TRILACICLIB, AN INTRAVENOUS CYCLIN-DEPENDENT KINASE 4/6 INHIBITOR, ENHANCES ANTITUMOR RESPONSES BY MODULATING T CELLS

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INTRODUCTION

- Administering trilaciclib (COSELA™, G1 Therapeutics, Inc.), an intravenous myeloprotection therapy, 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[1–7]
  - Trilaciclib has also been shown to favorably alter the tumor immune microenvironment via modulation of processes within the cancer-immunity cycle (**Figure 1**)[2,7–10]
- In an open-label, phase 2 trial in patients with metastatic triple-negative breast cancer (NCT02978716), administering trilaciclib prior to gemcitabine plus carboplatin (GCb) prolonged overall survival (a key secondary endpoint) compared with GCb alone (median 19.8 vs 12.6 months; $P < 0.0001$), potentially through protection and direct activation of immune function[9,10]
  - Efficacy outcomes were comparable, irrespective of CDK4/6 dependence status or immune-related gene expression[10]
  - There was a higher frequency of IFNγ-producing CD8+ T cells after *ex vivo* stimulation in the trilaciclib groups than in groups receiving GCb alone, suggesting that trilaciclib had a positive impact on T-cell function[9]
  - Administering trilaciclib resulted in an enrichment of new T-cell clones and decreased Simpson clonality in peripheral blood, suggesting enhanced antigen presentation and activation of T cells[10]
- The aim of this analysis was to evaluate the ability of trilaciclib to modulate key processes within the cancer-immunity cycle and to promote the differentiation of memory T cells

Figure 1. Potential Mechanistic Effects of Trilaciclib on the Cancer-Immunity Cycle

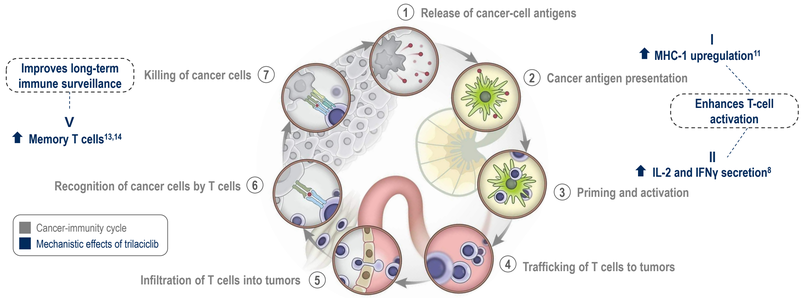


Figure adapted from Chen and Mellman (2013).[15]
CCL, chemokine ligand; CXCL, C-X-C motif chemokine ligand; IFNγ, interferon gamma; IL-2, interleukin 2; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; Treg, regulatory T cell.

METHODS

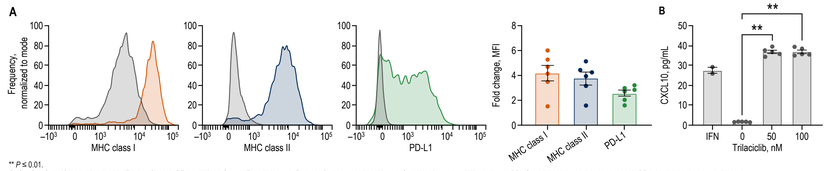
- Peripheral blood mononuclear cells (PBMCs) were isolated from 6 healthy human donors and activated with α-CD2/CD3/CD28 beads with or without trilaciclib
- To measure the expression of major histocompatibility complex (MHC) class I and class II proteins and programmed death-ligand 1 (PD-L1), supernatant from activated PBMCs was harvested after 72 hours and added to MCF-7 human breast cancer cells
- After 24 hours, levels of MHC classes I and II and PD-L1 were quantified by flow cytometry, and the secretion of C-X-C motif chemokine ligand 10 (CXCL10), which potentiates the recruitment of T cells to tumors, was measured by enzyme-linked immunosorbent assay
- To evaluate differentiation into memory T cells, naïve CD8+ T cells were isolated from PBMCs from 6 healthy human donors and activated with α-CD2/CD3/CD28 beads with or without trilaciclib
- Trilaciclib (50 or 100 nM) was added on day 0, 1, or 3 post activation to determine if the timepoint affected T-cell differentiation
- Activated T cells were collected and stained for flow cytometric analyses on days 3, 7, and 14 post activation, and clustering analysis was performed using FlowSOM[16] and ConsensusClusterPlus[17]

RESULTS

Trilaciclib Enhanced Antigen Presentation, T-Cell Activation, and T-Cell Recruitment to Tumors

- Compared with the control, the addition of trilaciclib increased the expression of MHC classes I and II and PD-L1 on the tumor cell surface (**Figure 2A**), and resulted in the increased secretion of CXCL10 (**Figure 2B**)
- Adding trilaciclib directly to tumor cells elicited no phenotypic changes, suggesting the effects of trilaciclib were indirect

Figure 2. Effect of Activating PBMCs With or Without 100 nM Trilaciclib on MHC Classes I and II and PD-L1 Expression (A), and on CXCL10 Secretion (B) in MCF-7 Human Breast Cancer Cells

