Intratumoral Administration of Low Volume Ultra-High Concentration Nitric Oxide and Immune Checkpoint Inhibitors in CT26 Tumor-Bearing Mice

#724

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Background and Study Aims: Previously, we reported treatment of CT26 tumor-bearing mice with high-volume (HV, 1 liter), ultra-high-concentration nitric oxide (UNO) and immune checkpoint inhibitors (ICIs) resulted in improved outcomes compared to UNO or anti-mPD-1. alone as UNO assists the immune system in overcoming anti-mPD-1 resistance (Epshtein Y 2023). This pilot study aim to evaluate low volume (LV, < 100 mL) compared to HV delivery of UNO in combination with ICIs.

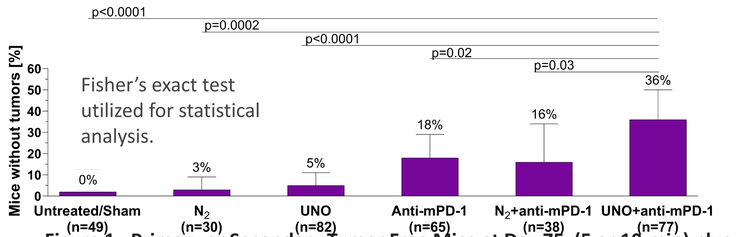

Figure 1: Primary or Secondary Tumor Free Mice at Day 75 (5 or 10-min) plus anti-mPD-1 (5 or 10 mg/kg)

Methods: The efficacy of LV delivery of UNO and ICIs was assessed by monitoring the primary tumor growth in BALB/c mice. The distribution of LV UNO in CT26 tumors was assessed by nitro-tyrosine (NT) staining of tumor slides (immunohistochemistry, IHC). Nitro-tyrosine is a post translational modification product resulting from the reaction of tyrosine with reactive nitrogen species.

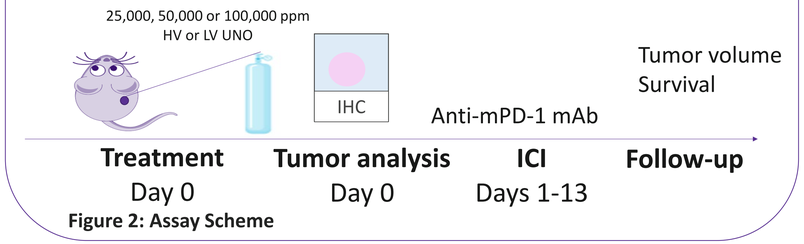

Figure 2: Assay Scheme

Results: Effect of LV UNO in Combination with Anti-mPD-1 mAb Reduced Primary Tumor Volume and Compares Favorably to HV

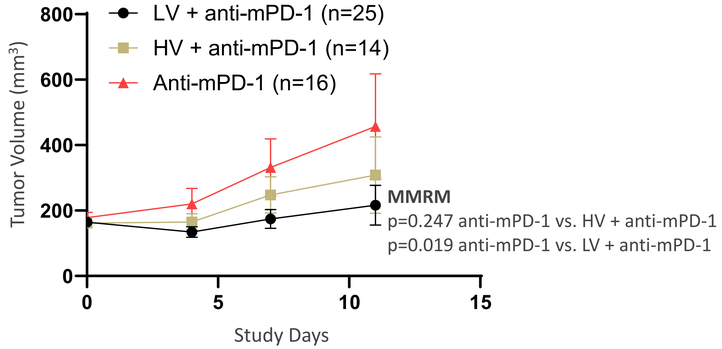

Figure 3: LV UNO in Combination with Anti-mPD-1 mAb Reduced Primary CT26 Tumor Volume

Results: Higher Levels of NT Post Exposure to UNO LV vs. UNO HV or Sham or Nitrogen (N2)

CT26 tumors were treated with HV or LV UNO. India ink staining (black) was utilized to mark the probe track during gas delivery. IHC nitro-tyrosine level (brown staining), a marker of NO distribution in the tumor, performed at x20 objective magnification, utilized the following scoring scale: Grade 0: No positive reaction; Grade 1: Few positive cells (<5 cells); Grade 2: Very mild reaction (5-15 cells); Grade 3: Mild reaction (15-25 cells); Grade 4: Moderate reaction (25-50 cells); Grade 5: Marked reaction (>50 cells). IHC analysis revealed that LV UNO demonstrated the highest increase in nitro-tyrosine level. The sham- or nitrogen-treated tumors showed the lowest levels of nitro-tyrosine staining.

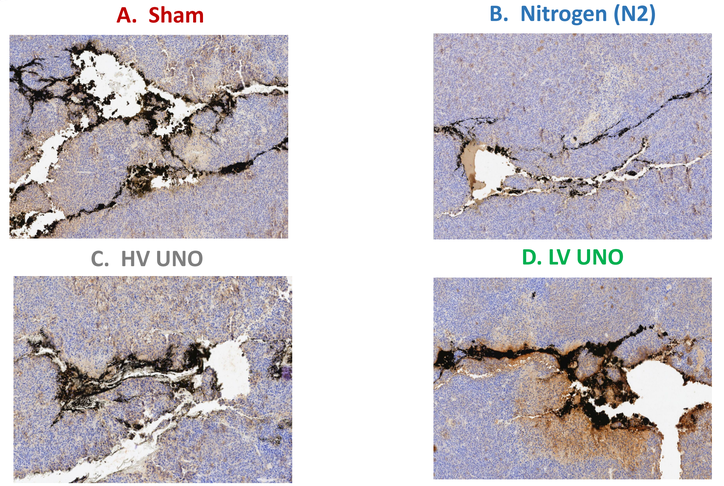

Figure 4: Nitro-tyrosine Analysis Post UNO Exposure in CT26 Tumors. (A-D; n=3 for all groups) Representative images of nitro-tyrosine staining. (A) Sham treatment (23G hypodermic needle, no gas), (B) N2 treated tumors, (C) High volume UNO treated tumors, (D) Low volume UNO treated tumors. (E) Summary of the IHC results (mean ± SD) for nitro-tyrosine staining, using a semi-quantitative scoring system. Statistical significance, ANOVA with post hoc by Tukey HSD: (*) = p<0.05; (**) = p<0.01.

Conclusions: HV UNO in combination ICIs resulted in tumor regression after treatment of CT26 tumors (Confino H 2023; Epshtein Y 2023). As presented herein, the experimental results of LV UNO compare favorably to HV UNO based on tumor regression results. Moreover, the improved tumor distribution of LV UNO, in the tumor model, appears superior to HV UNO, as demonstrated by nitro-tyrosine staining post treatment. The potential of an improved safety profile is anticipated, and the optimal UNO dose remains under investigation.