9.4.2. 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, 2018); 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 Investigator discretion regardless of hemoglobin levels.

Platelets

Platelet transfusion is recommended at a threshold of ≤10 x 10^9/L. Platelets should also be transfused in any patient who is bleeding with a platelet count <50 x 10^9/L (100 x 10^9/L for central nervous system or ocular bleeding) (Kaufman, 2015; Schiffer, 2001).

9.5. Prior/Concomitant Medications, Procedures, and Vaccinations

Medications and Procedures:

All prior medications and procedures will be collected from **21 days** prior to signing the ICF. All concomitant medications including prescription medications, over-the-counter preparations, growth factors, and blood products from informed consent through the End of Study Visit will be documented, where possible. The use of gonadotrophin-releasing hormone (GnRH) agonist therapy (e.g., goserelin acetate [Zolodex®]) for ovarian preservation and bisphosphonates or rank ligand inhibitors to prevent osteopenia or osteoporosis is allowed during chemotherapy.

Administration of other concomitant non-protocol anticancer therapies is not permitted while on this study. This includes any low-dose systemic chemotherapeutic agent given for a non-oncologic purpose (e.g., low-dose methotrexate for rheumatoid arthritis). Administration of other concomitant investigational agents for any indication is not permitted while on this study.

Any medication that is contraindicated when using doxorubicin, cyclophosphamide, paclitaxel, carboplatin or pembrolizumab (when applicable) is not permitted, and warnings and precautions for use of these study drugs should be observed.

Avoid concomitant use with certain OCT2, MATE1, and MATE-2K substrates where minimal concentration changes may lead to serious or life-threatening toxicities (Section 4.4.1.2).

Any diagnostic, therapeutic, or surgical procedure performed during the study period will be documented. Documentation will include information regarding the date(s), indication(s), description of the procedure(s), and any clinical or pathological findings, if available.

Vaccinations:

All prior vaccinations will be collected from **90 days** prior to first dose of study drug. Receipt of a live, attenuated vaccine within **30 days** prior to the first dose of study drug or anticipation that such a live, attenuated vaccine will be required during the study treatment period is prohibited.

Other vaccine types such as inactivated or nucleic acid vaccines (e.g., those given for COVID-19 or influenza) are permitted if given at one of these timepoints:

- > 21 days prior to first dose of study drug. In case of COVID-19 vaccinations requiring two doses, the second vaccine dose must be given > 21 days prior to first dose of study drug.
- Between C2D1 of chemotherapy (after pre-dose blood sample is obtained) and > 21 days prior to definitive surgery
- After definitive surgery

10. DISCONTINUATION OF STUDY TREATMENT AND PATIENT DISCONTINUATION/WITHDRAWAL

10.1. Discontinuation of Study Treatment

Study drugs 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 in a female patient during the study
- Significant noncompliance with protocol requirements
- The Sponsor or legal representative of the Sponsor requests the patient to withdraw
- If total time between chemotherapy treatments exceeds a total of >4 weeks, unless agreed to by the treating Investigator and Medical Monitor.
- Where permanent discontinuation of all study drugs is indicated in the toxicity management recommendations (Section 9.3).

Following study drug discontinuation, a Safety Follow-Up and End of Study Visit should be completed with assessments performed as shown in the Schedule of Assessments (Table 4). 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 drug, 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 electronic case report form (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 17.3).

10.2. Discontinuation/Withdrawal from the Study

A patient may withdraw from the study at any time at 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, a Safety Follow-Up and End of Study Visit should be completed (if they have not been done already) with assessments performed as shown in the Schedule of Assessments (Table 4).

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, she may request destruction of any samples taken and not tested, and the Investigator or designee must document this in the site study records.

10.3. Lost to Follow Up

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

In general, a patient is considered lost to follow-up after there are at least 3 documented attempts to contact the patient. It is recommended that 1 attempt is via certified letter to the patient.

10.4. Study and Site Start and Closure

The overall study begins when the first patient signs the ICF. The overall study ends when the last patient completes the last study-related phone call or visit, discontinues from the study, or is lost to follow-up (i.e., the patient is unable to be contacted by the Investigator).

A study site is considered eligible to start participation in the study once all regulatory approvals are in place, site agreement contract is fully executed, and any other required documents are in place as required by Sponsor.

The Sponsor reserves the right to close a study site(s) or terminate the study at any time for any reason. 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). 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 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.

Patients who discontinue from the study prior to receiving the first dose of study drug may be replaced.

11.1. Screening Assessments

The following information for screening failures should be recorded: patient identification, 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; only the qualifying result should be recorded.

Procedures conducted as part of the patient's routine clinical management (e.g., hematology, serum chemistry, baseline diagnostic tissue) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and timeframe.

11.1.1. ER-, PR-, HER2-Negative Status

Eligibility will be based on local ER-negative, PR-negative, HER2-negative status from a recent tumor biopsy report confirming TNBC prior to Screening.

