Therapeutic mRNA cancer vaccine for GBM reaches complete response in a pre-clinical model

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INTRODUCTION

Glioblastoma (GBM) is an aggressive primary brain tumor with a dismal prognosis. It is characterized by a limited number of neoantigens and a highly immunosuppressive tumor environment. A well-known GBM-associated driver mutation is EGFRvIII, which is detected in approximately 30% of patients at the time of diagnosis and plays a pivotal role in the emergence of GBM. Its high expression on the tumor cell surface makes it an ideal target antigen for vaccine development, as demonstrated by targeting therapies with antibodies and more recently CAR–T cells.

Providence Therapeutics has developed new lipid nanoparticles (LNPs) with promising safety and therapeutic profile for cancer vaccines. In this study, we evaluate two unique LNP formulations containing a novel proprietary ionizable lipid formulated with mRNA encoding EGFRvIII. These LNPs were used to vaccinate mice with established GBM, where the tumors resemble the human GBM pathology with dense presence of highly mitotic astrocytes progressing into vascular proliferation and necrosis. This GBM mouse model is not responsive to Temozolomide (TMZ) or a combination of TMZ and checkpoint inhibitors. However, the LNP vaccines developed by Providence demonstrated the ability to control GBM growth and induce a strong protective effect, showcasing their potential as anti-GBM immunotherapy.

RESULTS

1 New Lipid Nanoparticles (LNP) for EGFRvIII vaccine

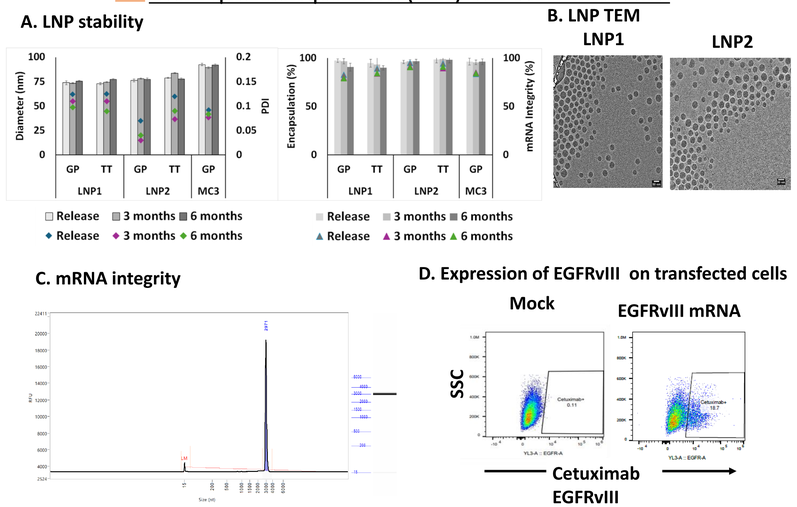

Figure 1. A. LNPs are stable for six months at –20°C and –80°C. No change in particle size (bars), PDI (diamonds), percentage encapsulation (bars) and mRNA integrity (triangles)were observed. **B.** LNPs transmission electron microscopy images (TEM). **C.** Integrity of EGFRvIII mRNA determined by CE indicates a single band of 2971nt and 100% purity. **D.** Expression of EGFRvIII on mRNA transfected 293T cells detected by Cetuximab.

2 Tumor model expressing human EGFRvIII resembles human GBM

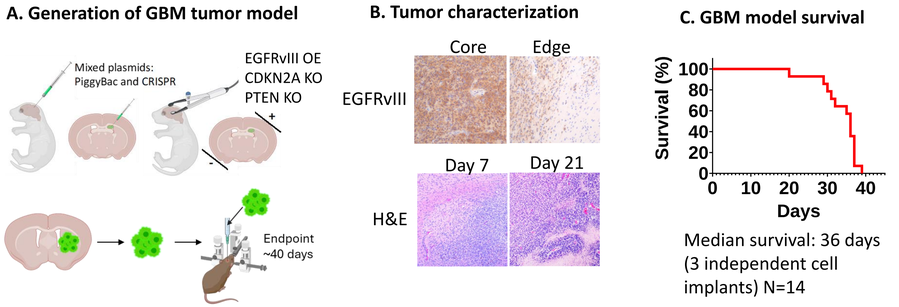

Figure 2. A. Generation of a transplantable GBM tumor model. Diagram of generation of implantable glioblastoma cell lines overexpressing EGFRvIII. **B.** EGFRvIII expression was detected by IHC in brain tumor sections. H&E images illustrate that the model shows typical features of glioblastoma. **C.** Survival in the absence of any therapeutic interventions, humane endpoint is reached at 35-40 days. Treatment with TMZ or in combination with checkpoint inhibitors do not control progression or alter survival.

