TRILACICLIB INDUCES IMMUNE CHANGES WITHIN THE TUMOR MICROENVIRONMENT IN EARLY-STAGE TRIPLE-NEGATIVE BREAST CANCER

Michael Danso[1]; Joyce O'Shaughnessy[2]; Lisa Wang[3]; Kailash Mosalpuria[4]; Sara A. Hurvitz[5]; Shom Goel[6]; Sarah Ahn[7]; Subing Cao[7]; John S. Yi[7]; Taofik Oyekunle[7]; Amanda Jacobson[7]; Andrew Beelen[7]; and Jeremy Force[8]

[1] Virginia Oncology Associates, Norfolk and Virginia Beach, VA, USA; [2] Texas Oncology-Baylor Charles A. Sammons Cancer Center, Dallas, TX, USA; [3] PIH Health Whittier Hospital, Whittier, CA, USA; [4] Nebraska Hematology-oncology P.C., Lincoln, NE, USA; [5] Division of Hematology/Oncology, David Geffen School of Medicine at UCLA/Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA; [6] The Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia; [7] G1 Therapeutics, Inc., Research Triangle Park, NC, USA; [8] Department of Medicine, Duke University, Durham, NC, USA

INTRODUCTION

- In early-stage triple-negative breast cancer (TNBC), there is accumulating evidence of a correlation between tumor-infiltrating lymphocytes in tumor tissue and favorable clinical outcomes, with a high CD8+/regulatory T-cell (Treg) ratio after neoadjuvant chemotherapy being predictive of overall survival and associated with pathologic complete response (pCR)[1,2]
- Administering trilaciclib (COSELA™; G1 Therapeutics, Inc.) prior to chemotherapy results in the transient arrest of cyclin-dependent kinase (CDK) 4/6–dependent hematopoietic stem and progenitor cells and immune cells in the G_1 phase of the cell cycle, thus protecting these cells from chemotherapy-induced damage and modulating antitumor immunity (**Figure 1**)[3–5]
- In preclinical studies, trilaciclib has been shown to enhance antitumor immunity by differentially arresting CD8+ T-cell and Treg subsets, characterized by a faster recovery of proliferation in CD8+ T cells compared with Tregs[5]
- In an open-label phase 2 trial for patients with metastatic TNBC (NCT02978716), administering trilaciclib prior to gemcitabine plus carboplatin (GCb) prolonged overall survival (a key secondary endpoint) compared with administering GCb alone (median 19.8 vs 12.6 months; $P < 0.0001$)[6,7]
  - Administering trilaciclib resulted in enriched T-cell diversity and decreased clonality in peripheral blood[7]
- This study was designed to determine an immune-based mechanism of action for neoadjuvant trilaciclib monotherapy followed by trilaciclib administered with standard-of-care treatment in patients with early-stage TNBC
  - Enrollment is complete

Figure 1. Proposed Immune Mechanism of Action of Trilaciclib

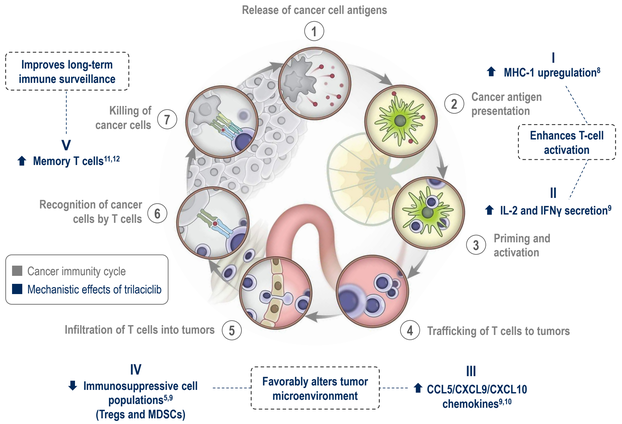
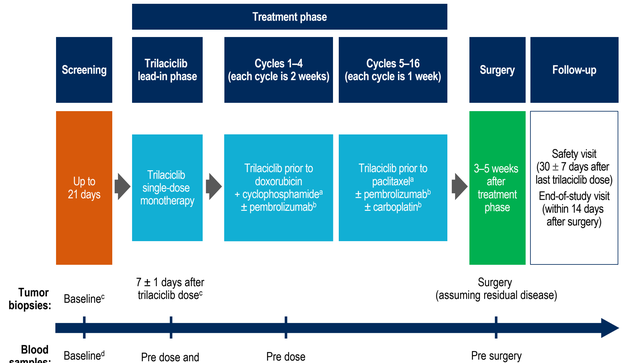


Figure adapted with permission from Chen and Mellman (2013).[13]
CCL, chemokine ligand; CXCL, C-X-C motif chemokine ligand; IFNγ, interferon gamma; IL-2, interleukin 2; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; Treg, regulatory T cell.

