Acta Neurologica Indonesia 2025; 03(04)

Acta Neurologica Indonesia
Department of Neurology, Faculty of Medicine Universitas Indonesia
ISSN 3032-6303

Review Article

POTENTIAL UTILIZATION OF MODIFIED MULTITARGET CAR-T CELLS AS AN ADVANCED IMMUNOTHERAPY FOR GLIOBLASTOMA: A SYSTEMATIC REVIEW

Elysia Gita Pascadea[1], Diondra Eka Rizkiawan[1]

[1]Dharmais National Cancer Center, Jakarta, Indonesia; elysiagita@gmail.com

ABSTRACT

Introduction : Glioblastoma (GBM) is the most common and aggressive malignant brain tumor, characterized by high morbidity and mortality. Standard treatments—surgery, radiotherapy, and chemotherapy—are often ineffective due to tumor heterogeneity and recurrence. Immunotherapy, particularly chimeric antigen receptor T (CAR-T) cell therapy, has emerged as a promising alternative. This systematic review evaluates the potential of modified multitarget CAR-T cells in addressing GBM's challenges.

Material and methods : A systematic review was conducted following PRISMA 2020 guidelines. Searches in PubMed, ScienceDirect®, ProQuest, EBSCOhost®, SAGE®, ClinicalKey®, and Scopus focused on studies from the past decade. Data were analyzed to assess the efficacy, safety, and feasibility of multitarget CAR-T therapies. Of 1,512 identified studies, 11 met the inclusion criteria. Findings indicate multitarget CAR-T cells enhance tumor recognition and immune activation, reducing immune evasion. CAR-T designs targeting EGFRvIII, IL13Rα2, HER2, and GD2 exhibited improved cytotoxicity, eradicated tumors, demonstrated long-term memory response, and were able to achieve complete tumor clearance. Studies found that CAR-T cells therapy in GBM enhanced tumor suppression and prolonged survival in glioblastoma patients.

Discussion : Originally developed for hematologic malignancies, CAR-T therapy is expanding to GBM by targeting tumor-associated antigens (TAAs) like CEA, GPC-3, MUC1, VEGFR2, EGFRvIII, IL13Rα2, HER2, and GD2. However, challenges such as the immunosuppressive tumor microenvironment (TME), antigen loss, and limited tumor penetration hinder efficacy. Strategies like multitarget CAR-T cells, immune checkpoint inhibition, and localized delivery methods may improve outcomes.

Conclusion : CAR-T therapy holds potential for GBM treatment, but further research is required to enhance its effectiveness and clinical applicability.

Keywords: glioblastoma, CAR-T cells, immunotherapy

INTRODUCTION

To date, cancer remains a disease with high morbidity and mortality rates, making it a significant public health concern that requires serious attention. According to the World Health Organization (WHO) through the International Agency for Research on Cancer (IARC), it is estimated that in 2022, there were

approximately 20 million new cancer cases globally, with 9.7 million cancer-related deaths.[1] Data from Globocan 2022 reported over 321,731 cases of brain and central nervous system cancers, resulting in approximately 248,500 deaths, highlighting the high morbidity and mortality associated with these tumors.[2] Epidemiologically, glioblastoma is the most common malignant primary brain tumor worldwide, affecting approximately 3.19 per 100,000 individuals. It accounts for 54% of all gliomas and 16% of all primary brain tumors.[3, 4]

Although the 2023 Basic Health Research (Riskesdas) survey does not provide specific data on the incidence of glioblastoma in Indonesia, data from Dharmais Cancer Hospital between 2011 and 2015 indicated that glioblastoma incidence was 64% in males and 36% in females. Glioblastoma represents a serious and complex medical challenge, not only because it affects the brain but also due to its potential to cause systemic complications. These complications may range from personality and organic behavioral disorders to life-threatening conditions such as perforation, severe hemorrhage, and paralysis.[5, 6]

The treatment of glioblastoma is equally challenging. Surgical intervention and solid tumor resection, which are among the primary therapeutic options, often result in recurrence within five years in many glioblastoma cases.[7] Radiofrequency ablation and chemotherapy are alternative therapies that also frequently prove ineffective against glioblastoma. Even advanced treatments such as molecular targeted therapy have shown poor response due to molecular heterogeneity.[8] In light of these challenges, there is a pressing need for more effective and efficient therapeutic approaches. One promising solution is immunotherapy, particularly the use of patient-derived T cells that have been engineered with chimeric antigen receptors (CAR-T cells).[9]

MATERIAL AND METHODS

This systematic review explores the potential of modified multitarget CAR-T cells as a cutting-edge immunotherapy for addressing organic personality and behavioral disorders caused by glioblastoma. The review systematically synthesizes data from multiple studies to assess the feasibility, safety, and efficacy of CAR-T cell therapies in targeting

glioblastoma-associated immune dysfunction and neurological sequelae.

Formulating the Research Questions

The primary objective of this review is to examine the therapeutic potential of CAR-T cells in treating glioblastoma. The specific research questions are:

1. What is the efficacy of multitarget CAR-T cells in modulating immune dysfunction associated with glioblastoma?
2. How can CAR-T cell therapies be optimized for neurological applications, especially in targeting glioblastoma-associated neuroinflammation?

Identifying Relevant Studies

The review adhered to PRISMA 2020 guidelines, with a systematic literature search conducted between September and October 2024. Databases searched included PubMed, ScienceDirect®, ProQuest, EBSCOhost®, SAGE®, ClinicalKey®, and Scopus. Search terms included Boolean combinations such as (("glioblastoma") OR ("GBM")) AND (("CAR-T cells") OR ("chimeric antigen receptor T cells") AND (("organic personality disorder") OR ("neurological disorder") OR ("neuroinflammation")). Studies published in the last decade focusing on advanced CAR-T cell designs for glioblastoma and their neurological impacts were prioritized.

By addressing these critical research areas, this review aims to contribute valuable insights into the emerging role of CAR-T cell immunotherapy in glioblastoma-induced neurological disorders.

