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Preliminary exploration of PSMA CAR-T combined with GD2 CAR-T for the treatment of refractory/relapsed gliomas

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Abstract

Background This study aimed to investigate the safety and efficacy of fourth-generation combined PSMA and GD2-targeted chimeric antigen receptor (CAR)-T cells in the treatment of refractory/relapsed gliomas.

Method This study employed a single-arm design, enrolling patients with confirmed refractory/relapsed gliomas at the Immuno-oncology Department of the Cancer Center at Clifford Hospital in Guangdong. Eligible patients received combined treatment with PSMA CAR-T and GD2 CAR-T cells via intravenous administration. The dose of reinfused CAR-T cells ranged from 1–5 × 10^6 cells/kg of body weight.

Results Six patients were included in the study, all of whom responded to the treatment. The overall response rate (ORR) was 50%, with three patients achieving complete response (CR) (50%) and three demonstrating stable disease (SD) (50%). The median progression-free survival (PFS) was 9.0 months (range, 1–56 months), and the median overall survival (OS) was 24.5 months (range, 13–63 months). Three patients (50%) developed cytokine release syndrome (CRS), all of which were classified as grade I CRS, and no patients experienced immune effector cell-associated neurotoxicity Syndrome (ICANS).

Conclusion Combined PSMA CAR-T and GD2 CAR-T cell therapy demonstrated significant efficacy and good tolerability in the treatment of refractory/relapsed gliomas, without severe adverse reactions.

Keywords Refractory/relapsed gliomas, PSMA CAR-T therapy, GD2 CAR-T therapy, Combination therapy

Background

Gliomas are the most common tumors of the central nervous system, accounting for approximately 47% of all malignant brain tumors and 81% of primary malignant tumors in this region [1–3]. Among gliomas, glioblastomas, classified as World Health Organization (WHO) Grade IV, represent 70% to 75% of cases. These tumors are particularly aggressive, with a median survival time of only 15 to 18 months after diagnosis and a five-year survival rate of just 10% [4–6]. Despite extensive research, the etiology of gliomas remains poorly understood, and standard treatment primarily consists of surgery, followed by targeted radiotherapy

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and chemotherapy with temozolomide [7, 8]. However, gliomas often develop resistance to radiation and chemotherapy, partly due to the protective blood–brain barrier [1, 9, 10]. As a result, even after aggressive treatment regimens, including surgery, radiation, and chemotherapy, many patients experience cancer recurrence within six months. For those with recurrent gliomas, the median survival time is limited to just 3 to 9 months [10–12], underscoring the urgent need for novel and more effective treatment strategies.

One promising approach is the use of chimeric antigen receptor-modified T (CAR-T) cells. These genetically engineered T cells express antigen-specific receptors, enabling direct recognition of tumor antigens, evasion of immune escape mechanisms, and prolonged survival in vivo [13]. CAR-T therapy has shown remarkable success in hematological malignancies, such as non-Hodgkin's lymphoma and chronic/acute lymphocytic leukemia. In particular, complete remission rates for relapsed diffuse large B-cell lymphoma range from 40 to 54% [14–16], while refractory mantle cell lymphoma has reached 67% [17] and indolent B-cell lymphomas exhibit remission rates between 69 and 74% [18, 19]. In leukemia, the overall remission rate three months after CAR-T therapy is reported to be 81% [20]. Despite its success in hematological cancers, CAR-T therapy in solid tumors, including gliomas, has encountered substantial challenges. These include the lack of tumor-specific antigens, difficulties in T cell trafficking and infiltration into tumor cells, and the immunosuppressive nature of the tumor microenvironment. As such, optimizing CAR-T cell functionality requires careful antigen selection.

Current clinical trials are exploring targeted therapies for gliomas, focusing on specific molecules with promising preliminary results. One such approach targets neuroblastoma cells that express high levels of the ganglioside GD2 [21], a molecule associated with high malignancy and limited expression in normal tissues. Another target is the prostate-specific membrane antigen (PSMA), which is found not only in prostate cancer but also in glioblastomas and other solid tumors [22, 23]. Additionally, a key trend in CAR-T therapy for solid tumors is the development of dual-target antigen CARs. This strategy involves selecting CAR-T cells that can target multiple tumor antigens simultaneously, reducing the likelihood of immune escape by tumor cells.

In this study, we conducted a clinical trial to evaluate the safety and efficacy of dual-targeted fourth-generation CAR-T therapy that combines GD2 and PSMA CAR-T cells in treating advanced or recurrent gliomas. We monitored CAR-T cell expansion, persistence, and anti-tumor effects to assess treatment outcomes. Our results support a rational clinical combination strategy based on CAR-T therapy, which may significantly improve therapeutic responses in patients with refractory or relapsed gliomas.

Methods

Study design and participants

Patients diagnosed with refractory or relapsed gliomas at the Immuno-oncology Department of Clifford Hospital in Guangdong were selected for this study, conducted from January 2020 to October 2021. Eligible participants exhibited high levels of both PSMA and GD2 (scores of 3+ or higher), as confirmed through pathology and immunohistochemistry tests, and met the specific inclusion and exclusion criteria outlined in Additional file 1: Appendices 1 and 2.

