

POSTER PRESENTATION

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Characterization and functional analysis of scFv-based CARs to redirect T cells to IL13R α 2-positive glioma

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Background

The goal of this project is to develop T cells that express chimeric antigen receptors (CARs) as an effective immunotherapy for glioblastoma (GBM), the most aggressive, primary brain tumor in humans, which outcome remains poor. IL13R α 2 is aberrantly expressed in GBM and therefore it is a promising candidate for CAR T cell immunotherapy. While other investigators have generated IL13R α 2-targeted CARs using mutated forms of IL13 as CAR binding domains, our studies indicate that these CARs also recognize IL13R α 1, raising significant toxicity concerns. To overcome this limitation we have developed a high affinity IL13R α 2-specific scFv (M47) that does not recognize IL13R α 1 and that was used to generate scFv-based IL13R α 2-specific CAR (M47-CAR T cells) and evaluate their function.

Methods

We constructed a panel of IL13R α 2-CARs containing M47 as an ectodomain, a short spacer or long spacer region (SSR, LSR), a CD28 transmembrane domain, and endodomains that consist of signaling domains derived from CD3 ζ and co-stimulatory molecules (CD28. ζ , CD28.OX40. ζ , CD28. ζ , CD28.41BB. ζ , 41BB. ζ) IL13R α 2-CAR T cells were generated by retroviral transduction, and their effector function was compared *in vitro* and in a GBM xenograft model.

Results

While all CARs were expressed as judged by Western blot analysis, CARs with a SSR and a CD28.41BB. ζ

endodomain were not expressed on the cell surface. In cytotoxicity assays, IL13R α 2-CAR T cells only killed target cells that expressed IL13R α 2 and not IL13R α 1 confirming specificity. While all IL13R α 2-CAR T cells secreted significant levels of IFN γ in co-culture assays with the IL13R α 2+ glioma cell line U373, only CAR T cells with a short spacer region (SSR) secreted significant amounts of IL2. In contrast, T cells expressing an IL13R α 2-CAR with a deleted endodomain (M47-CAR.D) did not recognize or kill any target cells. *In vivo*, injection of IL13R α 2.SSR.CAR T cells with CD28. ζ , CD28.OX40. ζ , or 41BB. ζ endodomains into U373-bearing mice resulted in regression of glioma xenografts and a significant survival advantage with M47-CAR.SSR.CD28. ζ T cell therapy providing the longest median overall survival (84 days vs 40 days for M47-CAR.SSR.D).

Conclusions

T cells redirected to IL13R α 2 with M47-CARs have potent anti-tumor activity against glioma cells *in vitro*, and induce the regression of established GBM xenografts. Our study adds to the growing literature that there is an intricate interplay between scFVs, spacer region, transmembrane domain and endodomain that determine CAR function, and that there is no single optimal configuration. M47-CARs may be of value in the treatment of not only IL13R α 2-positive GBMs but also other malignancies in which IL13R α 2 is expressed.

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