BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072484
Article Type:	Protocol
Date Submitted by the Author:	03-Feb-2023
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Keywords:	Neurology < INTERNAL MEDICINE, Neurobiology < NATURAL SCIENCE DISCIPLINES, Neurological oncology < NEUROLOGY, ONCOLOGY

Efficacy of Cell-based Immunotherapies on Patients with Glioma: an umbrella review of systematic reviews and meta-analysis

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Abstract:

Introduction: Glial brain tumors are highly mortal and are noted as major neurosurgical challenges due to frequent recurrence or progression. Despite standard-of-care treatment for gliomas, the prognosis of patients with higher-grade glial tumors is still poor, and hence empowering anti-tumor immunity against glioma is a potential future oncological prospect. This review is designed to improve our understanding of the efficacy of cell-based immunotherapies for glioma.

Methods and analysis: This systematic review will be performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive search of main electronic databases: PubMed/MEDLINE, Scopus, ISI web of Science EMBASE and ProQuest until 2 November (2022) on original articles, a by followed review manual of review articles. Only records in English and only clinical trials will be encountered for full-text review. All the appropriate studies that encountered the inclusion criteria will be screened, selected and then extracted data by two independent authors. For Meta-analyses, data heterogeneity for each parameter will be first evaluated by Cochran's Q and I2 statistics. In case of possible heterogeneity, a random-effects meta-analysis will be performed and for homogenous data, fixed-effects models will be selected for reporting the results of the proportional meta-analysis. Bias risk will be assessed through Beggs' and Eggers' tests and will also be visualized by Funnel plots.

Ethics and dissemination: As this study will be a systematic review without human participants' involvement, no ethical registration is required and meta-analysis will be presented at a peer-reviewed journal.

PROSPERO registration number: CRD42022373297

Keywords: Glioma, chimeric antigen receptor T cells (CAR T) cells, dendritic (DC) cells, Adoptive T cells, cytokine-induced killer (CIK) cells, natural killer (NK) cells

Strengths and limitations of this study

- This review is the first umbrella review of systematic reviews and meta-analyses evaluating the efficacy of Cell-based Immunotherapies on Patients with Glioma.
- Meta-analysis of studies according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.
- A comprehensive literature search from multiple databases was conducted.
- The search was restricted to English-language articles only.
- The Limited number of studies will be met the inclusion criteria.

Introduction:

Gliomas are among highly mortal neoplastic lesions which remain a major neuro-oncological concern due to their frequent recurrence/progression despite standard treatments [1]. Up to the present, numerous attempts have been devoted to improve the efficacy of the current standard-of-care treatment for gliomas which comprise concurrent chemo-radiation and surgical interventions [2]. The major challenges limiting the efficacy of the standard-of-care treatments for gliomas comprise the infiltrative nature of grade-high gliomas which limits the efficacy of total aggressive surgery due to residues remaining and also tumor heterogeneity. Another main concern is the mesenchymal-transformed cells referred to as cancer stem cells in the glioma tumor microenvironment (TME) which are resistant to chemo-radiation. This piece of evidence proves the ultimate need for designing treatment strategies with precision to individual characteristics of the tumor in each patient.

A key feature in the glioma pathogenesis is its immune-suppressed microenvironment due to the pauci nature of the brain as an immune-privileged site and also the overproduction of angiogenic signals in the glioma TME produced in the hypoxic central niche of highly-proliferating glioma cells which induces generation of tolerogenic dendritic cells (DCs) and impairs the antigen presentation process [3]. The gliomas TME comprise a low density of immune cells making it a "cold tumor" with limited immune contexture. Hence, re-empowering the immune system components (i.e. NK cells, cytotoxic T cells, and DC cells) against gliomas in a coordinated fashion and also transferring autologous/allogeneic immune cells (i.e. adoptive immunotherapy) to the tumor site to combat tumoral cells has been of particular interest as a highly precise therapy in the past decades [4]. Standing at the first and foremost stages of interest for such attempts in the previous literature are cellular immunotherapies (e.g, CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

Cellular immunotherapies can comprise both innate and adoptive immune cells. NK cells; granulocytic lymphocytes acting as powerful armamentaria of the innate immune system; are capable of eliminating abnormally-transformed cells without any need for prior sensitization. NK cells recruit to their action site in a chemokine-mediated manner. Some NK cells act as empowered soldiers able to kill numerous and diverse cells named "serial killers" which are noted as potent anti-tumor cells [5]. Moreover, the introduction of Chimeric Antigen Receptor

(CAR) NK cells also represented a step forward toward more efficient NK products [6] and efforts are underway to further clinically translate such immune products from benches to bedsides.

DCs are also key players in the immune system referred to as linkers of adaptive and innate immune responses. DCs enhance NK cell migration and recruitment to the tumor site by the production of numerous chemokines (e.g. CXCL8, CXCL9, and CXCL11) [7]. Further, DCs act as regulators of adaptive/cellular immune responses against tumoral cells mediated by CD8+ cytotoxic T cells by cross-presenting the tumoral antigens via major histocompatibility complex II (MHCII)-antigen complexes [8]. DCs are also responsible for coordinating the immune contexture in the TME by producing chemokines and cytokines which are responsible for an orchestrated migration of immune cells to the tumor site. DC therapy for gliomas has long been studied in clinical settings yielding acceptable results [9] and has introduced a paradigm shift toward more precise glioma management.

Further, the advent of adaptive T cell generation and clinical testing of such immune cell products has yielded promises toward glioma therapy. Early reports have suggested alloreactive T cells for glioma therapy [10]. Testing the autologous lymphocyte transfer has also opened a new toward more precision [11]. Such T cells were activated by several strategies against tumoral cells ex-vivo such as total tumor RNA pulsing. Further, mounting the previous literature, as earlier attempts generating antigen-specific T cells have been of particular interest (e.g. CMV-specific T cells) [12]. Recently, the advent of CAR T cells has revolutionized the advent of T cell therapy for gliomas as well as other neoplastic lesions [13-15]. Genetically engineered T-cells that express CARs can recognize tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs) presented by the MHCs resulting in a powerful anti-tumor immune response. Despite the potential limitations of CAR T cells for solid tumors, in gliomas promises have been obtained in early attempts possibly due to the cold nature of the glioma immune context [16]. CARs can be engineered to target various highly-expressed tumor antigens and can serve as next-generation adoptive cell therapies for gliomas [17]. As future prospects, using combination therapy regimens may yield substantial improvements in the field of glioma immunotherapy. Further, using adjuvants are also potential proposed strategies to improve the efficacy of adoptive immune cell therapy for gliomas [18-21].

Summarizing the results of the efficacy and limitations of the previous attempts on glioma immunotherapies opens door to the discovery of novel techniques and yields insight into the treatment failure causes and ways to overcome them. Herein, we aimed to discuss the main methods that will be applied in a comprehensive meta-analysis for assessing the response efficacy and survival of cell-based immunotherapies (e.g, CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma. The meta-analysis on cell-based immunotherapies aims to provide a hierarchical summary on the road to clinical translation of adoptive immunotherapies for gliomas and also discusses the technical limitations introducing variability in generating GMP-grade immune cell products. The review will also highlight the potential need for standardized protocols for more reproducible and scalable production techniques. Further, the review will discuss the potential strategies to enhance the efficacy of adoptive immunotherapies for gliomas. For instance, using adjuvants and also combination therapy.

Objectives:

This systematic review and meta-analysis aim to summarize the results of previous clinical trials on (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma patients regarding the number of patients, administered doses, adjuvants, antigens/targets, phases, submission dates, completion dates and allocation. Furthermore, this study aims to investigate the immunological efficacy of cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma. Also, this compares the administered dose of each therapy (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells), the survival outcome of the patients enrolled in treatment groups or control groups for each treatment. Moreover, the survival of the patients enrolled in different treatment groups. Further, the immunological response will be compared among the patients receiving each treatment and control groups for each therapy.

2. Methods and analysis:

2.1. Eligibility criteria:

This study follows the Population, Intervention, Comparison, Outcomes, and Study type (PICOS) format for conducting systematic reviews and meta-analyses [22]. According to PICO parts, the eligibility criteria will be met the following:

2.1.1. Participants/population

Inclusion criteria:

This umbrella review will consider systematic reviews that include the population for the current work consisting of patients and controls enrolled in clinical trials for glioma cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

Exclusion criteria:

Studies reporting patients with other cancers will be excluded.

