Jun-Xia Cao, Wei-Jian Gao, Jia You, Li-Hua Wu and Zheng-Xu Wang*

Assessment of the efficacy of passive cellular immunotherapy for glioma patients

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Abstract: To evaluate the therapeutic efficacy of passive cellular immunotherapy for glioma, a total of 979 patients were assigned to the meta-analysis. PubMed and the Cochrane Central Register of Controlled Trials were searched initially from February 2018 and updated in April 2019. The overall survival (OS) rates and Karnofsky performance status (KPS) values of patients who underwent passive cellular immunotherapy were compared to those of patients who did not undergo immunotherapy. The proportion of survival rates was also evaluated in one group of clinical trials. Pooled analysis was performed with random- or fixed-effects models. Clinical trials of lymphokine-activated killer cells, cytotoxic T lymphocytes, autologous tumor-specific T lymphocytes, chimeric antigen receptor T cells, cytokine-induced killer cells, cytomegalovirus-specific T cells, and natural killer cell therapies were selected. Results showed that treatment of glioma with passive cellular immunotherapy was associated with a significantly improved 0.5-year OS ($p = 0.003$) as well as improved 1-, 1.5-, and 3-year OS ($p \leq 0.05$). A meta-analysis of 206 patients in one group of clinical trials with 12-month follow-up showed that the overall pooled survival rate was 37.9% ($p = 0.003$). Analysis of KPS values demonstrated favorable results for the immunotherapy arm ($p < 0.001$). Thus, the present meta-analysis showed that passive cellular immunotherapy prolongs survival and improves quality of life for glioma patients, suggesting that it has some clinical benefits.

Keywords: autologous tumor-specific T lymphocytes; cytotoxic T lymphocytes; glioma; lymphokine-activated killer cells; meta-analysis; passive cellular immunotherapy.

***Corresponding author: Zheng-Xu Wang,** Biotherapy Center, The Seventh Medical Center of PLA General Hospital, No. 5 Nan Men Cang Road, Dongcheng District, Beijing 100700, China, e-mail: zhxuwang@qq.com, zhxwang18@hotmail.com
Jun-Xia Cao, Wei-Jian Gao, Jia You and Li-Hua Wu: Biotherapy Center, The Seventh Medical Center of PLA General Hospital, No. 5 Nan Men Cang Road, Dongcheng District, Beijing 100700, China

Introduction

Malignant gliomas (MGs) are the most common type of primary brain tumors, and glioblastoma multiforme (GBM) is one of the most lethal human cancers. The median survival for newly diagnosed GBM is still less than 2 years. The 5-year overall survival (OS) rates have remained at less than 4% for adults and less than 16% for children (Ishikawa et al., 2012; Ahmed et al., 2017). Attempts to improve the survival of these patients have led to the investigation of immunotherapy as a treatment for malignant brain tumors. Although the idea of using the immune system of the patient against the brain tumor is theoretically attractive, this technique has not yet been demonstrated to be an effective antitumor therapy. In a previous study, we showed that tumor antigen-pulsed dendritic cells (DC) for high-grade glioma (HGG) yielded encouraging results (Cao et al., 2014) for active immunotherapy. Active immunotherapy stimulates the host's immune system, but passive immunotherapy enhances the preexisting immune response. Thus, the term 'passive immunotherapy' classically includes antibody-mediated immunotherapy, cytokine immunotherapy, and adoptive T-cell transfer immunotherapy (Brody et al., 2011). We conducted a meta-analysis to evaluate the effects of lymphokine-activated killer (LAK) cells, cytotoxic T lymphocytes (CTLs), autologous tumor-specific T lymphocytes (ATTLs), cytokine-induced killer (CIK) cells, chimeric antigen receptor (CAR) T cells, and natural killer (NK) cells, collectively referred to as 'passive cellular immunotherapy', on the treatment of glioma patients in terms of survival, some of which have been reported in a recent meta-analysis, including passive immunotherapy, active immunotherapy, and autologous tumor cell immunotherapy (Hanaei et al., 2018).

Many studies have described passive cellular immunotherapy using LAK cells, CTLs, and tumor-infiltrating lymphocytes (TILs) for the treatment of patients with extracranial tumors, such as metastatic melanoma and renal cell carcinoma (Holladay et al., 1992; Quattrocchi et al., 1999; Avril et al., 2011). It was demonstrated that CTLs show higher cytotoxicity than LAK cells against tumors (Plautz et al., 1998). TILs are primarily CTLs that recognize proteolytically cleaved intracellular tumor

antigen fragments (epitopes) associated with specific major histocompatibility complex (MHC)-I antigens on the cell surface (Choi et al., 2014). Subsequently, the complex is recognized by the T-cell receptor of specific T lymphocytes. TILs are relatively specific in terms of their cytotoxicity for tumors due to their recognition of tumor antigen fragments that are associated with specific classes of MHC-I antigens and therefore have potential advantages over LAK cells in terms of tumor specificity and cytotoxicity but in an MHC-restricted manner (Chow et al., 2013). CIK cells are MHC-unrestricted cytotoxic lymphocytes that have been used in clinical trials for the treatment of MGs (Ryu et al., 2017).

A number of preclinical studies have also demonstrated that γδ T cells have potent cytolytic activity against GBM cells (Lamb et al., 2013). A more recent study showed that the absolute count of γδ T cells decreases, and their proliferative capacity is diminished in GBM patients. Moreover, NK cells have been reported to be useful for the immunotherapy of MGs (Ogbomo et al., 2011). In addition, ATTLs comprising $CD8^+$ and $CD4^+$ cells have also been reported to cure recurrent cases of MGs (Jeffes et al., 1993). Thus, various T-cell therapies have been developed for the treatment of glioma in the clinic.

