CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214200Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Integrated Review

NDA	214200
Link to EDR	\\CDSESUB1\evsprod\NDA214200\214200.enx
Submission Date	06/15/2020
PDUFA Date	02/15/2021 (Priority)
Submission Type	Original NME NDA
Generic Name	Trilaciclib
Dosage and Administration	240 mg/m^2 as a 30-minute intravenous infusion no more than 4 hours prior to chemotherapy on each day chemotherapy is administered
Dosage Form and Strength	For injection: 300 mg of trilaciclib as a sterile, preservative free, yellow, lyophilized cake in a single-dose vial for reconstitution and further dilution
Route of Administration	Intravenous (IV) infusion
Proposed Indication	(b) (4) chemotherapy-induced myelosuppression in adult patients with small cell lung cancer
Applicant	G1 Therapeutics, Inc.
Associated IND	119254
OCP Review Team	Xiaolei Pan, Sudharshan Hariharan (TL) Pharmacometrics: Eliford Kitabi, Justin C Earp (TL) QT IRT: Nan Zheng (TL)
OCP Final Signatory	Doanh Tran Deputy Director Division of Cardiometabolic and Endocrine Pharmacology (DCEP)

Contents

1. EXECUTIVE SUMMARY

This clinical pharmacology review is for an original new molecular entity (NME) 505(b)(1) NDA submitted by G1 Therapeutics on June 15, 2020. The applicant is seeking approval of trilaciclib for (b) (4) of chemotherapy-induced myelosuppression in adult patients with small cell lung cancer (SCLC). Trilaciclib is a selective, reversible, cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor that was designed to transiently maintain G1 cell cycle arrest of hematopoietic stem and progenitor cells (HSPCs) in the bone marrow, thus protecting the cells from damage by cytotoxic chemotherapy (myelopreservation). Due to its mechanism of action (MOA), trilaciclib is proposed to be administered prior to chemotherapy to ensure G1 arrest of HSPCs when those cells are exposed to cytotoxic chemotherapy. The proposed dosing regimen is 240 mg/m^2 trilaciclib as a 30-minute intravenous infusion no more than 4 hours prior to chemotherapy on each day chemotherapy is administered.

The clinical development program includes 7 Phase 1 clinical pharmacology studies in healthy subjects (single dose PK/PD evaluation, mass balance study, QT study, a bridging study in Japanese subjects, drug interaction studies) and 3 Phase 1b/2 trials in patients with SCLC. The submission also includes 2 reports for the development of population pharmacokinetic (PopPK) models for trilaciclib and topotecan, and 1 report for the development of exposure-response/safety relationship for trilaciclib. The applicant is seeking approval of trilaciclib relying on the efficacy and safety findings from three trials in patients with SCLC.

Key issues discussed in this review are:

1) (a) uncertainty with the primary metabolic pathway of trilaciclib; (b) uncertainty with the estimated plasma protein binding of trilaciclib; and (c) in vitro studies that are required to assess the drug interaction liability of the major inactive metabolite, M8.
2) Whether the proposed dosing regimen and instructions pertaining to time of administration relative to the chemotherapy agent, missing dose, and drug discontinuation are acceptable.
3) Dosing recommendations for concomitant use with renal drug transporter substrate(s).

The issue regarding QT prolongation risk of trilaciclib due to elevated exposure under different intrinsic or extrinsic factors is discussed separately in QT-IRT review (by Dr. Nan Zheng, 12/22/2020).

1.1 Recommendations

The Office of Clinical Pharmacology (OCP/DCEP) has determined that there is sufficient clinical pharmacology and biopharmaceutics information provided in the NDA to support an approval.

Decision	**Acceptable to OCP?**	**Comments**
Overall	☑ Yes ☐ No ☐ NA	Acceptable
General dosing instructions	☑ Yes ☐ No ☐ NA	240 mg/m^2 as a 30-minute intravenous infusion no more than 4 hours prior to

		chemotherapy on each day chemotherapy is administered
Dosing in patient subgroups (intrinsic and extrinsic factors)	☑ Yes ☐ No ☐ NA	1. Since body surface area (BSA) is determined to be a significant covariate for trilaciclib exposure, a BSA based dose is proposed for trilaciclib. 2. Hepatic impairment: A dedicated hepatic impairment study is ongoing. However, considering hepatic metabolism of trilaciclib, its use is not recommended in patients with moderate or severe hepatic impairment. 3. Drug interaction: Trilaciclib is an inhibitor of OCT2, MATE1 and MATE-2K. Co-administration of trilaciclib may increase the plasma concentration or net accumulation of OCT2, MATE1, and MATE-2K substrates in kidney (e.g., dofetilide, dalfampridine, and cisplatin). We recommend referring to the prescribing information for these concomitant medications for assessing the benefit and risk of concomitant use of trilaciclib.
Labeling	☑ Yes ☐ No ☐ NA	Acceptable

1.2 Post-Marketing Requirements and Commitments

Post-Marketing Requirements (PMR)

- PMR 1: Conduct an *in vitro* Drug-Drug Interaction (DDI) study to evaluate the major circulating metabolite of trilaciclib, M8, as an inhibitor for major CYP enzymes and drug transporters.
- PMR 2: Conduct a clinical trial to evaluate the effects of hepatic impairment on the pharmacokinetics and safety of trilaciclib.
- PMR 3: Conduct an in vitro metabolism study and CYP phenotyping study at clinically relevant concentrations to determine major metabolic pathway for trilaciclib. Characterize the formation of the major circulating metabolite of trilaciclib, M8, using the purified M8 compound with a validated bioanalytical method.

Post-Marketing Commitment (PMC)

- PMC 1: Conduct a plasma protein binding assay study for trilaciclib with appropriate validation of plasma stability, time to equilibrium and nonspecific binding at clinically relevant concentrations.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Summary of Clinical Pharmacology Findings

In the current submission, the applicant has submitted 11 clinical pharmacology studies (7 in healthy subjects and 4 in patients), and 13 in vitro studies. The submitted studies include 2 PK studies (1 single dose PK/PD study in healthy volunteers, 1 mass balance study in healthy volunteers), 1 intrinsic factor study evaluating the impact of race (Japanese subjects), 3 extrinsic factor studies evaluating drug interactions with rifampin, midazolam, metformin and itraconazole, 1 thorough QT study, and 3 efficacy studies. Population pharmacokinetic analysis was performed to evaluate the effect of body weight and sex on trilaciclib pharmacokinetics. The hepatic impairment study is on-going. Summarized below are the key clinical pharmacology findings from the submitted studies:

Absorption

- Not applicable since the intended route of trilaciclib administration is IV infusion.

Distribution

- Trilaciclib is extensively distributed into tissues (steady-state volume of distribution 1130 L) following intravenous administration.
- Plasma protein binding (PPB): protein binding of trilaciclib has not been fully characterized in vitro (refer to Appendix 4.5.1 for detailed discussion).
- In vitro partitioning of trilaciclib into human red blood cells was moderate (blood/plasma ratios 1.21 to 1.53) between 0.5 µg/mL to 50 µg/mL.

Elimination

- After C_{max} is reached by the end of IV infusion, the decline in plasma trilaciclib concentrations is multiphasic, with rapid decline in the initial 1 hour, and a mean terminal half-life ($t_{1/2}$) of approximately 14 hours. Clearance was estimated to be 158 L/hr.
- It is known from the mass balance study and clinical pharmacokinetic data that trilaciclib is primarily metabolized in the liver. The in vitro metabolism studies in hepatocytes and microsomes yielded discrepant results. Furthermore, the primary enzyme involved in the metabolic pathway of trilaciclib is unclear. Please refer to Appendix 4.5.2 for detailed discussion on these issues.
- Trilaciclib is the predominant circulating compound in plasma, representing ~50% of plasma total radiocarbon, and M8 represents ~25% of plasma total radiocarbon. Other circulating metabolites were less than ~5% of the plasma total radiocarbon.

  Trilaciclib is eliminated mainly via the fecal route, with a small contribution of the renal route. In healthy subjects, following a single IV infusion, 93.1% of the total administered

radioactive dose was recovered in 11 days (but >80% was recovered by 96 h); feces was the major route of excretion (79.1%), with 14% of the dose recovered in urine. Unchanged drug in feces and urine accounted for ~7% and ~2% of total radioactivity administered, respectively. In feces, M8 was the major drug-related material accounting for over 56% of the administered dose. M8 is not pharmacologically active.

***Intrinsic Factors*:**

- Age and sex: Clearance and volume of distribution of trilaciclib is not influenced by age and sex.
- Body surface area (BSA): The AUC in subjects with typical BSA values of 1.5 and 2.3 m^2 were 12.5% lower and 12% higher, respectively, relative to a subject with a BSA of 1.9 m^2.
- Hepatic impairment: Based on a population PK analysis, mild hepatic impairment had no effect on the exposure of trilaciclib. The PK of trilaciclib has not been studied in patients with moderate or severe hepatic impairment.
- Renal impairment: Based on a population PK analysis, mild and moderate renal impairment had no effect on the exposure of trilaciclib. The pharmacokinetics of trilaciclib has not been studied in patients with severe renal impairment, end stage renal disease or on dialysis.

***Drug Interactions*:**

- Strong CYP3A inhibitors (e.g., itraconazole): Two drug interaction studies in healthy subjects were conducted with itraconazole. Compared to trilaciclib alone, itraconazole (200 mg a day for 6 days) increased C_{max} of trilaciclib by 9.1% in one study and decreased C_{max} of trilaciclib by 28.9% in another study. AUC_{inf} did not change in one study and decreased by 14% in the other. The mechanism behind the decrease in trilaciclib exposure by itraconazole in one of the studies is not understood, although the extent of change in only modest.
- Strong CYP3A inducers (e.g., rifampin): Compared to trilaciclib alone, rifampin (600 mg daily for 10 days) decreased trilaciclib AUC_{inf} and C_{max} by approximately 17% and 20%, respectively.
- CYP3A substrates (e.g., midazolam): Compared to midazolam alone, multiple doses of trilaciclib (240 mg/m^2 once daily for 6 days) increased midazolam AUC_{inf} by approximately 7% and decreased C_{max} by approximately 17%.
- OCT2, Multidrug and Toxin Extrusion 1 (MATE1), and MATE-2K Substrates:
  - Compared to metformin alone, multiple doses of trilaciclib (240 mg/m^2 once daily for 6 days) increased metformin AUC_{inf} and C_{max} by approximately 65% and 81%, respectively. Renal clearance of metformin was decreased by 37%.
  - Topotecan: Data from 1 clinical trial in patients with SCLC and population PK analysis indicated no drug interaction between trilaciclib and topotecan (a MATE1 and MATE-2K substrate) following the administration of topotecan immediately after the administration of trilaciclib.
- Etoposide: Data from 2 clinical trials in patients with SCLC indicated no drug interaction between trilaciclib and etoposide following the administration of etoposide immediately after the administration of trilaciclib.

- Carboplatin: Data from 3 clinical trials in patients with SCLC indicated no drug interaction between trilaciclib and carboplatin following the administration of carboplatin immediately after the administration of trilaciclib.

In Vitro Studies:

- CYP metabolic Pathways: In vitro, trilaciclib is a time-dependent inhibitor of CYP3A4 at clinically relevant concentrations. In vitro evaluations indicated that trilaciclib has no potential to inhibit the activities of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 at clinically relevant concentrations. Trilaciclib is an inducer for CYP1A2, and is not an inducer for CYP2B6, and CYP3A4.
- Transporter Systems: Trilaciclib inhibits the renal transporters OCT2, MATE1, and MATE-2K at concentrations achievable at the recommended dose of 240 mg/m^2. At clinically relevant concentrations, trilaciclib does not inhibit the efflux transporters P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP); the hepatic uptake transporters organic anion transporting polypeptide 1B1 (OATP1B1), OATP1B3 or the renal uptake transporters OAT1 and OAT3. Trilaciclib is a substrate of human efflux transporters BCRP and P-gp but not a substrate of bile salt export pump (BSEP), multidrug and toxin extrusion (MATE)1, MATE2-K, or OCT2.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The recommended dose of trilaciclib is 240 mg/m^2 per dose. Administer as a 30-minute intravenous infusion within 4 hours prior to the start of chemotherapy on each day chemotherapy is administered.

Missed Treatment Session(s)

If the trilaciclib dose is missed, discontinue chemotherapy on the day the trilaciclib dose was missed. Consider resuming both trilaciclib and chemotherapy on the next scheduled day for chemotherapy.

Discontinuation of Treatment

If trilaciclib is discontinued, wait 96 hours from the last dose of trilaciclib before resumption of chemotherapy only.

2.2.2 Therapeutic individualization

BSA:

- BSA was determined to be a significant covariate for trilaciclib exposure; thus, a BSA based dose is proposed for trilaciclib.

Hepatic Impairment:

- No dosage adjustment is recommended for patients with mild hepatic impairment (total bilirubin ≤ upper limit of normal [ULN] and aspartate aminotransferase [AST] > ULN, or total bilirubin >1.0 to 1.5 × ULN and any AST) based on population PK analysis.

- The pharmacokinetics of trilaciclib has not been studied in patients with moderate or severe hepatic impairment. Considering hepatic metabolism of trilaciclib, its use is not recommended in patients with moderate or severe hepatic impairment.

Extrinsic factors:

- Trilaciclib is an inhibitor for OCT2, MATE1 and MATE-2K. Co-administration of trilaciclib may increase the plasma concentration or net accumulation of OCT2, MATE1, and MATE-2K substrates in kidney (e.g., dofetilide, dalfampridine, and cisplatin).

2.3 Outstanding Issues

- Since the metabolic pathway for trilaciclib has not been fully characterized, the DDI liability of trilaciclib as a victim drug when coadministered with CYP enzyme inhibitors or inducers, and the DDI liability of M8 as an inhibitor or inducer for major enzymes and/or transporters are not clear and 2 PMRs are being issued to address these issues.
- A hepatic impairment study in on-going. However, to ensure timely completion of the study and submission of report, a PMR is issued.
- Characterization of plasma protein binding of trilaciclib with appropriate validation methods is being requested in a PMC.

2.4 Summary of Labeling Recommendations

Based on the review, the review team have addressed the following issues in the package insert:

- *Section 2 Dosing and Administration*: Revised labeling language to ensure clarity in instructions related to missing dose(s) and drug discontinuation.
- *Section 7 Drug Interactions*: Recommendation to include cisplatin (and other important substrates) in Section 7 Drug interactions of the label, due to concern of OCT2, MATE1 and MATE2K-mediated inhibition by trilaciclib.
- *Section 8 Use in Specific Populations*: Recommendation to not use trilaciclib in patients with moderate and severe hepatic impairment.
- *Section 12 Clinical Pharmacology*: Recommendation to remove (b) (4) .
- *Section 12 Clinical Pharmacology*: Recommendation to remove statement about (b) (4) .

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Trilaciclib is a cyclin-dependent kinase (CDK) 4 and 6 inhibitor indicated (b) (4) chemotherapy-induced myelosuppression (CIM) in adult patients (b) (4) a platinum/etoposide-containing regimen or topotecan-containing regimen for SCLC. The development of trilaciclib was under IND 119254. The development of trilaciclib was granted breakthrough therapy designation in August 2019. The applicant filed this NDA via the 505(b)(1) regulatory pathway on June 15, 2020. The evidence in support of trilaciclib's

effectiveness for CIM is derived primarily from two Phase 1b/2a and one Phase 2 trials in patients with SCLC. The hepatic impairment study of trilaciclib is currently on-going.

3.2 General Pharmacological and Pharmacokinetic Characteristics

<table>
<tr><th colspan="2">Pharmacology</th></tr>
<tr><td>Mechanism of Action</td><td>Trilaciclib is a selective, reversible, CDK 4 and 6 inhibitor. Both hematopoietic stem and progenitor cells (HSPC) and lymphocyte populations are dependent on CDK4/6 activity for proliferation. Proliferating HSPCs are susceptible to chemotherapy damage resulting in myelosuppression.