Selection of Studies

This systematic review adhered to rigorous inclusion criteria to ensure the relevance of selected studies:

1. Published in English
2. Available in full text
3. Focused on glioblastoma and its immunotherapeutic approaches
4. Investigated the development, design, or clinical application of multitarget CAR-T cells
5. Clearly described outcomes related to glioblastoma treatment with CAR-T cells

Studies that were inaccessible in full text or did not align with the research

objectives were excluded. Initial screening of titles and abstracts identified potentially relevant studies, followed by full-text reviews to confirm their focus on CAR-T cell immunotherapy for glioblastoma.

Data Organization

Extracted data were systematically organized into a structured charting table, including critical information such as the author(s), publication year, study design, CAR-T cell targets, clinical outcomes, and conclusions.

Synthesis and Conclusion

The synthesized findings are presented in **Table 1**, highlighting the advancements and challenges in utilizing multitarget CAR-T cells for glioblastoma treatment. This review underscores the potential of modified CAR-T cell therapy in targeting glioblastoma's heterogeneous tumor microenvironment. By addressing key challenges such as tumor resistance and immunosuppression, multitarget CAR-T cells represent a promising avenue for improving therapeutic outcomes in glioblastoma patients.

Results Summary

During the initial phase of this systematic review, 1,512 studies and abstracts were identified through database searches. After removing 112 duplicate entries, 1,400 unique articles were screened for relevance based on their titles and abstracts. Of these, 78 studies were selected for further examination, while 1,322 articles were excluded due to their lack of alignment with the review's objectives. Following an in-depth review of the 78 studies, 32 were deemed to meet the inclusion criteria, while 46 were excluded for not providing sufficient data or failing to address glioblastoma or CAR-T cell therapy adequately. A comprehensive full-text review of the remaining 32 studies was conducted, resulting in the inclusion of 11 studies in this systematic review (**Figure 1**). This structured process ensured that only high-quality and relevant studies were included to evaluate the potential of multitarget CAR-T cells as a promising immunotherapy for glioblastoma.

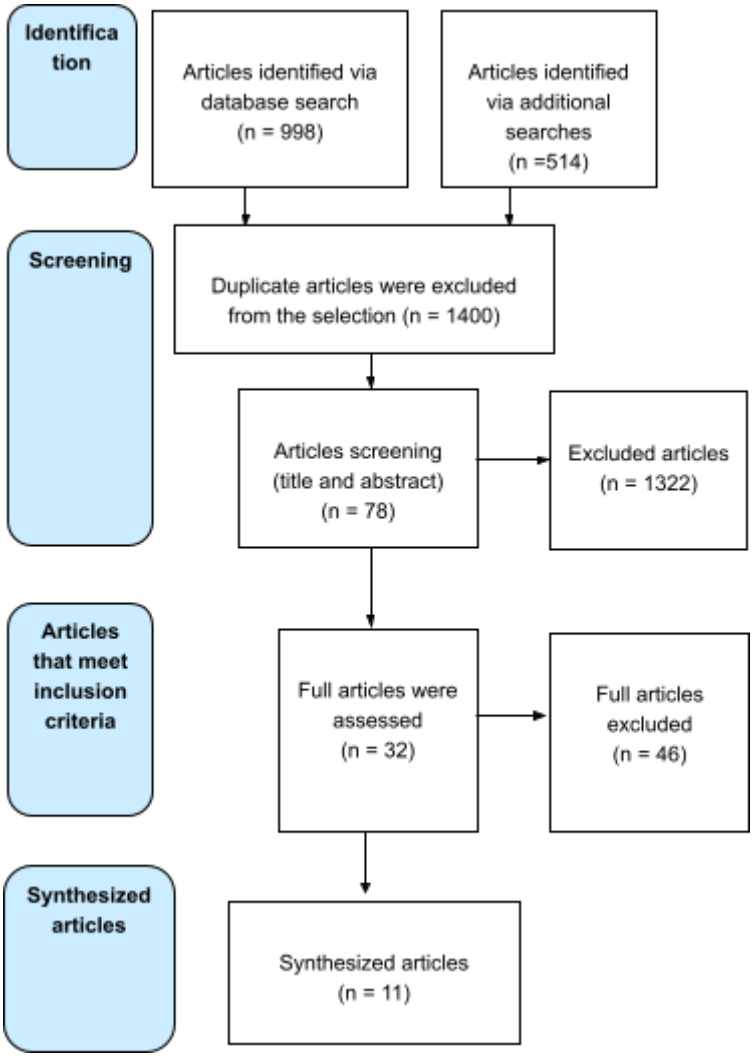

Figure 1. Systematic Review Flow Chart

Table 1 provides an overview of the key characteristics of the studies included in this review. The table details the research objectives, study designs, CAR-T cell targets, results, and conclusions. These studies investigated the effectiveness of multitarget CAR-T cells in addressing the challenges posed by glioblastoma's heterogeneous tumor microenvironment, including tumor resistance and immunosuppression. The findings emphasize the potential of multitarget CAR-T cells to enhance therapeutic outcomes, offering a promising approach to glioblastoma management, particularly in cases where conventional therapies have limited efficacy.

Table 1. Summary of Key Findings from the Included Studies.

