Next-generation mRNA-LNP vaccine prototype achieves tumor clearance in a GBM mouse model

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INTRODUCTION

1. **Glioblastoma (GBM) is the most prevalent and aggressive primary brain tumor type with an abysmal prognosis.**
   a. GBM features an immunologically dampened tumor microenvironment and limited neoantigen production.
   b. Novel therapeutic strategies for GBM are urgently needed.
2. **A well-characterized GBM-associated mutation can elicit an anti-tumor immune response: The EGFRvIII mutation.**
   a. The cancer-driver mutation is detected in approximately 30% of patients at the time of diagnosis and plays a pivotal role in the emergence of GBM.
   b. Its highly immunogenic nature represents an ideal focal point for cutting-edge mRNA-LNP candidate therapeutics.
   c. (CAR)–T cells directed against EGFRvIII have shown promise in a phase 1 study for GBM treatment, paving the way for a vaccination-based approach.

Methodology

1. **Evaluate the efficacy of proprietary lipid nanoparticle (LNP) – mRNA vaccines targeting EGFRvIII in a GBM mouse model.**
2. **Develop a murine transplantable GBM tumor model based on the C3H mouse strain.**
   a. C3H-associated MHC molecules bind the immunogenic EGFRvIII sequence to trigger anti-EGFRvIII T cell responses, unlike C57BL/6 mice.
   b. Somatically engineered glioblastomas harbor CRISPR double knockouts of Cdkn2a and Pten and stably overexpress EGFRvIII.
   c. Establishing transplantable neurosphere cultures for orthotopic tumor initiation in syngeneic and sex-matched recipients.
3. **One week after intracranial tumor cell implantation of 6-8 week-old female mice (day 0; n=10), half the animals were administered either buffer (control cohort) or our novel anti-EGFRvIII mRNA-LNP vaccine (experimental cohort).**
4. **Four intramuscular injections before monitoring tumor progression by MRI on day 30.**

Figure 1 - The EGFRvIII mutation

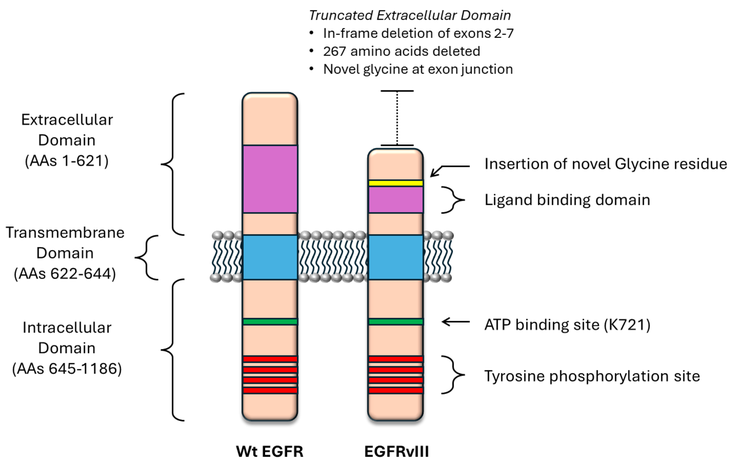


Figure 2 – Development of a murine transplantable GBM tumor model based on the C3H mouse strain.

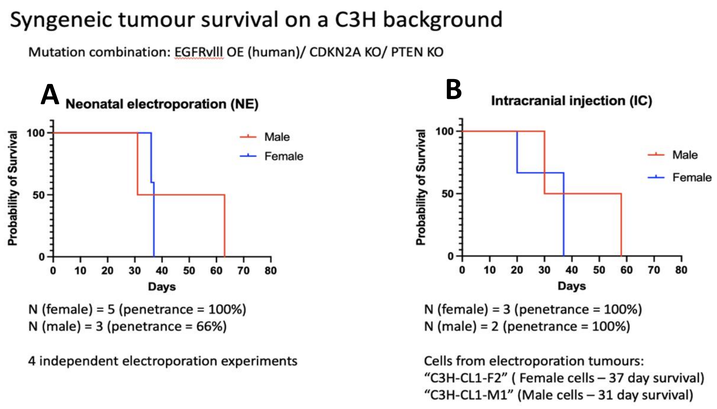


Figure 2 – Generation of a C3H-derived transplantable GBM tumor model

The parental tumors of these two, sex-discriminative, lines were generated by *in vivo* co-electroporation of constructs into the subventricular zone of early postnatal (P1-3) pups. Constructs used were pB-EGFRvIII-GFP and CRISPR constructs for knockouts of Cdkn2a and Pten. Survival curve of female and male electroporated pups (**A**). After animals reached the humane endpoint, tumors were dissected from the brains, and cell lines were grown as neurosphere cultures in defined serum-free media. The ability of lines CH3-CL1-F2 (female line) and CH3-CL1-M1 (male line) to initiate tumors orthotopically in sex-matched syngeneic mice was validated by injection of 1x10e5 cells into the right striatum of 6-8 week-old female and male mice, respectively (**B**).

