BMC Cancer

RESEARCH

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Efficacy of vaccination therapy in newly diagnosed and recurrent glioblastoma patients: a meta-analysis

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Abstract

Background Glioblastoma (GB) is the most common and fatal primary central nervous system malignancy in adults. Various immunotherapies, including vaccination, are under investigation for their potential to extend survival in GB patients. Vaccination therapy has shown variable but promising outcomes across studies. This meta-analysis aims to evaluate the efficacy of available vaccines for newly diagnosed and recurrent GB.

Methods We conducted a systematic search in PubMed, Scopus, and Web of Science to identify randomized or non-randomized double-arm studies involving adult GB patients treated with vaccines. Overall survival (OS) and progression-free survival (PFS) were the primary outcomes, with effect sizes represented as hazard ratios (HR) and calculated using a random-effects model. Funnel plots, Egger's and Begg-Mazumdar tests assessed publication bias, and the chi-square (Q) statistic, I^2 statistic, and tau-squared (T^2) parameter addressed heterogeneity.

Results A total of 2,792 patients from 23 clinical studies were included. Our findings showed significant improvements in PFS (HR, 0.64; $p < 0.001$) and OS (HR, 1.09; $p < 0.00001$) with minimal publication bias but notable heterogeneity. Meta-regression identified vaccine type and publication year as influential factors. Subgroup analysis demonstrated survival benefits with dendritic cell and viral vector vaccines, with a trend towards lower 6-methylguanine-DNA methyltransferase (MGMT) methylation rates. Sensitivity analysis confirmed the robustness of our results.

Conclusions Vaccination therapy showed potential survival benefits for GB patients; however, further phase III studies are needed to validate these results, elucidate biological mechanisms, and strive for improved trial designs and patient stratification.

Keywords Cancer vaccines, Glioblastoma, Brain cancer

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Introduction

Glioblastoma (GB) remains the most common and most aggressive malignant tumor of the central nervous system representing 50.9% of all malignant primary brain and tumors in adults. The overall incidence reaches 3–5 adults per 100.000 population while the median survival remains low at 14.6–16 months from the time of diagnosis [1]. Despite multidisciplinary efforts, the 5- year survival rate remains lower than 10%. GB incidence is slightly higher in males than females with a ratio of 1.58 to 1 [2]. It overall trends up from the age of 45 with the highest incidence observed in the age group of 75–79 years [3].

Glioblastoma exhibits disease relapse in almost all cases. If left untreated, median survival recedes to six months [4]. The current standard of care (SOC) treatment for newly diagnosed glioblastoma is multimodal, including maximum feasible surgical resection, radiation therapy, concomitant or adjuvant chemotherapy (usually temozolomide, an alkylating agent) and low intensity alternating electric field therapy [5, 6]. Correspondingly, treatment for recurrent disease may include reoperation, reirradiation, chemotherapy, tumour treating fields (i.e. small non-invasive portable devices damaging DNA in rapidly dividing cells) and therapy targeting angiogenic factors, such as the vascular endothelial growth factors (VEGF) [7].

According to the most recent classification of World Health Organization for malignant brain neoplasms which was introduced in 2021, an emphasis is given to the combination of histology and molecular biology for tumor diagnosis. More specifically, for the diagnosis of glioblastoma a variety of factors are required, including lack of mutations in IDH codons of interest (i.e. codon 132 and 172), TERT promoter deletion, EGFR amplification and gain of chromosome 7/loss of chromosome 10 genotype [8]. Thus, glioblastoma is now diagnosed even in cases that may histologically appear to be of lower grade [9]. Currently, the promoter methylation of the 6-methylguanine-DNA methyltransferase (MGMT) gene, which encodes a DNA-repair protein, is the only established prognostic and predictive biomarker for GB [10–12]. Likewise, it is generally accepted that a greater extent of resection is linked to improved local control and overall enhanced survival outcomes [13], establishing maximal safe resection as the surgery goal. Emerging research has further explored the concept of supramaximal tumor resection [14, 15].

Shared characteristics of GB tumors include rapid growth potential, marked heterogeneity, both in molecular patterns and oncogenic characteristics, insufficient response to immunotherapy, diffuse and microscopic tissue expansion, microvascular proliferation, and unavoidable disease recurrence. Simultaneously, blood brain barrier (BBB) hinders drug delivery to the brain parenchyma, by presenting variable permeability and various efflux transporters which prevent the entry of chemotherapeutic drugs, therefore reducing their efficacy [16, 17].

In recent years, immunotherapy stands out as an important treatment option for cancer patients, improving outcomes in a variety of malignancies with its role in GB therapy currently unraveling [7]. Immunotherapy research in glioblastoma includes cytokine therapy exploring cytokines like TNF-a and IFN-a [18, 19], immune checkpoint inhibitors [20, 21], chimeric antigen receptor T cell (CAR-T) therapy [22], oncolytic viruses like adenovirus [23] and herpes simplex virus [24], immunogenic vaccines, as well as combinations of these strategies. Despite the more recent advances, the utilization of immunotherapy in the treatment landscape of GB is not yet as promising as in other solid malignancies such as melanoma which can be attributed to the unique characteristics of gliomas and GB in particular [5, 25]. These include the 'cold', immunologically silent tumor microenvironment (TME), a low tumor mutation burden (TMB), the as mentioned physical barrier of BBB. TME in GB is known to have multiple immunosuppressive features. High levels of regulatory T cells (Tregs) are present, suppressing antitumor T-cell responses through immune checkpoint upregulation and immunosuppressive cytokine secretion [26]. IL-10 contributes by inhibiting antigen-presenting cells (APCs), promoting tolerogenic dendritic cells, and facilitating Treg differentiation. Tumor antigen recognition by Tregs can lead to clonal expansion and further suppression of effector T cells [27]. Tumor-associated myeloid cells (tumor-associated macrophages [TAMs] and myeloid-derived suppressor cells [MDSCs]) have been found to comprise up to half of the tumor mass and are key producers of PD-L1, dampening adaptive immune responses and promoting resistance to immune checkpoint blockade [28]. TAMs derived from the bone marrow tend to adopt immunosuppressive phenotypes, while resident microglia inhibit T-cell infiltration via mTOR signaling [29]. MDSCs, induced by GBM cells, accumulate both in tumors and circulation, suppressing T-cell function through IL-4Rα-mediated arginase production—effects that can be reversed by their depletion [30, 31]. Chronic IFN-γ signaling, especially during tumor vaccination and the resulted T cell accumulation in the vaccination site, leads to T cell anergy and immune checkpoint genes upregulation, ultimately promoting an exhausted T cell phenotype despite enhanced MHC I expression induced by the initial, short-term IFNγ secretion [32].

A decreased tumor mutation burden (TMB) limits the number of neoantigens available for presentation on MHC molecules, reducing the chances of effective T-cell recognition and activation. Since only a small fraction of mutations generate immunogenic neoepitopes, low TMB results in fewer targets for the immune system, hindering both direct T-cell responses and the process of antigen spreading critical for sustained immunotherapy efficacy [33].

Vaccines represent an immunotherapeutic approach that is increasingly gaining attention in glioblastoma clinical trials, categorized as active specific immunotherapy. Since their initial conception, various types and approaches have been proposed and put into practice. Based on the platform serving as a vehicle for vaccine transportation, the vaccines can be divided into four main categories. These include peptide vaccines [34], whole cell vaccines, mRNA vaccines and viral vector vaccines [35]. As for the antigen types, these include both endogenous and exogenous molecules. Endogenous antigens derive from intracellular tumor proteins and include Neoantigens such as tumor-specific antigen and tumor-associated antigens (TAAs). Exogenous antigens may originate from pathogens that have infected tumor cells such as nucleic acids and proteins of human cytomegalovirus (CMV) [36, 37] and bacteria-derived antigens. Specifically, regarding bacteria-derived antigens, they trigger tumor antigen recognition by CD4 + T cells and CD8 + cytotoxic T cells, potentially due to molecular mimicry [38]. Previous research has yielded mixed results in terms of vaccine efficacy in improving survival [39]. However, scientific interest remains high, mainly attributed to the tolerable safety profile of these therapies and the presence of long-term survivors in various cohorts, potentially delineating the biological advantage of patients treated with vaccines [40]. For instance, a study by Lepski et al. showcased in a phase II study a significant survival benefit of patients with recurrent GB treated with a dendritic cell vaccine [41].