METHODS

- The aims of this phase 2, single-arm, open-label, neoadjuvant study are to:
  1) Evaluate the impact of a single dose of trilaciclib on the immune microenvironment of early-stage TNBC, as measured by changes in the CD8+ T-cell/Treg ratio in tumor tissue (primary objective); and
  2) Assess additional exploratory immune biomarker endpoints, safety and tolerability, and pCR of trilaciclib plus dose-dense anthracycline/cyclophosphamide (AC) and taxane (T), with the option to add carboplatin and/or pembrolizumab per investigator discretion, in patients with early-stage TNBC (NCT05112536)
- Eligible patients had previously untreated, early-stage, confirmed TNBC (estrogen/progesterone receptor < 1% and human epidermal growth factor receptor 2–negative, per the American Society of Clinical Oncology/College of American Pathologists guidelines) and a primary tumor ≥ 1.5 cm with any nodal status, for which treatment with neoadjuvant dose-dense AC/T was suitable, and patients intended to undergo curative surgery
- Patients received a single dose of trilaciclib 240 mg/m^2 intravenous (IV) during the lead-in phase
- Systemic therapy began approximately 7 days after the single dose of trilaciclib and consisted of 4 cycles of doxorubicin 60 mg/m^2 IV plus cyclophosphamide 600 mg/m^2 IV every 2 weeks, followed by 12 weekly cycles of paclitaxel 80 mg/m^2 IV; pembrolizumab 400 mg IV every 6 weeks starting on cycle 1, day 1, and/or carboplatin AUC 1.5 IV every week starting on cycle 5, day 1, was allowed per investigator discretion (**Figure 2**)
  - Trilaciclib 240 mg/m^2 IV was administered prior to the first dose of systemic therapy for each cycle
  - Tumor biopsies and peripheral blood samples were collected prior to any treatment, 7 (±1) days post administration of trilaciclib monotherapy, and before surgery, with an additional blood sample collection on cycle 2, day 1
- Biopsies were prepared and analyzed using the following methods:
  - Formalin-fixed, paraffin-embedded 4-µm tissue sections were stained with hematoxylin and eosin for histological analysis, or with separate CD8 and FoxP3 antibodies for immunohistochemistry analysis
  - The relative area and density of CD8+ and FoxP3+ cells in the central tumor were quantified using Visiopharm® software

Figure 2. Study Design



[a] G-CSF was administered after each dose of doxorubicin + cyclophosphamide per investigator discretion; prophylactic G-CSF use during treatment with paclitaxel was allowed.
[b] Per investigator discretion.
[c] Tumor samples were assessed by IHC to analyze changes in the CD8+ T-cell/Treg ratio, and by RNA sequencing.
[d] PBMC samples were assessed by CyTOF and ICS.
C, cycle; CD8, cluster of differentiation 8; CyTOF, cytometry by time of flight; D, day; G-CSF, granulocyte colony-stimulating factor; ICS, intracellular cytokine staining; IHC, immunohistochemistry; PBMC, peripheral blood mononuclear cell; Treg, regulatory T cell.

RESULTS

Patient Disposition and Characteristics

- As of September 21, 2022, patients (N = 24) had received a median (range) of 7 (3–16) cycles of treatment, 19 (79%) patients had completed cycles 1–4, and evaluable paired biopsies were available for 23/24 patients
  - 21 (87.5%) patients had received pembrolizumab starting at cycle 1, and 17 (70.8%) patients had received carboplatin starting at cycle 5, per investigator discretion
  - Treatment is ongoing for 23/24 patients: 1 patient with TNBC and neuroendocrine features discontinued at cycle 3 owing to progressive disease
- Baseline patient demographics and clinical characteristics are summarized in **Table 1**