ER/PR-negative status should be histologically or cytologically confirmed by using an assay consistent with local standards (defined as <1% nuclei staining) and HER2 non-overexpressing status should be confirmed per 2018 ASCO College of American Pathologists criteria.

11.1.2. Baseline Characteristics and Demographics

Age (year only), sex, race (when reporting is allowed per local regulations), ethnicity, and menopausal status will be collected during the Screening period.

11.1.3. Medical and Surgical History

Medical and surgical history, including past and current conditions, will be collected. Concomitant medications taken from informed consent and vaccinations within 90 days prior to the first dose of study drug through the End of Study Visit will be recorded.

Documentation of TNBC diagnosis and status at baseline must be thoroughly evaluated by the Investigator or designee including: date of initial diagnosis, stage at diagnosis, tumor grade, primary tumor location and type (i.e., single lesion, multi-centric, multifocal), clinical TNM staging at baseline, primary and sentinel lymph node biopsies and results, and BRCA classification (if available).

11.2. Biomarker and Efficacy Assessments

To identify novel biomarkers and investigate the immune-based MOA of trilaciclib and its impact on the tumor microenvironment, tumor tissue and blood will be collected. Investigations may include, but are not limited to:

1. see Tumor RNA Analyses

Targeted messenger RNA (mRNA) expression profiling and sequencing in tumor tissue via NanoString technologies will be performed to define longitudinal molecular changes within the tumor microenvironment and identify gene signatures that correlate to clinical response to treatment with trilaciclib and the combinations. Trilaciclib induces a response in tumors that likely reflects an inflamed/immune phenotype. Specific immune-related gene sets (such as those capturing interferon-gamma signaling, cytotoxicity, inflammatory chemokines, and antigen processing) may be evaluated and new signatures may be identified. Individual genes related to the immune system may also be evaluated (e.g., interleukin-10, CTLA-4, PD-1, TIGIT, and LAG-3).

2. Immunohistochemistry using Tumor Tissue

Tumor samples from this study may undergo proteomic analyses via immunohistochemistry (IHC) to quantitate cells expressing PD-L1, CD8, and FOXP3, and assess the association with response to trilaciclib. Collectively, the data will provide insight into the presence and change in TIL infiltration from baseline to post-trilaciclib monotherapy, and at surgery.

3. Immune Profiling of Peripheral Blood

Peripheral blood mononuclear cells collection at baseline, on-treatment, and at surgery will be characterized by mass cytometry and flow cytometry. Quantitative assessment of major immune populations and subsets, cytokine production along with qualitative measurement of cell activation, maturation, and exhaustion status, and polyfunctionality will provide a comprehensive profile of the peripheral blood immune landscape, and the kinetics of the immune response following trilaciclib.

11.2.1. Tumor Biopsy Collection

To evaluate the impact of trilaciclib on changes of the tumor-associated immune response in TNBC, immunophenotypic changes will be compared in tumor biopsies at three separate timepoints (Table 4):

1. Screening: baseline tissue is obtained prior to any study treatment
2. Trilaciclib Lead-In Phase: on-treatment biopsy is obtained 7 (±1) days post single-dose administration of monotherapy trilaciclib (prior to initiation of chemotherapy)
3. Definitive Surgery: in patients who have not achieved a pCR, tissue will be obtained at time of surgery, which is to occur 3-5 weeks after last dose of chemotherapy

Core needle biopsies should consist of at least 3 separate tumor cores (ideally ≥ 4), utilizing at least a 14-gauge needle and multiple passes. Tumor samples will be analyzed from primary tumor in breast, not nodal tissue. For fresh biopsies, cores may be stored in paraffin blocks or embedded in optimal cutting temperature compound (see laboratory manual for details).

Archival tissue may be used for the baseline tissue sample and should consist of representative FFPE tumor specimens in paraffin blocks (75-micron). It is the Investigator's responsibility to confirm there is sufficient baseline tissue prior to first dose. If there is insufficient archival tissue, a fresh biopsy will be obtained. The patient should not have received any antineoplastic therapy (including systemic therapy or radiation) between the baseline tissue collection and first dose of study drug. The Sponsor must approve if archival tissue was taken > 30 days from signing informed consent. Patients who have adequate archival tissue may consent to optional fresh biopsy collection at baseline.

Tumor tissue will be used to analyze immune endpoints including exploratory markers of trilaciclib sensitivity such as genomic profiles, mRNA expression, CDK4/6 dependency, IHC staining including PD-L1, among others. Additional details regarding tumor tissue collection, processing, handling, and shipping may be found in the laboratory manual. Samples may be stored for up to 10 years if patients sign the consent form and allow their samples to be stored for any future research.

11.2.2. Blood Collection

To further evaluate the impact of trilaciclib on the immune response, immunophenotypic changes will be compared from serial peripheral blood samples at four separate timepoints (Table 4):

1. Trilaciclib Lead-In Phase: day of single dose monotherapy trilaciclib administration (pre-dose)
2. Trilaciclib Lead-In Phase: day of biopsy, prior to the biopsy procedure and initiation of chemotherapy
3. Treatment Phase: C2D1 of chemotherapy (pre-dose)
4. Definitive Surgery: day of surgery (prior to surgery); within 3 days of surgery permitted, on surgery day preferred.