No	Study Title	Objectives	Study Design, Sample Size, Methodology	Results
1	Enhancing anti-EGFRvIII CAR T cell therapy against glioblastoma with a paracrine SIRPγ-derived CD47 blocker	Overcome CAR T cell resistance by combining CAR T cells with SGRP to enhance phagocytosis and antitumor response.	Engineered anti-EGFRvIII CAR T cells secreting SGRP; In vivo GBM and CD19+ lymphoma mouse models; In vitro testing on GBM cell lines.	Anti-EGFRvIII-SGRP CAR T cells eradicated GBM tumors, promoted GAM phagocytosis, and showed improved efficacy in eliminating tumor cells compared to conventional CAR T cells.
2	Optimization of IL13Rα2-Targeted Chimeric Antigen Receptor T Cells for Improved Anti-tumor Efficacy against Glioblastoma	Enhance IL13Rα2-targeted CAR T cell efficacy for GBM therapy.	Developed 4-1BB co-stimulatory IL13BBζ-CAR; Tested on orthotopic human GBM models in NSG mice; Analyzed delivery routes (intracranial vs intravenous).	IL13BBζ-CAR T cells improved anti-tumor activity and T cell persistence. Intracranial delivery proved superior to intravenous delivery, with intraventricular infusions showing potential for multifocal disease treatment.
3	Glioblastoma-targeted CD4+ CAR T cells mediate superior antitumor activity	Evaluate efficacy of CD4+ vs CD8+ CAR T cells in GBM treatment.	Tested CD4+ and CD8+ CAR T cells targeting IL13Rα2 in orthotopic GBM models; Assessed T cell persistence and functionality.	CD4+ CAR T cells demonstrated superior long-term efficacy and persistence compared to CD8+ T cells, even when mixed populations were tested. Identified CD4+ T cells as critical for CAR therapy success.

No	Study Title	Objectives	Study Design, Sample Size, Methodology	Results
4	Selective Targeting of Glioblastoma with EGFRvIII/EGFR Bitargeted Chimeric Antigen Receptor T Cell	Develop EGFRvIII/EGFR bi-targeted CAR T cells to minimize off-tumor toxicity and improve efficacy.	Created M27-based CAR T cells targeting EGFR/EGFRvIII; Tested on orthotopic and heterogeneic glioblastoma mouse models.	M27-28BBZ CAR T cells effectively lysed EGFRvIII/EGFR-overexpressing tumor cells while sparing normal cells. Enhanced tumor suppression and prolonged survival in glioblastoma mouse models.
5	Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma	Investigate IL-12 as an adjuvant to enhance CAR-T therapy in immunosuppressive GBM TME.	Combined IL-12 delivery with EGFRvIII-targeting CAR T cells in an immunocompetent GBM mouse model; Analyzed immune microenvironment changes.	Local IL-12 delivery reshaped the TME, reducing Tregs, activating myeloid cells, and enhancing proinflammatory responses, resulting in durable antitumor responses with minimal systemic effects.
6	Anti-VEGF therapy improves EGFRvIII-CAR-T cell delivery and efficacy in syngeneic glioblastoma models in mice	Test the hypothesis that anti-VEGF therapy can improve CAR-T cell delivery and efficacy in GBM models.	Engineered EGFRvIII-CAR T cells and tested anti-mouse VEGF antibody (B20) in syngeneic mouse models of GBM; Studied tumor vessel normalization and TME impact.	Anti-VEGF therapy enhanced CAR-T cell infiltration and distribution, delayed tumor growth, and prolonged survival in GBM-bearing mice compared to CAR-T cell therapy alone, supporting clinical evaluation of this combination therapy for GBM patients.
7	EphA3-targeted chimeric antigen receptor T cells are effective in glioma and generate curative memory T cell responses	Identify and target EphA3, a prevalently expressed glioma antigen, using CAR-T cells.	Cell surface proteomic analysis of glioma samples; Developed EphA3-targeted CAR-T cells; Tested in vitro and in vivo using orthotopic xenograft models in mice.	EphA3 CAR-T cells eradicated tumors, demonstrated long-term memory response, and achieved complete tumor clearance on re-challenge, highlighting a strong potential for clinical translation in high-grade gliomas.

No	Study Title	Objectives	Study Design, Sample Size, Methodology	Results
8	T cells expressing NKG2D chimeric antigen receptors efficiently eliminate glioblastoma and cancer stem cells	Investigate the efficacy of NKG2D CAR-T cells against glioblastoma and cancer stem cells.	Constructed NKG2D-BBz CAR-T cells; Evaluated cytotoxicity, cytokine release, and therapeutic efficacy in vitro and in vivo using xenograft tumor models.	NKG2D CAR-T cells efficiently lysed glioblastoma and cancer stem cells, showed high cytokine production, eliminated tumors in vivo, and exhibited no treatment-related toxicity, supporting their potential for GBM therapy.
9	NKG2D-Based CAR T Cells and Radiotherapy Exert Synergistic Efficacy in Glioblastoma	Explore the synergy of NKG2D-based CAR-T therapy and radiotherapy in glioblastoma treatment.	Combined chNKG2D CAR-T cells with radiotherapy in immunocompetent orthotopic glioblastoma models; Analyzed migration, effector function, and persistence.	The combination demonstrated synergistic efficacy, prolonged survival, and tumor eradication. CAR-T cells migrated effectively to the tumor site, and long-term persistence provided protection against rechallenge, presenting a rationale for clinical translation.
10	GD2 CAR T cells against human glioblastoma	Investigate the antitumor potential of GD2-targeted CAR T cells against glioblastoma.	Isolated GBM cells with high GD2 expression; Tested GD2-CAR in 2D and 3D GBM models and orthotopic NOD/SCID mouse models; Compared delivery routes (intracerebral vs intravenous).	GD2 CAR-T cells demonstrated robust antitumor activity in vitro and in vivo. Intracerebral delivery significantly improved survival compared to intravenous delivery, with no observed side effects, providing a strong therapeutic candidate for glioblastoma.
11	GD2-targeting CAR-T cells enhanced by transgenic IL-15 expression are an effective and clinically feasible therapy for glioblastoma	Enhance GD2-CAR T cells with IL-15 expression to improve persistence and efficacy.	Engineered GD2-CAR T cells with transgenic IL-15 expression; Evaluated efficacy in glioblastoma models in vitro and in vivo.	GD2-CAR T cells with IL-15 exhibited enhanced persistence, superior antitumor activity, and a clinically feasible safety profile, highlighting their potential for glioblastoma treatment.

