CAR T-Cell Therapy: A Promising Alternative to Traditional Glioblastoma Treatment

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Glioblastomas (GBM) are a rare and difficult cancer to treat with an incidence rate less than 4 per 100,000 people in the US. Although the current treatments are the most effective ones in our arsenal, surgical intervention and chemotherapies carry significant burden on patients both in terms of post-operative care and the myriad of side effects from different types of therapeutic drugs. Chimeric antigen receptor T-cell (CAR-T) therapy has become a promising alternative treatment through multiple successful clinical trials on haematological cancers improving the median survival rates of lymphoma patients. It has also made huge advancements, both increasing its efficacy and reducing side effects. With the difficulties in location, rarity and survival rates of GBM patients, providing research on novel treatment options can expand the effective treatment options available for patient survival and morale. CAR-T cell therapy has shown to have an ever-improving track record and carries the potential to support the move from current GBM treatments to CAR-T being the standard.

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Introduction

Glioblastoma (GBM) is the most aggressive form of diffuse glioma of astrocytic lineage and is classified as a grade IV glioma based on the World Health Organization (WHO) classification, the most abnormal and aggressive classification based on microscopy. Although GBM is the most common malignant primary tumour consisting of 54% of all gliomas and 16% of all primary brain tumours, it remains as an incurable tumour with a median survival of only 15 months.[1] The most common treatment starts with surgical resection limited by safety parameters, followed by radiation therapy and concurrent chemotherapy.[2] Current standard GBM therapies brings substantial common side effects like nausea, vomiting, fatigue which limits the dosage of chemotherapeutic agents leading to both worse patient prognosis and quality of life. Considering the complexity of intracranial surgery and the side effects from concurrent chemotherapy, alternatives with lesser burdens to the patient are being explored.

Cancers develop as the result of cell proliferation no longer being controlled by regulatory genes, internal mechanisms, or immune cells. The fundamental problem that hinders the ability of the immune system from preventing the growth of cancer stems from the poor immunogenic nature of cancerous cells, being that most tumour-associated antigens are derived from self. As the main mechanism of immune detection is through cell surface receptors, and since such receptors are almost indistinguishable from normal cells, this leads to an inability of immune cells to identifying tumour cells among healthy cells in the hostile tumour microenvironment. Where the immune cells specifically tasked to detect and remove cancerous cells, such as natural killer and T cells, fall short, CAR-T cells are specifically designed to improve on these limitations.[3]

CAR-T cell therapy shifts the treatment modality from the disease to the host, taking advantage of the human immune system. The therapy involves encoding an artificial molecule that re-programs an antigen receptor of peripheral blood polyclonal T-cells against a predetermined cell surface target. Chimeric antigen receptors (CARs) are expressed fusion molecules that couple the binding of a native target, tumour cell surface proteins, to a predefined T-cell activating signal.[4] These engineered receptors allow the grafting of a specified specificity onto an effector cell (T cell). This method allows polyclonal T-lymphocytes to have antibody-like specificity, circumventing most immune evasion mechanisms employed by a multitude of tumours. With such high specificity and activation towards malignant targets, CAR-T cell therapy has seen remarkable success in treating select haematological malignancies. However, unlike a liquid tumour like in blood cancers, GBM poses immense challenge due to its extremely immunosuppressive tumour environment, the sensitive nature of the brain and the need to cross the blood brain barrier results in a high level of difficulty of treating GBM with CAR-T until now, where advances in CAR-T allowed for circumventions for these challenges.

Use of CAR-T in clinical settings

CAR T cell therapy targeting CD19 has shown many successes in the treatment of B-cell lymphomas.[5] It has shown remarkable success in contrast to the overall response rate of ∼20-30% with median survival of 6 months for patients. This is the next line of treatment for patients deemed unfit for transplant or with relapse after auto transplant and the main approach of chemotherapy such as the rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) regimen. The study ZUMA-1 enrolling 111 patients with diffuse large B-cell lymphoma undergoing CAR-T cell therapy reported an objective response rate (ORR) of 82% and a complete response (CR) rate of 54%. An objective response rate refers to the patient successfully reaching the study's target percentage of tumour cells responding to therapy while a complete response rate refers to all tumour cells responding to therapy. When compared to the 20-30% ORR of alternative regimens, CAR T-cell therapy demonstrates significant success. More importantly, there were no differences in response rates between subgroups of patients predicted to be responsive or less responsive to the treatment, making this an effective method for even severe and difficult tumors.[6]

