Targeted Cytotoxic Killing of Glioblastoma by Genetically Engineered Central Memory T-cells Expressing Monoclonal Antibody 806 Chimeric Antigen Receptor

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Introduction

Glioblastoma is the most common, incurable and fatal form of brain malignancy in adults with a median survival of less than two years from the time of diagnosis. Each year, approximately 13,000 patients die of this disease. Our current treatment regimen of surgical resection, limited chemotherapy and radiation has remained stagnant and terribly ineffective over decades. Among many molecular aberrations spawning this malignant phenotype is an over-expression of wild type and truncated, constitutively active, form of Epidermal Growth Factor Receptor (EGFR vIII). Approximately, 70% of glioblastomas harbor EGFR over-expression. EGFRvIII variant is expressed in 30% of glioblastomas. These findings make EGFR and its variant an ideal cell-surface antigen for immune-based therapy. To address this possibility, we designed a chimeric antigen receptor (CAR) using a previously defined monoclonal antibody originally developed against the EGFRvIII variant (mAb 806), which also binds over-expressed EGFR with high affinity.

Methods

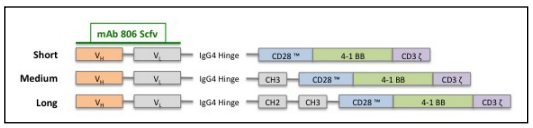
Lentivirus constructs were used to express three spacer variants of the mAb 806 CAR in primary human central memory CD8 T lymphoctyes (Tcm8). Chromium release assay was used to evaluate the cytotoxic ability of engineered Tcms.

Orthotopic xenograft mouse model was used to evaluate the cytotoxic potential of Tcms *in vivo.*

RESULTS

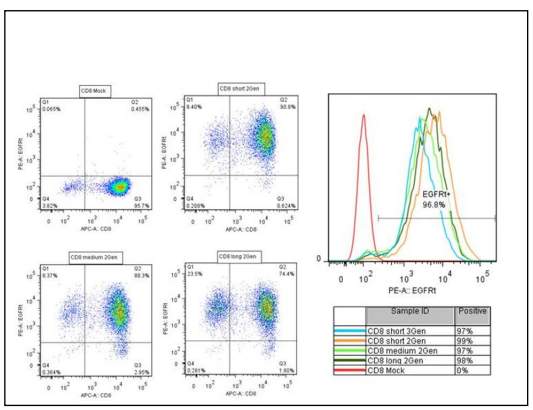
Generation of three mAB 806 CAR variants

The mAB 806 ORF was inserted upstream of three spacer variants of second generation CAR panel (with 4-1BB domain).



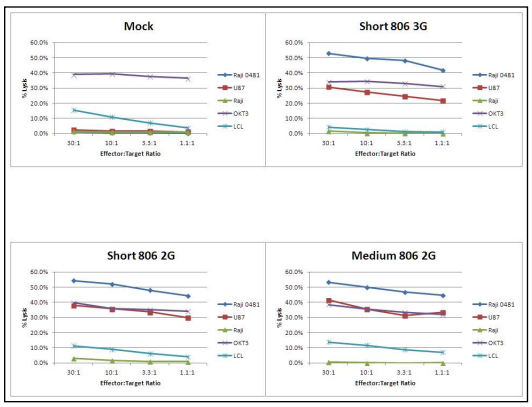
Generation of Central Memory T Cells Expressing mAb 806 CAR Variants

Central memory CD8 T cells are isolated from consenting adults. The cells are transduced with lentivirus constructs expressing the three mAB 806 CAR variants. Cell profile and expression pattern are confirmed using flow cytometry & western blot analysis.



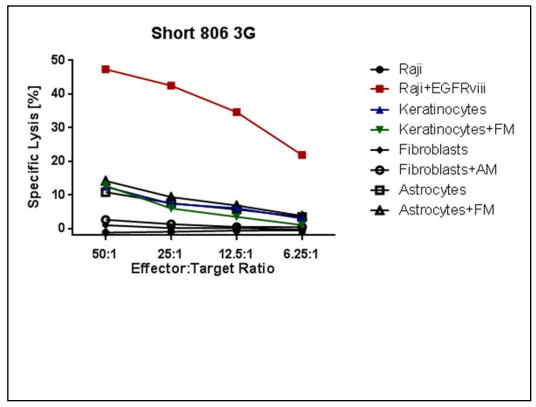
Functional Analysis of Engineered T Cells

In both Chromium release assay (Cytotoxic assay) and bioplex assay, all three mAb 806 CAR spacer lengths in Tcm8 cells showed robust cytotoxic effect against a number of target human glioblastoma permanent cell lines.



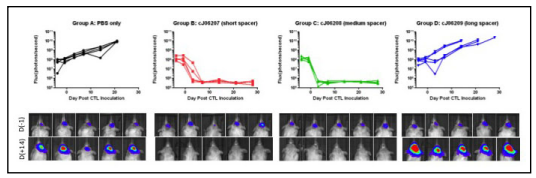
Target Specificity of mAB 806 CAR Against Tumor Cells

To ensure target specificity of mAB 806 CAR against tumor EGFR/EGFRviii, cytotoxic activity of the short variant was tested against various normal primary cell lines expressing wtEGFR. Inerestingly, Keratinocytes express higher levels of wt surface EGFR compared to glioblastoma U870 cells, but are a poor target for the mAb 806 CAR.



Targeted Cytotoxic Activity of the Short and Medium mAB 806 CAR Spacer Lengths in Mouse Model

In the orthotopic intracranial mouse xenograft glioblastoma model, the short and the medium spacer length mAb 806 CAR expressing Tcm8s obliterated existing tumors.



Conclusions

The short-spacer mAb 806 CAR in central memory CD8 cells has effective cytotoxic potential in a cell-based immune-mediated therapy against glioblastoma. Currently, the intracellular domain of the CAR short-spacer CAR is being refined with the goal of introducing this reagent into clinical trials as an adjunct post-resection mode of therapy.