Samples may be stored for up to 10 years if patients sign the consent form and allow their samples to be stored for any future research. Additional details regarding blood collection, processing, handling, and shipping may be found in the laboratory manual.

11.2.3. Pathologic Complete Response

Definitive surgery such as breast conservation surgery or mastectomy with or without axillary lymph node dissection will be performed as part of the local standard of care approximately 3 to 5 weeks following the completion or early discontinuation of the Treatment Phase. A thorough evaluation of breast cancer status, pathological staging per current American Joint Committee of Cancer (AJCC) Breast Cancer Staging, and assessment of surgical margins will be performed by the local pathologist on all the tissues removed during the surgery. Remaining tissue will be used for biomarker analysis.

pCR is defined as the absence of residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0/Tis ypN0 in the current AJCC staging system).

11.3. Safety Assessments

Unless specified otherwise, safety assessments should be conducted prior to study drug administration.

11.3.1. Vital Signs

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

- Pulse rate and blood pressure (diastolic and systolic)
- Height in centimeters (Screening visit only) and body weight in kilograms

Assessments may be performed by a physician or other qualified health care provider.

11.3.2. Physical Examination

Complete physical examination at Screening includes all major body systems, including general appearance, skin, neck, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, and neurological examinations. Subsequent symptom-directed physical exams should include body systems as appropriate (e.g., limited physician exam based on symptoms) and weight.

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.

11.3.3. ECOG Performance Status

The Investigator or designee will assess ECOG performance status during the Screening Period to assess for eligibility according to the inclusion and exclusion criteria (Table 10).

Table 10: ECOG Performance Status

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to be or chair
5	Dead

ECOG=Eastern Cooperative Oncology Group
Source: Oken, 1982.

11.3.4. Clinical Safety Laboratory Assessments

Hematology, serum chemistry, coagulation, and urinalysis will be performed at the site's local certified laboratory per the schedule outlined in the Schedule of Assessments (Table 4). A list of clinical laboratory tests to be performed is provided in Section 17.1.

Hematology may be obtained up to 24 hours and serum chemistry and urinalysis may be obtained up to 72 hours prior to each time point on Schedule of Assessments. For women of childbearing potential, pregnancy tests will be obtained at Screening and within 72 hours prior to chemotherapy treatment on C1D1, C5D1, C11D1, and C16D1 only using serum or urine beta human chorionic gonadotropin (ß-hCG).

Serum chemistry and hematology results shall be reviewed before dosing. Laboratory toxicities will be assessed using the NCI CTCAE v5.0.

An abnormal laboratory value is not an AE unless it is considered to be clinically significant by the Investigator. 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 the eCRF) 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 meets criteria for resumption of dosing (see Section 9.2).

11.3.5. Electrocardiogram

Standard 12-lead electrocardiograms (ECGs) will be performed at Screening (Table 4). Additional ECGs may be performed as clinically indicated at any time during the study. All 12-lead ECGs will be obtained after the patient has been resting for at least 10 minutes in the supine position and shall be recorded at 25 mm/sec.

Any ECG with a QTc value of >500 msec shall have the QTc value confirmed via manual read. Following confirmation, the Investigator should follow institutional guidelines for the appropriate dose modifications based on the grade of QTc prolongation and evaluate for any other potential causes of the prolongation (e.g., concomitant medications).

The Investigator or designee will review the ECGs locally.

11.3.6. Echocardiogram or MUGA

An ECHO or MUGA scan will be performed at Screening to evaluate adequate cardiac function and determine eligibility (Table 4). The assessment method will be at the Investigator's discretion and per local standard of care. Additional assessments may be performed as clinically indicated at any time during the study. If repeat cardiac assessments are necessary, the method (ECHO or MUGA) should be the same throughout the study.

11.3.7. Adverse and Serious Adverse Events

Study-procedure related SAEs will be collected after the informed consent form is signed; all AEs will be collected starting from the first dose of study drug through the End of Study Visit. Any AE, either directly or indirectly related to biopsy procedures, surgery, or the medication required to perform surgery and its sequelae should be reported. Toxicity will be assessed by Investigators using NCI CTCAE v5.0.

The Investigator and any 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 freely or in response to general, nondirected questioning. Adverse events (serious and non-serious) should be reported on the appropriate page of the eCRF from the first dose of study treatment through last patient visit.

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

AEs will be collected starting from the first dose of study drug through the End of Study Visit. 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. Any AEs that occur between the date of signing informed consent and the first dose of study drug should be recorded as Medical History.

All SAEs will be recorded and reported to G1 Therapeutics, Inc Pharmacovigilance (PVG) or designee immediately and should not exceed 24 hours after becoming aware of the event, as indicated in Section 17.2.

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 she considers the event to be reasonably related to the study intervention or study participation, the Investigator or designee must promptly notify G1 Therapeutics, Inc. PVG or designee.

11.3.7.2. 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 17.2.

