IN SITU VACCINATION UTILIZING INTRATUMORAL ELECTROPORATION OF PLASMIDS EXPRESSING IL-12 AND CD40 LIGAND (CD154) HAS EFFICACY AGAINST MURINE TUMORS

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Background Although the human body generates tumor-specific T cells, tumor-mediated immunosuppression protects tumors from antitumor immunity. Any immunotherapy must overcome this local/systemic immunosuppression. One strategy is in situ vaccination (ISV), where an immunostimulant is applied directly to an established tumor to disrupt the local immunosuppression, generating a robust local antitumor immunity, slowing or potentially eliminating the treated tumor. In addition, the rapid expansion of tumor-specific T cells induces antitumoral effects on distant, non-treated tumors known as the abscopal effect. While there are many therapeutic options under development for ISV, the optimal combination of immunostimulants needed for different tumor types remains unclear. Reported here are studies to express specific proteins by plasmid electroporation as ISV therapy. Previous studies have reported the benefit of expressing different molecules from plasmids in tumors, particularly IL-12, which has also been tested in phase II clinical trials. CD154 expression, (ligand for CD40), can mature antigen-presenting cells, lead to changes in cytokine expression, and support antigen presentation. We find that intratumoral CD154 electroporation has potent local antitumor effects and those effects are increased when CD154 is combined with IL-12. Presented studies will include abscopal effects on established but untreated tumors.

Methods Plasmids encoding for either CD154, IL-12, or control (Bgeo) were transformed into competent E.coli and inoculated into LB Miller cultures. Plasmid DNA was isolated and purified using Qiagen Plasmid Mega kit. Mice were anesthetized and injected with 2 x 10⁵ B16F10 cells into the right flank intradermally prior to electroporation treatment into C57BL6 mice. 50ug of each plasmid was injected intratumorally twice one week apart. Immediately following injection, mice were electroporated for 6 pulses at 1500v/cm for 6 pulses at 100us durations using BTX Harvard Gemini X2 electroporator.

Results We find that intratumoral CD154 electroporation has potent local antitumor effects and those effects are increased when CD154 is combined with IL-12. With the addition of the IL-12 plasmid, we are able to generate significant abscopal effects on established, untreated tumors.

Conclusions The addition of CD40 ligand expression in combination with IL-12 induces tumor clearance on the treated tumor. CD154 and IL-12 plasmid electroporation combination therapy provides superior protection on the treated tumor, in addition to providing protection on distant, untreated tumors.

REFERENCES

