TRILACICLIB, A CDK4/6 INHIBITOR, DOES NOT IMPAIR THE EFFICACY OF CHEMOTHERAPY IN CDK4/6-DEPENDENT TUMOR MODELS

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BACKGROUND

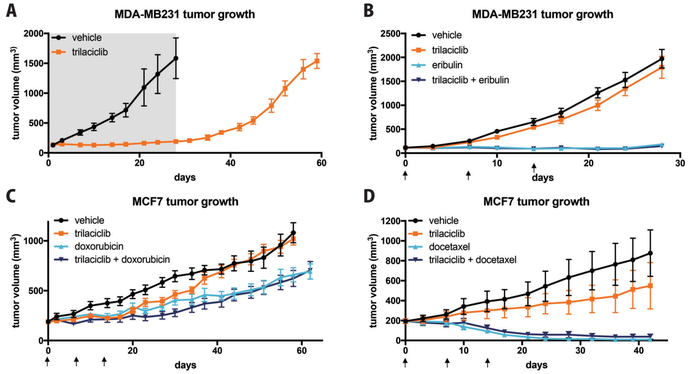
- Trilaciclib is a highly potent, selective, and reversible cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor in development to preserve hematopoietic stem and progenitor cells (HSPC) and immune system function during chemotherapy (myelopreservation)
- In preclinical studies, administration of trilaciclib prior to chemotherapy has been shown to induce transient cell-cycle arrest of HSPC, preserve HSPC and immune system function, protect against bone marrow exhaustion, improve complete blood counts (CBC) recovery, prevent myeloid skewing and consequent lymphopenia, and enhance T-cell effector function in the tumor microenvironment
- Trilaciclib has demonstrated proof-of-concept myelopreservation benefits in a recent randomized, placebo-controlled, double-blind Phase 2 trial (NCT02499770) in patients with small cell lung cancer (SCLC) receiving 1st-line chemotherapy, including reduced multi-lineage myelosuppression, reduced supportive care requirements, and reduced chemotherapy dose reductions
- There may be a theoretical risk that administration of trilaciclib prior to chemotherapy to patients with CDK4/6-dependent tumors may antagonize the intended anti-tumor efficacy of the chemotherapy
- The early clinical development of trilaciclib has focused on patient populations with CDK4/6-independent tumors through universal loss of Rb, such as SCLC, or have predominately Rb-null phenotypes, such as triple negative breast cancer (TNBC), thereby allowing assessment of trilaciclib's effects on the host without any potential direct effects on the tumor
- To assess whether trilaciclib may be used in patients with CDK4/6-dependent tumors that are receiving myelosuppressive chemotherapy, we evaluated the anti-tumor efficacy of trilaciclib plus chemotherapy compared to chemotherapy alone in CDK4/6-dependent tumor models

GOALS AND OBJECTIVES

To determine whether transient CDK4/6 inhibition with trilaciclib prior to chemotherapy administration antagonizes the intended anti-tumor effects of the chemotherapy in CDK4/6-dependent tumors.

