NEOADJUVANT SINGLE-DOSE TRILACICLIB PRIOR TO COMBINATION CHEMOTHERAPY IN PATIENTS WITH EARLY TRIPLE-NEGATIVE BREAST CANCER: SAFETY, EFFICACY, AND IMMUNE CORRELATE DATA FROM A PHASE 2 STUDY

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INTRODUCTION

- In early-stage triple-negative breast cancer (TNBC), there is accumulating evidence of a correlation between tumor-infiltrating lymphocytes (TILs) in tumor tissue and favorable clinical outcomes, with a high CD8+/regulatory T cell (Treg) ratio after neoadjuvant chemotherapy being predictive of overall survival (OS) 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 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 prolonged OS (a key secondary endpoint) compared with administering gemcitabine plus carboplatin alone (median 19.8 vs 12.6 months; $P < 0.0001$), regardless of programmed death-ligand 1 (PD-L1) status[6,7]
  - Enriched T-cell diversity and decreased clonality in peripheral blood were observed in trilaciclib-treated patients[7]
  - Meaningful benefit in OS rather than in progression-free survival suggests that trilaciclib may support the differentiation of memory CD8+ T cells for long-term efficacy
- Data from the current phase 2, single-arm, open-label study of neoadjuvant trilaciclib in TNBC (NCT05112536) showed that single-dose trilaciclib increased CD8+ T cell/Treg ratios within the tumor microenvironment (TME)[8]
- Here, we combine immune analysis with clinical outcomes to identify correlates of treatment response and present final clinical data

Figure 1. Proposed Immune Mechanism of Action of Trilaciclib

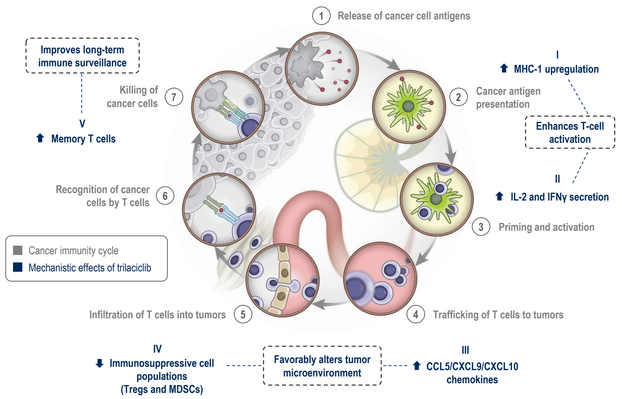
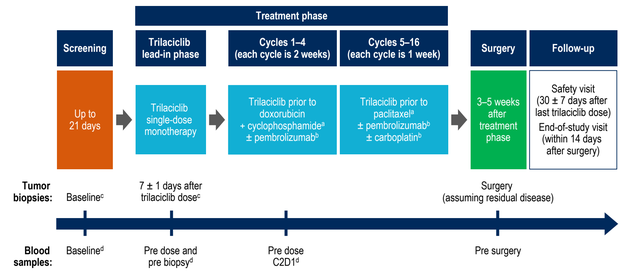


Figure adapted with permission from Chen and Mellman (2013).[9]
Roman numerals (I–V) show trilaciclib-induced modulation of the immune system.
CCL, chemokine ligand; CXCL, C-X-C motif chemokine ligand; IFNγ, interferon gamma; IL-2, interleukin 2; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; Treg, regulatory T cell.

METHODS

- The aims of this 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
- Eligible patients had previously untreated, early-stage, confirmed TNBC (estrogen/progesterone receptor < 1%, human epidermal growth factor receptor 2–negative, per the American Society of Clinical Oncology/College of American Pathologists guidelines) and a primary tumor of ≥ 1.5 cm with any nodal status, for which treatment with neoadjuvant dose-dense anthracycline/cyclophosphamide (AC) and taxane (T) was suitable, and for which patients intended to undergo curative surgery
- Patients received a single dose of intravenous (IV) trilaciclib 240 mg/m^2 during the lead-in phase. Systemic therapy began ~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. Trilaciclib 240 mg/m^2 IV was administered prior to the first dose of systemic therapy for each cycle (**Figure 2**)
- pCR was assessed at definitive surgery by a local pathologist, per the current American Joint Committee on Cancer staging system (ie, absence of invasive cancer in the breast and axillary nodes [ypT0/Tis ypN0])
- Biopsies from baseline and day 7 following trilaciclib monotherapy were prepared and analyzed using the following methods:
  - Formalin-fixed, paraffin-embedded (FFPE) 4-µm tissue sections were separately stained for hematoxylin and eosin, pan-cytokeratin-CD8, forkhead box P3 (FOXP3), PD-L1, and CD8/granzyme B (GZMB)/FOXP3 multiplex for immunohistochemical analysis (CellCarta)
  - RNA isolated from FFPE tissue was sequenced using the Tempus RNA sequencing (RNA-seq) assay[10]; differentially expressed genes were identified using the DESeq2 package[11] and gene set enrichment analysis was performed using GSEA_4.1.0 software[12,13]