DISCUSSION

The Role of the Immune System in Glioblastoma

Glioblastoma is a malignant tumor that primarily occurs in the brain. Like most carcinoma cases, glioblastoma has a multifactorial etiology, arising from complex interactions between genetic and environmental factors.[10] Fundamentally, glioblastoma is the end result of oncogenesis, a process in which normal cells transform into cancerous cells. This occurs due to an imbalance between the generation of new cells in the cell cycle and the loss of old cells through apoptosis. This imbalance results from genetic mutations in three groups of proteins: proto-oncogenes, tumor suppressor genes, and DNA repair genes. The continuous abnormal growth of these cells leads to a mass effect, exerting pressure within the confined intracranial compartment.[11]

Anti-cancer immunity is generally classified into innate and adaptive immune systems.[12] The key components of the innate immune system in cancer immunity include dendritic cells and natural killer (NK) cells, while the adaptive immune system primarily involves T lymphocytes. Dendritic cells play a crucial role in recognizing, processing, and presenting tumor antigens. Studies have shown defects in dendritic cell function in glioblastoma patients. Meanwhile, NK cells, which are part of the adaptive immune system, exhibit cytotoxic properties and regulate immune cell activity through cytokine release.[13,14]

A functional anti-cancer immune response can recognize and eliminate cancer cells expressing tumor-associated antigens (TAAs). These tumor antigens form complexes with human leukocyte antigen (HLA) molecules on the tumor cell surface.[15] For an anti-cancer response to be initiated, TAAs must be presented by dendritic cells. TAAs presented as HLA class I components activate cytotoxic T lymphocytes, while those presented as HLA class II components activate CD4+ T helper cells.[16]

Research indicates that glioblastoma cancer cells possess a unique ability to evade immune detection by concealing TAAs on their surface, a process known as immune tolerance induction.[17] This immune tolerance in glioblastoma involves three main stages: elimination, equilibrium, and escape.[18] During the elimination phase, immune cells recognize and destroy cancer cells. If elimination is incomplete, the equilibrium phase follows, in which cancer cells enter a dormant state and develop new immunogenic properties, while immune cells fail to completely suppress tumor growth. In the escape phase, glioblastoma cells employ various strategies to evade immune surveillance and establish immune tolerance.[19]

Immune tolerance can also be achieved through additional mechanisms, such as downregulating TAA expression on cancer cells and reducing HLA class I expression on dendritic cells via multiple signaling pathways. Cancer cells can also develop resistance to immune-mediated destruction by upregulating anti-apoptotic molecules such as Bcl-xL and FLIP.[20] Furthermore, glioblastoma cells evade immune responses by increasing the secretion of immunosuppressive factors such as TGF-β, IL-10, and CCL21 and by creating an immunosuppressive microenvironment.[21]

Immunodeficiency is observed in most glioblastoma patients and is associated with increased levels of regulatory T cells and decreased dendritic cell activity.[22, 23]

Utilization of CAR-T Cells as Cancer Immunotherapy

Chimeric antigen receptor (CAR) T-cell therapy is an advanced immunotherapy that utilizes the patient's own T cells, which are modified in the laboratory to recognize and attack cancer cells expressing specific tumor-associated antigens (TAA). Initially, T cells are extracted from the patient's blood and engineered to express a special receptor capable of recognizing cancer cell TAAs. This special receptor is called

CAR. The T cells equipped with CAR, henceforth referred to as CAR-T cells, are then infused back into the patient's body. Upon recognizing the TAA, CAR-T cells become activated, proliferate, and secrete cytokines. These activated CAR-T cells are then able to kill and eliminate cancer cells.[24] The mechanism of CAR-T cell formation can be seen in the following image.

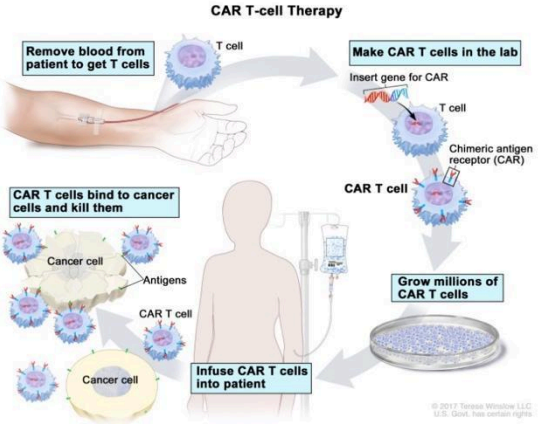

Figure 2. Mechanism of CAR-T cell formation[25]

The CAR consists of an extracellular domain, a hinge, a transmembrane domain, and an intracellular domain. The extracellular domain, which is typically composed of a single-chain fragment variable (scFv), is responsible for recognizing TAA and is therefore often referred to as the recognition domain. The hinge and transmembrane domain of the CAR function to maintain its stability.[26] The intracellular domain is usually derived from CD3 and serves to provide activation signals for the T cell.[27] An illustration of the CAR structure can be seen in the following image.

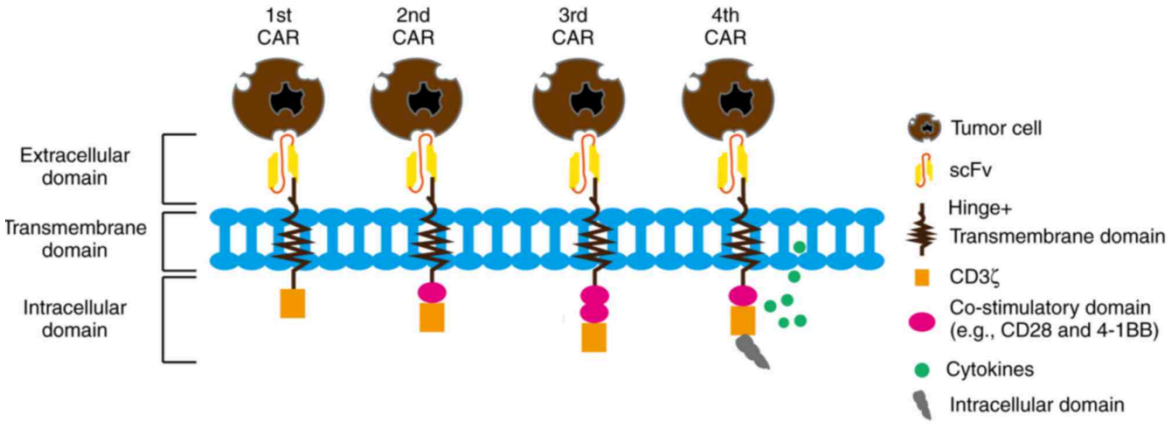

Figure 3. Components of the chimeric antigen receptor.[28]

