#1671P

TRILACICLIB PRESERVES AND ENHANCES IMMUNE SYSTEM FUNCTION IN EXTENSIVE-STAGE SMALL CELL LUNG CANCER (SCLC) PATIENTS RECEIVING FIRST-LINE CHEMOTHERAPY

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BACKGROUND

- Chemotherapy-induced damage of hematopoietic stem and progenitor cells (HSPCs) causes multi-lineage myelosuppression, including damage to various cell types of the immune system, potentially diminishing the activity of chemotherapy/immune checkpoint inhibitor (ICI) combinations
- No therapies exist to preserve HSPCs and multiple hematopoietic lineages, including the lymphocytic lineage, from chemotherapy-induced damage
- Trilaciclib is a highly potent, selective and reversible cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor in development to preserve HSPC and immune system function during chemotherapy (myelopreservation)
- SCLC was chosen as the first clinical setting to test the myelopreservation efficacy of trilaciclib because (1) of the high degree of myelotoxicity caused by the standard treatment regimens; (2) SCLC replicates independently of CDK4/6 allowing assessment of trilaciclib's effects on the host without any potential direct effects on the tumor; and (3) SCLC has a high response rate to first-line chemotherapy (etoposide/carboplatin (E/P)), thereby providing an optimal setting to demonstrate that trilaciclib does not antagonize the effects of chemotherapy
- This randomized, double-blind, placebo-controlled, two-part, Phase 1b/2 trial in SCLC demonstrated proof-of-concept for the potential myelopreservation benefits of trilaciclib including reduced multi-lineage myelosuppression (neutrophils, RBCs, lymphocytes) and reduced supportive care requirements and dose reductions (Dragnev et al ESMO 2018, Poster 1666PD)
- In addition to preserving HSPCs during chemotherapy, preclinical models have demonstrated trilaciclib can enhance anti-tumor response by augmenting T cell activation and modulating the function and/or differentiation of immune cell types in the tumor microenvironment, allowing for a more robust anti-tumor response when added to a chemotherapy/ICI combination (Sorrentino et al AACR 2017, Lai et al AACR 2018)
- Here, we evaluated the immunomodulatory properties of trilaciclib in lymphocyte subpopulations in SCLC patients that received E/P +/- trilaciclib

STUDY OBJECTIVE

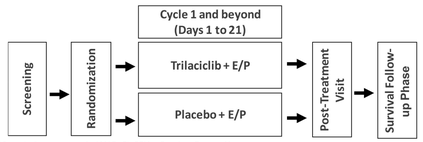
To assess the immunological changes in peripheral blood during E/P +/- trilaciclib treatment.

METHODS

- More comprehensive clinical trial details can be found on poster 1666PD; study schema can be found below in Figure 1.
- Whole blood was collected for analysis of absolute numbers and percentages of immune subsets by flow cytometry. Sample testing was performed at Covance Central Laboratories in accordance to the SOP for each validated assay panel.
- To assess the ability of T cells to produce cytokines, whole blood was stimulated with 5 ug/ml Staphylococcal Enterotoxin B overnight (15-18 hours) in the presence of Brefeldin A. Cells were then processed and labeled with antibodies against IFN-γ, IL-4, IL-2, CD45RA, IL-17A, CD4, and CD8 for flow cytometry analysis in accordance to the SOP established by Covance.
- Analyses included only those Part 2 patients who received 4+ cycles of chemotherapy and had successfully stained samples. Statistical outliers were excluded. Analyses were completed at Fios Genomics. Only data from C1D1, C3D1, C5D1 and Post-Treatment Visit (PTV) were graphed.
- Number of samples analyzed for flow cytometry analysis:

Treatment	C1D1	C3D1	C5D1	PTV	SFU
E/P	30	24	19	28	15
T/E/P	28	27	22	19	11

Figure 1. Study Schema

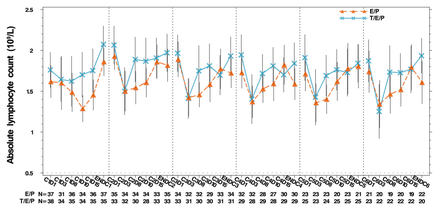


E/P = etoposide 100 mg/m² + carboplatin (AUC=5); SFU = Survival Follow-Up

- Trilaciclib + E/P continued until disease progression, unacceptable toxicity, or discontinuation by the patient or investigator (e.g., after completing 6 cycles). The tumor was assessed after every even cycle using Response Evaluation Criteria in Solid Tumors (RECIST), Version 1.1. Assessments were performed within 7 days of starting the subsequent cycle.
- Trilaciclib or placebo was administered prior to the administration of E/P on Day 1 and administration of etoposide on Days 2 and 3 of 21-day cycles.
- Patients returned to the study center for a Post-Treatment Visit at 30 + 3 days after the last dose of study drug.
- Peripheral blood samples were collected at predose on Day 1 of Cycles 1, 3, and 5; at the Post-Treatment Visit; and at first Survival Follow-Up Visit.
- The Survival Follow-up Phase will continue until at least 50% of the patients randomized to Part 2 of the study have died.

