Gaseous Nitric Oxide at High Concentrations is a Powerful Anti-Tumor Agent both *in-vitro* and *in-vivo*

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Introduction: Nitric oxide (NO) is a short-lived, endogenously produced gas that acts as a signaling molecule in the body in different pathologic conditions, including cancer.
Beyond Air is developing an innovative *in situ* gaseous NO (gNO) tumor ablation-based method.

Aims: (I) To test the ability of gNO to destroy cancer cells *in vitro* and solid tumors *in vivo* after short-term exposure to the gas at high concentrations.
(II) To assess the potency of gNO based ablation to stimulate an anti-tumor immune response *in-vivo.*

Methods: *In vitro*, mouse colon and breast cancer cell lines, CT26 and 4T1 respectively, were exposed to gNO in culture at 150-50,000 ppm for 10-180 seconds. Cell viability was measured 24hr later by XTT-based cell proliferation assay. *In vivo,* mouse colon tumors were treated with 10,000-200,000 ppm gNO and the percentage of tumor take was assessed. The immune response of tumor-bearing treated mice was evaluated by Challenge and Winn assays. Challenge assay: The tumors of CT26 tumor-bearing mice were treated with NO. Up to 14 days post NO treatment, mice were re-inoculated with CT26 cells and the percentage of tumor take was monitored. Winn assay: Immune splenocytes from a CT26 immunized mouse were mixed with CT26 cells and inoculated to naïve mice. The percentage of tumor take was monitored.

Results: According to our data, gNO at 10,000-200,000 ppm eradicates breast and colon cancer cells and tumors and triggers potent anti-tumor immunity *in-vivo*.

Conclusions: Our data demonstrate the potential utility of gNO as a treatment for cancer. In the current work, gNO at high concentrations showed a significant cytotoxic effect on cancer cells *in vitro* and *in vivo*. Moreover, our findings may indicate that tumor ablation with gNO stimulates an anti-tumor immune response.

Gaseous NO at high dose shows a potent cytotoxic effect on mouse cell line *in vitro*

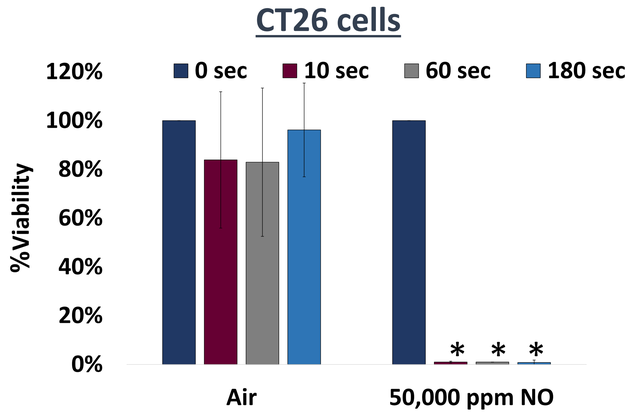

Figure 1: The viability of CT26 cells after exposure to 50,000ppm gNO or air for 10-180 seconds

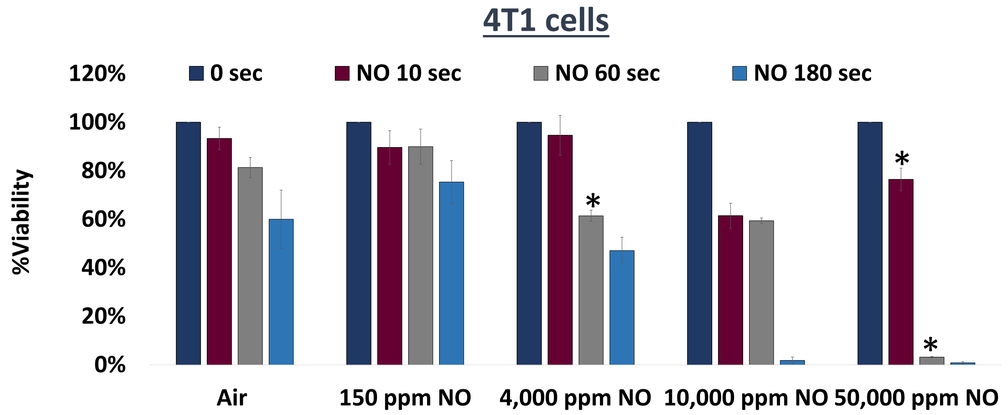

Figure 2: The viability of 4T1 cells after exposure to 150-50,000ppm gNO or air for 10-180 seconds

Gaseous NO at high dose demonstrates a potent anti-tumor effect on mouse colon tumors *in vivo*

