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Intratumoral Administration of Ultra High-Concentration Nitric Oxide (UNO) is more Efficacious than anti-mPD-1 Therapy in 4T1 Tumor-Bearing Mice

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[1]Beyond Cancer, [2]Beyond Air

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Background

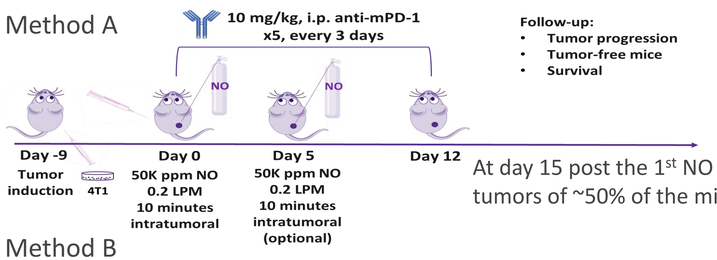
Chemotherapy is the standard first-line treatment for most metastatic triple-negative breast cancer (mTNBC) patients. However, treatment outcomes are poor. Immune checkpoint inhibitors (ICIs), anti–programmed death 1 (PD-1), and anti–programmed death ligand 1 (PD-L1) have demonstrated antitumor activity across various indications. In particular, pembrolizumab (Keytruda®) is approved for high-risk, early-stage TNBC in combination with chemotherapy in the neoadjuvant setting and in the recurrent unresectable or metastatic setting.

Previously we reported that delivering ultra-high concentrations of nitric oxide (UNO) to mouse colon tumors (CT26) stimulated innate and adaptive immune responses leading to the rejection of secondary-induced tumors. Moreover, adding a murine anti-PD-1 antibody (anti-mPD-1) to UNO treatment resulted in primary tumor regression in 53% of the mice, the rejection of distant tumors in all mice, and prolonged survival compared to control and anti-mPD-1 arms.

In this study we utilize the aggressive murine breast cancer model (4T1) to investigate single and repeat dosing of UNO as monotherapy and in combination with checkpoint inhibitors.

Methods

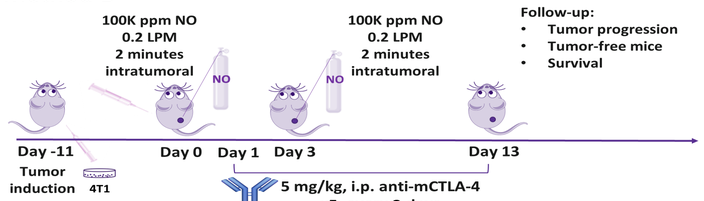
Method A



Follow-up:
- Tumor progression
- Tumor-free mice
- Survival

At day 15 post the 1st NO treatment the primary tumors of ~50% of the mice were resected

Method B



Follow-up:
- Tumor progression
- Tumor-free mice
- Survival

*All *in vivo* experimental procedures were carried out in accordance with the protocol approved by the Ethics Committee on the Use and Care of Animals of the IMOH (Israel).

Results

Primary tumor volume following 50K ppm gNO dosing combined with anti-mPD-1 (method A)

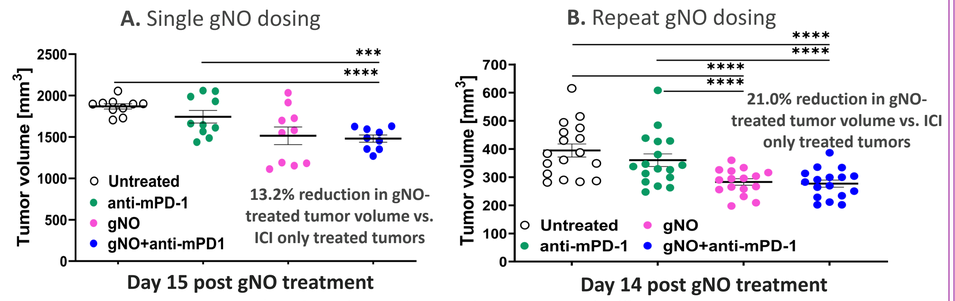


Figure 1: Average tumor volume (A) gNO+anti-mPD-1-treated tumors was significantly smaller compared to untreated and treated with only anti-mPD-1 tumors. N=9-10 mice per arm. (B) gNO-treated tumors with or without anti-mPD-1 was significantly smaller compared to untreated or treated with only anti-mPD-1 tumors. N=15-17 mice per arm. Statistical analysis for (A) and (B) Mixed model for repeated measures (MMRM) with Kenward-Rodger's method ***P<0.001 ****P<0.0001

