Journal of High School Science

CAR T-cell therapies: A Comparison of strategies for Glioblastoma Multiforme treatment

Sundar S

Received: December 27, 2024, Revised: version 1, February 20, 2025, version 2, February 25, 2025
Accepted: February 28, 2025

Abstract

Glioblastoma multiforme (GBM) is an aggressive form of brain cancer, with significant challenges in its treatment and with a poor prognosis. Current standard treatments, such as surgery, radiation, and chemotherapy, have demonstrated limited effectiveness in improving long-term outcomes. However, chimeric antigen receptor (CAR) T-cell therapy, is an immunotherapy that shows promise in treating certain blood cancers and is being explored as a potential treatment for solid tumors like GBM. This review examines various CAR T-cell therapies for GBM treatment, including general, SynNotch, and armored CAR T-cells, exploring their mechanisms, advantages, and limitations. The challenges posed by GBM are presented, such as tumor heterogeneity and the immunosuppressive tumor microenvironment. Although general and armored CAR T-cells have proven beneficial to the treatment of GBM, both face significant limitations, including a lack of specificity in targeting cancer cells, neurotoxicity, and the cytokine release syndrome. SynNotch CAR T-cells, because of their enhanced specificity and ability to overcome tumor heterogeneity, could be effective to target and eradicate GBM cells while sparing healthy brain tissue from damage. A new, “elapsed time” circuit CAR T-cell therapy is proposed where; based on the time elapsed between the last receptor engagement; gene circuits could be engineered to repress or de-repress the number or type of CAR T-cell receptors so as to morph *in vivo* and make tumor cell kill agnostic to the number or type of tumor antigens expressed.

Keywords

Glioblastoma Multiforme, Cimeric Antigen Receptor, CAR T-cell, SynNotch CAR T-cell, Armored CAR T-cell, Immunotherapy, Gene circuits, Transcription factor, Boolean gate, Tumor heterogeneity

---

Shwetha Sundar, Lynbrook High School, 1280 Johnson Avenue, San Jose, CA 95129, USA.
shwethaxsundar@gmail.com

Introduction

Glioblastoma multiforme (GBM) is one of the most frequent and rapidly growing malignant tumors originating from the central nervous system (1,2). GBM originates from glial cells and is considered a grade IV astrocytoma (1,2). It accounts for ~ 54% of all gliomas and ~ 16% of all primary brain tumors (2). The median age at the time of diagnosis is 65 years, and it is more common in males than females (1). GBM has a high mortality rate, accounting for more deaths than kidney cancer or melanomas (2). The five-year relative survival rate for GBM stands at only 7.2%, with a median survival following diagnosis of only ~ eight months.

Almost all of these tumors that initially respond to treatment will recur (1). Standard treatment currently available in GBM involves surgical resection of the tumor followed by radiotherapy and chemotherapy using temozolomide (1).

GBM is presently the leading cause of tumor-related deaths among children and young adults, which underscores the need for advances in treatment strategies (2). Aggressive GBM growth, high recurrence rates, and the brain's protective barrier all demonstrate the significant need for further research, with new, innovative therapeutic approaches for treatment (1,2). T lymphocytes, also known as T-cells, are white blood cells that are an integral part of the immune system. They are responsible for cell-mediated immunity, the part of the immune system that directly attacks and destroys infected or abnormal cells. Unlike other immune cells that circulate throughout the body, however, T-cells are primarily found in lymphoid organs, such as the thymus, lymph nodes, and spleen (3). T-cells emerge from hematopoietic stem cells in the bone marrow and then migrate to the thymus for maturation. During the process of maturation, T-cells begin to learn how to recognize foreign antigens by expressing T-Cell Receptors (TCRs) which activate T-cells following contact with a specific antigen presented on the surface of an antigen-presenting cell (3). Following their activation, T-cells then differentiate into effector T-cells and execute their specialized functions. Cytotoxic T-cells, a subset of CD8+ T-cells, directly kill infected or cancerous cells through cytotoxins, inducing apoptosis in target cells. Helper T-cells are CD4+ T-cells that, by secreting cytokines, soluble factors secreted by immune cells, including T-cells, act as signaling molecules to regulate the immune response (4). These cytokines stimulate and regulate other immune cells to effectively combat infections. While the immune system is effective against many infections and diseases, cancers often have immunosuppressive tumor microenvironments, thereby reducing the effectiveness of the immune system (5). Chimeric Antigen Receptor (CAR) T-cells are a type of genetically engineered T cell that is designed to be equipped with chimeric antigen receptors, modified to better recognize and eliminate cancerous cells. CAR T-cell therapies are based on T-cells genetically modified to express antigen-recognizing receptors (3). To initiate autologous CAR T-cell therapy, a collection of T-cells is taken from a patient’s peripheral blood (1,3). Thereafter, these T-cells

are genetically modified to express the engineered CAR on their surface either using viral vectors or through electroporation (3). The modified CAR T-cells are expanded in the laboratory and then re-introduced into the patient (1,3). If the tumor cell expresses the target antigen, then the extracellular domain of the CAR will bind to the antigen (3). This binding triggers the intracellular signaling domain, leading to T-cell activation and cytotoxic molecule release, eventually causing the death of the tumor cells (Figure 1) (1,3).