JULIET was an international, phase 2 study, used CAR-T cell therapy to treat adults with relapsed or refractory diffuse large B-cell lymphoma. This study consisted of 93 patients and showed a 52% ORR.[7] A sub-analysis of JULIET also showed patients who achieved CR sustained a health-related quality of life improvement in all aspects of the Functional Assessment of Cancer Therapy and showed improvement on 5 of 8 subscales in the Short Form-36 (SF-36, a set of generic, coherent ,and easy to administer quality-of-life measures[8]) health survey.

Both phase 2 clinical trials show promising results in the use of CAR T-cell therapy, having greater efficacy than pre-existing treatment regimens and improving the quality of life of relapsed patients.

Limitations of traditional therapies

Tumours of the central nervous systems (CNS), which GBM fall under, are responsible for almost 300,000 deaths per year between 1990 and 2016. It is also one of the most difficult cancers to treat due to its proximity to vital organs and tissue. The most common treatments for brain tumours are surgery, chemotherapy, and radiation therapy.[9] Recent studies have shown cognitive changes induced by the use of chemotherapeutic drugs, raising increasing concern among cancer survivors about the cost of this treatment in quality of life. Animal studies have demonstrated a multitude of mechanisms of chemotherapy causing irreversible brain damage, such as the reduction of formation of hippocampal neurons, direct oxidative damage to tissues and cells due to reactive oxygen species formed from chemotherapeutic drugs, myelinated neurons being irreversibly damaged, and reduced brain vascularization and blood flow, where fewer blood vessels form in the brain resulting in decrease in blood flow. All of these suggest a direct impact on cognitive abilities of patients after receiving chemotherapy. The data suggests a post-treatment cognitive rate of decline much higher than within the context of factors that influence the trajectory of normal aging. Tamoxifen, a commonly used chemotherapy drug, is one of the examples that has been shown to be genotoxic and may be associated with increased DNA damage and the shortening of telomeres.[10] This process has implicated a cognitive decline and the development of neurodegenerative diseases.

Radiotherapy is another commonly used treatment modality in all types of cancer, with approximately 60% of solid tumour patients receiving irradiation. As the target area for irradiation can only be focused so small, there are often adverse effects on neighbouring cells. Recent studies have also shown a systemic impact leading to secondary effects other than tissue necrosis and apoptosis.[11] The damage to nearby vasculature within the irradiated field can result in a condition coined radiation fibrosis syndrome. In radiation fibrosis syndrome, the first phase consists of the chronic inflammation of endothelial cells. This is followed by a phase of patchy areas of increased rates of fibrosis leading to high density myofibroblasts. The third phase is the fibroatrophic phase, which is characterized by the loss of parenchymal cells. This injury to the vascular endothelium is thought to play a role in the development of this condition, such that excessive fibrin accumulates in the intravascular, perivascular, and extravascular compartments. Radiation increases chronic free radical production and oxidative stress in treated tissues, upregulating numerous pathways significant to vascular disease.[12] These mechanisms play an important role in post-remission care and is one of the biggest challenges patients have to face.

Advancements in CAR-T

CAR Activation and Inactivation

Despite promising results on B cell malignancies, therapy-related severe adverse events such as cytokine release syndrome and neurotoxicity are common and possibly fatal.[13] Cytokine release syndrome is caused by a combination of activation of CAR T-cells and proliferation. Both activation and proliferation of CAR T-cells cause the release of cytokines due to the rapid clonal expansion where these cytokines signal the activation and proliferation of other non-CAR immune cells triggering a cascade leading to systemic inflammation. Such events could cause severe supraphysiologic cytokine production and massive in vivo T cell expansion and/or macrophage activation syndrome due to off-target tumour