Due to inhibition of CDK4/6, HSPC and lymphocyte population become arrested in the G1 phase of the cell cycle upon exposure to trilaciclib. Administration of trilaciclib as a myelopreservation therapy prior to myelosuppressive chemotherapy prevented chemotherapy-induced damage of the HSPCs and lymphocyte populations, resulting in faster recovery of complete blood cell counts, ability to receive more cumulative chemotherapy, and improved long-term bone marrow function.</td></tr>
<tr><td>QT Prolongation</td><td>A dose- and concentration-dependent increase in QTc was observed in the QT assessment of IV trilaciclib. There was, however, a time-delay between mean peak trilaciclib concentration and mean maximum increase in QTc. The mechanism for this delay is not known. At the therapeutic dose, no clinically meaningful increases in QTc interval was detected. Please refer to QT-IRT review by Dr. Nan Zheng for details.</td></tr>
<tr><th colspan="2">General Information</th></tr>
<tr><td>Bioanalysis</td><td>Three validated LC-MS/MS methods were developed to measure the concentrations of trilaciclib in serum and urine samples collected from both patients and healthy subjects in the clinical studies included in this NDA.</td></tr>
<tr><td>Healthy Volunteers vs. Patients</td><td>The exposure (C_{max} and AUC_{∞}) of trilaciclib in healthy subjects at 192 mg/m^2 were comparable to the exposure at 240 mg/m^2 in SCLC patients.</td></tr>
<tr><td>Drug Exposure at Steady-state Following the Therapeutic Dosing Regimen</td><td>The accumulation following repeat doses of trilaciclib is expected to be minimal.
<table>
<tr><th></th><th>C_{max} (ng/mL)
mean (CV%)</th><th>$AUC_{0\text{-}last}$ (ng*h/mL)
mean (CV%)</th></tr>
<tr><td>Single Dose</td><td>1270 (132)</td><td>2730 (45.3)</td></tr>
</table></td></tr>
</table>

	(240 mg/m^2)		
	Multiple Doses (240 mg/m^2)	1220 (120)	2890 (40)
Maximum Tolerated Dose or Exposure	Single Dose	700 mg/m^2 trilaciclib was the highest dose tested; and maximum tolerated dose (MTD) was not achieved.	
	Multiple Dose	280 mg/m^2 once daily dosing of trilaciclib was the highest dose tested; and MTD was not achieved.	
Dose Proportionality	Approximately dose proportional for C_{max}, and slightly greater than dose proportional for AUC_{0-24} and AUC_{0-last} between 6 to 700 mg/m^2		
Variability	The inter-subject variability of trilaciclib, across studies, ranges from 26 to 53% for C_{max} and 13 to 26% for AUC_{last} for healthy subjects, and ranges from 90%% to 287% for C_{max} and 23.7% to 75% for AUC_{last} in patients with SCLC at doses near to or equivalent to the recommended therapeutic dose of 240 mg/m^2.		
Accumulation	There was no accumulation of trilaciclib in plasma following repeated administration (once daily).		
Absorption			
T_{max}	30 minutes after IV infusion (end of infusion)		
Absolute Bioavailability	Not applicable		
Distribution			
V_d	1130 L following IV administration		
Protein Binding	The plasma protein binding of trilaciclib and M8 has not been fully characterized in vitro.		
Elimination			
$T_{1/2}$	The decline of trilaciclib concentrations in plasma is multiphasic, with rapid decline in the initial 1 hour, and the mean terminal $t_{1/2}$ is approximately 14 hours.		
Metabolism			

Primary Metabolizing enzymes	The primary metabolizing enzymes for trilaciclib has not been fully characterized in vitro.
Excretion	
Primary Excretion Pathways	Trilaciclib is primarily eliminated by hepatic metabolism followed by excretion of numerous metabolites in feces and urine.
In vitro DDI	
Transporter Systems	• Substrates: trilaciclib is a substrate of human efflux transporter BCRP and P-gp. Trilaciclib is not a substrate for BSEP, MATE1, MATE2/K, OCT2, OATP1B1 and OATP1B3 • Inhibitors: trilaciclib is an inhibitor of OCT2, MATE1 and MATE2/K. Trilaciclib does not inhibit P-gp, BCRP, OATP1B1, OATP1B3, OAT1 or OAT3
Enzymes	• Inhibitors: trilaciclib is a time-dependent inhibitor of CYP3A4. Trilaciclib does not have potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D5. • Inducer: Trilaciclib is an inducer of CYP1A2, but considering the intermittent dosing schedule of trilaciclib, the effects may not be clinically significant. Trilaciclib is not an inducer of CYP2B6 and CYP3A4.

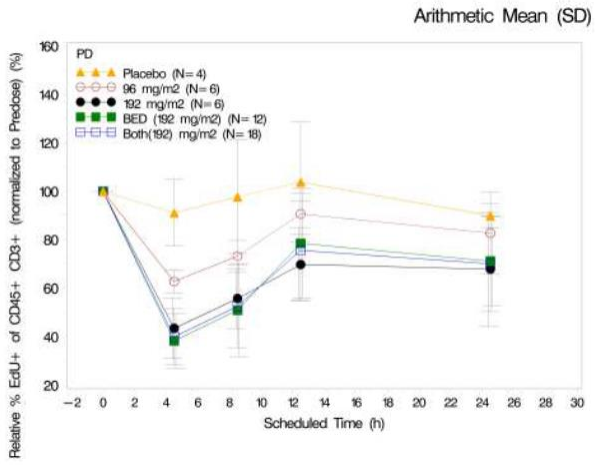
3.3 Clinical Pharmacology Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

Anti-Proliferative Activity on Lymphocytes and Bone Marrow

The anti-proliferative activity of trilaciclib was assessed in healthy subjects following a single infusion of trilaciclib (G1T28-1-01). A single IV infusion of trilaciclib resulted in a dose-dependent decrease in the percentage of EdU+ cells within the T-lymphocyte population, reflecting an inhibition of the proliferation of T-lymphocytes (Figure 1). A maximum mean inhibition of T-lymphocyte proliferation of 37.2% (at a dose of 96 mg/m^2) and 60% (at dose of 192 mg/m^2) was observed at 4 h after the end of infusion. Although T-lymphocyte proliferation started to recover from then on, inhibition of proliferation persisted until the last sampling time point at 24 hours after end of infusion.

Figure 1. Relative Percent EdU+ Cells of the CD45+/CD3+ Lymphocyte Population– Ex-vivo Blood Stimulation Data

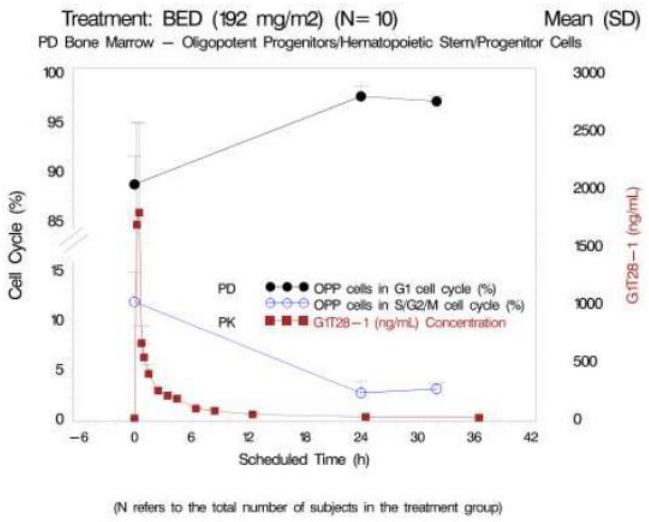


Source: Study G1T28-1-01 report, page 84, figure 9

In the bone marrow of subjects administered 192 mg/m^2 trilaciclib, an increase in the percentage of cells in the G1 cell cycle phase was observed up to the last sampling time point i.e., 32 hours post-dose, for all bone marrow progenitor subsets evaluated (HSC/MPP, OPP, monocyte lineage, granulocyte lineage, erythroid lineage, and megakaryocyte lineage, Figure 2).

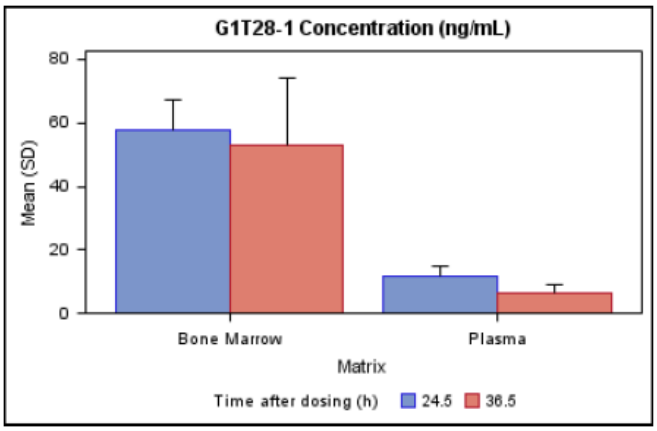
A subset of the bone marrow aspirate samples obtained from 5 subjects on Day 2 in the 192 mg/m^2 cohort was used for an explorative comparison between the trilaciclib concentration in bone marrow and blood plasma. The results showed that the concentration of trilaciclib on Day 2 appeared to be higher in bone marrow than in blood plasma (Figure 3). These results suggest that trilaciclib accumulated in bone marrow and half-life of trilaciclib may be longer in bone marrow than in plasma.

Figure 2. Percent HSPC/OPP Cell Populations in G1 or S/G2/M Cell Cycle Phase Including Mean PK Concentration Profile



Source: Study G1T28-1-01 report, page 98, figure 20

Figure 3. Concentration of Trilaciclib in Bone Marrow versus Blood Plasma after Intravenous Administration of 192 mg/m^2 Trilaciclib (N=5)



Source: Study G1T28-1-01 report, page 81, figure 6

Efficacy data supporting myelopreservation effects of trilaciclib

The evidence in support of trilaciclib effectiveness for CIM is derived primarily from three successful randomized, double-blind, placebo-controlled trials (studies 02, 03 and 05) in patients

with SCLC. The primary myelopreservation efficacy endpoint was the duration of severe (Grade 4) neutropenia (DSN) in Cycle 1 and occurrence of severe (Grade 4) neutropenia (SN). The administration of trilaciclib prior to SOC chemotherapy to patients with 1L and 2/3L SCLC in these trials consistently resulted in clinically meaningful and statistically significant reductions in CIM as measured by DSN in Cycle 1 and the occurrence of SN. The comparison of primary myelosuppression endpoints across studies is shown in Table 1. Please refer to clinical and statistical reviews by Drs. Andrew Dmytrijuk and Kate Dwyer, respectively, for more information.

Table 1. Comparison of primary myelosuppression endpoints across SCLC studies

Endpoint	Study G1T28-02 Part 2		Study G1T28-05		Study G1T28-03 Part 2	
	Plac + E/P (N=38)	Trila + E/P (N=39)	Plac + E/P/A (N=53)	Trila + E/P/A (N=54)	Plac + Topotecan 1.5 mg/m^2 (N=29)	Trila + Topotecan 1.5 mg/m^2 (N=32)
DSN in Cycle 1 (days), Strategy 1						
Mean (SD)	3 (3.9)	0 (0.5)	4 (4.7)	0 (1.0)	7 (6.2)	2 (3.9)
Difference (95% CI)	-2.5 (-3.8, -1.2)		-3.6 (-4.9, -2.3)		-5.5 (-8.2, -2.9)	
Adjusted 1-sided p-value[a]	—		<0.0001		<0.0001	
Raw 2-sided p-value[b]	<0.0001		—		—	
Occurrence of SN						
n (%)	16 (42.1)	2 (5.1)	26 (49.1)	1 (1.9)	22 (75.9)	13 (40.6)
aRR (95% CI)	0.127 (0.030, 0.534)		0.038 (0.008, 0.195)		0.536 (0.303, 0.948)	
Adjusted 1-sided p-value[a]	—		<0.0001		0.0160	
Raw 2-sided p-value[c]	0.0049		—		—	

aRR=adjusted rate ratio; CI=confidence interval; CSR=clinical study report; DCO=data cut-off; DSN=duration of severe (Grade 4) neutropenia; E/P=etoposide and carboplatin; E/P/A=etoposide, carboplatin, and atezolizumab; ISE=integrated summary of efficacy; ITT=intent-to-treat; plac=placebo; SAP=statistical analysis plan; SCLC=small cell lung cancer; SD=standard deviation; SN=severe (Grade 4) neutropenia; trila=trilaciclib.

a The p-value was generated from a Hochberg-based gatekeeping procedure that was utilized to control the global familywise error rate across the primary and key secondary endpoints in the strong sense at a 1-sided 0.025 level for Study G1T28-05 and 0.10 level for Study G1T28-03.

b The p-value was calculated using a nonparametric analysis of covariance.

c The p-value was calculated using a modified Poisson regression.

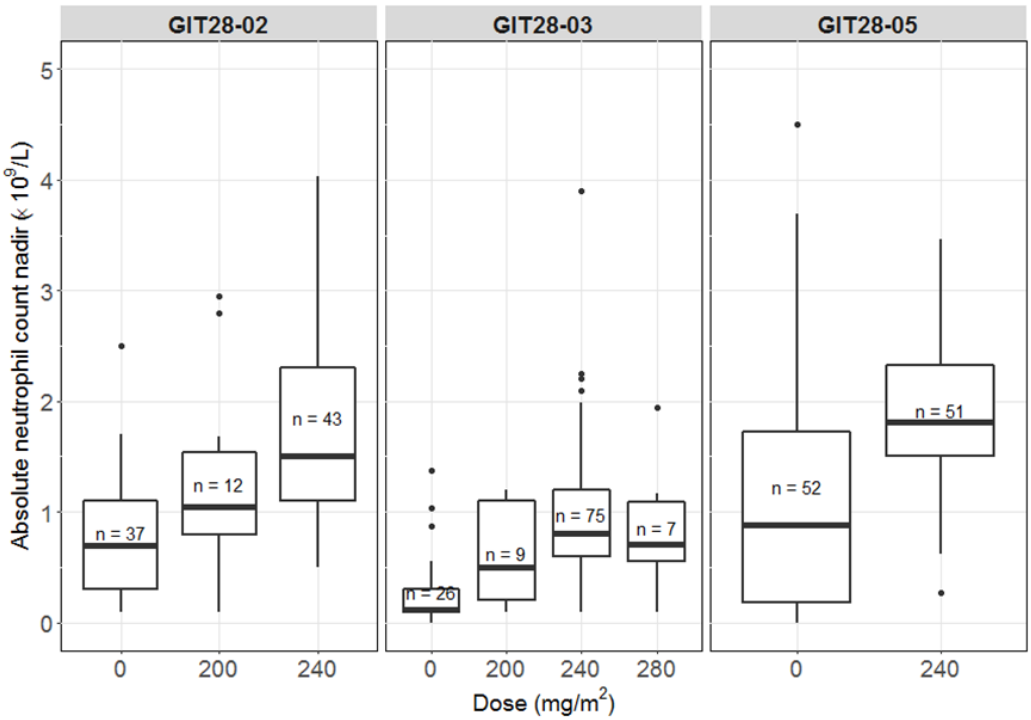
Source: Applicant's clinical efficacy summary (page 50, table 10)

3.3.2 Is the proposed general dosing regimen appropriate?

Yes, the proposed dosing regimen is appropriate. The review team performed graphical exploration of the relationship between trilaciclib dose/AUC and ANC nadir as shown in Figure 4. In general, the results indicate that ANC nadir increases with trilaciclib dose but seems to

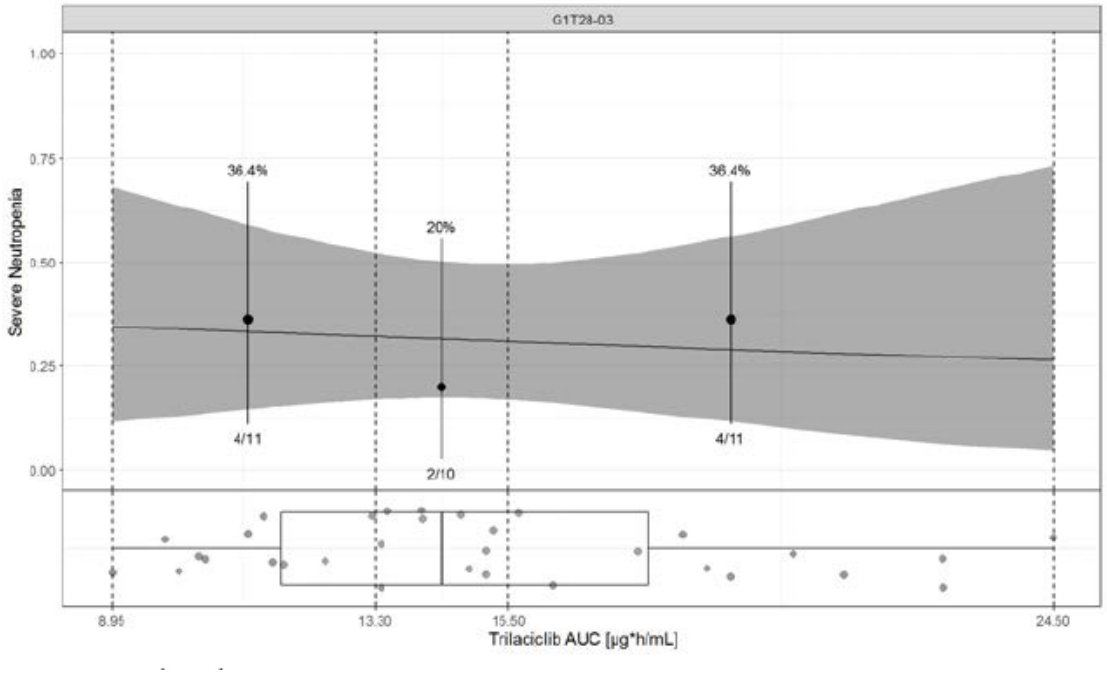
plateau at 240mg/m^2. The applicant performed several exposure-response analyses, including: AUC vs ANC nadir, AUC vs Area Under Effect Curve (AUEC), and AUC vs severe neutropenia. The results from the applicant's analyses indicates that at the dose of 240mg/m^2 there is no statistical or clinically meaningful relationships between AUC and the efficacy endpoints (see Figure 5). These results indicate that at the dose of 240mg/m^2, the individual AUCs are likely at the plateau of the dose response relationships.