2.1.2. Intervention(s), exposure(s)

The intervention (exposure) of this study will be cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

2.1.3. Comparator(s)/control

Administered doses, percentage of clinical trials targeting each tumoral antigen, immunological efficacy, and survival.

2.1.4. Main outcome(s)

The standardized mean difference for administered doses, the pooled effect size for each antigen for glioma immunotherapy, the pooled effect size of significant immunological responses for each therapy, and the overall survival benefit for each immunotherapy as an indicator of treatment efficacy.

2.1.5. Studies design

Inclusion Criteria:

Only systematic reviews and systematic review and meta-analysis studies will be included.

Exclusion Criteria:

Narrative reviews, commentaries, letters, case reports, case series, experimental studies, and research works in any other language rather than English are excluded from this review. Furthermore, studies suggesting a controversial result will be excluded. No time limit is for exclusion.

2.2. Information sources

The current work includes a comprehensive search of main electronic databases (PubMed, Scopus, ISI Web of Science, EMBASE, and Clinicaltrial.gov) and also is followed by a manual search of the reference lists of the previously-published review articles.

2.3. Search strategy

Search syntax for each main electronic database (PubMed, Scopus, ISI Web of Science, EMBASE, and Clinicaltrial.gov) will be generated according to their rules and Mesh terms [23-25]. An example of the PubMed/MEDLINE search strategy is presented in Table 1. A filter for study type, review, and clinical trial, will be used to minimize the presence of unrelated articles in the recovery search. All the retrieved references will be deposited in a single Endnote file, and after duplicate removal will undergo a title review for relevance.

Table 1. Representative example of the search syntaxes generated for the comprehensive search.

Search syntax for PubMed	
#1	((Glioma[tiab]) OR (Gliomas[tiab]) OR "Glial Cell Tumor*"[tiab] OR (Tumor*[tiab] AND Glial Cell[tiab]) OR "Mixed Glioma" [tiab] OR (Glioma*[tiab] AND Mixed[tiab]) OR "Mixed Glioma*"[tiab] OR "Malignant Glioma*"[tiab] OR (Glioma*[tiab] AND Malignant[tiab]) OR (glioblastoma[tiab]) OR "anaplastic astrocytoma" [tiab] OR "diffuse astrocytoma"[tiab] OR "anaplastic oligodendroglioma"[tiab] OR (oligodendroglioma[tiab]))
#2	((Immunotherapy[tiab] AND Adoptive[tiab]) OR "Cytokine-Induced Killer Cells"[tiab] OR "Dendritic Cells"[tiab] OR (Killer Cells AND Natural[tiab]) OR "cytokine induced killer"[tiab] OR "tumor infiltrating lymphocytes"[tiab] OR "lymphokine activated killer" [tiab] OR (autolymphocyte[tiab]) OR "activated T cells"[tiab] OR "activated killer cells" [tiab] OR "gamma delta T cells"[tiab] OR "γδ T cells" [tiab] OR "NKT cells" [tiab] OR "natural killer"[tiab] OR "NK cells" [tiab] OR "Adoptive Immunotherapy" [tiab] OR "Adoptive Immunotherapies"[tiab] OR (Immunotherapies[tiab] AND Adoptive[tiab]) OR ("Cellular Immunotherapy"[tiab] AND Adoptive[tiab]))

#3	(1992/01/01:2022/11/02[dp])
	#1 AND #2 AND #3

2.4. Selection process

After retrieval of relevant articles and duplicate removal, two individual authors; P.S. and M.N. will go through the title and abstracts of the relevant article to select the relevant qualified articles for the data mining process. In case of any disagreement between the two authors, it will be fixed via consensus and then will be checked by two other authors (S.M.M.Z and A.R.). Irrelevant studies and studies with controversial results will be excluded at this stage. D.A. and M.A. will be asked to build a consensus in cases where discrepant opinions exist.

2.5. Data collection process

Relevant qualified articles will undergo a full-text review in order to extract data from them. Two individual authors; P.S. and F.H.A. will extract data according to the checklist summarized in Excel from each study individually regarding the immunological responses and survival rates. A.M. and V.F.R. will do so for radiological response rates. In case of any disagreement between the two authors, it will be fixed via consensus and then will be checked by two other authors (S.M.M.Z and A.R.). At last, D.A. and M.A. will build consensus for discrepant reports.

The reports of data mining will be presented in tables for each cell-based immunotherapy (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) summarizing in detail the aforementioned parameters. The radiological responses reported according to the guidelines Response Assessment in Neuro-Oncology Criteria (RANO), immunotherapy response assessment for neuro-oncology (iRANO), Response evaluation criteria in solid tumors (RESICT), World Health Organization (WHO) oncology response criteria, Macdonald and AVAglio [26-31] will be summarized as depicted in table 1.

2.7. Quality assessment

The Cochrane Collaboration's tool will be used as the checklist of choice to assess the risk of bias among included studies which comprises 5 major domains including selection bias (random sequence generation and allocation), performance bias, detection bias, attribution bias, and

reporting bias. Each domain will be scored as high, low or unclear as implemented in our previous work [32-35].

2.8. Statistical analysis

For the assessment of heterogeneity among included studies, the I2 statistic defined as the fraction of variance that is due to heterogeneity will be used [36]. Heterogeneity will be categorized as negligible (I2=0–25%), low (I2=25–50%), moderate (I2=50–75%), or high (I2> 75%). Cochran's Q will also be encountered as a complementary measure for heterogeneity [37]. In presence of high heterogeneity, Random Effect Model will be applied by Dersimonian and Laird method and when the heterogeneity is low, the fixed effect model will be applied for meta-analysis [38]. Egger's and Begg's tests will be used to investigate the presence of publication bias [39, 40]. For dose estimation meta-analysis, as a continuous measure, the "Hedges g" statistic as a function for standardized mean difference (SMD) will be used at significant threshold of <0.05 [41]. For proportional data meta-analysis (for radiological and immune response assessment), Freeman-Tukey Transformation (arcsine square root transformation) will be used as the method of choice for meta-analysis [42]. For survival meta-analysis of survival rates (overall or PFS) at specific time points, also Freeman-Tukey Transformation will be performed however for survival meta-analysis with hazard ratios from KM analysis, the generic inverse variance method will be used [43]. Further, in order to visualize the data for better interpretation, the pooled effect size will be depicted by forest plots for each study and also funnel plots will be used for depicting the publication bias status [44]. The asymmetry of the funnel plot will show the presence of publication bias [45].

The results of the bias risk assessment through Cochrane Collaboration's tool and meta-analysis will be summarized in tables depicting each variable, heterogeneity parameters for (I^2 and Q) for the variable, and overall effect size with 95%Cis, and also the forest and funnel plot for each variable will be included.

2.9. Patient and public involvement

Patients and the public are not involved in the preparation of this protocol and will not be directly involved in the final systematic review.

3. Discussion:

In the discussion and conclusion parts, the results of the survival analyses performed will be discussed in detail and also the impact of using adjuvants on improving survival outcomes will be further discussed. In the later sections, previous adjuvants will be summarized and discussed. Regarding the immunological response rates, also a detailed discussion on the overall validity of each parameter for assessing the efficacy of immunotherapy will firstly be discussed and then the results will be compared for each therapy group.

Ethics and dissemination

This review will retrieve published data, so it will not require ethical approval. The findings of this systematic review and meta-analysis will be disseminated via an international peer-reviewed journal publication and several scientific conference presentations.

Ethics statements

Patient consent for publication

Not applicable.

Author Contributions:

D.A, M.N, A.M, and M.A developed the search strategy and participated in writing up the draft of the protocol and S.M.M.Z reviewed the manuscript and edited the final manuscript. All the authors read and approved the final draft. Data screening and selecting phases of the systematic review and meta-analysis will be performed by S.M.M.Z. and P.Sh. Quality assessment and Meta-analysis will be executed by P.Sh., F.H., and A.R. Data extraction and preparing the draft of the manuscript will be performed by S.M.M.Z., V.F., and AM. Moreover, P.Sh., M.N., and S.M.M.Z. will be responsible for reviewing the manuscript and editing the final manuscript.

Funding statement:

The current work was financially supported by the NIMAD institute under grant ID (973168).

Competing interest's statement

The authors declare that they have no conflict of interest.