Previous meta-analyses have examined the effect of a specific type of vaccine on patient survival for either newly diagnosed or recurrent disease [42, 43]. Therefore, the current meta-analysis aims to screen all the completed primary clinical trials regarding vaccination immunotherapy for both manifestations of glioblastoma, evaluate their clinical significance and the possibility of favorable outcomes, and make comparisons among the different types of vaccines.

Methods

Conception and methodology

This meta-analysis was conducted and reported in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [44], as shown in the PRISMA expanded checklist (Table S1) and registered in the International Prospective Register of Systematic Reviews (PROSPERO) database with the Registration Number CRD42024503505. No ethics committee approval was required as this study involved a retrospective analysis of publicly available data.

Criteria for eligibility and study selection

Included studies were all randomized or non- randomized clinical trials, with two arms, comparing clinical outcomes of GB patients who received SOC treatment versus those who received vaccine therapy on top of SOC treatment. In the case of multiple publications arising from the same trial, only the most recent publication with the largest sample size was included.

In order to be included in this systematic review and meta-analysis, a study should have met the following prespecified eligibility criteria: 1) Prospective randomized or non- randomized clinical trials with two arms of patients diagnosed with newly diagnosed and/or recurrent glioblastoma, where the control group received SOC treatment and the experimental arm received one specific type of vaccination regimen on top of SOC treatment, 2) Vaccination regimen included vaccines from one of the following classes: dendritic cell vaccines, peptide vaccines, viral vector vaccines and whole tumour cell vaccines, 3) Patients survival was assessed using overall survival (OS) and/or progression-free survival (PFS), with results presented as hazard ratios (HRs) or Kaplan–Meier survival curves, 4) Studies must include only adult patients (> 18 years of age), and 5) Studies written in English.

We excluded single- arm studies that did not have a control group, studies with non-adult cohorts, as well as studies that did not report either OS or PFS. Additionally, in vitro and animal studies, studies overlapping datasets, summary publications, literature reviews, editorials, commentaries, and opinion papers were not considered for publication.

Search strategy

An electronic search was performed on March 5th, 2025, by two researchers using single terms and phrases through three databases, namely PubMed (https://www.pubmed.ncbi.nlm.nih.gov/), Scopus (https://www.scopus.com/) and Web of Science (https://www.webofknowledge.com/), for articles published in the English language. Followingly, relevant search was conducted in ClinicalTrials.gov database for clinical trials. For PubMed, the predefined searching strategy was the following: (((high-grade glioma) OR (glioblastoma)) OR (glioblastoma multiforme)) AND (((((dendritic cell vaccines) OR (vaccine)) OR (Nucleic Acid Vaccines)) OR (vaccination))

OR (peptide vaccine)). Please see the complete search strategy in Table S2 for each respective database and registry.

Data extraction

The results from the predefined searching strategy of different databases were collectively imported to EndNote™ software, version 21 and each level of screening was performed by two independent researchers (IK and KK), based on the previously analysed inclusion and exclusion criteria. In case of discrepancies among researchers, they were resolved after discussion with a third author (KD). After removal of duplicates, screening was conducted based on title and abstract reviewing, followed by full text screening of the remaining studies. Additional details about the screening process are provided in the flowchart (Fig. 1).

Using a preconfigured template, data were independently extracted by two investigators from the included studies. Extracted data included: study characteristics (author, year of publication, sample size, vaccine arm, control arm), population characteristics (age, sex, baseline and supplementary treatment, tumour type), survival characteristics (OS, PFS, median PFS, median OS) and adverse events. For survival characteristics, hazard ratios for PFS and OS were extracted separately, as they are considered a more appropriate measure for analysing time-to-event outcomes compared to odds ratios or relative risk [45, 46]. When reports of hazard ratios (HR), standard errors (SE), or 95% confidence intervals (CIs)

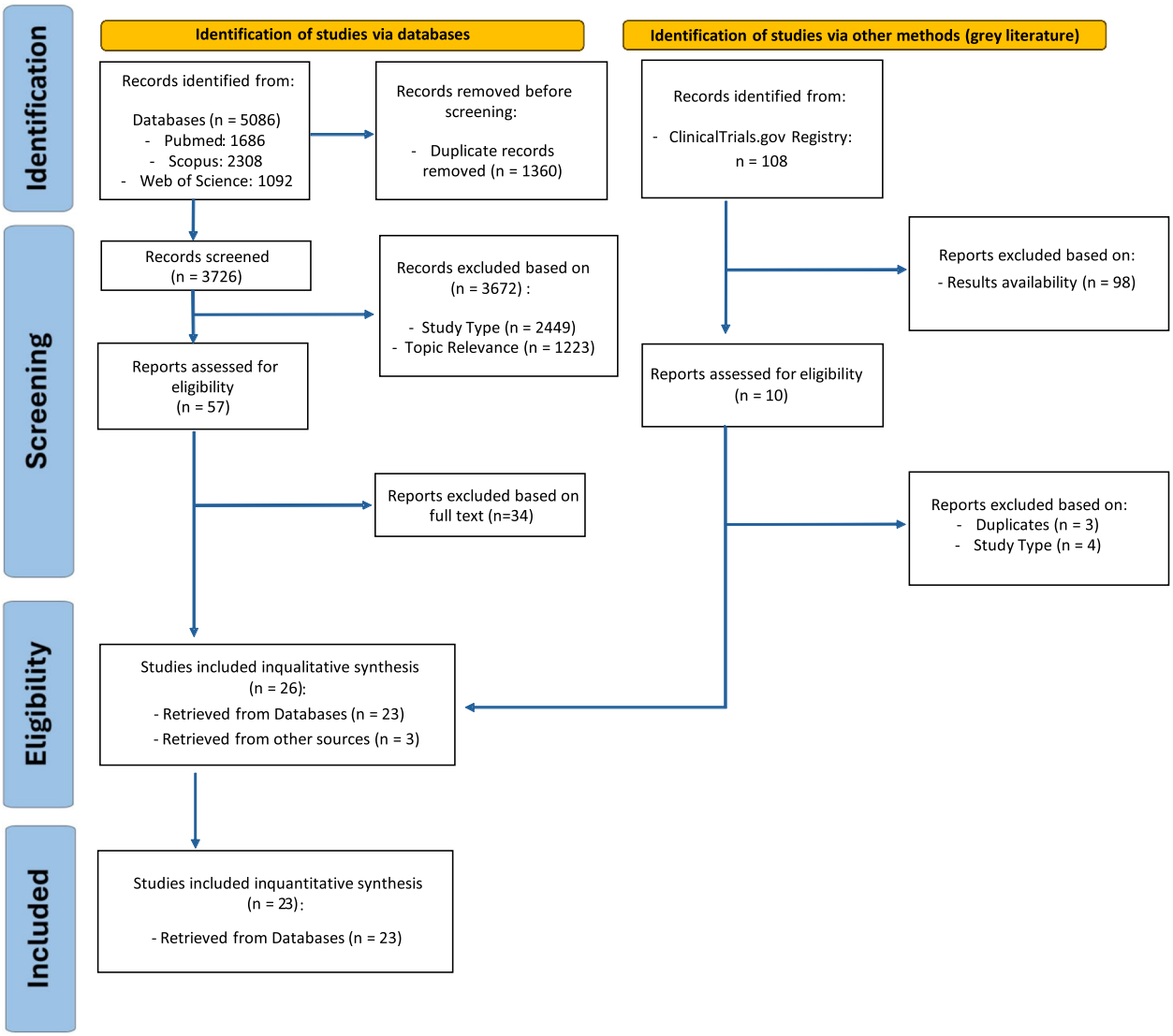


Fig. 1 Flow diagram of the screening process following Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA) guidelines

were not available, the estimated values were directly derived from Kaplan–Meier curves using the methodology described by Jayne F. Tierney [45]. Dot plots of the graphical data were extracted using PlotDigitizer software (https://plotdigitizer.com/app/). The final results were cross-verified and reached through a consensus discussion between two researchers. In the case of trials with not publicly available data at the time of data extraction (i.e. the three grey literature studies derived from ClinicalTrials.gov with yet unpublished results), the corresponding author was conducted up to 3 times before the exclusion of the study in case of no response. For studies with multiple publications, we compared the reports and selected the one with the most recent and comprehensive data.