Figure 4. Relationship between dose of trilaciclib and ANC nadir



Source: Pharmacometrics review report

Figure 5. Relationship between AUC of trilaciclib (at dosage of 240 mg/m^2) and probability of severe neutropenia



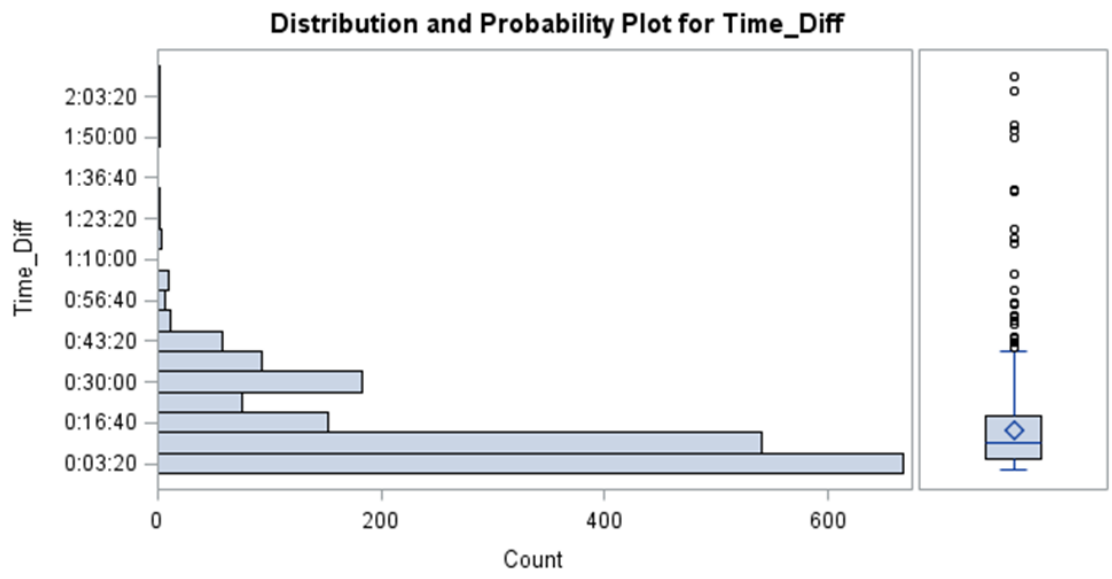
Source: Applicant's population PK and ER report (page 47)

Time window of dosing trilaciclib relative to chemotherapy

The applicant proposed that trilaciclib should be administered as 30-min IV infusion no more than 4 hours prior to chemotherapy on each day chemotherapy is administered. The review team assessed whether the proposed instructions are appropriate or whether spacing in between the time of administration of trilaciclib and chemotherapy agents is required. As some of these agents are substrate of OCT-2, MATE1, or MATE2/K, separating the time of administration may alleviate any potential drug interaction between the two.

An information request was sent to the applicant requesting the dataset for relative timing of administration of trilaciclib and each chemotherapy agent from all SCLC trials. The analysis showed that in approximately 65% of the patients, trilaciclib was administered almost within 15 min prior to the administration of the chemotherapy agent; and more than 90% of the patients received trilaciclib within 1 h prior to the administration of the chemotherapy agents. The data for the relative timing of administration from Study 03 (patients on topotecan) is shown in Figure 6. Similar trend was observed in Studies 02 and 05 (patients on etoposide and carboplatin; data not shown). These data show that trilaciclib was administered very close in time to the administration of chemotherapy agent and allows for evaluation of drug interaction from the clinical trial.

Figure 6. Distribution of dosing intervals between trilaciclib and topotecan (Study 03)



Source: Reviewer's analysis

The appropriateness of relative time of administration of trilaciclib and each chemotherapy agent is discussed separately below:

Topotecan

Topotecan is a substrate for MATE1 and MATE2/K. In Study 03, noncompartmental analysis demonstrated that total topotecan C_{max} and AUC_{0-4h}, and AUC_{0-last} were similar in the presence or absence of trilaciclib (Table 2). PopPK analysis of topotecan exposures also confirmed that trilaciclib administered prior to topotecan had no effect on topotecan PK. These results indicate that trilaciclib did not affect the PK of topotecan and the proposed instructions for relative time of administration is appropriate when dosed with topotecan.

Table 2. PK parameters for topotecan with/without trilaciclib

Dose (mg/m^2)	**C_{max} (ng/mL)**	**t_{max} (h)**	**AUC_{0-4} (ng•h/mL)**	**AUC_{0-last} (ng•h/mL)**
1.5 topotecan + 240 trilaciclib (n=27 or 29)	41.4 (46.9)	0.50 (0.25-3.47)	94.4 (43.9)	100 (48.1)
1.5 topotecan + placebo (n=24 or 25)	43.0 (39.0)	0.45 (0.00-0.67)	94.0 (28.0)	106 (27.3)

Values shown as mean (CV%); t_{max} as median (range)

Source: Applicant's clinical pharmacology summary

Etoposide and carboplatin

Etoposide and carboplatin are not substrates for OCTs or MATEs, and thus there is no potential of OCT- and MATE-mediated DDIs between trilaciclib and etoposide/carboplatin.

Further, noncompartmental analysis from the clinical trials 02 and 05 also confirmed that drug interactions between trilaciclib and etoposide or carboplatin was not observed. Therefore, the proposed instruction for relative time of administration with etoposide or carboplatin is also appropriate.

Cisplatin

Although the current clinical development program did not enroll patients with SCLC treated with cisplatin, it may potentially be used with trilaciclib. Cisplatin is a substrate for OCT2 and MATEs. Cisplatin product insert carries a box warning for nephrotoxicity which is dose-related and cumulative. Since trilaciclib is an inhibitor for OCT2, MATE1 and MATE2/K, there is potential that trilaciclib may change the net accumulation of cisplatin in renal proximal tubular cells and exacerbate renal toxicity.

Two approaches were considered for the management of the potential DDI risk: 1) (b) (4) or 2) recommend monitoring signs of nephrotoxicity in the 'Drug Interaction' section of the label. However, upon review, it was found that the first strategy would not be able to fully minimize the risk of drug interaction. Based on

the total plasma concentration of trilaciclib at 2-4 h post-dose (~ 0.5 µM) and the reported IC_{50} values of trilaciclib for OCT2 (IC_{50} = 0.152 µM), MATE1 (IC_{50} = 0.175 µM) and MATE2/K (IC_{50} = 0.071 µM), assuming the plasma protein binding of trilaciclib is ~72%, the $C_{u,max}/IC_{50}$ is still greater than the FDA recommended cut-off of 0.1 for in vivo DDI assessment. In addition, the dose separation strategy may also be considered impractical and inconvenient for patients. Based on these considerations, the review team recommended describing the risk along with the need for close monitoring for nephrotoxicity in the 'Drug Interaction' section of the label.

While oxaliplatin is also a substrate for OCT2 and MATEs, it is not used for the treatment of patients with SCLC.

Overall, the data indicate that the proposed time-window of trilaciclib dosing (no more than 4 hours prior to chemotherapy) is appropriate for use with chemotherapy agents that were tested in clinical trials - topotecan, etoposide and carboplatin. For use with cisplatin, the risk and the need for monitoring nephrotoxicity is described in the label, as separating the time of administration between trilaciclib and cisplatin may not completely alleviate the risk.

Dosing instructions for missed dose and drug discontinuation

The applicant proposed the following instructions to handle missed dose(s) and drug discontinuation in Section 2.1 of the label as shown below:

(b) (4)

(b) (4)

An information request was sent to the applicant to provide justification for the proposed instructions. The applicant stated in their response that these instructions were consistent with the trial conduct and were drafted to optimize the benefit of trilaciclib based on its mechanism of action.

Trilaciclib benefit is tied to administration prior to the dose of chemotherapy, which aims to arrest and maintain HSPC while chemotherapy is present i.e., myelopreservation. Suboptimal HSPC (i.e., for too short a duration) has the theoretical potential to release HSPCs into the S (DNA synthesis) phase of the cell cycle while chemotherapy is present, thereby potentially exacerbating myelosuppression. As a result, it was instructed in the SCLC protocols that if trilaciclib cannot be administered on Day 2, Day 3, etc. (i.e., the subsequent day of dosing), chemotherapy should also not be administered on that day. Therefore, if a dose of trilaciclib is missed, it should be administered prior to chemotherapy at the next scheduled dose.

For drug discontinuation, when a day of dosing is missed and chemotherapy is to be continued in the absence of trilaciclib, chemotherapy should be held until the effects of trilaciclib have diminished sufficiently so as to allow HSPCs to resume proliferation in an unsynchronized manner, which the applicant proposed to be at least 96 hours from last dose of trilaciclib. As

observed in clinical studies, the terminal $t_{1/2}$ of trilaciclib is about 14 hours and PD effects of trilaciclib on HSPCs lasted at least 32 hours. Therefore, a time frame of 96-hours ($\approx$ 4 days) was chosen based on clinical considerations, which is greater than 5-times the terminal $t_{1/2}$ and the known duration of trilaciclib PD effects in the bone marrow. For 3-day sequential regimens (e.g., platinum/etoposide containing regimens), if a patient misses a day of therapy, a 96-hours delay in resumption of chemotherapy would be expected to result in resumption of chemotherapy with the start of the next cycle. For example, if the patient receives trilaciclib and chemotherapy on Cycle 2, Day 1, then misses dosing on Cycle 2, Day 2, chemotherapy can resume with the start of Cycle 3. For 5-day sequential regimens (e.g., topotecan-containing regimens), if a patient misses a day of therapy, a 96-hour delay in resumption of chemotherapy could allow the patient to receive at least one more dose of chemotherapy in that cycle depending on which day was missed. For example, if the patient receives trilaciclib and chemotherapy on Cycle 2, Day 1, then misses dosing on Cycle 2, Day 2, chemotherapy could resume on Cycle 2, Day 5.

In summary, the review team determined that the instructions for missing doses and drug discontinuation are reasonable and appropriate. However, the exact language was modified to improve the clarity of the instruction and the final version is shown below.

Missed Treatment Session(s)

If the trilaciclib dose is missed, discontinue chemotherapy on the day the trilaciclib dose was missed. Consider resuming both trilaciclib and chemotherapy on the next scheduled day for chemotherapy.

Discontinuation of Treatment

If trilaciclib is discontinued, wait 96 hours from the last dose of trilaciclib before resumption of chemotherapy only.

3.3.3 Is an alternative dosing regimen and management strategy required for subpopulations based on intrinsic factors?

The impact of intrinsic factors on trilaciclib PK was evaluated via popPK analysis, including a full analysis of covariates. In addition, a study in Japanese healthy subjects was conducted to evaluate the impact of race and a hepatic impairment study is ongoing. The effect of these intrinsic factors on trilaciclib is summarized in the following subsections.

Body Weight

The proposed dosing regimen is based on BSA. The applicant's analysis determined that subjects with high body surface areas would have higher clearance. Specifically, the analyses show that typical subject with BSA of 1.26 m^2 and 2.43m^2 (corresponding to minimum and maximum BSA in the studied patients) would have 16% lower (133.0 L/h) and 11% higher (175.0 L/h) clearance compared to subjects with BSA of 1.9m^2 (158.0 L/h).

Renal Impairment

Trilaciclib appears to be cleared predominantly via nonrenal route. In the human mass balance study (Study 08), less than 3% of the dose was excreted unchanged in the urine.

A dedicated renal impairment study was not conducted. However, renal function, as defined by renal impairment categories, were tested as covariates in the popPK analysis to evaluate the effect of renal function on trilaciclib PK. The popPK dataset included 45 patients with mild renal impairment and 16 patients with moderate renal impairment according to FDA classification, and analysis indicated that mild to moderate renal impairment had no effect on the exposure of trilaciclib. Dosage adjustment are not recommended for patients with mild or moderate renal impairment.

The PK of trilaciclib has not been studied in patients with severe renal impairment. Considering minimal involvement of renal elimination for trilaciclib, a significant effect of severe renal impairment on trilaciclib exposure is not expected.

Hepatic Impairment

Trilaciclib appears to be cleared predominantly via nonrenal route. In the mass-balance study (Study 08), unchanged trilaciclib accounted for approximately 50% of total plasma radioactivity in plasma, whereas the major metabolite M8 represented approximately 25% of total plasma radioactivity. Unchanged trilaciclib accounted for 6.7% of radioactivity in feces, while M8 was the major drug-related material and accounted for >56% of the administered dose in feces. According to the FDA guidance, when hepatic metabolism and/or excretion accounts for a substantial portion (>20 % of the absorbed drug), a dedicated PK study in patients with impaired hepatic function is required.

While a dedicated hepatic impairment study was not available at the time of NDA submission, the study has been initiated by the applicant and is on-going.

PopPK analysis included liver function tests and NCI hepatic impairment category as potential covariates to evaluate the effect of hepatic function on trilaciclib exposure. The PK population included 30 patients with mild hepatic impairment according to NCI classification (total bilirubin ≤ upper limit of normal [ULN] and aspartate aminotransferase [AST] > ULN, or total bilirubin >1.0 to 1.5 × ULN and any AST). The results show that mild hepatic impairment had no effect on the exposure of trilaciclib. Thus, no dosage adjustment is recommended for patients with mild hepatic impairment.

The PK of trilaciclib has not been studied in patients with moderate and severe hepatic impairment. However, as trilaciclib seems to be primarily hepatically cleared, dosing of trilaciclib in patients with moderate and severe hepatic impairment is not recommended, at least until the results of the hepatic impairment study is available.

Race

The pharmacokinetics of a single dose of trilaciclib were assessed in healthy adult Japanese (Study 17) subjects at three dose levels (100, 200 and 240 mg/m^2). Japanese and non-Japanese subjects in Cohort 3 were matched by sex and body weight (± 20%). The results showed that there was a slightly greater than proportional increase in trilaciclib PK in Japanese subjects across the 100 to 240 mg/m^2 dose range. The exposure in terms of AUC_{inf} and C_{max} was 1.3- and 2.4-fold higher in Japanese subjects compared to non-Japanese subjects for the 240 mg/m^2 dose (Table 3).

The reason for higher systemic exposure of trilaciclib in Japanese subjects is not clear. Under the assumption that trilaciclib is driving the observed QTc effect, the predicted upper 90% CI of maximum QTc increase is below 10 msec at the 240 mg/m^2 dose level in the highest exposure scenario known in Japanese subjects (geomean C_{max} 2210 ng/mL) (refer to QT-IRT review by Dr. Nan Zheng). No dose adjustment is warranted for Japanese subjects.

Table 3. Comparison of trilaciclib exposure in Japanese vs non-Japanese subjects

Comparison	Geometric Mean		Ratio (test to reference)	90% CI
	Test	Reference		
C_{max} (ng/mL)	2457	1020	2.4	[1.87, 3.10]
AUC_{0-t} (ng*h/mL)	3882	2929	1.3	[1.17, 1.51]
AUC_{inf} (ng*h/mL)	3922	2969	1.3	[1.16, 1.50]

Test: Japanese 240 mg/m^2 trilaciclib

Reference: non-Japanese 240 mg/m^2 trilaciclib

Source: Applicant's clinical study report G1T28-17

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Food

Not applicable since the drug is administered intravenously.

DDI liability from in vitro studies

The DDI liability of trilaciclib has been assessed in in vitro studies and the results are summarized below.

Enzyme systems

- Trilaciclib is a time-dependent inhibitor of CYP3A4 (IC_{50} = 36.4 μM). The impact of trilaciclib on CYP3A4 substrate has been evaluated in clinical DDI studies with midazolam in healthy subjects, showing no major impact on midazolam exposure.
- Trilaciclib is not an inhibitor of CYP1A2, 2B6, 2C8, 2C9, 2C19 or 2D6 at clinically relevant concentrations.
- Trilaciclib is an inducer of CYP1A2, which increased the mRNA expression level by 2.2- to 6.3-fold at 3 μM. The reported C_{max} at steady-state following 240 mg/m^2 dose ranged from 1.7 to 4.3 μM in clinical studies, which indicate a clinically relevant drug interaction potential when concomitantly used with CYP1A2 substrate(s). However, considering the short, intermittent dosing regimen (3- or 5-day treatment out of 21 days) of trilaciclib, the

impact of trilaciclib on CYP1A2 substrate is not expected to be clinically significant. Further clinical DDI study with CYP1A2 substrate is not warranted.