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.

11.3.7.3. 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, Inc. 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 17.2.

11.3.7.4. Regulatory Reporting Requirements for Serious Adverse Events

Prompt notification of G1 Therapeutics, Inc. 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, Inc. 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, Inc. will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Central 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, Inc. 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, Inc. 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.

11.3.7.5. Pregnancy

Details of all pregnancies in female patients will be collected after the start of study intervention and until 30 days after the last dose of study drug.

If a pregnancy is reported, the Investigator or designee should inform G1 Therapeutics, Inc. PVG or designee within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 17.3.

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

11.3.8. Definitive Surgery

Approximately 3-5 weeks following completion or early discontinuation of the Treatment Phase, patients will undergo definitive surgery per local standard of care. Details regarding date of surgery, type of surgery, tumor resectability etc. will be recorded in the appropriate eCRF. Detailed pathological staging per current AJCC staging criteria and assessment of surgical margins will be performed by the local pathologist on all the tissues removed during the surgery and recorded in the appropriate eCRF.

11.4. Safety Monitoring Committee

An SMC will monitor accumulating safety data and will meet after 10 patients have completed 2 cycles of doxorubicin/cyclophosphamide, after 10 patients have completed 2 cycles of paclitaxel, and at the end of the study (or as defined in the SMC charter). Additional reviews may occur.

A single SMC charter will define the roles and responsibilities of the SMC and its members. Additional details regarding the committee's composition, scope, objectives, procedures, and policies, including the associated analysis plan and data to be reviewed for the Sponsor, Investigators, and patients, are described in the SMC charter. The SMC will monitor accumulating safety and disposition data and will be comprised of study team and independent members.

11.5. Safety Follow-up Visit

The patient should complete a Safety Follow-up Visit 30 days (+7 days) from last dose of study drug as outlined in Table 4. This visit may occur at the study site or can be via telephone and may coincide with the End of Study Visit.

11.6. End of Study Visit

The patient should complete an End of Study Visit within 14 days following surgery as outlined in Table 4. Pathology results should be recorded if available. The Investigator or designee should also ensure that the tumor sample from surgery was provided, if applicable. This visit may occur at the study site or can be via telephone and may coincide with the Safety Follow-up Visit.

12. STATISTICAL CONSIDERATIONS

A statistical analysis plan (SAP) will be developed and finalized prior to any analysis that will be performed for this study. While the SAP will include more details related to the statistical analyses for data collected from this study, this section is a summary of the key aspects of the planned statistical analyses.

12.1. Sample Size Determination

Sample size justification is based off of previously published data on the CD8+/Treg ratio from early stage TNBC patients who underwent neoadjuvant chemotherapy (Ahn, 2020). Using a 2-sided significance level of 0.05, and anticipating that 10% patients enrolled to this study will not have paired data (resulting in 27 patients with paired data), the power of the study to detect a respective mean of paired difference in CD8+/Tregs ratio using paired Wilcoxon signed-rank tests has been calculated using PASS 2019 (v19.0.3), and is presented in the table below.

Mean of Paired Difference in CD8+/Tregs	Power
1.8	72%
2.0	81%
2.3	90%
2.5	94%
Based on the assumption of an estimated standard deviation of paired differences of 3.4.	

12.2. Analysis Population

The Full Analysis Set - 1 (FAS1) population includes enrolled patients who have received trilaciclib during the Trilaciclib Lead-in Phase. This dataset will be used to assess immune response in tumor tissue.

The Full Analysis Set – 2 (FAS2) population includes enrolled patients who have received at least one dose of a study drug during the Treatment Phase of the study. This dataset will be used to assess safety and tolerability and the rate of pCR for trilaciclib in combination with neoadjuvant systemic therapies.

12.3. Statistical Analysis Methods

12.3.1. General Considerations

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

The categorical variables will be summarized by counts and percentages, the continuous variables will be summarized by mean, median, standard deviations, 25% and 75% percentiles, and minimum and maximum values by treatment group. For the immune-response measures and other biomarkers, median will be used to present the central point of the population been studied.

12.3.2. Patient Disposition

Patient disposition will be summarized for all patients. The summary will include number of all screened patients, and number and percentage of patients who were enrolled and received study drug, discontinued from each study drug and reasons, and discontinued from study and reasons.

12.3.3. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized. The summary will include age, age groups, gender, race, ethnicity, screening vital signs, ECOG status, region, and baseline disease characteristics.

12.3.4. Study Drug Exposure, Modification and Dose Intensity

Duration of study drug exposure will be defined for each chemotherapy drug (doxorubicin, cyclophosphamide, paclitaxel, carboplatin, and pembrolizumab, as applicable) and for trilaciclib.

The number of cycles that patients have received will be summarized by descriptive statistics as a continuous variable, while the number of cycles that are completed will be summarized as a categorical variable.