Quality assessment

We used the Cochrane Collaboration's tools RoB2 [47] and ROBINS-I [48] to assess the methodological quality and estimate the risk of bias among included RCTs (Fig. 1) and NRCTs (Figure S1) respectively. Two researchers (EK and KD) independently performed the assessments, while any discrepancies were resolved through consultation with the study supervisor (KG).

Statistical analysis

All data were analysed using the R statistical software, version 4.2.0. [49], with the "meta", "robumeta" and "metafor" packages [50]. The combined effect size was expressed as HRs with corresponding SEs and 95% CIs for each primary and secondary outcome, calculated using a random-effects model with the restricted maximum-likelihood (REML) adjustment. We selected the random effects model due to the clinical heterogeneity in our data, which arose from variations in patient age, study settings, WHO classification, cancer types, and vaccination therapies. Heterogeneity was assessed using the chi-square (Q) statistic, which reflects total dispersion, and the I^2 statistic, following Higgins'method, which indicates the percentage of excess dispersion relative to total dispersion. The tau-squared (T^2) parameter was also estimated as an absolute measure of heterogeneity. In cases of high heterogeneity (namely $I^2 > 50\%$), meta-regression analysis was conducted to identify potential variables that might explain the observed heterogeneity. Additionally, publication bias was tested visually by inspection of the Funnel plots (Fig. 5A and 5B) and statistically by performing Egger's and Begg- Mazumdar tests. When publication bias was detected as (marginally) statistically significant, the trim-and-fill method was applied to correct for funnel plot asymmetry (Fig. 5C and 5D). Furthermore, in order to evaluate the robustness of our findings and assess the influence of individual studies on the overall results, we conducted cumulative meta-analysis and sensitivity analysis (Figure S3 and S5). Subgroup analyses were performed based on several variables, including tumour type, vaccine type, MGMT methylation percentage, percentage of female patients, year of publication, WHO classification, temozolomide (TMZ) use, and recruiting areas, to explore potential differences in outcomes across these variable categories (Figs. 4 and 6 and S4 and S6). Finally, to further investigate heterogeneity and the robustness of our findings, we generated Baujat's plots and GOSH (Graphical Display of Study Heterogeneity) plots (Figure S2).

Results

Search results

Initially, we retrieved a total of 5,549 studies from all databases (PubMed, Scopus, Web of Science and Cochrane Library) and 112 from grey literature sources (ClinicalTrials.gov). After removing duplicates across all databases, a total of 3,030 articles remained. After title and abstract screening, a total of 65 articles were included, 23 of which were eventually selected after full text screening [33, 41, 51–71]. Of the 112 studies from ClinicalTrials.gov, only 3 met our inclusion criteria. However, these studies were included only in the qualitative synthesis due to difficulties in retrieving their results. Finally, this meta-analysis included 23 studies that assessed the safety (adverse events) and efficacy (OS, PFS) of vaccination therapy in addition to SOC treatment for patients with newly diagnosed or recurrent glioblastoma. Among these, 12 were RCTs and 11 were NRCTs (Fig. 1).

Study characteristics

The final 23 studies included in the meta-analysis encompassed a total of 2,792 glioblastoma patients from 12 countries, with 1,425 patients from non-randomized controlled trials (NRCTs) and 1,367 patients from randomized controlled trials (RCTs) (Table 1 and Table S3 and S4). In five studies involving a total of 814 patients, all participants had recurrent glioblastoma, whereas, in the remaining 18 studies including 1,978 patients, the majority of the patients had newly diagnosed glioblastoma (Table S3). Only three of these studies enrolled both newly diagnosed and recurrent cases (149 patients in total) (Table S3). Regarding vaccine types, dendritic cell vaccines were used in 14 studies, peptide vaccines were used in 5 studies, viral vector vaccines in 2 studies, and whole tumour cell vaccines in 2 studies (Table S3). In 12 studies, a recent WHO classification of glioblastomas was applied, while in the remaining 11 studies, either an older WHO classification was used, or the classification details were not available (Table S3). The median age in the vaccinated cohorts ranged from 45 to 64 years of age,

Table 1 Clinical Outcomes and Adverse Events of Glioblastoma Patients from NRCTs and RCTs Included in the Meta-analysis

Author	Year	Phase	PFS		OS		Median PFS (months)		Median OS (months)		Adverse Events		Completion of vaccination	Follow-up (months)	Intention-to-treat analysis
			Hazard Ratio	Standard Error SE(lnHR)	Hazard Ratio	Standard Error SE(lnHR)	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls			
Batich [44]	2017	I	HR: 0.28 95% CI: 0.13–0.60	0.40	NA	NA	25.3	8	41.1	19.2	grade III (1)	N/A	100%	N/A	N/A
Boydell [71]	2019	I/II	HR: 1.27 95% CI: 0.69–2.33	0.31	HR: 1.24 95% CI: 0.64—2.42	0.34	2.6	4.2	7.8	10	N/A	N/A	100%	N/A	N/A
Chang [57]	2011	I/II	NA	NA	HR: 0.57 95% CI: 0.34—0.97	0.27	N/A	N/A	17.5	12.7	grade I (11), grade II (2), grade III (0) IV (5), total (18)	N/A	100%	72	N/A
Lepski [41]	2023	I/II	N/A	NA	HR: 0.53, 95% CI: 0.36—0.78	0.40	N/A	N/A	27.6	16.3	grade I (1), grade II (1), total (2)	N/A	100%	N/A	N/A
Liau [59]	2023	III	N/A	N/A	HR: 0.80, 95% CI: 0.00—0.94	0.082280	N/A	N/A	19.3	16.5	5 serious adverse events grade II (1), grade III (4), 3 cases of intracranial edema (2 at grade 3; 1 at grade 2), 1 case of nausea (grade 3), 1 case of lymph node infection (grade 3)	N/A	98.70%	N/A	Yes
Rynda [62]	2024	N/A	HR: 2.98 95% CI: 1.71 - 5.14	0.28	HR: 2.39 95% CI: 1.43—3.97	0.26	13.8	7.9	21.7	15.8	total (1)	N/A	100%	N/A	N/A
Sampson [63]	2010	II	HR: 0.46 95% CI: 0.22–0.99	0.39	HR: 0.35 95% CI: 0.16—0.77	0.41	14.2	6.3	26	15	grade III—IV (2)	N/A	100%	N/A	N/A

Table 1 (continued)

Author	Year	Phase	PFS		OS		Median PFS (months)		Median OS (months)		Adverse Events		Completion of vaccination	Follow-up (months)	Intention-to-treat analysis
			Hazard Ratio	Standard Error SE(lnHR)	Hazard Ratio	Standard Error SE(lnHR)	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls			
Steiner [64]	2004	N/A	HR: 0.46 95% CI: 0.22–0.99	0.39	HR: 0.35 95% CI: 0.16—0.77	0.41	10	6.5	25	12.25	grade III—IV (0)	grade III—IV (0)	100%	59	N/A
Vik-Mo [65]	2013	II	HR: 0.46 95% CI: 0.22–0.99	0.39	HR: 0.39 95% CI: 0.17—0.86	0.41	23.1	7.87	25.3	19.5	grade I (19), grade II (3), grade III (1)	N/A	100%	N/A	N/A
Wheeler [68]	2004	I/II	NA	NA	HR: 0.74 95% CI: 0.31—1.79	0.45	N/A	N/A	N/A	N/A	N/A	N/A	N/A	48	N/A
Yamanaka [69]	2005	I/II	NA	NA	HR: 0.50 95% CI: 0.27—0.94	0.32	N/A	N/A	16	13.3	grade 1 (7)	N/A	100%	48	N/A
Bota [54]	2018	II	N/A	N/A	HR: 0.32 95% CI: 0.06—1.69	0.85	7.3	5.4	12.1	7.6	grade III (4), total (162)	grade III (8), total (58)	80%	N/A	N/A
Buchroithner [56]	2014	II	N/A	N/A	HR: 0.81 95% CI: 0.47—1.40	0.280258	N/A	N/A	14.6	12.7	N/A	N/A	100%	18	N/A
Buchroithner [55]	2018	II	HR: 1.09 95% CI: 0.54—2.19	0.354825	HR: 0.99 95% CI: 0.57—1.62	0.281746	6.8	6.9	18.8	18.93	grade II (48), grade III (0), IV (12), total (60)	grade II (86), grade III—IV (18), total (104)	100%	15	Yes
Cho [52]	2012	II	HR: 0.68, 95% CI: 0.32—1.44	0.381915	HR: 0.29, 95% CI: 0.14—0.61	0.379611	8.5	8	31.9	15	grade I (2), grade III (0), IV (3), total (5)	grade I (2), grade III-IV (2), total (5)	100%	14–52 (median follow-up: 33 m)	N/A
Ji [51]	2016	II	HR: 0.157, 95% CI: 0.062—0.398	0.474316	HR: 0.125, 95% CI: 0.04—0.36	0.533352	11.6	1.85	11.43	2.15	grade I (12), grade II (7), grade III (2), total (21)	grade I (23), grade II (7), grade III (3), total (33)	N/A	7.8	N/A