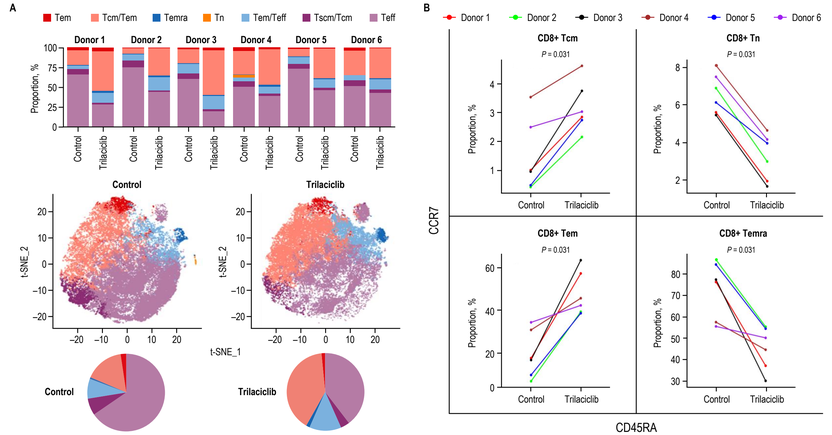


** $P \leq 0.01$.
CXCL, C-X-C motif chemokine ligand; IFN, interferon; MCF-7, Michigan Cancer Foundation-7; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; PD-L1, programmed death-ligand 1.

Addition of Trilaciclib Increased the Frequency of Memory T Cells

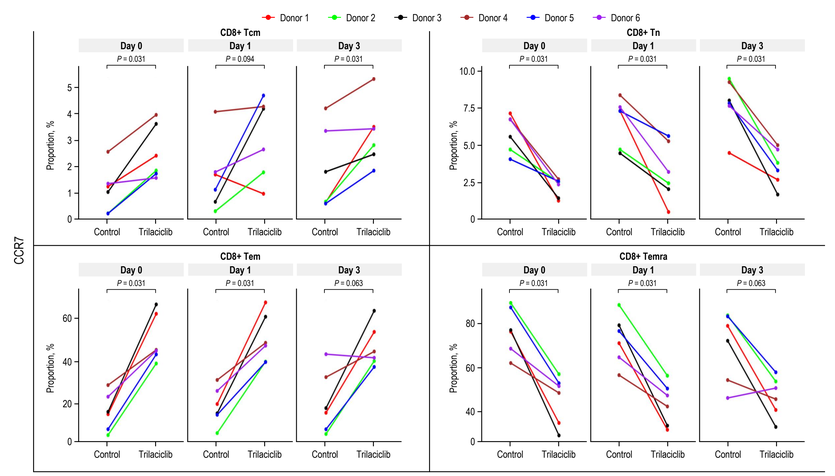
- Flow cytometric data from 14 days post activation showed that there was a proportional increase in central memory and effector memory T-cell populations when naïve CD8+ T cells were activated in the presence of 100 nM trilaciclib, compared with the control (**Figure 3**)
- An increased frequency of memory T-cell formation was observed, irrespective of the timepoint at which trilaciclib was added to activated CD8+ T-cell cultures (**Figure 4**)
- Changes in expression levels of the transcription factors Tox, Tcf-1, and T-bet supported the increase in the frequency of memory T cells following the addition of trilaciclib (**Figure 5**)

Figure 3. Proportional Change in T-Cell Populations When 100 nM Trilaciclib Was Added to CD8+ T-Cell Cultures on Day 0, 1, or 3 Post Activation, by Donor Sample (A) and by T-Cell Subset (B), Compared With Control



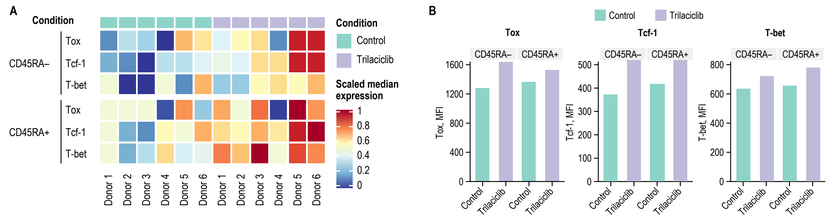
CCR, C-C motif chemokine receptor; CD45RA, cluster of differentiation 45 isoform RA; CD8+, cluster of differentiation 8 positive; t-SNE_1/2, t-distributed stochastic neighbor embedding dimension 1/2; Tcm, central memory T cell; Teff, effector T cell; Tem, effector memory T cell; Temra, effector memory T cell re-expressing CD45RA; Tn, naive T cell; Tscm, stem memory T cell.

Figure 4. Effect of Adding 100 nM Trilaciclib to CD8+ T-Cell Cultures at Day 0, 1, or 3 Post Activation on T-Cell Differentiation



CCR, C-C motif chemokine receptor; CD45RA, cluster of differentiation 45 isoform RA; CD8+, cluster of differentiation 8 positive; Tcm, central memory T cell; Tem, effector memory T cell; Temra, effector memory T cell re-expressing CD45RA; Tn, naive T cell.

Figure 5. Effect of 100 nM Trilaciclib on Expression of Transcription Factors Associated With T-Cell Differentiation: Heatmap (A) and Expression Levels (B) in CD45RA– and CD45RA+ T Cells



CD45RA–/+, cluster of differentiation 45 isoform RA negative/positive; MFI, mean fluorescence intensity; T-bet, T-box expressed in T cells; Tcf, T-cell factor; Tox, thymocyte selection-associated high mobility group box.

CONCLUSIONS

- Data showed skewing of naïve CD8+ T cells into memory T-cell populations upon exposure to trilaciclib
- Increased differentiation into memory T cells was observed, irrespective of when trilaciclib was added post activation
- Trilaciclib may induce a feedback loop that promotes antigen presentation and T-cell activation, while driving the recruitment of T cells to tumors

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