toxicity in 23-46% of patients.[14] Off-target tumour toxicity should be avoided at all costs in the case of brain tumours as even the most minute off-target response could cause permanent brain damage. A proposed solution to this is the regulation of CAR expression. Building upon the promising results of using hepatitis C virus NS3 protease (NS3p) and protease inhibitors to regulate diverse proteins, a team of researchers attempted at coexpressing the NS3 protease cleavage site and the NS3 protein and thereby cleaving the CARs at baseline rendering it inactive. Upon exposure to grazoprevir, an NS3p inhibitor, cleavage would be prevented and the CAR will remain functional. By integrating the NS3 protein between the single-chain variable fragment and the $CD8\alpha$ transmembrane domain, a proteolytic control of the CAR is achieved. A dose-responsive relationship was also observed between grazoprevir and interleukin-2 (IL-2) secretions. Through the use of protease and its inhibitor, the researchers were able to activate and deactivate the CAR through the use of proteases. The significance of this is both the activity of CAR-T cells and secretion of cytokines can be manipulated through controlling the dose of grazoprevir. The ability to activate and deactivate CAR-T cells with a moderate time delay (24hr) increases the safety and reduces the risk of permanent damage through close monitoring of cytokine release and cellular activity and the subsequent manipulation of protease inhibitor dose.[13] Another study showed an ON-switch system based on human retinol binding protein 4 (hRBP4), activated by a synthetic hRBP4-specific drug A1120 that triggers dissociation of hRBP4 from its carrier protein.[15] Through continued research into this area, more mechanisms would be discovered as to how the expression of CAR can be controlled with less time delay, improving treatment safety for patients. As this method of regulating the activation of CAR-T therapy is only tested in preclinical trials and mouse models, they are still needed to be further investigated in human clinical trials.

CAR-T cell therapy against solid tumour

In addition to being enclosed in a highly sensitive organ, GBM are also a type of carcinoma unlike haematological cancers. They are a subtype of cancer classified as solid tumours. Responses to solid tumours for CAR are often minimal, potentially due to the lack of sustained stimulation and proliferation of CAR T cells when binding to antigens in an extremely immunosuppressive tumour microenvironment. This is the exact opposite to the ability of antigen presenting cells (APCs) capable of providing T cells with strong stimulation in liquid tumours. Due to such conditions, significant grafting does not always occur. It is the initial interaction between T cell receptors (TCR) and major histocompatibility complex (MHC) that first signals the activation starts differentiation programs. In the case of solid tumours, most cell surface antigens are not expressed by APC, therefore the CAR T cells may not be able to fully engage with APCs in most solid cancers. A study has shown an increase in CAR T cell proliferation and effector function in response to enterotoxins. The study in question used staphylococcal enterotoxin B (SEB) to enhance CAR T cell antitumor activity. The interaction between SEB and TCR with a second-generation cytoplasmic domain composed of CD3 and CD28 signalling molecules led to significantly increased proliferation of CAR T cells. The results demonstrated SEB-stimulated CAR T cells secreted interferon-γ to a greater degree compared to the control. At the end of the mouse study, long-term surviving mice were also resistant to rechallenge with the tumours, suggesting immunological memory was established. This demonstrates the ability of the CAR T cells to continue fighting in the immunosuppressive tumour micro-environment without external support after initial exposure to enterotoxin and subsequent proliferation. The concept of CAR-T cell therapy was to allow the interaction of TCR and tumour cell-surface antigens independent of MHC haplotype. While this approach allows the successful interaction irrespective of MHC haplotype, it omits the interaction of T cells with APCs. The study used a strategy such that CAR T cells can still maintain the non-MHC dependency while allowing more APC interactions, increasing the likelihood of activation, differentiation and proliferation despite the profound immunosuppressive nature of the tumour micro-environment.[16]

CAR-T cell therapy and immune checkpoints

As CAR T cell therapy relies on the immune system to elicit a response against tumour cells, it is largely affected by the pathways tumours use to evade the immune system. A prominent problem in all cancer treatment is the ability of tumours to develop resistance against a certain treatment regimen over time, and CAR T cell therapy is no different. In recent years, passive cancer immunotherapies with monoclonal antibodies (mAbs) have gained more widespread use in treatment for a variety of carcinoma through the use of the antibody-dependent cellular cytotoxicity pathway and cellular phagocytosis, complement-dependent cytotoxicity, antibody-induced apoptosis and antibody-induced programmed cell death. Common types of immune checkpoint inhibitors are programmed cell death protein 1, programmed death-ligand 1, cytotoxic T lymphocyte-associated protein 4, all of which act on different pathways cancer cells use to suppress the immune system, enhancing the targeting and neutralisation of tumours. The passive administration of mAbs has proven to be clinically effective, indicating the potential of the combinational therapy of mAbs and CAR T cell therapy. The study showed an interesting ability of the FcγRIIIa-chimeric receptor (CD16-CR) to activate in engineered T cells only in the presence of mAbs opsonized target cells. This ability allows