Study drug modifications will be summarized in three categories: chemotherapy dose reduction, cycle delay, and infusion interruption. The number and percentage of patients who have any chemotherapy dose reduction or delay and have at least one dose reduction or delay for a particular chemotherapy will be summarized along with a summary of the number of dose reductions or delays for each chemotherapy; the number and percentage of patients who have at least one infusion interruption for trilaciclib, any chemotherapy, or pembrolizumab (if applicable) will be summarized along with a summary of the number of interruptions. Lastly, the primary reason for each form of study drug modification (chemotherapy dose reductions, cycle delay, and infusion interruption) will also be summarized.

For trilaciclib, doxorubicin, cyclophosphamide, paclitaxel, carboplatin and pembrolizumab cumulative dose, dose intensity, relative dose, and relative dose intensity will be derived and summarized.

12.3.5. Efficacy Analyses

12.3.5.1. Analyses for Primary Immune-Response Measure

Summary statistics for primary immune-response measure, the ratio of CD8+ TIL over FOXP3+ regulatory T cells (abbreviated as CD8+/Tregs), will be provided for measures taken at baseline (pre-trilaciclib), 7 ± 1 days after single dose trilaciclib (post-trilaciclib), and the change scores (post minus pre). Median will be used as the central point with Q1 and Q3 as the measure of spread. The significance of the magnitude of change will be tested by paired Wilcoxon Signed-Rank test. Analyses on the primary endpoint will be based on the FAS1 population.

12.3.5.2. Analyses for pCR

For the clinical efficacy endpoint, pCR rate will be reported with a 95% confidence interval using the Clopper-Pearson method based on FAS2 population. In addition, patients will be dichotomized by median value of changes in CD8+/Tregs ratio, and pCR rate will be summarized by the subgroup of patients with high or low change scores. The association between pCR status and high/low in ratio change will be tested using a Chi-square test. These analyses will be performed on the FAS2 population.

12.3.6. Safety Analyses

For trilaciclib alone, safety will be assessed by AE reporting during the Trilaciclib Lead-in Phase based on FAS1 population. For trilaciclib in combination with other systemic therapies, safety and tolerability will be assessed by AEs, dose modification, laboratory tests, vital signs, and ECGs based on FAS2 population.

12.3.6.1. Adverse Events

Adverse events are defined as those events occurring or worsening after treatment has begun on this study. Adverse event data will be coded to system organ class and preferred term using the latest version of Medical Dictionary for Regulatory Activities (MedDRA). The severity (toxicity grades 1-5) of AEs will be graded according to the NCI CTCAE Version 5.0 by investigators. The number and percentage of patients experiencing any AE overall, and by system organ class, preferred term and CTCAE grade will be tabulated. Adverse events considered by the Investigator to be related to treatment will also be by study drug (e.g., trilaciclib or each chemotherapy). 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. AESIs for trilaciclib, AEs leading to study drug discontinuation or study drug modification will be summarized by system organ class, preferred term and CTCAE grade, as appropriate. Concomitant medications will be coded to Anatomical Therapeutic Classification using the World Health Organization-Drug Dictionary (WHO-DD) and summarized.

12.3.6.2. Other Safety Endpoints

Observed values and changes from baseline in laboratory assessments of hematology and chemistry parameters, vital signs, and ECG parameters will be summarized for each scheduled visit at which the assessment is taken. Changes from baseline to the maximum and minimum values during treatment will also be summarized.

Clinical chemistry and hematology laboratory parameters will be characterized according to CTCAE toxicity grade from 1 to 5, Version 5.0, when possible. The number and percentage of patients within each CTCAE grade will be summarized for the overall treatment period as well as for each cycle. If a patient has multiple laboratory assessments in an interval of interest, the maximum grade will be reported.

For vital signs and ECG parameters, potentially clinically significant (PCS) findings will be summarized. The potentially clinically significant vital signs and ECG measures are defined either by post-baseline assessment or by the change from baseline with respect to the pre-specified thresholds. The criteria defining PCS for vital signs and ECG parameters will be detailed in the study SAP.

12.3.7. Exploratory Analyses

pCR by subgroup analysis will be performed based on PD-L1 status and CDK4/6 dependence signatures. Other exploratory analyses to evaluate trilaciclib's effect on immune response related biomarkers and their association with pCR status will be performed as deemed necessary.

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, 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 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 ICF. A copy of the signed ICF must be given to the patient or the patient's legally authorized representative where allowed by local regulation.

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 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 (SDV) 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.
- The Investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved 2 years following the discontinuance of the investigation of trilaciclib. If it becomes necessary for the Sponsor or the Regulatory Authority to review any documentation

relating to the study, the Investigator must permit access to such records. No records may be destroyed during the retention period without the written approval of the Sponsor. No records maybe transferred to another location or party without written notification to the Sponsor. The Investigator must ensure that the records continue to be stored securely for as long as they are maintained.

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. Dissemination of Clinical Study Data

The Sponsor fulfills its commitment to publicly disclose clinical study results through posting the results of studies on www.clinicaltrials.gov, the EudraCT, and other public registries in accordance with applicable local laws/regulations.

Data results are posted in an objective, accurate, balanced, and complete manner. Results are posted regardless of outcome of the study.