Protocol and registration

This systematic review has been registered **in the International Prospective Register of Systematic Reviews (PROSPERO)** (http://www.crd.york.ac.uk/PROSPERO), with the systematic review registration number: PROSPERO CRD42022373297 Available from: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=373297

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	P1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	P2, P12
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	P1, P11
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	-
Support:			
Sources	5a	Indicate sources of financial or other support for the review	P11
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	P4-P5-P6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	P6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	P6-P7
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	P7
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	P7-P8

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	P8-P9
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	P7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	P9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I², Kendall's τ)	P10
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	P10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	-

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

BMJ Open

Efficacy of Cell-based Immunotherapies on Patients with Glioma: an umbrella review of systematic reviews and Meta-analysis Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072484.R1
Article Type:	Protocol
Date Submitted by the Author:	22-May-2023
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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Neurology, Oncology

Keywords:	Neurology < INTERNAL MEDICINE, Neurobiology < NATURAL SCIENCE DISCIPLINES, Neurological oncology < NEUROLOGY, ONCOLOGY

Efficacy of Cell-based Immunotherapies on Patients with Glioma: an umbrella review of systematic reviews and Meta-analysis Protocol

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Abstract:

Introduction: Glial brain tumors are highly mortal and are noted as major neurosurgical challenges due to frequent recurrence or progression. Despite standard-of-care treatment for gliomas, the prognosis of patients with higher-grade glial tumors is still poor, and hence empowering anti-tumor immunity against glioma is a potential future oncological prospect. This review is designed to improve our understanding of the efficacy of cell-based immunotherapies for glioma.

Methods and analysis: This systematic review will be performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive search of main electronic databases: PubMed/MEDLINE, Scopus, ISI web of Science EMBASE and ProQuest until 2 November (2022) on original articles, a by followed review manual of review articles. Only records in English and only clinical trials will be encountered for full-text review. All the appropriate studies that encountered the inclusion criteria will be screened, selected and then extracted data by two independent authors. For Meta-analyses, data heterogeneity for each parameter will be first evaluated by Cochran's Q and I2 statistics. In case of possible heterogeneity, a random-effects meta-analysis will be performed and for homogenous data, fixed-effects models will be selected for reporting the results of the proportional meta-analysis. Bias risk will be assessed through Beggs' and Eggers' tests and will also be visualized by Funnel plots.

Ethics and dissemination: As this study will be a systematic review without human participants' involvement, no ethical registration is required and meta-analysis will be presented at a peer-reviewed journal.

PROSPERO registration number: CRD42022373297

Keywords: Glioma, chimeric antigen receptor T cells (CAR T) cells, dendritic (DC) cells, Adoptive T cells, cytokine-induced killer (CIK) cells, natural killer (NK) cells

Strengths and limitations of this study

- This review is the first umbrella review of systematic reviews and meta-analyses evaluating the efficacy of Cell-based Immunotherapies on Patients with Glioma.
- Meta-analysis of studies according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.
- A comprehensive literature search from multiple databases was conducted.
- The search was restricted to English-language articles only.
- The Limited number of studies will be met the inclusion criteria.

Introduction:

Gliomas are among highly mortal neoplastic lesions which remain a major neuro-oncological concern due to their frequent recurrence/progression despite standard treatments [1]. Up to the present, numerous attempts have been devoted to improving the efficacy of the current standard-of-care treatment for gliomas which comprise concurrent chemo-radiation and surgical interventions [2]. The major challenges limiting the efficacy of the standard-of-care treatments for gliomas comprise the infiltrative nature of grade-high gliomas which limits the efficacy of total aggressive surgery due to residues remaining and also tumor heterogeneity. Another main concern is the mesenchymal-transformed cells referred to as cancer stem cells in the glioma tumor microenvironment (TME) which are resistant to chemo-radiation. This piece of evidence proves the ultimate need for designing treatment strategies with precision to individual characteristics of the tumor in each patient.

A key feature in the glioma pathogenesis is its immune-suppressed microenvironment due to the pauci nature of the brain as an immune-privileged site and also the overproduction of angiogenic signals in the glioma TME produced in the hypoxic central niche of highly-proliferating glioma cells which induces generation of tolerogenic dendritic cells (DCs) and impairs the antigen presentation process [3]. The gliomas TME comprise a low density of immune cells making it a "cold tumor" with limited immune contexture. Hence, re-empowering the immune system components (i.e. NK cells, cytotoxic T cells, and DC cells) against gliomas in a coordinated fashion and also transferring autologous/allogeneic immune cells (i.e. adoptive immunotherapy) to the tumor site to combat tumoral cells has been of particular interest as a highly precise therapy in the past decades [4]. Standing at the first and foremost stages of interest for such attempts in the previous literature are cellular immunotherapies (e.g, CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

Cellular immunotherapies can comprise both innate and adoptive immune cells (Figure 1). NK cells; granulocytic lymphocytes acting as powerful armamentaria of the innate immune system; are capable of eliminating abnormally-transformed cells without any need for prior sensitization. NK cells recruit to their action site in a chemokine-mediated manner. Some NK cells act as empowered soldiers able to kill numerous and diverse cells named "serial killers" which are noted as potent anti-tumor cells [5]. Moreover, the introduction of Chimeric Antigen Receptor (CAR)

NK cells also represented a step forward toward more efficient NK products [6] and efforts are underway to further clinically translate such immune products from benches to bedsides.

DCs are also key players in the immune system referred to as linkers of adaptive and innate immune responses. DCs enhance NK cell migration and recruitment to the tumor site by the production of numerous chemokines (e.g., CXCL8, CXCL9, and CXCL11) [7]. Further, DCs act as regulators of adaptive/cellular immune responses against tumoral cells mediated by CD8+ cytotoxic T cells by cross-presenting the tumoral antigens via major histocompatibility complex II (MHCII)-antigen complexes [8]. DCs are also responsible for coordinating the immune contexture in the TME by producing chemokines and cytokines which are responsible for an orchestrated migration of immune cells to the tumor site. DC therapy for gliomas has long been studied in clinical settings yielding acceptable results [9] and has introduced a paradigm shift toward more precise glioma management.

Further, the advent of adaptive T cell generation and clinical testing of such immune cell products has yielded promises toward glioma therapy. Early reports have suggested alloreactive T cells for glioma therapy [10]. Testing the autologous lymphocyte transfer has also opened a new toward more precision [11]. Such T cells were activated by several strategies against tumoral cells ex-vivo such as total tumor RNA pulsing. Further, mounting the previous literature, as earlier attempts generating antigen-specific T cells have been of particular interest (e.g., CMV-specific T cells) [12]. Recently, the advent of CAR T cells has revolutionized the advent of T cell therapy for gliomas as well as other neoplastic lesions [13-15]. Genetically engineered T-cells that express CARs can recognize tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs) presented by the MHCs resulting in a powerful anti-tumor immune response. Despite the potential limitations of CAR T cells for solid tumors, in gliomas, promises have been obtained in early attempts possibly due to the cold nature of the glioma immune context [16]. CARs can be engineered to target various highly-expressed tumor antigens and can serve as next-generation adoptive cell therapies for gliomas [17] (Supplemental Table 1). As future prospects, using combination therapy regimens may yield substantial improvements in the field of glioma immunotherapy. Further, using adjuvants are also potential proposed strategies to improve the efficacy of adoptive immune cell therapy for gliomas [18-21].

Summarizing the results of the efficacy and limitations of the previous attempts on glioma immunotherapies opens the door to the discovery of novel techniques and yields insight into the

treatment failure causes and ways to overcome them. Herein, we aimed to discuss the main methods that will be applied in a comprehensive meta-analysis for assessing the response efficacy and survival of cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma. The meta-analysis on cell-based immunotherapies aims to provide a hierarchical summary on the road to clinical translation of adoptive immunotherapies for gliomas and also discusses the technical limitations introducing variability in generating GMP-grade immune cell products. The review will also highlight the potential need for standardized protocols for more reproducible and scalable production techniques. Further, the review will discuss the potential strategies to enhance the efficacy of adoptive immunotherapies for gliomas. For instance, using adjuvants and also combination therapy.