Significantly, recent genetically modified T cells have been readily adapted to GBMs, as CAR T cells show marked and durable efficacy in hematological malignancies. Now, GBM-associated specific tumor antigens such as interleukin (IL)-13 receptor subunit α-2 (IL-13Rα2), HER2, and EGFRvIII are available and can be used in the clinic (Ruzevick et al., 2012; Brown et al., 2016; Ahmed et al., 2017; O'Rourke et al., 2017). In a recent report, IL-13Rα2 CAR T cells were infused in one patient with metastatic GBM and induced complete regression. This clinical response continued for 7.5 months after the initiation of IL-13Rα2 CAR T-cell therapy (Brown et al., 2016). June et al. published the clinical data of 10 recurrent GBM patients treated with EGFRvIII CAR T cells. The study showed that EGFRvIII CAR T cells are feasible and safe to use, without any evidence of off-tumor toxicity or cytokine release syndrome. One patient had stable residual disease for more than 18 months of follow-up (O'Rourke et al., 2017). In another clinical trial, a total of 17 patients were included for the treatment with HER2 CAR T cells. For the entire study cohort, the median OS was 11.1 months from the first T-cell infusion and 24.5 months from the diagnosis (Ahmed et al., 2017). Currently, there are several reviews that have summarized passive cellular immunotherapy clinical trials. However, thus far, no proof of the efficacy of passive cellular immunotherapy with LAK cells, CTLs, ATTLs, CAR T cells, CIK cells, cytomegalovirus (CMV)-specific T cells, or NK cells has been shown using a meta-analysis. Thus, we address the proof of the efficacy of passive cellular immunotherapy with a data comparison in our meta-analysis.

Materials and methods

Participants

Patients with newly diagnosed and recurrent GBM, MG, and HGG were included in our analysis. Patients with glioma who received LAK, CTLs, ATTLs, TILs, CMV-specific T cells, NK, CIK, and CAR immunotherapy were considered eligible and included in this study.

Interventions

The intervention arms of the eligible studies were passive cellular immunotherapy with LAK cells, TILs, CTLs, ATTLs, CAR T cells, CIK cells, CMV-specific T cells, or NK cells.

Outcome measures

OS was defined as the time from the initiation of therapy. We included 0.5-, 1-, 1.5-, 2-, and 3-year OS to analyze the efficacy of passive cellular immunotherapy. Furthermore, the secondary endpoint was the Karnofsky performance status (KPS) that was used to assess the quality of life of glioma patients. We also assessed the 12-month follow-up survival rate in one group of clinical trials and evaluated the factors impacting OS.

Type of studies

The clinical trials containing data before and after therapy or with control were included in this study. Moreover, the proportions of OS in one group of clinical trials at 12-month follow-up were also analyzed in our study.

Literature search and inclusion and exclusion criteria

The trials analyzed in the present study were identified through an electronic search of the PubMed database,

the Cochrane Central Register of Controlled Trials, the Wanfang Database, the China Science and Technology Periodical Database, China Journal Net, reference lists of published trials, and relevant review articles. The search strategy included the medical subject headings 'glioma', 'immunotherapy', 'LAK', 'CTLs', 'TILs', 'NK', 'CIK', 'CARs', and free text searches. No language limitations were imposed. The initial search was performed in February 2018 and updated in April 2019. Furthermore, manual searches were performed based on the reference lists and conference proceedings of the American Society of Clinical Oncology Annual Meetings and the European Cancer Conference. We excluded abstracts that were never subsequently published as full papers and studies performed on animals and cell lines.

Data extraction

We collected various information, including the authors' names, journal and year of publication, sample size per arm, regimen used, median or mean age of patients, culture of cells, delivery route and dosage, and characteristics of the study design (i.e. whether the trial reported the mode of randomization, allocation concealment, description of withdrawals per arm, and blinding), for all trials included in the present study. Two reviewers independently screened the data.

Assessment heterogeneity

We also calculated the quantity I^2, which describes the percentage of variation across studies due to heterogeneity rather than chance. Generally, I^2 values of 25% represent low heterogeneity, and I^2 values of 50% and 75% are evidence of moderate and high heterogeneity, respectively. When no statistically significant heterogeneity existed, the odds ratio (OR) was calculated with a fixed-effect model; otherwise, a random-effect model was employed. $p < 0.05$ was considered statistically significant. All reported p values resulted from two-sided versions of their respective tests (Cao et al., 2014).

Statistical analysis

The analysis was performed using Review Manager version 5.0 (Nordic Cochran Centre, Copenhagen, Denmark). In the meta-analysis, we compared the immunotherapy arms of the selected trials to the respective non-immunotherapy arms. The treatment effects were evaluated based on the ORs for OS. OS data were extracted from each included study, and the pooled OR was calculated using the Mantel-Haenszel method. A pooled OR < 1 indicated a lower recurrence or lower survival in the immunotherapy arm. We used Cochran's Q test, which is χ^2 test with a df equal to the number of studies minus one, to test the null hypothesis, to demonstrate whether the difference among the studies based on the OR reflected chance, and to evaluate whether the results were homogeneous. SPSS 11.5 was also used to carry out the data analysis. Furthermore, we used Comprehensive Meta Analysis version 2.0 to estimate the proportions in one group at one time point.

Results

Selection of the trials

The electronic search yielded 214 references. After a title and abstract review, 144 publications were excluded for different reasons (9 review articles, 8 *in vitro* experiments, 77 animal models, 42 case reports, and 8 comparative studies, editorials). Thus, only 37 clinical trials of 979 patients were included in the present meta-analysis (Figure 1, Tables 1 and 2). All of the studies were published in English. Eventually, only 11 studies (Table 1), including 534 patients, were included as clinical data before and after therapy or with control in the present meta-analysis (Jacobs et al., 1986; Ingram et al., 1987; Kitahara et al., 1987; Vaquero et al., 1991; Jeffes et al., 1993; Hayes et al., 1995; Tsuboi et al., 2003; Dillman et al., 2004; Hongquan et al., 2004; Ishikawa et al., 2004; Kong et al., 2017). In addition, another 18 clinical trials, which did not report controls or

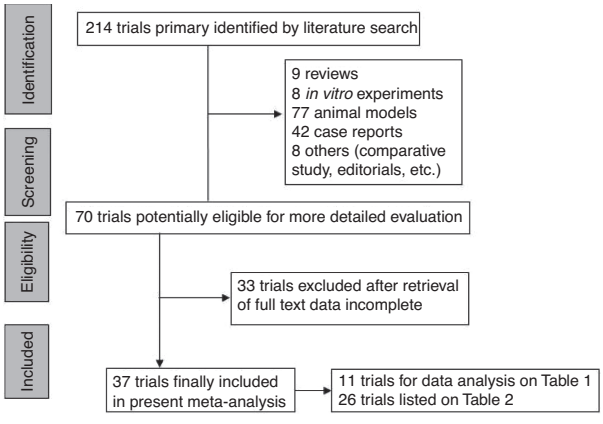

Figure 1: Flow diagram showing the record identification, screening, and study inclusion processes.

Table 1: Clinical information from eligible trials for the meta-analysis.