- Trilaciclib is not an inducer of CYP2B6 and 3A4.

Transporter systems

- Trilaciclib is an inhibitor of OCT1(IC_{50} = 0.6 μM), OCT2 (IC_{50} = 0.152 μM), MATE1 (IC_{50} = 0.175 μM), MATE2-K (IC_{50} = 0.071 μM), BCRP (IC_{50} = 31.1 μM), BSEP (IC_{50} = 10.8 μM), and OATP1B3 (IC_{50} = 22.9 μM). Trilaciclib is not an inhibitor of MRP1, P-gp, OAT1, OAT3, OATP1B1.

  The impact of trilaciclib on metformin (substrate of OCT2, MATE1 and MATE2-K) has been evaluated in healthy subjects, and with topotecan (substrate of MATE1 and MATE2-K) in patients with SCLC.

  Since the known clinically significant BCRP interactions are mediated by inhibition of the transporter in the gut, further evaluation of drug interaction between trilaciclib and a BCRP substrate was not warranted as trilaciclib is administered intravenously. There is no reported liver toxicity or increase in serum bile acid in non-clinical or clinical studies, and thus the impact of trilaciclib on BSEP may not be clinically significant. OCT1 and OATP1B2 are not listed in the current FDA guidance for in vitro DDI screening, and the clinical relevance of OCT1 of OATP1B2 inhibition is not fully clear. Therefore, further clinical evaluation for the DDI liability with these drug transporters (OCT1, OATP1B2, BSEP and BCRP) is not warranted.

- Trilaciclib is a substrate of BCRP and P-gp. However, since trilaciclib is administered via IV infusion and there are no potential safety concerns with trilaciclib distributing into tissues, the impact of BCRP and P-gp inhibitors on trilaciclib exposure is not expected to be clinically significant. No further clinical DDI studies with BCRP and P-gp inhibitors is warranted.

  Trilaciclib is not a substrate for BSEP, OCT2, MATE1, MATE2-K.

<u>DDI liability from clinical studies</u>

Impact of trilaciclib on other drugs

- *CYP3A4 substrate (midazolam)*

Clinical DDI study (Study G1T28-106) was conducted to assess the impact of multiple doses of trilaciclib (240 mg/m^2 QD for 5 days) on the exposure of single dose of midazolam (5 mg).

Coadministration of repeated doses of trilaciclib with midazolam resulted in no significant change in midazolam plasma AUC_{inf}, and the geometric mean C_{max} of midazolam was decreased by 16.6%. This result indicates no clinically significant effect of trilaciclib on the exposure of CYP3A4 substrate. No dose adjustment for CYP3A4 substrate is required when coadministered with trilaciclib.

- *OCT2, MATE1 and MATE2-K substrate (metformin)*

Clinical DDI study (Study G1T28-106) was conducted to assess the impact of multiple doses of trilaciclib (240 mg/m^2 QD for 6 days) on the exposure of single dose of metformin (5 mg).

Coadministration of metformin (an OCT2, MATE1, and MATE2-K substrate) with multiple doses of trilaciclib increased the metformin plasma AUC_{inf} by 65% and C_{max} by 81% in healthy subjects, compared to administration of metformin alone. Renal clearance of metformin decreased by 37% when coadministered with trilaciclib. However, the review team determined that dose modification for metformin is not warranted, due to the intermittent dosing scheme for trilaciclib. Unlike drugs that will be used chronically, trilaciclib will only be dosed for a short duration (3 to 5 days) in each treatment cycle (28 days), and the effect of transient increase in metformin exposure may not be clinically significant. Moreover, it is not practical to recommend frequent dose modification for patients on diabetic treatment.

Table 4. Statistical analysis of the effect of trilaciclib on metformin

	Metformin alone			Trilaciclib + Metformin		
Statistic	Ae (mg)	fe (%)	CL_R (L/h)	Ae (mg)	fe (%)	CL_R (L/h)
N	25	25	25	23	23	23
Mean (SD)	352 (89.6)	35.2 (9.0)	29.7 (8.11)	374 (131)	37.4 (13.1)	18.6 (4.58)
CV(%)	25.5	25.4	27.3	35.1	35.1	24.6
Min-Max	226-568	22.6-56.8	12.2-51.6	215-822	21.5-82.2	10.3-30.0
Geo Mean	342	34.2	28.6	356	35.6	18.1
Geo CV (%)	25.1	25.1	30.0	31.9	31.9	25.0

CV=coefficient of variation; Geo=geometric; Max=maximum; Min=minimum; N=number of subjects; PK=pharmacokinetic; SD=standard deviation

Source: Applicant's study report G1T28-106

Administration of trilaciclib prior to topotecan (a MATE substrate) did not affect the PK of either trilaciclib or topotecan in patients with SCLC. No dose adjustments for topotecan is required.

For other OCT2, MATE1 and MATE2-K substrates that are narrow therapeutic index drugs (e.g., dofetilide and dalfampridine), and cisplatin (which can be expected to be used in patients with SCLC), the following recommendations are proposed in 'Drug Interaction' section of the label (see 3.3.2 for details):

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	(b) (4) 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.

Impact of other drugs on trilaciclib

- *Strong CYP3A and P-gp inhibitor (itraconazole)*

Clinical DDI studies (Study G1T28-09 and Study G1T28-114) were conducted to assess the impact of multiple doses (200 mg QD, oral solution) of strong CYP3A4 inhibitor itraconazole on the single dose of trilaciclib (96 mg/m^2 or 200 mg/m^2). In study G1T28-09, itraconazole had minimal impact on the C_{max} (109.1%) and AUC_{inf} (100.3%) of trilaciclib; while in Study G1T28-114 itraconazole decreased C_{max} and AUC_{inf} of trilaciclib by 28.9% and 14.0%, respectively. Both studies confirmed that strong CYP3A4 inhibitor had no clinically significant impact on the exposure of trilaciclib. No dose adjustment is needed when trilaciclib is coadministered with CYP3A inhibitors.

In addition, these clinical DDI study results rule out the possibility that CYP3A4 is the primary enzyme involved in the metabolism of trilaciclib.

- *Strong CYP3A inducer (rifampin)*

Clinical DDI study (Study G1T28-106) was conducted to assess the effect of multiple oral doses of rifampin (600 mg QD) on the single dose PK of IV administered trilaciclib (240 mg/m^2). C_{max} and AUC_{inf} of trilaciclib were decreased by 19.9% and 17.3% when co-administered with repeated rifampin dosing for 10 days. This change of trilaciclib is not considered clinically significant. No dose adjustment is needed when trilaciclib is coadministered with CYP3A inducers. This result further rules out the possibility that the primary metabolic pathway of trilaciclib is via CYP3A.

3.3.5 Is the to-be-marketed formulation the same as the clinical trial formulation, and if not, are there bioequivalence data to support the to-be-marketed formulation?

Yes, the to-be-marketed formulation is the same as the clinical trial formulation.

4. APPENDICES

Appendix 4.1 Summary of Bioanalytical Method Validation and Performance

Trilaciclib concentrations in human plasma were measured by a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The method was first validated at a (b) (4) site in the (b) (4) over a range of 0.5 to 1000 ng/mL (method (b) (4)-NL-SML-1578) to support the first in human (FIH, Study 01) and mass balance (Study 08) studies conducted in the (b) (4). Subsequently, the assay was transferred to (b) (4) sites in the (b) (4) and fully validated at a similar range (1.0 to 1000 ng/mL) for K2-EDTA plasma samples to support other Phase 1 and Phase 2 studies (method (b) (4)-US-SML- 0114). Both methods consist of a protein precipitation sample preparation after addition of stable isotope labelled internal standards (trilaciclib-d3). The resulting extracts were evaporated to dryness, reconstituted and then injected on a reversed phase UHPLC C18 column using a gradient method. A triple quadrupole mass spectrometer (API Triple Quad 6500) equipped with a Turbo-ions pray source is used for detection in positive ion mode.

Trilaciclib concentrations in urine were also measured by a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The method was validated at a (b) (4) site in the (b) (4) over a range of 5.00 - 5000 ng/mL (method (b) (4)-NL-SML-1608) to support the FIH (Study 01) and mass balance (Study 08). Urine samples were prepared for analysis by dilution, and analysis was conducted using LC-MS/MS with a stable isotope internal standard (trilaciclibd3).

Summary of bioanalytical method and validation metrics is shown below. All validation results for the LC/MS/MS bioanalytical assay of trilaciclib appear acceptable and within the specification as recommended in the Bioanalytical Method Validation Guidance.

Table 5. Summary review of bioanalytical method measuring plasma and urine trilaciclib

Bioanalytical Method Review Summary	Method was adequately validated to support clinical studies
Method (b) (4)-NL-SML-1578 (Plasma)	
Method Description	An LC-MS/MS based bioanalytical method for the determination of the concentrations of trilaciclib in human plasma. This method was used in Studies 01 and 08.
Material for calibration curve & concentration	Trilaciclib (G1-T28-1, lot number: 1404309193; expiration date: 31 May 2015), Plasma
Internal Standard	Trilaciclib-d3
Validated Assay Range	0.500 - 1000 ng/mL
Recovery	Ranged from 103.6% to 116.1% for trilaciclib, 105.4% for G1T28-1-d3

Regression Model & Weighting	Linear Regression, $1/x^2$	
Validation Parameter	Method Validation Summary (Validation Report)	
Standard Curve Performance during accuracy and precision	Precision (CV%) Accuracy (Bias)	0.9% to 3.3% -3.0% to 2.5%
	Linearity	$R^2 \geq 0.99$
QC concentrations	0.500, 1.50, 50.0 and 800 ng/mL	
QCs performance during accuracy & precision	Intra-run accuracy (% bias)	-6.8% to 4.0%
	Intra-run precision (%CV)	1.7% to 4.4%
	Inter-run accuracy (%CV)	-3.2% to 2.4%
	Inter-run Precision (%CV)	1.6% to 4.2%
Dilution	10-fold dilution and quantification up to 10000 ng/mL is valid	
Plasma Stability	28 hours in plasma at room temperature, 42 days at -20°C and at -70°C	
Freeze/thaw Stability	6 cycles at -20°C and at -70°C	
Stock Stability	24 hours at room temperature 6 days at +4°C	
Stability in whole blood	• 2 hours at 0°C, • 2 hours at room temperature at 1.50 and 400 ng/mL only, • Whole blood stability at 4000 ng/mL up to 2 hours at room temperature was not demonstrated	
Carry-over	• Occasional carry-over occurred during method validation. Therefore, carry-over has be carefully monitored during (bio-)analysis and evaluated by the Study Director	
Incurred Sample Reanalysis (ISR)	ISR rate > 75% Passed	
Method (b) (4)-US-SML- 0114 (Plasma)		
Method Description	An LC-MS/MS based bioanalytical method for the determination of the concentrations of trilaciclib in human plasma. This method was used in Studies 02, 03, 05, 09, 11, 17, 106, and 114.	
Material for calibration curve & concentration	Trilaciclib (G1-T28-1, lot number: 1404309193; expiration date: 31 May 2017)	
Internal Standard	Trilaciclib-d3	

Validated Assay Range	1.00 - 1000 ng/mL	
Recovery	Ranged from 110.7% to 117.1% for trilaciclib, 97.5% for G1T28-1-d3	
Regression Model & Weighting	Linear Regression, $1/x^2$	
Validation Parameter	Method Validation Summary (Validation Report)	
Standard Curve Performance during accuracy and precision	Precision (CV%) Accuracy (Bias)	2.0% to 4.4% -6.3% to 3.5%
	Linearity	$R^2 \geq 0.99$
QC concentrations	0.500, 1.50, 50.0 and 800 ng/mL	
QCs performance during accuracy & precision	Intra-run accuracy (% bias)	-4.4% to 7.2%
	Intra-run precision (%CV)	1.0% to 7.9%
	Inter-run accuracy (% bias)	-2.4% to 5.3%
	Inter-run Precision (%CV)	3.0% to 7.4%
Dilution	10-fold dilution and quantification up to 10000 ng/mL is valid	
Plasma Stability	25 hours in plasma at room temperature; 591 days at -20°C and at -70°C	
Freeze/thaw Stability	5 cycles at -20°C and at -70°C	
Stock Stability	25 hours at room temperature; 155 days at -20°C	
Stability in whole blood	2 hours at 0°C and at ambient temperature	
Carry-over	No carry-over was observed.	
ISR	ISR rates > 80%, passed	
Method (b) (4)-NL-SML-1608 (Urine)		
Method Description	An LC-MS/MS based bioanalytical method for the determination of the concentrations of trilaciclib in human urine. This method was used in Studies 01 and 08.	
Material for calibration curve & concentration	Trilaciclib (G1-T28-1, lot number: 1404309193; retest date: 31 May 2016)	
Internal Standard	Trilaciclib-d3	

Validated Assay Range	5.00 - 5000 ng/mL	
Recovery	Ranged from 84.1% to 91.1% for G1T28-1, 98.4% for G1T28-1-d3	
Regression Model & Weighting	Linear Regression, $1/x^2$	
Validation Parameter	Method Validation Summary (Validation Report)	
Standard Curve Performance during accuracy and precision	Precision (CV%) Accuracy (Bias)	0.9% to 3.9% -4.6% to 2.5%
	Linearity	$R^2 \geq 0.99$
QC concentrations	0.500, 1.50, 50.0 and 800 ng/mL	
QCs performance during accuracy & precision	Intra-run accuracy (% bias)	-2.4% to 6.5%
	Intra-run precision (%CV)	1.6% to 5.1%
	Inter-run accuracy (% bias)	2.1% to 3.7%
	Inter-run Precision (%CV)	2.8% to 5.5%
Dilution	2-fold dilution and quantification up to 10000 ng/mL is valid	
Urine Stability	25 hours in urine at room temperature 24 hours at +4°C 11 days at -20°C and at -70°C (stability at -20°C and at -70°C for a longer period is ongoing)	
Freeze/thaw Stability	5 cycles at -20°C and at -70°C	
Stock Stability	25 hours at room temperature; 155 days at -20°C	
Urine pH stability	2 hours at 37°C (pH = 4.5, 6.5 and 8.5)	
Carry-over	No impact on method performance	
ISR	This method was used in Studies 01 and 08. ISR rates > 80%, passed	

Appendix 4.2 Population PK Analyses

4.2.1 Review Summary

The applicant's population PK analysis is acceptable for characterizing sources of PK variability and for exposure-response analyses to support trilaciclib dose selected (b) (4) of chemotherapy induced myelosuppression. The goodness-of-fit (GOF) plots and the prediction-corrected visual predictive check (PcVPC) indicate that the final population PK model is adequate in characterizing the PK profile of Trilaciclib in healthy subjects and in subjects with lung and breast cancers undergoing chemotherapy. The final population PK model was a 3-compartment model parameterized by clearance (CL), volume of the central compartment (V1), volume of 1st peripheral compartment (V2), volume of 2nd peripheral compartment (V3), transfer clearance between V1 and V2 (Q2), and transfer clearance between V1 and V3 (Q3). Identified sources of variability for CL were body surface area (BSA) and sex. In general females have 23% less CL compared to males; and subjects with BSA of 2.43m^2 have 11% higher CL compared to subjects with BSA of 1.9m^2. Plasma albumin was identified as a source of V1 variability; subjects with albumin levels of 27.0 and 52.0 g/L are expected to have 161% higher and 39% lower V1 compared to subjects with median albumin levels of 41.7 g/L. Sources of variability for V3 were Age and sex; subjects 19 and 80 years old are expected to have 50% lower and 24% higher V2 values compared to subjects with median age of 57 years. Females have about 27% lower V2 than males. The unexplained inter-individual variability (IIV) is small for CL (23.8%) but is large for V1 (90.2%), and v2 (42.7%). Eta Shrinkages for CL (4.2%), V1 (9.1%), and V2 (12.9) are small and support evaluation of covariates. The estimated PK parameters appear reasonable. The applicant's analyses were verified by the reviewer and no significant discordance were identified.

The developed model was used to support labelling of trilaciclib in the current submission as outlined in Table 6.