Objectives:

This systematic review and meta-analysis aim to summarize the results of previous clinical trials on (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma patients regarding the number of patients, administered doses, adjuvants, antigens/targets, phases, submission dates, completion dates and allocation. Furthermore, this study aims to investigate the immunological efficacy of cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma. Also, this compares the administered dose of each therapy (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells), the survival outcome of the patients enrolled in treatment groups or control groups for each treatment. Moreover, the survival of the patients enrolled in different treatment groups. Further, the immunological response will be compared among the patients receiving each treatment and control groups for each therapy. Furthermore, standardization of the protocols used to harvest cells, produce, and scale up the manufacturing process will hugely revolutionize the results obtained from each trial. There is a substantial need to improve guidelines for the GMP-level products moving from benches to bedsides to let the process be more reproducible and reliable. Additionally, standardizing the strategies to assess treatment efficacy will also hugely impact the results of trial pipelines (e.g., immunological response assessment, radiological response assessment criteria such as AVA Glio, RESICT, RANO, or iRANO). In the current meta-analyses, we will discuss the limitations on the way of clinical translation of the GMP level products in the trial pipelines for better outcome management and standardized results reporting.

2. Methods and analysis:

2.1. Eligibility criteria:

This study follows the Population, Intervention, Comparison, Outcomes, and Study type (PICOS) format for conducting systematic reviews and meta-analyses [22]. According to PICO parts, the eligibility criteria will be met the following:

2.1.1. Participants/population

Inclusion criteria:

This umbrella review will consider systematic reviews that include the population for the current work consisting of adult patients and controls enrolled in clinical trials for glioma cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

Exclusion criteria:

Studies reporting patients with other cancers will be excluded.

2.1.2. Intervention(s), exposure(s)

The intervention (exposure) of this study will be cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

2.1.3. Comparator(s)/control

Administered doses, percentage of clinical trials targeting each tumoral antigen, immunological efficacy, and survival.

2.1.4. Main outcome(s)

The standardized mean difference for administered doses, the pooled effect size for each antigen for glioma immunotherapy, the pooled effect size of significant immunological responses for each therapy, and the overall survival benefit for each immunotherapy as an indicator of treatment efficacy.

2.1.5. Studies design

Inclusion Criteria:

Only systematic reviews and systematic review and meta-analysis studies will be included.

Exclusion Criteria:

Narrative reviews, commentaries, letters, case reports, case series, experimental studies, and research works in any other language rather than English are excluded from this review. Furthermore, studies suggesting a controversial result will be excluded. No time limit is for exclusion. Controversies are among the unavoidable issues while collecting huge clinical data from diverse clinical centers worldwide testing a specific therapy in trial pipelines. To cope with, the systematic reviews, several strategies have been proposed such as removing the controversial reports. Herein, when meeting a controversy, the two independent authors reviewing the selected manuscripts will discuss the potential differences and diversities in the cell production process or obtain the efficacy results and will draw a certain conclusion by getting in touch with the corresponding authors. If the conflicting answer is due to inappropriate methodology, will not be considered in the meta-analysis stage. For instance, if the lack of adequate cell count to start the treatment is the reason for the trial failure, that study will not be considered in the meta-analysis stage but will be discussed in a separate section summarizing the failure reasons for each cell-based therapy and solutions to overcome will further be discussed.

2.2. Information sources

The current work includes a comprehensive search of main electronic databases (PubMed, Scopus, ISI Web of Science, EMBASE, and Clinicaltrial.gov) and also is followed by a manual search of the reference lists of the previously-published review articles.

2.3. Search strategy

Search syntax for each main electronic database (PubMed, Scopus, ISI Web of Science, EMBASE, and Clinicaltrial.gov) will be generated according to their rules and Mesh terms [23-25]. An

example of the PubMed/MEDLINE search strategy is presented in Table 1. A filter for study type, review, and clinical trial, will be used to minimize the presence of unrelated articles in the recovery search. All the retrieved references will be deposited in a single Endnote file, and after duplicate removal will undergo a title review for relevance.

Table 1. Representative example of the search syntaxes generated for the comprehensive search.

Search syntax for PubMed	
#1	((Glioma[tiab]) OR (Gliomas[tiab]) OR "Glial Cell Tumor*"[tiab] OR (Tumor*[tiab] AND Glial Cell[tiab]) OR "Mixed Glioma" [tiab] OR (Glioma*[tiab] AND Mixed[tiab]) OR "Mixed Glioma*"[tiab] OR "Malignant Glioma*"[tiab] OR (Glioma*[tiab] AND Malignant[tiab]) OR (glioblastoma[tiab]) OR "anaplastic astrocytoma" [tiab] OR "diffuse astrocytoma"[tiab] OR "anaplastic oligodendroglioma"[tiab] OR (oligodendroglioma[tiab]))
#2	((Immunotherapy[tiab] AND Adoptive[tiab]) OR "Cytokine-Induced Killer Cells"[tiab] OR "Dendritic Cells"[tiab] OR (Killer Cells AND Natural[tiab]) OR "cytokine induced killer"[tiab] OR "tumor infiltrating lymphocytes"[tiab] OR "lymphokine activated killer" [tiab] OR (autolymphocyte[tiab]) OR "activated T cells"[tiab] OR "activated killer cells" [tiab] OR "gamma delta T cells"[tiab] OR "γδ T cells" [tiab] OR "NKT cells" [tiab] OR "natural killer"[tiab] OR "NK cells" [tiab] OR "Adoptive Immunotherapy" [tiab] OR "Adoptive Immunotherapies"[tiab] OR (Immunotherapies[tiab] AND Adoptive[tiab]) OR ("Cellular Immunotherapy"[tiab] AND Adoptive[tiab]))
#3	(1992/01/01:2022/11/02[dp])
	#1 AND #2 AND #3

2.4. Selection process

After retrieval of relevant articles and duplicate removal, two individual authors; P.S. and M.N. will go through the title and abstracts of the relevant article to select the relevant qualified articles for the data mining process. In case of any disagreement between the two authors, it will be fixed via consensus and then will be checked by two other authors (S.M.M.Z and A.R.). Irrelevant studies and studies with controversial results will be excluded at this stage. D.A. and M.A. will be asked to build a consensus in cases where discrepant opinions exist.

2.5. Data collection process

Relevant qualified articles will undergo a full-text review in order to extract data from them. Two individual authors; P.S. and F.H.A. will extract data according to the checklist summarized in

Excel from each study individually regarding the immunological responses and survival rates. A.M. and V.F.R. will do so for radiological response rates. In case of any disagreement between the two authors, it will be fixed via consensus and then will be checked by two other authors (S.M.M.Z and A.R.). At last, D.A. and M.A. will build consensus for discrepant reports.

The reports of data mining will be presented in tables for each cell-based immunotherapy (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) summarizing in detail the aforementioned parameters. The radiological responses reported according to the guidelines Response Assessment in Neuro-Oncology Criteria (RANO), immunotherapy response assessment for Neuro-oncology (iRANO), Response evaluation criteria in solid tumors (RESICT), World Health Organization (WHO) oncology response criteria, Macdonald and AVAglio [26-31] will be summarized as depicted in Table 2.

Study features	**Patients feature**	**Treatment strategy features**	**Immunological response parameters**	**Survival features**	**Radiological response parameters**
first authors' surname	Estimated/actual number of enrolled patients	Immunotherapy strategy (innate or acquired)	INFγ increase	Overall survival rate	Complete response%
publication date	Tumor pathology and grade	Product type (e.g., CAR T, DC)	Induction of delayed type hypersensitivity (DTH)	Progression-free survival rate	Partial response%
study design, allocation and randomization		Adjuvants	Blood flow cytometry tests	Progression/recurrent rate	Stable disease%
University/institute		doses	TIL* flow cytometry tests	Mean/median overall survival (months)	Progression%
phase		boosters		Mean/median progression-free survival (months)	
Estimated/actual Study Completion Date		Antigens/ targeting moieties		Hazard ratio for overall survival	
Trial submission date				Hazard ratio for progression-free survival	
country					
Completion status					
Clinical trial submission number					

TIL*: Tumor-infiltrating lymphocyte

Table 2. Data extraction checklist for each study.

2.7. Quality assessment

The Cochrane Collaboration's tool will be used as the checklist of choice to assess the risk of bias among included studies which comprises 5 major domains including selection bias (random sequence generation and allocation), performance bias, detection bias, attribution bias, and

reporting bias. Each domain will be scored as high, low or unclear as implemented in our previous work [32-35].