14.4. 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. All data reported in the eCRF should be supported by source documents; direct entry of data into the eCRF is not permitted in this study.

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.

14.5. Audits and Inspections

Authorized representatives of G1 Therapeutics, Inc., 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, Inc. 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, Inc. 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, Inc. G1 Therapeutics, Inc. 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, Inc. shall determine order of authorship of any publication combining all clinical results of this study.

16. REFERENCES

Aapro MS, Bohlius J, Cameron DA, et al. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumors. Eur J Cancer. 2011;47:8-32.

Ahn S, Chung YR, Seo AN, et al. Changes and prognostic values of tumor-infiltrating lymphocyte subsets after primary systemic therapy in breast cancer. PLoS One. 2020;15(5):e0233037.

Asghar US, Barr AR, Cutts R, et al. Single-cell dynamics determines response to CDK4/6 inhibition in triple-negative breast cancer. Clin Cancer Res. 2017;23:5561-72.

Bisi JE, Sorrentino JA, Roberts PJ, et al. Preclinical characterization of G1T28: A novel CDK4/6 inhibitor for reduction of chemotherapy-induced myelosuppression. Mol Cancer Ther. 2016; 15:783–93.

Bonelli M, La Monica S, Fumarola C, et al. Multiple effects of CDK4/6 inhibition in cancer: from cell cycle arrest to immunomodulation. Biochem Pharmacol. 2019;170:113676.

Bracci L, Schiavoni G, Sistigu A, et al. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ. 2014 Jan;21(1):15-25.

Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019;30:1194-220.

Carey LA, Dees C, Sawyer L, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13(8):2329-34.

Carson JL, Guyatt G, Heddle NM, et al. Clinical Practice Guidelines From the AABB: Red Blood Cell Transfusion Thresholds and Storage. JAMA. 2016;316(19):2025-35.

Chaikovsky AC, Sage J. Beyond the cell cycle: enhancing the immune surveillance of tumors via CDK4/6 inhibition. Mol Cancer Res. 2018;16:1454-57.

Choe E, Cha YJ, Kim J-H, et al. Dynamic changes in PD-L1 expression and $CD8^+$ T cell infiltration in non-small cell lung cancer following chemoradiation therapy. Lung Cancer. 2019;136 :30-6.

Cimino-Mathews A, Foote JB, Emens LA. Immune targeting in breast cancer. Oncology (Williston Park). 2015;29:375–385.

Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014;384(9938):164-72.

Daniel D, Kuchava V, Bondarenko I, et al. Trilaciclib prior to chemotherapy and atezolizumab in patients with newly diagnosed extensive-stage small cell lung cancer: A multicentre, randomized, double-blind, placebo-controlled Phase II trial. Int J Cancer. 2020;148(10):2557-70.

De Laurentiis M, Cancello G, D'Agostino D, et al. Taxane-based combinations as adjuvant chemotherapy of early breast cancer: a meta-analysis of randomized trials. J Clin Oncol. 2008;26(1):44-53.

Demaria, S. et al. Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin. Cancer Res. 2001;7:3025–3030.

Deng J, Wang ES, Jenkins RW, et al. CDK4/6 inhibition augments anti-tumor immunity by enhancing T-cell activation. Cancer Discov. 2018;8:216-33.

Denkert C, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol.2010;28:105-113.

European Medicines Agency (EMA). The role of the pathological complete response as an endpoint in neoadjuvant breast cancer studies. London: European Medicines Agency, 2014.

Food and Drug Administration (FDA) Guidance for industry: pathological complete response in neoadjuvant treatment of high- risk early-stage breast cancer: use as an endpoint to support accelerated approval. Silver Spring, MD: Food and Drug Administration, October 2014.

Goel S, DeCristo MJ, Watt AC, et al. CDK4/6 inhibition triggers anti-tumor immunity. Nature 2017; 548(7668): 471-5.

Gonzalez H et al. Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev. 2018;32:1267-1284.

He S, Roberts PJ, Sorrentino JA, et al. Transient CDK4/6 inhibition protects hematopoietic stem cells from chemotherapy-induced exhaustion. Sci Transl Med. 2017a;9.

He Y, Rozeboom L, Rivard CJ, et al. MHC class II expression in lung cancer. Lung Cancer. 2017b;112:75-80.

Heckler M, Ali LR, Clancy-Thompson E, et al. Inhibition of CDK4/6 promotes CD8 T-cell memory formation. Cancer Discov. 2021;candisc.1540.2020.

Horsley V, Aliprantis AO, Polak L, et al. NFATc1 balances quiescence and proliferation of skin stem cells. Cell. 2008;132(2):299-310.

Huang AC, Orlowski RJ, Xu X, et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. Nat Med. 2019;25(3): 454-61.

Kaufman RM, Djulbegovic BD, Gernsheimer T, et al. Platelet Transfusion: A Clinical Practice Guideline From the AABB. Ann Intern Med. 2015; 162:205-213.