Table 1 (continued)

Author	Year	Phase	PFS		OS		Median PFS (months)		Median OS (months)		Adverse Events		Completion of vaccination	Follow-up (months)	Intention-to-treat analysis
			Hazard Ratio	Standard Error SE(lnHR)	Hazard Ratio	Standard Error SE(lnHR)	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls			
Jie [58]	2012	II	HR: 1.00, 95% CI: 0.46—2.18	0.398069	HR: 0.84, 95% CI: 0.53—1.33	0.232783	11.92	7.75	17.0	10.5	grade I (3)	N/A	100%	5.5—24	N/A
Muragaki [48]	2023	IIb	HR: 1.03 95% CI: 0.54—1.97	0.33	HR: 1.19, 95% CI: 0.57—2.47	0.374066	13.3	13.3	25.6	31.5	Grade I (50), grade II (20), grade III (6), total (76)	grade I (53) grade II (25) grade III (5) total (83)	93.75%	25	N/A
Narita [61]	2019	III	N/A	N/A	HR: 1.13, 95% CI: 0.6—1.9	0.294051	N/A	N/A	8.4	8	total (340)	total (122)	100%	7.7	N/A
Reardon [33]	2020	II	HR: 0.72, 95% CI: 0.43—1.21	0.263926	HR: 0.53, 95% CI: 0.32—0.88	0.258061	13.8	7.9	11.2	9.3	grade III (0), IV (11), total (84)	grade III—IV (17), total (67)	97.20%	N/A	Yes
Weller [66]	2017	III	HR: 0.94, 95% CI: 0.79—1.13	0.091311	HR: 0.89, 95% CI: 0.75—1.07	0.090648	7.1	5.6	17.4	17.4	grade I—II (2780), grade III (274), grade IV (60) grade V (7)	grade I—II (2963) grade III (295) grade IV (49) grade V (9)	99.50%	7.7	Yes
Wen [67]	2019	II	HR: 0.57, 95% CI: 0.33—0.98	0.277668	HR: 0.87, 95% CI: 0.52—1.45	0.261605	11.2	9	17	15	grade II (130), grade III (47)	grade II (103) grade III (34)	92.60%	40	Yes
Yao [70]	2018	II	HR: 0.73, 95% CI: 0.37—1.46	0.351917	HR: 0.40, 95% CI: 0.18—0.88	0.401923	7.7	6.9	13.7	10.7	N/A	N/A	100%	14	N/A
NCT03149003	2023	III	N/A	N/A	N/A	N/A	5.3 (3.9 to 5.6)	3.8 (3.7 to 5.6)	10.2 (8.2 to 11.4)	9.4 (7.4 to 10.3)	total serious (32)	total serious (14)	99%	24	Yes
NCT01814813	2023	II	N/A	N/A	N/A	N/A	3.7 (2.9 to 5.4)	5.3 (3.7 to 8.0)	6.6 (5.4 to 10.4)	10.7 (8.8 to 17.2)	total serious (8)	total serious (10)	100%	60	N/A

Table 1 (continued)

Author	Year	Phase	PFS		OS		Median PFS (months)		Median OS (months)		Adverse Events		Completion of vaccination	Follow-up (months)	Intention-to-treat analysis
			Hazard Ratio	Standard Error SE(lnHR)	Hazard Ratio	Standard Error SE(lnHR)	Vaccinated	Controls	Vaccinated	Controls	Vaccinated	Controls			
NCT03018288	2022	II	N/A	N/A	N/A	N/A	13.7 (9.92 to 17.48)	8 (5.94 to 10.06)	14.4 (9.9 to 18.9)	14.1 (11.5 to 16.7)	grade 1 (N/A), grade 2 (52), grade 3 (24), grade 4 (2), grade 5 (1), total (79)	grade 1 (N/A) grade 2 (41) grade 3 (22) grade 4 (2) grade 5 (0) total (65)	N/A	26	N/A

while in the control cohorts, the median age of participants ranged from 42 to 58 years of age. In the majority of the studies, there was adequate sex and age matching between vaccinated and control patients (Table S4). The total percentage of female patients in both arms of all studies was ranging from 20–30% to > 50%. More precisely, two studies enrolled 20–30% of female patients, seven studies enrolled 30–40% of female patients, while four had 40–50% of females and three of them had > 50% of female patients (Table S4). Regarding MGMT methylation percentage, it was found that only two studies had a relatively low percentage of MGMT methylation among recruited patients (0–20%), while four of them had 20 to 40% of methylation among participants, and three studies had 40 to 60% of methylation among participants. In the rest of the studies, no information regarding MGMT methylation was available (Table S3). Last, three of the studies were published before 2010, fifteen were published between 2010 and 2020 and five of them after 2020.

Quality assessment

For quality assessment of the 23 included studies, we used the Cochrane's Risk-of-Bias Tools, as detailed in Fig. 2 and Figure S1. For evaluating the risk of bias in the 12 RCTs, the RoB 2 tool was used (Fig. 2), while the ROBINS-I tool was applied to the remaining 11 NRCTs (Figure S1). As shown in Fig. 2, five out of the twelve randomized studies were judged to exhibit low risk of bias, while five exhibited intermediate risk of bias and two studies had high risk of bias (Fig. 2). Regarding NRCTs' quality assessment using ROBINS-I tool, it was shown that only one study exhibited moderate risk of bias, while seven of them had serious risk of bias and three had critical risk of bias (Figure S1).

Main results

Progression- Free Survival (PFS)

Of the 23 studies included in the meta-analysis, only 15 reported PFS, as shown in Fig. 3A. The pooled HR for PFS was 0.64 with 95% CIs ranging from 0.50 to 0.82, indicating a statistically significant reduction of 36% in hazard ($Z = -3.50$, $p < 0.001$).

Following the protocol, heterogeneity was assessed using the chi-square (Q) statistic and the I^2 statistic, as well as the tau-squared (T^2) parameter, as shown in Fig. 3. The I^2 value was estimated at 66%, indicating substantial heterogeneity ($\tau^2 = 0.1419$, $\chi^2 = 41.18$, $p < 0.01$). Consequently, we performed a meta-regression analysis based on various study-specific variables, to identify potential sources of heterogeneity (Table 2). Similarly to the PFS analysis, we found that vaccine type and year of publication variables significantly influenced the effect size across studies. These results prompted us to explore the impact of various variables on the overall effect size through specific subgroup analyses, as elaborated below.

To further explore heterogeneity, additional analysis, using the Baujat plot and the GOSH plot (Figure S2 A and S2B) was performed. Inspection of the GOSH plot revealed that various combinations of studies consistently exhibited similar trends in effect size and heterogeneity, with minimal outliers across different study groupings. According to the Baujat plot, it was shown that only one study had significant contribution to the overall heterogeneity and effect size [51], as also confirmed by the sensitivity analysis; However, after sequentially removing each individual study, the overall effect size remained statistically significant and its direction was unchanged, indicating the robustness of our findings (Figure S3 A). Likewise, the cumulative meta-analysis showed no significant temporal changes in the effect size with the gradual inclusion of more recent studies (Figure S3B).