This study followed a single-arm trial design (Fig. 1). Initially, patients were screened and enrolled after obtaining informed consent. The general condition of each patient was assessed, including Karnofsky Performance Status (KPS) scoring, and measurements of heart rate, respiration, blood pressure, and body temperature. Laboratory tests such as IL-6/TNF-a, C-reactive protein (CRP), routine blood tests, and coagulation function were performed, alongside an electrocardiogram (ECG) and brain magnetic resonance imaging (MRI). After confirming eligibility, blood was collected for the isolation of peripheral T cells. Lymphodepletion pre-treatment with fludarabine (25 mg/m^2/day) and cyclophosphamide (250 mg/m^2/day) was administered over three consecutive days, beginning five days prior to CAR-T cell infusion. Infusion dosages ranged from 1 to 5 million CAR-T cells per kilogram of body weight. The time from blood collection to CAR-T cell infusion spanned 7 days. Patients received CAR-T cell infusion on day 0. After the infusion, participants were monitored for adverse events, with weekly measurements of CAR-T cells and cytokines in peripheral blood. Brain MRI was performed after infusion to evaluate treatment efficacy. All patients were followed up at regular intervals. Patient follow-up data were collected until August 20, 2024.

Manufacture of PSMA CAR-T and GD2 CAR-T cells

In this study, CAR-T cells targeting both PSMA and GD2 antigens were manufactured by the Shenzhen Geno-Immune Medical Institute. The production process, spanning 7 days from cell collection to infusion, followed the regulatory guidelines for cell and gene therapy products [24–26]. Autologous PSMA- and GD2-specific CAR-T cells were generated from peripheral blood lymphocytes as our previous study [26]. Briefly, patient-derived T lymphocytes were first collected through leukapheresis and subsequently isolated using Ficoll-Paque Plus (GE Healthcare) for peripheral blood mononuclear cell (PBMC) separation. The isolated T cells were

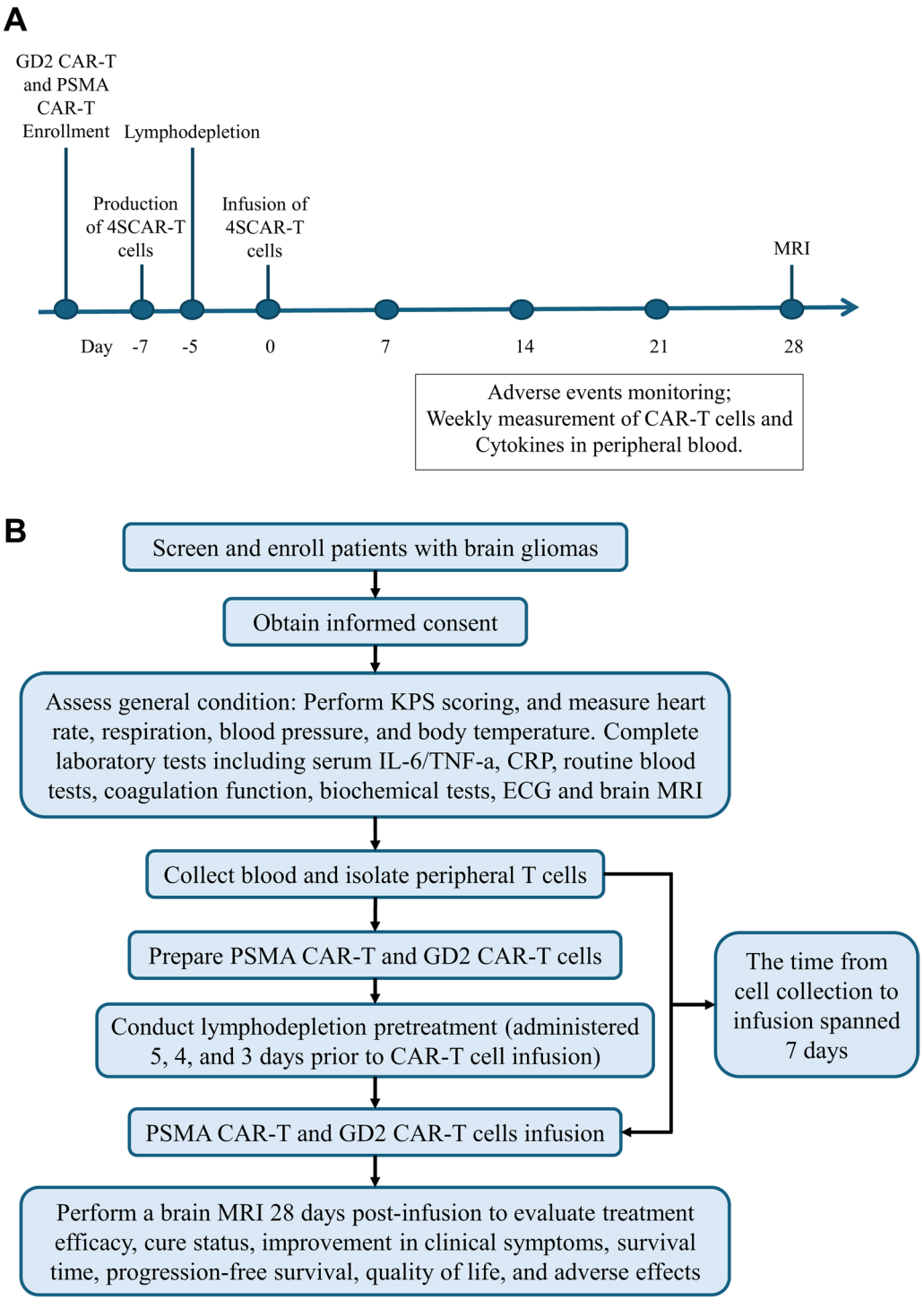

Fig.1 Clinical trial workflow of CAR-T cell therapy for patients with gliomas. **A** Protocol schema for enrollment, generation of fourth-generation safety-designed CAR T (4SCAR-T) cells, lymphodepletion, 4SCAR-T cell infusion, and follow-up. **B** This flowchart outlines the process of enrolling patients with gliomas for CAR-T cell therapy. It includes steps like patient screening, blood collection, preparation of PSMA and GD2 CAR-T cells, lymphodepletion pretreatment, cell infusion, and a follow-up brain MRI after infusion to assess treatment efficacy

