

## Short-Term Exposure of Cancer Cells to Ultra-High Concentrations Nitric Oxide Induces PDL-1 Upregulation

Yana Epshtein, Matan Goldshtein, Selena Chaisson, Jedd Monson, Matt Johnson, Amir Avniel, Steve Lisi, Hila Confino

### Background

We have previously shown that treating mouse colon carcinoma (CT26) tumor-bearing mice with ultra-high concentrations of nitric oxide (UNO) upregulates innate and adaptive immune cells both locally and systemically.

As immune-checkpoint molecules, such as the PD-L1 receptor PD-1, are expressed on immune cells, our group assessed the potential synergy of UNO and an anti-PD-1 antibody for treating tumors *in vivo*. Indeed, primary tumors regressed in 53% of the CT26 tumor-bearing mice treated with UNO and anti-PD-1. Moreover, these mice were primary and distant tumor-free and survived 100 days post-treatment.

In the current study, we assessed PD-L1 expression levels on cancer cells *in vitro* following short-term exposure to UNO.

### Methods

CT26 cells were exposed to 25,000 - 100,000 ppm NO for 10 - 60 seconds. The percentage of PD-L1-expressing cells was assessed by flow cytometry analysis.

### Results

1. Exposing CT26 cells to 100,000 ppm NO for 10 seconds upregulated PD-L1 expression. 85.1% of cells, which are still viable or at early apoptosis, expressed PD-L1 compared to 71% of untreated cells ( $p < 0.0001$ ). Exposure of CT26 cells to 25,000 ppm or 50,000 ppm NO for 10 seconds did not change the level of PD-L1 expression significantly.
2. Exposure to 100,000 ppm NO for 30 seconds further increased the percentage of cells expressing PD-L1 to 96.7% ( $p < 0.0001$  compared to untreated cells). For this duration, lower concentrations of NO also increased PD-L1-expression in viable early apoptotic CT26 cells to 82.8% - 92.3% when treated with 25,000 ppm or 50,000 ppm NO, respectively.
3. Finally, a 60 second exposure of CT26 cells to 25,000 ppm, 50,000 ppm, or 100,000 ppm NO resulted in the highest percentage of PD-L1 expressing cells. Approximately 95% of all viable or early apoptotic CT26 cells express PD-L1 under all NO conditions. This finding is significant compared to 71% of cells in the control arm that express PD-L1 ( $p < 0.0001$ ).

### Conclusion

Short-term exposure (10 – 60 seconds) to UNO upregulates PD-L1 expression on cancer cells in an NO concentration-dependent and exposure-dependent process. A 10-second exposure to 100,000 ppm



2600-character limit

NO is sufficient to significantly induce PDL-1 expression. Therefore, NO therapy can potentially be combined with anti-PD-L1 antibody treatment to improve therapeutic outcomes.