Trial reference	Tumor characteristic	No. patients (c)	Average/ median age (c)	M/F (c)	Immunotherapy arm	Control arm	Total cell ($\times 10^9$)	Culture of cells	Administration method	Study design
Dillman et al., 2004	Recurrent GBM II and III	31 (41)	50 (47)	18/13 (25/16)	LAK	Unknown	2.0 ± 1.0	IL-2	Ommaya reservoir or stereotactically placed	Nonrandomized
Hongquan et al., 2004	Recurrent or newly diagnosed glioma II–IV	19 (17)	Unknown	Unknown	DC-CTL + BCG	Unknown	Unknown	GM-CSF + IL-4	Intravenously	Randomized
Hayes et al., 1995	Recurrent GBM III and IV	15 (18)	46 (unknown)	7/8	IL-2 + LAK	Chemo	0.7–13	IL-2	Intracavitary	Historical control
Vaquero et al., 1991	Recurrent or newly diagnosed GBM I–IV	A 10 (25), B 14 (25), C 7 (50)	55 (unknown)	19/12	AL + HLBI	Unknown	0.86	Unknown	Intravenously	Historical control
Jacobs et al., 1986	Recurrent or newly diagnosed MG I–III	6 (4)	38.5 (57)	4/2 (4)	LAK	IL-2	0.05–10	IL-2	Intralesional	Nonrandomized
Kitahara et al., 1987	Recurrent or newly diagnosed GBM II–IV	5	41	5	CTL	–	0.5–1.04	IL-2	Ommaya reservoir	Before and after self-control
Ingram et al., 1987	Recurrent or newly diagnosed MG II–IV	29	47.3	21/8	LAK	–	0.11–0.97	IL-2	Intralesional	Before and after self-control
Jeffes et al., 1993	Recurrent HGG III and IV	19	45	16/3	LAK	–	6.3–93	IL-2	Intralesional	Before and after self-control
Tsuboi et al., 2003	Recurrent MG III and IV	10	50.5	5/5	ATTL	–	0.03–2.47	IL-1β + IL-2 + IL-4 + IL-6	Intralesional	Before and after self-control
Ishikawa et al., 2004	Recurrent MG II–IV	9	45.8	5/4	NK + IL-2	–	0.6–13.7	IL-2	Intravenously + intralesional	Before and after self-control
Kong et al., 2017	Newly diagnosed GBM	180 (91)	55 (54)	51 (40), 51 (38)	CIK + standard TMZ chemoradiotherapy	Standard TMZ chemoradiotherapy	10^9–2×10^{10}	IL-2, CD3	Intravenously	Multicenter, open-label, phase III study

Summary of basic information, including tumor stage, number of patients, patient age, and details of the immunotherapy, including cell type, dosage, and loading route. The third row from the end is the culture condition used for the cells. ASL, autologous stimulated lymphocytes (activated T lymphocytes).

Table 2: Clinical information of the eligible trials including 26 studies.

Trial reference	Tumor characteristics	No. patients	Average age	M/F	Immunotherapy arm	Total cells ($\times 10^9$)	Culture of cells	Administration method	Setting
Smith et al., 1996	Recurrent astrocytoma	6	45	2/5	TIL + IL-2	1.0 TIL		Intralesional	
Yoshida et al., 1988	Anaplastic astrocytoma	23	30	8/15	IL-2 + LAK	0.02–0.17	IL-2	Ommaya reservoir	
Schuessler et al., 2014	Recurrent GBM	19	50	12/7	CMV CTL	0.025–0.04	CMV peptide		Phase I
Sankhla et al., 1995	Recurrent malignant primary brain tumors	10	34	7/3	LAK	0.002–0.01		Reservoir catheter	
Holladay et al., 1996	Grade III/IV astrocytoma	15	49	9/6	Mononuclear	10–90	IL-2	Intravenous transfer	
Okamoto et al., 1988	Medulloblastoma	6	6	4/2	IL-2 + LAK	3–15	IL-2	Intrathecal	
Boiardi et al., 1994	Recurrent GBM	9	44	5/4	IL-2 + LAK	0.15	IL-2	Ommaya reservoir	
Brown et al., 2015	Recurrent GBM	3	18–70	12/8	IL13-zetakine CAR	0.375–1.06		Catheter reservoir	
Ghazi et al., 2012	GBM	20	50.1	19/14	CMV-specific T cells		Ad5f35-IE-I-pp65 and IL-2	Infusion	Phase I/II
Dillman et al., 2009	Primary GBM	40	57	6/4	LAK	1.75 ± 0.82	IL-2	Intralesional	
Barba et al., 1989	GBM	10	40	6/7	LAK + IL-2	0.9–21.0	IL-2	Reservoir/catheter	
Merchant et al., 1988	Recurrent GBM	13	48	6/4	LAK + IL-2	0.5–5	IL-2	Ommaya reservoir	Phase I
Lillehei et al., 1991	Recurrent MGs	20	44	15/4	LAK + ASL + IL-2	7.6	IL-2	Intracavitary implantation	Preliminary
Plautz et al., 1998	MG	10	49	17/10	Systemic T cells	0.9–150		Intravenous infusion	
Sloan et al., 2000	Recurrent MGs	19	52.3	8/4	Tumor vaccine + GM-CSF + anti-CD3-activated lymphocytes	20–80	IL-2	Infusion	
Crough et al., 2012	GBM	27	54	7/2	CMV-specific T cells	0.04	IL-2	Infusion	
Kruse et al., 1997	Recurrent glioma	5	29–46	3/1	CTL + IL-2			Catheter	
Quattrocchi et al., 1999	Recurrent MGs	6	49		TILs + IL-2			Ommaya reservoir	Prospective pilot
Plautz et al., 2000	Newly diagnosed gliomas	12	52		Tumor cells + GM-CSF + lymph nodes	6–55	IL-2	Intradermal	Phase I
Wood et al., 2000	Recurrent grade III/IV astrocytoma	9	53		Cancer cell vaccination and lymphocytes	10–100	IL-2	Intravenous infusion	Pilot study
Tsurushima et al., 1999	End-stage MG	4			CTLs + IL-2	0.03–0.21	IL-2	Ommaya tube	
Hayes et al., 2001	MGs	28			LAK + IL-2	0.5–5	IL-2	Ommaya reservoir	Phase I/II
Blancher et al., 1993	Recurrent GBM	5			LAK + IL-2		IL-2	Catheter	Nonrandomized pilot study
Brown et al., 2015	Recurrent multifocal GBM	1	50 (60)	1	IL-13Rα2-CAR T	2×10^6–10×10^6		Catheter device	Phase I
Ahmed et al., 2017	Progressive HER2-positive GBM	24	17 (14)	9/8	HER2-CAR VSTs	1×10^6–$1\times10^8/m^2$		Intravenous infusion	Phase I dose-escalation trial
O'Rourke et al., 2017	Recurrent GBM	10	7 (59.5)	5/5	EGFRvIII-CAR T	1×10^7		Infusions	Phase I study

