ABSTRACT #1752

TRANSIENT EXPOSURE TO TRILACICLIB, A CDK4/6 INHIBITOR, MODULATES GENE EXPRESSION IN TUMOR IMMUNE INFILTRATES AND PROMOTES A PRO-INFLAMMATORY TUMOR MICROENVIRONMENT

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BACKGROUND

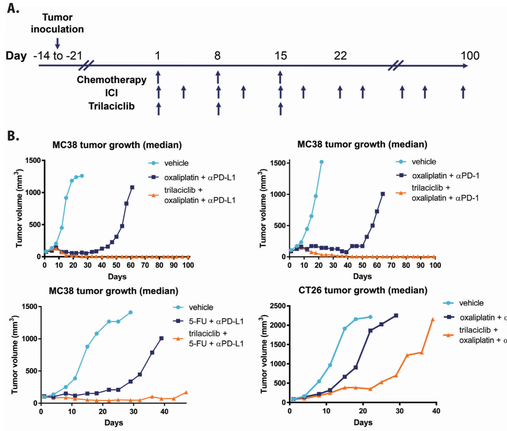
- While immune checkpoint inhibitors (ICIs) can lead to durable responses in patients with various cancers, only a minority of patients respond. An approach to increase the response rate of ICIs is to combine them with chemotherapy. Chemotherapy causes immunogenic cell death that can help to "prime" the immune system.
- A major drawback to many chemotherapeutic regimens is myelosuppression and immunosuppression, which may antagonize the efficacy of the ICIs 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/ICI combination regimens.
- Trilaciclib (G1T28) is a highly potent, selective, and reversible cyclin dependent kinase 4/6 (CDK4/6) inhibitor in clinical 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., *Mol Cancer Ther*, 2016; He et al., *Sci Transl Med*, 2017).
- In addition to preserving the host immune system during chemotherapy, trilaciclib and other CDK4/6 inhibitors have been shown to augment anti-tumor response through cell-cycle independent mechanisms, including enhancing T cell activation through modulation of NFAT activity (Deng et al., *Cancer Discovery*, 2017, Schaer et al., *Cell Reports*, 2018), as well as increasing antigen presentation by CDK4/6-sensitive tumor cells (Goel et al., *Nature*, 2017).
- In a placebo-controlled, double blind Phase 2 trial (NCT02499770) evaluating trilaciclib in patients undergoing chemotherapy for first-line small cell lung cancer (SCLC), the data demonstrated that trilaciclib reduced clinically relevant consequences of chemotherapy-induced myelosuppression versus placebo. In addition to demonstrating myelopreservation benefits across multiple hematopoietic lineages, trilaciclib showed favorable trends versus placebo for overall response rate (ORR), duration of response (DOR), and progression free survival (PFS).
- 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/ICI combinations could enhance anti-tumor activity.

OBJECTIVES

- Evaluate the addition of trilaciclib to chemotherapy/checkpoint inhibitor combination regimens through examination of various classes of chemotherapies (5-FU, oxaliplatin) and checkpoint inhibitors (αPD-1, αPD-L1) in syngeneic murine tumor models.
- Assess the role of trilaciclib in the enhancement of anti-tumor response in addition to preserving the host immune system during chemotherapy.
- Characterize the effect of transient exposure of trilaciclib on the tumor microenvironment, by examining the cellular composition, proliferation status, and gene expression of tumor immune infiltrates in preclinical models.

RESULTS

FIGURE 1. ADDITION OF TRILACICLIB TO CHEMOTHERAPY/ICI TREATMENT COMBINATIONS ENHANCES ANTI-TUMOR RESPONSE IN SYNGENEIC MURINE TUMOR MODELS



A. Dosing schedule to evaluate the effect of chemotherapy/immune checkpoint inhibitor (ICI) treatment combinations with or without trilaciclib in established tumors in MC38 or CT26 syngeneic murine tumor models. C57BL/6 mice were implanted with MC38 or CT26 tumor cells and treatment was initiated when mean tumor volume was ~100 mm³. 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. αPD-L1 (100 µg/animal, IP) or αPD-1 (5 mg/kg, IP) were given twice weekly continuously through the end of study.

B. Addition of trilaciclib to various chemotherapy/ICI treatment combinations enhanced tumor growth delay and the durability of anti-tumor response. The chemotherapy and ICI combination tested are indicated in the legend of each graph. Data represent the median tumor volume.

