STATISTICAL ANALYSIS PLAN

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 Version	2.0
Protocol Number:	G1T28-212
Compound:	Trilaciclib for Injection, 300 mg/vial
Study Phase:	Phase 2
Study Name:	MOA
Sponsor:	G1 Therapeutics, Inc.
Legal Registered Address:	700 Park Offices Dr. Suite 200 PO Box 110341 Research Triangle Park, NC 27709
Regulatory Agency Identifier Numbers:	IND: 145091
Statistical Analysis Plan Version:	V 2.0
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SAP SIGNATURE PAGE

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AC	anthracycline/cyclophosphamide
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransaminase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
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
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EOS	End of study
FAS	Full Analysis Set
FOXP3	Forkhead Box P3
ICF	informed consent form
IFN	interferon
IHC	immunohistochemistry
INR	international normalized ratio
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MOA	mechanism of action
mRNA	messenger ribonucleic acid
MUGA	multigated acquisition
NCI	National Cancer Institute

Abbreviation	Definition
pCR	pathologic complete response
PCS	potentially clinically significant
PD-L1	programmed death-ligand 1
PR	progesterone receptor
PT	Preferred term
Q1	25th percentile
Q3	75th percentile
Q3W	every 3 weeks
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SFU	Safety Follow-up
SOC	System organ class
TEAE	treatment-emergent adverse event
TIL	tumor-infiltrating lymphocyte
TNBC	triple negative breast cancer
Tregs	regulatory T cell
ULN	upper limit of normal
WHO-DD	World Health Organization Drug Dictionary

1. INTRODUCTION

This Statistical Analysis Plan (SAP) provides detailed statistical methods, variable definitions and derivations, and data handling that will be applied to analyze the clinical trial data collected during study 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)" based on the protocol Version 2.0.

The Statistical Programming Supportive Documents (SPSD) will be developed based on the SAP to serve as companion documents of the SAP to guide programming realization of the SAP. The SPSD contain three separate documents:

1. The table of contents (TOC) for planned analyses (in Excel Spreadsheet).
2. The reporting conventions (in Word).
3. The shells or specificities for tables, listings, and figures (TLFs) generation (in Word).

If there are differences between statistical analysis approaches described in this SAP and those in the protocol, the methods and approaches in this SAP will supersede those in the protocol.

Changes and additions to the last signed off version of this SAP will be documented with corresponding rationale in the clinical study report (CSR).

Statistical software SAS® (SAS Institute Inc., Cary, NC) Version 9.4 or later will be used to perform data analyses following the plan as laid out in this SAP.

1.1. Study Design

This is a Phase 2 multicenter, open-label, single-arm, neoadjuvant study with 4 phases:

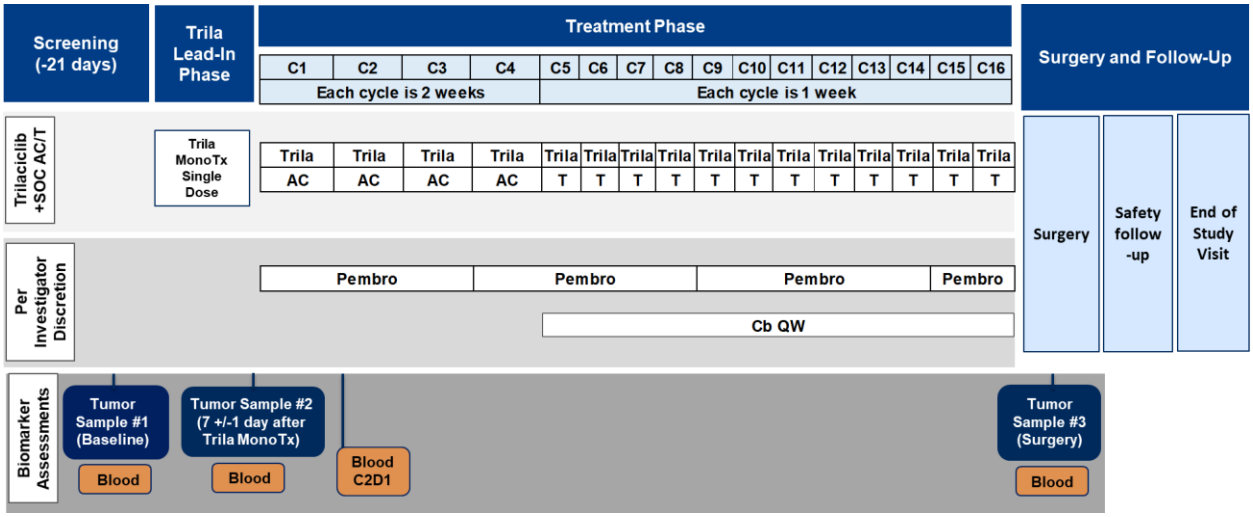
1. Screening Phase,
2. Trilaciclib Lead-in Phase,
3. Treatment Phase, and
4. Surgery and Safety Follow-Up Phase.

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 the 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 on the same visit.

Figure 1: G1T28-212 Study Design Diagram



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

1.2. Study Objectives

Primary:

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 (Tregs) ratio in tumor tissue.

Secondary:

- 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.

Specifically, the primary and secondary objectives and their associated endpoints are described in Table 1.

Table 1: Primary and Secondary 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/Tregs ratio in tumor tissue	• Change in CD8+ TILs/Tregs 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

CD8+=cluster of differentiation 8 positive; CTCAE=Common Terminology Criteria for Adverse Events; pCR=pathologic complete response; TIL=tumor-infiltrating lymphocyte; Tregs=regulatory T cells

Other objectives and endpoints of the study can be found in Section 5 of the study protocol.

1.3. Sample Size Consideration

Sample size justification is based off of previously published data on the CD8+/Tregs ratio from early stage TNBC patients who underwent neoadjuvant chemotherapy (Ahn). 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 Table 2.

Table 2: Statistical Power Calculation of Primary Endpoint

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.	

2. THE NUMBER OF PLANNED ANALYSES

There is only one planned analysis for this study. The planned analysis will be conducted to evaluate the immune-based mechanism of action of trilaciclib after a single dose as measured by the change in CD8+ TILs/Tregs ratio in tumor tissue. In addition, the pCR rate, at the time of definitive surgery as well as the safety and tolerability of trilaciclib in combination with standard neoadjuvant systemic therapies will be assessed.

3. ANALYSIS POPULATIONS

3.1. The Full Analysis Set – 1 (FAS1)

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

3.2. The Full Analysis Set – 2 (FAS2)

The Full Analysis Set – 2 (FAS2) population includes enrolled patients who have received at least one dose of any study drug during the Treatment Phase of the study.

3.3. The Full Analysis Set (FAS)

The Full Analysis Set (FAS) population includes enrolled patients who received at least one dose of any study drug during the treatment period, where treatment period consists of the Trilaciclib Lead-in Phase and the Treatment Phase. This population will be used to assess safety and tolerability and the rate of pCR for trilaciclib in combination with neoadjuvant systemic therapies.

4. GENERAL CONSIDERATIONS FOR DATA SUMMARY AND DISPLAY

4.1. Treatment Group Descriptions and Display in Table, Listings and Figures

This is a single-arm study in which all patients are treated with trilaciclib along with dose-dense anthracycline/cyclophosphamide followed by taxane chemotherapy (with optional administration of pembrolizumab and carboplatin). For TLFs that will be generated following SAP execution, the term *overall* will be used to describe all patients enrolled or all patients enrolled who have had at least one dose of any study drug, as applicable.