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; 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 April 3, 2023, patients (N = 24) had received a median (range) of 16 (3–16) cycles of treatment
  - 21 (87.5%) patients received pembrolizumab, and 21 (87.5%) patients received carboplatin, per investigator discretion
  - All patients completed the study; 5 patients underwent definitive surgery prior to completing planned study treatment (1 patient with TNBC and neuroendocrine features discontinued at cycle 3 owing to progressive disease)
- Baseline patient demographics and clinical characteristics have been previously reported[8]
  - Median (range) age was 57 (32–80) years; 71%, 21%, and 8% of patients were White, Black, and Asian, respectively; most patients (67%) were postmenopausal
  - At diagnosis, 79% of patients had stage II tumors and 88% had ductal carcinoma; 38% of patients had PD-L1+ tumors (assessed with the Ventana SP142 PD-L1 assay; positivity defined as ≥ 1% immune cells)

Safety and Tolerability

- During the trilaciclib lead-in phase, approximately half (54.2%) of the patients had an adverse event (AE; any causality); all trilaciclib-related AEs were grade 1/2
- Treatment-related AEs (TRAEs) are summarized in **Table 1**
  - The most common TRAEs (any grade) were fatigue (83.3%), nausea (66.7%), alopecia (62.5%), and neutropenia/neutrophil count decreased (58.3%)
  - Grade 3/4 TRAEs occurring in ≥ 2 patients were neutropenia (41.7%), anemia (8.3%), and peripheral neuropathy (8.3%)
- Serious AEs related to any study treatment occurred in 2 (8.3%) patients
  - 1 patient had febrile neutropenia related to doxorubicin and cyclophosphamide, and hypertransaminasemia related to pembrolizumab
  - 1 patient had colitis related to pembrolizumab, physical deconditioning related to paclitaxel, and urosepsis and pulmonary fibrosis related to trilaciclib and paclitaxel
- Treatment cycle delays due to hematologic events occurred in 13 (54.2%) patients and chemotherapy dose reductions occurred in 8 (33.3%) patients
- AEs leading to discontinuation of study drug occurred in 3 (12.5%) patients, all of whom continued with the remaining regimen of trilaciclib prior to chemotherapy
  - 1 patient with an AE of increased alanine transaminase leading to discontinuation of paclitaxel
  - 2 patients with AEs leading to discontinuation of pembrolizumab: 1 patient with colitis and lung neoplasm malignant, and 1 patient with hypertransaminasemia

Table 1. TRAEs Related to Any Study Drug Occurring in ≥ 20% of Patients During the Treatment Phase

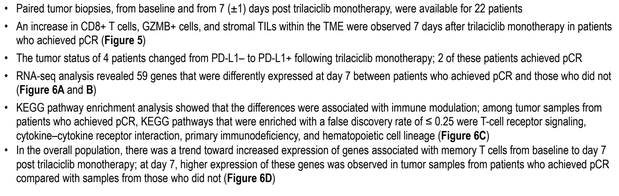
	Any Grade (N = 24)	Grade 3/4 (N = 24)
Patients with TRAE, n (%)	24 (100)	10 (41.7)
Fatigue	20 (83.3)	0
Nausea	16 (66.7)	0
Alopecia	15 (62.5)	0
Neutropenia[a]	14 (58.3)	10 (41.7)
Anemia	10 (41.7)	2 (8.3)
Neuropathy peripheral	10 (41.7)	2 (8.3)
Stomatitis	9 (37.5)	0
Dysgeusia	8 (33.3)	0
Decreased appetite	7 (29.2)	0
Diarrhea	7 (29.2)	0
Constipation	6 (25.0)	0
Headache	5 (20.8)	0

[a] Includes patients who had a TRAE of neutropenia and/or neutrophil count decreased.
TRAE, treatment-related adverse event.

