Combination Immunotherapies

**IMMUNE CHECKPOINT BLOCKADE ENHANCES CYCLOPHOSPHAMIDE INDUCED ANTI-TUMOR IMMUNITY IN A PRECLINICAL MELANOMA MODEL**

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**Background** Immune Checkpoint Blockade (ICB) with anti-programmed cell death protein (PD)-1 and anti-cytotoxic T lymphocyte associated protein (CTLA)-4 have had immense success in cancer treatment. However, primary and acquired resistance to these therapies limits their clinical benefit. Accumulating evidence indicates that chemotherapeutic agents such as Cyclophosphamide (CTX) can induce anticancer immunity and improves the efficacy of ICBs when used in combination. A combination strategy to reorchestrate the anti-tumor immune response could magnify the clinical benefit of ICBs. CTX is an alkylating chemotherapeutic agent which is directly tumoricidal and induces immunomodulatory properties. CTX preferentially depletes T cell subsets (such as T regulatory cells) and leads to homeostatic proliferation of T cells. We hypothesized that the addition of ICBs to CTX treatment will augment the immunomodulatory changes induced by CTX including but not limited to the homeostatic proliferation of T cells which will reset the T cell receptor (TCR) repertoire to favor tumor antigen-specific T cells. The murine melanoma tumor model, B16-F10, is known to be refractory to treatment with ICBs, particularly anti-PD-1, as it only modestly slows tumor growth.

**Methods** Mice were given a single dose of CTX one day prior to starting an ICB (anti-PD-1 and/or anti-CTLA-4) regimen.

**Results** The treatment slows tumor progression of B16-F10 compared to CTX or ICB alone and significantly prolongs the survival of mice. Further, the combination of CTX with anti-PD-1 and anti-CTLA-4 increased the number of activated and proliferating CD8+ tumor infiltrating lymphocytes (TILs). This increase in cytotoxic T cells was accompanied by a decrease in Tregs, which further augments the tumor control. Additionally, we observed a significant increase in a highly cytolytic population of CD4+ CD8+ double positive TILs. The depletion of CD8+ cells but not CD4+ cells abrogates the therapeutic effect of the triple combination suggesting that the anti-tumor effect is CD8+ T cell dependent. The tumor control effect of the triple combination (CTX+ anti-PD-1 + anti-CTLA-4) extends to other murine tumor models namely, MC38 (colon adenocarcinoma) and 4T1 (mammary carcinoma).

**Conclusions** Our results suggest that the combination of CTX with ICBs, anti-PD-1 and anti-CTLA-4, is a potent combinatorial approach that can prime an anti-tumor response and promote robust control of tumor growth. These findings form the basis for further investigations in understanding the mechanisms of combinatorial cancer therapies in tumor models that are refractory to ICB therapies and can inform the design of future therapeutic interventions that combine ICB with chemotherapy.