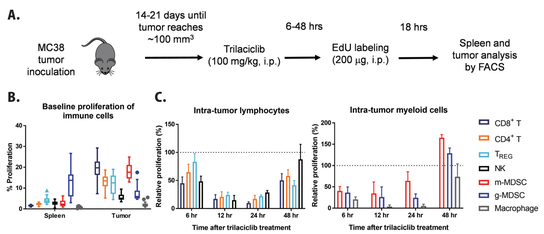
TABLE 1. ADDITION OF TRILACICLIB TO CHEMOTHERAPY/ICI TREATMENT COMBINATIONS ENHANCES COMPLETE RESPONSE (CR) AND MEDIAN OVERALL SURVIVAL (OS)

Model	Treatment	PR%	CR%	ORR%	OS (days)
MC38	Vehicle (n=30)	0	0	0	18
	Oxaliplatin + αPD-L1 (n=14)	7	36	43	52
	Trilaciclib + oxaliplatin + αPD-L1 (n=14)	7	79	86	Not reached
	Oxaliplatin + αPD-1 (n=15)	0	40	40	64
	Trilaciclib + oxaliplatin + αPD-1 (n=15)	0	60	60	Not reached
	5-FU + αPD-L1 (n=14)	0	29	29	39
	Trilaciclib + 5-FU + αPD-L1 (n=14)	0	50	50	76.5
CT26	Vehicle (n=10)	0	0	0	18
	Oxaliplatin + αPD-L1 (n=15)	0	0	0	25
	Trilaciclib + oxaliplatin + αPD-L1 (n=15)	7	13	20	39

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

PR%, CR%, ORR%, and OS (days) were calculated from data shown in Figure 1.

FIGURE 2. MAJOR INTRA-TUMOR IMMUNE CELL TYPES ARE HIGHLY PROLIFERATIVE AND SENSITIVE TO CDK4/6 INHIBITION

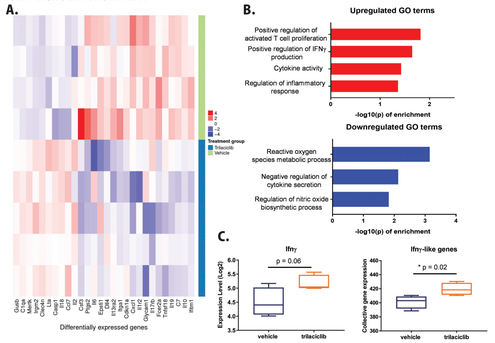


A. Experimental design to assess proliferation of immune cell types. MC38 tumor-bearing mice were treated with trilaciclib and 5-ethynyl-2′-deoxyuridine (EdU) at the indicated times. Splenocytes and CD45⁺ enriched cells from tumors were stained for lineage-specific cell surface markers, and EdU incorporation was detected using Click-iT chemistry followed by flow cytometric analysis.

B. The majority of immune cell types have increased proliferation in tumors compared to their spleen counterparts. % Proliferation is represented as the proportion of EdU⁺ cells in each cell population.

C. Trilaciclib treatment led to transient and reversible inhibition of proliferation in intra-tumor lymphoid and myeloid immune cell types. Relative proliferation is represented as the (%EdU⁺ in trilaciclib treated) / (%EdU⁺ in vehicle treated) x 100 for each cell population at each time point. Data represent mean ± SD.

FIGURE 3. TRANSIENT EXPOSURE OF TRILACICLIB LEADS TO CHANGES IN THE INTRA-TUMOR GENE EXPRESSION PROFILE CONSISTENT WITH ENHANCEMENT OF PRO-INFLAMMATORY TUMOR MICROENVIRONMENT



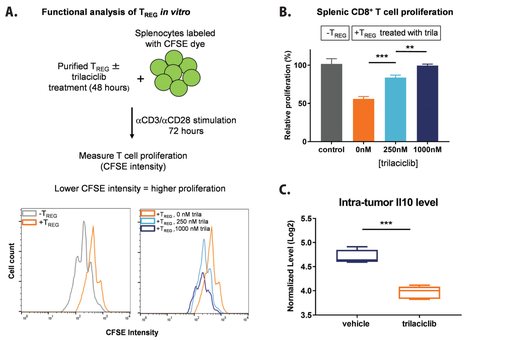
MC38 tumor-bearing mice were treated with two weekly doses of trilaciclib (100 mg/kg) and tumors were harvested 24 hours after the last dose for analysis (n=5 per group). Gene expression profiling was performed using the PanCancer Immune Profiling Panel (NanoString). Normalized and Log2 transformed expression values were used for identification of differentially expressed genes and Gene Ontology (GO) term enrichment analysis.