Keytruda® (pembrolizumab) Injection Prescribing Information. Merck. 2021

Kim KH Cho J, Ku BM, et al. The first-week proliferative response of peripheral blood PD-1+ $CD8^+$ T cells predicts the response to anti-PD-1 therapy in solid tumors. Clin Cancer Res. 2019;25(7):2144-54.

Klein ME, Kovatcheva M, Davis LE, et al. CDK4/6 inhibitors: the mechanism of action may not be as simple as once thought. Cancer Cell. 2018;34:9-20.

Kozar K, Ciemerych MA, Rebel VI, et al. Mouse development and cell proliferation in the absence of D-cyclins. Cell. 2004;118(4):477–91.

Ladoire S, Mignot G, Dabakuyo S, et al. In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival. J Pathol 2011;224:389–400.

Lai AY, Sorrentino JA, Strum JC, et al. Transient exposure to trilaciclib, a CDK4/6 inhibitor, modulates gene expression in tumor immune infiltrates and promotes a pro-inflammatory tumor microenvironment. American Association for Cancer Research Annual Meeting 2018; Chicago, IL; April 14–18, 2018 (abstr 1752). https://www.g1therapeutics.com/file.cfm/34/docs/tr-G1_AACR2018_Lai.pdf.

Lai AY, Sorrentino JA, Dragnev KH, et al. CDK4/6 inhibition enhances anti-tumor efficacy of chemotherapy and immune checkpoint inhibitor combinations in preclinical models and enhances T-cell activation in patients with SCLC receiving chemotherapy. J Immunother Cancer. 2020; 8.

Lehmann BD, Jovanocic B, Chen X, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. PLoS One. 2016;11e0157368.

Lesterhuis WJ, et al Dynamic versus static biomarkers in cancer immune checkpoint blockade: unravelling complexity. Nat Rev Drug Discov 2017;16:264-72.

Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol. 2008 Mar 10;26(8):1275-81.

Malumbres M, Sotillo R, Santamaria D, et al. Mammalian cells cycle without the D-type cyclin-dependent kinases Cdk4 and Cdk6. Cell. 2004;118(4):493-504.

McDonnell AM, Nowak AK, Lake RA. Contribution of the immune system to the chemotherapeutic response. Semin Immunopathol. 2011;33(4):353-67.

Merck Press Release: Merck announces Phase 3 KEYNOTE-522 trial met dual primary endpoint of event-free survival (EFS) in patients with high-risk early-stage triple-negative breast cancer (TNBC). Merck. Published May 13, 2021.

National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology Breast Cancer. Version 4. 2021. NCCN, Fort Washington, PA.

Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5(6):649-55.

Ono M, et al. Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer. Breast Cancer Res Treat. 2012;132:793-805.

O'Shaughnessy J, Wright GS, Thummala AR, et al. Trilaciclib improves overall survival when given with gemcitabine/carboplatin in patients with metastatic triple-negative breast cancer: Final analysis of a randomized Phase 2 trial. San Antonio Breast Cancer Symposium 2020; San Antonio, TX; December 8-11, 2020 (Poster #PD1-06).

Park JH, Ahn JH, Kim SB. How shall we treat early triple-negative breast cancer (TNBC): from the current standard to upcoming immune-molecular strategies. ESMO Open 2018;3(suppl 1):e000357.

Park YH, Lal S, Lee JE, et al. Chemotherapy induces dynamic immune responses in breast cancers that impact treatment outcome. Nat Commun. 2020;11(1):6175.

Petrelli F, Coinu A, Borgonovo K, et al. The value of platinum agents as neoadjuvant chemotherapy in triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat. 2014;144(2):223-32.

Prat A, Bianchi G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. Clin Cancer Res. 2014;20:511-21

Ramsey MR, Krishnamurthy J, Pei XH, et al. Expression of p16Ink4a Compensates for p16Ink4a Loss in Cyclin-Dependent Kinase 4/6–Dependent Tumors and Tissues. Cancer Res 2007;67:4732-41.

Schaer DA, Beckmann RP, Dempsey JA, et al. The CDK4/6 inhibitor abemaciclib induces a T cell inflamed tumor microenvironment and enhances the efficacy of PD-L1 checkpoint blockade. Cell Rep. 2018;22:2978-94.

Schiffer CA, Anderson KC, Bennett CL, et al. Platelet transfusion for patients with cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol. 2001;19(5):1519-38.

Schmid P, Cortes J, Pusztai L, et al. KEYNOTE-522 Investigators. Pembrolizumab for early triple-negative breast cancer. N Engl J Med. 2020a;382(9):810-21.

Schmid P, Salgado R, Park YH, et al. Pembrolizumab plus chemotherapy as neoadjuvant treatment of high-risk, early-stage tiple-negative breast cancer: results from the phase 1b open-label multicohort KEYNOTE-173 study. Ann Oncol. 2020b;31(5):569-81.

Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol. 2015;33(1):13-21.

Smith TJ, Bohlke K, Lyman GH, et al. Recommendations for the Use of WBC Growth Factors: American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol. 2015;33:3199-212.