Summary of basic information, including tumor stage, number of patients, patient age, and details of the immunotherapy, including cell type, dosage, loading route, and culture conditions.

comparisons of the data before and after therapy, with a total of 206 patients, were selected for the analysis of 12-month survival rate in one group (Table 2; Merchant et al., 1988; Okamoto et al., 1988; Yoshida et al., 1988; Barba et al., 1989; Boiardi et al., 1994; Holladay et al., 1996; Sankhla et al., 1995; Smith et al., 1996; Kruse et al., 1997; Plautz et al., 1998; Quattrocchi et al., 1999; Sloan et al., 2000; Wood et al., 2000; Dillman et al., 2009; Schuessler et al., 2014; Brown et al., 2015, 2016; Ahmed et al., 2017).

Characteristics of passive cellular immunotherapy

The tumor grades of the included patients were primarily grades II to IV. Patients included in the study had both newly diagnosed and recurrent GBM, MG, and HGG, but most of the cases were recurrent. The clinical data for the trials are shown in Table 1. The median age of the included patients was 46.7 years according to the available data. Additionally, most of the included patients received LAK cell and CTL therapy without other simultaneous treatments, and controls were primarily nonrandomized; before and after self-control cohorts, only three studies included historical and randomized cohorts, respectively (Vaquero et al., 1991; Hayes et al., 1995; Kong et al., 2017).

Culturing of LAK cells, CTLs, and NK cells is now well-established, and a sufficient number of cells can be generated by IL-2, granulocyte-macrophage colony stimulating factor (GM-CSF), IL-4, IL-1β, and IL-6 but are primarily generated by IL-2. In Tables 1 and 2, we summarize the patient information concerning cell treatment. The number of injected cells ranged from 3×10^7 to 9×10^{10} cells. The control arms included chemotherapy and IL-2. The routes of cell administration included intralesional instillation in seven clinical trials (Jacobs et al., 1986; Ingram et al., 1987; Jeffes et al., 1993; Smith et al., 1996; Tsuboi et al., 2003; Ishikawa et al., 2004; Dillman et al., 2009), intravenous instillation in 11 clinical trials (Ingram et al., 1987; Vaquero et al., 1991; Holladay et al., 1996; Plautz et al., 1998; Sloan et al., 2000; Wood et al., 2000; Hongquan et al., 2004; Crough et al., 2012; Ghazi et al., 2012; Ruzevick et al., 2012; Kong et al., 2017), intracavitary instillation in two clinical trials (Lillehei et al., 1991; Hayes et al., 1995), stereotactically placed Ommaya reservoir or catheter in another 15 clinical trials (Kitahara et al., 1987; Merchant et al., 1988; Okamoto et al., 1988; Yoshida et al., 1988; Barba et al., 1989; Blancher et al., 1993; Boiardi et al., 1994; Sankhla et al., 1995; Kruse et al., 1997; Quattrocchi et al., 1999; Tsurushima et al., 1999; Hayes et al., 2001; Dillman et al., 2004; Brown et al., 2015; Ahmed et al., 2017), and intradermal instillation in one clinical trial (Plautz et al., 2000). In particular, we included three clinical trials, including 28 patients treated with CAR T cells, which have achieved rapid use in hematological malignancies (Brown et al., 2016; Ahmed et al., 2017; O'Rourke et al., 2017). The specific tumor targets were IL-13Rα2, HER2, and EGFRvIII, and the CAR T-cell dose was 1×10^6 to 1×10^7 cells.

Survival

0.5-, 1-, 1.5, and 2-year OS

Information on 0.5-year survival was available for five trials (Jacobs et al., 1986; Vaquero et al., 1991; Hayes et al., 1995; Dillman et al., 2004; Hongquan et al., 2004). These five trials contained 282 patients (102 patients who received immunotherapy and 180 patients who did not receive immunotherapy as controls). The 0.5-year OS rates were 75% (76/102) for glioma patients who received immunotherapy treatment and 48% (87/180) for control cohorts. The estimated pooled OR for these five trials showed a significantly improved 0.5-year OS for patients who received LAK and CTL passive immunotherapy compared to the nonimmunotherapy group [OR, 3.47; 95% confidence interval (95% CI), 1.54–7.86; $p=0.003$; Figure 2A]. Data regarding 0.5-year survival could not be estimated in one trial (Hongquan et al., 2004).

Information on 1-year survival was also available for six trials (Jacobs et al., 1986; Vaquero et al., 1991; Hayes et al., 1995; Dillman et al., 2004; Hongquan et al., 2004; Kong et al., 2017), and the included patients did not fully overlap with those in the 0.5-year OS analysis. These six trials contained 462 patients (193 patients who received immunotherapy and 269 patients who did not receive immunotherapy as controls). The 1-year OS rates were 63% (122/193) for glioma patients who received immunotherapy treatment and 40% (108/269) for controls. The meta-analysis showed a significantly improved 1-year OS for patients who received immunotherapy compared to those who did not (OR, 1.91; 95% CI, 1.12–3.26; $p=0.02$; Figure 2A). Data regarding 1-year survival was zero in one trial and could not be estimated (Vaquero et al., 1991).

