**ABSTRACT**

Response to anti-PD1 blockade has been associated with the presence of “partially exhausted” T cells in distant tumors. This phenomenon is associated with higher IL-12 expression in the tumor microenvironment, which can drive differentiation of CD8+ T cells into Short-Lived Effector T cells (SLEC). We hypothesized that intratumoral electroporation (IT-pIL12-EP) may enhance tumor immunogenicity and drive anti-tumor responses in distant tumors. To test this hypothesis, we generated a syngeneic mouse model of B16-F10 tumors. We intratumorally electroporated one tumor with mIL12P2A, while the other tumor was left untreated. Both tumors were measured 1-2 times a week.

**RESULTS**

Gene expression changes in electroporated lesions assessed by NanoString nCounter technology. Pro-inflammatory cytokines and immune genes (fold change) over no treatment levels at 7 days post treatment assessed by NanoString nCounter technology (p<0.05). c) Graph showing heat map of immune genes fold change (log2 scale) over untreated tumor at indicated time points after treatment; **p value < 0.005 (Mann-Whitney).

**CONCLUSIONS**

IT-pIL12-EP enhances the immunogenicity in the poorly immunogenic, low TIL, B16-F10 syngeneic mouse model, leading to the generation and dissemination of a TAA-specific KLRG1hiCD127lo CD8 T cell population. The emergence of this population correlates with the increased inflammatory signature.

**Figure 2** Gene expression changes in non-electroporated lesions assessed by NanoString nCounter technology. a) Hierarchical clustering and heat map (Z score) representation of all genes, b) induction in expression of immune genes (fold change) over no treatment levels at 7 days post treatment assessed by NanoString nCounter technology (p<0.05). c) IHC contra-tumor control 16 day post mIL12-EP.

**Figure 3** Gene expression changes in electroporated lesions assessed by NanoString nCounter technology. a) Hierarchical clustering and heat map (Z score) representation of all genes. b) Induction in expression of immune genes (fold change) over no treatment levels at 7 days post treatment assessed by NanoString nCounter technology (p<0.05). c) IHC contra-tumor control 16 day post mIL12-EP.

**Figure 4** Treatment of primary tumor with pIL12 leads to increased inflammatory infiltrate and conversion of treated tumor into pro-immunogenic tumor microenvironment.

**Figure 5** Gene expression changes in non-electroporated lesions assessed by NanoString nCounter technology. a) Hierarchical clustering and heat map (Z score) representation of all genes. b) Induction in expression of immune genes (fold change) over no treatment levels at 7 days post treatment assessed by NanoString nCounter technology (p<0.05). c) IHC contra-tumor control 16 day post mIL12-EP.

**Summary and Conclusions**

- Intratumoral mouse pIL122A EP results in systemic anti-tumor responses in B16-F10 and multiple other murine tumor models [10, 32, and 41] (data not shown).
- Mouse pIL12 EP1 tumors in mouse convert immunologically ‘cold’ tumors to ‘hot’. Increased tumor infiltrating lymphocytes (TIL) and immune activation.
- Induction of interferon-γ expression and dependent gene activation, including antigen presentation and processing machinery.
- Unilateral IT-mIL12 EP treatment intratumoral (mouse) - Increased IL-12 and mIL12 driven immune activation consistent with the induction of anti-PD1 immunoresponsiveness.
- IL-12 EP results in the generation of circulating antigen-specific CD8 T cells (B16-OVA model).
- Interestingly, Anti-OVA SIFKEK+CD8+ T cells are KLH-GCD+127+ SLECs both in spleen and untreated distant tumor.
- IL-12 primes the systemic immune and is likely synergistic with anti-checkpoint therapies like anti-PD-1.

**Rationale for combination of IT-pIL12-EP and anti-PD1 blockade**