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Mukhopadhyay, A**. Wright, J.H*., Shirley, S.A*., Burkart, C*., Canton, D.A., Connolly, R.J., Campbell, J.S., and Pierce, R.H. Oncosec Medical Inc., INTRATUMORAL ELECTROPORATION-MEDIATED IL-12 GENE THERAPY

...induction of INF-γ immunogenic, low TIL B16.F10 syngeneic mouse model, leading to the generation and dissemination of...

CONCLUSIONS: 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) driven CD8+ CD8 TILs was observed. The generation of these CD8 T cells correlated with growth inhibition of the contralateral, untreated tumor. Transcriptional and flow cytometric analysis of the untreated tumor showed the presence of TAA-specific CD8 populations, and an increased INF-γ signature (i.e. Ifng, Cxcl9, Stat1, Tgfb) 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 KLRG1+2IDTC1IFN γ+ T cell population. The emergence of this population correlates with the induction of an IFNγ-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 immunoologically cold tumors.

Figure 2: Gene expression changes in electroporated lesions assessed by NanoString nCounter technology (i) Clinical staining and heat map (2 scores) representation of all genes, (ii) Induction in expression of immunogenic tumor microenvironment in treated lesions. (iii) Bioinformatics analysis was performed on spleen and both treated and untreated tumors. RESULTS: 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 response in mice. The strong IL-12 anti-tumor response depends on SLECs.

Figure 3: Gene expression changes in non-electroporated lesions assessed by NanoString nCounter technology. a) Transcriptional and heat map (2 scores) representation of all genes; b) Induction of tumor antigen-specific immune genes (fold change) over no treatment levels at 7 days post treatment assessed by NanoString nCounter technology (p<0.05); c) H&E contralateral tumor 16 day post mIL12-EP induction.

Figure 4: a) Intratumoral electroporation of mouse pIL12 leads to systemic anti-tumor response in mice bearing B16-OVA+ tumors. b) Intratumoral electroporation of mouse pIL12 leads to systemic anti-tumor response in mice bearing B16-OVA+ tumors. c) Uninfected TILs. d) Untreated TILs.

Figure 5: Representative flow cytometry graphs of immune phenotyping of CD8+ T cells are shown from the experiment shown in Figure 6 (n=6 per cohort). The percentage of CD8+ T cells that are CD44+ (blue) and OVA (SINFEKL-antigen-positive, red) are shown in boxes a) (left panel) and b) (right panel). In both splenocytes and contralateral TIL, a specific population of CD8+ T cells that are OVA+ and KLRG1- are induced in B16-OVA 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 CD8+ T cells into Short-Lived Effector Cells (SLECs).

Summary and Conclusions

• Intratumoral mouse pIL12EP2A results in systemic anti-tumor responses in B16-F10 and multiple other murine tumor models (MC38, CT26, and 4T1, data not shown).
• Mouse pIL12 EP tumors in mouse convert immunologically ‘cold’ tumors to ‘hot’.
• Increased tumor infiltrating lymphocytes (TILs) and immune activation.
• Induction of interferon-γ expression and GC-dependent gene activation, including antigen presentation and processing machinery.
• Uninfected contralateral tumors (control). Increased TIL and INF driven immune activation consistent with de novo adoptive immunogenic response.
• IL-12 EP results in the generation of circulating antigen-specific CD8 T cells (B16-OVA model).
• Interestingly, Anti-OVA SINFEKL+CD8+ T cells are KLRG1+CD127− SLECs both in spleen and untreated distal tumor.
• IL-12 primes the immune system and is likely synergistic with anti-checkpoint therapies like anti-PD-1.

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

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