for mAbs within specific therapeutic windows to maximize cytotoxic killing or to mitigate unwanted side effects. It is also important that these "cocktails" are also able to limit or reduce development of tumour resistance to therapy while at the same time increasing sensitivity, improving long-term effects of treatment in cancer patients.[17] Similar to the protease inhibitor approach, this use of mAbs allow a finer control over the proliferation and activity of CAR expressing cells, improving on efficacy and minimizing side effects based on tumour and patient response. However, it must also be noted immune-related adverse events (irAE) after the administration of anti-PD-1 or anti-PD-L1 is 66%, and with a grade 3 or higher in the Common Terminology Criteria for Adverse Events v5.0 of 14%.[18] A novel method is then used to include both benefits of CAR-T and immune checkpoint therapy. For example, using CRISPR/Cas9 to create PD-1 disrupted CAR-T cells prevents patients from severe irAE.[19] Using this method, CAR-T can be more safely combined with immune checkpoint therapy to bring a higher efficacy to this therapy.

The recent advancements CAR T-cell therapy highlighted above allowed for combinational therapies that would greatly benefit GBM. As noted in this review, GBM is a solid tumour with an extremely immunosuppressive tumour microenvironment located in a highly sensitive organ where we need to be extremely careful about inflammation and off-target responses. The potential synergies explained above can allow for a finer control over CAR expression and therefore the level of inflammation; the use of enterotoxins reduces the impact of the immunosuppressive nature of GBM; and the use of mAb in combination with CAR T-cells can increase its efficacy in such immunosuppressive environments.

Clinical trials with CAR T cells for GBM

Predominant clinical experience in CNS cancers is focused on GBM due to its lethality across all demographics.[20] There are 29 ongoing clinical studies as of early 2023 on CAR Immunotherapy in GBM.[21] Among them, 3 trials targeting 3 different molecules showed promising preliminary results.

Interleukin-13 receptor alpha 2 (*IL*13*R*α2)

This CAR therapy leverages the overexpression of antigen *IL*13*R*α2 in GBM but not in healthy brain tissue.[22] After preclinical mouse models showing *IL*13*R*α2 CAR T could lead to regression of adult glioma patient-derived xenografts, a current Phase I clinical trial is determining the effects of cellular immunotherapy in conjunction with chemotherapy in *IL*13*R*α2-positive recurrent or refractory (R/R) brain cancer in children21. Apart from this, encouraging results were published in an adult patient treated with autologous *IL*13*R*α2 CAR T as part of a clinical trial investigating the efficacy of *IL*13*R*α2 CAR T cells. This clinical trial also highlights a delivery method that could solve one of the most intricate problems in the adaptation of CAR T cell-based immunotherapy into CNS tumours by directly delivering CAR T cells into the tumour or into the ventricles of the brain via an intraventricular catheter.[21–23]

Disialoganglioside (GD2)

GD2 is a specific ganglioside with a more restricted neuroectodermal expression and is highly expressed in multiple cancers. This CAR T-cell therapy hinges on the high expression of GD2 in the select cancer types[20]. One such cancer is glioma cells with a H3K27M mutation on the gene coding for histone protein 3. In earlier mouse studies in a model that exhibit a dense pattern of growth, the control group with glioma is consistently lethal within one month, while the group infused with GD2-CAR plateaus at a percent survival of 75% after 13 days post-treatment.[24] With this promising preclinical study, a clinical trial was conducted for H3K27M-mutated diffuse midline glioma. The trial has shown elevated levels of cell-free mutant K27M allele containing DNA in cerebrospinal fluid in 50% of all patients 100 days after intravenous infusion showing tumoricidal activity. After further data analysis, the researchers found a strong correlation between the infusion of GD2-CAR cells and the elevated levels of cell-free DNA in cerebrospinal fluid, showing K27M allele containing cell lysis correlates to the infusion of GD2-CAR cells. This CAR T-cell therapy has doubled the average life expectancy of patients with glioma cells with the H3K27M mutation, showing promising results.[25]

