INTRATUMORAL ELECTROPORATION-MEDIATED IL-12 GENE THERAPY CAN ENHANCE TUMOR IMMUNOGENICITY

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ABSTRACT

BACKGROUND: Response to anti-PD1 blockade has been associated with the presence of "partially exhausted" PD-1+ CD8 tumor-infiltrating lymphocytes (TILs) and 'adaptive immune resistance', characterized by INFγ-driven upregulation of PD-L1. Poorly immunogenic tumors are associated with a low probability of response to this class of agents. Conversion of 'cold' poorly immunogenic tumors into 'hot' inflamed tumors, therefore, represents a major therapeutic goal. **HYPOTHESIS:** Electroporation-mediated IL-12 gene therapy (IT-pIL12-EP) will enhance immunogenicity in the low TIL/PD-1 refractory B16 model, leading to the generation of a systemic anti-tumor immune response and increased TILs. **METHODS:** To assess the ability of IT-pIL2-EP to generate a systemic anti-tumor effect, we developed a two-tumor model (B16.F10 or B16-OVA), where only one tumor receives IT-pIL12-EP treatment. Histologic, transcriptional (Nanostring®) and flow cytometric analysis was performed on spleen and both treated and untreated tumors. **RESULTS:** IT-pIL12-EP led to tumor necrosis, leukocyte infiltration, up-regulation of pro-inflammatory genes and regression of most treated lesions. In the B16-OVA model, a significant enrichment of tumor antigen-specific (SIINFEKL-tetramer+) KLRGhiCD127low CD8 T cells was observed. The generation of these CD8 T cells correlated with growth inhibition of the contralateral, untreated tumor. Transcriptional and flow cytometric analyses of the untreated tumor showed the presence of TAA-specific CD8 populations, and an increased INFγ signature (i.e. Ifng, Cd274, STAT1, Cxcl10). **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 KLRGhiCD127low CD8 T cell population. The emergence of this population correlates with the induction of a INFγ-gene signature consistent with the induction of 'adaptive immune resistance' in the distant, untreated tumors. Based on these data, we predict that IT-pIL12-EP will increase the response rates of anti-PD1 blockade in immunologically 'cold' tumors.

Murine model to study mechanism of action of IL12 Electroporation

Groups
Untreated
pUMVC3 (50µg)
pUMVC3 – mIL 12P2A (50µg)
At time of treatment
Primary Lesion Size: 125-160 mm³
Contralateral Lesion Size: 30-35 mm³
Electroporation Conditions
400 V/cm, 8 pulses, 10ms each

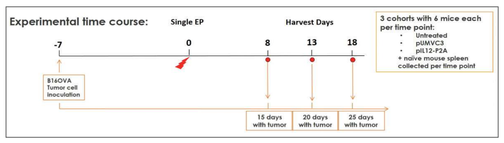
Test for regression of distant lesion following local intratumoral therapy

Contralateral Model: B16.F10 or B16.F10 OVA+ cells were implanted on both flanks of C57Bl/6J 6-8 wk. old female mice at different densities. Tumor on one flank was treated while the tumor on the other flank was left untreated. Both tumors were measured 2-3 times a week.

Gene Expression: Primary and Contralateral Tumors were collected 7 days Post Treatment (Tx). RNA was extracted using Trizol® and 50ng of total RNA was used for NanoString®

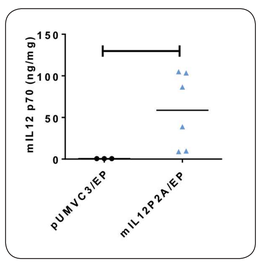
Regression: Poorly immunogenic B16 cell line (C57Bl/6J mice) were implanted and 10 days post implantation, tumors were treated with above mentioned groups once. Tumors were measured 3 times a week and mice were sacrificed when they reached max allowed tumor burden (1500 mm3).

Immuno-Phenotyping:

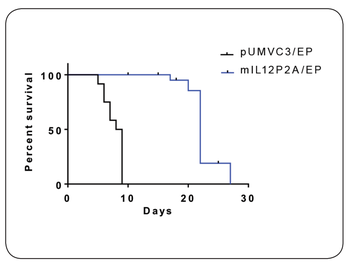


Intratumoral Electroporation of mouse pIL12 leads of Systemic Anti-Tumor Response in Mice

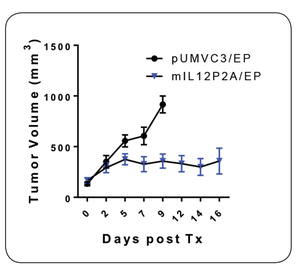
a) **Intratumoral Expression**



b) **Kaplan-Meir**



c) **Treated Lesion**



d) **Untreated Contralateral Lesion**

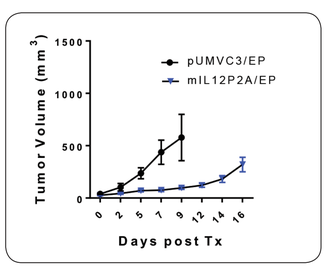
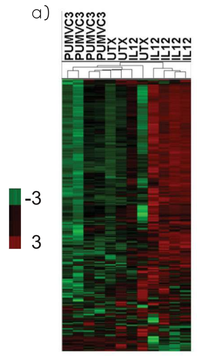