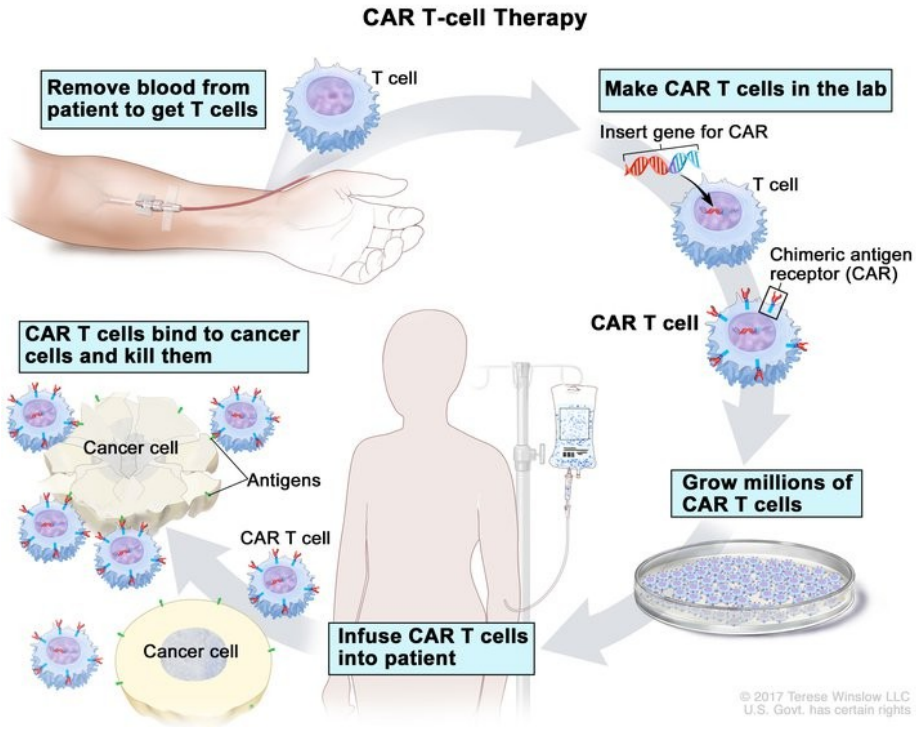

Figure 1. This schematic depicts a patient receiving CAR T cell therapy– first, blood is drawn to harvest the patient's T cells for modification; then, the T cells are engineered and grown to make millions of copies of the CAR T cells; finally, the newly created CAR T cells are reintroduced to the patient. This image was taken from the National Cancer Institute's Visuals Online.

Since TCRs can only target human leukocyte antigen-peptide complexes (HLA complexes), while CARs can attack antigens on tumor cells regardless of their previous HLA processing, CAR T-cells are more effective than T-cells with TCRs (2). This means that CAR T-cells can attach to and kill tumor cells that express specific proteins (2). CARs can also be engineered to target glycolipids and carbohydrates, while TCRs can only target peptide antigens (2). However, treating GBM with CAR T cell therapy still poses multiple challenges. GBM tumors have both high inter and intratumoral heterogeneity, meaning the

tumors either express an antigen that is also expressed on normal, healthy tissue, or the expression of the antigen is not uniform across the tumor cells (6).

Additionally, even if an antigen is identified, solid tumors can evade CAR-T-cells by repressing the expression of the target antigen. The tumor microenvironment is also hypoxic, meaning oxygen is deficient, thereby decreasing immune cell functionality (7). These two challenges make GBM difficult to treat with CAR T cell therapy, thereby hampering treatment progress. In conclusion, although CAR T cell therapy holds the potential to be effective in the future, addressing the considerable obstacles that remain in its use for treating solid tumors such as GBM is crucial to its success.

General CAR T-cells

Chimeric antigen receptor T-cells express CARs that are artificially introduced receptors, enabling T-cells to recognize and target a variety of proteins expressed on the surface of tumor cells (8,9). Various antigens have been explored as putative targets for CAR T-cell therapy in glioblastoma. The antigens chosen for this purpose depend on their minimal expression in normal tissues and are highly expressed in glioblastoma to minimize the potential killing of normal cells. EGFRvIII is one of the most prevailing mutations in glioblastoma; half of amplified EGFR glioblastoma patients have EGFRvIII, meaning that such antigens can be targeted. Its restricted tumor expression and role in cancer development make it a compelling CAR target (9). Similarly, IL13Rα2 is highly expressed on glioblastoma cells but absent in normal brain tissue and most healthy tissues, making it an important antigen for CAR T cell therapy as well. IL13Rα2 has been explored as a target due to its restricted expression and high affinity binding to IL-13 (10). Clinical trials using IL13Rα2-redirected CAR T-cells have shown promising but short-term anti-tumor responses (9,10). Clinical trials are underway to explore more antigens specific to cancer cells (9).