then activated with phytohemagglutinin (PHA, 5 μg/mL) for 2–3 days and maintained in TexMACS medium supplemented with IL-2 (40 U/mL), IL-7 (20 U/mL), and IL-15 (10 U/mL) to promote proliferation. Activated T cells were transduced with PSMA- and GD2-specific fourth-generation safety-designed CAR (4SCAR) vectors and subsequently expanded. Fourth-generation CAR-T cells, incorporating the CD28 transmembrane and cytoplasmic domains, the co-stimulatory 4-1BB intracellular TRAF binding domain, the CD3z chain intracellular domain, and an inducible caspase 9 suicide gene, represent a significant advancement over earlier versions, improving both efficacy and safety. The 4SCAR vectors was created using the NHP/TYF lentiviral vector system [27, 28]. Chemically synthesized CAR DNA sequences were cloned into the pTYF vector with the human EF1α promoter. The constructed lentiviral CAR underwent extensive validation through restriction enzyme analysis and DNA sequencing. After expansion, the cells were tested for sterility, transduction efficiency, and killing function. The availability of manufactured CAR-T cells was defined by a transduction efficiency of ≥30% and a viability rate of ≥90%.

Assessment of cytotoxic effects

Adverse events and toxicities were carefully monitored and documented following the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were assessed using the criteria established by the American Society for Transplantation and Cellular Therapy (ASTCT), as outlined in Additional file 1: Appendices 3 and 4.

Measurement of response

Tumor response to treatment was evaluated using MRI scans after CAR-T cell infusion. Efficacy was classified according to RECIST v1.1 criteria as partial response (PR), complete response (CR), stable disease (SD), or progressive disease (PD). Additionally, clinical symptom improvement, overall survival (OS), and progression-free survival (PFS) were monitored to evaluate the long-term benefits of the treatment.

Measurement of CAR copy number

Peripheral blood samples were collected before and at specified time points post-CAR-T cell infusion (days 7, 14, 21, and 28). Genomic DNA was extracted from peripheral blood using a genomic DNA purification kit (Promega). The copy number of GD2 and PSMA CAR-T cells was quantified utilizing quantitative SYBR green real-time PCR (RT-qPCR).

Statistical analysis

Descriptive statistics were employed to summarize patient characteristics, treatment outcomes, and the overall effectiveness of CAR-T therapy. Statistical analyses were conducted using SAS 9.2 or GraphPad Prism 9.0. Kaplan–Meier survival analysis was performed for PFS and OS, and statistical significance was determined using the log-rank test. Pearson correlation analyses were performed to assess the relationships between tumor burden and clinical outcomes, as well as CAR-T cell persistence and clinical outcomes.

Results

Patient characteristics

This study enrolled six patients (five males and one female) diagnosed with either glioblastoma (GBM; grade IV) or diffuse midline glioma (DMG; grade IV) who received treatment between January 2020 and October 2021. All patients demonstrated high expression levels of GD2 and PSMA, confirmed by immunohistochemistry, with median copy numbers of GD2 and PSMA being 1.36×10^8 and 1.32×10^8, respectively (Table 1). The median age of the participants was 37.5 years, with two patients being under the age of 18. Four of the six patients underwent surgical resection, and all received radiation therapy and temozolomide. Additionally, two patients (Patients 03 and 04) received Bevacizumab (Bev), and one (Patient 06) underwent tumor-treating fields (TTFields). All enrolled patients were IDH1/2 wild-type, and methylation of O^6-Methylguanine-DNA methyltransferase (MGMT) was observed in one patient (Patient 02). At the time of enrollment, all patients had relapsed, except for one patient (Patient 06), who was disease-free at baseline (Table 1).

Safety and side effects of infusion

Hematological issues and CRS are common adverse events in the acute phase following CAR T-cell infusion [29]. Within the first four weeks after the initial infusion of PSMA and GD2 CAR-T cells, three out of six patients (50%) developed grade 1 CRS after the first infusion, but none required the administration of the intervention drug tocilizumab (Table 2). Hematologic toxicities were observed in five patients (83%), with four patients (66.7%) experiencing grade 2 or higher thrombocytopenia, and three (50%) developing grade 2 or higher neutropenia (Table 2). Regarding other adverse events, three patients developed infections associated with bone marrow suppression (Additional file 2: Supplementary Table 1). One patient (Patient 03) experienced fever on day 11 post-infusion, accompanied by a confirmed lung infection on chest CT. Empirical piperacillin therapy was ineffective, prompting a switch to imipenem, which resulted