4.2. Data Summary and Precision

General Principles of Data Summary

Data will be summarized in table format for the respective analysis populations described in Analysis Populations. In general, continuous variables will be summarized based on number of patients with non-missing data (indicated by n), by mean, standard deviation (SD), median, Q1, Q3, minimum, and maximum values. Categorical variables will be summarized by number (n) and percentage of patients in each category.

General Principle of Data Listing

All collected data and derived variables will be included in patient data listings. An indicator will be provided for any imputed data element (e.g., imputed adverse event [AE] onset date). Columns in listings will be ordered by study site, patient identification (ID), visit, and assessment or event date, if applicable, and then data elements.

General Principle of Precision for Summary Statistics and Calculated Statistical Quantities

The precision of summary statistics for continuous variables, including mean, median, Q1, Q3, minimum, and maximum, will be consistent with the precision of the variable as collected. Calculated quantities of variability (e.g., standard deviation, standard error [SE]) will be presented with one more decimal place than the precision of the variable that is collected. The boundaries of a confidence interval (CI) will keep the same precision as the point estimate.

When the collected data are integers (including counting variables), the mean, median, Q1, and Q3 will be presented with one decimal place.

For percentages, 1 digit will be presented after the decimal point (e.g., 10.1%). A percentage value less than 0.1% will be displayed as "<0.1%." A percentage value that is >99.9% and <100% will be displayed as ">99.9%."

P-values, in general, will be displayed in 3 decimal places except for when a p-value is less than < 0.001, it will be presented as "<0.001". If a p-value is greater than 0.999, it will be displayed as ">0.999".

Rounding will take place after all calculation steps are completed prior to result display.

4.3. Definitions for Analysis Related Timepoint and Time Interval

Timepoint and time intervals related to efficacy and safety data collected during the study will be summarized. For each specific category of data, the time interval by which the data will be

analyzed or summarized will be specified in the respective section in which the data analysis plan is described.

To clarify data inclusion for each time interval of interest and baseline or end values for each given time interval, Table 3 presents definitions for timepoints, timepoint related assessments, and time intervals involved in statistical data analysis or summary.

Table 3: Definitions for Timepoints, Timepoint Related Assessments and Time Intervals

Term	Definition
Start of study (date)	Date of first dose of any study drug.
Study baseline	The last non-missing value prior to or on the date of the first dose of any study drug at the time that is before the time of the first study drug administration. If the event occurs on the same day as date of first dose of study drug but does not have the exact time captured, the event is assumed to have occurred prior to the first dose of study drug.
Pre-trilaciclib	The period before lead-in monotherapy trilaciclib administration when the first tumor biopsy (sample #1) will be obtained
Post-trilaciclib	The date that is 7 (±1) days after lead-in monotherapy trilaciclib administration when the second tumor biopsy (sample #2) is obtained
Day 1 of Cycle X (date)	The date, during the Treatment Phase, when the first dose of any study drug for cycle X is administered.
End of cycle (date)	The date of Day 1 of the subsequent cycle, if there is a subsequent cycle. The end of the last cycle is the date of Safety Follow-up visit. If Safety Follow-up visit does not occur for a patient after the last dose of study drug, the End of cycle for the patient will be defined as 30 days post the last dose of study drug.
Duration of a cycle (days)	Total number of days from Day 1 of the cycle to End of cycle, that is, End of cycle – Day 1 of the cycle + 1.
Duration of study drug exposure (weeks)	The duration (in weeks) between the date of lead-in dose of monotherapy trilaciclib and End of the last cycle in the study. That is, (End of the last study cycle – Day 1 of lead-in dose of monotherapy trilaciclib + 1) / 7.

4.4. Study Day

Study Day will be calculated for an event date or an assessment date to provide additional information for interpretation of the event occurrence. Study Day is calculated as:

- The start date of the event (visit date, onset date of an event, assessment date etc.) – date of first dose of any study drug + 1, if the event occurred on or after the reference date.

- The start date of the event (visit date, onset date of an event, assessment date etc.) − date of first dose of any study drug, if the event occurred prior to the reference date.

4.5. General Principles of Missing Data Handling

For primary efficacy endpoint the observed data are used for data summary and analyses. That is, no missing imputation will be performed. However, imputation of missing onset or stop dates for AEs and concomitant medication will be adapted to determine the status of each AE and the prior/concomitant/subsequent status of each non-study treatment medication. Please refer to 9.2.2 for the rules of imputation of missing AE onset or stop date and 5.4 for the rules of imputation of missing medication onset or stop dates.

For demographic and baseline characteristics, continuous variables will be summarized based on non-missing observations with the sample size of patients with non-missing data indicated, and a category of "Missing" will be included in the summary for each categorical variable.

5. DISPOSITION AND BASELINE CHARACTERISTICS

5.1. Patient Disposition

A summary of patient disposition will be generated using all accumulated data as of the database lock date.

In general, patient disposition summary will include the following 4 major sections:

1. Disposition of all screened patients who signed informed consent
2. Study drug disposition for patients who received at least one dose of any study drug
3. Study disposition for patients who were enrolled
4. Deaths among patients who were enrolled

The specific details for each section are described below.

1. Disposition of all screened patients

The total number of screened patients who signed informed consent will be presented as two mutually exclusive groups: those who were screen failures and those who were enrolled. For those who were enrolled, patients who received no study drug and those who receive a single dose of monotherapy trilaciclib during the Lead-in Phase are presented overall. Further, among patients who received a single dose of monotherapy trilaciclib, the number who had at least one dose of any study drug during the Treatment Phase will be summarized. Unless otherwise stated, number of enrolled patients will be the denominator for calculating percentages of patients in each of the categories.

2. Study drug disposition

For each study drug (trilaciclib, doxorubicin, cyclophosphamide, pembrolizumab, paclitaxel and carboplatin), the number and percentage of patients who had at least one dose will be summarized. In addition, the number of patients who were discontinued from the study drug will be summarized. The number of patients who received at least one dose of study drug will be the denominator for the percentage calculation of number of patients who discontinued for the respective drug. For patients who discontinued the study drug, the primary reasons for study drug discontinuation will be presented with the percentage of patients in each reason being calculated based on the number of patients who discontinued the study drug.

3. Study disposition

The number and percentage of patients who discontinued from the study will be summarized. The number of patients who were enrolled will be the denominator for the percentage calculation. The primary reason for study discontinuation will also be summarized, and the number of patients who discontinued the study will be the denominator for the percentage calculation for each reason of discontinuation.

4. Death

The number and percentage of patients who died during the study will be summarized overall for all enrolled patients along with the primary reason of death (Progressive Disease, Adverse Event, Other). The number of enrolled patients will be the denominator for calculating percentages of

death, and the number of patients who died will be the denominator for calculating the percentage of patients within each reason for death.

Study drug and study disposition information will be provided in data listings for all enrolled patients.

5.2. Demographics and Baseline Disease Characteristics

Demographics and baseline disease characteristics will be summarized on the FAS population.

Demographics including age at screening visit, age group (18-65, > 65), sex at birth, child-bearing potential, ethnicity, and race will be summarized. In addition, baseline body mass index (BMI) and Eastern Cooperative Oncology Group (ECOG) score will be summarized. Further, baseline disease characteristics such as stage at diagnosis, histologic grade, histopathological type at diagnosis, BRCA mutation and PD-L1 status will be summarized for all patients in the FAS population.

Data listing for demographics and baseline disease characteristics will be provided for all enrolled patients.

5.3. Medical/Surgical History and Ongoing Conditions

Surgical history and medical history and ongoing medical conditions at the Screening Visit will be summarized on the FAS population.

Non-breast cancer related medical history and ongoing medical conditions as collected at the Screening Visit will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 25.1 or later, and then summarized overall. Medical history and ongoing conditions will be presented by system organ class (SOC) and preferred term (PT), with both SOC and PT sorted in descending frequency based on the overall column. A patient will only be counted once within a particular SOC (or PT) even if the patient had multiple conditions/diseases in the same SOC (or PT).