We performed subgroup analysis based on several variables, but we mainly focused on those that could provide clinical and biological insights, namely vaccine types, MGMT methylation percentage, and tumour types (Fig. 4). We found that the viral vector vaccine subgroup exhibited the most protective effect on PFS with a HR of 0.30, followed by the dendritic cell subgroup (HR = 0.58) and the peptide vaccine subgroup (HR = 0.88). Notably, only the dendritic cell subgroup demonstrated statistically significant results (HR = 0.58, 95% CIs: 0.43–0.79) (Fig. 4A). Regarding tumour type, only one study had enrolled patients with recurrent glioblastoma [33]. The overall effect size for the subgroup of newly diagnosed glioblastoma was HR = 0.62, which was comparable to the overall effect size across all studies (HR = 0.63), indicating minimal difference between the subgroups (Fig. 4B). Regarding MGMT methylation percentage, we found that the subgroup with moderate methylation percentage (i.e. 20 to 40% of all patients) had 46% hazard reduction of disease progression, in a statistically significant level. Conversely, the subgroup with high methylation percentage (40 to 60%) exhibited a relatively diminished reduction of hazard at 18%, in a non- statistically significant level (Fig. 4C). Subgroup analysis based on additional variables were performed and are reported in the Supplementary material section (Figure S4 A to S4 F).

Next, we examined the publication bias through Funnel plot inspection (Fig. 5A) and statistical tests (Egger's and Begg's tests). Funnel plot inspection revealed a relatively symmetrical result, with some exceptions where studies appeared outside the expected range, suggesting potential publication bias. While the Begg's test was not statistically significant ($p = 0.0619$), the Egger's test was

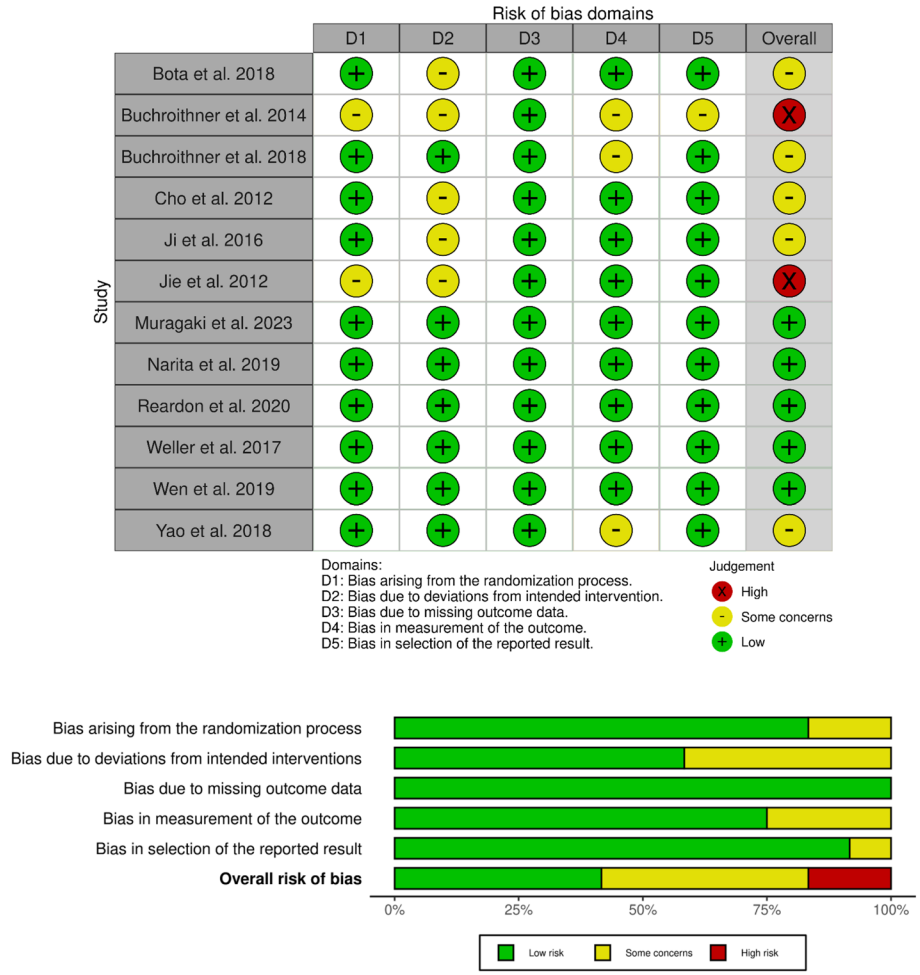


Fig. 2 Quality assessment of included RCTs using the RoB-2 Cochrane's Risk-of-Bias Assessment Tool

marginally not statistically significant (t = −2.1763, p = 0.0502), thus prompting us to perform trim-and-fill analysis. The latter indicated that no additional studies were necessary to correct for potential asymmetry observed in the Funnel plot (Fig. 5C).

Overall Survival (OS)

Of the 23 studies included in the meta-analysis, 22 of them reported OS, as shown in Fig. 3B. The pooled HR for PFS was 0.64 with 95% CIs ranging from 0.53 to 0.77, indicating a statistically significant reduction of 36% in hazard (Z =—4.73, $p < 0.00001$).

Regarding heterogeneity, the I^2 value was estimated at 58%, indicating significant heterogeneity ($\tau^2 = 0.0967$, $\chi^2 = 49.81$, $p < 0.01$). Consequently, we performed a meta-regression analysis based on various study-specific variables, to identify potential sources of heterogeneity (Table 3). Similarly to the PFS analysis, we found that vaccine type and year of publication variables significantly influenced the effect size across studies. These results prompted us to explore the impact of various variables on the overall effect size through specific subgroup analyses, as elaborated below.

Heterogeneity was further explored using the Baujat plot and the GOSH plot (Figure S2 C and S2D). Inspection of the GOSH plot revealed that various combinations of studies consistently exhibited similar trends in effect size and heterogeneity, with minimal outliers across different study groupings. According to the Baujat plot, it was shown that only one study had significant contribution to the overall heterogeneity and effect size [51], which was also confirmed by the sensitivity analysis. However, after sequentially removing each individual study, the overall effect size remained statistically significant and its direction was unchanged, indicating the robustness of our findings (Figure S5 A). Likewise, the cumulative meta-analysis showed no significant temporal changes in the effect size with the gradual inclusion of more recent studies (Figure S5B).