Table 6. Reviewer's specific comments on population PK model

Utility of the final model			**Reviewer's Comments**
Support applicant's proposed labeling statements about intrinsic and extrinsic factors	**Intrinsic factor**	Trilaciclib dosing is individualized based on BSA. This is consistent with BSA dependent CL	No clinically meaningful exposure-efficacy relationship was identified indicating that at the dose of 240 mg/m^2, exposures (AUC) in the indicated population is at the plateau of exposure response relationship.
	Extrinsic factor	No extrinsic factor was identified as a covariate on PK parameters	Furthermore, shallow exposures versus safety relationships (headache, phlebitis and injection-site reactions) were identified. But this relationship does not support any further dose adjustments.

Derive exposure metrics for Exposure-response analyses		The use of predicted exposures in E-R analyses is acceptable since the model performance was reasonable as indicated by GOF and PcVPC
Predict exposures at alternative dosing regimen		The model was not used to assess predicted exposures at other doses

4.2.2 Introduction

The primary objectives of this analysis were to:

- Characterize the population pharmacokinetics of Trilaciclib.
- Identify sources of PK variability that may impact exposure to Trilaciclib.
- Generate post-hoc PK parameters through non-compartmental analyses of predicted concentrations for individual patients in phase 2 studies that can be used for subsequent exposure-response analyses

4.2.3 Model development

Data

The analyses were based on PK data from 6 studies. The study design, study population, and timing of blood samples varied among the 6 clinical studies. Brief descriptions of the studies included are presented in Table 7.

The final NONMEM data file for analysis contained 2333 PK observations from 197 subjects. Table 8 provide summary statistics of the baseline demographic covariates in the analysis dataset.

Table 7. Summary of Studies with PK Sampling Included in Population PK Analysis

Study Number	Study Design, N	Trilaciclib and Chemotherapy Dose Regimens
G1T28-1-01	A Phase 1 first-in-human safety, PK and PD study of trilaciclib in healthy subjects N = 53 SAD Cohorts 1-6: N = 33 BED Cohort 7: N = 12 PO Cohort 8: N = 8	**Trilaciclib** SAD (Cohorts 1 – 6): 30-min IV infusion: 6, 12, 24, 48, 96, and 192 mg/m^2 BED Cohort (single dose prior to bone marrow aspirates): 192 mg/m2 30-min IV infusion Cohort 8 (single dose oral): Period 1: 100 mg solution Period 2: 400 mg solution Period 3: 400 mg capsule (Cohort 8 will not be included in the analysis)
G1T28-02	Phase 1b/2a randomized, double-blind, placebo-controlled study to assess PK, safety, and efficacy of trilaciclib in combination with etoposide/carboplatin in 1st line SCLC patients Part 1: 19 patients Part 2: 77 patients • Placebo: 38 • Trilaciclib: 39	Trilaciclib QD on Days 1-3 + etoposide QD on Days 1-3 + carboplatin on Day 1 of 21-day chemotherapy cycles **Trilaciclib** Part 1 (Phase 1b): 30-min IV infusion: 200 mg/m2, 240 mg/m^2 Part 2 (Phase 2a): 30-min IV infusion: 240 mg/m2 or matching placebo (1:1) **Chemotherapy** Parts 1 and 2: Etoposide: 100 mg/m2 IV Carboplatin: IV administration to achieve target AUC = 5 mg·min/mL (maximum 750 mg).
G1T28-03	A Phase 1b/2a randomized, double-blind, placebo-controlled safety and PK study for trilaciclib in combination with topotecan in 2nd/3rd line SCLC patients Part 1: 32 patients Part 2: 91 patients • Placebo + topotecan (1.5 mg/m^2) (n=29) • Trilaciclib + topotecan (0.75 mg/m^2) (n=30) • Trilaciclib + topotecan (1.5 mg/m^2) (n=32)	30-min IV infusion of trilaciclib and 30-min IV infusion of topotecan, both QD on Days 1-5 of 21-day topotecan cycles Part 1 (Phase 1b): Cohort 1: 200 mg/m^2 trilaciclib + 1.5 mg/m2 topotecan Cohort 2: 200 + 1.25 Cohort 3: 200 + 0.75 Cohort 4: 240 + 0.75 Cohort 5: 280 + 0.75 Cohort 6: 240 + 0.75 Cohort 7: 240 + 1.0 Part 2A (Phase 2a; 2:1 trilaciclib:placebo): Arm 1: 240 mg/m2 trilaciclib + 0.75 mg/m2 topotecan Arm 2: placebo + 1.5 Part 2B (Phase 2a; 2:1 trilaciclib:placebo): Arm 1: 240 mg/m2 trilaciclib + 1.5 mg/m2 topotecan Arm 2: placebo + 1.5
Study Number	**Study Design, N**	**Trilaciclib and Chemotherapy Dose Regimens**
G1T28-04	A Phase 2 randomized, open label, placebo-controlled safety and tolerability study of trilaciclib in combination with gemcitabine/carboplatin in 1st/2nd/3rd line TNBC patients Randomized: 102 patients • Group 1 (34 patients) • Group 2 (33 patients) • Group 3 (35 patients)	Doses Trilaciclib dose: 240 mg/m2 30-min IV Gemcitabine dose: 1000 mg/m2 IV on Days 1 and 8 (Groups 1 and 2) or Days 2 and 9 (Group 3) of each 21-day cycle Carboplatin dose: IV administration to achieve target AUC = 2 mg·min/mL (maximum 300mg) Study Treatments (21-day cycles) Group 1: GC therapy Group 2: GC therapy on Days 1 and 8 + trilaciclib on Days 1 and 8 Group 3: GC therapy on Days 2 and 9 + trilaciclib on Days 1, 2, 8, and 9
G1T28-05	Patients with newly diagnosed extensive-stage SCLC • 107 patients randomized (1:1 ratio) • Sites in US & Europe	Double blinded, randomized at 1:1 ratio to trilaciclib or placebo IV QD, prior to chemotherapy **Group 1**: Placebo IV QD with E/P/A therapy on Days 1 to 3 of each 21-day chemotherapy cycle for up to 4 cycles followed by atezolizumab monotherapy every 21 days **Group 2:** Trilaciclib (240 mg/m^2) IV QD with E/P/A therapy on Days 1 to 3 of each 21-day chemotherapy cycle for up to 4 cycles followed by atezolizumab monotherapy every 21 days Note: E/P/A therapy comprises standard of care etoposide (100 mg/m^2) IV on Days 1, 2, and 3, carboplatin AUC = 5 (calculated dose, maximum of 750 mg) on Day 1, with the addition of atezolizumab (1200 mg) IV on Day 1 of each 21-day chemotherapy cycle.
G1T28-11	Positive-Controlled, Single-Dose, Dose-Escalation Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics (Including Assessment of Potential Electrocardiographic Effects) of Intravenous Trilaciclib (G1T28) in Healthy Volunteers • 78 healthy subjects: Male/Female • Single-center (US)	Double-blind, double-dummy, placebo- and positive-controlled, randomized at 6:4:3 ratio Single IV infusion of trilaciclib (200, 260, 330, 440, 550, 700 mg/m^2) + moxifloxacin placebo (oral) Single 400 mg of moxifloxacin (oral) + trilaciclib placebo (IV infusion) trilaciclib placebo (IV infusion) + moxifloxacin placebo (oral)

QD = once daily

Source: Applicant's population PK and ER report (page 18 - 19)

Table 8. Summary of Baseline Demographic Covariates. Source: Applicant's population PK and ER report (page 31 - 33)

Characteristics	Study ID						Overall (N=197)
	G1T28-1-01 (N=36)	G1T28-02 (N=9)	G1T28-03 (N=89)	G1T28-04 (N=14)	G1T28-05 (N=13)	G1T28-11 (N=36)	
Disease Status							
Healthy Subjects	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36 (100%)	72 (36.5%)
Oncology Patients	0 (0%)	9 (100%)	89 (100%)	14 (100%)	13 (100%)	0 (0%)	125 (63.5%)
Tumor Type							
Healthy Subject	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36 (100%)	72 (36.5%)
Small Cell Lung Cancer (SCLC)	0 (0%)	9 (100%)	89 (100%)	0 (0%)	13 (100%)	0 (0%)	111 (56.3%)
Triple Negative Breast Cancer (TNBC)	0 (0%)	0 (0%)	0 (0%)	14 (100%)	0 (0%)	0 (0%)	14 (7.1%)
ECOG							
0	0 (0%)	2 (22.2%)	26 (29.2%)	9 (64.3%)	1 (7.7%)	0 (0%)	38 (19.3%)
1	0 (0%)	6 (66.7%)	52 (58.4%)	5 (35.7%)	11 (84.6%)	0 (0%)	74 (37.6%)
2	0 (0%)	1 (11.1%)	10 (11.2%)	0 (0%)	1 (7.7%)	0 (0%)	12 (6.1%)
Missing	36 (100%)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)	36 (100%)	73 (37.1%)
Chemotherapy Combination							
Topotecan	0 (0%)	0 (0%)	89 (100%)	0 (0%)	0 (0%)	0 (0%)	89 (45.2%)
Carboplatin and Etoposide	0 (0%)	9 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (4.6%)
Carboplatin, Etoposide, and Atezolizumab	0 (0%)	0 (0%)	0 (0%)	0 (0%)	13 (100%)	0 (0%)	13 (6.6%)
Carboplatin and Gemcitabine	0 (0%)	0 (0%)	0 (0%)	14 (100%)	0 (0%)	0 (0%)	14 (7.1%)
No Chemotherapy	36 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36 (100%)	72 (36.5%)

ECOG = Eastern Cooperative Oncology Group; N=number of subjects.

Characteristics	Study ID						Overall (N=197)
	G1T28-1-01 (N=36)	G1T28-02 (N=9)	G1T28-03 (N=89)	G1T28-04 (N=14)	G1T28-05 (N=13)	G1T28-11 (N=36)	
Sex							
Male	22 (61.1%)	4 (44.4%)	56 (62.9%)	1 (7.1%)	8 (61.5%)	20 (55.6%)	111 (56.3%)
Female	14 (38.9%)	5 (55.6%)	33 (37.1%)	13 (92.9%)	5 (38.5%)	16 (44.4%)	86 (43.7%)
Race							
White	32 (88.9%)	7 (77.8%)	83 (93.3%)	8 (57.1%)	13 (100%)	11 (30.6%)	154 (78.2%)
Black or African American	0 (0%)	2 (22.2%)	4 (4.5%)	3 (21.4%)	0 (0%)	23 (63.9%)	32 (16.2%)
Asian	0 (0%)	0 (0%)	1 (1.1%)	1 (7.1%)	0 (0%)	1 (2.8%)	3 (1.5%)
multiple or other	4 (11.1%)	0 (0%)	1 (1.1%)	2 (14.3%)	0 (0%)	1 (2.8%)	8 (4.1%)
Ethnicity							
Hispanic or Latino	1 (2.8%)	1 (11.1%)	0 (0%)	3 (21.4%)	0 (0%)	4 (11.1%)	9 (4.6%)
Not Hispanic or Latino	35 (97.2%)	8 (88.9%)	88 (98.9%)	11 (78.6%)	13 (100%)	32 (88.9%)	187 (94.9%)
Not reported	0 (0%)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
Age Group							
< 65 years	36 (100%)	3 (33.3%)	49 (55.1%)	12 (85.7%)	6 (46.2%)	36 (100%)	142 (72.1%)
≥ 65 years	0 (0%)	6 (66.7%)	40 (44.9%)	2 (14.3%)	7 (53.8%)	0 (0%)	55 (27.9%)
Hepatic Impairment, NCI Classification							
Normal	34 (94.4%)	3 (33.3%)	75 (84.3%)	11 (78.6%)	10 (76.9%)	34 (94.4%)	167 (84.8%)
Mild	2 (5.6%)	6 (66.7%)	14 (15.7%)	3 (21.4%)	3 (23.1%)	2 (5.6%)	30 (15.2%)
Renal Impairment, FDA Classification							
Normal	35 (97.2%)	3 (33.3%)	47 (52.8%)	8 (57.1%)	7 (53.8%)	36 (100%)	136 (69.0%)
Mild	1 (2.8%)	3 (33.3%)	32 (36.0%)	4 (28.6%)	5 (38.5%)	0 (0%)	45 (22.8%)
Moderate	0 (0%)	3 (33.3%)	10 (11.2%)	2 (14.3%)	1 (7.7%)	0 (0%)	16 (8.1%)

FDA=Food and Drug Administration; NCI=National Cancer Institute; N=number of subjects.

Characteristics	G1T28-1-01 (n=36)	G1T28-02 (n=9)	G1T28-03 (n=89)	G1T28-04 (n=14)	G1T28-05 (n=13)	G1T28-11 (n=36)	Overall (n=197)
Age (years)							
Mean (CV%)	37.9 (40.6%)	66.4 (18.0%)	63.6 (13.1%)	57.1 (21.1%)	64.8 (12.6%)	34.1 (22.0%)	53.3 (31.5%)
Median [Min, Max]	30.5 [20.0, 60.0]	72.0 [45.0, 80.0]	63.0 [46.0, 79.0]	60.5 [39.0, 78.0]	66.0 [50.0, 75.0]	33.0 [19.0, 48.0]	57.0 [19.0, 80.0]
Body Weight (kg)							
Mean (CV%)	78.8 (14.5%)	72.2 (22.9%)	75.4 (24.9%)	72.0 (17.7%)	82.0 (30.5%)	79.6 (18.6%)	76.8 (22.1%)
Median [Min, Max]	78.4 [54.7, 111]	71.5 [52.8, 109]	74.0 [42.7, 125]	70.0 [48.0, 91.0]	73.0 [55.0, 134]	79.5 [40.0, 108]	76.2 [40.0, 134]
BMI (kg/m^2)							
Mean (CV%)	25.3 (12.7%)	26.3 (21.8%)	25.8 (20.2%)	26.5 (16.4%)	28.7 (30.8%)	26.3 (13.2%)	26.1 (18.9%)
Median [Min, Max]	24.7 [19.7, 30.9]	25.7 [16.7, 37.8]	24.5 [17.5, 40.9]	26.5 [20.6, 33.4]	26.4 [16.9, 50.8]	26.0 [20.0, 32.0]	25.2 [16.7, 50.8]
BSA (m^2)							
Mean (CV%)	1.95 (8.62%)	1.81 (11.1%)	1.86 (13.6%)	1.79 (9.18%)	1.91 (13.6%)	1.93 (12.3%)	1.89 (12.3%)
Median [Min, Max]	1.96 [1.59, 2.40]	1.77 [1.56, 2.20]	1.87 [1.29, 2.43]	1.75 [1.42, 2.00]	1.78 [1.58, 2.32]	1.94 [1.26, 2.30]	1.89 [1.26, 2.43]
Albumin (g/L)							
Mean (CV%)	46.8 (5.32%)	37.2 (10.6%)	39.5 (10.2%)	42.2 (5.96%)	39.1 (11.0%)	42.7 (5.84%)	41.5 (10.9%)
Median [Min, Max]	47.0 [40.0, 51.0]	39.0 [32.0, 42.0]	40.0 [27.0, 49.0]	43.0 [37.0, 47.0]	40.0 [30.0, 47.5]	42.8 [33.7, 47.8]	41.7 [27.0, 51.0]
ALP (U/L)							
Mean (CV%)	70.3 (26.6%)	133 (80.7%)	99.8 (86.7%)	90.8 (28.9%)	76.1 (26.9%)	59.3 (26.1%)	86.3 (76.7%)
Median [Min, Max]	68.5 [33.0, 144]	98.0 [74.0, 414]	86.0 [33.0, 757]	83.5 [55.0, 151]	71.0 [45.0, 108]	56.5 [29.0, 110]	74.0 [29.0, 757]
ALT (U/L)							
Mean (CV%)	23.9 (49.7%)	44.4 (56.7%)	25.7 (77.4%)	30.4 (50.2%)	25.3 (63.1%)	16.7 (42.7%)	24.9 (69.7%)
Median [Min, Max]	21.5 [8.00, 63.0]	38.0 [13.0, 86.0]	20.0 [5.00, 107]	24.0 [13.0, 62.0]	23.0 [9.00, 67.0]	15.0 [7.00, 43.0]	20.0 [5.00, 107]
AST (U/L)							
Mean (CV%)	23.2 (27.9%)	46.6 (55.7%)	28.1 (82.8%)	29.9 (38.6%)	25.8 (78.8%)	19.4 (33.5%)	26.4 (71.0%)
Median [Min, Max]	22.0 [13.0, 43.0]	48.0 [20.0, 108]	21.0 [9.00, 169]	27.5 [15.0, 58.0]	18.1 [9.60, 81.0]	18.5 [11.0, 38.0]	22.0 [9.00, 169]
Bilirubin (µmol/L)							
Mean (CV%)	11.5 (69.8%)	8.93 (48.6%)	8.90 (49.3%)	8.79 (43.7%)	8.42 (38.7%)	9.44 (37.8%)	9.44 (53.9%)
Median [Min, Max]	8.50 [6.00, 42.0]	8.55 [3.42, 18.8]	7.20 [2.00, 25.7]	9.41 [1.71, 15.4]	8.89 [1.71, 14.0]	8.47 [4.79, 19.2]	8.55 [1.71, 42.0]
CrCL (mL/min)							
Mean (CV%)	134 (20.1%)	75.7 (34.1%)	92.5 (30.3%)	106 (48.4%)	93.7 (29.5%)	128 (18.6%)	107 (32.4%)
Median [Min, Max]	131 [69.2, 191]	67.2 [45.0, 114]	92.2 [38.6, 179]	106 [44.0, 249]	99.8 [56.1, 162]	128 [93.6, 206]	105 [38.6, 249]
Neutrophil Count ($10^9/L$)							
Mean (CV%)	2.98 (32.5%)	6.04 (52.6%)	5.23 (45.6%)	5.86 (41.3%)	9.42 (47.5%)	2.90 (31.9%)	4.46 (58.2%)
Median [Min, Max]	2.75 [1.70, 5.60]	5.03 [3.20, 13.6]	4.88 [2.00, 13.1]	5.94 [3.14, 8.40]	8.20 [5.30, 15.8]	2.53 [1.50, 5.39]	3.60 [1.50, 15.8]
Missing	0 (0%)	0 (0%)	18 (20.2%)	10 (71.4%)	6 (46.2%)	0 (0%)	34 (17.3%)

ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; BSA=body surface area; CV=coefficient of variation; Max=maximum; Min=minimum; N=number of subjects.

