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 UNO (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.

**Methods**

- **5-10 mg/kg anti-PD1**
  - Follow-up: Primary tumor volume
  - Primary and secondary tumor-free mice
  - Survival
- **Day -2**
- **Day 0**
- **Day 6**
- **Day 100**
- **2nd tumor induction**
  - 5-10 min
  - 50k ppm NO

**Results: CT26 Primary Tumor Growth**

<table>
<thead>
<tr>
<th>Group</th>
<th>Adjusted Mean (SEM)</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-PD1</td>
<td>16</td>
<td>446.28 (68.73)</td>
<td>NA</td>
</tr>
<tr>
<td>NO 10 min + Anti-PD1</td>
<td>15</td>
<td>100.43 (68.74)</td>
<td>345.85</td>
</tr>
</tbody>
</table>

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

Adjusted mean is estimated via PROC MIXED; CI = Confidence Interval; NA = Not Applicable; SEM = Standard Error of the Mean.

**Results: CT26 Primary & Secondary Tumor-Free mice**

- **Mixed model repeated measures (MMRM) with fixed effects for baseline tumor volume, study day, treatment by study day interaction.**

**Results: Increase in Mice Survivability**

- **Statistical analysis:** Fisher’s Exact Test: p-value = 0.1489

**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.