TRANSIENT INHIBITION OF CYCLIN-DEPENDENT KINASE 4/6 WITH TRILACICLIB ENHANCES INHIBITORY RECEPTOR IMMUNOTHERAPY TO IMPROVE ANTITUMOR EFFICACY

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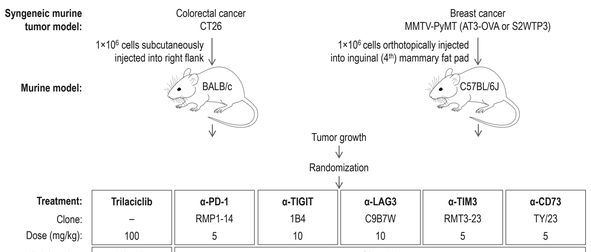
INTRODUCTION

- Trilaciclib (COSELA™, G1 Therapeutics, Inc.) is an intravenous myeloprotection therapy, approved by the US Food and Drug Administration to decrease the incidence of chemotherapy-induced myelosuppression in adult patients when administered prior to a platinum/etoposide-containing or topotecan-containing chemotherapy regimen for extensive-stage small cell lung cancer[1]
  - Administering trilaciclib prior to chemotherapy transiently arrests cyclin-dependent kinase (CDK)4/6-dependent hematopoietic stem and progenitor cells and immune cells in the G_1 phase of the cell cycle during exposure to chemotherapy, thus protecting them from chemotherapy-induced damage[1–7]
  - Trilaciclib has also been shown to favorably alter the tumor immune microenvironment through transient T-cell inhibition[2,7–10]
  - Preclinical data suggest that, following the transient arrest of intratumoral T cells, cytotoxic T cells recover faster than regulatory T cells[7]
- In an open-label phase 2 trial in patients with metastatic triple-negative breast cancer (NCT02978716), administering trilaciclib prior to gemcitabine plus carboplatin (GCb) improved 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]
  - Administering trilaciclib prior to GCb prolonged overall survival versus GCb alone, irrespective of programmed death-ligand 1 (PD-L1) status, but had greater benefit in the PD-L1–positive population (PD-L1–positive: median 32.7 vs 10.5 months, respectively; PD-L1–negative: median 17.8 vs 13.9 months, respectively)[10]
  - Efficacy outcomes were comparable regardless of CDK4/6 dependence status or immune-related gene expression[10]
  - Compared with GCb alone, there was a higher frequency of IFNγ-producing CD8+ T cells after *ex vivo* stimulation in the trilaciclib groups, 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 T-cell activation[10]
- Based on its broad effects on the cancer-immunity cycle (including enhanced antigen presentation and effector CD8+ T-cell function, and sensitivity of regulatory T cells to transient CDK4/6 inhibition), we hypothesized that trilaciclib may enhance the antitumor efficacy of inhibitory receptor immunotherapy
- We investigated the effect of combining trilaciclib with checkpoint inhibitors (α-PD-1, α-TIGIT, α-LAG3, and α-TIM3) or an adenosine pathway inhibitor (α-CD73) on tumor volume and survival

METHODS

- Syngeneic murine models of breast cancer (MMTV-PyMT-AT3-OVA or MMTV-PyMT-S2WTP3) and colorectal cancer (CT26) were utilized to evaluate the synergy between trilaciclib and inhibitory receptor immunotherapy (**Figure 1**)
- Trilaciclib 100 mg/kg was administered weekly, and α-PD-1 (5 mg/kg; clone RMP1-14), α-TIGIT (10 mg/kg; clone 1B4), α-LAG3 (10 mg/kg; clone C9B7W), α-TIM3 (5 mg/kg; clone RMT3-23), and α-CD73 (5 mg/kg; clone TY/23) were administered biweekly
- Treatment was administered intraperitoneally and continued until animals reached humane or study endpoint
- Tumor volume and weight were measured 2–3 times per week

FIGURE 1. STUDY DESIGN



α, anti; AT3-OVA, ovalbumin-expressing AT-3; CD73, cluster of differentiation 73; LAG3, lymphocyte-activating gene 3; MMTV-PyMT, mouse mammary tumor virus-polyoma middle tumor-antigen; PD-1, programmed death protein-1; TIGIT, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; TIM3, T-cell immunoglobulin and mucin domain 3.