Base Model

The final base model was a 3-compartment PK model with zero order intravenous infusion to the central compartment. Clearance was allometrically scaled by BSA using a power model. No other parameters were allometrically scaled. Inter-individual variability was estimated for 3 structural model parameters, namely CL, V1, and V2. Data below limit of quantification (BLQ) were excluded from analysis. Outlying data points (defined as data points with absolute conditional weighted residuals CWRES > 5) were also excluded from further model development steps.

Inter-individual variability (IIV) was modelled assuming a log-normal distribution for patient level random effects. Residual variability was tested as additive, proportional or both on the dependent variable. Model evaluation and selection of the base model were based on standard statistical criteria of goodness-of-fit such as a decrease in the minimum objective function value (OFV), accuracy of parameter estimation (i.e., 95% confidence interval excluding 0), successful model convergence, and diagnostic plots.

Covariate Analysis

Graphical exploration of ETA-vs- covariate plots were used selection of covariates to include into univariate analysis of parameter-covariate relationships. The following covariates were assessed: Disease status (patient vs health), tumor type, ECOG status, subject demographics (age, sex, race), body size (weight, BSA and BMI), renal impairment status, hepatic impairment status and serum chemistry (creatinine clearance, total bilirubin, alanine aminotransferase (ALT), aspertate aminotransferase (AST), alkaline phosphatase (ALP), albumin). Only parameter-covariate relationships significant at 0.01 level in univariate testing were taken forward into the full automated stepwise covariate search. Continuous covariates were evaluated using a power function and categorical covariates were parameterized as a fractional change. In the automated stepwise covariate model building, forward step included covariate-parameter relationships that decreased objective function value (OFV) by more than 6.63 ($p<0.01$). In a backward step, covariate-parameter relationship was to be excluded from the model if doing so resulted in less than 10.828 increase in OFV.

4.2.4 Final Model

The stepwise covariate model building included the following relationships: Sex as covariate on CL and V2; albumin as covariate on V1; and age as covariate on V2. The parameter estimates for the final covariate model are listed in Table 9. The goodness-of-fit plots for the final covariate model for all data are shown in Figure 7. The Visual Predictive Check (VPC) plot for the final covariate model with all data is shown in Figure 8.

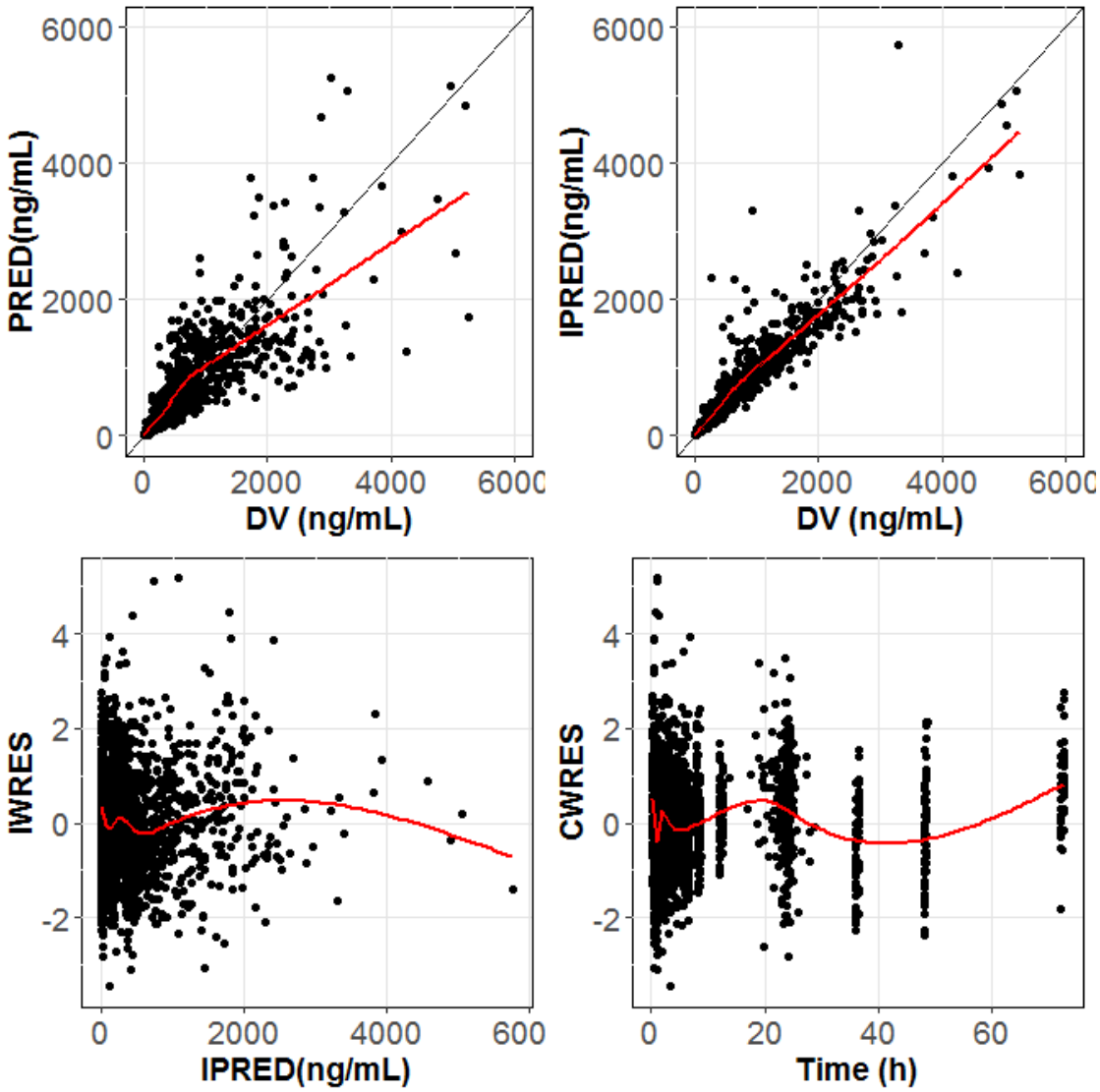
Table 9. Parameter estimates from the Applicant's final population PK model

Parameter	Estimate (RSE)	BSV (RSE)	Shrinkage
CL (L/h)	**158** (4.15%) × (BSA/1.9)$^{0.425}$ (32.1%) × **0.873** if Female (3.78%)	**23.8%** (6.76%)	**4.2%**
V1 (L)	**177** (15.7%) × (Albumin/41.7)$^{-2.21}$ (23.2%)	**90.2%** (8.61%)	**9.1%**
V2 (L)	**533** (7.66%) × (Age/57)$^{0.629}$ (11.5%) × **0.731** if Female (7.72%)	**42.7%** (12.9%)	**11.3%**
V3 (L)	**420** (7.90%)	NA	NA
Q2 (L/h)	**254** (10.3%)	NA	NA
Q3 (L/h)	**32.5** (13.0%)	NA	NA
Error Model			
Additive Term (ng/mL)	**0.5** Fixed (NA)	NA	NA
Proportional Term (%)	**23.1** (2.90%)	NA	NA

Note: Population PK parameters are for a typical 57-year male adult subject with a BSA of 1.9 m^2 and albumin of 41.7 g/dL. BSA = body surface area, BSV = between-subject variability, CL = clearance, V1 = central volume of distribution, V2 = first peripheral volume of distribution; V3 = second peripheral volume of distribution; Q2 = first distribution clearance; Q3 = second distribution clearance. Additional PK parameters are presented in Appendix 2, Section 14.30.

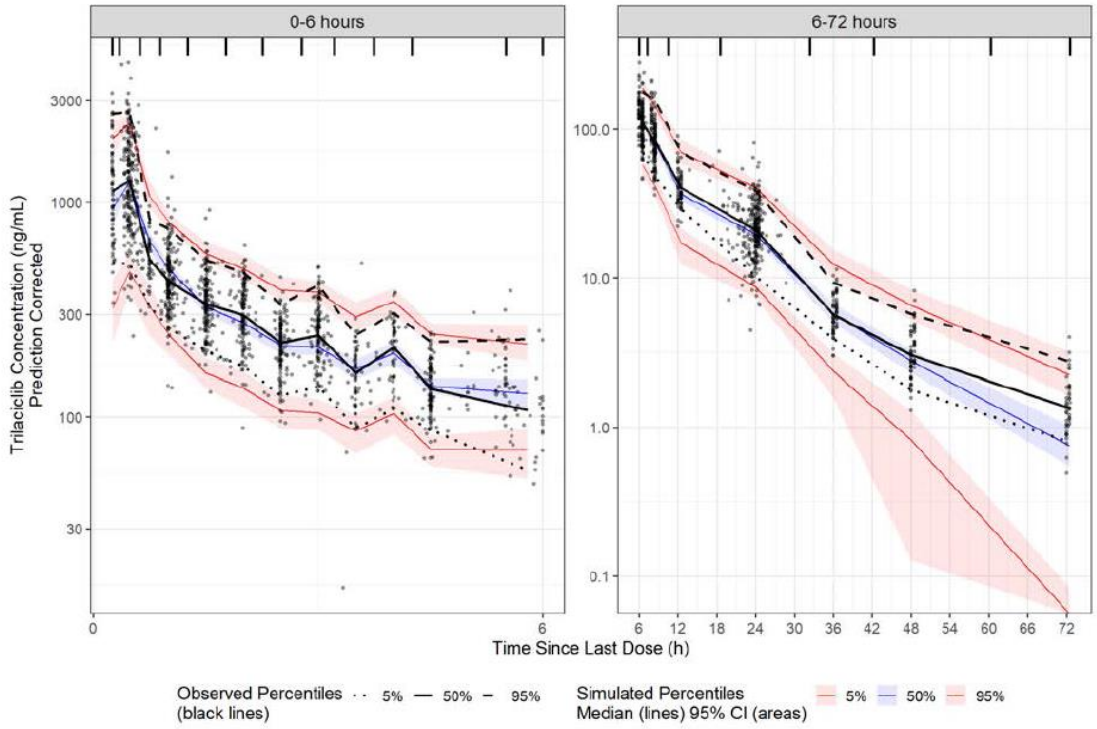
Source: Applicant's population PK and ER report (page 38)

Figure 7. Goodness-of-fit plots for final covariate model



Source: Reviewer's post-processing of NONMEM outputs of the Applicant's pop PK model

Figure 8. Visual predictive check of the final model: All cycles and all time points



Source: Applicant's population PK and ER report (page 41)

4.2.5 Reviewer's comments

The reviewer finds the applicant's model development steps and identification of covariate effects to be acceptable. Therefore, the reviewer did not perform independent exploration of covariate effects. The reviewer repeated the applicant's analyses and found similar results as those reported by the sponsor.

Appendix 4.3 Exposure-Response for Efficacy Analysis

4.3.1 Review Summary

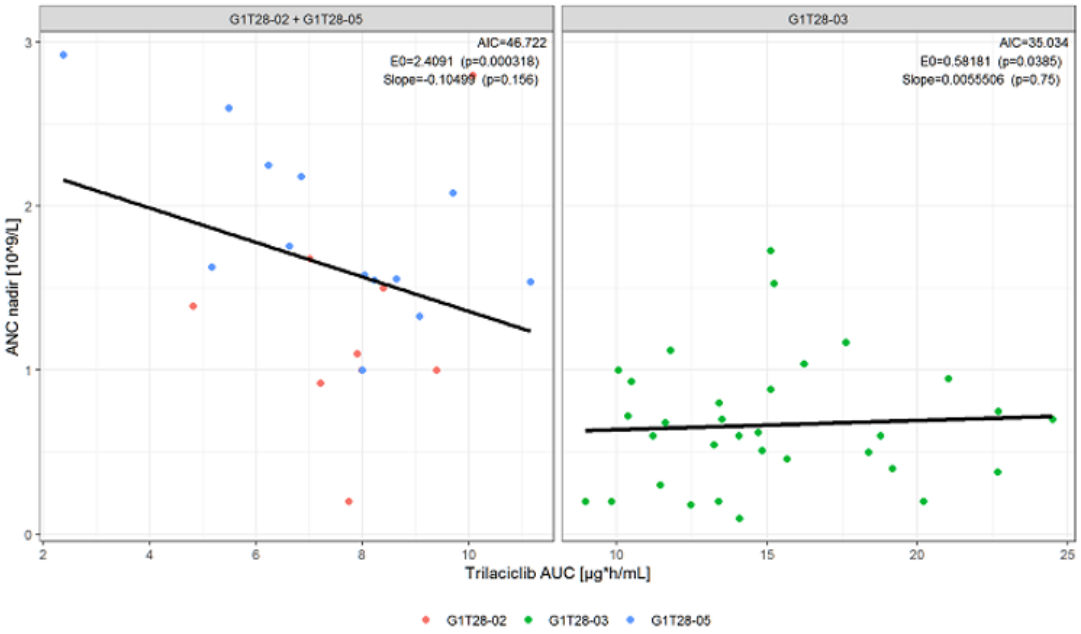
The applicant's exposure-response analyses for efficacy are acceptable for describing the relationship between efficacy and exposure for the recommended dose of trilaciclib. The applicant used trilaciclib AUC derived from post-hoc PK parameters of the population PK modelling to assess linear and non-linear relationships between exposure and efficacy endpoints. The evaluated efficacy endpoints included neutrophil nadir (cells/µL), proportions with severe neutropenia (SN), area under effect curve (AUEC, Area between baseline and ANC curve). The applicant also assessed if trilaciclib treatment impacted the antitumor activity of chemotherapies. The endpoints for this assessment were overall response rate (ORR), progression free survival (PFS), and overall survival (OS). Logistic regression was used to assess the relationships between trilaciclib AUC and probability of SN or ORR. Kaplan-Meier curves or Cox-proportional hazard modeling were used to assess the relationship between trilaciclib AUC and PFS or OS. The applicant's linear models showed none or implausible relationships between trilaciclib AUC and ANC nadir. In general, relationships were not clinically meaningful as it indicated that subjects with lower AUC had higher neutrophil nadir than subjects with high AUC. Similarly, the applicant found no relationship between Trilaciclib AUC and probability of severe neutropenia or anemia. Also, the applicant found no relationship between trilaciclib AUC and probability of overall survival. The applicant's assessment of the impact of trilaciclib on survival found no apparent relationships between trilaciclib AUC and progression free survival nor overall survival. Also, none of the covariates tested (race, region, sex, age, albumin, BSA, BMI, baseline platelet count, neutrophil count, hemoglobin, and ECOG) explained variability in the probability of PFS or OS. The lack of apparent exposure-response relationships is a potential indication that at the recommended trilaciclib dose of 240 mg/m^2, individual AUCs are at the plateau of the assessed exposure-response relationships.

4.3.2. Applicant's exposure-response analyses

4.3.2.1. Trilaciclib AUC vs ANC nadir

This assessment was performed using data from patients with small cell lung cancer in studies GIT28-02, GIT28-03, and GIT28-05. In studies GIT28-02 and GIT28-05, patients received trilaciclib (plus chemotherapy consisting of etoposide and carboplatin) in the first 3 days of the 21-day treatment cycle. In study GIT28-03, patients received trilaciclib (plus topotecan) for the first 5 days of the 21 days treatment cycle. Data from studies GIT28-02 and GIT28-05 (3-days dosing studies) were therefore analyzed separately from study GIT28-03 (5-days dosing study). Results from linear regression analyses are summarized in figure 3. The figure shows no relationship between AUC and ANC nadir for patients in GIT28-03 but shows a trend for low ANC nadir in patients with high AUC in studies GIT28-02 and GIT28-05. Baseline neutrophil count was the only predictor associated with ANC nadir being high for subjects with high neutrophil nadir.