A data listing for medical history and ongoing medical conditions collected at the Screening Visit will be provided for all enrolled patients. In addition, a data listing for prior surgeries/procedures will be generated for all patients in the FAS population.

5.4. Prior and Concomitant Medications

A summary of concomitant, non-cancer medications will be generated on the FAS population. Prior medications will be presented in a data listing. All prior medications will be collected from 21 days prior to signing the informed consent form (ICF).

Concomitant medications are those protocol-permitted medications that were given during the time interval from informed consent through the End of Study Visit. Vaccinations administered within 90 days prior to the first dose of study drug through the End of Study Visit will also be included as concomitant medications. Medications collected through electronic case report forms (eCRFs) will be coded to Anatomical Therapeutic Classification (ATC) and PT, where applicable, using the most recent World Health Organization Drug Dictionary (WHO-DD) version WHODrug-Global-B3 202209.

Concomitant medications will be summarized by ATC and PT and presented in a descending order of frequency for ATC and PT within an ATC based on the overall group. If a patient took multiple medications within the same ATC, the patient will only be counted once for that ATC. Similar logic applies to PT summaries. The number and percentage of patients receiving any concomitant medications will be summarized overall.

Handling of Missing Start and/or End Date for Medications Entered into EDC

Medications with incomplete start and/or end dates will be imputed according to the specifications described below. Those with incomplete start and/or end dates will be assumed to be concomitant if it cannot be shown that the medication was taken outside of the treatment period.

For completely missing or partially missing start dates:

- If the start date has month and year but day is missing, the first dose date will be used if the month and year is the same as the first dose date, otherwise, the first day of the month will be used.
- If the start date has year, but day and month are missing, then the first dose date will be used if the year is the same as the first dose date, otherwise January 1st will be used.
- If the start date is completely missing, then it will be imputed as the first dose date of study drug.

After the imputation, the imputed start date will be compared with corresponding stop date, if available. If the imputed start date is later than the stop date, the start date will be imputed with the stop date instead.

For completely missing or partially missing stop dates for concomitant medications that are not ongoing at the time of study database lock:

- If the stop date has month and year but day is missing, the last day of the month will be used.
- If the stop date has year, but day and month are missing, December 31st will be used.
- If the stop date is completely missing, the last dose date will be used.

After the imputation, the imputed stop date will be compared against the death date for patients who died. If the date is later than the death date, the date of death will be used to impute the stop date instead.

Prior and concomitant medications will be listed with an indicator for prior or concomitant among all patients in the FAS population.

5.5. Summary of Protocol Deviations

5.5.1. Definitions and Process for Identifying Protocol Deviations

Protocol deviation refers to situations where a patient's eligibility for study entry or a specific data collection deviate from the entry criteria or study procedure as specified in the protocol. Protocol deviation cases at the patient level with specific data elements of concern need to be summarized and reported in the CSR. Protocol deviations will be categorized as major or minor. Major protocol deviations are those that could affect the integrity of the data or adversely affect patients' safety. Criteria that define major or minor protocol deviations will be specified, documented, and signed off prior to study database lock. Specifically, a protocol deviation specifications document that describes the criteria defining major and minor protocol deviations, the categories of major protocol deviations, and the list of patients who had at least one protocol deviation case with the classification of major or minor will be created and signed off prior to study database lock.

5.5.2. Summary of Protocol Deviations

A protocol deviation summary will be generated for the final analysis.

The number and percentage of patients in the FAS population with protocol deviations will be tabulated for each of the following categories.

- Patients with at least one protocol deviation (major or minor)
- Patients with at least one major protocol deviation
- Patients with at least one protocol deviation in each of the major deviation categories

Protocol deviations will be listed with details and flags for major or minor for all enrolled patients.

6. CLASSIFICATION OF PRIOR SURGERIES AND PROCEDURES

Prior surgeries and procedures refer to those treatments that patients received prior to the Screening Visit. Summaries of prior surgeries and procedures will be based on the FAS population. The number and percentage of patients with any surgeries and procedures will be summarized. In addition, the number and percentage of patients with any surgeries and procedures related to the disease under study will be summarized.

A data listing for prior surgeries and procedures collected at the Screening Visit will be provided for all patients in the FAS population.

7. STUDY DRUG EXPOSURE, DOSE INTENSITY AND MODIFICATION

Analyses described in this section will be based on the FAS population unless otherwise specified.

7.1. Duration of Study Drug Exposure

Duration of study drug exposure (weeks) is defined as the duration from date of lead-in dose of monotherapy trilaciclib to date of End of cycle for the last cycle. That is, duration of study drug exposure (weeks) = (Date of End of cycle for the last cycle – date of lead-in dose of monotherapy trilaciclib + 1)/7, where the definition for End of cycle for the last cycle is provided in 4.3.

For each study drug, the number of patients who received the study drug at each cycle (including the Lead-in Phase) will be summarized. In addition, the total number of cycles that a patient received will be summarized as a continuous variable, as well as a categorical variable. That is, descriptive summary statistics will be provided for the total number of cycles that patients received, and the number and percentage of patients who received exactly 0, 1, 2…, to the maximum number of cycles will be summarized.

Corresponding data listings will be provided.

7.2. Cumulative Dose and Dose Intensity

The definitions for cumulative delivered dose, delivered dose intensity, relative cumulative dose, and relative dose intensity at the patient level, along with other parameters involved in the calculation of these variables are presented in Table 4. All collected dose information will be used in the calculation.

The calculated cumulative delivered dose, delivered dose intensity, relative cumulative dose, and relative dose intensity will be summarized as continuous variables for each study drug.

All variables described in Table 4 will be included in the data listing except for the study drug administration schedule.

Table 4: Dose Administration Parameters for Study G1T28-212

Parameter	Meaning	Trilaciclib	Doxorubicin	Cyclophosphamide	Paclitaxel	Pembrolizumab	Carboplatin
Study drug administration schedule	Drug dose and schedule per protocol	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 once every 2 weeks for 4 cycles (Cycles 1-4)	600 mg/m^2 once every 2 weeks for 4 cycles (Cycles 1-4)	80 mg/m^2 once every week for 12 cycles (Cycles 5-16)	400 mg once every 6 weeks starting on Day 1 of Cycle 1 and continue through Treatment Phase	AUC 1.5 every week starting on Day 1 of paclitaxel chemotherapy (Cycles 5-16)
Cumulative delivered dose (mg/m^2 or mg or AUC)	Sum of actual doses administered for each study drug	Sum of doses over the duration of trilaciclib administration [mg/m^2]	Sum of doses over the duration of doxorubicin administration [mg/m^2]	Sum of doses over the duration of cyclophosphamide administration [mg/m^2]	Sum of doses over the duration of paclitaxel administration [mg/m^2]	Sum of doses over the duration of pembrolizumab administration [mg]	Sum of doses over the duration of carboplatin administration [AUC]
Delivered Dose Intensity (mg/m^2/week or mg/week or AUC/week)	Cumulative dose administered per week	Cumulative delivered dose / EXP [mg/m^2/week]	Cumulative delivered dose / EXP2 [mg/m^2/week]	Cumulative delivered dose / EXP2 [mg/m^2/week]	Cumulative delivered dose / EXP3 [mg/m^2/week]	Cumulative delivered dose / (EXP2 + EXP3) [mg/week]	Cumulative delivered dose / EXP3 [AUC/week]
Relative cumulative dose (%)	(Cumulative delivered dose / Cumulative prescribed dose) ×100	[Cumulative delivered dose / (240 × (1+CYC+CYC2))] ×100	[Cumulative delivered dose / (60 × CYC)] ×100	[Cumulative delivered dose / (600 × CYC)] ×100	[Cumulative delivered dose / (80 × CYC2)] ×100	{Cumulative delivered dose / [400 × CEL((EXP2 + EXP3) / 6)]} ×100	[Cumulative delivered dose / (1.5 × CYC2)] ×100
Relative dose intensity (%)	(Delivered dose intensity / prescribed dose intensity) ×100	{Delivered dose intensity / [(240 × (1 + CYC + CYC2)) / (1 + 2CYC + CYC2)]} ×100	[Delivered dose intensity / (60/2)] ×100	[Delivered dose intensity / (600/2)] ×100	(Delivered dose intensity / 80) ×100	[Delivered dose intensity / (400/6)] ×100	(Delivered dose intensity / 1.5) ×100