Currently, there are four CAR designs. The first-generation CAR consists of an extracellular domain, a hinge, a transmembrane domain, and an intracellular domain. The second-generation CAR is similar to the first but includes a co-stimulatory domain, such as CD28/B7, which enhances IL-2 synthesis and optimizes T cell activation. The third-generation CAR combines multiple co-stimulatory domains, such as CD3ζ-CD28-OX40 or CD3ζ-CD28-41BB, to enhance potency by increasing cytokine production. The fourth-generation CAR introduces an additional intracellular domain that co-expresses certain small molecules (such as IL-12, IL-18 and programmed cell death protein 1), which can trigger cytokine-induced signaling or block signaling pathways that affect CAR-T cell function, aiming to improve therapeutic effects.[28]

CAR-T cell therapy was initially designed and developed for the treatment of hematologic malignancies, one of which is CAR-T cell therapy for acute lymphoblastic leukemia (ALL). A study conducted at Memorial Sloan Kettering Cancer Center reported that out of 16 patients with acute lymphoblastic leukemia treated with CAR-T cells, 14 achieved a complete response and remission. In this study, acute lymphoblastic leukemia had CD19 as its TAA, which is expressed across all B-cell malignancies.[29]

Similarly, GUCY2C is another potential TAA target for CAR-T cell immunotherapy. GUCY2C is a glioblastoma-associated antigen expressed on the tumor cell surface.[39] Studies on CAR-T cell therapy targeting GUCY2C in other solid tumors have shown that CAR-T cells effectively suppress tumor growth and eliminate metastases, indicating their potential application in glioblastoma.[40]

MUC1 is a transmembrane glycoprotein that is overexpressed in glioblastoma and serves as a promising TAA for CAR-T cell therapy.[41] First- and third-generation CAR-T cell therapies targeting MUC1 in carcinoma have demonstrated that CAR-T cells can recognize and eliminate cancer cells overexpressing MUC1 without harming normal cells.[42] Similar studies targeting GPC-3 with first- and third-generation CAR-T cells in glioblastoma have yielded comparable results, demonstrating effective suppression of tumor growth.[43]

Potential Utilization of Multitarget CAR-T Cells as Immunotherapy for Glioblastoma

Based on the explanation above, CAR-T cells, in addition to being used as immunotherapy for hematologic malignancies, should also be considered for use in solid tumors, such as glioblastoma. In this approach, T cells are harvested from the patient and genetically engineered to express receptors specific to glioblastoma antigens, then reinfused into the patient's body. Recent data on CAR-T cell activity targeting GAD2 in diffuse intrinsic pontine glioma indicate that CAR-T cell therapy has the potential to elicit effective responses against primary brain tumors.[30]

Based on the discussion above, CAR-T cells, besides being used as immunotherapy for hematologic malignancies, should also be applicable as immunotherapy for solid tumors, including glioblastoma.

The initial step in developing CAR-T cell immunotherapy is identifying tumor-associated antigens (TAAs) expressed on the surface of cancer cells. Marcella et al. summarized the essential criteria for selecting TAAs suitable for CAR-T cell targeting in solid tumors: the target must be expressed on the tumor cell surface, should not exhibit ectopic expression even at low levels, and must be present in all tumor cells.[31]

One of the identified TAAs that meets these criteria for CAR-T cell therapy in glioblastoma is carcinoembryonic antigen (CEA).[32] CEA is a 180-kDa carcinoembryonic antigen produced during fetal development but ceases production after birth. As an adhesion molecule, CEA overexpression in glioblastoma accelerates tumor growth and induces metastasis. Studies have shown that glioblastoma patients possess T cells specific to CEA epitopes.[33] Research on CAR-T cell therapy targeting CEA in glioblastoma has demonstrated high therapeutic efficacy, resulting in significant tumor size reduction.[34]

Other identified TAAs in glioblastoma include glypican-3 (GPC-3), guanylyl cyclase C (GUCY2C), and mucin-1 (MUC1).[35, 36] GPC-3 is a 65-kDa protein composed of 580 amino acids, functioning as a heparan sulfate proteoglycan anchored to the cell membrane via glycosylphosphatidylinositol. Like CEA, GPC-3 is expressed during fetal development but ceases production postnatally.[37] Although its exact role in glioblastoma progression is not fully understood, GPC-3 is believed to contribute to neoplastic transformation by regulating growth factors. Given these characteristics, GPC-3 is a specific and promising TAA for CAR-T cell therapy.[38]

Additionally, the abundant vascularization in glioblastoma tumors presents another promising target for CAR-T cell therapy. Extensive blood vessel networks enable cancer cells to secrete immunosuppressive molecules into circulation. Poor prognosis and tumor metastasis are associated with the expression of vascular

endothelial growth factor (VEGF) and its receptors in the tumor microenvironment.[44] The presence of VEGF receptor-2 (VEGFR2) as a TAA suggests its potential as a target for CAR-T cell therapy in glioblastoma.[45]

Although CAR-T cell therapy has shown high cure rates in many patients, some reports indicate therapy failure due to diminished patient response. One proposed explanation for reduced immunotherapy efficacy is the downregulation or loss of TAA expression on cancer cell surfaces following CAR-T cell therapy. This theory is based on sequencing data from leukemia patients treated with CAR-T cells targeting CD19, where relapse occurred due to CD19 antigen loss post-therapy. A similar mechanism of TAA downregulation and loss may also occur in solid tumors like glioblastoma.[46]

One strategy to overcome TAA downregulation and loss following CAR-T cell therapy is the use of multitarget CAR-T cells, which target multiple antigen receptors simultaneously. This can be achieved through two approaches: co-transduction, which involves engineering CAR-T cells to express two different CARs, and tandem CAR, which encodes two different extracellular domains within a single chimeric protein. The target TAAs for multitarget CAR-T cells may include a combination of glioblastoma-associated TAAs such as CEA, GPC-3, MUC1, and GUCY2C.[47] The schematic representation of CAR-T cell activity using the co-transduction and tandem CAR mechanisms, which enable the recognition of multiple tumor-associated antigens simultaneously, can be seen in the following image.

