

Peer review

Sundar, Shwetha. 2025. "CAR T-Cell Therapies: A Comparison of Strategies for Glioblastoma Multiforme Treatment." *Journal of High School Science* 9 (1): 158–72.

The manuscript as written presents information in the public domain and does not therefore contribute significantly to the corpus of existing knowledge in the field. As written, it is not suitable for acceptance as a review paper: see <https://jhss.scholasticahq.com/for-authors>, types of manuscript, review papers.

The author will need to propose at least one idea or a non-obvious extrapolation or point out an implicit incorrect assumption that does not yet exist in the public domain. I propose the following, although the author is free to propose her own:

Incorporate an 'elapsed time' into the logic circuits that will enable a CAR-T cell population (in vivo) to automatically 'switch' between three gates: a monovalent gate, a divalent "AND" gate and a trivalent "AND-AND" gate, depending on what population of the cancer cells carry one, two or three cancer specific markers respectively. This can be accomplished by a gene circuit that keeps track of 'elapsed time' for a logic promoter (I don't know if such a circuit can be engineered). For example, if there is no population of cancer cells with 3 cancer specific antigens, the elapsed time for the "AND-AND" circuit promoter will be large (top third of cells in pdf). Once the elapsed time exceeds a certain limit, the CAR-T abandons the "AND-AND" (trivalent) circuit (for a certain time) and switches to an "AND" (divalent) circuit. This allows the CAR-T cells to kill cancer cells that express varying numbers of cancer cell receptors within a tumor population.

see pdf attached. Some other manuscripts of interest appear below.

<https://doi.org/10.1007/s00018-024-05112-7>

<https://doi.org/10.1158/1078-0432.CCR-18-1211>

<https://doi.org/10.3390/cancers16162858> (references 164-173) and more therein.

<https://doi.org/10.1016/j.clim.2022.109030>

Elapsed Time Circuits: A Novel Approach

Although SynNotch CAR T-cell therapy successfully overcomes tumor heterogeneity, it still fails to address the diversity of tumor cells in that it cannot adapt to the different antigens expressed in different locations of the tumor.

Tumor cells often express varying levels of target antigens, leading to some cells escaping CAR T-cell recognition and contributing to relapse.²³ This heterogeneity is a significant obstacle in using CAR T-cell therapy to treat tumors because CAR T-cells engineered to target a single antigen may be effective against some tumor cells but ineffective against other cells that lack or express low levels of that antigen. This phenomenon of antigen escape highlights the need for more sophisticated CAR T-cells that can effectively reach a broader range of tumor cells.^{12,23}

To address this challenge, I propose a novel approach incorporating a "time-elapsed" circuit within CAR T-cells that would allow the CAR T-cell to switch between different levels of specificity based on the locations of tumor cells and the cells' expression of varying antigen combinations.²⁴

Specifically, CAR T-cells could be programmed as a circuit that would go through trivalent ("AND-AND"), divalent ("AND"), and monovalent targeting modes.²⁵ The "time-elapsed" segment would look at the time for which the CAR-T cell stays in a targeting mode. If the CAR T-cell is in the "AND-AND" mode and proves ineffective because finds few or no cells that express all three target antigens, the circuit would switch to a less strict mode, such as the "AND" mode or even a single-antigen targeting mode in regions with a lower amount of non-cancerous cells.²⁶ This method of using

different levels of specificity based on efficacy would allow CAR T-cells to adjust to the heterogeneous and diverse landscape of GBM; the CAR T-cell could effectively eliminate a wider range of tumor cells, including those with many antigen profiles. The “time-elapsed” part of the circuit would prevent the CAR-T cell from being stuck in one, ineffective targeting mode because the antigen combination is infrequent in that particular region of cells. Because like SynNotch CAR T-cells, this method would target multiple antigens, it would improve specificity; with its different stages of specificity, it would also cater to a broader range of cells. This means that they are more likely to be able to find and kill tumor cells, even if the tumor cells are heterogeneous. Therefore, the development of this novel approach to CAR T-cell therapy would prove incredibly useful for the future of GBM treatment.

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Please resubmit the word file with the changes. I look forward to your revised submission.

CAR T-Cell Therapies: A Comparison of Strategies for Glioblastoma Multiforme Treatment

Shwetha Sundar

Summary of changes to manuscript

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AAAS MATERIAL:

Figure 1a, b from Joseph H. Choe et al. ,SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. *Sci. Transl. Med.* 13,eabe7378(2021).DOI:10.1126/scitranslmed.abe7378

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 8. Remove subjective words such as immense, incredibly, tremendous, superior..... from the manuscript. - **DONE**

Thank you for addressing my comments. Accepted. Please check the attached galley proof for errors and revert within 48 hours to ensure a timely publication. Note that I have changed some text, expanded on the legend of Figure 3 (as well as changed it slightly). Please check thoroughly.