Table 1 Characteristics of the patient at baseline

No.	Diagnosis (WHO)	Gender	Age	Disease status	MGMT methylation	IDH1/2 status	ECOG	Previous treatment				CD2 copy (1×10^8)	PSMA copy (1×10^8)
								Operation	Radiation	TMZ	Others		
01	GBM grade IV	Male	12Y	Relapsed	0	WT	3	1	1	1	0	0.92	1.1
02	DMG grade IV	Male	14Y	Relapsed	1	WT	3	0	1	1	0	1.42	1.38
03	GBM grade IV	Male	61Y	Relapsed	0	WT	2	1	1	1	Bev	1.5	1.78
04	GBM grade IV	Female	32Y	Relapsed	0	WT	4	0	1	1	Bev	1	1.26
05	GBM grade IV	Male	43Y	Relapsed	0	WT	0	1	1	1	0	2.4	2
06	GBM grade IV	Male	47Y	Disease-free	0	WT	0	1	1	1	TTFields	1.3	1.2

GBM glioblastoma, *DMG* diffuse midline glioma, *Y* years, *MGMT* O^6-Methylguanine-DNA methyltransferase, *IDH* isocitrate dehydrogenase, *ECOG* Eastern Cooperative Oncology Group, *TMZ* Temozolomide, *WT* Wildtype, *Bev* Bevacizumab, *0* NO,*1* YES, *TTFields* tumor-treating fields

Table 2 Acute adverse events in six patients during the first four weeks after the first infusion of GD2 + PSMA CAR-T cells

Event	Number of patients			
	Grade1	Grade2	Grade3	Grade4
Cytokine release syndrome	3	0	0	0
Central neurotoxic effects	0	0	0	0
Hematologic toxic effects				
Anemia	2	0	0	0
Neutropenia	2	2	1	0
Thrombocytopenia	0	3	1	0
Leukocytopenia	0	0	0	0
Nervous system				
Epilepsy	0	0	0	0
Encephalalgia	0	0	0	0
Respiratory system				
Pneumonia	0	2	0	0
Dyspnea	0	0	0	0
Abnormal laboratory values				
Elevated alanine aminotransferase level, aspartate aminotransferase level, or both	0	0	0	0
Elevated bilirubin level	0	0	0	0
Electrolyte abnormalities	0	0	0	0
Miscellaneous				
Infection	3	0	0	0
Fever	3	0	0	0
Nausea	0	0	0	0
Diarrhea	0	1	0	0
Bellyache	0	1	0	0

Hematologic toxic effects, anomalies caused by the disease or by previous treatments were not included

in symptom resolution and radiographic improvement. Another patient (Patient 06) developed fever, diarrhea, and vomiting on day 9, suspected to be of bacterial origin with concurrent viral prophylaxis. A combination of cefoperazone-sulbactam and oseltamivir led to resolution of symptoms. The third patient (Patient 04) exhibited fever and respiratory symptoms on day 2, with a confirmed *Pseudomonas aeruginosa* infection from sputum culture. This patient received levofloxacin and tazocin (piperacillin-sulbactum), leading to symptomatic improvement, though recurrent ventilator-associated pneumonia was noted due to mechanical ventilation. All three patients responded to appropriate antibiotic therapy, with resolution of infection-related symptoms. No central nervous system toxicity, including central neurotoxic effects or encephalopathy, was observed in any of the patients. Additionally, there were no significant abnormalities in laboratory values, including liver enzymes, bilirubin levels, or electrolytes. During subsequent long-term follow-up, no cases of hematological issues and CRS were reported and no significant abnormalities in laboratory values were detected (Additional file 2: Supplementary Table 2). Regarding other delayed adverse events, one patient developed grade 1 encephalalgia, and one patient experienced grade 2 pneumonia accompanied by dyspnea and fever. No severe toxicities (grade 3 or higher) were observed. Overall, the adverse reactions observed in this cohort were largely consistent with the expected effects of the chemotherapy drugs, with no unexpected toxicities beyond what is typically observed with such treatments.

Clinical responses and outcomes after GD2 and PSMA CAR-T infusion

To assess the expansion of GD2 and PSMA CAR-T cells, blood samples were collected from all participants at multiple time points after infusion and analyzed using RT-qPCR. As shown in Fig. 2, expansion of GD2 CAR-T and PSMA CAR-T cells was observed in the peripheral blood of all six patients, although with differences in peak timing and persistence of CAR-T cell levels over time. Among the available data, four patients (Patients 01, 02, 03, and 04) still had GD2 CAR-T cells in their bodies four weeks post-infusion, and three patients (Patients 02, 03,