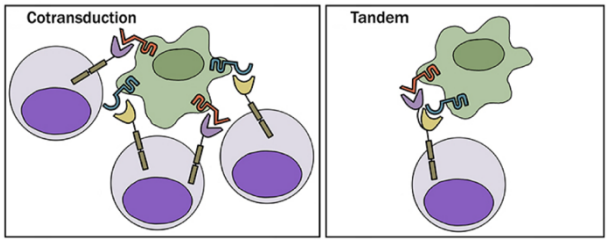

Figure 4. Multitarget CAR-T cells utilizing co-transduction and tandem mechanisms to recognize multiple tumor-associated antigens (TAAs) simultaneously.[48]

Co-transduction is performed by integrating two or more CARs targeting different TAAs into a single CAR-T cell during production. Meanwhile, tandem CAR-T cells modify the extracellular domain of a single CAR molecule, enabling it to recognize multiple antigens. This is achieved by linking two distinct single-chain variable fragments (scFv) in the extracellular domain of a single CAR molecule, allowing recognition of two or more different TAAs.[48]

Modifications in the Use of CAR-T Cells for Glioblastoma

One of the challenges in developing CAR-T cells as immunotherapy for solid tumors like glioblastoma is ensuring that T cells can reach the tumor site by penetrating the tumor microenvironment. One of the barriers to infiltration is the extracellular matrix, which contains heparan sulfate proteoglycans. Unfortunately, T cells lack the ability to express the enzyme heparanase to degrade heparan sulfate proteoglycans. Therefore, CAR-T cell design for solid tumors like glioblastoma must include the ability to secrete heparanase to enhance the effectiveness of CAR-T cells within the tumor.[49]

Another challenge in using CAR-T cells for glioblastoma is that, unlike hematologic malignancies, tumor-associated antigens (TAAs) in solid tumors are rarely present in the circulatory system. In addition, immune system modifications and abnormal cytokine secretion within

the solid tumor microenvironment limit CAR-T cell function and tumor elimination. To address this issue, CAR-T cells for solid tumors can be administered via intratumoral injection. Studies have shown that intratumoral administration enhances the efficacy of immunotherapy while reducing systemic toxicity.[50]

Glioblastoma, as a solid tumor, is also associated with elevated levels of immune checkpoint molecules such as programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4).[51] Chronic inflammation of the brain and surrounding tissues due to glioblastoma can significantly increase checkpoint molecule expression in most cases, and these levels may serve as prognostic indicators for patients.[52]

Checkpoint molecules play a crucial role in preventing autoimmunity. T cell maturation occurs in the thymus, where immature T cells proliferate and differentiate into mature T cells with diverse receptors that recognize antigens.[53] During this process, T cells with strong reactivity against self-antigens are eliminated to prevent autoimmune responses. However, some T cell receptors may still cross-react with self-antigens. To mitigate this risk, the immune system employs immune checkpoint molecules, such as CTLA-4 and PD-1, to inhibit T cell activation. Cancer cells exploit this mechanism by expressing immune checkpoint molecules, preventing T cells from recognizing abnormal TAAs and becoming activated.[54]

To overcome this challenge, CAR-T cell immunotherapy for glioblastoma can be combined with immune checkpoint inhibitors, such as CTLA-4 and PD-1 inhibitors. Although most combination therapy studies involving CAR-T cells and immune checkpoint inhibitors remain in the preclinical stage, this modification is expected to enhance the effectiveness of CAR-T cell therapy in solid tumors like glioblastoma.[55]

CONCLUSION

The high morbidity and mortality rates of glioblastoma cases worldwide, including in Indonesia, highlight the urgency of effective disease management. Surgical therapy, radiofrequency ablation, chemotherapy, and molecular targeted therapy are often ineffective and associated with high recurrence rates. This has driven the development of alternative and more effective treatments, such as immunotherapy. The foundation of immunotherapy is the utilization of the immune system to eliminate and destroy cancer cells through a series of processes, including recognition, immune cell activation, and cancer cell elimination. One promising immunotherapy approach involves the use of T cells engineered with multitarget chimeric antigen receptors (CAR) along with several modifications. These CAR-modified T cells can recognize glioblastoma-specific tumor-associated antigens (TAAs), such as CEA, GPC3, MUC1, and GUCY2, leading to T cell activation and the involvement of other immune components, ultimately facilitating the elimination of glioblastoma cancer cells.

Several modifications are required for CAR-T cells to function effectively against glioblastoma, which is a solid tumor. These modifications include intra-tumoral administration of CAR-T cells, the addition of heparanase to enable CAR-T cells to penetrate the solid glioblastoma tumor microenvironment, and the incorporation of immune checkpoint inhibitors, such as CTLA4 and PD1 inhibitors. Further research on the effectiveness of modified CAR-T cells as a glioblastoma therapy, along with in vivo studies, should be conducted to enable clinical trials. In the future, this approach could be utilized in clinical settings to reduce glioblastoma-related mortality both in Indonesia and globally.

ACKNOWLEDGEMENT

There are no acknowledgements for this research work.