RESULTS

FIGURE 1. TRILACICLIB DOES NOT DECREASE CHEMOTHERAPY EFFICACY IN CDK4/6-DEPENDENT CELL-BASED XENOGRAFT MODELS

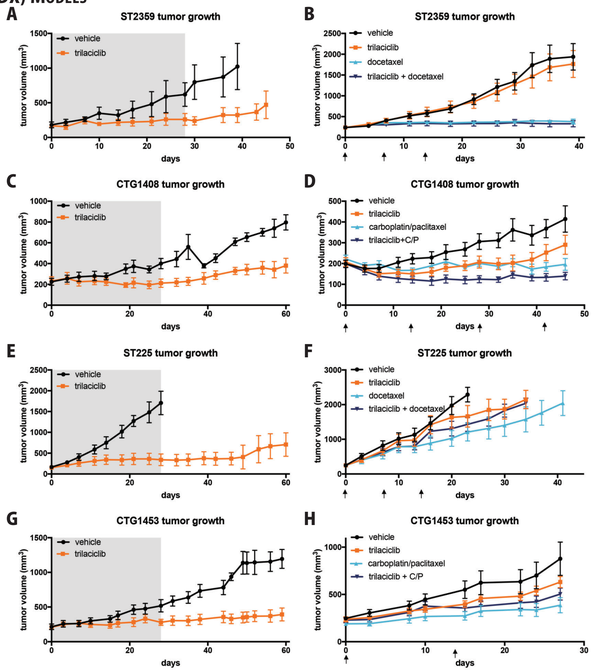


(A) MDA-MB231 tumor-bearing mice were treated with daily trilaciclib (IP, 100 mg/kg, n=6) for 28 days to confirm CDK4/6 dependence. (B) MDA-MB231 tumor-bearing mice were treated with eribulin (IV, 0.5 mg/kg) +/- trilaciclib once weekly for three doses (n=10). (C) MCF7 tumor-bearing mice, well-established to be CDK4/6 dependent, were treated with doxorubicin (IV, 5 mg/kg) +/- trilaciclib once weekly for three doses (n=10). (D) MCF7 tumor-bearing mice were treated with docetaxel (IV, 20 mg/kg) +/- trilaciclib once weekly for three doses (n=10). In all experiments, tumor volume was calculated twice weekly. Trilaciclib was given 30 minutes prior to chemotherapy treatment (B-D). Shading represents daily dosing. Arrows represent days of treatment administration starting on Day 0. Graphs represent mean tumor volume over time. Error bars represent SEM. The MDA-MB231 model was performed at Charles River Labs (Research Triangle Park, NC) and the MCF7 model was performed at START (San Antonio, TX).

- MDA-MB231 and MCF7 are well-established breast tumor models known to be highly sensitive to CDK4/6 inhibition (Bisi et al. 2017)
- Transient CDK4/6 inhibition prior to chemotherapy (eribulin, doxorubicin, docetaxel) did not antagonize the intended anti-tumor effects of the chemotherapy in these CDK4/6-dependent tumor models

RESULTS

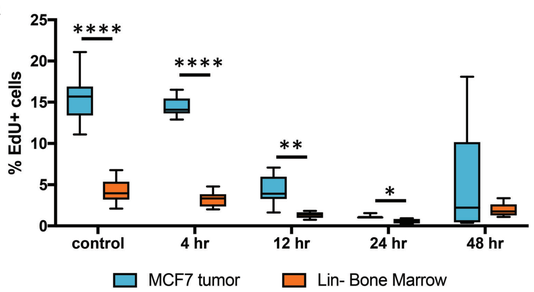
FIGURE 2. TRILACICLIB DOES NOT DECREASE CHEMOTHERAPY EFFICACY IN CDK4/6-DEPENDENT PATIENT-DERIVED XENOGRAFT (PDX) MODELS



Breast cancer PDX models were treated with daily trilaciclib (IP, 100 mg/kg, n=3-6 per cohort) for 28 days to identify models that were CDK4/6 dependent, which was defined as having a > 58% tumor growth inhibition (TGI) at day 28. Using this criterion, four CDK4/6-dependent tumor models (A, C, E, G) were selected to evaluate the effects of transient trilaciclib administration prior to chemotherapy on the anti-tumor efficacy of the chemotherapy (B, D, F, H). In each model, trilaciclib (IP, 100 mg/kg) or vehicle control was administered 30 minutes before chemotherapy. (B) ST2359 tumor-bearing mice were treated with docetaxel (IP, 10 mg/kg) +/- trilaciclib once weekly for three doses (n=8). (D) CTG1408 tumor-bearing mice were treated with carboplatin (IP, 50 mg/kg)/paclitaxel (IV, 10 mg/kg) +/- trilaciclib once every two weeks for four doses (n=8). (F) ST225 tumor-bearing mice were treated with docetaxel (IP, 10 mg/kg) +/- trilaciclib once weekly for three doses (n=8). (H) CTG1453 tumor-bearing mice were treated with carboplatin (IP, 50 mg/kg)/paclitaxel (IV, 10 mg/kg) +/- trilaciclib once every two weeks for two doses (n=8). In all experiments, tumor volume was calculated twice weekly. Shading represents daily dosing. Arrows represent days of treatment administration starting on Day 0. Graphs represent mean tumor volume over time. Error bars represent SEM. PDX models were performed at START (ST2359 and ST225; San Antonio, TX) or Champions Oncology (CTG1408 and CTG1453; Rockville MD).