Figure 1: a) Amount of intratumoral mIL12(p70)in 1mg of total protein 48hrs post intratumoral electroporation of mouse pIL12P2A(n=7, *p=0.02). mIL12 p70 DuoSet Elisa kit(DY419) was used to measure mIL12 levels. b) Survival of mice, bearing B16-F10 tumors, upon intratumoral electroporation of mIL12P2A compared to empty vector (pUMVC3) (Kaplan-Meier plots, *p<0.05). Tumor volumes (short axis measurement (squared) X long axis measurement)/2) of c)primary and d) contralateral B16-F10 lesions upon intratumoral electroporation of mIL12P2A and pUMVC3.

IT-mouse pIL12-EP leads to increased inflammatory infiltrate and conversion of treated tumor into pro-immunogenic tumor microenvironment

a)



b)

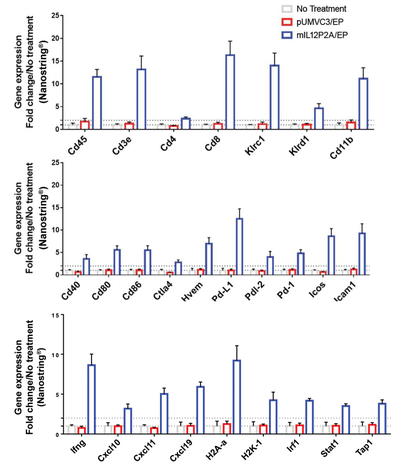
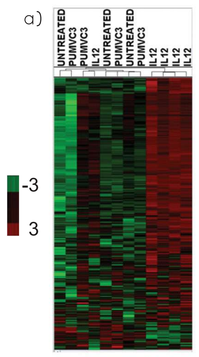


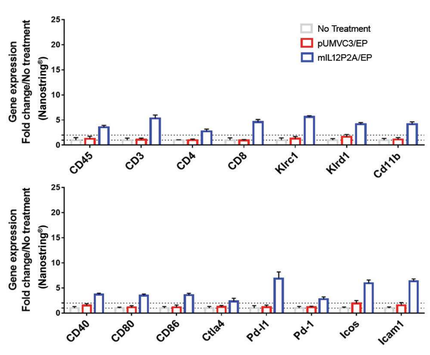
Figure 2: Gene expression changes in electroporated lesions assessed by NanoString nCounter technology. a) Hierarchical clustering and heat map (Z scores) representation of all genes. b) Induction in expression of immune genes (fold change) and INFγ responsive genes (fold change) over no treatment levels at 7 days post treatment assessed by NanoString nCounter technology (p<0.05).

IT-mouse pIL12-EP increases TILs in untreated contralateral B16. F10 tumors

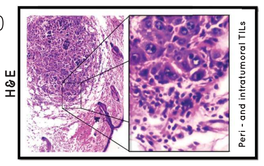
a)



b)



c)

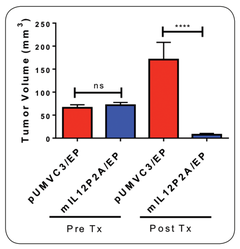


Pathological Assessment
Day 18 (3/5 mice with TIL infiltrate and histological changes c/w regression

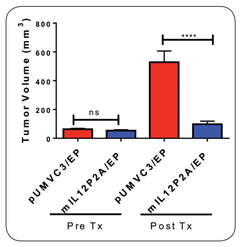
Figure 3: Gene expression changes in non electroporated lesions assessed by NanoString nCounter technology. a) Hierarchical clustering and heat map (Z scores) representation of all genes. b) Induction in expression of immune genes (fold change) over no treatment levels at 7 days post treatement assessed by NanoString nCounter technology (p<0.05. c) H&E contralateral tumor 18 day post mIL12 EP.

Intratumoral Electroporation of mouse pIL12 leads to Systemic Anti-Tumor Response and enrichment of SIINFEKL+ CD8 T cells in mice bearing B16-OVA+ tumors

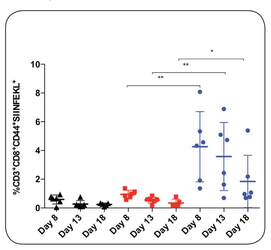
a) **Treated Lesion**



b) **Contralateral Lesion**



c) **Splenocytes**



d) **Contralateral TIL**

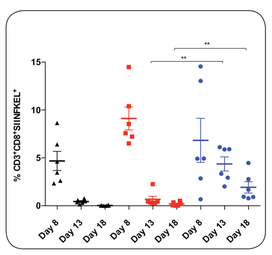
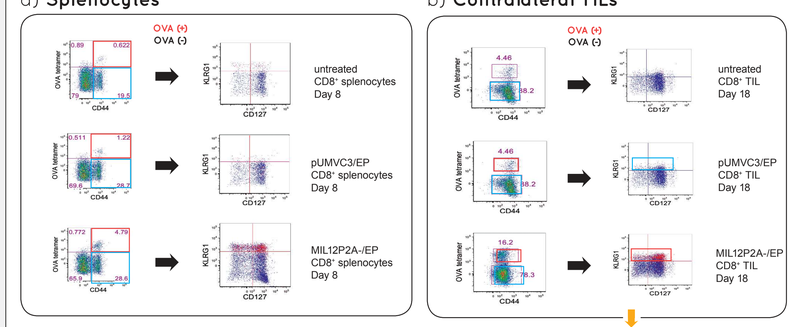