RESULTS

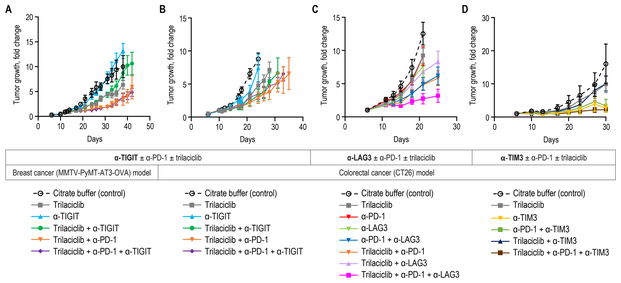
ADDITION OF TRILACICLIB IMPROVED EFFICACY OF INHIBITORY RECEPTOR IMMUNOTHERAPY TREATMENT

- In both MMTV-PyMT and CT26 models, adding trilaciclib to inhibitory receptor immunotherapy delayed tumor growth and improved survival compared with treatment with inhibitory receptor immunotherapy alone (**Figures 2–5**)

EFFECT ON TUMOR VOLUME

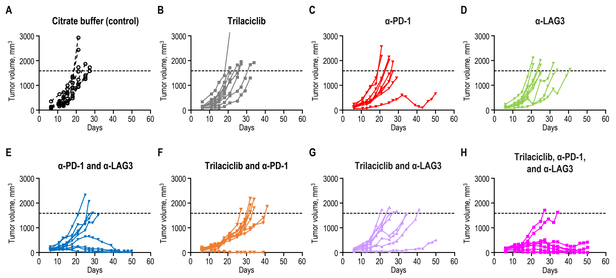
- When trilaciclib was added to α-TIGIT therapy, tumor growth was delayed in both the MMTV-PyMT-AT3-OVA ($P = 0.007$) and CT26 models compared with α-TIGIT alone (**Figures 2A** and **2B**, respectively)
- For the CT26 model, data from individual mice are shown to illustrate the effect of combining trilaciclib with α-PD-1 and/or α-LAG3 on tumor growth (**Figure 3**)
- When evaluating the combination of trilaciclib with multiple inhibitory receptor immunotherapies in the CT26 model, a delay in tumor growth was observed with trilaciclib plus α-PD-1 and α-LAG3 ($P = 0.006$) (**Figure 2C**)
  - There was no difference in tumor growth when trilaciclib was added to α-PD-1 plus α-TIM3 (**Figure 2D**)
- A delay in tumor growth was observed when trilaciclib was added to α-PD-1 plus α-CD73 in the CT26 model (**Figure 4A**)

FIGURE 2. EFFECT OF COMBINING TRILACICLIB WITH INHIBITORY RECEPTOR IMMUNOTHERAPY ON TUMOR GROWTH: α-PD-1 AND/OR (A, B) α-TIGIT (N = 6–7), (C) α-LAG3 (N = 5–8), AND (D) α-TIM3 (N = 5–8)



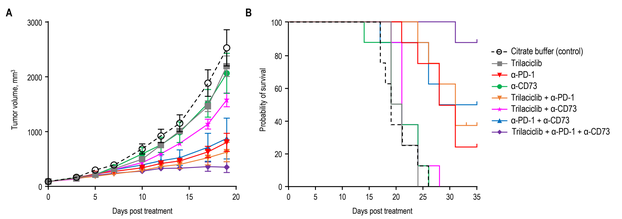
α, anti; AT3-OVA, ovalbumin-expressing AT-3; LAG3, lymphocyte-activating gene 3; MMTV-PyMT, mouse mammary tumor virus-polyoma middle tumor-antigen; PD-1, programmed death protein-1; TIGIT, T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains; TIM3, T-cell immunoglobulin and mucin domain 3.

FIGURE 3. EFFECT OF COMBINING TRILACICLIB WITH α-PD-1 AND/OR α-LAG3 ON TUMOR GROWTH IN INDIVIDUAL MICE, IN THE CT26 MODEL (N = 5–8)



α, anti; LAG3, lymphocyte-activating gene 3; PD-1, programmed death protein-1.

FIGURE 4. EFFECT OF COMBINING TRILACICLIB WITH α-PD-1 AND/OR α-CD73 (N = 8) ON (A) TUMOR VOLUME AND (B) PROBABILITY OF SURVIVAL IN THE CT26 MODEL

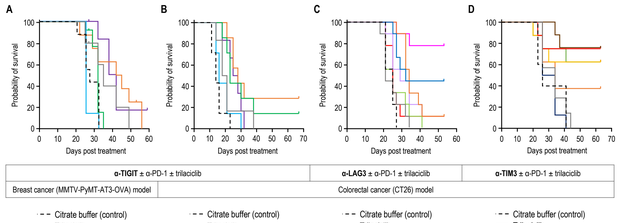


α, anti; CD73, cluster of differentiation 73; PD-1, programmed death protein-1.

