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Short-Term Exposure of Cancer Cells to Ultra-High Concentrations of Nitric Oxide (UNO) Activates the mPD-1/mPD-L1 Axis and Immune Response

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Background

We have previously shown that treating mouse colon carcinoma (CT26) tumor-bearing mice with ultra-high concentrations of nitric oxide (UNO) upregulates innate and adaptive immune cells both locally and systemically. Furthermore, we demonstrated that co-treatment of mice with mPD-1 antibody and UNO results in long term tumor regression and survival (Figure 1). In the current study, we assessed mPD-L1 and immune response following short-term exposure to UNO.

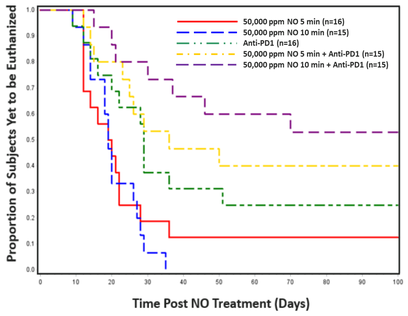


Figure 1: Increase in survivability of CT26 tumor-bearing mice following 10-min 50,000 ppm NO and anti-mPD-1. Comparison Hazard Ratio, 50,000 ppm NO 10 min + Anti-mPD1 vs Anti-mPD1.

HR = 0.41, p-value = 0.0653,[95% CI] = [0.16, 1.06] (Cox proportional hazard model)

Methods

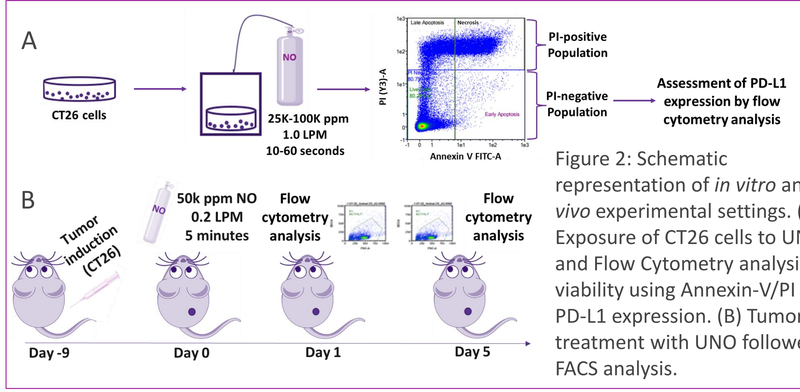
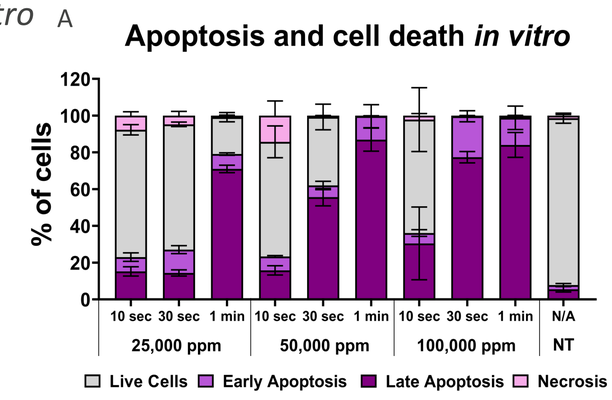


Figure 2: Schematic representation of *in vitro* and *in vivo* experimental settings. (A) Exposure of CT26 cells to UNO and Flow Cytometry analysis of viability using Annexin-V/PI and PD-L1 expression. (B) Tumor treatment with UNO followed by FACS analysis.

Results

In vitro



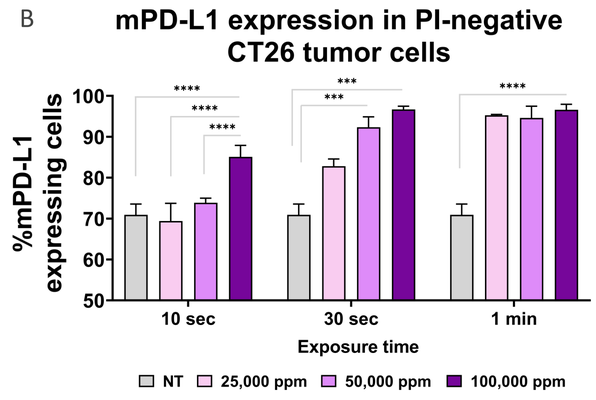


Figure 3: Cell death mechanism and mPD-L1 expression after exposure to UNO. (A) Apoptotic cell mechanism analysis using Annexin V/PI intracellular staining and (B) mPD-L1 expression on PI-negative cells. Two-way ANOVA, multiple comparison test, ***P<0.001, ****P<0.0001.

In vivo

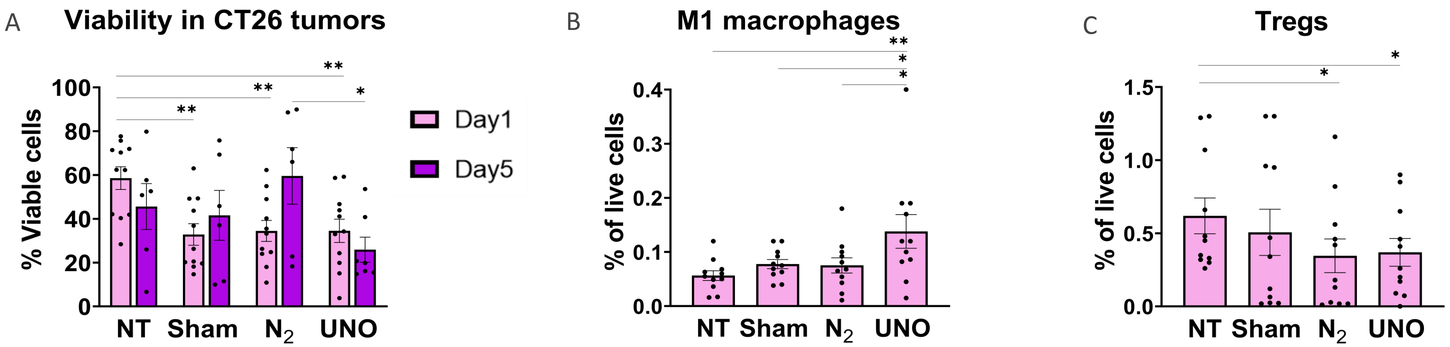


Figure 4: Cell viability and immune profiling of CT26 mice treated with NO 50,000 ppm for 5 minutes. (A) Viability of tumor cells in CT26 tumors treated with 50,000 ppm NO at 1- and 5-days post treatment, (B) Levels of blood M1 macrophages at day 1 after treatment, (C) Tregs in CT26 tumors 1 day after treatment. A,B were analyzed by One-way ANOVA, multiple comparison test, and C was analyzed by Two-way ANOVA *P<0.001, **P<0.0001.

Conclusions

Short exposure of CT26 cells to UNO results in the upregulation of mPD-L1, suggesting that local treatment with UNO in solid tumors may sensitize “cold” tumor cells within the tumor mass to become responsive to immune checkpoint blockade. In addition, UNO prompts a more favorable local and systemic immune environment that may further enhance anti-tumor response.