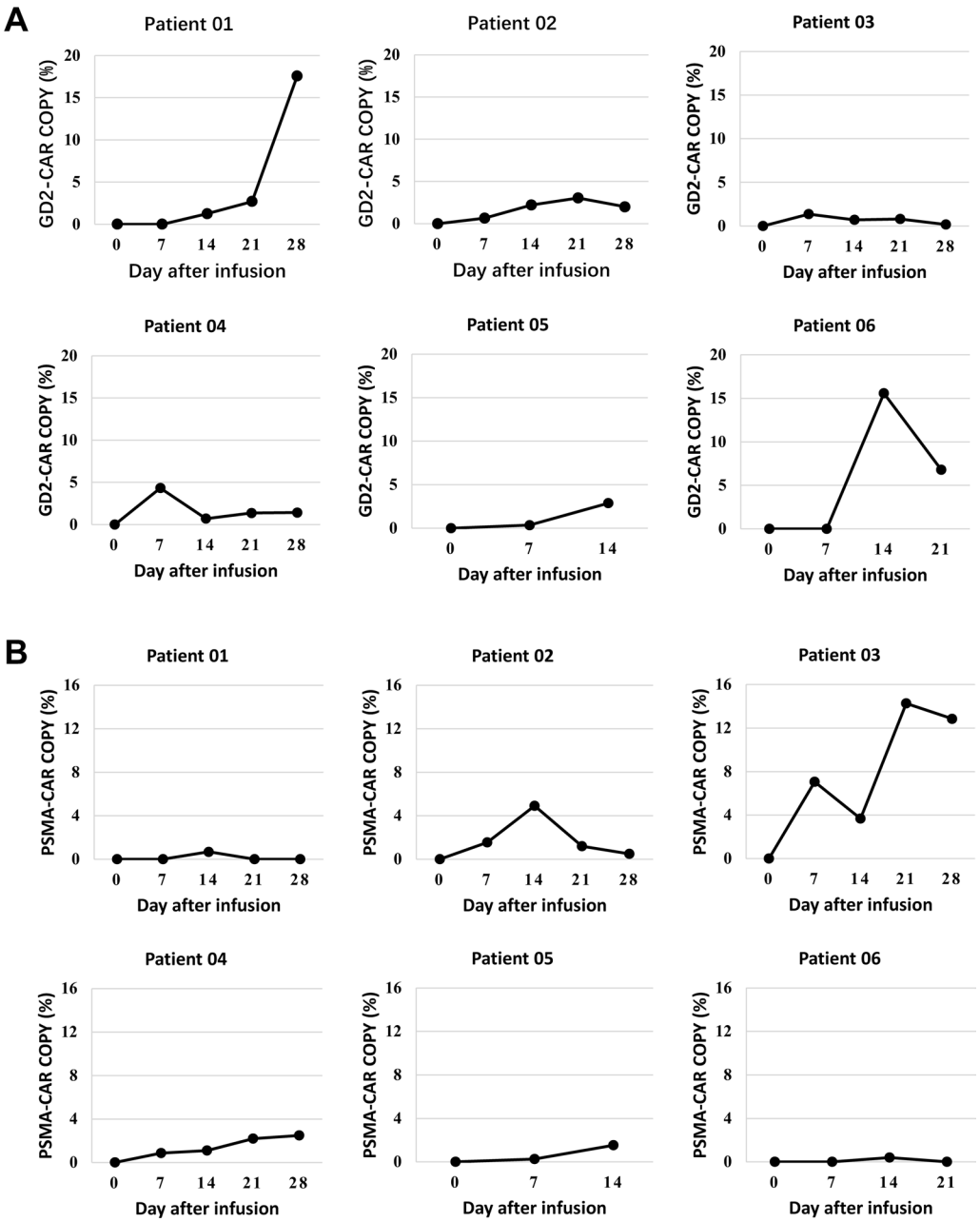

Fig. 2 Expansion and persistence of CAR-T cells determined by RT-qPCR after infusion. **A** Copy number of GD2 CAR-T cells in peripheral blood of six patients. **B** Copy number of PSMA CAR-T cells in peripheral blood of six patients

and 04) retained PSMA CAR-T cells at four weeks post-infusion (Fig. 2A, B). Higher CAR T cell levels following infusion are usually associated with improved responses. However, in this study, no significant effect of exposure, as assessed by peak level and area under the curve (AUC), was observed on clinical outcomes (Additional file 2: Supplementary Fig. 1). Notably, long-term follow-up revealed that GD2 CAR copies (0.77%) were detected in Patient 01 on day 407 post-infusion, both GD2 (0.05%) and PSMA CAR-T copies (0.21%) were detected in Patient 02 on day 202, and PSMA CAR-T copies (1.19%) were identified in Patient 04 on day 292 (Additional file 2: Supplementary Table 3). These results indicated that CAR-T cells could expand rapidly after cell infusion, reach a peak level and then persist at a lower level for an extended period after treatment.

To assess the activity of GD2 and PSMA CAR-T cells, MRI was performed on the brains of six patients after

CAR T cell infusion (Fig. 3A-F). MRI scans revealed a significant reduction in tumor size for Patients 01 and 05 following CAR-T infusion (Fig. 3A and E). All six patients experienced a clinical benefit, defined as either a CR or SD (Table 3), according to the criteria. Four weeks post-infusion, two (Patients 05 and 06) of the six patients achieved CR, representing 33.3% of the cohort. Patient 01 attained CR four months post-infusion, increasing the overall CR rate to 50%. The remaining three patients (Patients 02, 03, and 04) exhibited SD, lasting for 1 month, 5 months, and 9 months, respectively, followed by progressive disease (Table 3). In detail, Patient 03 experienced a 46% reduction in tumor size and an improvement in muscle strength 6 months after infusion (Fig. 3C). Patient 02, who was previously dependent on a ventilator due to a medullary tumor, exhibited a 12% increase in tumor size four weeks post-infusion (Fig. 3B), yet the patient demonstrated significant improvement in muscle strength. Despite disease progression at a later stage, a second CAR-T infusion helped regain disease control, extending survival to 13 months (Fig. 4A). In Patient 04, the presence of diffusely distributed lesions involving the midbrain, bilateral inferior and superior colliculi, brainstem, and medulla oblongata—extending to the C1 spinal segment—precluded comparative analysis using single imaging slices. Therefore, the baseline prominent medullary lesion was selected for longitudinal evaluation (Fig. 3D). Follow-up imaging at seven weeks revealed significant necrosis within the tracked lesion, with no evidence of apparent enhancing foci. However, at nine months, the tumor showed enlargement, leading to a diagnosis of PD. Overall, although all patients eventually developed progressive disease during the follow-up period, and five succumbed to the disease, all survived for more than 13 months post-infusion (Fig. 4A). As of the data cutoff date (August 2024), the median PFS was 9 months (range, 1–56 months), and the median OS was 24.5 months (range, 13–63 months) (Table 3). Notably, patients who achieved CR exhibited a lower tumor burden (< 3 cm) (Fig. 3A, E, and F), and we observed a strong negative relationship between tumor burden and clinical outcomes (Additional file 2: Supplementary Fig. 2), indicating that a higher tumor burden is associated with reduced treatment efficacy. Additionally, patients with lower tumor burdens had significantly longer OS compared to those with higher tumor burdens (Fig. 4B). The two-year OS rate was 100% for patients with low tumor burdens, whereas no patients with high tumor burdens survived beyond two years.