2.8. Statistical analysis

For the assessment of heterogeneity among included studies, the I2 statistic defined as the fraction of variance that is due to heterogeneity will be used [36]. Heterogeneity will be categorized as negligible (I2=0–25%), low (I2=25–50%), moderate (I2=50–75%), or high (I2> 75%). Cochran's Q will also be encountered as a complementary measure for heterogeneity [37]. In the presence of high heterogeneity, Random Effect Model will be applied by Dersimonian and Laird method and when the heterogeneity is low, the fixed effect model will be applied for meta-analysis [38]. Egger's and Begg's tests will be used to investigate the presence of publication bias [39, 40]. For dose estimation meta-analysis, as a continuous measure, the "Hedges g" statistic as a function for standardized mean difference (SMD) will be used at a significant threshold of <0.05 [41]. For proportional data meta-analysis (for radiological and immune response assessment), Freeman-Tukey Transformation (arcsine square root transformation) will be used as the method of choice for meta-analysis [42]. For survival meta-analysis of survival rates (overall or PFS) at specific time points, also Freeman-Tukey Transformation will be performed however for survival meta-analysis with hazard ratios from KM analysis, the generic inverse variance method will be used [43]. Further, in order to visualize the data for better interpretation, the pooled effect size will be depicted by forest plots for each study and also funnel plots will be used for depicting the publication bias status [44]. The asymmetry of the funnel plot will show the presence of publication bias [45].

The results of the bias risk assessment through Cochrane Collaboration's tool and meta-analysis will be summarized in tables depicting each variable, heterogeneity parameters for (I^2 and Q) for the variable, and overall effect size with 95%Cis, and also the forest and funnel plot for each variable will be included.

2.9. Patient and public involvement

Patients and the public are not involved in the preparation of this protocol and will not be directly involved in the final systematic review.

3. Discussion:

In the discussion and conclusion parts, the results of the survival analyses performed will be discussed in detail and also the impact of using adjuvants on improving survival outcomes will be further discussed. In the later sections, previous adjuvants will be summarized and discussed. Regarding the immunological response rates, also a detailed discussion on the overall validity of each parameter for assessing the efficacy of immunotherapy will firstly be discussed and then the results will be compared for each therapy group.

Ethics and dissemination

This review will retrieve published data, so it will not require ethical approval. The findings of this systematic review and meta-analysis will be disseminated via an international peer-reviewed journal publication and several scientific conference presentations.

Ethics statements

Patient consent for publication

Not applicable.

Author Contributions:

D.A., M.N, A.M, and M.A developed the search strategy and participated in writing up the draft of the protocol and S.M.M.Z reviewed the manuscript and edited the final manuscript. All the authors read and approved the final draft. Data screening and selecting phases of the systematic review and meta-analysis will be performed by S.M.M.Z. and P.S. Quality assessment and Meta-analysis will be executed by P.S., M.R., F.H., and A.R. Data extraction and preparing the draft of the manuscript will be performed by S.M.M.Z., V.F., and AM. Moreover, P.S., M.N., and S.M.M.Z. will be responsible for reviewing the manuscript and editing the final manuscript. E.N. also contributed to designing the schemas and also revising the protocol.

Funding statement:

The current work was financially supported by the NIMAD institute under grant ID (973168).

Competing interest's statement

The authors declare that they have no conflict of interest.

Protocol and registration

This systematic review has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) (http://www.crd.york.ac.uk/PROSPERO), with the systematic review registration number: PROSPERO CRD42022373297 Available from: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=373297

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Figure 1. A schema of different cell-based immunotherapy strategies to combat glioma growth.

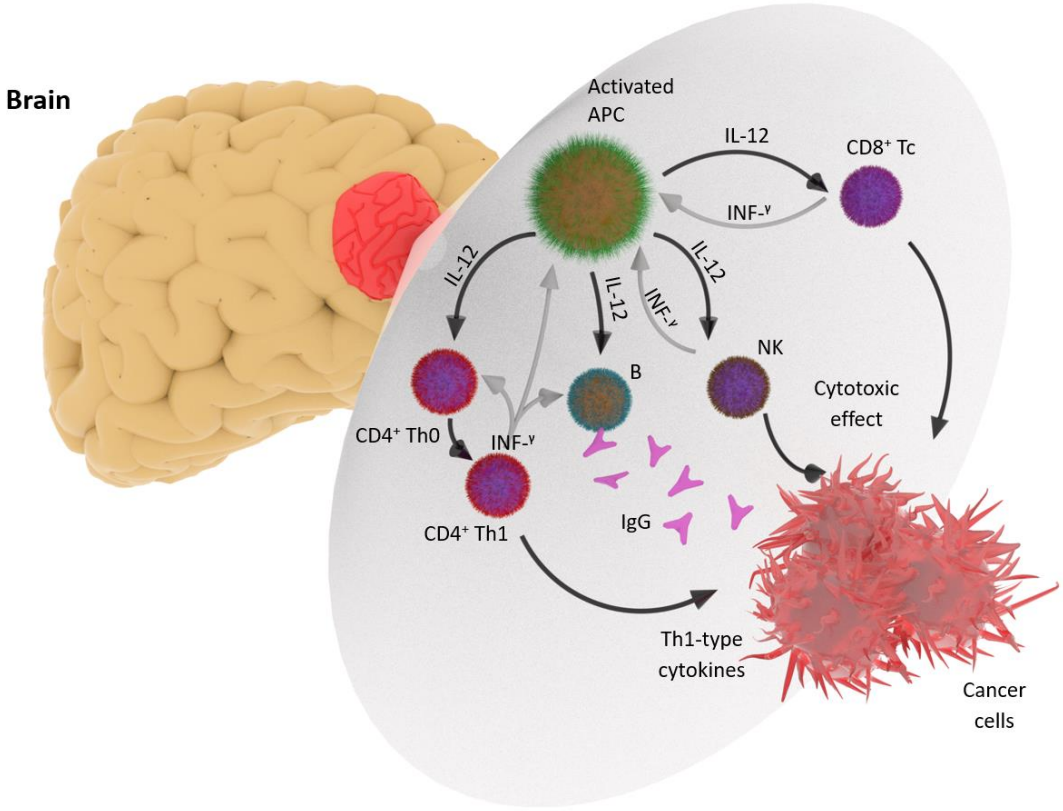

Figure 1. A schema of different cell-based immunotherapy strategies to combat glioma growth.

Supplemental Table 1. Some Examples of Cell-based Immunotherapy Strategies (DC) for Glioma

Cells used	Year published	Adult/Childhood gliomas	First author	Affiliated as	ref
DC cells	2020	adult	Jeremy D. Rudnick	Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, United States	1
autologous dendritic cell vaccine	2018	adult	Linda M. Liau	University of California Los Angeles (UCLA) David Geffen School of Medicine & Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA	2
Dendritic cell-based immunotherapy targeting Wilms' tumor 1	2015	adult	Keiichi Sakai	Department of Neurosurgery, National Hospital Organization, Shinshu Ueda Medical Center, Ueda, Nagano, Japan	3
Intraventricular B7-H3 CAR T Cells	2023	Childhood (DIPG*)	Nicholas A. Vitanza	Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, Washington.	4
IL13Rα2 CAR T cell	2016	Adult	Christine E. Brown	Department of Hematology and Hematopoietic Cell Transplantation, T Cell Therapeutics Research Laboratory, City of Hope Beckman Research Institute and Medical Center, Duarte, CA	5
Autologous CMV-specific T cells	2020	Adult	Corey Smith	QIMR Berghofer Centre for Immunotherapy and Vaccine Development and Tumor Immunology Laboratory, Department of Immunology, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia. 2 NEWRO Foundation, Brisbane, Queensland, Australia	6
Autologous HER2 CMV bispecific CAR T cells	2015	Adult	Nabil Ahmed	Department of Pediatrics, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA	7
EGFRvIII CAR T Cell	2021	Adult	Joseph S. Durgin	Glioblastoma Translational Center of Excellence, The Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, United States	8
HER2-Specific CAR T cells	2017	Adult	Nabil Ahmed	Center for Cell and Gene Therapy, Texas Children's Hospital, Houston Methodist Hospital, Baylor College of Medicine, Houston	9
EGFRvIII-directed CAR T cells	2017	Adult	DONALD M. O'ROURKE	Department of Neurosurgery, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA.	10

* DIPG: Diffuse Intrinsic Pontine Glioma

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	P1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	P2, P13
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	P1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	-
Support:			
Sources	5a	Indicate sources of financial or other support for the review	P13
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	P4-P5-P6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	P6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	P6
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	P8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	P8-P9

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	P9-P10
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	P7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	P10
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	P11
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	P11
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	-

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

BMJ Open

Efficacy of Cell-based Immunotherapies on Patients with Glioma: an umbrella review of systematic reviews and Meta-analysis Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072484.R2
Article Type:	Protocol
Date Submitted by the Author:	07-Oct-2023
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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Neurology, Oncology

Keywords:	Neurology < INTERNAL MEDICINE, Neurobiology < NATURAL SCIENCE DISCIPLINES, Neurological oncology < NEUROLOGY, ONCOLOGY

Efficacy of Cell-based Immunotherapies on Patients with Glioma: an umbrella review of systematic reviews and Meta-analysis Protocol

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Abstract:

Introduction: Glial brain tumors are highly mortal and are noted as major neurosurgical challenges due to frequent recurrence or progression. Despite standard-of-care treatment for gliomas, the prognosis of patients with higher-grade glial tumors is still poor, and hence empowering anti-tumor immunity against glioma is a potential future oncological prospect. This review is designed to improve our understanding of the efficacy of cell-based immunotherapies for glioma.

