

AlloECAR-NKT: New hope for GBM patients

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Chimeric antigen receptor (CAR)-T cell therapy (CAR-T) has achieved remarkable success in treating hematological malignancies. However, its efficacy against solid tumors, including glioblastoma (GBM), is significantly hampered by the complexity of autologous cell manufacturing, tumor heterogeneity, the immunosuppressive tumor microenvironment (TME), and adverse effects such as cytokine release syndrome (CRS) and immune cell-associated neurotoxicity syndrome.¹ In this issue of *Molecular Therapy*, Li et al. describe the use of hematopoietic stem and progenitor cell gene engineering combined with a feeder-free, *ex vivo* differentiation protocol to generate allogeneic EGFRvIII-specific CAR-engineered invariant natural killer T (AlloECAR-NKT) cells containing a soluble human interleukin-15 (IL-15) transgene through a clinically guided method.² These AlloECAR-NKT cells demonstrated potent antitumor efficacy and an improved safety profile in both subcutaneous and orthotopic GBM humanized mouse models.

Compared with conventional ECAR-T cells, AlloECAR-NKT cells exhibited high purity and stable CAR expression that exceeded 99%, which eliminated the need for further CAR cell enrichment.² This powerful platform has the potential to produce 1,000–10,000 doses of therapeutic drugs in a single batch, whereas allogeneic CAR-T cells derived from donor peripheral blood mononuclear cells generally produce >100 doses per batch.³ One potential limitation is that EGFRvIII is expressed in only ~30% of GBM tumors, making it difficult to achieve complete tumor elimination by solely targeting EGFRvIII. In a previous study, bivalent CAR-T

cells targeting EGFR and IL-13 receptor $\alpha 2$ were used to treat GBM to address tumor antigen heterogeneity leading to antigen escape, and an encouraging early efficacy signal was observed.⁴ However, AlloECAR-NKT cells rely on their unique CAR-dependent and NKT-dependent targeting mechanisms to kill tumor cells and thereby carry a reduced risk of antigen escape in principle.²

The immunosuppressive GBM TME is a pivotal factor in immunotherapy resistance. To overcome this barrier, previous approaches have included the use of a transforming growth factor β (TGF- β) trap or the dominant-negative receptor of TGF- β in CAR-T cells to treat GBM and prostate cancer, respectively.^{5,6} However, the AlloECAR-NKT cells used in the present study² were characterized by increased secretion of Th1-related cytokines, indicating that no additional modifications are required to effectively activate the body's immune system.⁷ In addition, the NKT cell receptor recognizes the CD1d ligand, which is highly expressed on myeloid-derived suppressor cells and tumor-associated macrophages (TAMs). Therefore, AlloECAR-NKT cells can overcome TAM-mediated immunosuppression and remodel the TME while preserving their effector function.

Due to the specific administration method used in CAR-T cell therapy for GBM, patients are prone to adverse reactions. In a phase 1 clinical trial, all 6 patients experienced toxic reactions and severe neurotoxicity in the early stage of treatment.⁴ In a previous preclinical study, a team knocked out IL-6 in CAR-T cells to reduce CRS.⁸ By contrast, in the present study, AlloECAR-NKT cells exhibited increased tumor

infiltration, superior tumor-clearing capacity, and prolonged survival, with minimal off-target trafficking to peripheral organs.² Consequently, AlloECAR-NKT cells did not induce graft-versus-host disease or CRS in the xenograft models, indicating a favorable safety profile. These findings support AlloECAR-NKT cells as a viable cancer immunotherapy for GBM, which warrants further clinical investigation of their therapeutic potential.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Commentary

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