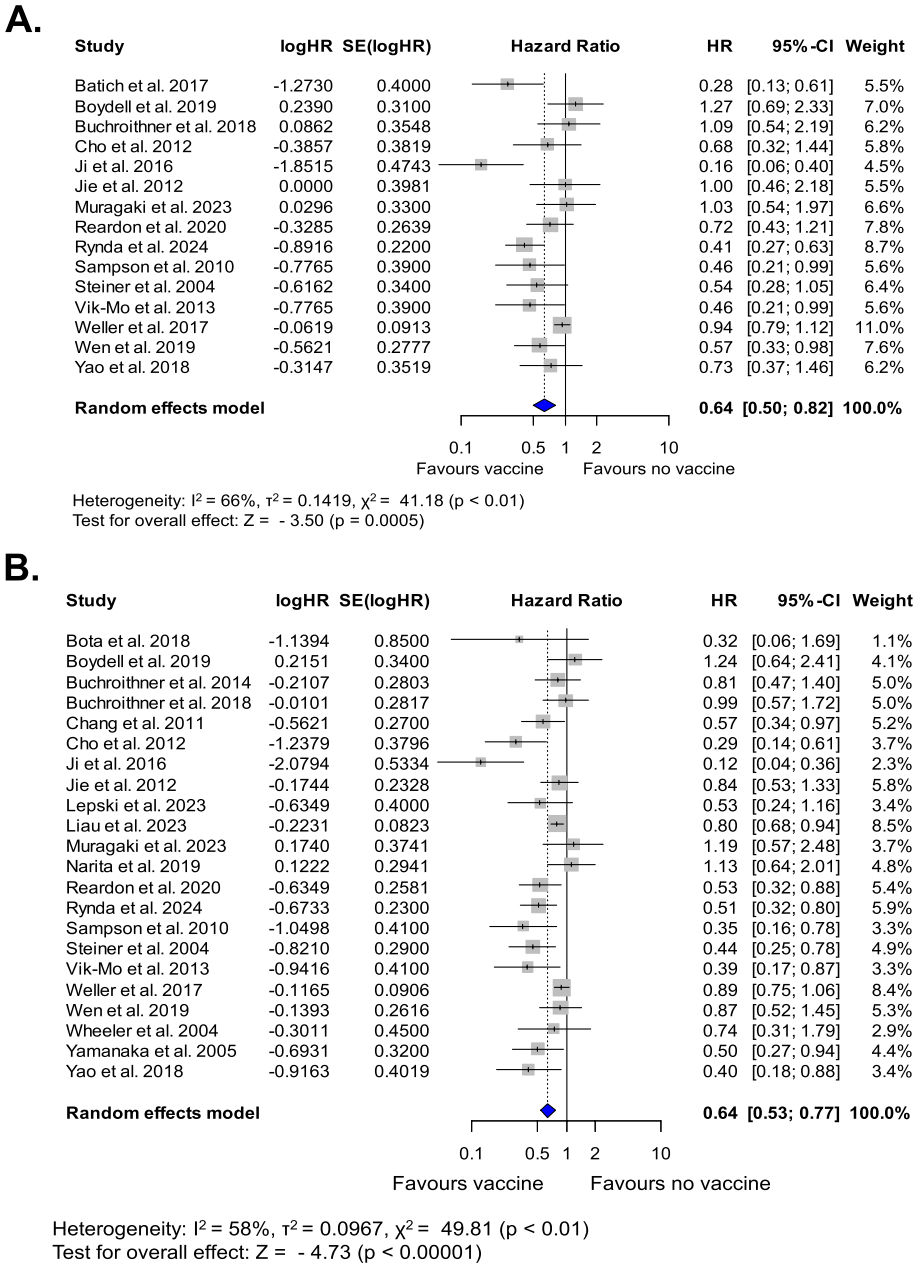


Fig. 3 Forest Plots of Progression-Free Survival **(A)** and Overall Survival **(B)** in Glioblastoma Patients Treated with Vaccine Therapy Plus Standard of Care versus Standard of Care Alone

We performed subgroup analysis based on several variables, but we mainly focused on those that could provide clinical and biological insights, namely vaccine types, MGMT methylation percentage, and tumour types (Fig. 6). We found again that the viral vector vaccine subgroup exhibited the most protective effect on PFS with a HR of 0.25, followed by the dendritic cell subgroup (HR =0.65), the whole tumour cell vaccine subgroup (HR =0.77) and the peptide vaccine subgroup (HR =0.78). Notably, the viral vector vaccine subgroup (HR =0.25, 95% CIs: 0.07–0.86) and the dendritic cell subgroup (HR =0.65, 95% CIs: 0.54–0.78) demonstrated statistically significant results (Fig. 6A). Regarding tumour type, five of the 23 total studies had included patients with recurrent glioblastoma. The overall effect sizes between the subgroups of newly diagnosed glioblastoma (HR =0.63) and the recurrent glioblastoma (HR =0.62) did not differ significantly, while both were statistically significant

Table 2 Meta-regression analysis for Progression-Free Survival (PFS) and Overall Survival (OS) in Glioblastoma Patients Treated with Vaccine Therapy Plus Standard of Care versus Standard of Care Alone

Progression Free Survival (PFS)									
Variable	Variable category tested	β estimate	SE	Z- value	*P*- value	Test of Moderators	R^2	Test for Residual Heterogeneity	I^2
Randomized status	Randomized	0.3543	0.2475	1.4317	0.1522	0.1522	20.35%	0.0048	57.78%
	intercept	−0.6626	0.1941	−3.4144	0.0006				
Tumor type	Recurrent GBM	0.1516	0.5312	0.2854	0.7754	0.7754	0.00%	<.0001	71.40%
	intercept	−0.4801	0.1519	−3.1595	0.0016				
Vaccine type	Dendritic Cell Vaccine (baseline variable)	−0.5355	0.1587	−3.3747	0.0007	0.0002	-	0.0300	49.14%
	Peptide Vaccine	−0.1765	0.1935	−0.9121	0.3617				
	Viral Vector Vaccine	−1.1021	0.3498	−3.1509	0.0016				
	Whole Tumor Cell Vaccine	0.0296	0.4415	0.0669	0.9466				
Use of temozolomide in standard of care treatment	TMZ	0.1737	0.5420	0.3205	0.7486	0.7486	0.00%	0.0001	68.19%
	intercept	−0.6162	0.5242	−1.1755	0.2398				
Year of publication	2010 to 2020 (baseline variable)	−0.4520	0.1672	−2.7026	0.0069	0.0136	-	0.0002	69.92%
	after 2020	−0.4309	0.2955	−1.4578	0.1449				
	before 2010	−0.6162	0.5516	−1.1170	0.2640				
WHO classification	Recent WHO classification	0.0064	0.2618	0.0245	0.9804	0.9804	0.00%	0.0026	58.51%
	intercept	−0.4534	0.1872	−2.4217	0.0154				
Overall Survival (OS)									
Variable	Variable category tested	β estimate	SE	Z- value	*P*- value	Test of Moderators	R^2	Test for Residual Heterogeneity	I^2
Randomized status	Randomized	0.1708	0.1860	0.9181	0.3586	0.3586	4.19%	0.0005	58.01%
	intercept	−0.5339	0.1366	−3.9075	<.0001				
Tumor type	Recurrent GBM	−0.0516	0.2780	−0.1855	0.8528	0.8528	0.00%	0.0002	75.21%
	intercept	−0.4445	0.1358	−3.2734	0.0011				
Vaccine type	Dendritic Cell Vaccine (baseline variable)	−0.4490	0.1078	−4.1657	<.0001	<.0001	-	0.0075	50.05%
	Peptide Vaccine	−0.2373	0.1640	−1.4471	0.1479				
	Viral Vector Vaccine	−1.1988	0.3246	−3.6931	0.0002				
	Whole Tumor Cell Vaccine	−0.0984	0.4045	−0.2433	0.8078				
Use of temozolomide in standard of care treatment	TMZ	−0.0282	0.2745	−0.1027	0.9182	0.9182	0.00%	0.0003	66.93%
	intercept	−0.4252	0.2533	−1.6791	0.0931				
Year of publication	2010 to 2020 (variable)	−0.4289	0.1250	−3.4326	0.0006	<.0001	-	0.0006	61.47%
	after 2020	−0.4024	0.1891	−2.1276	0.0334				
	before 2010	−0.6474	0.2771	−2.3360	0.0195				
WHO classification	Recent WHO classification	0.0895	0.2120	0.4223	0.6728	0.6728	0.00%	0.0002	63.43%
	intercept	−0.5489	0.1646	−3.3356	0.0009				

