Designing CAR T cells for glioblastoma

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Chimeric antigen receptor (CAR)-modified T cells directed against CD19 can mediate long-term durable remissions in B cell malignancies, but bringing a new target antigen to the clinic requires extensive modeling to avoid on-target and off-target toxicity. We recently described a systematic approach to test a new CAR directed against EGFR variant III.

Immunotherapies that engage T cells have the potential to induce long-term durable remissions of cancer. In the treatment of hematologic malignancies, allogeneic hematopoietic stem cell transplantation can be curative partly due to T-cell mediated antitumor immunity. Among immune-based therapies against solid tumors, checkpoint blockade with anti-CTLA-4 or anti-PD-1 monoclonal antibodies can mediate long-term responses by releasing T cells from tightly controlled peripheral tolerance. Chimeric antigen receptors (CARs) are synthetic molecules designed to re-direct T cells to specific antigens. Re-directing T cells with CARs is an alternative method of overcoming tolerance and can be performed in the autologous setting. In B cell malignancies, CAR T cells directed to CD19 can mediate long-term remissions, and are associated with on-target toxicity (B cell aplasia), and cytokine release syndrome.1–3 However, CAR immunotherapy in solid tumors remains challenging, largely due to the lack of appropriate surface antigens whose expression is confined to malignant tissue. Off-tumor expression of the antigen target has potential to cause on-target toxicity with varying degrees of severity depending on the affected organ tissue.4–6

The ideal antigen target for a CAR T cell is expressed on the surface of tumor cells and has a role in maintaining the tumor phenotype, attributes that would make the CAR T cell effective. However, in order to synthesize a safe CAR T cell, it is also important that normal tissues lack expression of the antigen (to avoid on-target toxicity), and for the CAR to be highly specific to only the desired antigen (to avoid off-target toxicity). Because re-directed T cells have antigen receptors that have not undergone thymic selection, their specificity requires extensive laboratory validation to ensure that there is no cross-reactivity to self-antigens.6–9 The variant III mutation of the epidermal growth factor receptor (EGFRvIII) results from an in-frame deletion of a portion of the extracellular domain, thus creating a neoepitope. The EGFRvIII mutation is oncogenic in glioblastoma, portends a poor prognosis and is purportedly enriched in glioblastoma stem cells. However, because the neoepitope of EGFRvIII is based on a small peptide sequence, an antibody or single-chain variable fragment (scFv) directed to this epitope must be rigorously tested to confirm lack of cross-reactivity to the ubiquitously expressed wild-type EGFR. We recently described a systematic approach to pre-clinical testing of a new humanized CAR specific for EGFRvIII (Fig. 1).10 We began by choosing a vector backbone based on xenograft glioblastoma models treated with a panel of murine-based CAR T cells. We then generated a large panel of humanized scFv’s, and performed extensive in silico (biophysical), in vitro (cellular assays), and in vivo (xenograft models) assays to characterize the function and reactivity of the CAR.

The vector backbone chosen was based on a lentiviral vector encoding a second-generation CAR composed of an EGFRvIII-specific scFv fused to the human CD8, 4–1BB (TNFRSF9), and CD3 zeta chains. Genetically modified T cells encoding this CAR protein were the fastest to clear an orthotopic xenograft model of glioblastoma. Due to concerns of anti-mouse immune responses in humans, we also generated a panel of humanized scFv’s for testing and further development. This panel of 8 scFv’s was tested for their specificity and function as soluble proteins binding to cells, namely via assaying soluble scFv’s binding to membrane-bound EGFR and EGFRvIII, and soluble EGFR and EGFRvIII binding to CAR T cells engineered with the 8 scFv’s. We noted that some of the higher affinity scFv’s bound to wild type EGFR, which we wished to avoid for safety reasons. We therefore selected a relatively low affinity scFv as the lead candidate, based on its specificity for EGFRvIII over wild type EGFR. Further biophysical characterization determined the affinity of the lead scFv candidate to soluble EGFR and EGFRvIII by BIACore (surface plasmon resonance) analysis.

The lead candidate scFv was tested in vitro for its ability to direct CAR-transduced T cells to specifically lyse, proliferate, and secrete cytokines in response to antigen-bearing targets. In all experiments, we verified specificity of the CAR T cells to EGFRvIII over wild type EGFR. However, most tumor cell lines, including glioblastoma cell lines such as the U87 MG model that we used, actually overexpress EGFR. We wished to confirm the lack of reactivity to EGFR by testing normal

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human tissues that naturally express EGFR, particularly keratinocytes in the skin. We used cultured keratinocytes and a novel model of mice grafted with normal human skin to test off-target toxicity. We also developed cetuximab-based CAR to serve as a positive control, as cetuximab binds to both EGFR and EGFRvIII.

We confirmed that the cetuximab-based CAR bound to both soluble EGFR and EGFRvIII without discrimination, and lysed human keratinocytes and EGFR-expressing glioblastoma cells in vitro. When cetuximab-CAR T cells were injected into mice grafted with human skin, the skin was infiltrated with T cells and caused keratinocyte apoptosis. In contrast, the optimal candidate EGFR-vIII-directed CAR T cells did not bind EGFR, did not lyse EGFR-expressing cells and did not cause keratinocyte apoptosis in the skin.

EGFRvIII-directed CAR-T cells were also able to control tumor growth in xenogeneic subcutaneous and orthotopic models of human EGFRvIII+ glioblastoma. We also noted that MRI imaging of tumor-bearing mice revealed that EGFR-vIII-directed CAR T cells induced remarkable tumor regressions. Histologic examination of mouse tissues demonstrated infiltration of brain tissue with CAR T cells, indicating appropriate trafficking to the site of antigen.

Based on these results, we have opened a Phase I clinical study of CAR T cells transduced with humanized scFv directed to EGFRv III in patients with either residual or recurrent glioblastoma (NCT02209376).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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