A. Heatmap displaying genes that were significantly upregulated (red) and downregulated (blue) in tumors after trilaciclib treatment. Twenty-eight differentially expressed genes were identified, defined using a p-value < 0.05 and absolute fold-change ≥ 1.3.

B. Enrichment of immune-related Gene Ontology (GO) terms in differentially expressed genes between vehicle and trilaciclib treatment groups. Upregulated (red) or downregulated (blue) genes were analyzed for enrichment of GO terms across all three GO ontologies using a hypergeometric test. Enrichment was defined as p < 0.05.

C. Ifnγ, a pro-inflammatory cytokine critical for CD8⁺ T cell anti-tumor response, and Ifnγ-like genes were elevated after trilaciclib treatment. Fifty-nine genes whose expression positively correlated with Ifnγ (Ifnγ-like genes) were identified in the dataset by Pearson's correlation (p < 0.05, correlation > 0). Statistical significance was assessed using Student's t-test.

FIGURE 4. TRILACICLIB ATTENUATES THE IMMUNOSUPPRESSIVE FUNCTION OF T_{REG} *IN VITRO* AND *IN VIVO*

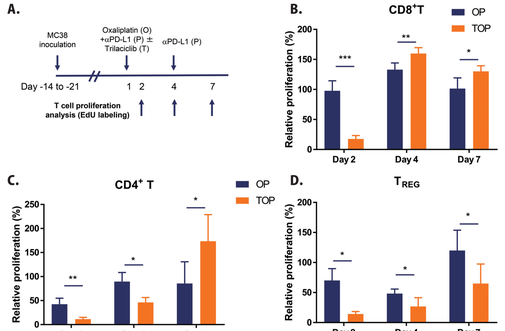


A. Experimental design to assess the effect of trilaciclib on the immuno-suppressive function of regulatory T cells (T_{REG}) *in vitro*. CD8⁺ T cell proliferation was quantified by the level of CFSE dilution by flow cytometric analysis.

B. Proliferation of CD8⁺ T cells was enhanced when co-cultured with trilaciclib-treated T_{REG}. The level of T cell proliferation was normalized to the control, where T cells were stimulated in the absence of T_{REG}. Data represent mean ± SD. Statistical significance was assessed using Student's t-test (**p < 0.01, ***p < 0.001).

C. Trilaciclib treatment in MC38 tumor-bearing mice led to a significant decrease in the intra-tumor transcript level of Il-10, a cytokine produced by T_{REG} to mediate immuno-suppression. Gene expression of tumors from vehicle or trilaciclib treated animals were analyzed as described in Fig. 3A. Data represent normalized Log2 transcript levels. Statistical significance was assessed using Student's t-test (***p < 0.001). This result is consistent with the ability of trilaciclib to attenuate T_{REG} function.

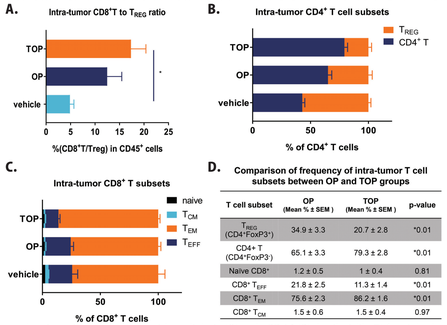
FIGURE 5. ADDITION OF TRILACICLIB TO CHEMOTHERAPY/ICI COMBINATION SELECTIVELY PROLONGS PROLIFERATION ARREST OF T_{REG}, BUT NOT CD4⁺ AND CD8⁺ T CELL POPULATIONS



A. Experimental design to assess proliferation of immune cell types in tumors after oxaliplatin+αPD-LI (OP) or trilaciclib+oxaliplatin+αPD-L1 (TOP) treatments. MC38 tumor-bearing mice were dosed at the timepoints indicated. Tumors were harvested 18 hours after EdU labeling for analysis.

B-D. Addition of trilaciclib to oxaliplatin+αPD-L1 treatment combination resulted in transient proliferation arrest followed by a faster recovery of CD8⁺ and CD4⁺ T cells compared with T_{REG}. The relative proliferation is determined as (% EdU⁺ in trilaciclib treated) / (%EdU⁺ in vehicle treated) x 100 for each cell population at each time point. Data represent mean ± SD. Statistical significance was assessed using Student's t-test (*p < 0.05).