AUC = area under curve; IV = intravenous; CYC = number of cycles during cycles 1-4; CYC2 = number of cycles during cycles 5-16; EXP = duration of study drug exposure in weeks including lead-in; EXP2 = duration of study drug exposure in weeks during cycles 1-4; EXP3 = duration of study drug exposure in weeks during cycles 5-16; CEL = ceiling (smallest integer ≥ the resulting decimal number)

7.3. Study Drug Modifications

Overall study drug modifications will be summarized on the FAS population. There are three types of study drug modification: dose reduction, cycle delay or infusion interruption. Protocol permitted dose reductions for each study drug are summarized in Table 5.

Table 5: Protocol Permitted Dose Reduction by Study Drug

Study Drug	Maximum Number of Dose Reductions Allowed
Trilaciclib	0
Doxorubicin	2
Cyclophosphamide	2
Paclitaxel	2
Carboplatin	2
Pembrolizumab	0

The overall number of patients with any study drug modification will be summarized based on the FAS. In addition, study drug modifications will be summarized in each of the following categories: chemotherapy dose reduction, treatment cycle delay, and infusion interruption. The number and percentage of patients who had any chemotherapy dose reduction, any cycle delay, or any infusion interruption will be summarized.

More detailed summaries as outlined below will be provided.

Dose reduction for a specific chemotherapy. Four chemotherapies (doxorubicin, cyclophosphamide, paclitaxel, and carboplatin) can have dose reduction per protocol (Table 5). The number and percentage of patients who had at least one dose reduction during the treatment period will be summarized overall. Dose reduction is counting for all the reductions across these four study drugs during the treatment period. In addition, the number and percentage of patients in the following mutually exclusive categories: no chance to have any dose reduction (Cycle 1 treatment only), 0, 1, 2, up to the maximum number of reductions for the drug will be summarized. The reasons for dose reduction as collected in the eCRF will also be summarized for each of these chemotherapies.

Cycle delay. Information regarding whether a cycle was delayed was collected in the eCRF for each cycle. The number of cycles that have been delayed will be summarized as a continuous variable. In addition, the number and percentage of patients in each of the following mutually exclusive categories will be summarized: no chance to have any cycle delay (Cycle 1 treatment only), 0, 1, 2, and 3 or more cycle delays.

The reasons for cycle delay collected in eCRF are as follows:

- Low Neutrophil Count
- Low Platelet Count
- BOTH Low Neutrophil Count AND Low Platelet Count
- Other Hematologic Toxicity
- Non-hematologic toxicity
- Other

The number and percentage of patients in each of the categories will be calculated based on the number of patients in the FAS population.

Infusion interruption. Infusion interruption was captured in the eCRF for each respective study drug with the reasons. In addition to summarizing the number and percentage of patients who had at least one infusion interruption for any study treatment, the summary statistics for the total number of infusion interruptions and the number and percentage of patients in each of the following mutually exclusive categories will be summarized: 0, 1, 2, and 3 or more infusion interruptions. The number of interruptions is the sum of infusion interruptions for all study drugs for which the interruption occurred at least once during the infusion across all cycles in the Treatment Phase and Trilaciclib Lead-in Phase. Multiple interruptions for a drug during an infusion will only be counted once. The reasons for infusion interruption as collected in the eCRF will also be summarized.

Corresponding data listings will be provided.

8. EFFICACY ANALYSIS

The parameters needed for the primary immune-response measure – the ratio of CD8+ TIL over Forkhead Box P3 (FOXP3)+ regulatory T cells (abbreviated as CD8+ TILs/Tregs) will be measured by our bio-marker vendor and a detailed analytical plan for the quantification of CD8+ TILs and FOXP3 is included in Appendix 3. Briefly, CD8+ TILs and FOXP3+ cells will be quantitated on baseline and Day 7 tumor samples using two independent immunohistochemistry (IHC) assays, PanCK/CD8 IHC and FOXP3 IHC, respectively. The IHC slides are evaluated by a pathologist and the derived density (/mm2) of CD8+ TILs and FOXP3+ cells will be used to determine the ratio of CD8+ TILs/Tregs.

8.1. Definitions of Efficacy Endpoints

8.1.1. Primary Efficacy Endpoints – Change in CD8+ TILs/Tregs ratio from baseline to 7 ± 1 days after single-dose monotherapy trilaciclib administration

From biopsy samples (archival or fresh) obtained, the ratio of CD8+ TILs to Tregs will be calculated at pre-trilaciclib and post-trilaciclib. Among patient with paired results, the difference in the ratio from pre-trilaciclib to post-trilaciclib will be calculated as

$$\text{Change } in \left(\frac{\text{CD8 + TILs}}{\text{Tregs}}\right) = \left(\frac{\text{CD8 + TILs}}{\text{Tregs}}\right)_{post-trilaciclib} - \left(\frac{\text{CD8 + TILs}}{\text{Tregs}}\right)_{pre-trilaciclib}$$

For patients with missing value at baseline or day 7, no imputation will be carried out.

Histograms and Box plot for the distribution of the change in CD8+ TILs/Tregs will be generated. In addition, a scatter plot for the correlation between pre and post trilaciclib ratio of CD8+ TILs/Tregs will also be generated.

The change in the ratio of CD8+ TILs to Tregs will be categorized as positive (change in [CD8+ TILs/Tregs] > 0), negative (change in [CD8+ TILs/Tregs] < 0), unchanged (change in [CD8+ TILs/Tregs] = 0) or not evaluable. Further, categories for the incremental units of 1.5 will be created for positive and negative changes. In situations where 2 or more consecutive units of 1.5 yield zero counts, such categories will be combined until the next unit of 1.5 with > 0 count.

Summary statistics for CD8+ TILs/Tregs among patients with both pre- and post-trilaciclib data will be provided for measurements taken at baseline (pre-trilaciclib), 7 ± 1 days after single dose trilaciclib (post-trilaciclib), and the change scores (post minus pre). The significance of the magnitude of change will be compared using paired Wilcoxon Signed-Rank test. As sensitivity analysis, patients with neuroendocrine features of the disease will be excluded and the comparison analysis will be repeated.

The primary efficacy endpoint defined in this section will be derived based on FAS1 population.

8.1.2. Secondary Efficacy Endpoints – Pathologic complete response (pCR) rate at the time of definitive surgery

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. The pCR rate will be reported with a 95% confidence interval using the Clopper-Pearson method based on FAS population.

In addition, subgroup analyses will be conducted on the pCR rate by classifying patients into 2 distinct groups using the following criteria:

- High change in CD8+ TILs/Tregs from pre- to post-trilaciclib (> median value) vs. Low change in CD8+ TILs/Tregs from pre- to post-trilaciclib (≤ median value).
- Positive vs. negative changes in CD8+ TILs/Tregs (unchanged CD8+ TILs/Tregs will be grouped with positive changes).

pCR rate will be summarized by subgroup and Fisher's exact test will be used to assess the association between pCR status and subcategories above.

