ABSTRACT #5628

TRILACICLIB (G1T28), A CDK4/6 INHIBITOR, ENHANCES THE EFFICACY OF COMBINATION CHEMOTHERAPY AND IMMUNE CHECKPOINT INHIBITOR TREATMENT IN PRECLINICAL MODELS

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BACKGROUND

- Immune checkpoint inhibitors such as anti-PD-L1 or anti-PD-1 antibodies inhibit the interaction between programmed death ligand 1 (PD-L1) on the surface of the tumor and antigen-presenting cells with programmed death 1 (PD-1) on the surface of activated T lymphocytes and subsequently release the PD-L1/PD-1 mediated inhibition of the immune response.
- While checkpoint inhibitors can lead to durable responses in patients with various cancers, only a minority of patients respond. An approach to increase the response rate of checkpoint inhibitors is to combine them with chemotherapy. Chemotherapy causes immunogenic cell death that can help "prime" the immune system.
- A major drawback to many chemotherapeutic regimens is myelosuppression and immunosuppression, which may antagonize the efficacy of the checkpoint inhibitors by reducing both the number and function of lymphocytes and the generation of a sustained anti-tumor immune response. Therefore, an approach to maintain immune system function while administering cytotoxic chemotherapy is needed to fully exploit the therapeutic potential of chemotherapy/checkpoint inhibitor combination regimens.
- Trilaciclib (G1T28) is a highly potent, selective, and reversible cyclin-dependent kinase 4/6 (CDK4/6) inhibitor in development to preserve bone marrow and immune system function (including lymphoid progenitors and lymphocytes) from damage by chemotherapy.
- In preclinical animal models, trilaciclib induces a transient G1 cell cycle arrest of the hematopoietic stem and progenitor cells (HSPCs) and administration of trilaciclib prior to chemotherapy results in improved recovery of complete blood counts (CBCs), preservation of the immune system, maintenance of long-term bone marrow function, prevention of myeloid skewing, and enhancement of anti-tumor efficacy. (Bisi et al., MCT, 2016; He et al., STM, in press)
- In Phase 1a/2b SCLC trials (first-line, carboplatin-etoposide, NCT02499770; and second-line, topotecan, NCT02514447), early results suggest trilaciclib preserves HSPC function as evidenced by improvements in multi-lineage blood cell counts, reduced hematological adverse events, and reduced need for medical management of hematological toxicities. Tumor efficacy in these studies is encouraging.
- In a retrospective chart review of SCLC patients, baseline lymphocyte counts were lower at the start of second-line treatment compared to the start of first-line treatment (1116 vs 1481 cells/μL, respectively; p=0.0002), demonstrating persistent lymphopenia months after first-line chemotherapy. In the ongoing trilaciclib SCLC studies, lymphocyte counts have remained relatively unchanged through repeated cycles of both first- and second-line chemotherapy, demonstrating preservation of this important cell population. (EORTC-NCI-AACR Symposium, Roberts et al., 2016)
- Based on the ability of trilaciclib to preserve the HSPC compartment and enhance immune system function during chemotherapy, we tested whether the addition of trilaciclib to chemotherapy/checkpoint inhibitor combinations could enhance anti-tumor activity.

OBJECTIVES

- Evaluate the addition of trilaciclib to chemotherapy/checkpoint inhibitor combination regimens through examination of various classes and schedules of chemotherapies (5-FU, oxaliplatin) and checkpoint inhibitors (anti-PD-1, anti-PD-L1) in syngeneic murine tumor models.
- Assess the ability of trilaciclib to preserve and enhance the function of the immune system when administered in combination with chemotherapy and checkpoint inhibitors and characterize immune system changes in the tumor microenvironment.