Table 1. Demographic and Clinical Characteristics at Baseline

Characteristic		Patients With Early-Stage TNBC (N = 24)
Age	Median (range), years	57 (32–80)
	18–65 years, n (%)	22 (91.7)
	> 65 years, n (%)	2 (8.3)
Sex, n (%)	Male	0
	Female	24 (100)
Race, n (%)	White	17 (70.8)
	Black or African American	5 (20.8)
	Asian	2 (8.3)
ECOG PS, n (%)	0	22 (91.7)
	1	2 (8.3)
Stage at diagnosis, n (%)	I	2 (8.3)
	II	19 (79.2)
	III	3 (12.5)
Histological grade, n (%)	1	1 (4.2)
	2	3 (12.5)
	3	20 (83.3)
Histopathological type at diagnosis, n (%)	Ductal	21 (87.5)
	Lobular	1 (4.2)
	Other[a]	2 (8.3)
PD-L1 status, n (%)[b,c]	Negative	15 (62.5)
	Positive	9 (37.5)
***BRCA* mutation status (n/N known)[b]**	Negative	7/8
	BRCA1/2	1/8

[a] Includes 1 patient with neuroendocrine differentiation and 1 patient with invasive, poorly differentiated disease (clinically grouped with ductal disease).
[b] PD-L1 and *BRCA* mutation status were not required per protocol.
[c] PD-L1 status was determined from submitted baseline tumor samples with the Ventana SP142 PD-L1 assay (positivity defined as ≥ 1% immune cells).
BRCA(1/2), breast cancer gene (*1/2*); ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death-ligand 1; TNBC, triple-negative breast cancer.

Safety and Tolerability

- During the trilaciclib lead-in phase, approximately half (54.2%) of patients had an adverse event (AE; any causality)
  - Trilaciclib-related AEs (all grade 1/2) included headache (3 [12.5%] patients), nausea (2 [8.3%] patients), and metallic taste in mouth, flushing, hypersensitivity reaction, and mouth sores (1 [4.2%] patient each)
- Treatment-related AEs (TRAEs) that occurred during the treatment period are summarized in **Table 2**
  - The most common TRAEs (any grade) were fatigue (79.2%), nausea (62.5%), alopecia (62.5%), and neutropenia (50.0%)
  - Grade 3/4 TRAEs occurring in ≥ 2 patients were neutropenia (33.3%) and anemia (8.3%)
- Serious AEs related to any study treatment occurred in 2 (8.3%) patients
  - 1 patient had 1 event of febrile neutropenia related to doxorubicin and cyclophosphamide, and 1 event of increased transaminases related to pembrolizumab
  - 1 patient had colitis related to pembrolizumab
- Treatment cycle delays due to hematologic events occurred in 9 (37.5%) patients, and chemotherapy dose reductions occurred in 3 (12.5%) patients
- AEs leading to treatment discontinuation of study drug occurred in 2 (8.3%) patients, both resulting in discontinuation of pembrolizumab only
  - 1 patient had colitis and new primary lung neoplasm and 1 patient had transaminitis; both patients continued with the remaining chemotherapy regimen

Biomarker Assessments

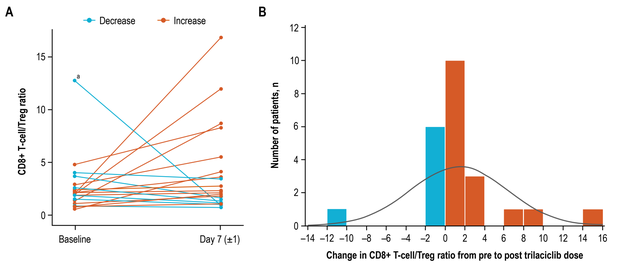
- Paired tumor biopsies, from baseline and from 7 (±1) days post trilaciclib monotherapy, were available for 23 patients
- For all patients with available paired biopsy data, the median CD8+ T-cell/Treg ratio was 2.0 at baseline compared with 2.2 at 7 (±1) days post trilaciclib monotherapy (median change, 0.3 [$P = 0.101$]; mean change, 1.3; **Figure 3**)
  - In patients with lobular or invasive ductal TNBC (n = 22), the median change in CD8+ T-cell/Treg ratio was 0.3 ($P = 0.033$)
  - In the patient who had TNBC with neuroendocrine features, and subsequently discontinued the study on day 6 of cycle 3, there was an overall decrease in the CD8+ T-cell/Treg ratio in the tumor microenvironment

Table 2. TRAEs Related to Any Study Drug Occurring in ≥ 15% of Patients During the Treatment Phase