Baseline Correlates of Clinical Outcome

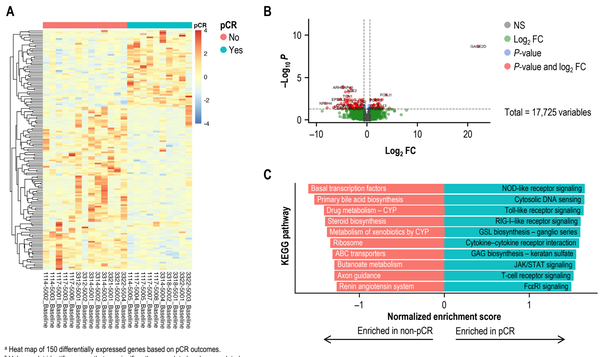
- All 24 patients underwent surgery, and residual tumor was assessed; pCR (ypT0/Tis ypN0) in the total study population and by subgroup at baseline is presented in **Figure 3**
  - pCR was achieved in 10 (41.7%; 95% CI, 22.1–63.4) patients, of whom 9 received pembrolizumab
  - pCR rates were increased in patients who had PD-L1+ tumors (77.8%) at baseline compared with those who had PD-L1– tumors (20.0%)
  - The pCR rate was higher in patients with an immune-inflamed TME (75.0%) than in those with immune-excluded (35.7%) or immune-desert (33.3%) TMEs
- At baseline, RNA-seq analysis revealed 150 genes that were differently expressed based on pCR outcomes (**Figure 4A** and **B**); the transcriptional differences observed were not associated with pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways database (**Figure 4C**)
- There was a trend toward increased numbers of CD8+ T cells and GZMB+ cells in baseline tumor samples among patients who achieved pCR compared with those who did not (**Figure 5A** and **B**)

Figure 3. pCR by Baseline Disease and Tumor Characteristics



[a] pCR was achieved in 6/7 (86%) patients with PD-L1+ tumors who received pembrolizumab.
pCR, pathologic complete response; PD-L1, programmed death-ligand 1.

Figure 4. Differential Gene Expression at Baseline, Between Patients Who Achieved pCR and Those Who Did Not: (A) Heat Map[a]; (B) Volcano Plot[b]; and (C) Gene Set Enrichment Analysis[c]

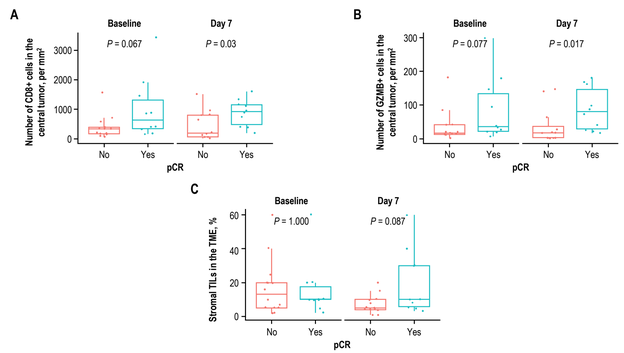


[a] Heat map of 150 differentially expressed genes based on pCR outcomes.
[b] Volcano plot identifies genes that are significantly upregulated or downregulated.
[c] Gene set enrichment analysis of the top 10 pathways differentially expressed at baseline.
ABC, adenosine triphosphate-binding cassette; CYP, cytochrome P450; FC, fold change; FcεRI, high-affinity immunoglobulin E receptor; GAG, glycosaminoglycan; GSL, glycosphingolipid; JAK/STAT, Janus kinase/signal transducers and activators of transcription; KEGG, Kyoto Encyclopedia of Genes and Genomes; NOD, nucleotide oligomerization domain; NS, not significant; pCR, pathologic complete response; RIG-I, retinoic acid-inducible gene I.