METHODS

EVALUATION OF ANTI-TUMOR ACTIVITY

Anti-tumor activity of trilaciclib in combination with checkpoint inhibitors and chemotherapies was evaluated in the MC38 syngeneic murine colon carcinoma model. For all xenograft studies, nine-week old female C57BL/6 mice (C57BL/6NCrl) were implanted with MC38 tumor cells and treatment was initiated when mean tumor volume was ~100 mm^3. A summary of treatment combinations and schedules can be found in Figure 1. Briefly, trilaciclib (100 mg/kg), oxaliplatin (10 mg/kg), or 5-fluorouracil (5-FU, 75 mg/kg) were administered intraperitoneally (IP) once weekly for three doses. Anti-PD-L1 (100 μg/animal, IP) or anti-PD-1 (5 mg/kg, IP) were given twice weekly for two weeks starting on Day 1 (induction [I]), starting on Day 15 and continuing through the end of study (maintenance [M]), or starting on Day 1 and continuing through the end of study (induction + maintenance [IM]). Single, two, and three drug combinations were tested and trilaciclib was administered 30 minutes prior to chemotherapy treatment. Complete response (CR) and partial response (PR) were calculated using standard Charles River Laboratories (CRL; RTP, NC) criteria. Body weight (BW) and health were monitored, and tumor volume was measured twice weekly. The individual tumor volume endpoint was 1000 mm^3 or Day 100, whichever came first. All xenograft studies were completed at CRL, and all protocols were approved through CRL IACUC committee.

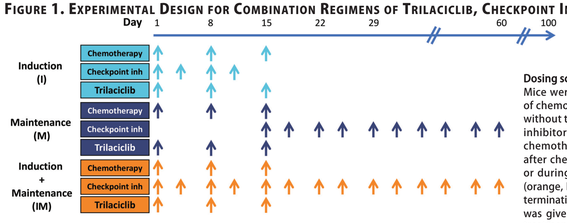
ANALYSIS OF INTRA-TUMOR T_{REG} POPULATIONS

MC38 tumor-bearing C57BL/6 mice were treated with oxaliplatin (10 mg/kg, IP) and anti-PD-L1 (100 μg/mouse, IP) ± trilaciclib (100 mg/kg, IP) for either four or eight days. Twenty-four hours post final dose, mice were euthanized and tumors were harvested. Tumors were then processed and stained for CD45, CD3, CD4, CD25, and FOXP3. The CD25$^+$FOXP3$^+$ population was measured within the CD45$^+$CD3$^+$CD4$^+$ population through flow cytometric analysis.

T LYMPHOCYTE STIMULATION ASSAY

C57BL/6 mice were treated with 3 daily IP doses of 50 mg/kg 5-FU ±100 mg/kg trilaciclib. Two and seven days after the final treatment, mice were euthanized and spleens were harvested. Splenocytes were stimulated *ex vivo* with anti-CD3/CD28 antibodies for 72 hours and interferon gamma (IFNγ) or interleukin-2 (IL-2) levels were measured via ELISA (R&D systems).

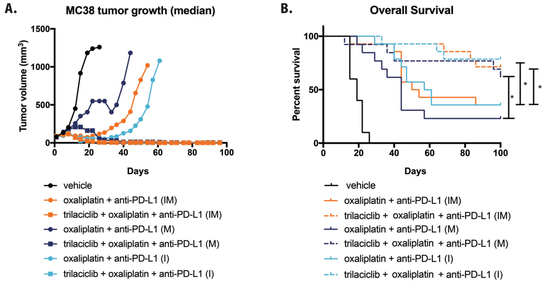
FIGURE 1. EXPERIMENTAL DESIGN FOR COMBINATION REGIMENS OF TRILACICLIB, CHECKPOINT INHIBITOR AND CHEMOTHERAPY



Dosing schedules for murine xenograft studies
Mice were given one of the dosing schedules of chemotherapy/checkpoint inhibitor with or without trilaciclib. Specifically, the checkpoint inhibitor was either given only during chemotherapy treatment (light blue, I), only after chemotherapy treatment (dark blue, M) or during and after chemotherapy treatment (orange, IM) until complete response or animal termination. In experimental cohorts, trilaciclib was given 30 minutes prior to chemotherapy treatment.