3 EGFRvIII vaccine controls GBM

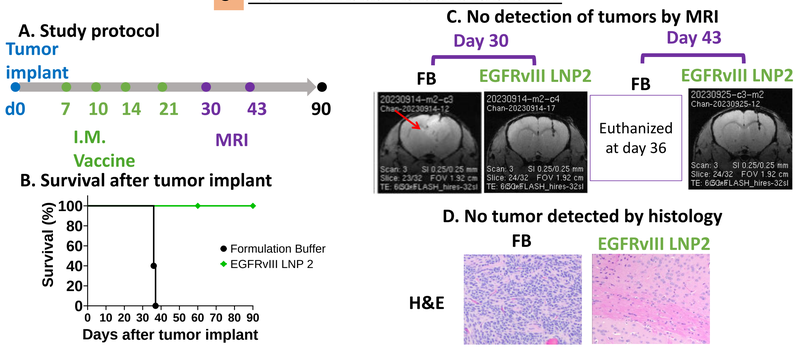

Figure 3. A. Study protocol. C3H/HeJ female mice were injected with $1x10^5$ cells into the right striatum and were I.M vaccinated with 10 µg of vaccine or formulation buffer (FB) four times starting on day 7 until day 21. MRI imaging was done on day 30 and 43. **B.** Survival after tumor implant. **C.** MRI images of brains from each group. **D.** H&E images show densely cellular tumors in the formulation buffer and no presence of tumor cells were detected in EGFRvIII vaccinated mice at endpoint.

4 EGFRvIII vaccine induced long lasting protective immunity

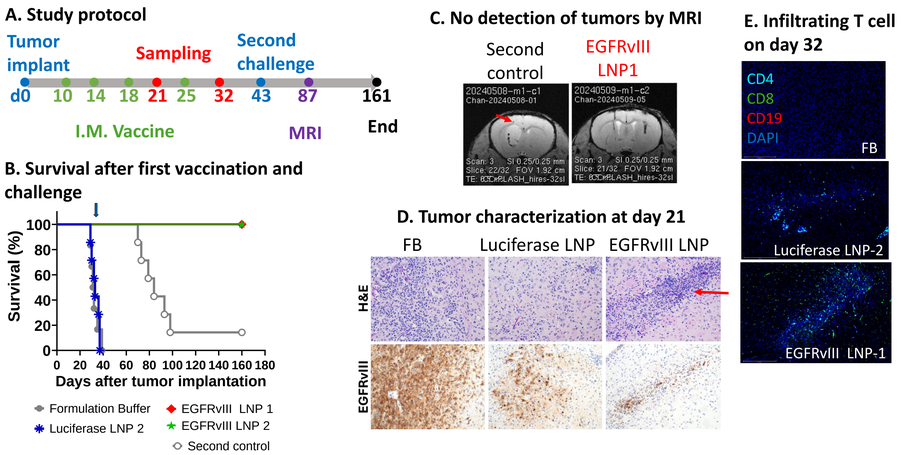

Figure 4. A. Study protocol**.** C3H/HeJ female mice were injected with $10E^5$ cells into the right striatum and were vaccinated I.M. with 10 µg of vaccine or formulation buffer starting on day 10 and until day 25 . Mice that survived were challenged with $10E^5$ cells on the left striatum on day 43. **B.** Survival after tumor implant. **C.** MRI images of one set of mice of each group after the second challenge. Experiment stopped by day 161. **D**. H&E and EGFRvIII labeling. Control or Luciferase show dense presence of tumor cells. Arrow indicates lymphocyte infiltration. **E.** Tumor fixed sections from day 32 were process for OPAL multiplex immunofluorescence labeling with antibodies against CD4, CD8, CD19 and using DAPI for nuclear stain.

5 EGFRvIII mRNA vaccine induced strong T cell and antibody responses

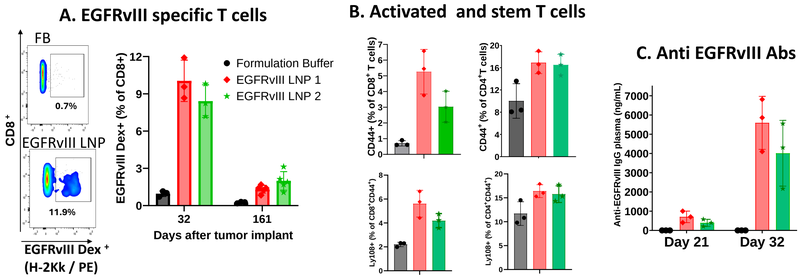

Figure 5. A. Splenocytes EGFRvIII MHC I H-$2K^k$ tetramer detection by flow. **B.** CD44+ and Ly 108 T cells from d32 detected in spleen by flow . **C.** Plasma anti-EGFRvIII antibodies determined by ELISA.

CONCLUSIONS

New EGFRvIII-LNPs have promising stability profile and are effective at inducing tumor control in 100% of mice with established GBM. Moreover, EGFRvIII LNPs vaccination provides long lasting protection to a second GBM challenge where no residual tumors were identified by MRI or histology at endpoint in protected animals. Lymphocyte infiltration with $CD4^+$ and $CD8^+$ T cells was detected in brain tumor sections of vaccinated animals that correlates with reduced tumor burden. EGFRvIII specific $CD8^+$ T cells were detected in high numbers in the spleen and an increase in activated T cells with steam phenotype. Vaccination also induced high levels of anti-EGFRvIII antibodies. These studies strongly support the use of Providence's new LNPs for the development of a GBM vaccine and other cancer therapeutic vaccines.

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