Intratumoral Administration of Ultra High-Concentration Nitric Oxide (UNO) and Anti PD-1 Treatment Leads to High Tumor Regression Rates and Prolonged Survival in Tumor-Bearing Mice

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Background

Immune checkpoint inhibitors have transformed clinical oncology. However, their use is limited to a subset of tumors as response is observed in only ~20% - 50% of patients.

Nitric Oxide (NO) is a signaling molecule, found to have a key function in multiple diseases, including cancer. Furthermore, it has been shown to activate anti-tumor immune responses. Previously, we reported that treating CT26 tumor-bearing mice with ultra-high-concentration gaseous NO (UNO) followed by tumor resection stimulated antitumor immune responses.

In a previous study, we showed that treatment of 50,000 ppm NO for 10 minutes in combination with anti-mouse-PD-1 (anti-mPD-1) resulted in primary and secondary tumor complete regression in 53% of treated mice at day 100 post-UNO treatment(1).

Here, we investigated a higher dose NO over a shorter delivery time to evaluate the hypothesis of improved efficacy of a combination NO and programmed cell death protein-1 (PD-1) antibody in treating CT26 tumor-bearing mice. In this study, we tested the potential efficacy of 100,000 ppm NO administered for 5-minutes in combination with anti-mPD-1.

Methods

At day zero, 0.5 X 10^6 CT26 cells were injected to the right flank of Balb/c male mice (n=8 per group) and at Day three, CT26 cells were injected to the contralateral flank. On Day twelve, tumors (average size 75-100 mm^3) were treated intratumorally with UNO (100,000 ppm, 5 minutes, flow rate ~0.2 liter per minute). Day thirteen anti-mPD-1 injections (5mg/kg, q3d, x 5) commenced. Post-treatment tumor volume and survival were monitored thereafter.

Results

Complete regression of the primary tumor occurred in 2/8 (25%) of mice treated with combination of 5-minute UNO and anti-mPD-1, 26 days post-treatment. This is compared to 1/8 (12.5%) of controls treated with anti-mPD-1 alone and 0/8 (0%) treated with UNO alone. In the nitrogen + anti-mPD-1 group primary tumor regression occurred in 1/8 mice (12.5%), showing no benefit over anti-mPD-1.

Six of eight (~75%) of the mice in the UNO+anti-mPD-1 arm are distant tumor free, compared to 3/8 – 4/8 (37.5% - 50%) in all control arms, resulting in 25% tumor-free mice in the UNO+anti-mPD-1 arm.

Survival was increased in the UNO/anti-mPD-1 combination arm compared to anti-mPD-1 alone, 26 days post-treatment.

Conclusion

UNO in combination with PD-1 blockade resulted in an increase in the proportion of mice that demonstrate regression of primary tumors, an increase in the number of tumor-free mice, and prolonged survival.

Here we present a second study showing similar results at day 26 using a shorter duration of UNO. The results imply that UNO sensitizes CT26 tumors to anti-mPD-1 therapy with improved outcomes compared to each therapy alone.

1. Confino H, Dirbas FM, Goldshtein M, Yarkoni S, Kalaora R, Hatan M, et al. Gaseous nitric oxide tumor ablation induces an anti-tumor abscopal effect. Cancer Cell Int. 2022;22(1):405.