Brown et al. (11) evaluated the safety and efficacy for recurrent glioblastoma treated with CAR T-cells. Treatment involved the genetic engineering of a patient's T-cells to express a CAR specific for IL13Rα2, a cell surface antigen expressed on glioblastoma cells, thereby enhancing the immune system's capability to recognize tumor cells and eliminate them (11). The trial included one 50-year-old male patient with recurrent multifocal glioblastoma, consisting of multiple tumors in the brain and spine who had failed to respond to first-line standard-of-care treatments. The patient's tumors showed a high expression of IL13Rα2, making him a suitable candidate for this treatment. The trial utilized two delivery routes for the CAR T-cells–intracavitary and intraventricular. In the intracavitary phase, the first dose consisted of two million CAR T-cells infused into the cavity that was created by surgically removing one of the brain tumors. Subsequently, 5 more doses of ten million CAR T-cells were administered to the patient. While this approach appeared to prevent tumor recurrence at the local injection site, it did not halt the progression of tumors in other areas of

the brain and spine (11). The researchers switched to an intraventricular delivery route, infusing CAR T-cells into the brain's ventricular system, allowing for broader distribution within the cerebrospinal fluid. Notably, this approach led to a complete regression of all detectable tumors, both in the brain and spine, after six infusions (11). The patient experienced a significant improvement in quality of life, including the discontinuation of steroid medications and the ability to return to work (11).

This clinical response persisted for 7.5 months, after which the patient experienced tumor recurrence at new sites (11). Further investigation revealed that these new tumors showed decreased IL13Rα2 expression, potentially explaining the treatment's eventual failure (11). Thus, although general CAR T cell therapy shows notable improvement in the quality of life, research is needed to improve long-term outcomes.

SynNotch CAR T-cells: creating increased specificity

Synthetic Notch, also known as SynNotch, is a technique used to regulate and enhance the specificity of CAR expression in CAR T cell treatment (Figure 2) (12).

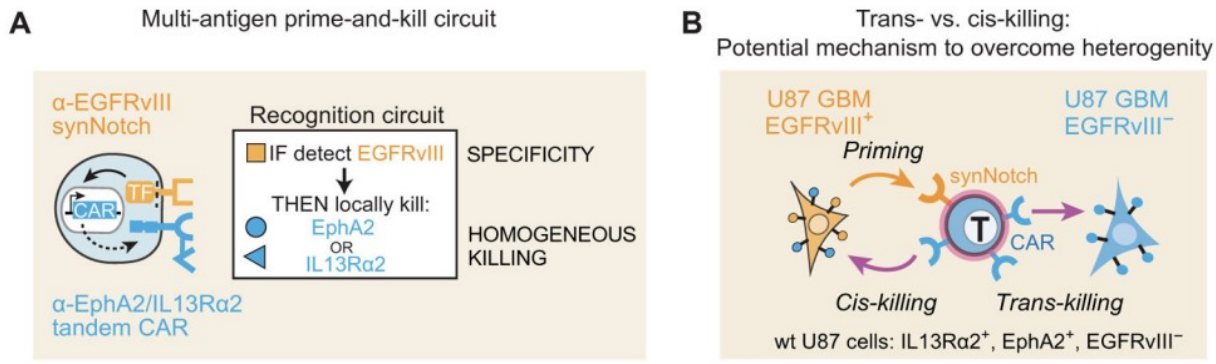

Figure 2. A. The synNotch receptor design triggers the expression of a tandem α-EphA2/IL13Rα2 CAR (Chimeric Antigen Receptor) upon encountering the EGFRvIII neoantigen. This means the engineered T-cells with this receptor will only be activated to eliminate target cells expressing EphA2 or IL13Rα2 if they have been previously exposed to cells carrying the EGFRvIII antigen (6) **B.** This system holds the potential to address the challenge of antigen heterogeneity in tumors. Specifically, it allows for "trans-killing," where the antigen required for initial T cell priming and the antigen present on the target cells designated for killing can be expressed on separate but adjacent cells (6). This figure was derived from Figure 1 in Choe et al. 2021 (6).

A synthetic receptor expressed by SynNotch CAR T-cells detects a priming antigen and thereafter triggers a transcription factor (12). Consequently, the development of a CAR that targets a different, "killing" antigen promotes this triggering (Figure 2) (12). The SynNotch CAR T-cells are only able to destroy target cells that express the killing antigen and the

priming antigens as a result of this mechanism. This is analogous to a Boolean "AND" gate, which only produces an output when two conditions are met (6,12).