Further, summary statistics for the change in CD8+ TILs/Tregs (from pre- to post-trilaciclib) will be provided for patients who had pCR and those who did not have pCR (pCR=Yes vs. pCR=No vs. pCR=Missing). Finally, where applicable, boxplots for the distribution of change in CD8+ TILs/ Tregs by pCR subcategories (Yes vs. No vs. Missing) will be presented.

The secondary efficacy endpoint defined in this section will be derived based on the FAS population.

8.1.3. Exploratory Endpoints

The following endpoints will be explored. The description of the detailed steps involved in the analyses of these endpoints is outside the scope of this SAP.

- 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 Tregs infiltration
- 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
- 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

9. SAFETY ANALYSIS

9.1. General Consideration of Safety Analysis

Unless otherwise specified, all safety data collected during the study will be summarized. Hence, safety data summaries will be based on the FAS as defined in 3.3 of this SAP.

Safety data will be summarized using descriptive statistics for all patients when appropriate. All safety data collected through scheduled or non-scheduled visits during the study will be included in the safety data analyses. Missing safety data will generally not be imputed, unless otherwise specified.

Baseline assessment is, in general, defined as the last non-missing observation prior to receiving the first dose of any study drug.

9.2. Adverse Events

9.2.1. Definition and Classification of Adverse Events

AEs are defined as those AEs occurring on or worsening in severity after the first dose of any study drug (i.e., the conventional treatment-emergent AEs). Only AEs as described above are collected in the study database. All AEs are reported from the first dose of any study drug until end of study (EOS) visit. If EOS visit does not exist, then AEs until safety follow-up (SFU) visit date will be reported, if SFU date does not exist then AEs collected until, and including, 30 days after the last dose of study drug will be reported. SAEs thought to be related to a study specific procedure are also collected between the time the patient signs the informed consent and the first dose of any study drug.

AEs will be coded from verbatim text to PT and grouped by primary SOC according to MedDRA version 25.1. The severity (toxicity grades 1-5) of AEs will be graded according to the NCI CTCAE version 5.0 by the Investigator.

Hematologic Adverse Events

AEs related to hematologic toxicity will be collapsed based on the PTs from MedDRA version 25.1 and will be summarized separately (see 9.2.3). Table 6 outlines those PTs that will be collapsed.

Table 6: Hematologic Preferred Terms to be Collapsed

Term presented in the Output	Preferred Term
Neutropenia	Neutropenia
	Neutrophil count decreased
Anemia	Anemia
	Anaemia
	Red blood cell count decreased
	Hemoglobin decreased

Thrombocytopenia	Thrombocytopenia
	Platelet count decreased
Lymphocytopenia	Lymphocytopenia
	Lymphopenia
	Lymphocyte count decreased
Leukopenia	Leukopenia
	White blood cell count decreased

Trilaciclib Adverse Events of Special Interest

AEs of special interest (AESI) for trilaciclib have been identified, reflecting either the findings in the AEs from the previous studies of trilaciclib or class effects for CDK 4/6 inhibitors. AESI for trilaciclib will be identified by searching MedDRA PTs based on the Customized MedDRA Queries as detailed in Appendix 2.

Specifically, trilaciclib AESI include the following 5 categories:

- Injection site reaction/Phlebitis/Thrombophlebitis
- Acute drug hypersensitivity reaction
- Hepatotoxicity
- Interstitial lung disease /Pneumonitis
- Embolic and thrombotic events, venous

9.2.2. Imputation Rules for Missing Start or Stop Date for Adverse Events

AEs with start/stop dates that are partially or completely missing that are not ongoing at the time of data cutoff will be imputed according to the specifications below in order to classify AEs.

For completely missing or partially missing AE start date:

- If the start date has month and year but day is missing, the first dose date will be used if the month and year is the same as the first dose date, otherwise, the first day of the month will be used.
- If the start date has year, but day and month are missing, then the first dose date will be used if the year is the same as the first dose date, otherwise January 1st will be used.
- If the start date is completely missing, then it will be imputed as the first dose date of study drug.

After the imputation, the imputed start date will be compared with AE stop date, if available. If the imputed start date is later than the stop date, the start date will be imputed with the stop date instead.

For completely missing or partially missing AE stop dates:

- If the stop date has month and year but day is missing, the last day of the month will be used.
- If the stop date has year, but day and month are missing, December 31st will be used.
- If the stop date is completely missing, then the date of EOS visit will be used; if EOS visit does not exist, then the SFU visit date is used, if SFU date does not exist then the last dose date + 30 days will be used.

After the imputation, the imputed AE stop date will be compared against the death date for patients who died. If the date is later than the death date, the date of death will be used to impute the stop date instead.

Every attempt will be made to obtain complete information for AEs regarding severity (i.e., CTCAE Grade) and relationship to drug. However, in the rare case of missing data, the following conservative approach will be taken for summary purpose. The non-imputed raw data will be presented in AE listings.

- Missing AE grade will be classified as 'Grade 3'
- Missing AE relationship will be classified as "Related"

9.2.3. Analysis for Adverse Events

AEs will be summarized by number and percentage of patients having at least one occurrence at the PT and SOC level overall. Patients with more than one occurrence of the same SOC (PT) will be counted only once within the SOC (PT) categorization. In general, the percentage of patients with an event will be calculated using the number of patients in the FAS as the denominator. For tables summarizing AEs related to study drugs the percentages will instead be calculated using the number of patients who received the drug as the denominator.

AEs will also be summarized by CTCAE grade and relationship to study drug (to any study drugs and to each individual study drug). Should a patient experience more than one occurrence of the same SOC (PT), the patient's worst occurrence (highest grade or highest related causality) will be used for the analysis and reporting.

In AE summaries, the SOC and PT within a SOC will be presented in descending order based on the incidence from all patients. If the incidence for two or more PTs is equal, these PTs will be presented in alphabetical order.

The AEs by decreasing frequency in PT during the trilaciclib Lead-in Phase will be summarized based on FAS1.

An overall AE summary table will be generated to present general information related to AEs including the following categories: number and percentage of patients with any AE, Serious AEs, AEs with CTCAE Grade ≥ 3 or 4, AEs leading to discontinuation of any study drug, AEs leading to death, AEs related to study drug (to any study drug, to trilaciclib, and to each other study drug).

In addition, the following summary tables will be generated, and they will be, in general, presented by SOC and PT unless otherwise specified.

1. AEs by decreasing frequency of PT during trilaciclib Lead-in Phase
2. AEs by decreasing frequency of PT during treatment period
3. AEs by PT and CTCAE Grade
4. AEs with CTCAE Grade 3 or 4 by PT
5. AEs leading to discontinuation of any study drug
6. AEs leading to death
7. AEs related to any and each study drug
8. Hematological AEs by collapsed PT and CTCAE grade
9. Serious AEs
10. Serious AEs related to any and each study drug
11. AESI for trilaciclib will be summarized.

In the above, hematological AEs will be reported using their coded PT with the exception of item 8 (Hematological AEs by collapsed PT and CTCAE grade), for which the collapsed terms as specified in Table 6 (9.2.1) will be reported in place of coded PT.

Corresponding AE listings will be provided to clearly indicate, at the patient level, the AE and SAE occurrence, start/stop date, relative study days to onset or stop, grade and causality for each AE. AESI for trilaciclib will also be listed with a similar level of detail. The AE listings will be generated based on FAS.

9.3. Clinical Laboratory Data

9.3.1. Laboratory Parameters

Blood and urine samples for the determination of clinical chemistry, hematology, urinalysis and other laboratory variables described in Table 7 will be measured according to Schedule of Assessments in Appendix 1.