RESULTS

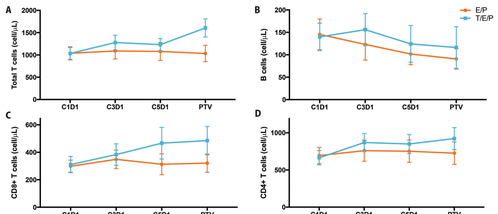
Figure 2. Lymphocyte Assessments Over Time



Hematological assessments were scheduled on days 1, 3, 8, 10, 15, and 22 of each cycle. Mean counts over time for lymphocytes are displayed above. Timing of assessments and number of patients at risk are shown on the X axis. The dotted line represents a CTCAE grade 3 lymphocyte count decreased value, i.e. 0.5 x 10⁹/L. C = cycle; D = day; ENDC = end of cycle which is defined as the last value measured prior to the first day of dosing in the subsequent cycle. Error bars represent 95% CI.

- **Patients that received trilaciclib prior to E/P demonstrated faster recovery of lymphocytes when compared to placebo**

Figure 3. Lymphocyte Subpopulations



Mean absolute cell count for (A) T cells (CD3+), (B) B cells (CD19+), (C) CD8+ T cells, (D) CD4+ T cells in whole blood was analyzed by flow cytometry at the indicated time points. Error bars represent 95% CI.

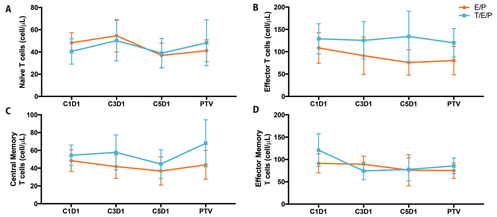
Table 1. Evaluation of Lymphocyte Subpopulations

Cell Population	Treatment Group	Slope Value	p-value
Total T Cells	Placebo slope (units/week)	0.016	0.996534
Total T Cells	**Trilaciclib slope (units/week)**	**11.872**	**0.00539**
Total T Cells	Difference (Trilaciclib-Placebo)	81.934	0.254362
B Cells	**Placebo slope (units/week)**	**-3.195**	**1.00E-06**
B Cells	Trilaciclib slope (units/week)	-1.048	0.118053
B Cells	Difference (Trilaciclib-Placebo)	24.925	0.172018
CD8+ T Cells	Placebo slope (units/week)	1.157	0.430809
CD8+ T Cells	**Trilaciclib slope (units/week)**	**6.990**	**1.50E-05**
CD8+ T Cells	**Difference (Trilaciclib-Placebo)**	**83.222**	**0.037337**
CD4+ T Cells	Placebo slope (units/week)	0.993	0.726558
CD4+ T Cells	**Trilaciclib slope (units/week)**	**9.487**	**0.002674**
CD4+ T Cells	Difference (Trilaciclib-Placebo)	92.308	0.148707

Linear mixed-effect model was fitted on the longitudinal data for each of the cell-types to evaluate the change of a specific cell population over time.

- **E/P decreased the number of circulating B cells over time but did not affect the number of other lymphocyte populations in the peripheral blood**
- **The addition of trilaciclib to E/P preserved the number of circulating B cells as well as increased the number of circulating CD8+ and CD4+ T cells compared to placebo**

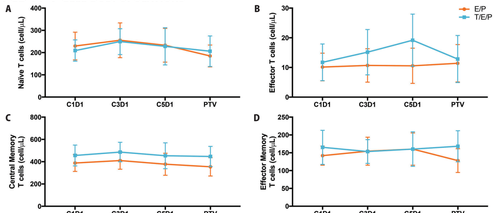
Figure 4. CD8+ T Cell Subpopulations



Mean absolute T cell count for (A) naïve cells (CD45RA+CCR7+CD3+CD8+), (B) effector cells (CD45RA+CCR7-CD3+DC8+), (C) central memory cells (CD45RA-CCR7+CD3+CD8+), and (D) effector memory cells (CD45RA-CCR7-CD3+CD8+), in whole blood was analyzed by flow cytometry at the indicated time points. Error bars represent 95% CI.

- **With the addition of trilaciclib to E/P, the increase in number of circulating CD8+ T cells is most pronounced in the effector T cell population and, to a lesser extent, in the central memory T cell population**

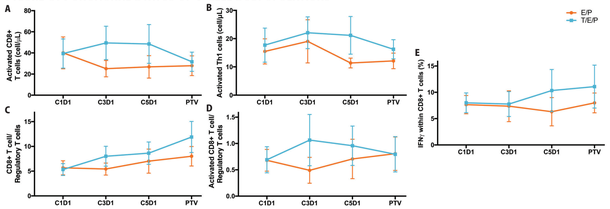
Figure 5. CD4+ T Cell Subpopulations



Mean absolute T cell count for (A) naïve cells (CD45RA+CCR7+CD3+CD4+), (B) effector cells (CD45RA+CCR7-CD3+CD4+), (C) central memory cells (CD45RA-CCR7+CD3+CD4+), and (D) effector memory cells (CD45RA-CCR7-CD3+CD4+), in whole blood was analyzed by flow cytometry at the indicated time points. Error bars represent 95% CI.