EFFECT ON SURVIVAL

- When trilaciclib was added to α-TIGIT therapy, survival was significantly improved in both the MMTV-PyMT-AT3-OVA ($P = 0.002$) and CT26 ($P = 0.04$) models compared with α-TIGIT alone (**Figures 5A** and **5B**, respectively)
- In the CT26 model, improved survival was observed when trilaciclib was added to α-LAG3 compared with α-LAG3 alone (**Figure 5C**)
- When evaluating the combination of trilaciclib with multiple inhibitory receptor immunotherapies in the CT26 model, increased survival was observed with the triplet combinations of trilaciclib plus α-PD-1 and α-LAG3, α-TIM3, or α-CD73, compared with doublet therapies in the absence of trilaciclib (**Figures 5C**, **5D**, and **4B**, respectively)

FIGURE 5. EFFECT OF COMBINING TRILACICLIB WITH INHIBITORY RECEPTOR IMMUNOTHERAPY ON SURVIVAL: α-PD-1 AND/OR (A, B) α-TIGIT (N = 6–7), (C) α-LAG3 (N = 5–8), AND (D) α-TIM3 (N = 5–8)



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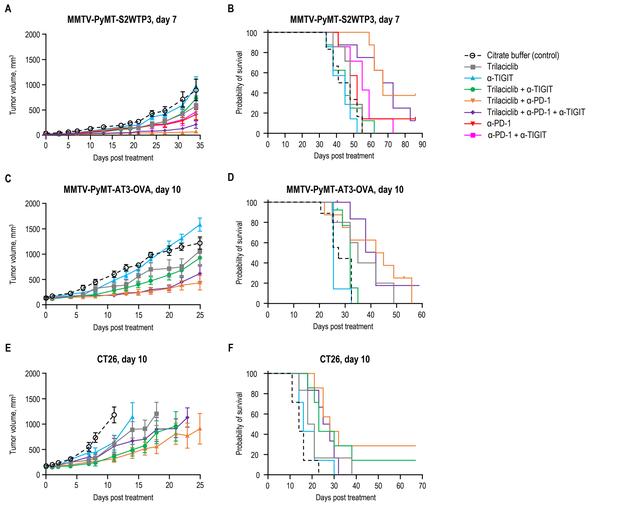
COMBINING TRILACICLIB WITH INHIBITORY RECEPTOR IMMUNOTHERAPY IS EFFECTIVE, IRRESPECTIVE OF TUMOR MODEL OR DAY OF TREATMENT INITIATION

- In the MMTV-PyMT-S2WTP3 model, addition of trilaciclib to α-PD-1 significantly delayed tumor growth ($P = 0.004$) and improved survival ($P = 0.02$) compared with α-PD-1 monotherapy (**Figures 6A** and **6B**, respectively)
  - Treatment with a combination of trilaciclib and α-PD-1 plus α-TIGIT resulted in delayed tumor growth and improved survival compared with α-PD-1 plus α-TIGIT (**Figures 6A** and **6B**, respectively)
- Compared with α-TIGIT monotherapy, trilaciclib plus α-PD-1 demonstrated efficacy, irrespective of tumor model or starting day of treatment:
  - In the MMTV-PyMT-S2WTP3 model, in which treatment was initiated on day 7 post inoculation, tumor growth was significantly delayed ($P = 0.004$) and survival was significantly improved ($P = 0.00006$) (**Figures 6A** and **6B**, respectively)
  - In the MMTV-PyMT-AT3-OVA model with day 10 treatment start, there was significant delay in tumor growth ($P = 0.0012$) and improvement in survival ($P = 0.01$) (**Figures 6C** and **6D**, respectively)
  - In the CT26 model with day 10 treatment start, tumor growth was delayed and survival was significantly improved ($P = 0.01$) (**Figures 6E** and **6F**, respectively)

FIGURE 6. EFFECT OF COMBINING TRILACICLIB WITH α-PD-1 AND/OR α-TIGIT ON TUMOR VOLUME AND PROBABILITY OF SURVIVAL, IN (A, B) THE MMTV-PYMT-S2WTP3 MODEL WITH DAY 7 TREATMENT START, (C, D) THE MMTV-PYMT-AT3-OVA MODEL WITH DAY 10 TREATMENT START, AND (E, F) THE CT26 MODEL WITH DAY 10 TREATMENT START (N = 6–7)



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CONCLUSIONS

- Adding trilaciclib to inhibitory receptor immunotherapy combinations enhanced antitumor activity
- Combining trilaciclib with α-PD-1 was consistently effective, irrespective of when treatment was initiated or the tumor model used
- The data suggest that trilaciclib provides complementary immune modulatory benefits that support the mechanisms of inhibitory receptor immunotherapy
  - Findings provide a rationale for combining trilaciclib with inhibitory receptor immunotherapy to enhance clinical efficacy, including in populations resistant to checkpoint blockade or who have received prior treatment with inhibitory receptor immunotherapy

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