1142 MRNA LNP VACCINATION WITH THE TUMOR-SPECIFIC ANTIGEN EGFRVIII BOLSTERS CAR T CELL ACTIVITY IN VIVO

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Background CAR T cell persistence is the only known clinical correlate of activity. Often in solid tumors, CAR T cells fail to persist in patients shortly after infusion. Modulation and maintenance of CAR T cell in patients is crucial for this type of therapy to work. We have completed two CAR T cell clinical trials targeting the tumor specific antigen EGFR variant III (EGFRvIII) in glioblastoma (GBM). Clinical trial data indicated that CAR T cell persistent suffered shortly after infusion. One mechanism to maintain and modulate CAR T cell levels is through mRNA vaccination with the CAR T cell antigen.

Methods We designed a lipid nanoparticle (LNP) carrying the mRNA for the EGFRvIII antigen to boost CAR T cell levels *in vivo*. We used a combination of *in vitro* and *in vivo* assays to test the ability of a truncated EGFRvIII or the corresponding negative control truncated CD19 to activate and boost CAR T cells. Two CARs directed to EGFR were used to evaluate the efficacy of boosting CAR T cell via mRNA LNP vaccination.

Results *In vitro* gene transfer assays were used to determine the ability of mRNA LNPs to transiently transfect target cells. The truncated EGFRvIII and truncated CD19 expressed in transfected cells. In coculture, CAR T cells were able to recognize and be activated by target cells transfected with their cognate antigen. Cytokine production in CAR T cell demonstrated antigen specific activation in vitro. We next evaluated the ability of mRNA LNPs to boost CAR T cells in a murine tumor model. Tumor bearing mice were treated with sub-therapeutic doses of CAR T cells and subsequently boosted twice using mRNA vaccines to EGFRvIII or CD19. Only mice boosted with the EGFRvIII saw a delay in tumor growth and a conferred survival advantage.

Conclusions Taken together, these data demonstrate that mRNA LNP vaccination with CAR T cell antigens can be an effect way to bolster CAR T cells activity in tumor bearing mice. The antigen specific activation of CAR T cell both *in vivo* and *in vitro* highlight the utility of this approach in modulating CAR T cell activity. Furthermore, this technology could be used to rescue poor engraftment of CAR T cell in patients.

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