FIGURE 6. ADDITION OF TRILACICLIB TO CHEMOTHERAPY/ICI COMBINATION GENERATES INTRA-TUMOR T CELL SUBSETS FAVORING AN ENHANCED CYTOTOXIC T CELL RESPONSE



D. Comparison of frequency of intra-tumor T cell subsets between OP and TOP groups

T cell subset	OP (Mean % ± SEM)	TOP (Mean % ± SEM)	p-value
T_{REG} ($CD4^+FoxP3^+$)	34.9 ± 3.3	20.7 ± 2.8	*0.01
CD4+ T ($CD4^+FoxP3^-$)	65.1 ± 3.3	79.3 ± 2.8	*0.01
Naive $CD8^+$	1.2 ± 0.5	1 ± 0.4	0.81
$CD8^+ T_{EFF}$	21.8 ± 2.5	11.3 ± 1.4	*0.01
$CD8^+ T_{EM}$	75.6 ± 2.3	86.2 ± 1.6	*0.01
$CD8^+ T_{CM}$	1.5 ± 0.6	1.5 ± 0.4	0.97

MC38 tumor-bearing mice were treated with oxaliplatin (O) and αPD-L1 (P) ± trilaciclib (T) for eight days as shown in Fig. 1A. Twenty-four hours post final dose, tumors were harvested and processed for flow cytometric analysis to assess the proportion of intra-tumor CD4⁺ and CD8⁺ T cell subsets.

A. The ratio of CD8⁺ T to T_{REG} in tumor is elevated in TOP treated animals, consistent with a tumor microenvironment favoring an enhanced cytotoxic T cell response. Data is presented as mean ratio ± SEM. Statistical significance was assessed using Student's t-test (*p < 0.05).

B. The proportion of immuno-suppressive regulatory T cells (T_{REG}) within total CD4⁺ cells in tumors is significantly decreased in TOP treated animals compared to the OP treatment group. Data is presented as mean proportion of $CD4^+FoxP3^+$ T_{REG} and $CD4^+FoxP3^-$ T cells ± SEM.

C. The proportion of Effector memory T cells (T_{EM}) within CD8⁺ T cells in tumors is significantly increased in TOP treated animals compared to the OP treatment group, with a concomitant decrease in effector T cells (T_{EFF}). CD8⁺ T cells were divided into four subsets using CD62L and CD44 markers: naïve T cells ($CD62L^+CD44^-$), effector (T_{EFF}, $CD62L^+CD44^-$), central memory (TCM, $CD62L^+CD44^+$), and effector memory (T_{EM}, $CD62L^-CD44^+$). Data is presented as mean proportion of each subset ± SEM.

D. Comparison of frequency of intra-tumor T cell subsets between OP and TOP groups in B and C. Statistical analysis was performed using Student's t-test.

SUMMARY

- Addition of trilaciclib to chemotherapy (oxaliplatin or 5-FU) and checkpoint inhibitor (αPD-1 or αPD-L1) combinations enhances the anti-tumor activity in MC38 and CT26 syngeneic tumor-bearing mice.
- In addition to preserving the host immune system during chemotherapy, trilaciclib can enhance anti-tumor response through multiple mechanisms, including augmenting T cell activation and modulating the function and/or differentiation of immune cell types in the tumor microenvironment.
- Within the tumor microenvironment:
  - Pulsatile dosing of trilaciclib can induce transient cell-cycle arrest in highly proliferative intra-tumor immune cells, leading to gene expression changes that promote a pro-inflammatory tumor microenvironment.
  - When combined with oxaliplatin and αPD-L1 treatment combination, the addition of trilaciclib resulted in transient proliferation arrest followed by a faster recovery of $CD8^+$ and $CD4^+$ T cells compared with T_{REG} in tumors. This resulted in T cell subsets within the tumor microenvironment with an enhanced cytotoxic T cell response.
- Peripheral blood immunophenotyping from a recently completed Phase 2a trial of chemotherapy +/- trilaciclib in 1st-line SCLC (NCT02499770) is ongoing and will be presented in 4Q18.
- A randomized, placebo-controlled, double-blind Phase 2 trial to assess the safety and efficacy of trilaciclib or placebo with carboplatin, etoposide, and atezolizumab in first-line extensive stage SCLC patients completed enrollment in 1Q18 (NCT03041311).

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