Figure 4: a) Tumor volumes of primary (treated) and b) contralateral (untreated) B16-OVA lesions before and after intratumoral electroporation of mIL12P2A, pUMVC3 at day 11 ****p<0.001. c) Flow cytometric analysis of isolated splenocytes from treated and untreated mice at indicated time points; *p value < 0.05, **p value < 0.005 (Mann-Whitney) d) Flow cytometric analysis of isolated lymphocytes from untreated, contralateral tumors at indicated time points after treatment; **p value < 0.005 (Mann-Whitney).

IT-pIL12-EP drives systemic expansion of CD8 Short Lived Effector Cells (SLECs)

a) **Splenocytes**

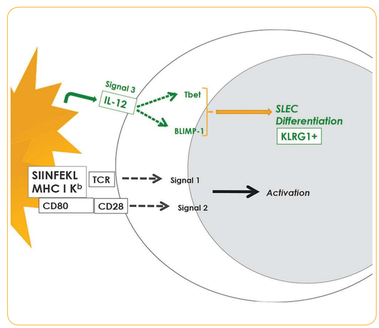
b) **Contralateral TILs**



Parent population:
Singlet/ lymphocyte/ LIVE/ CD3+/ CD19-/ NK1.1-/ CD8+

Figure 5: Representative flow cytometry graphs of immune phenotyping of CD8+ T cells are shown from the experiment shown in Figure 4 (n=6 per cohort). The percentage of CD8+ T cells that are CD44+ (blue) and OVA+ (SIINFEKL-tetramer positive; red) are shown in boxes in a) left panels, and as corresponding colored dots in b) right panels. In both splenocytes and contralateral TIL, a specific population of CD8+ T cells that are OVA+ and KLRG1+ are induced in IL-12/EP treated mice. In the TIL, this population (red box) is also lower for PD-1 expression than the corresponding KLRG1+ cells in control mice (blue and green boxes), as shown in the histogram.

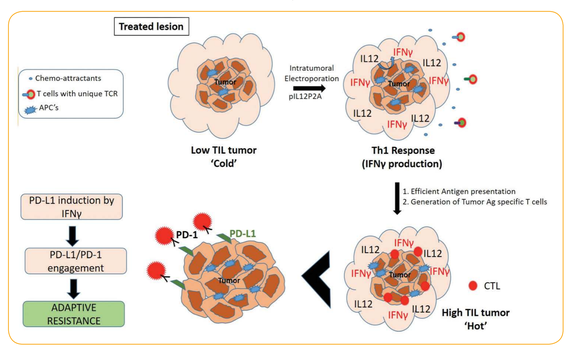
IL-12 drives differentiation of CD8s into Short-Lived Effector



Summary and Conclusions

- Intratumoral mouse pIL-12P2A EP results in systemic anti-tumor responses in B16-F10 and multiple other murine tumor models (MC38, CT26, and 4T1, data not shown).
- Mouse pIL-12 EP'd tumors in mouse convert immunologically 'cold' tumors to 'hot'.
  - Increased tumor infiltrating lymphocytes (TIL) and immune activation
  - Induction of Interferon-γ expression and IFNγ dependent gene activation, including antigen presentation and processing machinery (APM)
- Untreated contralateral tumors (mouse) : Increased TIL and IFNγ driven immune activation consistent with de novo adaptive immune response.
- IL-12 EP results in the generation of circulating antigen-specific CD8 TILs (B16-OVA model).
- Interestingly, Anti-OVA SIINFEKL+CD8+T cells are KLRG1+CD127-SLECs both in spleen and untreated distal tumor.
  - Is the strong IL-12 anti-tumor response dependent on SLECs?
  - Do these SLECs become 'exhausted' TILs (Adaptive resistance)?
  - What is the nature of the KLRG1+ CD127+ CD8 T cells in IT-mIL12P2A EP treated mice.
- Poorly immunogenic/low TIL tumors are unlikely to respond to PD-1 inhibition (tremendous unmet medical need).
  - IL12 primes the immune system and is likely synergistic with anti-checkpoint therapies like anti-PD1.

Rationale for combination of IT-pIL12-EP and anti-PD-1 blockade



Ongoing combination trial of IT-pIL12-EP and Pembrolizumab in low-TILmetastatic melanoma will test IT-pIL12-EP priming. (UCSF- A. Algazi; HCI-R. Andtbacka Abstract 78, ASCO/SITC 2017)

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