Immunomodulatory Effects of Trilaciclib (Changes From Baseline to 7 Days Post Trilaciclib Monotherapy)

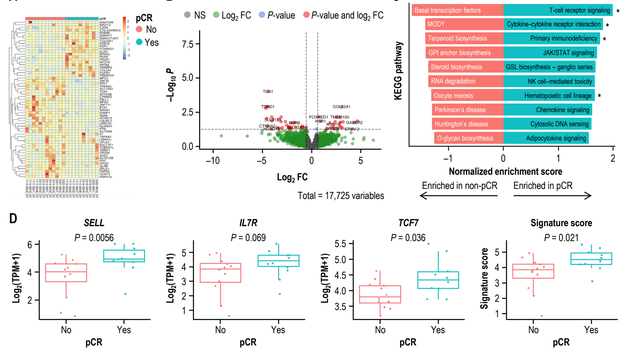
- Paired tumor biopsies, from baseline and from 7 (±1) days post trilaciclib monotherapy, were available for 22 patients
- An increase in CD8+ T cells, GZMB+ cells, and stromal TILs within the TME were observed 7 days after trilaciclib monotherapy in patients who achieved pCR (**Figure 5**)
- The tumor status of 4 patients changed from PD-L1– to PD-L1+ following trilaciclib monotherapy; 2 of these patients achieved pCR
- RNA-seq analysis revealed 59 genes that were differently expressed at day 7 between patients who achieved pCR and those who did not (**Figure 6A** and **B**)
- KEGG pathway enrichment analysis showed that the differences were associated with immune modulation; among tumor samples from patients who achieved pCR, KEGG pathways that were enriched with a false discovery rate of ≤ 0.25 were T-cell receptor signaling, cytokine–cytokine receptor interaction, primary immunodeficiency, and hematopoietic cell lineage (**Figure 6C**)
- In the overall population, there was a trend toward increased expression of genes associated with memory T cells from baseline to day 7 post trilaciclib monotherapy; at day 7, higher expression of these genes was observed in tumor samples from patients who achieved pCR compared with samples from those who did not (**Figure 6D**)

Figure 5. Change in Levels of CD8+ T Cells (A), GZMB+ Cells (B), and Stromal TILs (C) in the TME, From Baseline to 7 (±1) Days Post Trilaciclib Single-Dose Monotherapy



GZMB, granzyme B; pCR, pathologic complete response; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment.

Figure 6. Differential Gene Expression at Day 7 Post Trilaciclib Monotherapy, Between Patients Who Achieved pCR and Those Who Did Not: (A) Heat Map[a]; (B) Volcano Plot[b]; (C) Gene Set Enrichment Analysis[c]; and (D) Gene Signature Analysis of Memory T Cells[d]



[a] Heat map of 59 differentially expressed genes based on pCR outcomes.
[b] Volcano plot identifies genes that are significantly upregulated or downregulated.
[c] Gene set enrichment analysis of the top 10 pathways differentially expressed at day 7.
[d] Expression of genes associated with memory T cells in tumor samples.
* False discovery rate ≤ 0.25.
FC, fold change; GPI, glycosylphosphatidylinositol; GSL, glycosphingolipid; JAK/STAT, Janus kinase/signal transducers and activators of transcription; KEGG, Kyoto Encyclopedia of Genes and Genomes; MODY, maturity onset diabetes of the young; NK, natural killer; NS, not significant; pCR, pathologic complete response; TPM, transcripts per million.

CONCLUSIONS

- Trilaciclib in combination with AC/T ± pembrolizumab ± carboplatin in the neoadjuvant setting for early-stage TNBC has a similar safety and tolerability profile as standard neoadjuvant regimens[14]
- In this small study, pCR rate was comparable with that of standard neoadjuvant therapy in the overall population[14]; increased pCR rates were observed in patients who had PD-L1+ tumors or an immune-inflamed TME
- Changes in immune-related gene expression within the TME, from baseline to 7 days post trilaciclib monotherapy, demonstrate the immunomodulatory effects of trilaciclib, including a trend toward increased expression of genes associated with memory T cells
- In addition to the long-term OS benefit observed in the earlier phase 2 study in metastatic TNBC, data from this study further support the role of trilaciclib in increasing the pool of memory T cells that could potentially contribute to long-term immune surveillance and efficacy
- Future activities will focus on expanding the exploratory dataset to identify patients who may derive long-term benefit from the addition of trilaciclib to their treatment regimen for TNBC

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