Ultra-High Concentration Nitric Oxide (UNO) Enhances Anti-CTLA-4 Treatment Activity and Induces a Durable Anti-Tumor Immune Response

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Background

We previously demonstrated that intratumoral administration of ultra-high concentration nitric oxide (UNO) favorably augments the immune response in CT26 tumor-bearing mice. Five to 14 days after treatment, antigen-presenting cells inform the adaptive immune response by presenting antigens to immature T cells. At day 14, treatment with 50,000 ppm UNO resulted in higher levels of T-cell, B-cell, macrophage, and dendrocyte infiltration compared to both nitrogen and 20,000 ppm UNO (Figure 1).

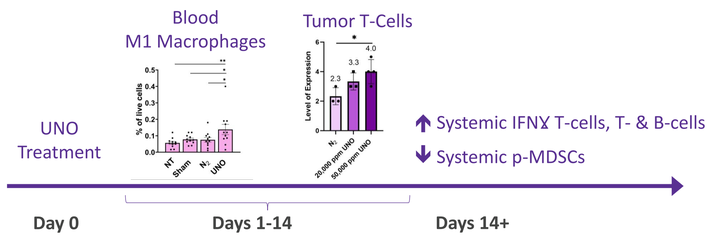

Figure 1: Timeline Showing the MOA Following UNO Treatment

Methods

In this study, we assessed the systemic and local tumor response of CD8+ T-cells, T-cells, and systemic central memory T-cells, recognizing the AH-1 antigen following 50,000 or 100,000 ppm UNO and 5mg/kg anti-mCTLA-4 treatment given for 2 to 5 doses in CT26 (mouse colon cancer model) tumor-bearing BALB/c mice.

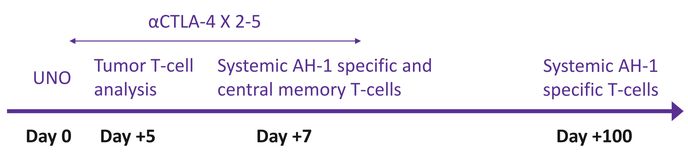

Figure 2: Study Scheme

Tumor T-cell Response Following UNO and Anti-mCTLA-4

CD8+ cytotoxic T-cells (Figure 3A) and CD4+ helper T-cells (Figure 3B) are elevated in the tumor at day 5 post-treatment. In addition, more T-helper cells are active following UNO and anti-mCTLA-4 treatment as seen by IFNɣ upregulation (Figure 3C).

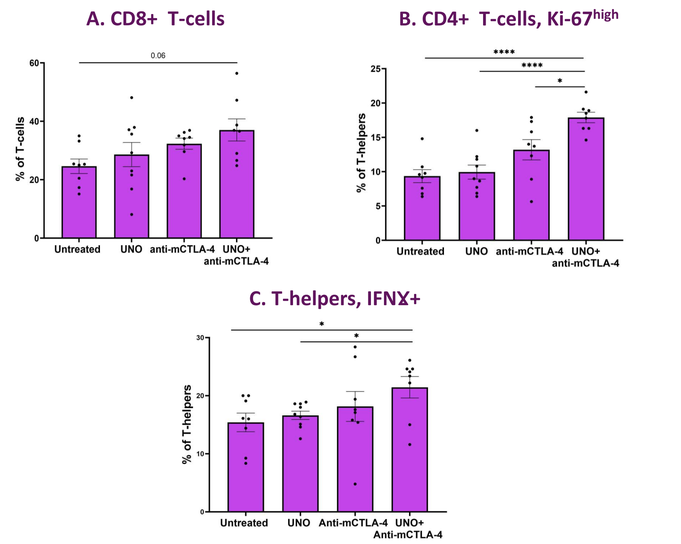

Figure 3: Tumor T-cell Levels Following UNO and Anti-mCTLA-4 Treatment at Day 5. (A) Tumor T-cytotoxic cells (B) Proliferating Tumor T-helper cells, (C) IFNɣ+ expressing tumor T-helper cells. *p<0.05, ****p<0.0001

Systemic T-cell Response Following UNO and Anti-mCTLA-4

The immunodominant AH-1 antigen is associated with anti-tumor immune response to CT26 tumors. A systemic antigen-specific and central memory (CM) response is observed by day 7 (Figure 4A and 4B). Durable upregulation in systemic AH-1-specific T-cells is observed in cured mice at day 55 (Figure 4C) and day 100 (Figure 4D).

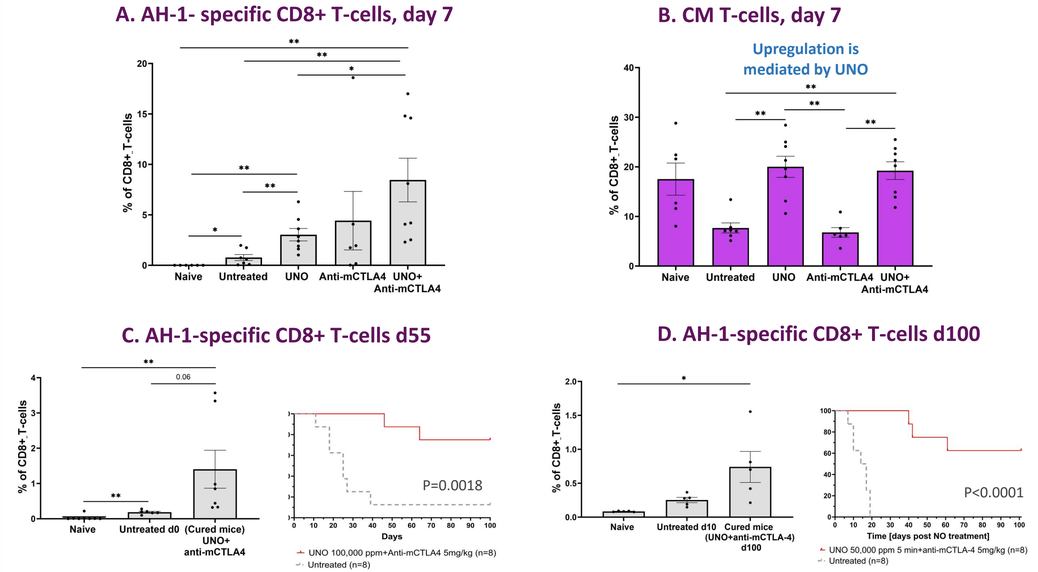

Figure 4: Systemic Antigen-specific and Memory T-cells Following UNO and Anti-mCTLA-4 Treatment at Days 7, 55, and 100. (A) Blood AH-1 T-cells at day 7, (B) Blood CM T-cells at day 7, (C,D) Blood AH-1-specific T-cells (Left), and mouse survival (Right) at day 55 and 100. *p<0.05, **p<0.01

Conclusions

Intratumorally, at day 5 post-UNO treatment, T-helper and T-cytotoxic cells are significantly increased in the combination arm relative to the anti-mCTLA-4 arm alone. Systemically, UNO stimulates a significantly higher central memory T-cell response than anti-mCTLA-4 and synergizes with this drug by day 7 to generate a higher antigen-specific T-cell response. Durable upregulation in systemic AH-1-specific T-cells is still seen at day 55 post-therapy and has been confirmed as late as day 100. A clinical study investigating intratumoral administration of UNO has been initiated (BA-ONC-01 clinicaltrials.gov NCT identifier: NCT05351502).