Figure 3 – Flow cytometric evaluation of mRNA-encoded EGFRvIII expression in 293T cells

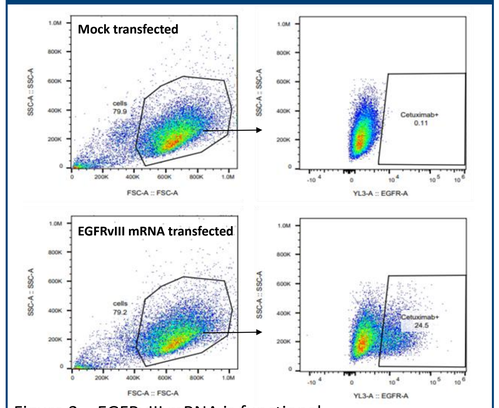
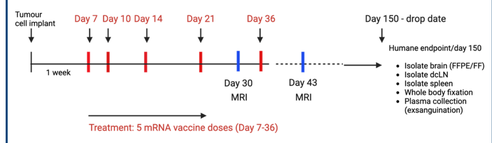


Figure 3 – EGFRvIII mRNA is functional

293T cells were transfected using the EGFRvIII-encoding mRNA and mMessengerMax. 60 hrs later, cells were stained with an anti-EGFRvIII antibody (Cetuximab).

Figure 4 – Schematic Experimental Setup



CONCLUSIONS

1. **A preclinical pilot study investigating a novel anti-EGFRvIII vaccine for therapeutic cancer treatment yielded remarkable efficacy in an engineered GBM mouse model.**
2. **100% of vaccine-treated animals survived orthotopic GBM transplantation, whereas all control mice succumbed to tumor-associated symptoms by day 38.**
3. **MRI scans (days 30 and 43) and IHC analyses (at day 150, endpoint) revealed no detectable tumor cells in vaccine-protected mice.**
4. **Additional POC for anti-GBM vaccines are ongoing as Providence Therapeutics' program moves toward clinical trials 2025.**

Figure 6 – Vaccine-treated mice survive tumor cell transplantation

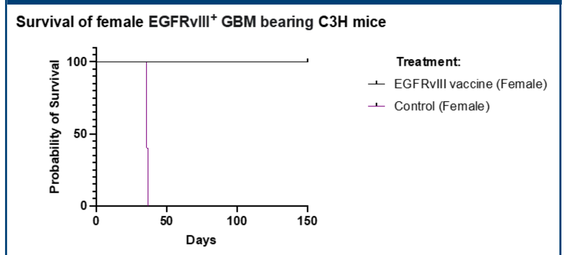


Figure 6 – Survival Data

All buffer-treated control mice died by day 38, whereas vaccine-treated animals survived the tumor cell transplantation with no signs of tumor development.

Figure 5 – MRI analysis shows no tumor signs in EGFRvIII vaccine-treated mice

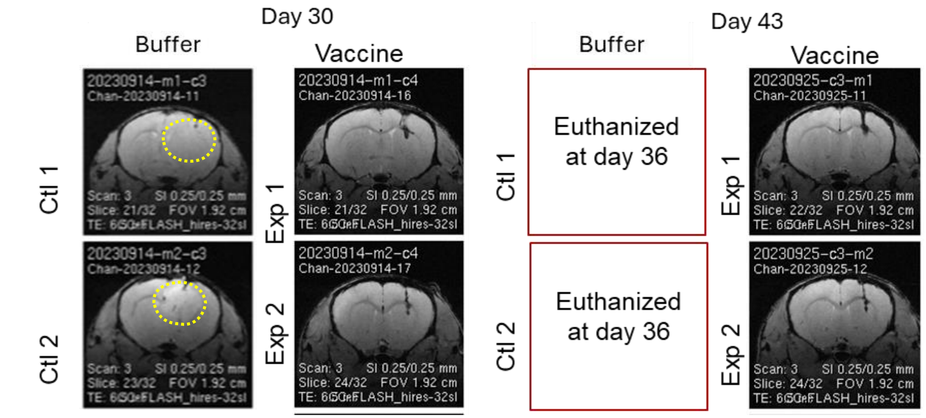


Figure 5 – Generation of a C3H-derived transplantable GBM tumor model

MRI assessment of C3H female recipients at day 30 and day 43. Images are shown at the level of the injection needle tract. EGFRvIII vaccine-treated animals show no evidence of tumor. Buffer-treated animals showed bulky tumors (iso-intense compared to the normal cortex and striatum; dashed line).