Human epidermal growth factor receptor (HER)

The HER family has four transmembrane receptors with HER2 being the focus of this study. HER2 is already an established immunotherapeutic target in both breast and ovarian cancer. In paediatric GBM malignancies, HER2 is another receptor that is overexpressed.[20] Currently, HER2-CAR T cells are tested in clinical trials for children with brain tumours that is HER2+. These trials utilize either the same method of delivery in the *IL*13*R*α2 study of intraventricular delivery or a direct injection into the tumour resection cavity. The ongoing study has recently published an interim report, indicating promising results. The interim has confirmed the safety point of view of the treatment as it has shown the treatment is feasible without dose-limiting toxicity; it has also shown that the HER2-CAR T cells are able to activate local immune response. These two findings from the interim report have proven the safety and efficacy of the HER2-CAR T cell therapy and the possibility of progressing into a phase 2 clinical trial with a larger cohort.[26]

Discussion

Until recently, GBM were mainly treated with surgical resection, radiotherapy and chemotherapy. As outlined in this paper, all three types of treatment carry significant burden on both the patient and care provider of the patient due to the profound side effects of radiotherapy and chemotherapy as well as the stringent care required post-operation. CAR T-cell therapy has proven to be the next generation of all cancer treatment, with high targeting specificity, efficacy as well as greatly improved quality of life post-remission.

For the therapy to have any substantial effect, it must be able to pass the blood brain barrier (BBB). Because of this and the possible regiment-induced neuroinflammation, the development of this therapy for brain tumours is greatly hindered. However, it is also important to note a newly discovered EphA2-specific CAR T-cells can cross the BBB and proliferate into the tumour microenvironment.[27] The discovery of novel delivery methods like the intraventricular delivery method highlighted in this review and the EphA2-specific CAR T-cells proves CAR-T therapy can either bypass the BBB or pass through the BBB. A novel classification of CAR-T has also been demonstrated to be able to cross the BBB and penetrate the brain parenchyma. The strategies for enhancing such homing of T cells from blood to the brain include VLA-4 and CXCR10 expressions[28]. This leaves a lot of room for future development into delivery methods of CAR-T or even the engineering of CAR-T to be able to pass through the BBB.

With the advancements in reducing its side effects, the immunosuppressive nature of solid tumours through the use of enterotoxins, as well as novel delivery methods to bypass the BBB, together with successful Phase I clinical trials, the use of CAR should be more widely applied to GBM patients considering its invariable lethality. This technology allows healthcare providers to shift the paradigm of cancer treatment from a direct approach to utilizing the sophisticated immune system that has evolved over millions of years. Despite all the advancements in this treatment, there are still a lot of logistical challenges due to its multidisciplinary nature, carrying a toxicity profile not fully understood and requires specialised expertise. With its early successes, future research can be built onto what we already know about it, other mechanisms to control inflammation from CAR T-cell proliferation, novel delivery method based on the new classification of CAR, new synergistic combinational therapy and more. As revolutionary as this technology is, it does have its caveats. As this is a highly personalized treatment, the cost to engineer cells are extremely high, more research must be done on how to economically scale up the use of such technologies as well as how cost could be reduced to make this radically new technology accessible to all patients.

Conclusion

Recent advancements in CAR T-cell therapy have allowed it to be better equipped to combat GBM and reducing the most severe and prominent side effects. Studies have also shown why we want to steer away from traditional treatment regimens due to their profound side effects. Overall, the studies reviewed had shown promising results for CAR-T cell therapy being an effective tool in reducing patient mortality and improving patient quality of life. However, more research is needed to fully understand other possible side effects of such treatments when a larger sample is used, how healthcare providers can systematically scale up the number of patients treated with CAR T-cell therapy, as well as how cost could be reduced to make this technology accessible to all patients. Despite these limitations, evidence reviewed shows an ever-improving track record of CAR T-cell therapy and its potential to become a mainstream treatment option, providing a strong basis for further exploration in applying CAR T-cell therapy to GBM patients.

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