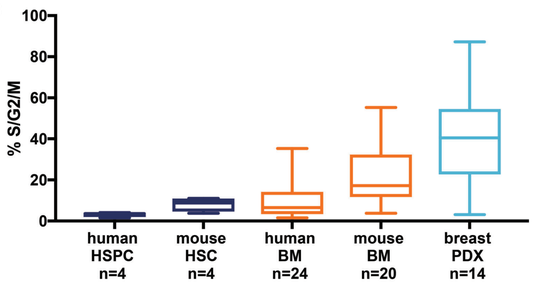
- To confirm the findings from cell line-based xenograft models shown in Figure 1, a panel of CDK4/6-dependent PDX models was used to compare the anti-tumor activity of chemotherapy alone versus chemotherapy plus trilaciclib
- The addition of trilaciclib to chemotherapy did not negatively impact the anti-tumor activity of the chemotherapy in three out of four models (ST2359, CTG1408, CTG1453) as measured by linear regression analysis
- In the ST225 model, which was the least sensitive to chemotherapy, the addition of trilaciclib showed a modest separation between the docetaxel and docetaxel plus trilaciclib arms after completion of chemotherapy, however the combination arm still demonstrated tumor growth inhibition

FIGURE 3. TUMORS HAVE A GREATER PERCENTAGE OF ACTIVELY DIVIDING CELLS COMPARED TO BONE MARROW



(A) MCF7 tumor-bearing mice were treated with a single dose of trilaciclib (IP, 100 mg/kg) or vehicle control. 4, 12, 24, and 48 hours post-treatment, animals were pulsed with 5 ethynyl-2′-deoxyuridine (EdU; IP, 200 μg). Tumors and femurs from each animal were then harvested after 4 hours of EdU dosing and processed to single cell suspensions for detection of EdU+ cells by flow cytometry. HSPC in bone marrow is defined as cell populations negative for lineage markers Mac-1, Gr-1, Ter119, B220, CD4, and CD8. The mean percentage of cycling MCF7 tumor cells at baseline (15.57%) is significantly higher than the percentage of cycling cells in lineage negative (Lin-) bone marrow (4.1%) as measured by EdU incorporation.

****p≤ 0.0001, **p≤ 0.01, *p≤ 0.05



(B) To further evaluate the difference in cell cycle kinetics between hematopoietic stem cells (HSC), HSPC, bone marrow (BM), and PDX tumor cells in mice and humans, the mean differences in baseline proliferation rates were examined using flow cytometric analysis of the cell cycle (He et al. 2017). Error bars represent the minimum and maximum.

- Direct comparison of cell cycle kinetics following trilaciclib administration shows a higher proportion of cycling cells (cells in S/G2/M) through 24 hours post-treatment in MCF7 tumor cells compared to bone marrow
- At baseline, a higher proportion of breast PDX and MCF7 tumor cells are cycling when compared to total bone marrow or the HSPC compartment from both mice and humans

CONCLUSIONS

- Trilaciclib, a highly potent, selective, and reversible CDK4/6 inhibitor, does not decrease chemotherapy efficacy in CDK4/6-dependent xenograft and PDX models
- Trilaciclib maintains G1-arrest of HSC and HSPC (He et al. 2017) while a significant fraction of tumor cells are past the G1 checkpoint (in S/G2/M; shown above), thereby creating a therapeutic window for the selective protection of bone marrow compared to CDK4/6-dependent tumor cells from the cytotoxic effects of chemotherapy
- The addition of trilaciclib to multiple chemotherapy treatments in both CDK4/6-dependent and -independent tumors suggests trilaciclib can be used in both chemotherapy and tumor agnostic manners
- Trilaciclib is being evaluated for myelopreservation in four randomized Phase 2 trials: 1st-line SCLC (+etoposide/carboplatin; NCT02499770), 1st-line SCLC (+atezolizumab/etoposide/carboplatin; NCT03041311), 2nd/3rd-line SCLC (+topotecan; NCT02514447), and TNBC (+gemcitabine/carboplatin; NCT02978716)