RESULTS

FIGURE 2. TRILACICLIB INCREASES EFFICACY WHEN ADDED TO OXALIPLATIN/ANTI-PD-L1 COMBINATION THERAPY



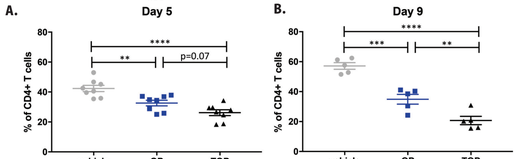
(A) Mice treated with trilaciclib (n=9-14) in combination with oxaliplatin and anti-PD-L1 (TOP) showed enhanced tumor regression and an extended duration of response when compared to mice treated with oxaliplatin and anti-PD-L1 (OP). **(B)** This resulted in improved survival of TOP-treated mice relative to OP-treated mice in all schedules evaluated. Additionally, TOP treatment was well tolerated and did not cause significant weight loss during and after treatment (data not shown). * p≤0.05

TABLE 1. TRILACICLIB ENHANCES COMPLETE RESPONSE (CR) AND OVERALL SURVIVAL (OS) WHEN ADDED TO OXALIPLATIN/ANTI-PD-L1 COMBINATION THERAPY

TREATMENT	PR %	CR %	ORR %	OS (days)
Vehicle (n=20)	0	5	5	19
Trilaciclib (T) (n=9)	0	0	0	19
Anti-PD-L1 (P) (n=9)	0	11	11	33
Oxaliplatin (O) (n=9)	0	22	22	36
TO (n=10)	10	10	20	37
OP (IM) (n=14)	7	36	43	52
TOP (IM) (n=14)*	7	79	86	not reached
OP (I) (n=24)	13	33	46	59
TOP (I) (n=24)*	8	67	75	not reached
OP (M) (n=13)	0	15	15	44
TOP (M) (n=13)*	8	62	70	not reached

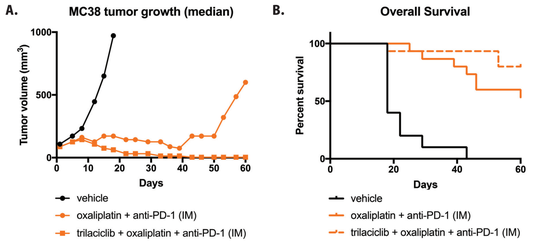
Abbreviations: PR, partial response; CR, complete response; ORR, objective response rate; OS, overall survival. Data shown in Table 1 is from two independent experiments. Statistical significance comparing CR rate of TOP to OP within each treatment schedule was evaluated using Fisher's exact test. * p≤0.05

FIGURE 3. ADDING TRILACICLIB TO OXALIPLATIN/ANTI-PD-L1 COMBINATION THERAPY FURTHER DECREASES INTRA-TUMOR T_{REG} POPULATIONS



Tumors were harvested for flow cytometric analysis of T_{reg} populations (CD45$^+$CD3$^+$CD4$^+$CD25$^+$FoxP3$^+$) in immune cell infiltrates after 5 days **(A)** and 9 days **(B)** of treatment. The population of intra-tumor T_{reg} cells within the CD4$^+$ T cell fraction was significantly decreased in TOP-treated mice when compared to vehicle- and OP-treated mice. Error bars represent SEM. Statistics were evaluated using one-way ANOVA. **p<0.01, ***p<0.001, ****p<0.0001

FIGURE 4. TRILACICLIB INCREASES EFFICACY WHEN ADDED TO OXALIPLATIN/ANTI-PD-1 COMBINATION THERAPY (ONGOING)



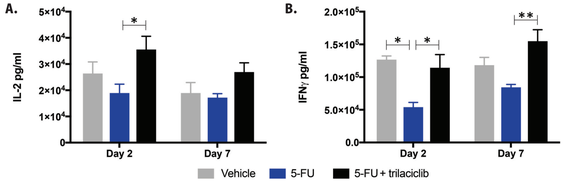
(A) Mice treated with trilaciclib (n=10-15) in combination with oxaliplatin and anti-PD-1 (TOP) showed enhanced tumor regression and an extended duration of response when compared to mice treated with oxaliplatin and anti-PD-1 (OP). **(B)** This resulted in improved survival of TOP-treated animals relative to OP-treated mice. Additionally, TOP treatment was well-tolerated and did not cause significant weight loss during and after treatment (data not shown).

