Intratumoral Administration of High-Concentration Nitric Oxide and Anti PD-1 Treatment Leads to Higher Tumor Regression Rates and Prolonged Survival in CT26 Tumor-Bearing Mice

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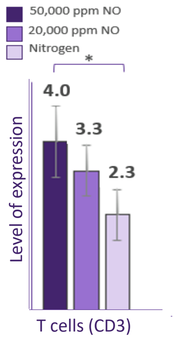
Background

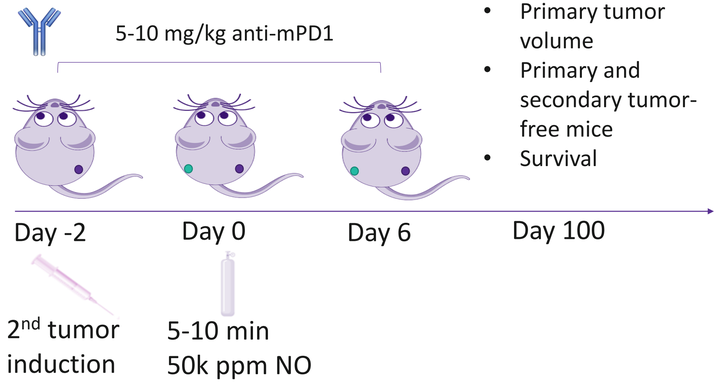
- The activity of immune checkpoint inhibitors is dramatic, however, limited to a subset of highly sensitive tumors, showing a limited response.
- Nitric Oxide (NO) is a signaling molecule in multiple diseases, including cancer.
- Previously, we reported that treatment of CT26 tumor-bearing mice with ultra high-concentration NO (UNO) stimulated anti-tumor immune responses leading to the rejection of a secondarily-induced tumor and an increase in T and B cells 14-21 days post-UNO treatment.



TILs 14 days post treatment

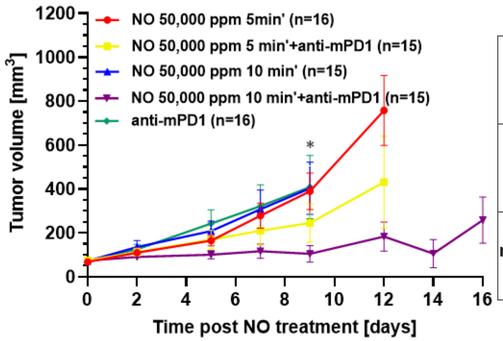
* p-value < 0.05, one-way ANOVA

Methods



Follow-up:
- Primary tumor volume
- Primary and secondary tumor-free mice
- Survival

Results: CT26 Primary Tumor Growth

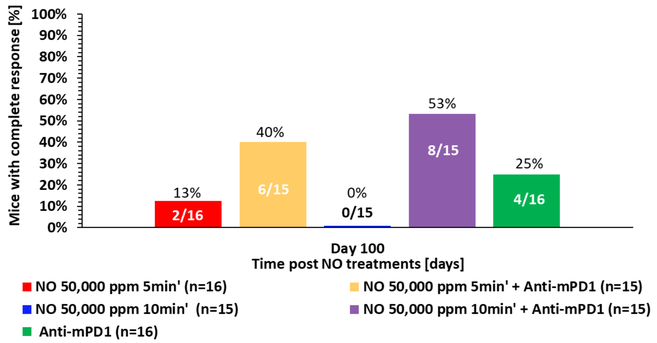


*Tumor Volume at Study Day 9 - Difference Between Treatment Groups[a]

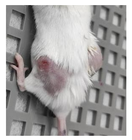
Group	n	Adjusted Mean (SEM)	Adjusted Mean	95% CI	p-value
Anti-mPD1	16	446.28 (68.73)	NA	NA	NA
NO 10 min+Anti-mPD1	15	100.43 (68.74)	-345.85	(-538.09, -153.62)	0.0005

Adjusted mean is estimated via PROC MIXED; CI=Confidence Interval; NA=Not Applicable, SEM=Standard Error of the Mean. [a]Analysis via mixed model repeated measures (MMRM) with fixed effects for baseline tumor volume, study day, treatment by study day interaction.

Results: CT26 Primary & Secondary Tumor-Free mice



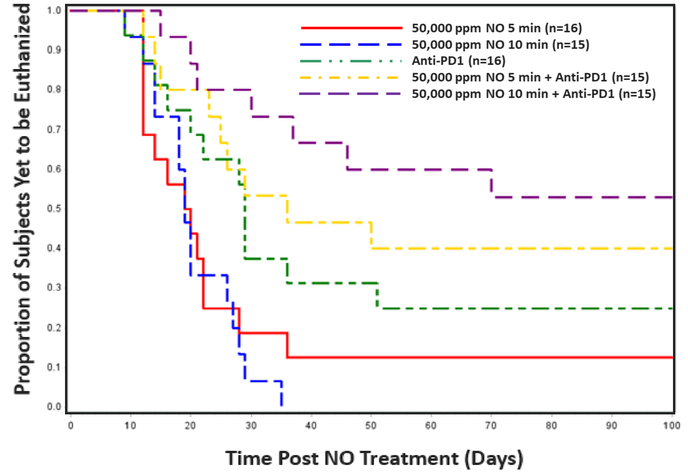
UNO + anti-mPD1, Complete Response



Control, Primary & secondary tumors

Statistical analysis: Fisher's Exact Test: p-value = 0.1489, Pairwise Treatment Group Comparison - 50,000 10 min + Anti-mPD1 vs Anti-mPD1

Results: Increase in Mice Survivability



Statistics – Kaplan Meier	HR	p-value[1]	[95% CI]
Comparison Hazard Ratio, 50,000 ppm NO 10 min + Anti-mPD1 vs Anti-mPD1	0.41	0.0653	[0.16, 1.06]

[1]Hazard ratio and p-value derived from Cox proportional hazard model.

Survival = not being euthanized due to tumor volume reaching a pre-determined size

Conclusions

Combination of UNO with anti-PD-1 significantly improved outcomes compared with UNO or anti-PD-1 alone. Since anti-PD-1 was administered prior to NO treatment, it was given an advantage over NO. Yet, the combination of NO and anti-PD1 was superior to anti-PD1 alone. A strong possibility is that high-concentration NO assists the immune system in overcoming anti-PD-1 resistance. Thus, the combination of ultra high-concentration NO and immune checkpoint inhibitors such as anti-PD-1 can be a breakthrough therapy with important clinical implications.