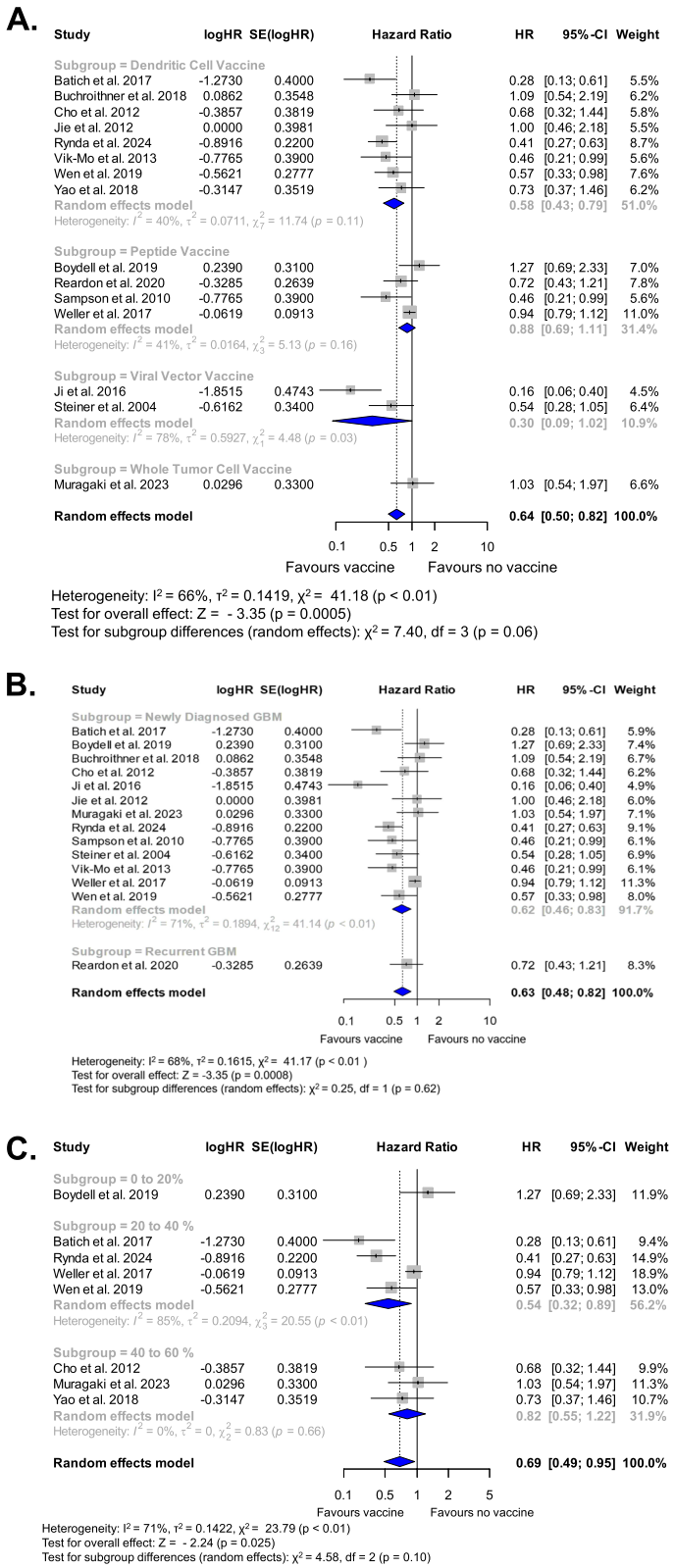


Fig. 4 Subgroup analysis of Progression-Free Survival in Glioblastoma Patients Treated with Vaccine Therapy Plus Standard of Care versus Standard of Care Alone Based on Vaccine Type **(A)**, Tumour Type **(B)** and MGMT Methylation Percentage **(C)**

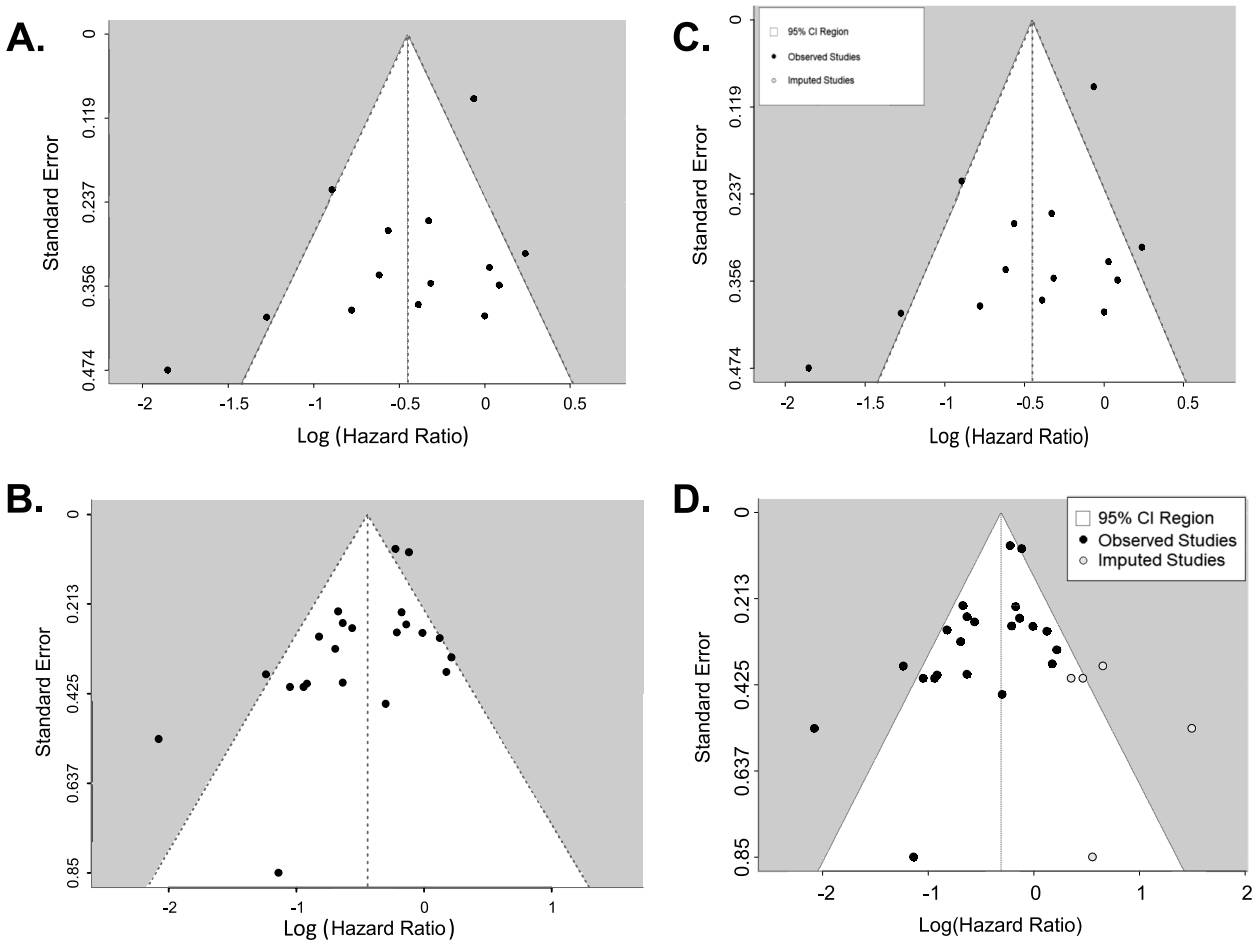


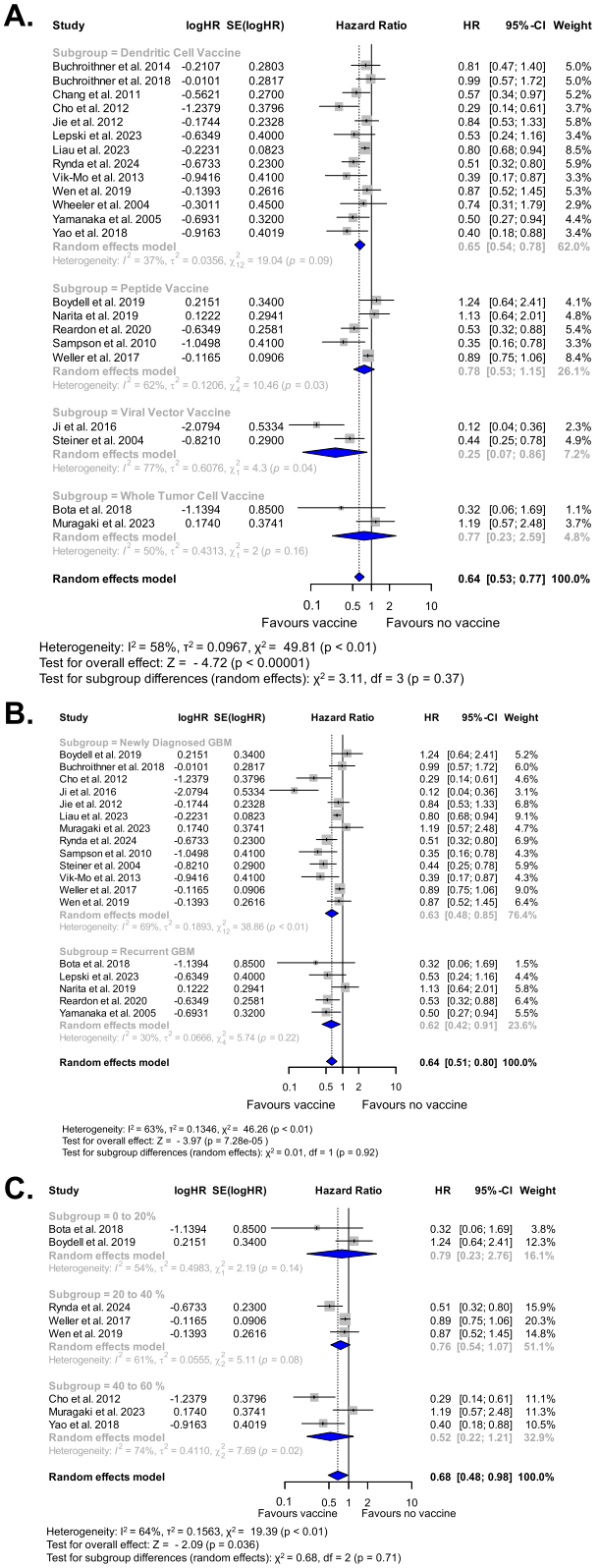
Fig. 5 Publication Bias Analysis: Funnel Plots **(A, B)** for Progression-Free Survival **(A)** and Overall Survival **(B)** and Trim-And-Fill analysis **(C, D)** for PFS **(C)** and OS **(D)** in Glioblastoma Patients Treated with Vaccine Therapy Plus Standard of Care versus Standard of Care Alone

