

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

Background

Immune checkpoint inhibitors have shown dramatic activity transforming clinical oncology [1]. Yet, their activity is limited to a subset of highly sensitive tumors (e.g., melanoma). Even then response is observed in only 50% of patients [2].

Nitric Oxide (NO) is a signaling molecule in multiple diseases, including cancer and has been shown to activate anti-tumor immune responses [3]. Previously, we reported that treatment of CT26 tumor-bearing mice with high-concentration NO (UNO) stimulated anti-tumor immune responses leading to the rejection of a secondarily-induced tumor. More specifically, a significant increase of tumor-infiltrating T-cells and blood and spleen B and T-cells were observed 14-21 days post-UNO treatment.

In this study we investigated the ability of UNO to improve the efficacy of anti-PD-1 antibody.

Methods

Day zero, 5.0×10^6 CT26 cells were injected to the right flank of Balb/c mice (n=15-16 per group). Day six, CT26 cells were injected to the contralateral flank and anti-PD1 injections (5mg/kg mouse, q2d, x 5) commenced.

Day eight, tumors (average size $71.9 \pm 37.2 \text{mm}^3$) were treated intratumorally with UNO (50,000 ppm, 5 or 10 minutes, flow rate ~ 0.2 liter per minute). Post-treatment tumor volume and survival were monitored thereafter.

Results

1. Complete regression of the primary tumor occurred in 9/15 (60%) of mice treated with combination 10-minute NO and anti-PD-1, post-treatment day 26. This compared to 4/16 (25%) of controls treated with anti-PD-1 alone ($p=0.13$) and 0/15 (0%) treated with UNO alone ($p=0.0027$).
2. Survival was drastically increased in the 10-minute UNO/anti-PD-1 combination arm-compared to anti-PD-1 alone ($p<0.05$), post-treatment day 32.
3. Secondary, contralateral flank tumor take in the anti-PD-1 alone arm was 21.4% but reduced by 38% to 13.3% in the 10-min combination arm and reduced by 67% to 7.14% in the 5-min combination arm, post-treatment day 19.
4. Survival was significantly improved for both the 5- and 10-minute combination arms compared to the 5- and 10-minute UNO controls ($p=0.02$ and $p<0.0001$, respectively).

Conclusion

Combination of UNO with anti-PD-1 significantly improved outcomes compared with UNO or anti-PD-1 alone. A strong possibility is that high-concentration NO assists the immune system in

overcoming anti-PD-1 resistance. Thus, the combination of high-concentration NO and immune checkpoint inhibitors such as anti-PD-1 can be a breakthrough therapy with important clinical implications.

References

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