Methods and analysis: This systematic review will be performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive search of main electronic databases: PubMed/MEDLINE, Scopus, ISI web of Science EMBASE and ProQuest until 2 November (2022) on original articles, a by followed review manual of review articles. Only records in English and only clinical trials will be encountered for full-text review. All the appropriate studies that encountered the inclusion criteria will be screened, selected and then extracted data by two independent authors. For Meta-analyses, data heterogeneity for each parameter will be first evaluated by Cochran's Q and I2 statistics. In case of possible heterogeneity, a random-effects meta-analysis will be performed and for homogenous data, fixed-effects models will be selected for reporting the results of the proportional meta-analysis. Bias risk will be assessed through Beggs' and Eggers' tests and will also be visualized by Funnel plots.

Ethics and dissemination: As this study will be a systematic review without human participants' involvement, no ethical registration is required and meta-analysis will be presented at a peer-reviewed journal.

PROSPERO registration number: CRD42022373297

Keywords: Glioma, chimeric antigen receptor T cells (CAR T) cells, dendritic (DC) cells, Adoptive T cells, cytokine-induced killer (CIK) cells, natural killer (NK) cells

Strengths and limitations of this study

- This review is the first umbrella review of systematic reviews and meta-analyses evaluating the efficacy of Cell-based Immunotherapies on Patients with Glioma.
- Meta-analysis of studies according to Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines.
- A comprehensive literature search from multiple databases was conducted.
- The search was restricted to English-language articles only.
- A limited number of studies will meet the inclusion criteria.

1. Introduction:

Gliomas are among highly mortal neoplastic lesions which remain a major neuro-oncological concern due to their frequent recurrence/progression despite standard treatments [1]. Up to the present, numerous attempts have been devoted to improving the efficacy of the current standard-of-care treatment for gliomas which comprise concurrent chemo-radiation and surgical interventions [2]. The major challenges limiting the efficacy of the standard-of-care treatments for gliomas comprise the infiltrative nature of grade-high gliomas which limits the efficacy of total aggressive surgery due to residues remaining and also tumor heterogeneity. Another main concern is the mesenchymal-transformed cells referred to as cancer stem cells in the glioma tumor microenvironment (TME) which are resistant to chemo-radiation. This piece of evidence proves the ultimate need for designing treatment strategies with precision to individual characteristics of the tumor in each patient.

A key feature in the glioma pathogenesis is its immune-suppressed microenvironment due to the pauci nature of the brain as an immune-privileged site and also the overproduction of angiogenic signals in the glioma TME produced in the hypoxic central niche of highly-proliferating glioma cells which induces generation of tolerogenic dendritic cells (DCs) and impairs the antigen presentation process [3]. The gliomas TME comprise a low density of immune cells making it a "cold tumor" with limited immune contexture. Hence, re-empowering the immune system components (i.e. NK cells, cytotoxic T cells, and DC cells) against gliomas in a coordinated fashion and also transferring autologous/allogeneic immune cells (i.e. adoptive immunotherapy) to the tumor site to combat tumoral cells has been of particular interest as a highly precise therapy in the past decades [4]. Standing at the first and foremost stages of interest for such attempts in the previous literature are cellular immunotherapies (e.g, CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

Cellular immunotherapies can comprise both innate and adoptive immune cells (Figure 1). NK cells; granulocytic lymphocytes acting as powerful armamentaria of the innate immune system; are capable of eliminating abnormally-transformed cells without any need for prior sensitization. NK cells recruit to their action site in a chemokine-mediated manner. Some NK cells act as empowered soldiers able to kill numerous and diverse cells named "serial killers" which are noted as potent anti-tumor cells [5]. Moreover, the introduction of Chimeric Antigen Receptor (CAR)

NK cells also represented a step forward toward more efficient NK products [6] and efforts are underway to further clinically translate such immune products from benches to bedsides.

DCs are also key players in the immune system referred to as linkers of adaptive and innate immune responses. DCs enhance NK cell migration and recruitment to the tumor site by the production of numerous chemokines (e.g., CXCL8, CXCL9, and CXCL11) [7]. Further, DCs act as regulators of adaptive/cellular immune responses against tumoral cells mediated by CD8+ cytotoxic T cells by cross-presenting the tumoral antigens via major histocompatibility complex II (MHCII)-antigen complexes [8]. DCs are also responsible for coordinating the immune contexture in the TME by producing chemokines and cytokines which are responsible for an orchestrated migration of immune cells to the tumor site. DC therapy for gliomas has long been studied in clinical settings yielding acceptable results [9] and has introduced a paradigm shift toward more precise glioma management.

Further, the advent of adaptive T cell generation and clinical testing of such immune cell products has yielded promises toward glioma therapy. Early reports have suggested alloreactive T cells for glioma therapy [10]. Testing the autologous lymphocyte transfer has also opened a new toward more precision [11]. Such T cells were activated by several strategies against tumoral cells ex-vivo such as total tumor RNA pulsing. Further, mounting the previous literature, as earlier attempts generating antigen-specific T cells have been of particular interest (e.g., CMV-specific T cells) [12]. Recently, the advent of CAR T cells has revolutionized the advent of T cell therapy for gliomas as well as other neoplastic lesions [13-15]. Genetically engineered T-cells that express CARs can recognize tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs) presented by the MHCs resulting in a powerful anti-tumor immune response. Despite the potential limitations of CAR T cells for solid tumors, in gliomas, promises have been obtained in early attempts possibly due to the cold nature of the glioma immune context [16]. CARs can be engineered to target various highly-expressed tumor antigens and can serve as next-generation adoptive cell therapies for gliomas [17] (Supplemental Table 1). As future prospects, using combination therapy regimens may yield substantial improvements in the field of glioma immunotherapy. Further, using adjuvants are also potential proposed strategies to improve the efficacy of adoptive immune cell therapy for gliomas [18-21].

Summarizing the results of the efficacy and limitations of the previous attempts on glioma immunotherapies opens the door to the discovery of novel techniques and yields insight into the

treatment failure causes and ways to overcome them. Herein, we aimed to discuss the main methods that will be applied in a comprehensive meta-analysis for assessing the response efficacy and survival of cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma. The meta-analysis on cell-based immunotherapies aims to provide a hierarchical summary on the road to clinical translation of adoptive immunotherapies for gliomas and also discusses the technical limitations introducing variability in generating GMP-grade immune cell products. The review will also highlight the potential need for standardized protocols for more reproducible and scalable production techniques. Further, the review will discuss the potential strategies to enhance the efficacy of adoptive immunotherapies for gliomas. For instance, using adjuvants and also combination therapy.

Objectives:

This systematic review and meta-analysis aim to summarize the results of previous clinical trials on (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma patients regarding the number of patients, administered doses, adjuvants, antigens/targets, phases, submission dates, completion dates and allocation. Furthermore, this study aims to investigate the immunological efficacy of cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) for glioma. Also, this compares the administered dose of each therapy (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells), the survival outcome of the patients enrolled in treatment groups or control groups for each treatment. Moreover, the survival of the patients enrolled in different treatment groups. Further, the immunological response will be compared among the patients receiving each treatment and control groups for each therapy. Furthermore, standardization of the protocols used to harvest cells, produce, and scale up the manufacturing process will hugely revolutionize the results obtained from each trial. There is a substantial need to improve guidelines for the GMP-level products moving from benches to bedsides to let the process be more reproducible and reliable. Additionally, standardizing the strategies to assess treatment efficacy will also hugely impact the results of trial pipelines (e.g., immunological response assessment, radiological response assessment criteria such as AVA Glio, RESICT, RANO, or iRANO). In the current meta-analyses, we will discuss the limitations on the way of clinical translation of the GMP level products in the trial pipelines for better outcome management and standardized results reporting.