Diverse glioblastoma tumors were targeted by Choe et al. (6) using SynNotch CAR T-cells that had been engineered with a priming receptor against EGFRvIII as well as a killing CAR against IL13Rα2 and EphA2 (12). The method used was especially specific because it only killed target cells *in vitro* when no less than 10% of them expressed EGFRvIII. In murine studies, constitutively expressed EGFRvIII CAR T cell therapy caused tumor recurrence, whereas the SynNotch system produced total tumor control and remission (12). Subsequent studies found that the SynNotch CAR T cell promoted a stem central memory phenotype in T-cells and decreased the expression of exhaustion markers on T-cells (12).

These traits let SynNotch CAR T-cells target heterogeneous tumors more effectively and have stronger anticancer effects (12). The studies found that the antigens EphA2 and IL13Rα2 on GBM could be detected by a CAR (6). This system was then primed with either a normal CNS-specific antigen or a tumor-specific but heterogeneous neoantigen (EGFRvIII) (6).

The continuous expression of CARs is a prerequisite for traditional CAR T-cell therapy, which has various limitations for treating solid tumors like GBM. On the other hand, SynNotch CAR T-cells use an inducible CAR and a SynNotch receptor to control the production of CARs (6). Because of antigen heterogeneity, using the conventional type of CAR T-cell to target individual antigens may cause tumor escape, which is when cancer cells "escape" the treatment, in this case, because they are too diverse to be targeted by one specific antigen receptor (6,12). However, the receptors on SynNotch CAR T-cells are capable of identifying a particular priming antigen, such as the tumor-specific EGFRvIII (6). This identification triggers the expression of a CAR that targets a second, more widely expressed antigen, such as IL13Rα2 or EphA2, by activating a transcription factor (6). SynNotch CAR T-cells are capable of "trans-killing," which is the process of priming one cell expressing the priming antigen (EGFRvIII+) to kill nearby cells that display just the killing antigen (such as EphA2+ or IL13Rα2+), thereby addressing the challenge of tumor antigen diversity (6).

Choe et al. (6) discovered that SynNotch CAR T-cells engineered with an EGFRvIII - targeting priming receptor and an IL13Rα2 and EphA2 - targeting killing CAR were extremely effective in targeting diverse GBM tumors (6). As discussed earlier, this system only eliminated target cells *in vitro* if at least 10% displayed EGFRvIII, emphasizing its high specificity (12). The SynNotch system also brought about the complete control of the tumor and remission in murine models, different from the EGFRvIII CAR T-cell therapy that resulted in tumor recurrence, thereby highlighting the SynNotch CAR T-cell

therapy's potential for the future of treating GBM (12).

Armored CAR T-cell therapy: enhancing T-cell function

Armored CAR T-cells have significantly advanced CAR T-cell therapy because they address the limitations that general CAR T-cells face in treating solid tumors. General CAR T-cells have shown substantial effectiveness in treating hematologic malignancies; however, their effectiveness in solid tumors is compromised by GBM's immunosuppressive tumor microenvironment (TME) (13,14). Armored CAR T-cells, on the other hand, are modified further to function even within the immunosuppressive TME (14).

Armored CAR T-cells can be engineered to secrete cytokines. Modifying CAR T-cells to secrete certain cytokines can change the TME and make it more receptive to these immunotherapies. IL-12, for example, is a cytokine that increases cytotoxic activity in CD8+ T-cells, significantly boosting the immune system (13,14). In preclinical models (12,15,16), CAR T-cells that secrete IL-12 were shown to have improved proliferation, lowered apoptosis, and increased the expression of the IL-2 receptor on CD8+ cells, thereby increasing their ability to kill tumor cells (13–17). These results emphasize the ability of these engineered CAR T-cells to significantly increase efficacy in such environments (13). Additionally, by using and activating macrophages, increasing antigen cross-presentation, and reprogramming myeloid-derived suppressor cells (MDSCs), IL-12 has been shown to help overcome tumor escape (13,14). Koneru et al. (18) also demonstrated the efficacy of engineering CAR T-cells to secrete cytokines by showing that IL-12 produced by CAR T-cells in an ovarian cancer murine model eradicated disseminated disease (13,18). Similar to IL-12, IL-18 can also cause an immune response against tumors (14).

Importantly, a study comparing IL-12 and IL-18 in TCR transfer therapy, wherein the tumors received regressive treatment, did not induce toxicities that were observed with IL-12, which thus makes IL-18 a safer option to consider upon infusion into patients (14). In summary, IL-12 is recognized for its ability to enhance the cytotoxic capabilities of CD8+ cells, impede tumor evasion by engaging macrophages, and reprogram MDSCs (13,14). Similarly, IL-18 is noted for its capacity to enhance the cytotoxic function of CAR T-cells, particularly against solid tumors (14).