Figure 9. Relationship between AUC of trilaciclib and ANC nadir

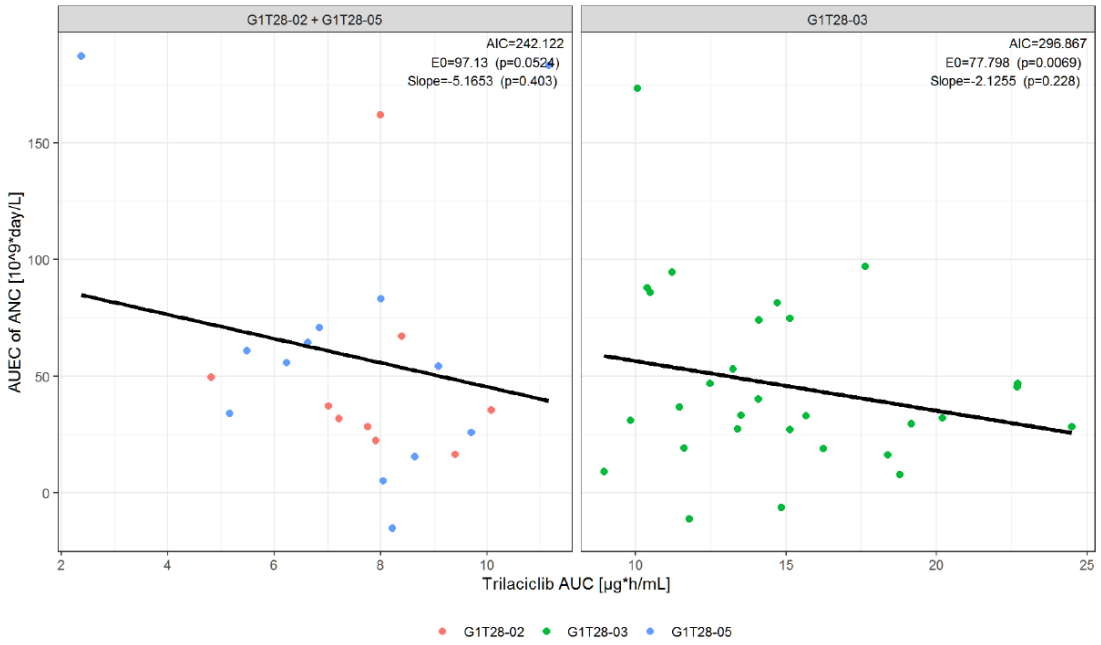


Source: Applicant's population PK and ER report (page 45)

4.3.2.2. Trilaciclib AUC vs AUEC

Assessments of relationships between trilaciclib AUC and AUEC were done separately between 3-days and 5-days dosing studies. Results from linear regression analyses of AUC vs AUEC are summarized in Figure 10. The figure shows trends towards negative relationship between AUC and AUEC for both 3-days and 5-days dosing studies. The figure suggests that subjects with high AUC have lower AUEC which is consistent with expected protection from myelosuppression by trilaciclib. These relationships were however not statistically significant. Baseline neutrophil count was the only predictor associated with AUEC being high for subjects with low AUEC.

Figure 10. Relationship between AUC of trilaciclib and AUEC

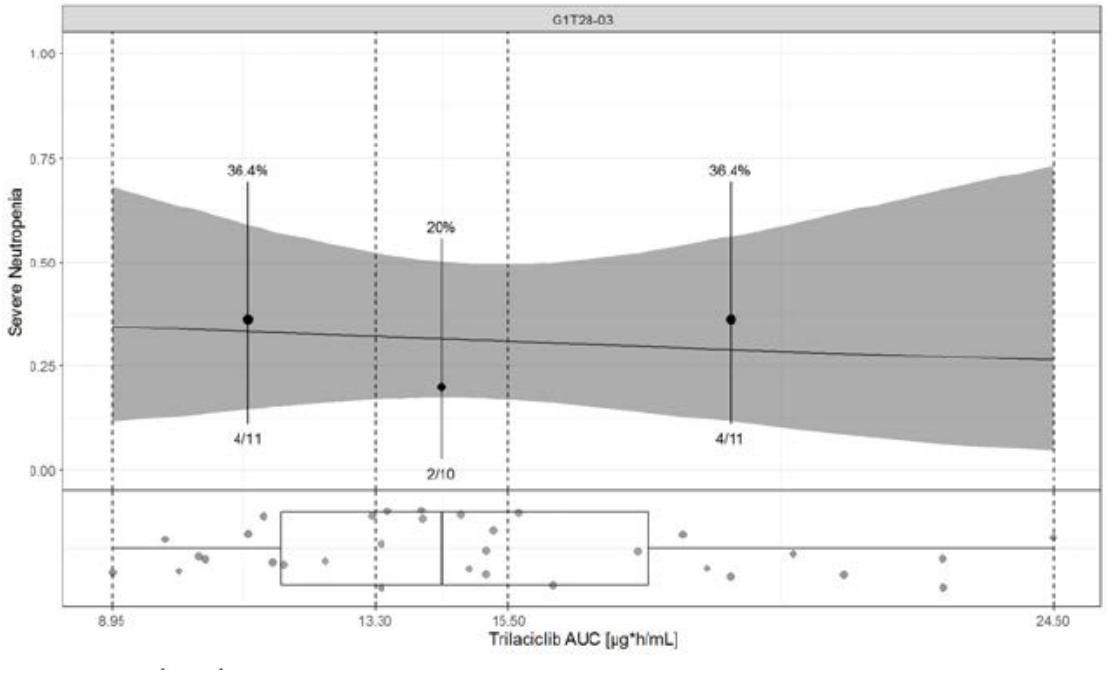


Source: Applicant's population PK and ER report (page 134)

4.3.2.3. Trilaciclib AUC vs Severe Neutropenia

The applicant performed logistic regression analysis for relationship between AUC and probability of severe neutropenia for patients in study GIT28-03. This analysis was not possible to 5-days dosing studies as there was only 1 subject with severe neutropenia. Results from this analysis are presented in Figure 11. The figure shows no significant relationship between AUC and probability of severe neutropenia at the range of AUCs studied.

Figure 11. Relationship between AUC of trilaciclib and probability of severe neutropenia

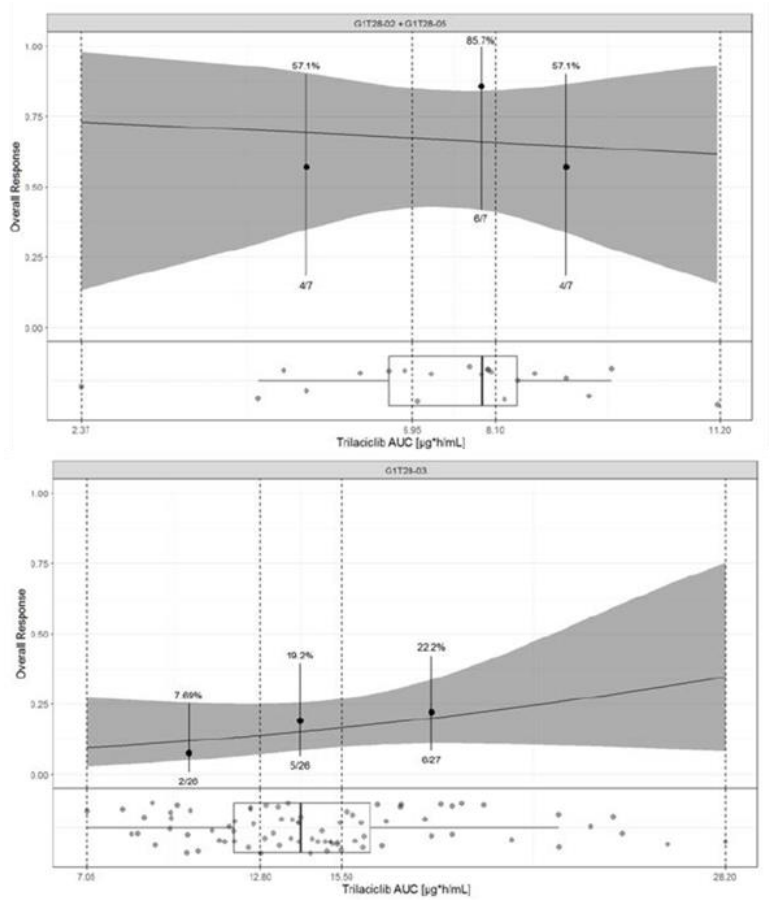


Source: Applicant's population PK and ER report (page 47)

4.3.2.4. Trilaciclib AUC vs ORR

Analyses were performed separately between 3-days and 5-days dosing studies. Results from these analyses are summarized in Figure 12. The figure shows no significant relationship between AUC and ORR at the range of AUCs studied.

Figure 12. Relationship between AUC of trilaciclib and overall response rate

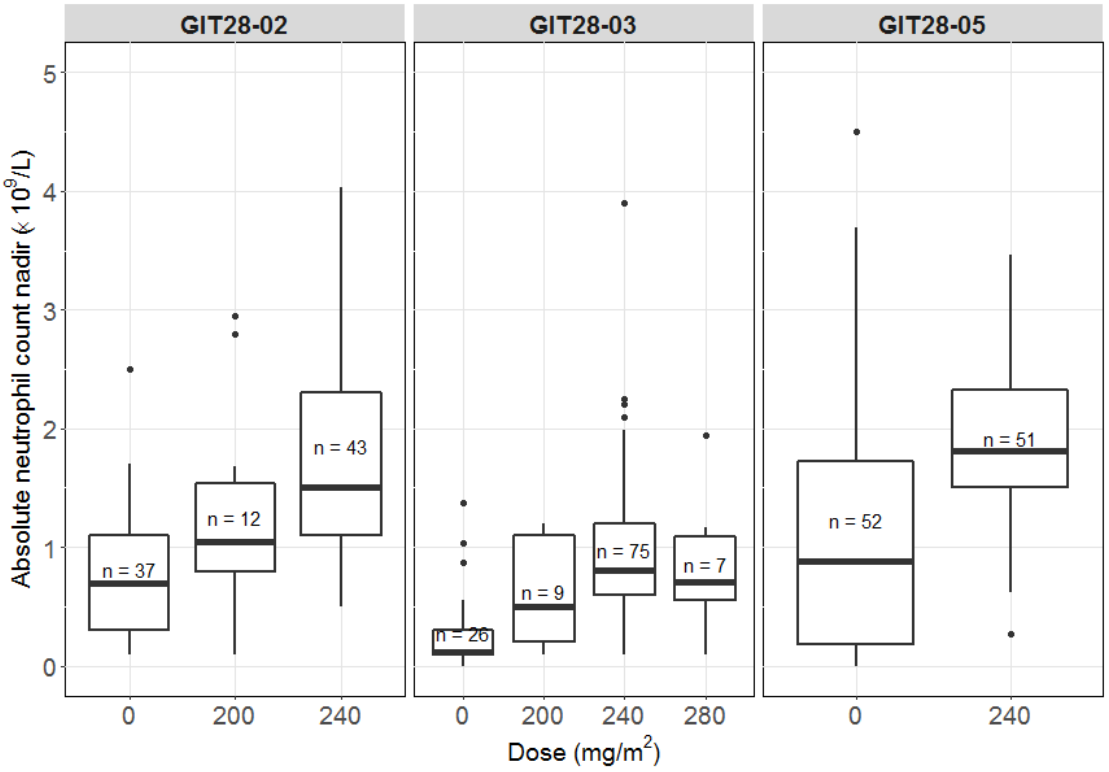


Source: Applicant's population PK and ER report (page 50 - 51)

4.3.3. Reviewer's comments

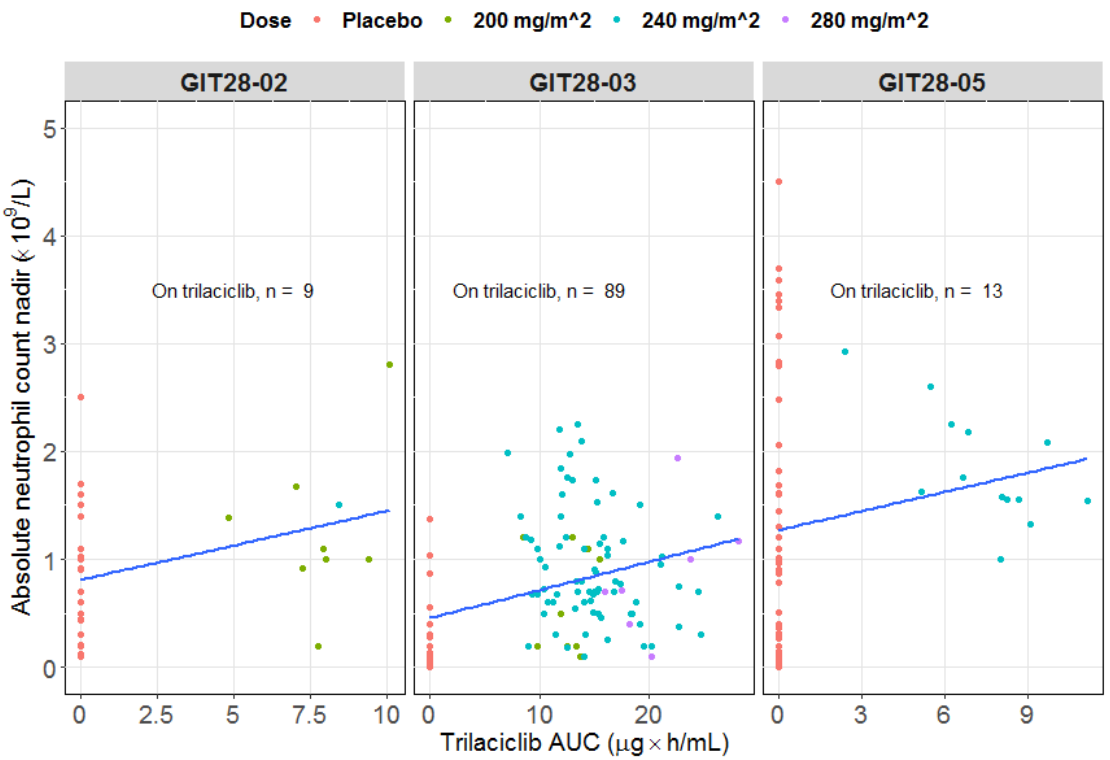
The applicant's analyses are acceptable for making conclusions on exposure-response relationships at the recommended trilaciclib doses. The reviewer performed additional assessments of exposure-response relationships by pooling data from additional dose groups investigated in studies GIT28-02, GIT28-03, and GIT28-05. The reviewer's analyses are graphical exploration of correlation between trilaciclib dose/AUC and ANC nadir. The results of these analyses are presented in Figure 13 and Figure 14. The figures shows that ANC nadir increases with trilaciclib dose and seems to plateau at 240mg/m^2. In Figure 14 AUC vs ANC nadir scatter plots indicates shallow but positive relationship after including placebo group into the analysis. It is noted however that many subjects (studies GIT28-02, and GIT28-05) did not have PK parameters, therefore excluded from this analysis. Subjects in study GIT28-03 had overall higher AUC (because of 5-days dosing) compared to subjects in studies GIT28-02, and GIT28-05 (Studies with 3-days dosing). Nevertheless, ANC nadir levels are comparable between the 3-days and 5-days dosing studies.