2. Methods and analysis:

2.1. Eligibility criteria:

This study follows the Population, Intervention, Comparison, Outcomes, and Study type (PICOS) format for conducting systematic reviews and meta-analyses [22]. According to PICO parts, the eligibility criteria will be met the following:

2.1.1. Participants/population

Inclusion criteria:

This umbrella review will consider systematic reviews that include the population for the current work consisting of adult patients and controls enrolled in clinical trials for glioma cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

Exclusion criteria:

Studies reporting patients with other cancers will be excluded.

2.1.2. Intervention(s), exposure(s)

The intervention (exposure) of this study will be cell-based immunotherapies (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells).

2.1.3. Comparator(s)/control

Administered doses, percentage of clinical trials targeting each tumoral antigen, immunological efficacy, and survival.

2.1.4. Main outcome(s)

The standardized mean difference for administered doses, the pooled effect size for each antigen for glioma immunotherapy, the pooled effect size of significant immunological responses for each therapy, and the overall survival benefit for each immunotherapy as an indicator of treatment efficacy.

2.1.5. Studies design

Inclusion Criteria:

Only systematic reviews and systematic review and meta-analysis studies will be included.

Exclusion Criteria:

Narrative reviews, commentaries, letters, case reports, case series, experimental studies, and research works in any other language rather than English are excluded from this review. Furthermore, studies suggesting a controversial result will be excluded. No time limit is for exclusion. Controversies are among the unavoidable issues while collecting huge clinical data from diverse clinical centers worldwide testing a specific therapy in trial pipelines. To cope with, the systematic reviews, several strategies have been proposed such as removing the controversial reports. Herein, when meeting a controversy, the two independent authors reviewing the selected manuscripts will discuss the potential differences and diversities in the cell production process or obtain the efficacy results and will draw a certain conclusion by getting in touch with the corresponding authors. If the conflicting answer is due to inappropriate methodology, will not be considered in the meta-analysis stage. For instance, if the lack of adequate cell count to start the treatment is the reason for the trial failure, that study will not be considered in the meta-analysis stage but will be discussed in a separate section summarizing the failure reasons for each cell-based therapy and solutions to overcome will further be discussed.

2.2. Information sources

The current work includes a comprehensive search of main electronic databases (PubMed, Scopus, ISI Web of Science, EMBASE, and Clinicaltrial.gov) and also is followed by a manual search of the reference lists of the previously-published review articles.

2.3. Search strategy

Search syntax for each main electronic database (PubMed, Scopus, ISI Web of Science, EMBASE, and Clinicaltrial.gov) will be generated according to their rules and Mesh terms [23-25]. An

example of the PubMed/MEDLINE search strategy is presented in Table 1. A filter for study type, review, and clinical trial, will be used to minimize the presence of unrelated articles in the recovery search. All the retrieved references will be deposited in a single Endnote file, and after duplicate removal will undergo a title review for relevance.

Table 1. Representative example of the search syntaxes generated for the comprehensive search.

Search syntax for PubMed	
#1	((Glioma[tiab]) OR (Gliomas[tiab]) OR "Glial Cell Tumor*"[tiab] OR (Tumor*[tiab] AND Glial Cell[tiab]) OR "Mixed Glioma" [tiab] OR (Glioma*[tiab] AND Mixed[tiab]) OR "Mixed Glioma*"[tiab] OR "Malignant Glioma*"[tiab] OR (Glioma*[tiab] AND Malignant[tiab]) OR (glioblastoma[tiab]) OR "anaplastic astrocytoma" [tiab] OR "diffuse astrocytoma"[tiab] OR "anaplastic oligodendroglioma"[tiab] OR (oligodendroglioma[tiab]))
#2	((Immunotherapy[tiab] AND Adoptive[tiab]) OR "Cytokine-Induced Killer Cells"[tiab] OR "Dendritic Cells"[tiab] OR (Killer Cells AND Natural[tiab]) OR "cytokine induced killer"[tiab] OR "tumor infiltrating lymphocytes"[tiab] OR "lymphokine activated killer" [tiab] OR (autolymphocyte[tiab]) OR "activated T cells"[tiab] OR "activated killer cells" [tiab] OR "gamma delta T cells"[tiab] OR "γδ T cells" [tiab] OR "NKT cells" [tiab] OR "natural killer"[tiab] OR "NK cells" [tiab] OR "Adoptive Immunotherapy" [tiab] OR "Adoptive Immunotherapies"[tiab] OR (Immunotherapies[tiab] AND Adoptive[tiab]) OR ("Cellular Immunotherapy"[tiab] AND Adoptive[tiab]))
#3	(1992/01/01:2022/11/02[dp])
	#1 AND #2 AND #3

2.4. Selection process

After retrieval of relevant articles and duplicate removal, two individual authors; P.S. and M.N. will go through the title and abstracts of the relevant article to select the relevant qualified articles for the data mining process. In case of any disagreement between the two authors, it will be fixed via consensus and then will be checked by two other authors (S.M.M.Z and A.R.). Irrelevant studies and studies with controversial results will be excluded at this stage. D.A. and M.A. will be asked to build a consensus in cases where discrepant opinions exist.

2.5. Data collection process

Relevant qualified articles will undergo a full-text review in order to extract data from them. Two individual authors; P.S. and F.H.A. will extract data according to the checklist summarized in

Excel from each study individually regarding the immunological responses and survival rates. A.M. and V.F.R. will do so for radiological response rates. In case of any disagreement between the two authors, it will be fixed via consensus and then will be checked by two other authors (S.M.M.Z and A.R.). At last, D.A. and M.A. will build consensus for discrepant reports.

The reports of data mining will be presented in tables for each cell-based immunotherapy (e.g., CAR T cells, DC cells, Adoptive T cells, CIK cells and NK cells) summarizing in detail the aforementioned parameters. The radiological responses reported according to the guidelines Response Assessment in Neuro-Oncology Criteria (RANO), immunotherapy response assessment for Neuro-oncology (iRANO), Response evaluation criteria in solid tumors (RESICT), World Health Organization (WHO) oncology response criteria, Macdonald and AVAglio [26-31] will be summarized as depicted in Table 2.

Table 2. Data extraction checklist for each study.

Study features	**Patients feature**	**Treatment strategy features**	**Immunological response parameters**	**Survival features**	**Radiological response parameters**
first authors' surname	Estimated/actual number of enrolled patients	Immunotherapy strategy (innate or acquired)	INFγ increase	Overall survival rate	Complete response%
publication date	Tumor pathology and grade	Product type (e.g., CAR T, DC)	Induction of delayed type hypersensitivity (DTH)	Progression-free survival rate	Partial response%
study design, allocation and randomization		Adjuvants	Blood flow cytometry tests	Progression/recurrent rate	Stable disease%
University/institute		doses	TIL* flow cytometry tests	Mean/median overall survival (months)	Progression %
phase		boosters		Mean/median progression-free survival (months)	
Estimated/actual Study Completion Date		Antigens/ targeting moieties		Hazard ratio for overall survival	
Trial submission date				Hazard ratio for progression-free survival	
country					
Completion status					
Clinical trial submission number					

TIL*: Tumor-infiltrating lymphocyte

2.7. Quality assessment

The Cochrane Collaboration's tool will be used as the checklist of choice to assess the risk of bias among included studies which comprises 5 major domains including selection bias (random sequence generation and allocation), performance bias, detection bias, attribution bias, and reporting bias. Each domain will be scored as high, low or unclear as implemented in our previous work [32-35].