Sorrentino J, Lai A, Strum J, Roberts PJ. Trilaciclib (G1T28), a CDK4/6 inhibitor, enhances the efficacy of combination chemotherapy and immune checkpoint inhibitor treatment in preclinical models. American Association for Cancer Research Annual Meeting. Cancer Res. 2017;77(13):5628.

Sorrentino JA, Bisi JE, Thompson D, et al. Trilaciclib, a CDK4/6 inhibitor, does not impair the efficacy of chemotherapy in CDK4/6-dependent tumor models. 30th EORTC-NCI-AACR Symposium. 13-16 November 2018. Dublin, Ireland.

Sparano JA, Zhao F, Martino S, et al. Long-term follow-up of the E1199 Phase III trial evaluating the role of taxane and schedule in operable breast cancer. JCO. 2015;33(21):2353-60.

Tan AR, Wright GS, Thummala AR, et al. Trilaciclib plus chemotherapy versus chemotherapy alone in patients with metastatic triple-negative breast cancer: a multicentre, randomized, open-label, Phase 2 trial. Lancet Oncol. 2019;20:1587-601.

Tarantino P, Gandini S, Trapani D, et al. Immunotherapy addition to neoadjuvant chemotherapy for early triple negative breast cancer: A systematic review and meta-analysis of randomized trials. Crit Rev Oncol Hematol. 2021;159:103223.

Teh JLF, Aplin AE. Arrested Developments: CDK4/6 Inhibitor Resistance and Alterations in the Tumor Immune Microenvironment. 2019;25(3):921-7.

Vasievich EA et al. The suppressive tumor microenvironment: a challenge in cancer immunotherapy. Mol Pharm 2011;8:635-641.

von Minckwitz G, Untch M, Nuesch E, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German neo-adjuvant chemotherapy trials. Breast Cancer Res Treat. 2011;125(1):145-56.

von Minckwitz G, Schneeweiss A, Loibl S, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol. 2014;15(7):747-56.

Yamaguchi R, et al. Tumor-infiltrating lymphocytes are important pathologic predictors for neoadjuvant chemotherapy in patients with breast cancer. Hum Pathol. 2012;43:1688-94.

Zhang J, Bu X, Wang H, et al. Cyclin DCDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. Nature. 2018;553:91-5.

Zitvogel L, Apetoh L, Ghiringhelli F, et al. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008;8:59-73.

17. APPENDICES

17.1. Clinical Laboratory Tests

- The timing and laboratory tests detailed in Schedule of Assessments (Table 4) 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 11: Protocol-Specified Safety Laboratory Assessments

Laboratory Assessment	Parameters			
Hematology	WBC		Hemoglobin	
	Platelets		Absolute neutrophil count	
Serum Chemistry	Sodium	Urea	Magnesium	Bilirubin
	Potassium	Phosphorus	Total protein	Albumin
	Chloride	Creatinine	Aspartate aminotransferase	
	Bicarbonate	Calcium	Alanine aminotransferase	
Coagulation	Prothrombin time and/or international normalized ratio			
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 WOCBP only)			

hCG=human chorionic gonadotropin; RBC=red blood cell; WBC=white blood cell; WOCBP=woman of childbearing potential

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

17.2.1. 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, serum 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.

17.2.2. 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.

17.2.3. 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, Inc. (or designee) in lieu of completion of the AE/SAE eCRF page or Paper SAE Report Form.
- There may be instances when copies of medical records for certain cases are requested by G1 Therapeutics, Inc. (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 Inc. (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, Inc. PVG (or designee) within 24 hours of notification on an SAE Form in the eCRF. Any relevant source data related to the SAE should also be emailed or faxed to G1 Therapeutics, Inc. PVG (or designee):

  G1 Therapeutics, Inc. 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 (Related or Not Related); i.e., is there a "reasonable possibility" the study intervention caused the event (yes/no).
- 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 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, Inc. 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, Inc. 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, Inc. PVG (or designee; SAEs only) 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 on the paper SAE Report Form to G1 Therapeutics, Inc. PVG (or designee) within 24 hours of receipt of the information:

  G1 Therapeutics Inc. Pharmacovigilance

  Email: safetyreporting@g1therapeutics.com

  Fax: +1-984-285-7131

17.2.4. 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

17.3. 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 should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

- All females of childbearing potential must have a negative serum or urine β-hCG test result at Screening and on Day 1 of each chemotherapy cycle.
- 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 study drug. 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). The vasectomized male partner should be the sole partner for the 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.

In addition, patients are to comply with the following guidelines:

- Contraceptive use is required for the duration of the study and for at least 6 months after the last dose of study drug.

Collection of Pregnancy Information

- 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 (Pregnancy – Initial Report 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 collected on the Pregnancy – Follow-up Report Form and forwarded to G1 Therapeutics, Inc. 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, Inc. 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.

  Contact for Pregnancy reporting:
  G1 Therapeutics Inc. Pharmacovigilance
  Email: safetyreporting@g1therapeutics.com
  Fax: +1-984-285-7131