REFERENCES

1. The International Agency for Research on Cancer (IARC). Global cancer burden growing, amidst mounting need for services. doi:10.3390/cancers14215331
2. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2022;74(3):229-263. doi:10.3322/caac.21834
3. Tebha SS, Ali Memon S, Mehmood Q, Mukherjee D, Abdi H, Negida A. Glioblastoma management in low and middle-income countries; existing challenges and policy recommendations. *Brain and Spine*. 2023;3:101775. doi:https://doi.org/10.1016/j.bas.2023.101775
4. Juweid AFT and M. Epidemiology and Outcome of Glioblastoma. In: *Glioblastoma*. Vol 8. ; 2017. doi:10.1097/NCC.0000000000000139.Adherence
5. Davis ME. Glioblastoma: Overview of Disease and Treatment. *Clin J Oncol Nurs*. 2016;20(5 Suppl):S2-S8. doi:10.1188/16.CJON.S1.2-8
6. Amelot A, Baronnet-Chauvet F, Fioretti E, et al. Glioblastoma complicated by fatal malignant acute ischemic stroke: MRI finding to assist in tricky surgical decision. *Neuroradiol J*. 2015;28(5):483-487. doi:10.1177/1971400915598073
7. Jackson C, Westphal M, Quiñones-Hinojosa A. Complications of glioma surgery. *Handb Clin Neurol*. 2016;134:201-218. doi:10.1016/B978-0-12-802997-8.00012-8
8. Touat M, Idbaih A, Sanson M, Ligon KL. Glioblastoma targeted therapy : updated approaches from recent biological insights. *Response Eval criteria lymphoma (RECIL 2017)*. 2017;28(7):1457-1472. doi:10.1093/annonc/mdx106
9. Xu D, Jin G, Chai D, Zhou X, Gu W. The development of CAR design for tumor CAR-T cell therapy. 2018.
10. Berger MS, Bruce JN, Chen TC, Zadeh G. Introduction: Glioblastoma: an update on pathophysiology and management strategies. *Neurosurg Focus FOC*. 2014;37(6):Introduction. doi:10.3171/2014.9.FOCUS14677
11. Rajaratnam V, Islam MM, Yang M, Slaby R, Ramirez HM, Mirza SP. Glioblastoma: Pathogenesis and Current Status of Chemotherapy and Other Novel Treatments. *Cancers (Basel)*. 2020;12(4):937. doi:10.3390/cancers12040937
12. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev*. 2018;32(19-20):1267-1284. doi:10.1101/gad.314617.118
13. Marcus A, Gowen BG, Thompson TW, et al. Recognition of tumors by the innate immune system and natural killer cells. *Adv Immunol*. 2014;122:91-128. doi:10.1016/B978-0-12-800267-4.00003-1
14. Pandya PH, Murray ME, Pollok KE, Renbarger JL. The Immune System in Cancer Pathogenesis: Potential Therapeutic Approaches. *J Immunol Res*. 2016;2016:4273943. doi:10.1155/2016/4273943
15. Pardoll D. Cancer and the Immune System: Basic Concepts and Targets for Intervention. *Semin Oncol*. 2015;42(4):523-538. doi:10.1053/j.seminoncol.2015.05.003
16. Alatrash G, Jakher H, Stafford PD, Mittendorf EA. Cancer immunotherapies, their safety and toxicity. *Expert Opin Drug Saf*. 2013;12(5):631-645. doi:10.1517/14740338.2013.795944
17. Krebs S, Rodríguez-Cruz TG, Derenzo C, Gottschalk S. Genetically modified T cells to target glioblastoma. *Front Oncol*. 2013;3:322. doi:10.3389/fonc.2013.00322
18. Nurieva R, Wang J, Sahoo A. T-cell tolerance in cancer. *Immunotherapy*. 2013;5(5):513-531. doi:10.2217/imt.13.33
19. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting : Integrating Suppression and Promotion. (i).
20. Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. *Aging (Albany NY)*. 2016;8(4):603-619. doi:10.18632/aging.100934
21. Pombo Antunes AR, Scheyltjens I, Duerinck J, Neyns B, Movahedi K, Van Ginderachter JA. Understanding the glioblastoma immune microenvironment as basis for the development of new immunotherapeutic strategies. *Elife*. 2020;9:e52176. doi:10.7554/eLife.52176