- **With the addition of trilaciclib to E/P, the increase in number of circulating CD4+ cells is most pronounced in the effector T cell population**
- **There were no changes in number of circulating regulatory T cells in either treatment arm**

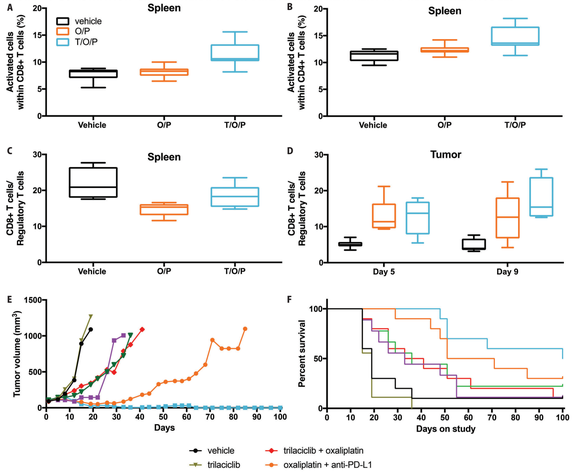
Figure 6. Functional Status of T Cell Subpopulations



Mean absolute cell count for (A) activated CD8+ T cells (CD38+HLA-DR+CD3+CD8+) and (B) activated Th1 cells (CXCR3+CXCR6-CD38+HLADR+CD3+CD4+). Mean ratios of absolute (C) CD8+ T/regulatory T cells and (D) activated CD8+ T/regulatory T cells. (E) Mean frequency of IFNγ+ population of CD8+ T cells after *ex vivo* stimulation (IFNγ+IL-17A-(CD3+CD8+). Cell populations in whole blood were analyzed by flow cytometry at the indicated time points. Error bars represent 95% CI.

- **The addition of trilaciclib to E/P preserved number of circulating activated CD8+ T and Th1 cells**
- **Trilaciclib also increased the ratio of total CD8+ T and activated CD8+ T cells to regulatory T cells**
- **After *ex vivo* stimulation, there is a higher frequency of CD8+ T cells producing IFNγ in patients that received trilaciclib prior to E/P compared to placebo, suggesting a more functional lymphocyte population**

Figure 7. Combination Chemotherapy/ICI/Trilaciclib Treatment in Preclinical Models



MC38 tumor bearing mice were treated intraperitoneally with trilaciclib (T, 100 mg/kg, D1, 8, 15), oxaliplatin (O, 10 mg/kg, D1, 8, 15), and/or αPD-L1 (P, 100 µg/animal, D1, 4, 8, 11) and T cell populations were evaluated in spleens and tumors. (A-B) Mean frequency of activated CD8+ T (CD8+CD4-CD69+) and CD4+ T(CD8-CD4+CD69+) populations in spleens on day 5. Mean ratio of CD8+ T (CD4-CD8+) to regulatory T cells (CD8-CD4+FoxP3+) on day 5 in (C) spleens and at indicated timepoints in (D) tumors. (E-F) Median tumor volume and survival analysis of mice treated as previously described.

- **The addition of trilaciclib to O/P increased the number of activated CD8+ and CD4+ T cells in spleens**
- **There is a higher ratio of CD8+ T to regulatory T cells in the spleens and tumors of animals that received T/O/P vs O/P**
- **Addition of trilaciclib to O/P treatment enhances tumor growth delay and durability of anti-tumor response leading to an increase in overall survival**

CONCLUSIONS

- Trilaciclib is the first therapeutic approach in clinical development that has demonstrated proof-of-concept for the potential to preserve HSPC and immune system function during chemotherapy (myelopreservation), including reduced multi-lineage myelosuppression and reduced supportive care requirements and dose reductions
- Peripheral blood immunophenotyping from this Phase 2 trial in SCLC (NCT02499770) demonstrated that trilaciclib potentially enhances lymphocyte function as measured by:
  - Preservation of circulating B cells and increased number of circulating T cells
  - Preservation of circulating activated CD8+ T and Th1 cells
  - Increased circulating CD8+ T/regulatory T cell ratio
- Collectively, these data suggest that during chemotherapy treatment, co-administration of trilaciclib can preserve multiple arms of the host immune system, as well as enhance CD8+ T cell function
- The myelopreservation benefits of trilaciclib demonstrated here, in addition to the preclinical murine data, suggest there is potential to enhance the anti-tumor response of a checkpoint inhibitor + chemotherapy regimen with trilaciclib. This hypothesis is being assessed in a randomized Phase 2 1st line SCLC trial (+atezolizumab/etoposide/carboplatin; NCT03041311)
- Trilaciclib is also being evaluated in two additional randomized Phase 2 trials: 2nd/3rd line SCLC (+topotecan; NCT02514447) and triple negative breast cancer (+gemcitabine/carboplatin; NCT02978716)

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