Table 7: Laboratory Categories and Parameters

Lab Category	Lab Parameters
Hematology	white blood cell (WBC), platelets, hemoglobin, absolute neutrophil count (ANC).
Serum Chemistry	Sodium, Potassium, Chloride, Bicarbonate, Urea, Phosphorus, Creatinine, Calcium, Magnesium, Total Protein, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Bilirubin, Albumin.
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

For hematology parameters, if absolute counts are not provided, those values will be derived from the differential percentage by multiplying differential percentage value with leukocyte value from the same sample. The normal ranges will be left missing in those cases.

Clinical chemistry and hematology assessments will be graded according to NCI CTCAE criteria, Version 5.0 or later. The determination of CTCAE grade for each measurement will be based on the collected laboratory values and will not involve clinical judgement. For laboratory parameters that CTCAE toxicity grade are not available, they will not be included in the analyses in which toxicity grades are reported. Instead, these parameter results will be classified and reported by the low/normal/high based on the laboratory normal reference ranges.

Abnormal Hepatic Laboratory Values

Abnormal hepatic laboratory values are defined in the following categories including any occurrence among all on-treatment, post-baseline assessments including scheduled and unscheduled values.

- Hy's Law: alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) > 3x the upper limit of normal (ULN), alkaline phosphatase (ALP) < 2xULN, and total bilirubin ≥ 2xULN
- AST: > 3 and ≤ 5x ULN, > 5 and ≤ 8x ULN, > 8 and ≤ 10x ULN, > 10 and ≤ 20x ULN, and > 20x ULN; AST > 5x ULN for more than 5 weeks.
- ALT: >3 and ≤ 5x ULN, > 5 and ≤ 8x ULN, > 8 and ≤ 10x ULN, > 10 and ≤ 20x ULN, and > 20x ULN; ALT > 5x ULN for more than 5 weeks.
- Total bilirubin > 1.5xULN and < 2xULN, ≥ 2xULN

9.3.2. Analysis for Laboratory Parameters

Laboratory data from all central and local laboratories will be included in the analyses. Different laboratories are likely using slightly different normal reference ranges, which should not affect the planned analysis since they are all categorical and reported based on CTCAE toxicity grade or relationship to the normal ranges. The default convention for reporting of laboratory units will be standard international (SI) units. If a lab value is reported using an inequality symbol e.g., less than (<) a certain value, or greater than (>) a certain value, the given numeric value will be used in the summary. Data will be presented in listings with their inequality symbol.

For each parameter in the clinical chemistry and hematology laboratory group, respectively, CTCAE toxicity grading is used to classify patients into a toxicity grade from 1 to 4 for each timepoint assessment. The number and percentage of patients with highest grade will be

summarized for each grade from 1 to 4 and Grade 3-4, along with such summary for the value collected at baseline.

For the laboratory parameters that cannot be classified by CTCAE grade, the number and percentage of patients in the categories of treatment-emergent low or treatment-emergent high based on the normal reference range associated with the parameter will be summarized.

In addition, shift in CTCAE grade from baseline to the worst post-baseline value will be produced. The shift tables will include patients who had non-missing baseline and at least one non-missing post-baseline value for the parameter of interest.

The number and percentage of patients in each category of abnormal hepatic laboratory values will be summarized.

Laboratory parameters will be listed by the group of chemistry, hematology, coagulation, and other tests. In the data listing, flags that indicate the corresponding CTCAE grades and the classifications relative to the laboratory reference ranges will be included. In addition, a separate listing will be prepared for patients who met Hy's law.

9.4. Vital Signs

9.4.1. Vital Sign Parameters

Vital signs including pulse rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), weight and height will be measured according to Schedule of Assessments in Appendix 1.

Baseline vital signs refer to the measurements taken on the date of the first dose of any study drug (only if time of vital measurement is prior to the time of dosing) or, in the event vitals are not taken on the date of first dose of study drug, at the Screening visit. Post-baseline assessments refer to the measurements taken after the first dose of any study drug during the study. Change from baseline to the highest/lowest value across all post-baseline measurements for each vital sign parameter will be calculated. Patients who had a non-missing baseline and at least one non-missing measurement post-first dose for a given parameter will be included in the calculation.

Patients are classified with respect to the criteria of potentially clinically significant (PCS) findings of vital signs, which are defined by the highest/lowest value among post-baseline assessments and/or the change from baseline to the highest/lowest observed value. Details of PCS criteria for vital signs can be found in Table 8.

Table 8: Criteria for Potentially Clinically Significant Vital Signs

Parameter	Direction	Highest/Lowest Observed Value	Change from Baseline to the Highest/Lowest Observed Value
SBP	High	≥180 mmHg	Increase ≥40 mmHg
	Low	≤ 90 mmHg	Decrease ≥40 mmHg
DBP	High	≥105 mmHg	Increase ≥ 20 mmHg
	Low	≤ 50 mmHg	Decrease ≥20 mmHg
Heart Rate	High	≥ 120 bpm	Increase ≥40 bpm

	Low	≤ 50 bpm	Decrease ≥40 bpm
Weight	High	--	Increase ≥10%
	Low	--	Decrease ≥10%

bpm=beats per minute; DBP-diastolic blood pressure; SBP=systolic blood pressure

9.4.2. Analysis for Vital Signs

Number and percentage of patients who meet any PCS criteria for each vital sign parameter as well as for each criterion will be summarized.

All observed vital sign values, change from baseline at each post-first dose assessment, and PCS flag will be listed.

9.5. ECOG Performance Status

ECOG performance status was assessed at the Screening Visit and at Day 1 of each treatment cycle during the study.

A shift table that tabulates ECOG status at baseline and the worst status post first dose of any study drug will be generated.

All ECOG data will be included in data listing.

9.6. 12-lead Electrocardiograms

9.6.1. Electrocardiograms Parameters

The standard 12-lead Electrocardiogram (ECG) will collect heart rate, PR interval, QRS interval, RR interval, and QT interval at screening visit only. Additional ECGs may be performed as clinically indicated at any time during the study (Appendix 1).

Since either QTcF or QTcB could be collected by different study sites, QTcF (using Fridericia's method) will be calculated from the QT and RR (converted from collected ms to sec) intervals based on the formula:

QTcF = Uncorrected QT/ (RR Interval)^1/3, if QT and/or RR are missing, the QTcF will be left as missing.

A summary table of ECG parameters at baseline will be generated. In addition, ECG data will be included in data listing.

10. CHANGES FROM THE PROTOCOL

In section 12.2 of the protocol, FAS1 and FAS2 are defined as the study populations. In this SAP, a FAS population is defined to ensure that safety data for patients who dropped from the study after receiving a single dose of trilaciclib in the Lead-in Phase are adequately captured and summarized where necessary. The FAS population also includes patients who did not receive the single dose of trilaciclib in the Lead-in Phase before proceeding to the Treatment Phase.

11. REFERENCES

Soomin Ahn, Yul Ri Chung, An Na Seo, Milim Kim, Ji Won Woo, So Yeon Park. Changes and prognostic values of tumor-infiltrating lymphocyte subsets after primary systemic therapy in breast cancer. PLoS One. 2020;15(5):e0233037.

