TRILACICLIB (G1T28), A CDK4/6 INHIBITOR, PRESERVES T LYMPHOCYTE FUNCTION FROM DAMAGE BY CYTOTOXIC CHEMOTHERAPY

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BACKGROUND

While chemotherapy-induced tumor cell death can be immunogenic (e.g. through the release of neoantigens), many chemotherapeutic regimens negatively impact the immune system leading to reduced anti-tumor efficacy. Lymphopenia is a common consequence of chemotherapy that can occur from the direct killing of lymphocytes, as well as through hematopoietic stem cell (HSC) damage and subsequent myeloid-biased differentiation (bone marrow exhaustion). Persistent lymphopenia is associated with worse clinical outcomes following chemotherapy, and may result in long-term clinical complications and impaired anti-tumor immunity.

Efforts to maximize the anti-tumor efficacy of immune checkpoint inhibitors have led to the clinical development of combinations with chemotherapy. Most chemotherapeutic agents cause immunogenic cell death that can prime an anti-tumor immune response. These chemotherapeutic effects can augment the therapeutic benefit of immune checkpoint inhibitors. However, in clinical practice, repeated cycles of chemotherapy reduces both the number and function of lymphocytes, which may antagonize the intended benefit of both chemotherapy and checkpoint inhibitors. Trilaciclib (G1T28) is an IV CDK4/6 inhibitor that preserves HSCs and enhances immune system function during chemotherapy. Trilaciclib transiently arrests HSCs and T lymphocytes and protects them from damage by chemotherapy. Trilaciclib has the potential to increase the tolerability and efficacy of chemotherapy and synergize with immune checkpoint inhibitors.

METHODS

CLINICAL LYMPHOCYTE COUNTS

As a clinical biomarker of immune system function, we evaluated the baseline lymphocyte counts from patients with small cell lung cancer (SCLC) who received first line carboplatin/etoposide (n=134) or second line topotecan therapy (n=78). The dataset included a retrospective chart review and data from two ongoing SCLC clinical trials testing the combination of trilaciclib with the following chemotherapy regimens (1st line, carboplatin-etoposide, NCT02499770; and 2nd line, topotecan, NCT02514447). In addition, serial lymphocyte counts were evaluated from patients enrolled into the two trilaciclib SCLC trials.

MICE

Mice were housed in the AAALAC-accredited, specific-pathogen-free animal care facility operated by the Division of Laboratory Animal Medicine at the University of North Carolina in Chapel Hill in accordance with protocols approved by the Institutional Animal Care and Use Committee.

SERIAL 5 FLUOROURACIL (5FU) TREATMENT AND COMPETITIVE BONE MARROW (BM) RECONSTITUTION ASSAY

Eight week-old female B6.SJL-Ptprca/BoyAiTac (CD45.1) mice were treated with vehicle or 150 mg/kg 5FU ± 150 mg/kg trilaciclib (oral gavage, 30 minutes prior to 5FU) every 21 days for 4 cycles. Eight weeks after the last dose of 5FU, BM cells were harvested and a competitive long-term BM reconstitution assay was performed by transplanting 1:1 of CD45.1+ BM (donor) and CD45.2+ (competitor) cells into lethally irradiated C57BL/6 (CD45.2+) recipients. Peripheral blood (PB) was collected from recipient mice at 4-week intervals for at least 16 weeks after transplantation. 32 weeks after initial transplantation, BM cells were harvested from each primary recipient mouse, analyzed for donor cell contribution to each hematopoietic lineage, and competitively re-transplanted into individual lethally irradiated secondary recipient (CD45.2+). The frequencies of donor CD45.1+ cells in each blood lineage were monitored by analyzing the PB of secondary recipients at 4-week intervals for at least 16 weeks post transplantation.

T LYMPHOCYTES STIMULATION ASSAY

C57Bl/6 mice were treated with 3 daily intraperitoneal (IP) doses of 50 mg/kg 5FU ±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 (IFNg) or interleukin-2 (IL-2) levels were measured via ELISA (R&D systems).

ACKNOWLEDGEMENTS

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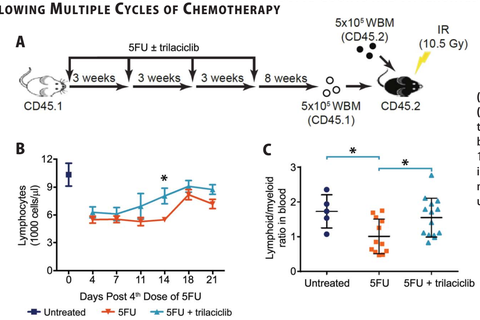
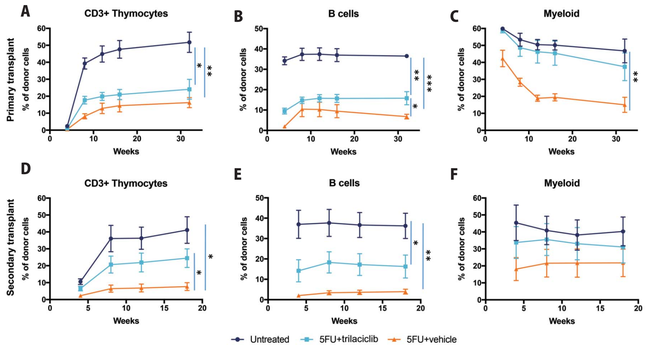
RESULTS