IL-15 proliferates and enhances the cytotoxic capabilities of CD8+ T-cells and natural killer (NK) cells, both being critical components of antitumor immunity (14). IL-15 was shown to improve the anti-tumor functionality of adoptively transferred CD8+ tumor-reactive T-cells (14). Engineering CAR T-cells to secrete IL-15 resulted in enhanced tumor cytotoxicity and expansion compared to CAR T-cells lacking IL-15 secretion (14). Notably, IL-15 represents a cytokine previously described to promote the persistence of CAR T-cells through stimulation of expansion of tumor-reactive CD8+ T-cells and also by contributing

to homeostasis of certain subsets of CD8+ T-cells (14).

IL-7 plays a crucial role in the survival and function of T-cells (14). In the context of CAR T-cell therapy, IL-7 has an advantage over IL-2, which is commonly used to support T-cell function. While IL-2 can also promote the survival of regulatory T-cells (Tregs) that suppress immune responses, IL-7 selectively supports the expansion and function of CAR T-cells without enhancing Treg activity (19). This selective support makes IL-7 a potentially important cytokine in the improvement of CAR T-cell therapy, especially in solid tumors, where the immunosuppressive environment is maintained by Tregs (14,19). Furthermore, IL-7 has also become an essential factor to counteract the suppressive action of TGF- β, a cytokine implicated both in impeding T-cell differentiation and in promoting regulatory T-cell development (14,19).

Another approach to engineering armored CAR T-cells involves equipping them to secrete antibody-like proteins, such as T-cell–engaging antibody molecules (TEAMs) (19). The primary focus for glioblastoma treatment with armored CAR T cell therapy centers on the application of CARv3-TEAM-E T-cells (19). TEAMs can simultaneously bind to a tumor antigen and a T-cell activating receptor, facilitating the destruction of tumor cells by T-cells (19). For instance, CARv3-TEAM-E T-cells were designed to target EGFRvIII through a CAR and secrete TEAMs against wild-type EGFR (19). Wild-type EGFR is not expressed in the normal brain but is nearly always expressed in glioblastoma. This simultaneous targeting of both EGFRvIII and wild-type EGFR led to significant tumor regression in patients with recurrent glioblastoma (19). The secreted TEAMs work locally at the tumor site, redirecting T-cells and even regulatory T-cells, which normally suppress the immune response, against the tumor (19). This dual-targeting capability is a key advantage of armored CAR T-cells in addressing tumor heterogeneity and potentially leading to better treatment outcomes. Therefore, armored CAR T-cells represent a method for treating GBM that overcomes the challenges pertaining to the tumor microenvironment by secreting cytokines.

Discussion

There are different types of CAR T cell therapy available for various cancers, some being more effective than others. CAR T-cell therapy has proven successful against certain hematological malignancies, but translation to treating solid tumors, including glioblastoma, largely remains curtailed by tumor-specific challenges. These include heterogeneity in antigen expression among tumor cells, a highly immunosuppressive tumor microenvironment, and the potential for toxicity to healthy tissues expressing the target antigen (7). General, SynNotch, and Armored CAR T-cells, each have their benefits and limitations.

General CAR T-cell therapy

General CAR T-cell therapy is the foundational type of CAR T-cell therapy. It involves engineering T-cells to express a CAR that targets a single tumor-associated antigen

(12,14,20). This strategy exploits the ability of the engineered CAR to recognize target antigens independently of the major histocompatibility complex, thereby triggering strong T-cell activation and subsequent tumor cell destruction (12,20). While this approach is effective against certain blood cancers, it faces challenges when applied to solid tumors, including tumor cells which escape the immune response by downregulating the expression of the targeted antigen, limited migration of the CAR T-cells to the tumor site, and difficulty penetrating the solid tumor mass (6,20). Although this approach has been effective for some blood cancers, for solid tumors like GBM, successes are fewer (7). General CAR T-cell therapy is limited in its specificity because it only targets a single antigen; therefore, it can also destroy non-cancerous tissue (6,12). Target antigens are often selected for CAR T-cell therapy because they are usually overexpressed on tumor cells; however, these same antigens can also be expressed; albeit to a lesser extent, on healthy cells (20). For instance, a clinical trial by Rutkowska et al. targeted EGFRvIII in glioblastoma but showed limited success, partially due to EGFRvIII's heterogeneous expression in the tumor; this heterogeneity not only increased the chance of tumor escape but also risked potential harm to healthy cells expressing EGFRvIII (8,21). A clinical trial by Morgan et al., targeting other tumor-associated antigens HER2/neu and EphA2, which were overexpressed in glioblastoma, also resulted in poor outcomes because the antigens were also expressed on healthy cells (8,22). These examples demonstrate general CAR T-cell therapy's inability to differentiate between cancerous and healthy cells expressing the same target antigen, which leads to on-target off-tumor toxicity.

