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

Hila Confinö, Steve Lisi¹, Pam Golden¹, Rinat Kalaora², Matan Goldshein², Shay Yarkoni², Amir Avnien¹,²
¹Beyond Air Inc., ²Beyond Air Ltd. Contact information: hconfino@beyondair.net

**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.