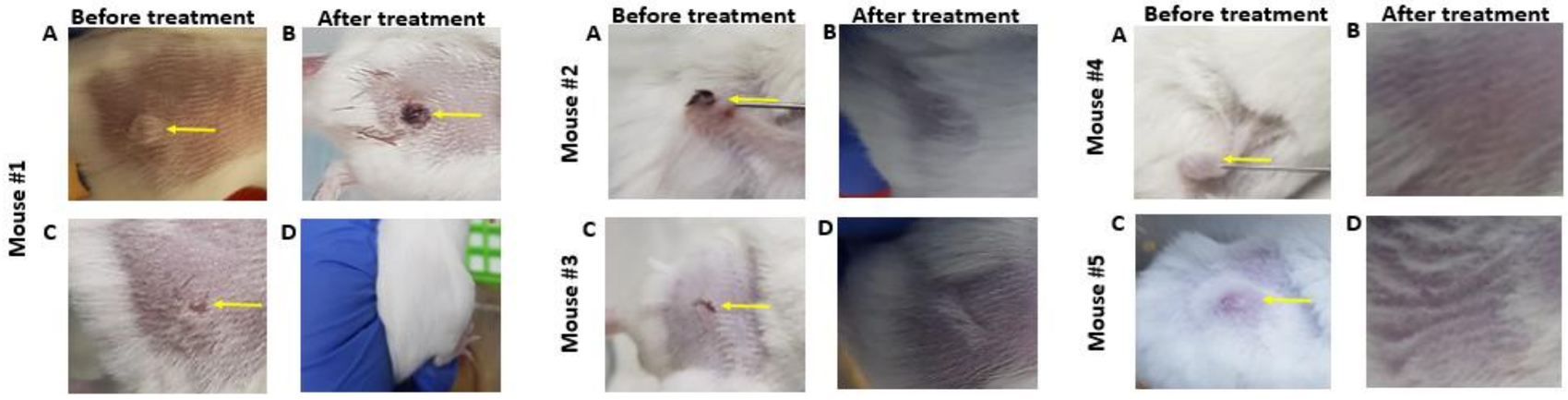

Figure 3: Treatment with 10,000ppm-200,000ppm NO for 35 seconds to 30 minutes

Gaseous NO at high dose stimulates an anti-tumor immune response *in vivo*

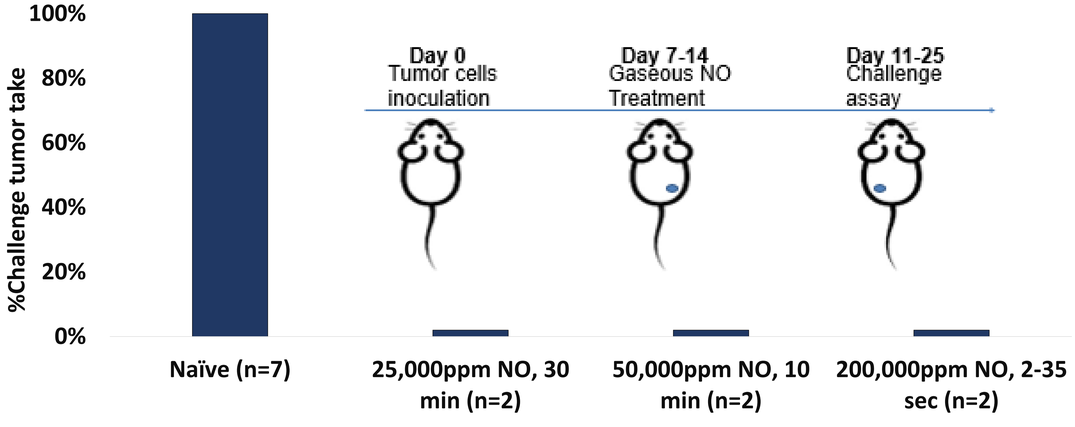

Figure 4: Challenge assay

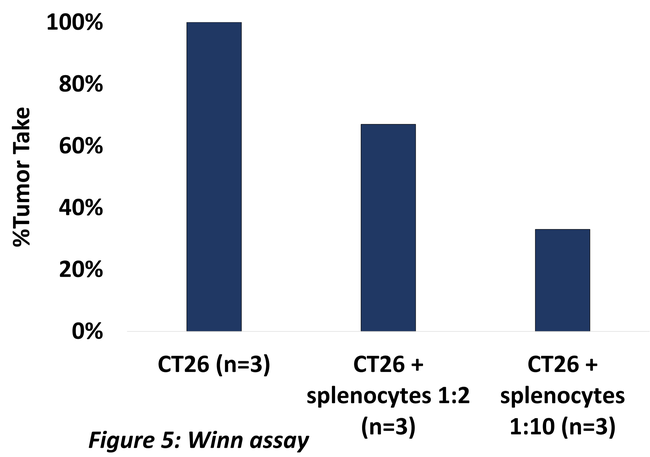

Figure 5: Winn assay