Figure 13. Relationship between dose of trilaciclib and ANC nadir



Source: Reviewer's independent analyses

Figure 14. Relationship between AUC of trilaciclib and ANC nadir



Source: Reviewer's independent analyses

Appendix 4.4 Exposure-vs-Safety Analysis

4.4.1 Review Summary

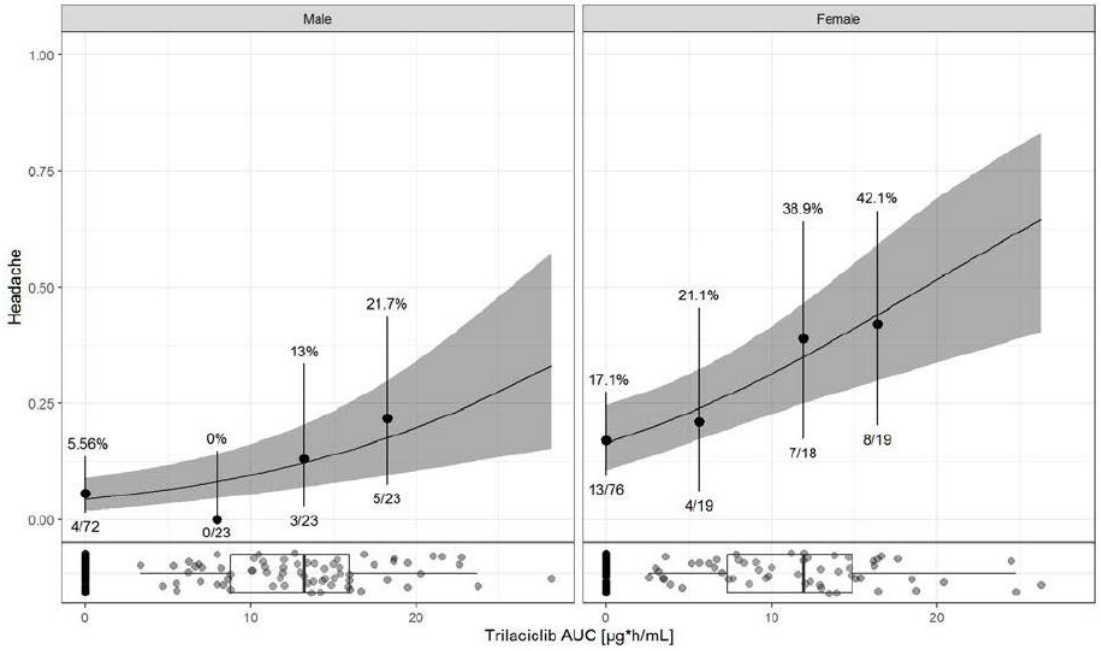
The applicant's exposure -vs - safety analyses are acceptable for describing relationships between trilaciclib exposures and side effects particularly headache and phlebitis but not injection site reaction. Using post-hoc trilaciclib AUC, the applicant assessed the relationship between Trilaciclib exposure and the following safety end points: headache, phlebitis, and injection site reactions. Statistical analyses identified statistically significant relationships between trilaciclib AUC and the investigated safety endpoints (p-value<0.05).

4.4.2 Applicant's exposure-safety analyses

4.4.2.1. Trilaciclib AUC vs headache

Assessments of trilaciclib AUC vs probability of headache was based on studies in small cell lung cancer patients (SCLC) (studies GIT28-02, GIT28-03, and GIT28-05), and in triple-negative breast cancer patients (TNBC) (study GIT28-04). The AUC vs probability of headache relationship was best described by a logistic regression model. Results are summarized in Figure 15. In addition to trilaciclib AUC, probability of headache was also dependent on sex with female subjects have 16.4% higher baseline probability of headache than male subjects. The odds for a 10 unit increment in AUC (μg▪h/mL) of trilaciclib relative to placebo treatment was 0.106 (which corresponds to a 9.6% higher probability of headache. It should be noted however that cumulative AUC was used in these analyses. Subjects with 5-days dosing (study GIT28-03) have relative higher AUC compared to those receiving 3-days dosing. The applicant did not find the chemotherapy combination (study effect) to be a significant predictor of headache in this analysis. It can be seen from the figure that the background rate of headache in this patient population is relatively high compared to the general population.

Figure 15. Relationship between AUC of trilaciclib and probability of headache

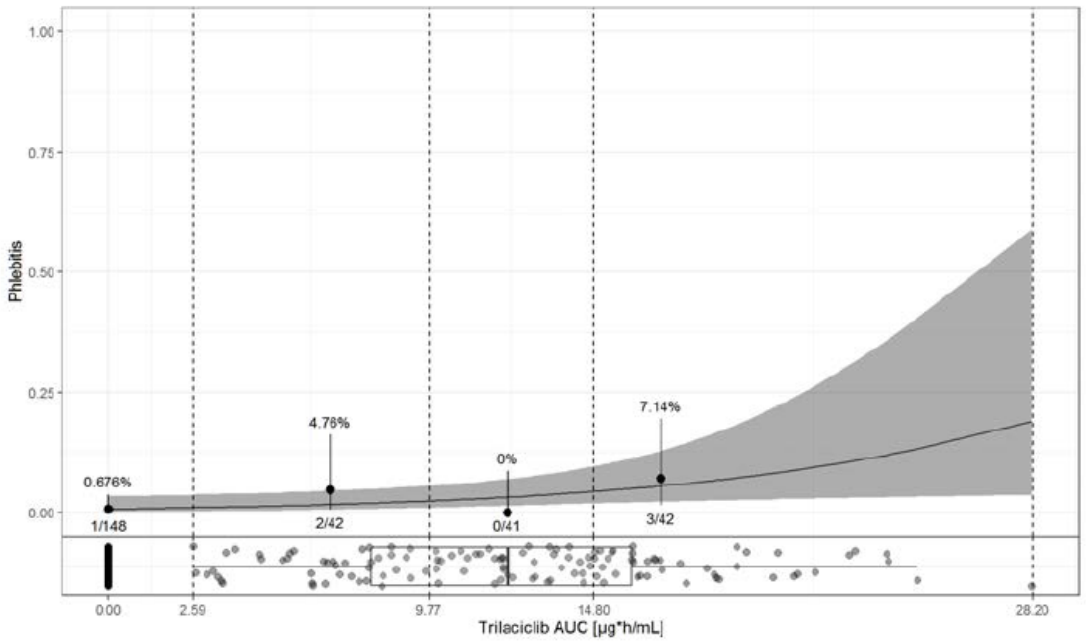


Source: Applicant's population PK and ER report (page 57)

4.4.2.2 Trilaciclib AUC vs phlebitis/thrombophlebitis

Assessments of trilaciclib AUC vs probability of phlebitis/thrombophlebitis was based on studies in SCLC and TNBC patients. Logistic regression model provided a better description of the relationship between AUC and probability of phlebitis/thrombophlebitis. Results are summarized in Figure 16. No other predictor of phlebitis was identified by this analysis. The odds of phlebitis in placebo treatment was 0.00730 (which corresponds to a 0.7% probability of phlebitis/ thrombophlebitis). The odds of phlebitis for a 10 unit increment in AUC (µg▪h/mL) of trilaciclib relative to the placebo treatment was 0.0250 which corresponds to a 2.4% higher probability of phlebitis/thrombophlebitis.

Figure 16. Relationship between AUC of trilaciclib and probability of phlebitis/thrombophlebitis

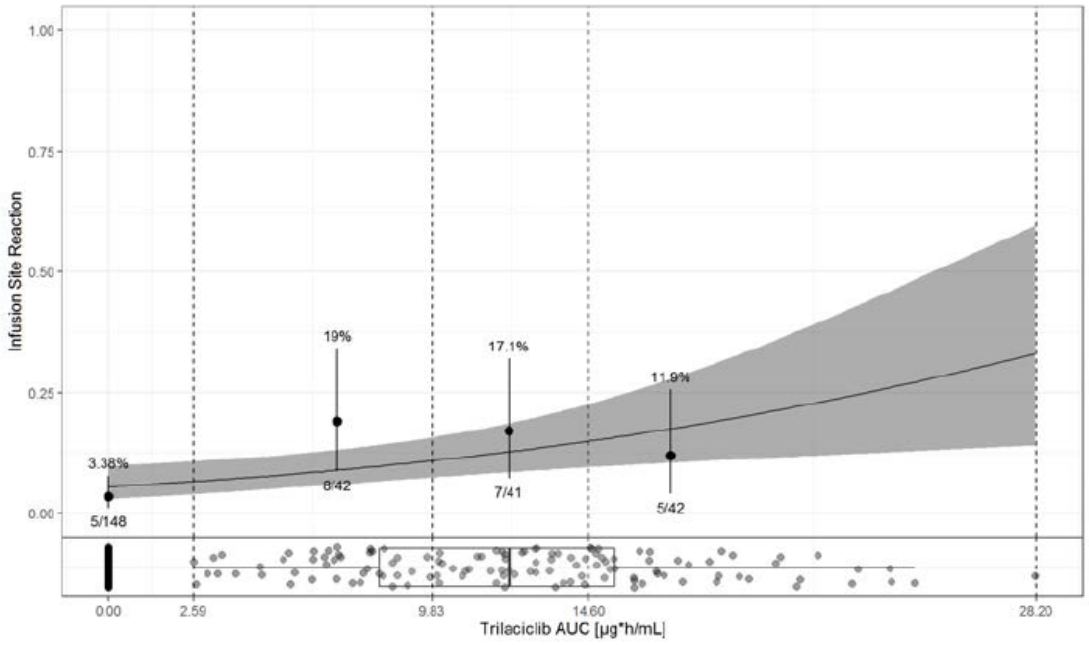


Source: Applicant's population PK and ER report (page 58)

4.4.2.3. Trilaciclib AUC vs injection-site reaction

Assessments of trilaciclib AUC vs probability of Injection-site reaction was based on studies in SCLC and TNBC patients. Logistic regression model provided a better description of the relationship between AUC and probability of injection-site reaction. Results are summarized in Figure 17. No other predictor of injection-site reaction was identified by this analysis. The odds of injection-site reaction in placebo treatment was 0.0578 (which corresponds to a 5.5% probability of injection site reaction). The odds of injection-site reaction for a 10 unit increment in AUC (µg▪h/mL) of trilaciclib relative to the placebo treatment was 0.124 which corresponds to a 11.0% higher probability of injection-site reaction. However, despite the statistical significance, this positive correlation lacks physiological explanation. In fact, as seen in Figure 17, there seems to be no positive relationship between proportion of injection-site reactions and trilaciclib AUC if patients on placebo are excluded from the analysis. Nevertheless, the plot indicates higher incidence of injection-site reaction among subjects on trilaciclib than subjects on placebo which is biologically plausible; trilaciclib may induce reactions at the site of administration which is independent of the final extent of exposure (AUC) to the drug.

Figure 17. Relationship between AUC of trilaciclib and probability of injection-site reaction



Source: Applicant's population PK and ER report (page 60)

4.4.3. Reviewer's comments

The applicant's analyses are acceptable for making conclusions on exposure-safety relationships at the recommended trilaciclib doses. Although significant relationships are identified by these analyses, the side effects were tolerated by most patients, did not lead to drug discontinuations, and were reversible after completing treatment within a chemotherapy cycle. Therefore, dose adjustments are not necessary.

Appendix 4.5 Outstanding Review Issues

4.5.1 Plasma protein binding

The plasma protein binding of trilaciclib has been determined in two individual studies with different results: 90.1% to 91.3% in Study (b) (4)-221501 and 71.7% in Study 20G1THP2. The applicant explained that the plasma protein binding value (71.7%) determined in Study 20G1THP2 should override the result from Study (b) (4)-221501 because the buffer in Study 20G1THP2 was modified and improved. An information request was sent to the applicant to provide further justification for choosing one study over the other, and why the report for Study 20G1THP2 lacked details regarding the performance of the plasma protein assay.

The applicant, in their response, justified that the (b) (4)-221501 study was conducted with a phosphate buffer strength of 10 mM in accordance with the vendor's SOP. This is lower than the device manufacturer's as well as industry-standard recommended buffer strength of 100 mM for the type of assay used (i.e., rapid equilibrium dialysis). This issue was identified after the submission of the nonclinical data in NDA Sequence 0001 on 11/20/2019. Therefore, the assay was repeated with 100 mM phosphate buffer by a different vendor in Study 20G1THP2. In addition, the applicant justified that plasma stability, time to equilibrium, and nonspecific binding have been established in Study (b) (4)-221501, and there was not a need to repeat these assays.

Regardless of the difference in buffer strength between the two studies, the review team found that there are deficiencies in both studies that preclude reporting a value in the product insert:

- Study (b) (4)-221501: As shown in Table 10, the concentration of trilaciclib did not reach equilibrium after 6 hours of dialysis at 50 µg/mL in the assay to determine equilibrium dialysis incubation time. The applicant subsequently conducted a plasma protein binding assay following 6 hours of equilibration time in triplicate at 3 concentrations: 0.5, 5 and 50 µg/mL, and the reported human plasma protein binding ranged from 90.1% to 91.3% . However, based on the trend seen from Table 1, it may be possible that the reported plasma protein binding evaluated at 6 hours is an overestimation.
- Study 20G1THP2: The plasma stability, time to equilibrium, and nonspecific binding were not evaluated in Study 20G1THP2, and thus it is not possible to evaluate the performance and robustness of this modified plasma protein binding assay.

Table 10. Determination of trilaciclib equilibrium dialysis time in Study (b) (4)-221501

Species	Incubation Time (h)	Mean concentration (µg/mL)		Percent Bound	Percent recovery (%)
		Plasma	Dialysis Buffer		
Human	0.5	46.8	0.476	99.0	94.8
	1	44.3	0.965	97.8	90.8
	3	38.3	3.19	91.7	83.1
	6	30.5	5.05	83.4	71.3

Percent Bound = (Plasma Concentration - Dialysis Buffer Concentration)/Plasma Concentration × 100 Percent Recovery = (Plasma Concentration + Dialysis Buffer Concentration)/Starting Concentration ×100
Source: Nonclinical study report (b) (4)*-221501*

Due to deficiencies will both the studies, the review team recommends a post-marketing commitment (PMC) for conducting a new plasma protein binding assay with appropriate validation of plasma stability, time to equilibrium and nonspecific binding.

4.5.2 The metabolic pathway for trilaciclib

The metabolism of trilaciclib has been assessed in multiple hepatocyte and microsome studies. However, there are deficiencies in some of these in vitro studies that also yielded contradictory results. While one set of studies showed trilaciclib is metabolically stable, the other studies showed disappearance of the parent drug, suggesting hepatic metabolism of trilaciclib. The results are summarized below:

- Studies showing trilaciclib was stable in in vitro metabolism studies
  - Study 8320101: 89.9% of trilaciclib remained unchanged after incubation with human hepatocytes by 120 min.
  - Study 8320098 (CYP phenotyping study): The integrated peak area for trilaciclib did not decrease following incubating with human liver microsomes and recombinant enzymes.
- Studies showing trilaciclib was metabolized in in vitro metabolic studies
  - Study 14F220G1TH: By 60 min and 120 min, 77% and 61% of G1T28-1 remained unchanged after incubation with human hepatocytes, with estimated $t_{1/2}$ of 165 min
  - Study 13F106G1TH: By 60 min, 69.5% of trilaciclib remained unchanged after incubation with liver microsomes, with estimated $t_{1/2}$ of 119 min.
  - Study 13F120G1TH: By 60 min, 56% of trilaciclib remained unchanged after incubation with liver microsomes, with estimated $t_{1/2}$ of 66 min

In spite of the discrepancy noted above, the applicant postulated that trilaciclib is hepatically metabolized and CYP3A4 is the primary enzyme involved in its metabolic pathway. While it is clear from human mass balance study that trilaciclib is predominantly hepatically metabolized, the review team disagrees with the role for CYP3A4 in trilaciclib metabolism. It is noted from completed drug interaction studies (Studies G1T28-09 and G1T28-114) that the exposure to trilaciclib is not altered when coadministered with itraconazole, a strong CYP3A inhibitor. Consequently, the team believes that the major metabolizing enzyme contributing to the formation of M8 from trilaciclib is yet to be determined.

An information request was sent to the applicant to clarify the uncertainty in the role of CYP3A4 as the primary metabolizing enzyme. The applicant explained that M8 as a major metabolite in human systemic circulation was discovered recently in the human mass-balance study G1T28-08, and the structure of M8 has not been fully characterized at this point. The applicant proposed that some non-CYP enzymes may be involved in the formation of M8, such as flavin-containing monooxygenase (FMO) or aldehyde oxidase (AO) enzymes. Another possibility is that trilaciclib is oxidized by gut bacteria/microbes to M8 after being excreted/effluxed directly into the

gastrointestinal tract, with a small fraction of M8 absorbed into systemic circulation. All these hypotheses are yet to be determined.

The review team acknowledges these possibilities of alternative metabolic pathways for M8. However, these hypotheses cannot address the contradictory findings in the in vitro metabolic studies listed above. Specifically, in the CYP phenotyping study (8320098), the sponsor did not explain 1) whether metabolites other than M2 and M6 (e.g., M8) were formed or not, possibly due to unawareness of other potential metabolites, and 2) why the parent drug was stable in this study. It is also noted that the trilaciclib concentration (15 μM) used in this study was 10-15 fold higher than clinically relevant concentration, which may lead to a different metabolic profile than observed in vivo due to concentration dependent kinetics.

Overall, the review team concludes that the major metabolic enzyme(s) involved in trilaciclib's metabolism has not been adequately determined. A PMR is proposed for conducting an in vitro metabolism study and CYP phenotyping study at clinically relevant concentrations to determine major metabolic pathway for trilaciclib and to characterize the formation of the major circulating metabolite of trilaciclib, M8, using the purified M8 compound with a validated bioanalytical method.

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