Information on 1.5-year survival was available for four trials (Vaquero et al., 1991; Hayes et al., 1995; Dillman et al., 2004; Hongquan et al., 2004). These four trials contained 321 patients (156 patients who received immunotherapy and 165 patients who did not receive immunotherapy as controls). The 1.5-year OS rates were 58% (91/156) for glioma patients who received immunotherapy treatment and 39% (64/165) for controls. The meta-analysis showed a

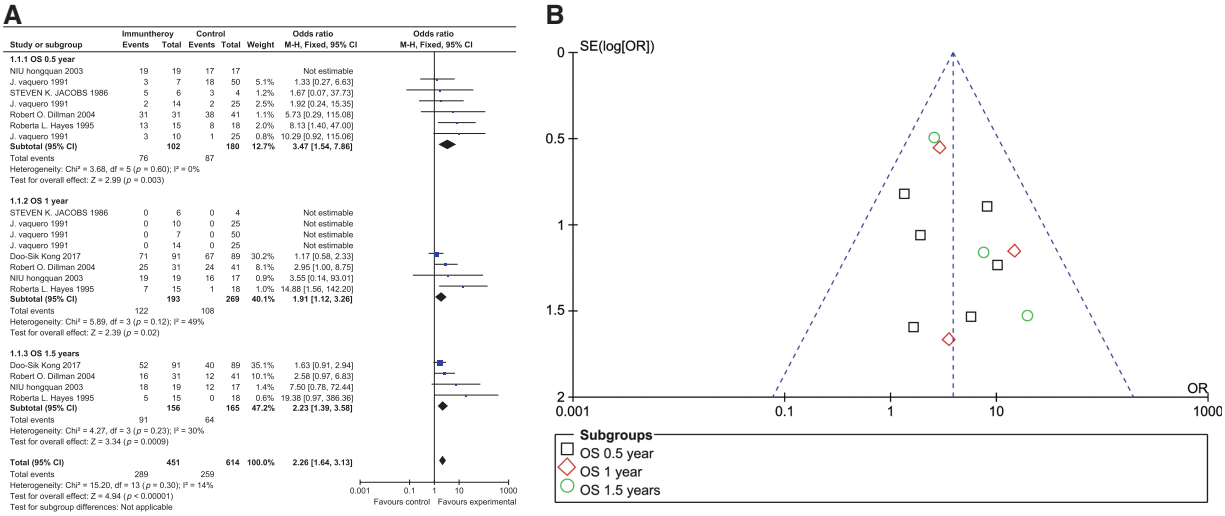

Figure 2: Comparison of 0.5-, 1-, and 1.5-year OS between immunotherapy and control groups.
(A) Forest plot of OS. Fixed-effects meta-analysis model (Mantel-Haenszel method) was used. Immunotherapy and control groups. Each trial is represented as a square, and the OR for each trial is shown in the center. The size of the square is proportional to the information in that trial. The ends of the horizontal bars denote 95% CI. The black diamond shows the overall OR for the combined results of all trials. (B) The funnel plot represents the selected data for 0.5-, 1-, and 1.5-year OS for a separate study, indicating the publication bias of the test. Log[OR], natural logarithm of OR. Horizontal line, mean effect size.

significant improvement in 1.5-year OS in glioma patients who received immunotherapy compared to those who did not (OR, 2.23; 95% CI, 1.39–3.58; $p = 0.0009$; Figure 2A).

Information on 2-year survival was available for four trials (Vaquero et al., 1991; Hayes et al., 1995; Dillman et al., 2004; Hongquan et al., 2004) that contained 321 patients who fully overlapped with patients in the 1.5-year OS analysis. The 2-year OS rates were 42% (66/156) for glioma patients who received immunotherapy treatment and 31% (51/165) for controls. The estimated pooled OR for these three trials showed no significantly increased 2-year OS for patients who received immunotherapy compared to those who did not (OR, 1.58; 95% CI, 0.98–2.54; $p = 0.06$; Figure 3).

Cochran's Q test yielded $p > 0.05$, and the corresponding I^2 quantity was nearly 50%, indicating that the degree of variability between the trials was consistent with that expected to occur by chance alone (Figures 2A and 3); therefore, a fixed-effect model was employed. In addition, the black square of the funnel plot (Figure 2B) denoted the 0.5-, 1-, and 1.5-year OS, and the symmetry of relative risk among the included studies indicated a potential selection bias, but the results showed no bias in the present analysis.

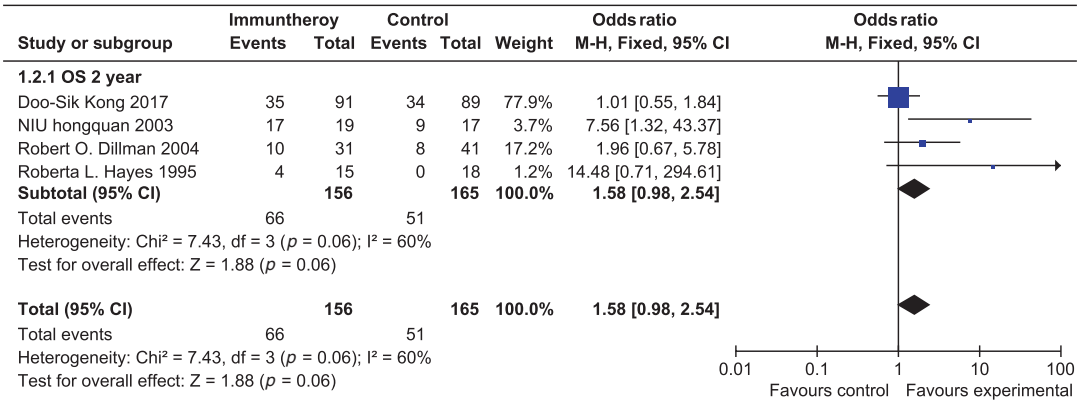

Figure 3: Comparison of 2-year OS between immunotherapy and control groups.
Fixed-effects meta-analysis model (Mantel-Haenszel method) was used in the present analysis.

Three-year OS

Information on 3-year survival was available for three trials (Hayes et al., 1995; Dillman et al., 2004; Hongquan et al., 2004) that contained 141 patients who did not fully overlap with patients from the 1.5- and 2-year OS analyses. These three trials included 141 patients (65 patients who received immunotherapy and 76 patients who did not receive immunotherapy as controls). The 3-year OS rate was 29% (19/65) for glioma patients who received immunotherapy and 3% (2/76) for controls. The meta-analysis showed a significantly longer 3-year OS for patients who received immunotherapy than for those who did not (OR, 11.36; 95% CI, 3.01–42.87; $p = 0.0003$). Cochran's Q test had $p = 0.86$, and the corresponding I^2 quantity was 0% (Figure 4).

To clearly depict the results, we combined all of the survival intervals into one table (Table 3) that demonstrates the number of included patients, OR, P, and heterogeneity separately for the 0.5-, 1-, 1.5-, 2-, and 3-year OS analyses.