12. APPENDICES

APPENDIX 1. 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 **Error! Reference source not found.**
ER-/PR-/HER2- status	X											Section **Error! Reference source not found.**
Inclusion/ exclusion criteria	X											Section **Error! Reference source not found.**
Baseline characteristics and demographics	X											Section **Error! Reference source not found.**
Medical and surgical history	X											Section **Error! Reference source not found.**
Concomitant medications and procedures	X	X		X	X	X	X	X (C5, 8, 11, 16 only)	X	X	X	Section **Error! Reference source not found.**
Complete physical exam	X											Section **Error! Reference source not found.**

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
		Single dose mono. trila.	On-tx biopsy	Doxorubicin/ Cyclophosphamide +/- pembrolizumab *Each cycle = 2 weeks*				Paclitaxel +/- carboplatin [b] +/- pembrolizumab [b] *Each cycle = 1 week*				
				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**	
Directed physical exam		X		X	X	X	X	X (C5, 8, 11, 16 only)				Section **Error! Reference source not found.**
Vitals	X	X		X	X	X	X	X				Section **Error! Reference source not found.**
ECOG performance status	X	X		X				X (C5 and 16 only)			X	Section **Error! Reference source not found.**
Adverse event reporting	X	X		X	X	X	X	X	X	X	X	Section **Error! Reference source not found.**
Hematology	X	X		X	X	X	X	X				Section **Error! Reference source not found.**
Serum chemistry	X	X		X	X	X	X	X				Section **Error! Reference source not found.**
Coagulation (PT or INR)	X											Section **Error! Reference source not found.**
12-lead ECG	X											Section **Error! Reference**

Protocol Activity	Screening	Trilaciclib Lead-in Phase		Treatment Phase					Definitive Surgery	Safety Follow-up Visit	End of Study Visit	
		Single dose mono. trila.	On-tx biopsy	Doxorubicin/ Cyclophosphamide +/- pembrolizumab *Each cycle = 2 weeks* C1 D1[a]	C2 D1	C3 D1	C4 D1	Paclitaxel +/- carboplatin [b] +/- pembrolizumab [b] *Each cycle = 1 week* 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**	**See Protocol Section for Additional Details**
												source not found.
Echocardiogram or MUGA for LVEF assessment	X											Section **Error! Reference source not found.**
Urinalysis	X											Section **Error! Reference source not found.**
Urine/serum HCG (WOCBP only)	X			X				X (C5, 11, 16 only)				Section **Error! Reference source not found.**
Biomarker and Efficacy Assessments												
Blood samples for biomarkers		X (pre-dose)	X (pre-biopsy)		X (pre-dose)				X (-3 day)			Section **Error! Reference source not found.**
Tumor sample #1 (Baseline)	X											Section **Error! Reference source not found.**
Tumor sample #2 (On treatment)			X									Section **Error! Reference source not found.**
Tumor sample #3 (Surgery)									X			Section **Error! Reference**

Protocol Activity	Screening	Trilaciclib Lead-in Phase		Treatment Phase					Definitive Surgery	Safety Follow-up Visit	End of Study Visit	
				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**	**See Protocol Section for Additional Details**
												source not found.
pCR assessment									X			
Treatment												
Trilaciclib		X		X	X	X	X	X				Section **Error! Reference source not found.**
Doxorubicin/ cyclophosphamide				X	X	X	X					Section **Error! Reference source not found.**
Paclitaxel								X				Section **Error! Reference source not found.**
Carboplatin AUC 1.5 (Investigator discretion)								X				Section **Error! Reference source not found.**
Pembrolizumab (Investigator discretion)				X			X	X (C9 and C15 only)				Section **Error! Reference source not found.**
Definitive surgery									X			Section **Error! Reference source not found.**

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.

APPENDIX 2. CUSTOMIZED MedDRA QUERIES FOR TRILACICLIB AESIs

AESI Categories	Preferred Terms	
Injection Site Reaction/ Phlebitis/ Thrombophlebitis	Administration site phlebitis	Periphlebitis
	Application site phlebitis	Phlebitis
	Catheter site phlebitis	Phlebitis deep
	Chemical phlebitis	Phlebitis infective
	Infusion site phlebitis	Septic phlebitis
	Infusion site thrombosis	Thrombophlebitis
	Injection site phlebitis	Thrombophlebitis septic
	Injection site thrombosis	Thrombophlebitis superficial
		Vascular access site thrombosis
	Administration related reaction	Infusion site erythema
	Administration site dermatitis	Infusion site hypersensitivity
	Administration site hypersensitivity	Infusion site pain
	Administration site pain	Infusion site rash
	Administration site rash	Infusion site reaction
	Administration site recall reaction	Infusion site recall reaction
	Administration site urticaria	Infusion site urticaria
	Administration site vasculitis	Infusion site vasculitis
	Application site dermatitis	Injection related reaction
	Application site hypersensitivity	Injection site dermatitis
	Application site pain	Injection site erythema
	Application site rash	Injection site hypersensitivity
	Application site recall reaction	Injection site pain
	Application site urticaria	Injection site rash
	Application site vasculitis	Injection site reaction
	Catheter site dermatitis	Injection site recall reaction
	Catheter site hypersensitivity	Injection site urticaria
	Catheter site pain	Injection site vasculitis
	Catheter site rash	Installation site urticaria
	Catheter site urticaria	instillation site hypersensitivity
	Catheter site vasculitis	instillation site pain
	Immediate post-injection reaction	instillation site rash
	Infusion related reaction	Skin reaction
	Infusion site dermatitis	Vessel puncture site rash
		Vessel puncture site vesicles
Acute drug hypersensitivity reaction	Allergic bronchitis	Laryngitis allergic
	Allergic cough	Laryngospasm
	Allergic eosinophilia	Laryngotracheal oedema

AESI Categories	Preferred Terms	
	Allergic oedema	Lip edema
	Allergic pharyngitis	Lip swelling mast cell degranulation present
	Allergic reaction to excipient	Mouth swelling
	Allergic respiratory disease	oedema mouth
	Allergic respiratory symptom	Oropharyngeal oedema
	Anaphylactic reaction	Oropharyngeal spasm
	Anaphylactic shock	Oropharyngeal swelling
	Anaphylactic transfusion reaction	Palatal oedema
	Anaphylactoid reaction	Palatal swelling
	Anaphylactoid shock	Periorbital oedema
	Anaphylaxis treatment	Periorbital swelling
	angioedema	Pharyngeal oedema
	Bronchospasm	Pharyngeal swelling
	Circulatory collapse	Pruritus allergic
	Circumoral oedema	Reaction to excipient
	Circumoral swelling	Shock
	Distributive shock	Shock symptom
	Documented hypersensitivity to administered product	Swelling face
	Drug hypersensitivity	Swelling of eyelid
	Drug reaction with eosinophilia and systemic symptoms	Swollen tongue
	Epiglottic oedema	Therapeutic product cross-reactivity
	Eye oedema	Tongue oedema
	Eye swelling	Tracheal oedema
	Eyelid oedema	Type I hypersensitivity
	Face oedema	Urticaria
	Hypersensitivity	Urticaria contact
	Immune-mediated adverse reaction	Urticaria popular
	Infusion related hypersensitivity reaction	Urticarial dermatitis
	Laryngeal oedema	Urticarial vasculitis
Hepatotoxicity	Acute hepatic failure	Hepatitis acute
	Acute on chronic liver failure	Hepatitis cholestatic
	Acute yellow liver atrophy	Hepatitis chronic active
	Allergic hepatitis	Hepatitis chronic persistent
	Autoimmune hepatitis	Hepatitis fulminant
	Cholestatic liver injury	Hepatitis toxic
	Chronic hepatic failure	Hepatocellular foamy cell syndrome