SynNotch CAR T-cell therapy

SynNotch CAR T-cell therapy offers a more advanced strategy to address the limitations of general CAR T-cell therapy. By relying on a two-antigen recognition system, rather than the single target antigen used in general CAR T-cell therapy, SynNotch receptors trigger a response only when two specific antigens are present (6,9,12). This dual-antigen recognition approach significantly enhances specificity and lessens the likelihood of CAR T-cells attacking healthy tissues that may express one of the target antigens, a significant limitation posed by SynNotch's general counterpart (6,12). Glioblastoma, which often has heterogeneous antigens in both healthy and cancerous tissues, may substantial benefit from such an approach (6,9,12). By requiring the recognition of two antigens, SynNotch CAR T-cells can target tumor cells more precisely, which minimizes the damage done to the surrounding healthy brain tissue (6). Additionally, because SynNotch CAR T-cells are engineered to recognize two different antigens expressed on the tumor cells, it is harder for the tumor to escape by downregulating a single antigen (6). However, SynNotch CAR T-cell therapy is a relatively new development, hence further research and clinical trials are needed to evaluate its long-term safety and efficacy in humans.

Armored CAR T-cell therapy

Armored CAR T-cell therapy represents an effort to augment the function of the immune system through the use of CAR T-cells in highly immunosuppressive tumor microenvironments (14). The tumor microenvironment is capable of suppressing the action of immune cells, including CAR T-cells (14). Armored CAR T-cells are redesigned to overcome this suppression by secreting cytokines, expressing cytokine receptors, and releasing antibody-like proteins that neutralize the immunosuppressive factors within the tumor microenvironment (4,14). Certain armored CAR T-cells are designed to produce cytokines, including IL-12, to enhance the activities of other immune cells, developing a pro-inflammatory environment that enhances anti-tumor response (4,13,14). Armored CAR T-cell therapy is specifically tailored to improve survival and function by placing CAR T-cells in the unfavorable tumor microenvironment. These armored CAR T-cells can overcome such limitations imposed by the tumor microenvironment through the secretion of cytokines that stimulate anti-tumor immune responses, expression of cytokine receptors that confer reduced sensitivity to immunosuppressive signals, or secretion of antibody-like proteins with the capability to neutralize suppressive factors (13). Nevertheless, similar to general CAR T-cell therapy, armored CAR T-cell therapy may cause cytokine release syndrome and neurotoxicity because of the potential to overactive the immune system. Such overactivation may cause inflammation, hence leading to irreversible destruction of various organs (8). Therefore, more research about overcoming these limitations is required.

SynNotch CAR T-cells: enhanced specificity and potential in Glioblastoma therapy

Among these approaches, SynNotch CAR T-cell therapy appears particularly promising for treating glioblastoma due to its capacity to address several key challenges associated with this disease. Unlike general CAR T-cell therapy and armored CAR T-cell therapy, which usually only target a single antigen, making them ineffective against tumor escape mechanisms, SynNotch CAR T-cells can be designed to recognize two different antigens expressed on the surface of GBM cells (6). By incorporating an "AND" gate, where T-cells only become activated after recognizing both a tumor antigen and a glioblastoma-associated antigen, SynNotch CAR T-cells show enhanced specificity compared to general or armored CAR T-cell therapies because they offer a solution to tumor heterogeneity, which is a significant obstacle in glioblastoma treatment (6). This improved specificity creates a lower risk of off-target toxicity, which is a critical factor when considering treatments for a disease like glioblastoma, where maintaining healthy brain tissue is crucial (6). In addition to increased specificity and the ability to overcome tumor heterogeneity, SynNotch CAR T-cells also exhibit less exhaustion (6,12). This persistence within the tumor microenvironment is necessary for long-term disease control (12). Because SynNotch CAR T-cells are designed to remain longer in the tumor associated environment than their general counterparts, long-lasting responses in patients with

glioblastoma are also more likely (12). While challenges remain in taking this technology to clinical trials, SynNotch CAR T-cell therapy shows a significant advancement in immunotherapy.

Elapsed Time Circuits: A Novel Approach

Although SynNotch CAR T-cell therapy effectively overcomes tumor heterogeneity, it still fails to address the diversity of tumor cells in that it cannot adapt to the different number and/or types of antigens expressed in different tumor locations or on different tumor cells.

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 (i.e. number of expressed antigen receptors) based on the locations of tumor cells and the cells' expression of varying antigen numbers and/or combinations (Figure 3) (24). Specifically, CAR T-cells could be programmed (as an example) as a circuit that would go through trivalent ("AND-AND"), divalent ("AND"), and monovalent targeting modes (25). The "time-elapsed" segment would clock 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 is less effective because it 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 and adapt *in vivo* in real-time 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 diverse 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 kill a broader range of tumor 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 useful for the future of GBM treatment. It is not currently known if such time-monitoring gene circuits exist, and whether they can be harnessed.