Furthermore, the factors influencing the OS of the patients in these six included trials, such as age (≤50, >50 years), recurrence (with recurrence or no recurrence), various cell products [LAK cells, DC-CTLs, AL + human lymphoblastoid interferon (HLBI) and CIK cells], and administration mode (intravenous, by Ommaya reservoir, intracavitary, and intralesional) were analyzed by χ^2 test (Table 4). There was no statistically significant difference in age, with all Ps > 0.05. However, the cell type and administration mode did impact the OS of cell therapy based on the χ^2 test (Table 4). Furthermore, other clinical information from the trials, such as tumor grade and performance status, were not analyzed due to insufficient data.

Survival rate in one group of clinical trials with 12-month follow-up

The survival rates for patients treated with passive cellular immunotherapy in each clinical trial varied widely, ranging from 8.7% (Yoshida et al., 1988) to 87.5% (Dillman et al., 2009). Figure 5 shows the overall estimate of survival rate at 12 months of follow-up after immunotherapy and the 95% CI from the individual studies. Meta-analysis of all 18 studies yielded an overall pooled survival rate with 12-month follow-up of 37.9% (95% CI, 30.5–45.8; $p = 0.003$).

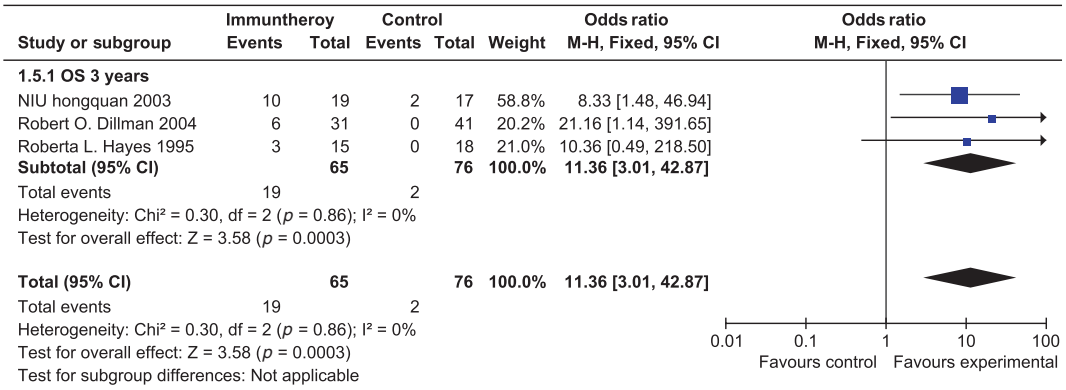

Figure 4: Comparison of 3-year OS between immunotherapy and control groups.
Fixed-effects meta-analysis model (Mantel-Haenszel method) was used in the present analysis.

Table 3: Comparison of OS between passive immunotherapy and control groups.

Event	No. of trials	No. of patients		OR (95% CI)	*p*-Value	Heterogeneity (I^2)
		Passive immunotherapy	Control			
0.5-year OS	5	102	180	3.47 (1.54–7.86)	0.003	0%
1-year OS	6	193	269	1.91 (1.12–3.26)	0.02	49%
1.5-year OS	4	156	165	2.23 (1.39–3.58)	0.0009	30%
2-year OS	4	156	165	1.58 (0.98–2.54)	0.06	60%
3-year OS	3	65	76	11.36 (3.01–42.87)	0.0003	0%

Forest plot comparing the 0.5-, 1-, 1.5-, 2-, and 3-year OS between passive cellular immunotherapy and control groups. Due to the low heterogeneity detected, fixed-effect model was used in the OS meta-analysis.

Table 4: Factors impacting OS assessment in the different therapy groups.

Factors (χ^2 test, p-value)	0.5-year OS	1-year OS	1.5-year OS	2-year OS	3-year OS
Age (≤50, >50 years)	0	0.406	0.137	0.232	–
Recurrence	0	0.072	0.029	0.038	0.01
Various cells (LAK/others)	0.132	0.072	0.029	0.038	0.01
Administration (intravenous/others)	0.132	0.072	0.029	0.038	0.01

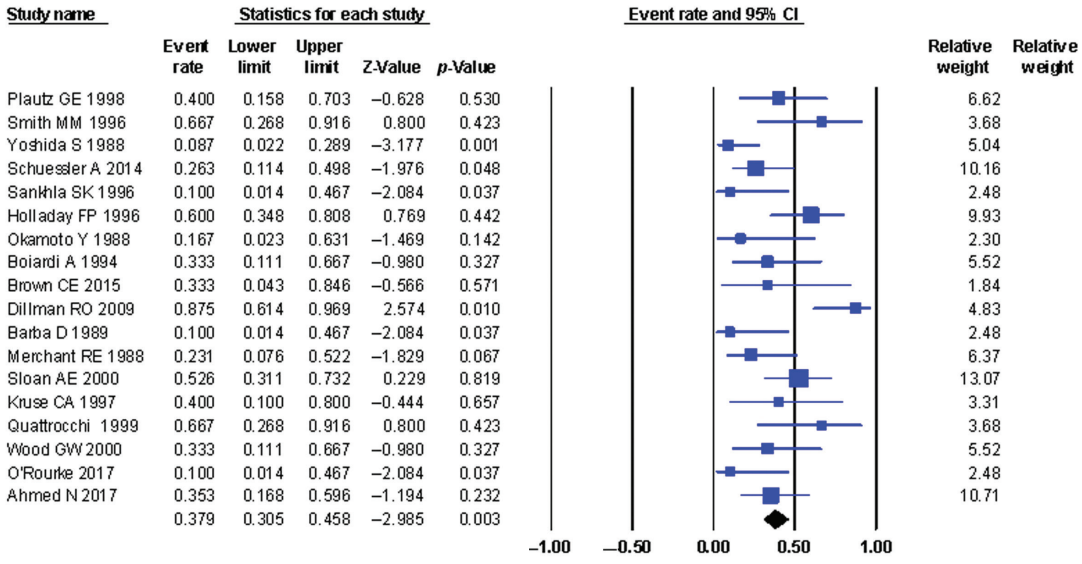

Figure 5: Forest plot showing the survival rates 12 months after passive immunotherapy and the CIs in each study and overall.

