

**IMMUNE CHECKPOINT BLOCKADE AUGMENTS
CYCLOPHOSPHAMIDE INDUCED ANTI-TUMOR
IMMUNITY BY EXPANDING EFFECTOR CD8⁺ T CELL
CLONES IN A PRECLINICAL MELANOMA MODEL**

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Background Cancer treatment using immune checkpoint blockade (ICB) with anti-programmed cell death protein (PD)-1 and anti-cytotoxic T lymphocyte associated protein (CTLA)-4 has been successful, but primary and acquired resistance limits their clinical benefit. Chemotherapeutic agents such as Cyclophosphamide (CTX) are known to induce anticancer immunity and improves efficacy of ICBs when used in combination. Exploring means to improve the clinical benefit of ICB therapies by reorchestrating anti-tumor immunity with mechanism-based drug combinations is warranted. The alkylating chemotherapeutic agent, CTX, has direct tumoricidal and immunomodulatory properties that preferentially depletes T cell subsets (such as T regulatory cells) and induces homeostatic proliferation of T cells. We hypothesized that the addition of ICBs to CTX treatment could augment immunomodulatory changes induced by CTX including homeostatic proliferation of antigen specific T cells which will reset the T cell receptor (TCR) repertoire in favor of tumor specific T cells.

Methods We used a murine melanoma tumor model, B16-F10 known to be refractory to ICBs, particularly, anti-PD-1.

Results Here, we show that a single dose of CTX one day prior to starting an ICB (anti-PD-1 and anti-CTLA-4) regimen slows tumor progression compared to CTX or ICB alone and significantly prolongs host survival. The triple combination (CTX+ anti-PD-1 + anti-CTLA-4) increased the number of activated and proliferating CD8⁺ tumor-infiltrating lymphocytes (TILs) and increased the CD8/Treg ratio. A decrease in dysfunctional CD8⁺ TILs further augmented tumor control by the triple combination therapy. The depletion of CD8⁺ but not CD4⁺ cells abrogates the therapeutic effect of the triple combination suggesting that the anti-tumor effect is CD8⁺ T cell dependent. Through TCR sequencing platforms, we show that the expanding CD8⁺ TCR clones possess a majority effector phenotype following triple combination therapy compared to control groups that possessed more dysfunctional TCR clones. The therapeutic effect of the triple combination extends to other murine tumor models namely, MC38 (colon adenocarcinoma) and E0771 (triple negative mammary carcinoma).

Conclusions These findings suggest that the combination of CTX with ICBs is a potent combinatorial approach that can prime an anti-tumor response and promote robust control of tumor growth. Further investigations are required to understand the mechanisms of combinatorial cancer therapies in refractory tumor models and to inform the design of future therapeutic interventions combining ICB with chemotherapy.

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.0789>