Survival following 50K ppm gNO repeat dosing in combination with anti-mPD-1 (method A)

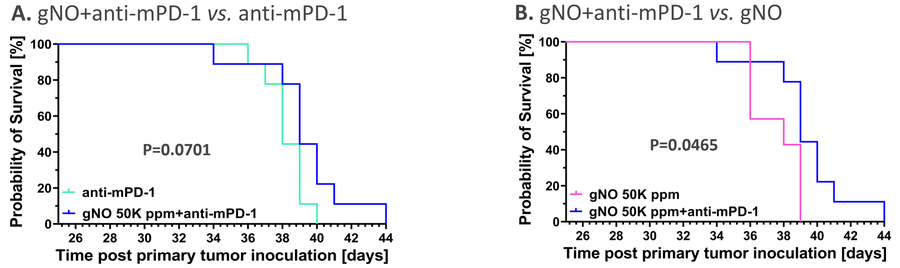
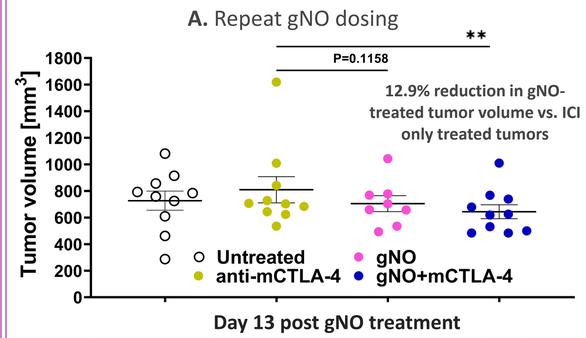


Figure 2: Survival following repeated 50K ppm NO dosing and anti-mPD-1. (A) gNO+anti-mPD-1 vs. anti-mPD-1, N=9 mice per arm, HR=0.35, [1]P=0.0701, Log-Rank P=0.0723, [95% CI]=[0.11, 1.09]. (B) gNO+anti-mPD-1 vs. gNO, N=7-9 mice per arm, HR=0.22, 1P=0.0465, Log-Rank P=0.0475, [95%CI]=[0.05,0.98]. [1]Hazard ratio and p-value derived from Cox proportional hazard model.

Primary tumor volume and survival following repeat 100K ppm gNO dosing combined with anti-mCTLA-4 (method B)



B. UNO prolongs mice survival compared to anti-mCTLA-4

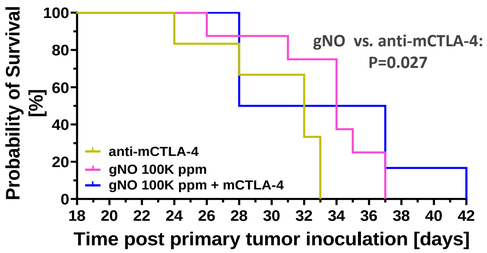


Figure 3: (A) Average tumor volume. N=8-10 mice per arm. Analysis via mixed model for repeated measures (MMRM) on day 13 after 1st treatment with Kenward-Rodger's method. **P<0.01 (B) Survival curves, N=6-10 mice per arm. gNO vs. anti-mCTLA-4: HR= 0.16, [1]P=0.027, Log-Rank P = 0.0173, [95% CI]=[0.03, 0.81]. [1]Hazard ratio and p-value derived from Cox proportional hazard model. *8 mice died at day 13 due to toxic reaction to an anti-mCTLA-4 antibody and were excluded from this analysis.

Conclusions

Single and repeat dosing of UNO as monotherapy improved outcomes compared to checkpoint inhibitor monotherapy. Average tumor volume was 13.2% smaller after single gNO treatment and 21.0%/12.9% smaller with repeat gNO treatment. Repeat dosing of UNO monotherapy significantly prolonged mice survival compared to anti-mCTLA-4 monotherapy. Repeat dosing of UNO in combination with anti-mPD-1 prolonged mice survival compared to anti-mPD-1 monotherapy. These findings suggest that local administration of UNO, either alone or in combination with ICIs, can be a viable treatment option for patients not responding to immune checkpoint inhibitors.