2.8. Statistical analysis

For the assessment of heterogeneity among included studies, the I2 statistic defined as the fraction of variance that is due to heterogeneity will be used [36]. Heterogeneity will be categorized as negligible (I2=0–25%), low (I2=25–50%), moderate (I2=50–75%), or high (I2> 75%). Cochran's Q will also be encountered as a complementary measure for heterogeneity [37]. In the presence of high heterogeneity, Random Effect Model will be applied by Dersimonian and Laird method and when the heterogeneity is low, the fixed effect model will be applied for meta-analysis [38]. Egger's and Begg's tests will be used to investigate the presence of publication bias [39, 40]. For dose estimation meta-analysis, as a continuous measure, the "Hedges g" statistic as a function for standardized mean difference (SMD) will be used at a significant threshold of <0.05 [41]. For proportional data meta-analysis (for radiological and immune response assessment), Freeman-Tukey Transformation (arcsine square root transformation) will be used as the method of choice for meta-analysis [42]. For survival meta-analysis of survival rates (overall or PFS) at specific time points, also Freeman-Tukey Transformation will be performed however for survival meta-analysis with hazard ratios from KM analysis, the generic inverse variance method will be used [43]. Further, in order to visualize the data for better interpretation, the pooled effect size will be depicted by forest plots for each study and also funnel plots will be used for depicting the publication bias status [44]. The asymmetry of the funnel plot will show the presence of publication bias [45].

The results of the bias risk assessment through Cochrane Collaboration's tool and meta-analysis will be summarized in tables depicting each variable, heterogeneity parameters for (I^2 and Q) for the variable, and overall effect size with 95%Cis, and also the forest and funnel plot for each variable will be included.

2.9. Patient and public involvement

Patients and the public are not involved in the preparation of this protocol and will not be directly involved in the final systematic review.

3. Discussion:

In the discussion and conclusion parts, the results of the survival analyses performed will be discussed in detail and also the impact of using adjuvants on improving survival outcomes will be further discussed. In the later sections, previous adjuvants will be summarized and discussed. Regarding the immunological response rates, also a detailed discussion on the overall validity of each parameter for assessing the efficacy of immunotherapy will firstly be discussed and then the results will be compared for each therapy group.

Ethics and dissemination

This review will retrieve published data, so it will not require ethical approval. The findings of this systematic review and meta-analysis will be disseminated via an international peer-reviewed journal publication and several scientific conference presentations.

Ethics statements

Patient consent for publication

Not applicable.

Author Contributions:

D.A., M.N., A.M., and M.A. developed the search strategy and participated in writing up the draft of the protocol and S.M.M.Z reviewed the manuscript and edited the final manuscript. All the authors read and approved the final draft. Data screening and selecting phases of the systematic review and meta-analysis will be performed by S.M.M.Z. and P.S. Quality assessment and Meta-analysis will be executed by P.S., M.R., F.H., and A.R. Data extraction and preparing the draft of the manuscript will be performed by S.M.M.Z., V.F., and AM. Moreover, P.S., M.N., and

S.M.M.Z. will be responsible for reviewing the manuscript and editing the final manuscript. E.N. also contributed to designing the schemas and also revising the protocol.

Funding statement:

The current work was financially supported by the NIMAD institute under grant ID (973168).

Competing interest's statement

The authors declare that they have no conflict of interest.

Protocol and registration

This systematic review has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) (http://www.crd.york.ac.uk/PROSPERO), with the systematic review registration number: PROSPERO CRD42022373297 Available from: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=373297

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Figure 1. A schema of different cell-based immunotherapy strategies to combat glioma growth.

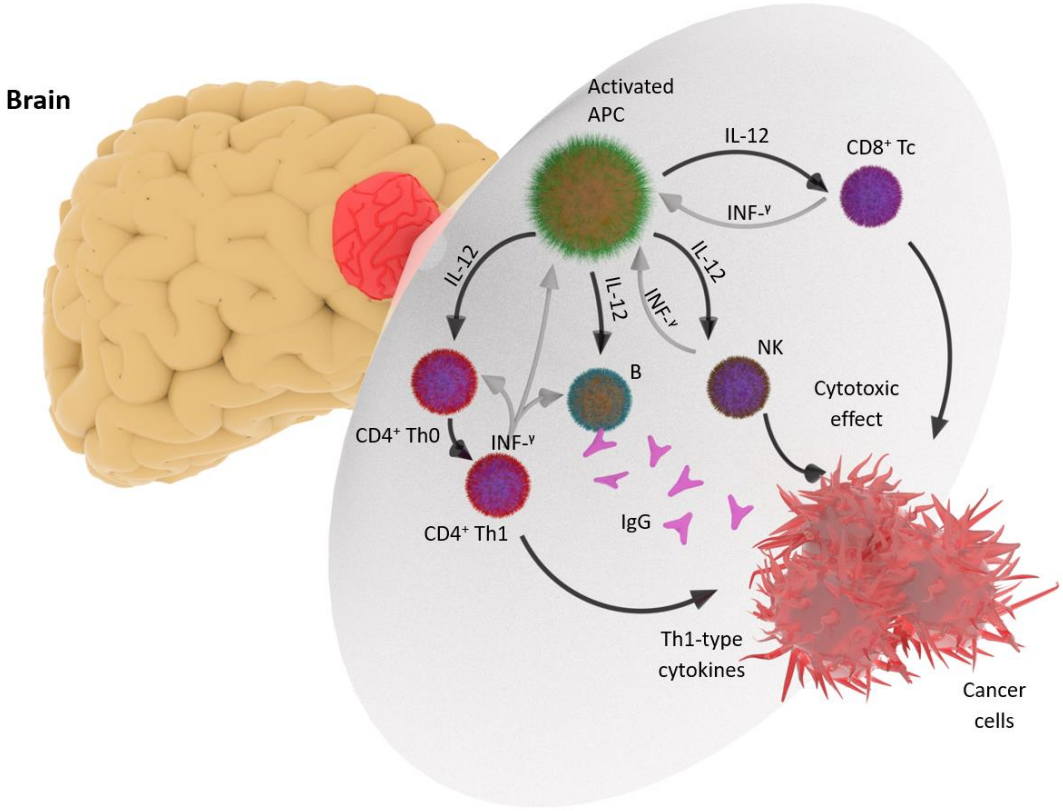

Figure 1. A schema of different cell-based immunotherapy strategies to combat glioma growth.

Supplemental Table 1. Some Examples of Cell-based Immunotherapy Strategies (DC) for Glioma

Cells used	Year published	Adult/Childhood gliomas	First author	Affiliated as	ref
DC cells	2020	adult	Jeremy D. Rudnick	Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, United States	1
autologous dendritic cell vaccine	2018	adult	Linda M. Liau	University of California Los Angeles (UCLA) David Geffen School of Medicine & Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA	2
Dendritic cell-based immunotherapy targeting Wilms' tumor 1	2015	adult	Keiichi Sakai	Department of Neurosurgery, National Hospital Organization, Shinshu Ueda Medical Center, Ueda, Nagano, Japan	3
Intraventricular B7-H3 CAR T Cells	2023	Childhood (DIPG*)	Nicholas A. Vitanza	Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, Washington.	4
IL13Rα2 CAR T cell	2016	Adult	Christine E. Brown	Department of Hematology and Hematopoietic Cell Transplantation, T Cell Therapeutics Research Laboratory, City of Hope Beckman Research Institute and Medical Center, Duarte, CA	5
Autologous CMV-specific T cells	2020	Adult	Corey Smith	QIMR Berghofer Centre for Immunotherapy and Vaccine Development and Tumor Immunology Laboratory, Department of Immunology, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia. 2 NEWRO Foundation, Brisbane, Queensland, Australia	6
Autologous HER2 CMV bispecific CAR T cells	2015	Adult	Nabil Ahmed	Department of Pediatrics, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA	7
EGFRvIII CAR T Cell	2021	Adult	Joseph S. Durgin	Glioblastoma Translational Center of Excellence, The Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, United States	8
HER2-Specific CAR T cells	2017	Adult	Nabil Ahmed	Center for Cell and Gene Therapy, Texas Children's Hospital, Houston Methodist Hospital, Baylor College of Medicine, Houston	9
EGFRvIII-directed CAR T cells	2017	Adult	DONALD M. O'ROURKE	Department of Neurosurgery, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA.	10

* DIPG: Diffuse Intrinsic Pontine Glioma

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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	P1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	P2, P13
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	P1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	-
Support:			
Sources	5a	Indicate sources of financial or other support for the review	P13
Sponsor	5b	Provide name for the review funder and/or sponsor	
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	P4-P5-P6
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	P6
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	P6
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	P8
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	P8-P9

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	P9-P10
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	P7
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	P10
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	P11
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	P11
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	-

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.