KPS

Immunotherapy may also improve the quality of life of postoperative patients. Information on KPS values was available for eight trials, including a total of 110 glioma patients (Ingram et al., 1987; Kitahara et al., 1987; Merchant et al., 1988; Okamoto et al., 1988; Jeffes et al., 1993; Hayes et al., 1995; Tsuboi et al., 2003; Ishikawa et al., 2004). Analysis of KPS values demonstrated favorable results for the immunotherapy arm (OR, 5.47; 95% CI 4.54–6.40; $p < 0.001$; Figure 6). Cochran's Q test had $p = 0.13$, and the corresponding I^2 quantity was 39%, indicating that the

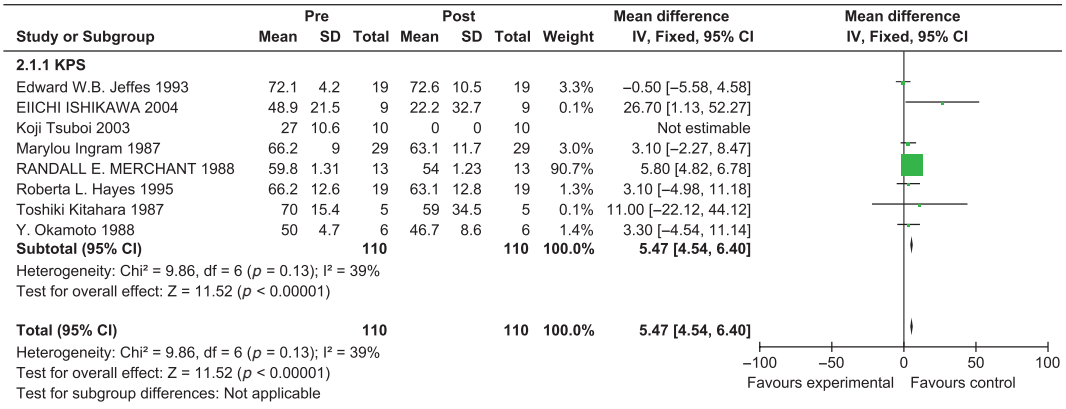

Figure 6: Forest plot for the KPS assessment.
Data were collected from patients before and after immunotherapy. Fixed-effects meta-analysis model (Mantel-Haenszel method) was used in the present analysis.

degree of variability between the trials was consistent with that expected to occur by chance alone.

Discussion

Because of the high rate of local recurrence, there has also been interest in other local therapies and immunotherapies that might improve the survival rate of patients with glioma (Chung et al., 2014; Dixit, 2014). Numerous centers have studied various forms of immunotherapy, including LAK cells, CTLs, TILs, NK cells, ATTLs, CIK cells, CAR T cells, and autologous lymphocyte (ALs) cells, for brain neoplasms (Nagasawa et al., 2012; Brown et al., 2016; O'Rourke et al., 2017; Rodriguez et al., 2017). Until recently, there have been four meta-analysis studies evaluating the effects of active immunotherapies with DCs for glioma (Cao et al., 2014; Wang et al., 2014; Artene et al., 2018; Vatu et al., 2018); now, we conducted a meta-analysis only to address passive cellular immunotherapy for glioma patients.

The present systematic meta-analysis yielded three major findings. First, passive cellular immunotherapy can significantly improve the 0.5-, 1-, and 1.5-year OS ($p < 0.05$) of glioma patients compared to controls. A meta-analysis of the data for patient outcomes revealed that immunotherapy significantly influences the 3-year OS ($p < 0.001$). Second, KPS values, which reflect patient quality of life, of glioma patients were significantly increased after immunotherapy ($p < 0.001$). Third, the analysis of 12-month follow-up after immunotherapy showed an overall pooled survival rate of 37.9% ($p = 0.003$) in one group and in one time point. Overall, according to the present analysis, passive cellular immunotherapy can prolong OS to some degree, and lead to a significant improvement in KPS values for glioma patients, consistent with the results of a previous study reporting on DC therapy for HGG patients (Cao et al., 2014; Wang et al., 2014; Artene et al., 2018; Vatu et al., 2018). In addition, for passive immunotherapy, a meta-analysis included nine studies, in which three studies of LAK cell immunotherapy have been contained in our meta-analysis (Hayes et al., 1995; Hayes et al., 2001; Dillman et al., 2009), but they still included another six studies of monoclonal antibody immunotherapy, which would be different with our analysis (Hanaei et al., 2018).

In the present meta-analysis, the comprehensive results showed that the 0.5-, 1-, 1.5-, 2-, and 3-year OS rates were 75%, 63%, 58%, 42%, and 29%, respectively, whereas controls had 0.5-, 1-, 1.5-, 2-, and 3-year OS rates of 48%, 40%, 39%, 31%, and 3%, respectively. The 2-year OS rates were 42% for the immunotherapy group and 31% for the control group, which included four trials, and there was no significant improvement in 2-year OS. However, the 3-year OS rates (29% for the immunotherapy group and 3% for the control group) contained three trials and showed a significant improvement in 3-year OS; therefore, analysis of 2- and 3-year OS from different trials accounts for the observed bias. However, the meta-analysis still showed significant improvement for 0.5-, 1-, and 1.5-year OS; thus, it would be better to implement passive cellular immunotherapy, as it can prolong the OS of glioma patients. Furthermore, the 4- and 5-year OS rates were only reported in one clinical trial each at 26.3% (Hongquan et al., 2004). These rates were higher than the rates reported for patients who underwent DC treatment in a previous study, which were approximately 20% for 4-year OS and 14% for 5-year OS (Cao et al., 2014). Thus, although the present analysis demonstrated a positive effect of passive immunotherapy on the OS of glioma patients, additional long-term follow-up studies are needed.

Regarding KPS, the median survival of glioma patients with KPS ≥ 60 ranged from 22 to 32 weeks, with less than 5% surviving for more than 1 year after reoperation (Hayes et al., 1995; Ammirati et al., 1987; Hervey-Jumper and Berger, 2014). In the present meta-analysis, the KPS values of the patients included in the selected papers at the time of treatment ranged from 27 to 72. Significant improvement in KPS values between pretreatment and posttreatment was observed in the selected papers, which included 110 patients (Kitahara et al., 1987; Ingram et al., 1987; Merchant et al., 1988; Okamoto et al., 1988; Jeffes et al., 1993; Hayes et al., 1995; Tsuboi et al., 2003; Ishikawa et al., 2004). The highest KPS value for the included patients was 72, but it did not increase after immunotherapy (Jeffes et al., 1993), and the lowest KPS value for the included patients was 27, which decreased to zero after treatment (Tsuboi et al., 2003). The KPS values of the recruited patients in most of the clinical trials were ≥60. An increase in KPS ≥ 30 points is correlated with improved or stable neurological function, but a decrease in KPS = 50 reflect worsening clinical symptoms (Hayes et al., 1995; Tsuboi et al., 2003). In the present meta-analysis, we propose that passive cellular immunotherapy may also improve the quality of life of glioma patients ($p < 0.001$), which have been demonstrated in Figure 6. Moreover, the follow-up time was varied to evaluate the KPS values in each trial, which may have induced bias into the analysis of the results; thus, these data limit the analysis and impact of the potential significance of the immunotherapy. Furthermore, passive cellular immunotherapy was safe and feasible and no complications or significant side

effects were reported in the clinical trials, and more randomized trials are needed to establish therapeutic benefit.