Discussion

Gliomas, particularly aggressive and invasive brain tumors, are typically treated with surgery, radiation, and temozolomide chemotherapy. Despite these interventions, glioblastomas often recur, and no definitive standard of care exists for managing these recurrences. CAR-T cell immunotherapy, which has proven effective in treating hematologic cancers, is also showing potential in the treatment of glioblastomas.

A landmark application of CAR-T therapy in 2016 by Christine E. Brown and her team targeted the interleukin-13 receptor alpha 2 (IL13α2) in a patient with recurrent multifocal glioblastoma, resulting in complete tumor regression in both the brain and spinal cord for 7.5 months [30]. Following this, a Phase I clinical trial utilizing GD2-specific CAR-T cells for glioblastoma patients with histone H3 mutations (H3 K27M) demonstrated clinical and radiographic improvements in three out of four patients [31]. These responses were accompanied by increased levels of pro-inflammatory cytokines in plasma and cerebrospinal fluid, indicating both safety and potential therapeutic efficacy. Additionally, Vitanza et al. confirmed the safety of direct brain administration of CAR-T cells, with patient samples showing active and sustained immune responses [32]. The largest CAR-T study to date, involving 65 patients with recurrent glioblastoma, reported that half of the participants achieved stable disease or better, with a median overall survival of 7.7 months [33]. Notably, those receiving higher doses of CAR-T cells had an extended median survival of 10.2 months [33].

Beyond conventional glioblastoma targets, PSMA has emerged as a promising candidate for CAR-T therapy. Initially identified in prostate cancer cells [34, 35], PSMA is also expressed in approximately one-third of glioblastomas [36]. Its expression has been correlated with higher malignancy on positron emission tomography (PET) imaging [36, 37] and shorter patient survival [38], making it a compelling target for high-grade glioblastomas. Similarly, ganglioside GD2, a well-established marker in neuroblastomas [39, 40], is overexpressed in certain glioblastomas. Preclinical studies utilizing GD2 CAR-T cells in glioblastoma models have demonstrated effective tumor infiltration and control, achieving a 50% objective response rate [41, 42].

Based on the available data and a limited number of studies, both GD2 and PSMA have demonstrated therapeutic potential as targets for gliomas. However, single-target therapies for gliomas have yielded suboptimal survival outcomes, with a median PFS of approximately 7 months. This study established a clinical trial specifically targeting refractory or relapsed gliomas with high PSMA and GD2 expression (Fig. 1). The trial assessed

