# 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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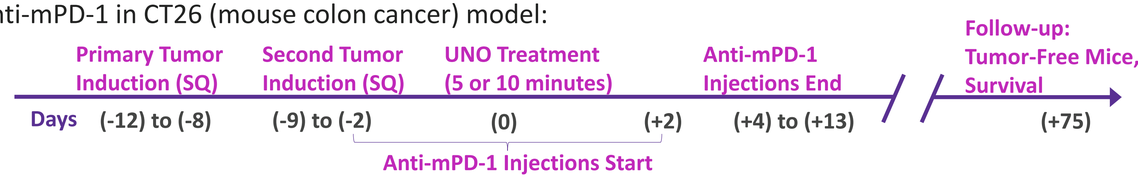
AACR-NCI-EORTC INTERNATIONAL CONFERENCE ON MOLECULAR TARGETS AND CANCER THERAPEUTICS
October 11-15, 2023

BEYOND CANCER™ Next level immuNO-oncology

## Background

Nitric Oxide (NO) has been shown to activate anti-tumor immune responses. Previously, we reported that combining 10-min UNO with anti-mouse PD-1 (anti-mPD-1) in CT26 (mouse colon cancer) model resulted in higher rates of primary and secondary tumor-free mice compared to all control arms.
Here we present a pooled data analysis across many combination studies using 5 or 10-min UNO and anti-mPD-1 in CT26 (mouse colon cancer) model:




- Experimental model: CT26; Mouse model: Balb/c mice.
- UNO treatment regimen: 50,000 or 100,000 ppm injected for 5 or 10 minutes, at 0.2 LPM.
- Anti-mPD-1 dosing started at days (-2) to (+2). 5 or 10 mg/kg doses injected every 2-3 days, 4-5 doses in total.
- All studies were conducted under approved IACUC protocols.

## Effect of Single UNO Treatment and Anti-mPD-1 on % Tumor-Free Mice

Combination UNO and anti-mPD-1 doubled the number of cured mice compared to the antibody alone. This improvement in the percentage of cured mice was not observed when the tumors were treated with nitrogen and anti-mPD-1 combination.

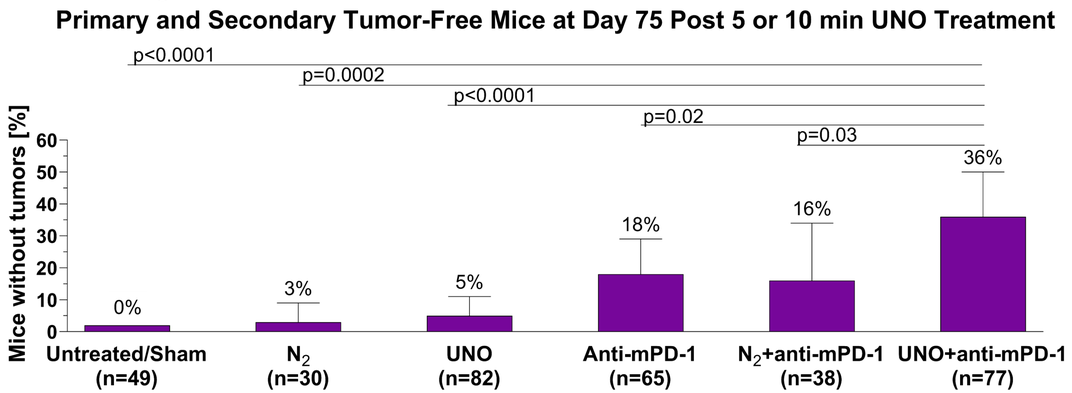
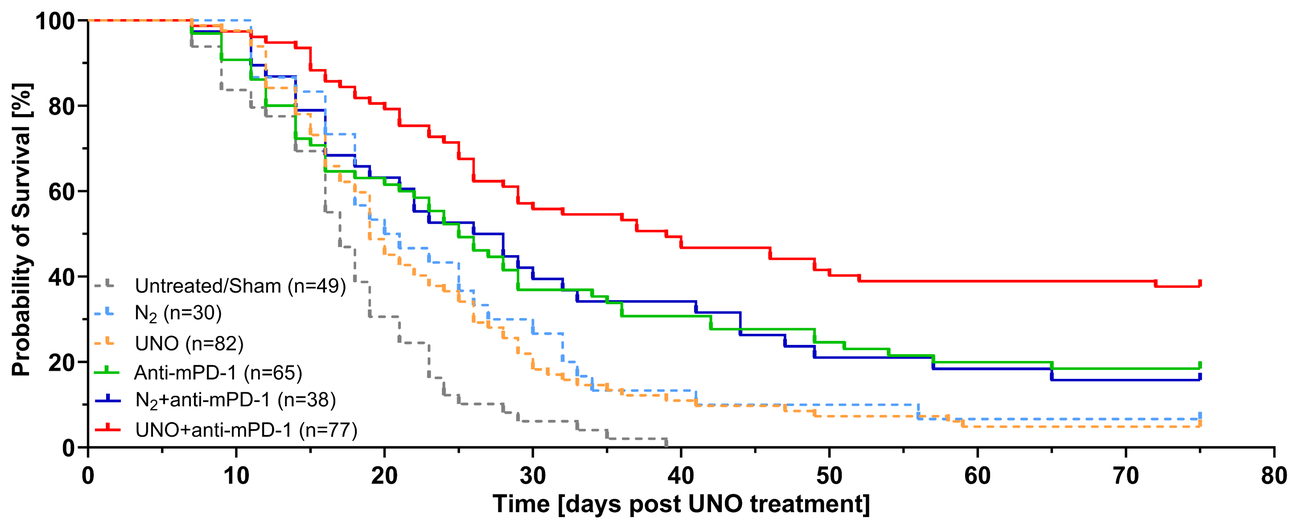



**Figure 1: Pooled data across studies of 5 or 10-min UNO and anti-mPD-1 (5 or 10 mg/kg) treatment – cured mice.** N per group indicated on the graph. Bars represent weighted mean ± SD. Fisher's exact test was used for statistical analysis.

## Effect of Single UNO Treatment and Anti-mPD-1 on Mice Survival




| Statistics – Kaplan Meier | HR[1] | p-value[1] | [95% CI] |
|---|---|---|---|
| UNO + Anti-mPD-1 vs Anti-mPD-1 | 0.561 | 0.0038 | [0.379, 0.830] |
| UNO + Anti-mPD-1 vs $N_2$ + Anti-mPD-1 | 0.555 | 0.01 | [0.354, 0.869] |
| UNO + Anti-mPD-1 vs Untreated/Sham | 0.181 | <0.0001 | [0.115, 0.286] |
| $N_2$ + Anti-mPD-1 vs Anti-mPD-1 | 0.999 | ns | [0.644, 0.1.551] |

- Survival = not being euthanized due to tumor volume reaching a pre-determined size.
- One mouse from UNO+anti-mPD-1 and one mouse from N2+anti-mPD-1 arms survived at day 75 although not being tumor-free at this time point.

**Figure 2: Pooled data across studies of 5 or 10-min UNO and anti-mPD-1 (5 or 10 mg/kg) treatment – survival data.**
[1]Hazard ratio and p-value derived from Cox proportional hazard model.

## Summary

The combination of Ultra-high concentration NO with anti-mPD-1 improved outcomes and mice survival compared to UNO or anti-mPD-1 alone, as UNO assists the immune system in overcoming anti-mPD-1 resistance. Therefore, the combination of UNO and immune checkpoint inhibitors such as anti-PD-1 is worthy of further evaluation in the clinical setting which may lead to important clinical implications and potential modifications to standard of care.