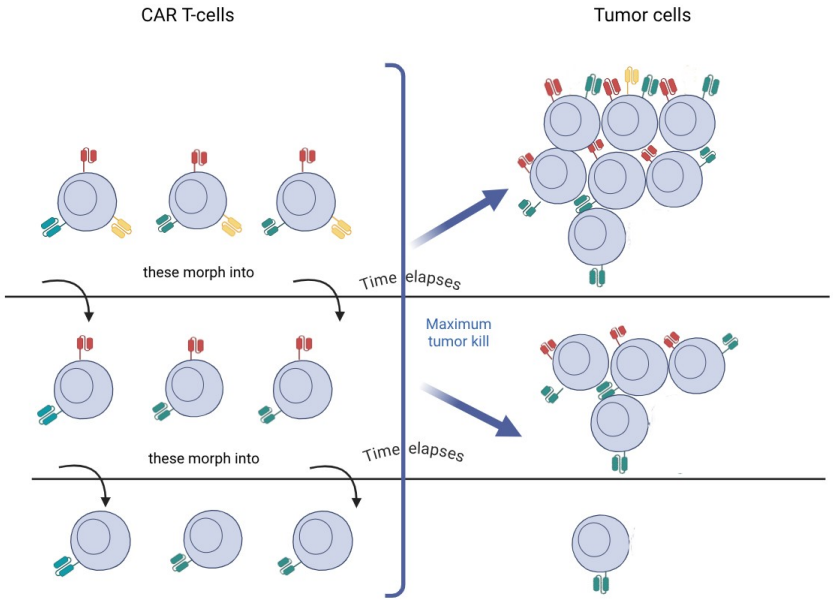

Figure 3. This type of CAR T-cell would effectively maximize the tumor cells killed by morphing from expressing (for example) three to two antigens because the tumor in this case maximally expresses two antigens. The three to two antigen morphing occurs because the third (yellow) antigen's elapsed time circuit is triggered based on no antigen engagement for a long time. For the same reason, the two antigen CAR-T cell would experience a slow conversion into a one-antigen (green only) cell because both the red and green antigen receptors circuits are not triggered (they are engaged with the tumor cell antigens). This allows for differing levels of specificity based on the location of the tumor and the prevalence of the specific antigen combinations. The elapsed time circuit can also work in reverse; i.e. express another antigen if that antigen's circuit is not triggered for a long time. The key is to discover such 'elapsed time' genetic circuits.

Conclusion

Glioblastoma multiforme is an aggressive form of brain cancer with high recurrence rates. It is a significant challenge to treat and hence needs innovative strategies for treatment. Chimeric Antigen Receptor T-cell therapy, is an immunotherapy that has demonstrated success in treating certain blood cancers and is being explored as a potential treatment for solid tumors like GBM. General, SynNotch, and armored CAR T-cells are the various mechanisms explored in this paper by contrasting their advantages, and limitations. The SynNotch CAR T-cells improve the targeting, activation, and longevity of T cells fighting glioblastoma, which could lead to a broadly applicable strategy for treating other solid tumors. However, further research is needed to evaluate their long-term safety and efficacy in humans. The "elapsed time" circuit represents a new approach to refine CAR T-cell therapy for GBM or for solid tumors in general.

It prevents the CAR-T cell from being stuck in one, ineffective targeting mode because that antigen combination is infrequent in those particular cancer cells. Similar to SynNotch CAR T-cells, this method would target multiple antigens, it would improve specificity; with its different stages of specificity, it would be able to change *in vivo* so as to able to kill to a broader range of cells. This means that the 'elapsed time' CAR T-cells are more likely to be able to find and kill tumor cells, even if the tumor cells are heterogeneous.

References

1. Mahmoud AB, Ajina R, Aref S, Darwish M, Alsayb M, Taher M, et al. Advances in immunotherapy for glioblastoma multiforme. Front Immunol. 2022, 13:944452. https://doi.org/10.3389/fimmu.2022.944452

2. Shen SH, Woroniecka K, Barbour AB, Fecci PE, Sanchez-Perez L, Sampson JH. CAR T cells and checkpoint inhibition for the treatment of glioblastoma. Expert Opin Biol Ther. 2020, (6):579–91. https://doi.org/10.1080/14712598.2020.1727436

3. Sun L, Su Y, Jiao A, Wang X, Zhang B. T cells in health and disease. Signal Transduct Target Ther. 2023, 8(1):235. https://doi.org/10.1038/s41392-023-01471-y

4. Ma S, Li X, Wang X, Cheng L, Li Z, Zhang C, et al. Current Progress in CAR-T Cell Therapy for Solid Tumors. Int J Biol Sci. 2019, 15(12):2548–60. https://doi.org/10.7150/ijbs.34213

5. Yasinjan F, Xing Y, Geng H, Guo R, Yang L, Liu Z, et al. Immunotherapy: a promising approach for glioma treatment. Front Immunol. 2023, 14:1255611. https://doi.org/10.3389/fimmu.2023.1255611

6. Choe JH, Watchmaker PB, Simic MS, Gilbert RD, Li AW, Krasnow NA, et al. SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma. Sci Transl Med. 2021, 13(591):eabe7378. https://doi.org/10.1126/scitranslmed.abe7378