22. Desai R, Suryadevara CM, Batich KA, Farber SH, Sanchez-Perez L, Sampson JH. Emerging immunotherapies for glioblastoma. *Expert Opin Emerg Drugs*. 2016;21(2):133-145. doi:10.1080/14728214.2016.1186643
23. Arrieta VA, Cacho-Díaz B, Zhao J, Rabadan R, Chen L, Sonabend AM. The possibility of cancer immune editing in gliomas. A critical review. *Oncoimmunology*. 2018;7(7):e1445458-e1445458. doi:10.1080/2162402X.2018.1445458
24. Wang Z, Chen W, Zhang X, Cai Z, Huang W. A long way to the battlefront : CAR T cell therapy against solid cancers. 2019;10. doi:10.7150/jca.30406
25. PDQ Pediatric Treatment Editorial Board. Childhood Acute Lymphoblastic Leukemia Treatment (PDQ®). In: *PDQ Cancer Information Summaries*. ; 2019. https://www.cancer.gov/types/leukemia/patient/child-all-treatment-pdq.
26. Guedan S, Calderon H, Posey Jr AD, Maus M V. Engineering and Design of Chimeric Antigen Receptors. *Mol Ther Methods Clin Dev*. 2018;12:145-156. doi:10.1016/j.omtm.2018.12.009
27. Stoiber S, Cadilha BL, Benmebarek M, Lesch S, Endres S, Kobold S. Limitations in the Design of Chimeric Antigen Receptors for Cancer Therapy. 2019:1-26.
28. Sun D, Shi X, Li S, Wang X, Yang X, Wan M. CAR-T cell therapy: A breakthrough in traditional cancer treatment strategies (Review). *Mol Med Rep*. 2024;29(3):47. doi:10.3892/mmr.2024.13171
29. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. 2014;6(224):224ra25-224ra25.
30. Luksik AS, Yazigi E, Shah P, Jackson CM. CAR T Cell Therapy in Glioblastoma: Overcoming Challenges Related to Antigen Expression. *Cancers (Basel)*. 2023;15(5). doi:10.3390/cancers15051414
31. Maus M V, June CH. Zoom zoom: racing CARs for multiple myeloma. *Clin Cancer Res*. 2013;19(8):1917-1919.
32. Price RL, Chiocca EA. Evolution of malignant glioma treatment: from chemotherapy to vaccines to viruses. *Neurosurgery*. 2014;61 Suppl 1(0 1):74-83. doi:10.1227/NEU.0000000000000390
33. Holdhoff M, Yovino SG, Boadu O, Grossman SA. Blood-based biomarkers for malignant gliomas. *J Neurooncol*. 2013;113(3):345-352. doi:10.1007/s11060-013-1144-0
34. Akhavan D, Alizadeh D, Wang D, Weist MR, Shepphird JK, Brown CE. CAR T cells for brain tumors: Lessons learned and road ahead. *Immunol Rev*. 2019;290(1):60-84. doi:10.1111/imr.12773
35. Shimizu Y, Suzuki T, Yoshikawa T, et al. Cancer immunotherapy-targeted glypican-3 or neoantigens. *Cancer Sci*. 2018;109(3):531-541. doi:10.1111/cas.13485
36. Schijns VEJC, Pretto C, Strik AM, et al. Therapeutic Immunization against Glioblastoma. *Int J Mol Sci*. 2018;19(9):2540. doi:10.3390/ijms19092540
37. Azizpour S, Ezati R, Saidijam M, et al. The Expression of Glypican-3 in Colorectal Cancer. *Cytol Genet*. 2019;53:430-440. doi:10.3103/S0095452719050037
38. Moek KL, Fehrmann RSN, Vegt B Van Der, Vries EGE De, Groot DJA De. Glypican 3 Overexpression across a Broad Spectrum of Tumor Types Discovered with Functional Genomic mRNA Pro fi ling of a Large Cancer Database. *Am J Pathol*. 2018;188(9):1973-1981. doi:10.1016/j.ajpath.2018.05.014
39. Zhu H, Li JT, Zheng F, et al. Restoring soluble guanylyl cyclase expression and function blocks the aggressive course of glioma. *Mol Pharmacol*. 2011;80(6):1076-1084. doi:10.1124/mol.111.073585
40. Bagley SJ, Desai AS, Linette GP, June CH, O'Rourke DM. CAR T-cell therapy for glioblastoma: recent clinical advances and future challenges. *Neuro Oncol*. 2018;20(11):1429-1438. doi:10.1093/neuonc/noy032
41. Fecci PE, Sampson JH. The current state of immunotherapy for gliomas: an eye toward the future. *J Neurosurg JNS*. 2019;131(3):657-666. doi:10.3171/2019.5.JNS181762
42. Ma YD, Wang Z, Gong RZ, et al. Specific cytotoxicity of MUC1 chimeric antigen receptor-engineered Jurkat T cells against hepatocellular carcinoma. *Acad J Second Mil Med Univ*. 2014;35:1177-1182.
43. Yazdanifar M, Zhou R, Mukherjee P. Emerging immunotherapeutics in adenocarcinomas: A focus on CAR-T cells. *Curr Trends Immunol*. 2016;17:95-115.

https://pubmed.ncbi.nlm.nih.gov/28659689.
44. Chen J-C, Chang Y-W, Hong C-C, Yu Y-H, Su J-L. The role of the VEGF-C/VEGFRs axis in tumor progression and therapy. *Int J Mol Sci*. 2012;14(1):88-107. doi:10.3390/ijms14010088
45. US National Library of Science. clinicaltrials.gov[online], https://clinicaltrials.gov.ct2.show/NCT01218867.
46. Zah E, Lin M, Silva-benedict A, Jensen MC, Chen YY. T Cells Expressing CD19 / CD20 Bispeci fi c Chimeric Antigen Receptors Prevent Antigen Escape by Malignant B Cells. 2016;4(June). doi:10.1158/2326-6066.CIR-15-0231
47. Majzner RG, Mackall CL. Tumor antigen escape from CAR T-cell therapy. *Cancer Discov*. 2018;8(10):1219-1226.
48. Shah NN, Maatman T, Hari P, Johnson B. Multi Targeted CAR-T Cell Therapies for B-Cell Malignancies. 2019;9(March):1-7. doi:10.3389/fonc.2019.00146
49. Caruana I, Savoldo B, Hoyos V, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat Med*. 2015;21(5):524-529. doi:10.1038/nm.3833
50. van der Stegen SJC, Davies DM, Wilkie S, et al. Preclinical in vivo modeling of cytokine release syndrome induced by ErbB-retargeted human T cells: identifying a window of therapeutic opportunity? *J Immunol*. 2013;191(9):4589-4598. doi:10.4049/jimmunol.1301523
51. Huyghe N, Baldin P, Van den Eynde M. Immunotherapy with immune checkpoint inhibitors in colorectal cancer: what is the future beyond deficient mismatch-repair tumours? *Gastroenterol Rep*. 2019;8(1):11-24. doi:10.1093/gastro/goz061
52. Huang J, Liu F, Liu Z, et al. Immune Checkpoint in Glioblastoma: Promising and Challenging. *Front Pharmacol*. 2017;8:242. doi:10.3389/fphar.2017.00242
53. Kondo K, Ohigashi I, Takahama Y. Thymus machinery for T-cell selection. *Int Immunol*. 2019;31(3):119-125. doi:10.1093/intimm/dxy081
54. Brahm CG, van Linde ME, Enting RH, et al. The Current Status of Immune Checkpoint Inhibitors in Neuro-Oncology: A Systematic Review. *Cancers (Basel)*. 2020;12(3):586. doi:10.3390/cancers12030586
55. Ma S, Li X, Wang X, et al. Current Progress in CAR-T Cell Therapy for Solid Tumors. *Int J Biol Sci*. 2019;15(12):2548-2560. doi:10.7150/ijbs.34213