Direct intracerebral delivery by infusion at the tumor site at the time of surgery or using catheter/reservoir systems is often used for immunotherapy with LAK cells and CTLs. Indeed, this particular route bypasses the finely regulated migration through the blood-brain barrier and therefore enables a high number of effector cells at the tumor site (Sankhla et al., 1995; Marchi et al., 2010). In the present meta-analysis, 2 of the 10 trials used reservoir systems (Kitahara et al., 1987; Dillman et al., 2004), 7 of the trials used an intralesional injection route, and a few used an intravenous route; thus, the method of administration of immunotherapy also impacts the efficacy of the treatment and the prognostic results. Furthermore, we also analyzed the patient age, recurrence, cell type and methods of administration using χ^2 test, and the results showed that some of the factors influenced the OS of patients treated with passive immunotherapy ($p < 0.05$). However, comprehensive analysis and recruitment of different studies did not produce any heterogeneity, as demonstrated in Figures 2–4, and according to the present meta-analysis, the 0.5-, 1-, 1.5-, and 3-year OS rates were significantly improved in patients treated with immunotherapy compared to controls. In the present analysis, we examined cellular immunotherapy used to treat gliomas over a decade from as early as 1986 to as late as 2015, including 37 clinical trials covering 979 cases, which has not been reported in previous studies (Chow et al., 2013; Cao et al., 2014; Chung et al., 2014; Dixit, 2014).

T-lymphocyte-mediated cancer immunotherapy is now a clinical reality. CAR-engineered T cells targeting the tumor-associated antigen IL-13Rα2 (ClinicalTrials.gov number, NCT02208362) were reported in December 2016 (Brown et al., 2016). In April and July 2017, NCT01109095 and NCT02209376 have also been published (Ahmed et al., 2017; O'Rourke et al., 2017). A total of 28 patients received CAR T-cell treatments in these three clinical trials and are listed in Table 2, and the 12-month survival rate was analyzed in one group on Figure 5 ($p = 0.003$). As combination targeting of these tumor-associated antigens may offset the immunity escape mechanism (Hegde et al., 2013), recent trials with CMV-specific T cells are also listed in Table 2 (Crough et al., 2012; Ghazi et al., 2012; Schuessler et al., 2014) that included 66 glioma patients, and after treatment with CMV peptide-specific T cells, these patients demonstrated expression of transgenic cytokines, chimeric cytokine/chemokine receptors, or signaling molecules to evade tumor immune suppression and improve homing efficacy. In addition, one multicenter, open-label, randomly assigned phase III study has been performed, and the addition of CIK cell immunotherapy to standard chemoradiotherapy with temozolomide (TMZ) improved progression-free survival (clinicaltrials.gov NCT00807027). Thus, CAR and CMV T cells, including CIK, CTL, LAK, and NK cells, targeting tumor-specific antigen may be a promising approach for the treatment of invasive brain tumors and would be a development direction for future cellular immunotherapy.

Briefly, passive cellular immunotherapy, including the use of CTLs, LAK cells, ALs, CIK cells, CAR T cells, CMV T cells, and NK cells, has yielded encouraging results with clinical benefits for glioma patients based on the present meta-analysis, but we must address the likelihood of selection and evaluation biases.

Limitations of the study

Although several early-phase clinical trials have demonstrated promising therapeutic outcomes thus far, clinical immunotherapy trials for gliomas have not yet demonstrated objective results in randomized and controlled, double-blinded, or multicenter studies. In our meta-analysis, only one multicenter, open-label, phase III study including 180 patients was included, which had the largest sample size of all the selected clinical trials (Kong et al., 2017). Although it strengthened the results of the meta-analysis, it may have introduced some bias. Thus, the limitations of the present analysis should be considered when interpreting the results. In the present meta-analysis, not all of the included studies reported random allocation concealment, and in most cases, we collected data from nonrandomized controls or historical cohorts. In particular, a randomized, double-blind, phase III trial showed that rindopepimut did not increase survival in patients with newly diagnosed GBM. Combination approaches that include rindopepimut might be required to show efficacy of immunotherapy in GBM (Weller et al., 2017). Thus, early-phase clinical trials of rindopepimut based on historical controls may skew the results in favor of immunotherapy. Additionally, to maintain consistency in the present systematic review, we only selected assessable patients for analysis; thus, the literature selection process may have had great potential to skew the findings.

Moreover, the numbered of cells infused varied from 3×10^7 to 9×10^{10}, and the cell phenotypes included CTLs, LAK cells, ALs, CAR T cells, CIK cells, CMV T cells, and NK cells. Thus, the present analysis may have led to an overestimation of the treatment effects. In addition, the recruited patients for every included clinical trial may have

had less dense disease, better immune function, less prior chemotherapy, etc., which needs to be addressed before any conclusions can be drawn. However, there was no significant heterogeneity in the extracted data, as shown in Figures 2–5. Therefore, we expect that the present study will be valuable for the design of more comprehensive, larger, controlled clinical trials of immunotherapy with CTLs, LAK cells, ALs, CIK cells, NK cells, and CAR T cells, as previous meta-analyses have reported the results of DC immunotherapy in glioma patients (Cao et al., 2014; Ahmed et al., 2017).

Taken together, these data suggest that passive cellular immunotherapy with LAK cells, CTLs, CAR T cells, CIK cells, CMV T cells, and NK cells has great potential for clinically efficacious therapy in the treatment of advanced-stage glioma patients who exhibit poor tolerance for chemotherapy or radiotherapy, but the results should be verified more stringently before application in the clinic.

Compliance with ethical standards

Ethical approval: This article does not contain any studies involving human participants or animals performed by any of the authors.

Data sharing statement: The authors confirm that all data underlying the findings are fully available without restriction. All the data underlying the results described in our manuscript can be found and are freely available to other researchers in the body of the manuscript and the supplementary. No additional data are available.

Declaration of conflict of interest: None.

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