The use of immunomodulatory cytokines has been shown effective in regressing a wide range of tumors. However, systemic delivery of recombinant cytokines can result in serious adverse effects, often limiting its use. Intratumoral delivery of cytokines avoids these concerns and is well tolerated and well-studied in Phase I/II clinical trials in metastatic melanoma and Merkel cell carcinoma. We sought to improve the systemic anti-tumor response of intratumoral (IT) gene electrotransfer (GET) of Interleukin-12 (IL-12), a potent immunomodulatory cytokine, has demonstrated an acceptable safety profile of delivering cytokines to target tissues. Intratumoral (IT) gene electrotransfer (GET) of Interleukin-12 (IL-12), a potent immunomodulatory cytokine, has demonstrated an acceptable safety profile of delivering cytokines to target tissues.

**Abstract**

Intratumoral delivery of a P2A-linked bicistronic IL-12 construct leads to high intratumoral expression and systemic anti-tumor response.

**Low Voltage electrotransfer conditions improve intratumoral transfection efficiency and transgene expression.**

- **Figure 2** Schematic depicting the electroporation parameters of a high Voltage generator and Low Voltage generator. (a) High Voltage generator (n = 10 per treatment group). (b) Low Voltage generator. Data plotted as mean +/- SEM for each time point (n = 10 per treatment group).

**Contralateral B16.F10 tumor regression model to assess the therapeutic effect of IT-pIL12-EP.**

- **Figure 3** Gene expression changes in treated and untreated lesions were assessed by NanoString nCounter technology. Fold change in transcript levels of immune genes signaling a systemic immune response normalized to no treatment and displayed as mean +/- SEM. (a) IT-pIL12-EP leads to statistically significant improvement of tumor control on the contralateral side. (b) Low Voltage conditions lead to statistically significant improvement of tumor control on the contralateral side.

**IT-pIL12-EP with p40-P2A and Low Voltage conditions generates a systemic IFN-γ gene signature and antigen-specific splenic CD8 T cells.**

- **Figure 4** Gene expression changes in treated and untreated lesions were assessed by NanoString nCounter technology. Fold change in transcript levels of immune genes signaling a systemic immune response normalized to no treatment and displayed as mean +/- SEM. (a) IT-pIL12-EP leads to statistically significant improvement of tumor control on the contralateral side. (b) Low Voltage conditions lead to statistically significant improvement of tumor control on the contralateral side.

**Summary and Conclusions**

- Our novel p12-L2-P2A plasmid leads to increased expression of IL-12 p70 and its downstream effector molecule IFN-γ both in vitro and in vivo.
- Applying Low Voltage electrotransfer parameters results in enhanced transgene expression in vivo by improving transfection efficiency.
- Modifications to plasmid design (P2A) or electrotransfer parameters (Low Voltage) can improve the contralateral (systemic) anti-tumor response of IT-pIL12-EP in a mouse melanoma model.
- IT-pIL12-P2A-EP with Low Voltage conditions results in the generation of circulating antigen-specific CD8 T cells (B16-OVA model).
- P2A-linked multigene constructs in combination with a Low Voltage electrotransfer generator will provide the platform for our future clinical studies.

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