TABLE 2. INTERIM DATA DESCRIBING THE EFFECTS OF TRILACICLIB WHEN ADDED TO OXALIPLATIN/ANTI-PD-1 COMBINATION THERAPY

TREATMENT	PR %	CR %	ORR %	OS (days)
Vehicle (n=10)	0	0	0	18
Trilaciclib (T) (n=10)	10	10	20	22
Anti-PD-1 (P) (n=10)	0	0	0	22
Oxaliplatin (O) (n=10)	0	10	10	31
TO (n=10)	0	20	20	44.5
TP (n=10)	10	0	10	31
OP (IM) (n=15)	7	33	40	not reached
TOP (IM) (n=15)	7	53	60	not reached

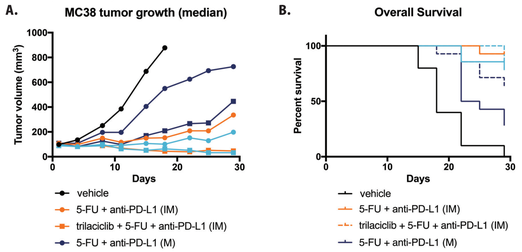
Abbreviations: PR, partial response; CR, complete response; ORR, objective response rate; OS, overall survival.

FIGURE 5. TRILACICLIB PRESERVES LYMPHOCYTE FUNCTION WHEN ADDED TO 5-FU TREATMENT



After *ex-vivo* splenocyte stimulation in C57BL/6 mice, trilaciclib enhances IL-2 (A) and preserves IFN γ production (B) after 5-FU treatment suggesting that trilaciclib preserves immune function in T lymphocytes from chemotoxicity. Error bars represent SEM. Statistics were evaluated using two-way ANOVA. *p≤0.05, **p<0.01

FIGURE 6. TRILACICLIB INCREASES EFFICACY WHEN ADDED TO 5-FU/ANTI-PD-L1 COMBINATION THERAPY (ONGOING)



(A) Mice treated with trilaciclib (n=10-14) in combination with 5-FU and anti-PD-L1 (TFP) showed enhanced tumor regression compared to mice treated with 5-FU and anti-PD-L1 (FP). **(B)** Preliminary data suggests an improved survival of TFP-treated animals relative to FP-treated mice. Additionally, TFP treatment was well-tolerated and did not cause significant weight loss during and after treatment (data not shown).

TABLE 3. INTERIM DATA DESCRIBING THE EFFECTS OF TRILACICLIB WHEN ADDED TO 5-FU/ANTI-PD-L1 COMBINATION THERAPY

TREATMENT	PR %	CR %	ORR %	PD %	OS @ D29 %
Vehicle (n=10)	0	0	0	100	0
Anti-PD-L1 (P) (n=10)	0	0	0	90	40
5-FU (F) (n=10)	0	0	0	100	30
TF (n=10)	0	0	0	90	50
TP (n=14)	0	0	0	93	43
FP (IM) (n=14)	14	14	28	71	93
TFP (IM) (n=14)	29	14	43	36	93
FP (I) (n=14)	36	0	36	50	79
TFP (I) (n=14)	36	7	43	21	86
FP (M) (n=14)	0	0	0	93	29
TFP (M) (n=14)	0	0	0	71	64

Abbreviations: PR, partial response; CR, complete response; ORR, objective response rate; PD, progressive disease; OS, overall survival. Percent overall survival at 29 days is shown. PD is defined as the percent of tumors that have doubled in size by Day 29.

SUMMARY

- Addition of trilaciclib to chemotherapy (oxaliplatin or 5-FU) and checkpoint inhibitor (anti-PD-1 or anti-PD-L1) combinations enhances the response and survival of MC38 syngeneic tumor-bearing mice.
- Potential cell cycle dependent and independent mechanisms by which trilaciclib enhances anti-tumor activity include:
  - reducing intra-tumor T_{reg} populations
  - preservation of T lymphocyte function from chemotoxicity
  - direct activation of T-effector cells
- Studies to evaluate the added benefit of trilaciclib in other syngeneic tumors models including CT26 colon carcinoma are ongoing.
- Based on these results, a Phase 2 study to assess the safety and efficacy of trilaciclib or placebo with carboplatin, etoposide, and atezolizumab in first-line extensive stage SCLC patients will be initiated in Q2 2017 (NCT03041311).