AESI Categories	Preferred Terms	
	Chronic hepatitis	Hepatocellular injury
	Coma hepatic	Hepatotoxicity
	Drug-Induced Liver Injury	Immune-mediated hepatitis
	Hepatic failure	Liver disorder
	Hepatic infiltration eosinophilia	Liver injury
	Hepatic necrosis	Mixed liver injury
	Hepatic steato-fibrosis	Non-alcoholic steatohepatitis
	Hepatic steatosis	Steatohepatitis
	Hepatitis	Subacute hepatic failure
Interstitial Lung Disease (ILD) /Pneumonitis	Acute interstitial pneumonitis	Idiopathic interstitial pneumonia
	Acute lung injury	Idiopathic pneumonia syndrome
	Acute respiratory distress syndrome	Idiopathic pulmonary fibrosis
	Alveolar lung disease	Immune-mediated pneumonitis
	Alveolitis	Interstitial lung disease
	Alveolitis necrotizing	Necrotizing bronchiolitis
	Autoimmune lung disease	Obliterative bronchiolitis
	Diffuse alveolar damage	Pneumonitis
	Eosinophilic pneumonia	Pneumonitis chemical
	Eosinophilic pneumonia acute	Progressive massive fibrosis
	Eosinophilic pneumonia chronic	Pulmonary fibrosis
	Granulomatous pneumonitis	
	Hypersensitivity pneumonitis	Pulmonary toxicity
		Restrictive pulmonary disease
Embolic and thrombotic events, venous	Axillary vein thrombosis	Pulmonary thrombosis
	Brachiocephalic vein occlusion	Pulmonary vein occlusion
	Brachiocephalic vein thrombosis	Pulmonary veno-occlusive disease
	Deep vein thrombosis	Pulmonary venous thrombosis
	Embolism venous	Subclavian vein occlusion
	Iliac vein occlusion	Subclavian vein thrombosis
	Obstructive shock	Superior vena cava occlusion
	Pelvic venous thrombosis	Thrombosis
	Peripheral vein occlusion	Vena cava embolism
	Peripheral vein thrombus extension	Vena cava thrombosis
	Phlebectomy	Venous occlusion
	Pulmonary embolism	Venous thrombosis
	Pulmonary microemboli	Venous thrombosis limb

AESI Categories	Preferred Terms	
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	Anaphylactoid reaction	Palatal swelling
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	Acute yellow liver atrophy	Hepatitis chronic active
	Allergic hepatitis	Hepatitis chronic persistent
	Autoimmune hepatitis	Hepatitis fulminant
	Cholestatic liver injury	Hepatitis toxic
	Chronic hepatic failure	Hepatocellular foamy cell syndrome

AESI Categories	Preferred Terms	
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	Coma hepatic	Hepatotoxicity
	Drug-Induced Liver Injury	Immune-mediated hepatitis
	Hepatic failure	Liver disorder
	Hepatic infiltration eosinophilia	Liver injury
	Hepatic necrosis	Mixed liver injury
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	Hepatitis	Subacute hepatic failure
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	Alveolar lung disease	Immune-mediated pneumonitis
	Alveolitis	Interstitial lung disease
	Alveolitis necrotizing	Necrotizing bronchiolitis
	Autoimmune lung disease	Obliterative bronchiolitis
	Diffuse alveolar damage	Pneumonitis
	Eosinophilic pneumonia	Pneumonitis chemical
	Eosinophilic pneumonia acute	Progressive massive fibrosis
	Eosinophilic pneumonia chronic	Pulmonary fibrosis
	Granulomatous pneumonitis	
	Hypersensitivity pneumonitis	Pulmonary toxicity
		Restrictive pulmonary disease
Embolic and thrombotic events, venous	Axillary vein thrombosis	Pulmonary thrombosis
	Brachiocephalic vein occlusion	Pulmonary vein occlusion
	Brachiocephalic vein thrombosis	Pulmonary veno-occlusive disease
	Deep vein thrombosis	Pulmonary venous thrombosis
	Embolism venous	Subclavian vein occlusion
	Iliac vein occlusion	Subclavian vein thrombosis
	Obstructive shock	Superior vena cava occlusion
	Pelvic venous thrombosis	Thrombosis
	Peripheral vein occlusion	Vena cava embolism
	Peripheral vein thrombus extension	Vena cava thrombosis
	Phlebectomy	Venous occlusion
	Pulmonary embolism	Venous thrombosis
	Pulmonary microemboli	Venous thrombosis limb

APPENDIX 3. ANALYTICAL PLAN FOR QUANTIFICATION OF CD8+ TIL AND FOXP3+ CELLS

Assay Name	Tissue Type (skin/tumor/...)	Test SOP	Analysis SOP	Analysis Form	Remarks
PD-L1 SP142 IHC	Tumor	TE-IHC-198	TE-IQ-16	FO-390	
PanCK/CD 8 IHC	Tumor	TE-IHC-153	TE-IQ-36 TE-IQ-40	FO-525 FO-526	
CD8/FoxP3 /GZMB	Tumor	TE-IHC-246	TE-IQ-137	FO-760	
FoxP3	Tumor	TE-IHC-324	TE-IQ-62	FO-729	

PD-L1 SP142 will be scored according to SP142 algorithm (TE-IQ-16). The fraction of viable tumor cells (%) that express PD-L1 (discernible membrane staining of any intensity) can be scored. Cytoplasmic staining is not included in the scoring. In addition, immune cells are scored as a relative area estimate, i.e. the percentage of the tumor area that is covered by PD-L1-positive immune cells.

The PanCK-CD8 IHC slides will be evaluated by a pathologist for CD8 using the density proportion score algorithm (TE-IQ-40). The output of this analysis is the relative surface area (%) of the tumor with a CD8-positive immune cell density that belongs to one of the 8 density bins, and for each of the 8 density bins (8 data fields per IHC slide). The density bins are linked to reference images that are used by the pathologist. The reference images are marker-specific (CD8). There are 4 density bins (0 – 3) for intra-stromal CD8-positive immune cells and 4 density bins (0- 3) for intra-epithelial CD8-positive immune cells. In addition, the immune phenotype will be scored as 'desert', 'inflamed', 'excluded' in the PanCK-CD8 IHC slides based on the raw density proportion scores.

For the automated quantitative analysis (TE-IQ-36), a central tumor region is delineated by a pathologist. If an interface is present with adjacent normal tissue, an a 1000-µm wide invasive margin will be drawn. Visiopharm software is used to generate an epithelial carcinoma mask denoting the tumor region. The relative surface area (%) as well as density (#/mm²) of CD8-positive TILs can then be measured in both stromal and epithelial (carcinoma cell nests) compartments . If possible, these analyses will be performed for both the invasive margin and in the center of tumor samples. A 'tumor-stroma ratio' between the PanCK-positive carcinoma and the PanCK-negative stromal areas will be calculated, and areas will be provided for each region of interest.

CD8/GZMB/FoxP3 IHC will be scored according to TE-IQ-137. An assay to measure relative marker area and densities of CD8, FOXP3 and GZMB in solid tumor samples, within two regions of interest (ROIs): Central Tumor (CnTumor) and Invasive Margin (IM). The tumor region is delineated by a pathologist on the image of a serial HE-stained slide. The tumor annotation is copied manually onto the CD8-GZMB-FOXP3 image. If an interface is present with adjacent normal tissue, a 1000-µm wide invasive margin will be drawn. Single CD8-positive, single GZMB-positive, single FOXP3-positive, double CD8-GZMB-positive and double CD8-FOXP3-positive cells are automatically detected in the annotated regions through image analysis. The relative surface area (%) and density (#/mm²) of these different cell types can then be quantified as well as the proportion in relation to the CD8 population.

FoxP3-stained slides will be analyzed by the TE-IQ-62 method. A central tumor region is delineated by a pathologist. If an interface is present with adjacent normal tissue, an a 1000-µm wide invasive margin will be drawn. The relative area and the density of FoxP3-positive cells in central tumor and, if present, the invasive margin are quantified.