	Any Grade (N = 24)	Grade 3/4 (N = 24)
Patients with TRAE, n (%)	24 (100)	10 (41.7)
Fatigue	19 (79.2)	0
Nausea	15 (62.5)	0
Alopecia	15 (62.5)	0
Neutropenia[a]	12 (50.0)	8 (33.3)
Dysgeusia	8 (33.3)	0
Headache	7 (29.2)	0
Anemia	6 (25.0)	2 (8.3)
Stomatitis	5 (20.8)	0
Decreased appetite	5 (20.8)	0
Constipation	4 (16.7)	0
Diarrhea	4 (16.7)	0
Neuropathy peripheral	4 (16.7)	1 (4.2)

[a] Includes preferred terms of neutropenia and decreased neutrophil count; 4 patients had grade 4 events.
TRAE, treatment-related adverse event.

Figure 3. Change in the CD8+ T-Cell/Treg Ratio in Tumor Tissue From Baseline to 7 (±1) Days Post Trilaciclib Single-Dose Monotherapy (N = 23), per Patient (A) and Overall (B)



CD8+ T-Cell/Treg Ratio	Baseline, Pre Trilaciclib Dose (n = 23)	Day 7 (±1), Post Trilaciclib Dose (n = 23)	Change, Post – Pre Dose (n = 23)
Median (Q1, Q3)	2.0 (1.4, 2.6)	2.2 (1.4, 4.0)	0.3 (–0.5, 2.7)
P-value[b]	-	-	0.101
Mean (SD)	2.6 (2.436)	3.8 (3.979)	1.3 (4.784)

[a] Patient had TNBC with neuroendocrine features.
[b] Calculated using the Wilcoxon signed-rank test.
CD8, cluster of differentiation 8; Q, quarter; TNBC, triple-negative breast cancer; Treg, regulatory T cell.

CONCLUSIONS

- Safety and tolerability data are encouraging for trilaciclib in combination with AC/T ± pembrolizumab ± carboplatin in the neoadjuvant setting for early-stage TNBC
  - Clinical efficacy will be assessed once patients have completed curative surgery
- A trend toward increased CD8+ T-cell/Treg ratios within the tumor microenvironment was observed following treatment with a single 240 mg/m^2 IV dose of trilaciclib
  - An exploratory analysis of patients with lobular/invasive ductal TNBC showed a significant increase in CD8+ T cells and/or a decrease in Tregs
  - Together, this evidence supports previous data suggesting that trilaciclib enhances T-cell infiltration
- The data support the hypothesis that transient CDK4/6 inhibition by trilaciclib may modulate the composition of immune cells in the tumor microenvironment to support antitumor immune responses
- Correlative biomarker analysis is underway to assess RNA signatures in tumor biopsies, and peripheral phenotype and function using high-dimensional immune cell profiling

San Antonio Breast Cancer Symposium (SABCS)
December 6–10, 2022 | San Antonio, TX, USA

REFERENCES:
1. Ladoire S, et al. *Br J Cancer*. 2011;105:366–71.
2. Park YH, et al. *Nat Commun*. 2020;11:6175.
3. COSELA™ (trilaciclib). Prescribing Information. https://www.g1therapeutics.com/cosela/pi/. Accessed October 17, 2022.
4. He S, et al. *Sci Transl Med*. 2017;9:eaal3986.
5. Lai A, et al. *J Immunother Cancer*. 2020;8:e000847.
6. Tan AR, et al. *Lancet Oncol*. 2019;20:1587–601.
7. Tan AR, et al. *Clin Cancer Res*. 2022;28:629–36.
8. Goel S, et al. *Nature*. 2017;548:471–5.
9. Deng J, et al. *Cancer Discov*. 2018;8:216–33.
10. Uzhachenko RV, et al. *Cell Rep*. 2021;35:108944.
11. Lelliott EJ, et al. *Cancer Discov*. 2021;11:2582–601.
12. Heckler M, et al. *Cancer Discov*. 2021;11:2564–81.
13. Chen DS, Mellman I. *Immunity*. 2013;39:1–10.

ACKNOWLEDGMENTS:
- We thank all the investigators and site staff, with special thanks to the patients and their families.
- Study sponsored by G1 Therapeutics, Inc. Medical writing assistance was provided by Alligent Europe (Envision Pharma Group), funded by G1 Therapeutics, Inc.