7. Dapash M, Hou D, Castro B, Lee-Chang C, Lesniak MS. The Interplay between Glioblastoma and Its Microenvironment. Cells. 2021, 10(9):2257. https://doi.org/10.3390/cells10092257

8. Maggs L, Cattaneo G, Dal AE, Moghaddam AS, Ferrone S. CAR T Cell-Based Immunotherapy for the Treatment of Glioblastoma. Front Neurosci. 2021, 15:662064. https://doi.org/10.3389/fnins.2021.662064

9. Ma K, Hu P. Chimeric Antigen Receptor T-Cell Therapy for Glioblastoma. Cancers. 2023, 15(23):5652. https://doi.org/10.3390/cancers15235652

10. Li L, Zhu X, Qian Y, Yuan X, Ding Y, Hu D, et al. Chimeric Antigen Receptor T-Cell Therapy in Glioblastoma: Current and Future. Front Immunol. 2020, 11:594271. https://doi.org/10.3389/fimmu.2020.594271

11. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N Engl J Med. 2016, 375(26):2561–9. https://doi.org/10.1056/nejmoa1610497

12. Zhang J, Siller-Farfán JA. Current and future perspectives of chimeric antigen receptors against glioblastoma. Immunother Adv. 2022, 2(1):ltac014. https://doi.org/10.1093/immadv/ltac014

13. Yeku OO, Purdon TJ, Koneru M, Spriggs D, Brentjens RJ. Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. Sci Rep. 2017, 7(1):10541. https://doi.org/10.1038/s41598-017-10940-8

14. Hawkins ER, D'Souza RR, Klampatsa A. Armored CAR T-Cells: The Next Chapter in T-Cell Cancer Immunotherapy. Biol Targets Ther. 2021, 15:95–105. https://doi.org/10.2147/btt.s291768

15. Wilkie S, Picco G, Foster J, Davies DM, Julien S, Cooper L, et al. Retargeting of Human T Cells to Tumor-Associated MUC1: The Evolution of a Chimeric Antigen Receptor. J Immunol. 2008, 180(7):4901–9. https://doi.org/10.4049/jimmunol.180.7.4901

16. Zhao J, Zhao J, Perlman S. Differential Effects of IL-12 on Tregs and Non-Treg T Cells: Roles of IFN-γ, IL-2 and IL-2R. Unutmaz D, editor. PLoS ONE. 2012, 7(9):e46241. https://doi.org/10.1371/journal.pone.0046241

17. Zhong XS, Matsushita M, Plotkin J, Riviere I, Sadelain M. Chimeric Antigen Receptors Combining 4-1BB and CD28 Signaling Domains Augment PI3kinase/AKT/Bcl-XL Activation and CD8+ T Cell–mediated Tumor Eradication. Mol Ther. 2010, (2):413–20. https://doi.org/10.1038/mt.2009.210

18. Koneru M, Purdon TJ, Spriggs D, Koneru S, Brentjens RJ. IL-12 secreting tumor-targeted

chimeric antigen receptor T cells eradicate ovarian tumors *in vivo*. OncoImmunology. 2015, 4(3):e994446. https://doi.org/10.4161/2162402X.2014.994446

19. Choi BD, Gerstner ER, Frigault MJ, Leick MB, Mount CW, Balaj L, et al. Intraventricular CARv3-TEAM-E T Cells in Recurrent Glioblastoma. N Engl J Med. 2024, 390(14):1290–8. https://doi.org/10.1056/nejmoa2314390

20. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 2021, 11(4):69. https://doi.org/10.1038/s41408-021-00459-7

21. Rutkowska A, Stoczyńska-Fidelus E, Janik K, Włodarczyk A, Rieske P. EGFR^{vIII}: An Oncogene with Ambiguous Role. J Oncol. 2019, 16:1–20. https://doi.org/10.1155/2019/1092587

22. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing ERBB2. Mol Ther. 2010, 18(4):843–51. https://doi.org/10.1038/mt.2010.24

23. Nobili A, Kobayashi A, Gedeon PC, Novina CD. Clutch Control: Changing the Speed and Direction of CAR-T Cell Therapy. J Cancer Immunol. 2022, 4(2). https://doi.org/10.33696/cancerimmunol.4.066

24. Singh V. Recent advances and opportunities in synthetic logic gates engineering in living cells. Syst Synth Biol. 2014, 8(4):271–82. https://doi.org/10.1007/s11693-014-9154-6

25. Han X, Wang Y, Wei J, Han W. Multi-antigen-targeted chimeric antigen receptor T cells for cancer therapy. J Hematol OncolJ Hematol Oncol. 2019, 12(1):128. https://doi.org/10.1186/s13045-019-0813-7

26. Wang B, Kitney RI, Joly N, Buck M. Engineering modular and orthogonal genetic logic gates for robust digital-like synthetic biology. Nat Commun. 2011, 2(1):508. https://doi.org/10.1038/ncomms1516