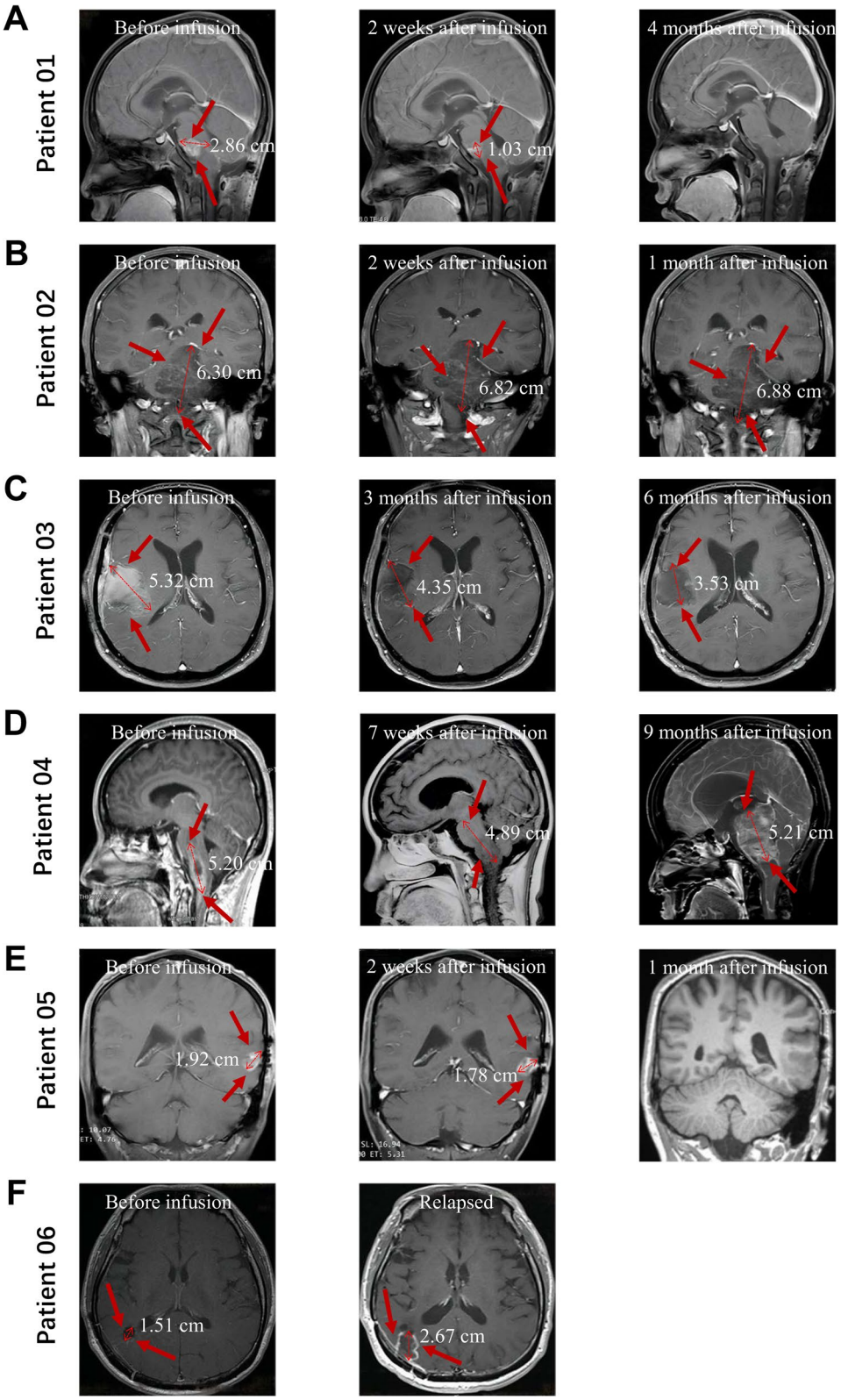

Fig. 3 Magnetic resonance imaging (MRI) scan of brain. **A-F** Brain MRI scans of six patients before and after CAR T cell infusion. Tumors are marked with red arrows. No visible tumor is present in A-right, and E-right, so no red arrows are indicated. Tumor burden was measured by MRI and shown in the corresponding image

Table 3 Patient outcomes

Patient No.	Disease response	Time to progression (m; from infusion)	Survival (m)		Outcome
			From diagnosis	From infusion	
01	CR	9	29	26	DOD
02	SD	1	19	13	DOD
03	SD	5	25	23	DOD
04	SD	9	18	14	DOD
05	CR	28	36	31	DOD
06	CR	56	67	63	Alive

M Months, *SD* stable disease, *CR* complete response, *DOD* Died of disease

the safety and efficacy of a dual-targeting strategy using PSMA CAR-T and GD2 CAR-T cells, with the goal of improving therapeutic responses in these challenging cancer cases. As of August 2024, preliminary results appear promising, with a median OS of 24.5 months (range, 13–63 months) and a median PFS of 9 months (range, 1–56 months) (Table 3). Among the six patients enrolled in the trial, the objective response rate (ORR) was 50%, with all patients achieving at least SD, and half demonstrating a CR (Fig. 4A). Notably, patients who achieved CR exhibited significantly prolonged survival (Fig. 4B), with a median PFS of 28 months and a median OS of 32 months, exceeding typical survival rates for refractory or relapsed glioblastomas.

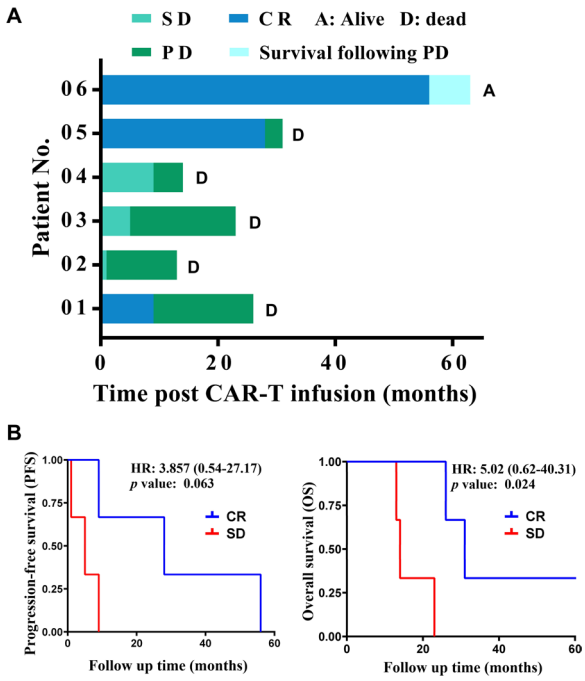

Fig. 4 Overall survival post CAR-T cell infusion. **A** Swimmer plot showing the responses of six patients. **B** PFS and OS of patients who achieved CR and those who exhibited SD following infusion. *CR* complete response, *SD* stable disease, *PD* progressive disease, *PFS* progression-free survival, *OS* overall survival

In comparative analyses involving similar products from the same research center's fourth-generation CAR-T platform, a cohort of eight patients received GD2 single-target CAR-T therapy for gliomas. Among these patients, four achieved partial remission (PR), while three maintained SD for up to 23 months, with a median OS of 10 months [26]. In contrast, our dual-target CAR-T therapy demonstrated extended PFS and OS compared to single-target approaches. These preliminary findings suggest that the dual-target strategy is highly feasible and holds promise for improving treatment outcomes in this patient population.

The current results highlight the effectiveness and manageable safety of combining PSMA CAR-T and GD2 CAR-T therapies for treating gliomas. A critical factor contributing to this success is the incorporation of radiotherapy, which all patients received prior to CAR-T therapy (Table 1). Radiotherapy, a standard initial treatment for gliomas, has been shown to enhance CAR-T efficacy, as supported by both retrospective studies and expert opinions [43, 44]. Research indicates that even low-dose radiation can increase tumor cell apoptosis, promote T cell migration, and improve the overall immune response. Additionally, radiotherapy's ability to penetrate the blood–brain barrier facilitates the targeted delivery of CAR-T cells to the tumor site [44, 45]. However, variations in patient outcomes suggest that further investigation is needed into the immune microenvironment, the modalities and dosages of radiotherapy, and the optimal timing between radiotherapy and CAR-T treatment.