FIGURE 1. TRILACICLIB PRESERVES ACUTE LYMPHOCYTE COUNTS AND ATTENUATES MYELOID BIASED DIFFERENTIATION FOLLOWING MULTIPLE CYCLES OF CHEMOTHERAPY



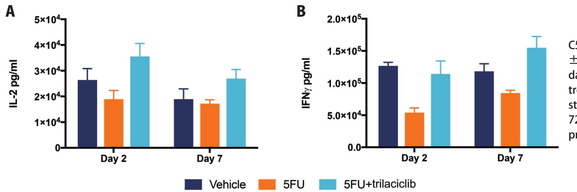
(A) Treatment and BM transplantation schematic. **(B)** Serial lymphocyte counts from 4 to 21 days after the fourth dose of 5FU treatment. **(C)** Peripheral blood lymphoid/myeloid cell ratios were determined 13 months after the 4th 21-day cycle of 5FU treatment in mice that were not transplanted. Data represent mean ± SD. Statistical significance was assessed using two-tailed Student's t-test (*P < 0.05).

FIGURE 2. TRILACICLIB PRESERVES HSC FUNCTION FROM PROLIFERATIVE EXHAUSTION



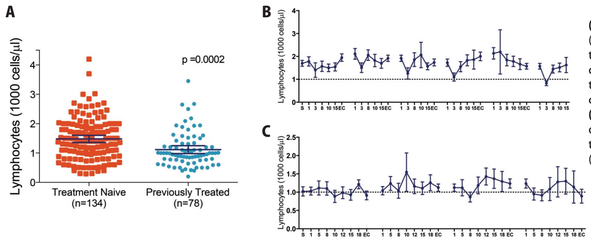
Mice treated as described in Figure 1A. The percentage of donor derived cells (CD45.1+) in T lymphocyte (CD3+), B lymphocyte (B220+), and myeloid cell (Mac1+) fractions 1 to 4 months after primary **(A-C)** or secondary **(D-F)** BM transplantation. Statistical significance were assessed using unpaired, two-tailed Student's t-test (*P<0.05, **P < 0.01, ***P < 0.001)

FIGURE 3. TRILACICLIB PRESERVES LYMPHOCYTE FUNCTION



C57BL/6 mice were treated with 50 mg/kg 5FU ± trilaciclib (100 mg/kg, IP) or vehicle for three days. Two and seven days after the final treatment, splenocytes were isolated and stimulated with α-CD3/CD28 antibodies for 72 hours to measure **(A)** IL-2 and **(B)** IFN γ production by ELISA.

FIGURE 4. RETROSPECTIVE AND PROSPECTIVE LYMPHOCYTE COUNTS IN PATIENTS WITH SCLC RECEIVING CHEMOTHERAPY



(A) Baseline lymphocyte counts from 1st line (treatment naïve) and 2nd line (previously treated) SCLC patients. **(B)** Serial lymphocyte counts from an ongoing 1st line SCLC clinical trial testing trilaciclib 240 mg/m² plus carboplatin-etoposide (n=10; NCT02499770). **(C)** Serial lymphocyte counts from an ongoing 2nd/3rd line SCLC clinical trial testing trilaciclib 240 mg/m² plus topotecan (n=8, NCT02514447).

CONCLUSIONS

1. Chemotherapy-associated bone marrow toxicity results from HSC damage and premature exhaustion.
2. Trilaciclib administered prior to chemotherapy preserves HSC function, thereby ameliorating the long-term toxicity associated with serial exposure to chemotherapy agents: i.e. "exhaustion", myeloid-biased differentiation, and consequent lymphopenia.
3. In addition to preserving lymphocyte number, trilaciclib preserves lymphocyte function following exposure to chemotherapy.
4. Chemotherapy-induced lymphopenia is a well-known phenomenon which we show persists for months after completion of 1st line chemotherapy in patients with SCLC.
5. In the clinic, trilaciclib maintains lymphocyte counts in patients receiving multiple cycles of 1st or 2nd line chemotherapy for the treatment of SCLC.
6. Trilaciclib-mediated preservation of immune system function during chemotherapy may enhance the efficacy of chemotherapy. Clinical trials testing trilaciclib administered with chemotherapy are ongoing, and novel combination(s) with chemotherapy and immune checkpoint inhibitors are planned.