Among the three patients who achieved SD following infusion, Patient 02 had the shortest PFS and OS (Fig. 4A). This patient had a grade IV DMG in the pons, characterized by the H3 K27M mutation (Table 1). This mutation is associated with a poor prognosis,

with a typical survival of less than 12 months. DMG harbors a tumor microenvironment characterized by low levels of infiltrating lymphocytes and pro-inflammatory molecules, which contributes to its resistance to conventional therapies such as radiotherapy and chemotherapy [46]. While preclinical studies have demonstrated some efficacy, early clinical trials suggest that treatment remains challenging due to severe potential side effects and only transient therapeutic benefits [47]. In addition to the tumor's immunosuppressive microenvironment, tumor burden is another critical factor that may contribute to the lack of disease remission in patients following infusion. In this study, patients with a lower tumor burden achieved better clinical benefits (Fig. 3 and Fig. 4), demonstrating a strong negative correlation between tumor burden and treatment response (Additional file 2: Supplementary Fig. 2). This finding highlights the importance of early intervention and tumor control in improving patient outcomes in CAR-T therapy. A high tumor load, a well-established risk factor for CAR-T therapy in other cancers such as lymphoma, is also associated with an increased risk of CRS and poorer clinical outcomes, including reduced PFS and OS. Strategies aimed at reducing tumor burden prior to CAR-T therapy are currently under investigation. Notably, Patient 5, who achieved CR for six years until tumor progression in March 2024, had received tumor-treating fields (TTFields) prior to CAR-T therapy. TTFields, which disrupt critical cellular functions and may enhance anti-tumor immunity [48], combined with radiotherapy and TMZ, represent a promising therapeutic approach. However, the synergistic effects of TTFields with CAR-T therapy warrant further investigation [49]. In particular, CAR T cell levels following infusion are also an important factor in predicting response durability [13]. B cell lymphoma patients in the majority of studies, though not all, consistently show improved responses when experiencing higher peak CAR-expressing cell levels and higher levels of CAR-expressing cells during the first month of infusion [13]. In this study, no apparent effect of exposure on clinical outcome was observed in patients with refractory or relapsed gliomas (Additional file 2: Supplementary Fig. 1). The sample size of this cohort (n = 6) may have limited the statistical power to detect a significant correlation between CAR-T cell kinetics and clinical outcomes. Interpatient variability in baseline tumor burden, and immune status could further contribute to heterogeneous responses, obscuring potential associations. Therefore, further studies with larger cohorts are warranted to elucidate the impact of CAR-T cell expansion kinetics and functional persistence on therapeutic efficacy in gliomas.

The dual-target design of CAR-T cells aims to prevent antigen escape by tumors, potentially extending the durability of treatment. Clinical data suggest that dual-target CAR-T cells may offer a longer duration of response and improved overall survival compared to single-target approaches, with potentially fewer adverse reactions [45, 50]. In this study, the development of PSMA CAR-T and GD2 CAR-T cells, which target specific antigens in gliomas, has demonstrated significant efficacy in a small cohort of patients, all of whom achieved stable disease or better, with no severe therapy-related adverse effects. A recent meta-analysis reported that the average OS for refractory or relapsed gliomas rarely exceeds 5–7 months [51]. Although the sample size (n = 6) of this study is limited, our findings demonstrated a median PFS of 9.0 months (range, 1–56 months) and a median OS of 24.5 months (range, 13–63 months), showing promising preliminary results. Encouraged by these findings, we are planning a follow-up study with an expanded cohort (n = 44) to further investigate the efficacy and safety of PSMA CAR-T combined with GD2 CAR-T cells. The sample size was determined based on the PFS of 9 months observed in our study and the reported PFS of 3 months for the cohort receiving cediranib treatment [52]. Using a two-sided test with $\alpha = 0.05$, 80% power, and an estimated dropout rate of 10%, we calculated the necessary sample size to ensure statistical robustness. We are optimistic that our ongoing efforts will contribute to a more comprehensive understanding of this novel treatment approach.

Conclusions

In conclusion, the dual-target PSMA and GD2 CAR-T cell therapy shows promising efficacy and safety in treating refractory or relapsed glioblastomas, offering improved PFS and OS compared to single-target approaches. However, challenges such as tumor microenvironment resistance and high tumor burden remain. Further investigation, including larger multi-center trials, is necessary to validate these findings and optimize treatment strategies for glioma patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06523-1.

Additional file 1

Additional file 2

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Author contributions
G.J.S. and L.J.S. conducted the research and summarized the data. Z.G., X.J.Y., J.R., C.W., and L.Z.F. collected the clinical cases. C.C.W and C.L.J. performed and supervised CAR-T cell manufacturing. G.J.S., L.J.S., X.Y., Z.G., X.J.Y., J.R., C.W., and L.Z.F. analyzed and interpreted the data. W.H.J. provided critical advice and discussed the work. C.Q.C., M.H.J., and C.L.J. designed and directed the entire study. G.J.S., L.J.S., and C.Q.C. wrote the manuscript with input from the other authors.

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Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Clifford Hospital. All patients included in this study provided written informed consent.

Consent for publication
Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare that they have no competing interests.

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