(Fig. 6B). Regarding MGMT methylation percentages, we observed that the subgroup with low methylation (0–20%) experienced a 21% reduction in mortality hazard, though this was not statistically significant. Interestingly, the subgroup with moderate methylation (20–40%) showed a slightly greater reduction in hazard at 24% (HR = 0.76). Notably, the subgroup with the highest methylation percentage (40–60%) derived the most significant survival benefit from the vaccine, with a hazard ratio of 0.52 (Fig. 6C). These findings might suggest a progressively increased benefit of vaccination therapy on overall survival with higher levels of MGMT methylation. These results align and further support previous studies that have associated MGMT methylation with improved prognosis in glioblastoma patients [10, 11, 72]. Subgroup analysis based on additional variables were performed and are reported in the Supplementary material section (Figure S6 A to S6 F).

Next, we examined the publication bias through Funnel plot inspection (Fig. 5B) and statistical tests (Egger's and Begg's tests). Funnel plot inspection revealed a relatively symmetrical result, with some exceptions where studies appeared outside the expected range, suggesting potential publication bias. While the Begg's test was not statistically significant ($p = 0.0710$), the Egger's test was statistically significant ($t = -2.9177$, $p = 0.0085$), thus prompting us to perform trim-and-fill analysis, which indicated 5 missing studies on the right side of the funnel plot, suggesting potential publication bias (Fig. 5D).

Adverse events

Out of the 23 studies included in the meta-analysis, only seven adequately reported adverse events in a manner that allowed for extraction and statistical analysis. For that reason, we acknowledge the presence of significant bias and therefore, safe conclusions cannot be made. Even in the absence of bias, any observed differences in adverse events incidence between the vaccinated and control groups would be most likely correlative and not causal, given the influence of multiple potential



◄ **Fig. 6** Subgroup analysis of Overall Survival in Glioblastoma Patients Treated with Vaccine Therapy Plus Standard of Care versus Standard of Care Alone based on vaccine type **(A)**, Tumour Type **(B)** and MGMT Methylation Percentage **(C)**

confounders. In terms of outcomes, the pooled HRs for adverse events of more than grade II and grade III were 1.63 (95% CI: 1.45–1.83, $P < 0.01$) and 1.95 (95% CI: 1.73–2.21, $P < 0.01$) respectively, indicating a higher rate of statistically significant adverse events in the vaccine arm. However, these results had significant heterogeneity (grade ≥ II: I2 = 92%, grade ≥ III: I2 = 97%) and should be interpreted cautiously. However, in the supplementary material that accompanies this study, we provide our meta-analysis findings regarding adverse events, having divided them into three categories according to Common Terminology Criteria for Adverse Events (CTCAE) v6.0: adverse events of ≤ grade II, ≥ grade III and events of any grade (Figure S7 A – S7 C).

Discussion

In recent years, immunotherapy has emerged as a groundbreaking anticancer therapeutic approach that harnesses the immune system's innate ability to target and destroy cancer cells through both cellular and humoral mechanisms. Over the past two decades, there has been a significant intensification of research efforts in the field of immunotherapy for glioblastoma, with the goal of improving outcomes for patients with newly diagnosed and recurrent diseases. Among the various immunotherapeutic approaches, vaccines have shown encouraging preliminary results [35]. These vaccines are designed to elicit a targeted antitumor immune response against specific antigens associated with glioblastoma and have been extensively evaluated in numerous clinical trials as potential treatments aimed at prolonging patient survival. Our meta-analysis aimed at the systematic evaluation of all relevant clinical trials exploring vaccine immunotherapy for glioblastoma patients in terms of safety and efficacy. Following on, we summarize the key findings of our work, while providing a comparative interpretation based on current literature.

Summary of key findings and comparative interpretation

OS and PFS

Vaccines have proven efficacious in our meta-analysis to extend both the overall survival of patients and increase their progression-free intervals. These observations pertain to both newly diagnosed and recurrent disease, reducing the hazard of death and recurrence.

Vaccine types: dendritic cell, peptide, viral vector vaccines

Regarding the DCV, our study demonstrates a potential survival benefit for both PFS and OS exhibiting a hazard reduction of 42% and 35% respectively. Dendritic cells constitute the most potent antigen-presenting cells (APCs) to provoke adaptive immunity but lack maturation in the immunosuppressive tumor microenvironment (TME) of glioblastoma impeding the response of effector T cells. Therefore, sensitized dendritic cells could play a primordial role in inducing a rigorous antitumor effect by CD8 + T lymphocytes, which potentially explains why dendritic cell vaccination the most extensively studied vaccine type is. A meta-analysis by Cozzi et al. emphasized the time needed for the DCVs to be able to develop their therapeutic potential, showcasing an increase to 1 and 2-year survival of patients treated with DCV by 1.9 and 3.6 times, respectively, compared to control patients [73]. In accordance, in a meta-analysis performed by Vatu et al. [43], the DCV resulted in improvements in OS and PFS (35% and 41%, respectively) being superior to viral therapy (four clinical trials on herpes simplex virus thymidine kinase/ganciclovir gene therapy were included) in both outcome measures. Notably, combined therapies are currently being evaluated in addition to vaccine therapy, like immune checkpoint inhibitors (ICIs). There are several clinical trials, such as NCT04201873 (using pembrolizumab) and NCT03014804 (using nivolumab), designed to investigate the efficacy of combined treatment with DCV and immune checkpoint inhibitors (ICIs).

Concerning peptide vaccines, they did not demonstrate any combined statistically significant benefit either for OS or for PFS. At the forefront of peptide vaccine research is EGFRvIII, a deletion mutation that creates an extracellular tumor-specific epitope not expressed in normal tissue, thus presenting as an ideal neoantigen for vaccine targeting [74] Among the individual studies, Reardon et al., Weller et al., Sampson et al., and Boydell et al. (four of our included peptide vaccine studies) [33, 63, 66, 71] all utilized EGFRvIII targeting. However, improved outcomes in both OS and PFS were observed only in the studies by Reardon and Sampson [33, 63]. Notably, the only phase III trial, conducted by Weller, did not demonstrate any survival benefit for glioblastoma patients who received the vaccine [66].

Regarding viral vector vaccines, our analysis showcased OS benefit and marginally statistically significant improvement for PFS. However, we must acknowledge that the viral vector vaccine group consists of only two studies thus leading to widened confidence intervals [51, 64].

Newly diagnosed and recurrent disease

We observed a risk reduction in both groups, both for newly diagnosed and recurrent glioblastoma associated with increased overall survival. The combined vaccination therapy proved beneficial across both disease presentations, despite their significantly different underlying pathophysiologies [74].

As for PFS, vaccines resulted in increased progression-free intervals and reduced risk of progression for the newly diagnosed glioblastoma group. The impact of vaccination therapy on patients with recurrent GB could not be statistically evaluated, as this group includes only a single study reporting patient data on PFS.

MGMT methylation status

MGMT is a highly evolutionarily conserved DNA repair enzyme that removes alkylated guanine residues at the DNA level, thereby antagonizing the effects of alkylating therapeutic agents [75]. Methylation of CpG islands in the MGMT promoter leads to suppressed enzyme transcription, enhancing vulnerability of cancer cells to alkylating chemotherapies, like temozolomide.

Our findings on the impact of MGMT on overall survival demonstrated a progressively greater reduction in hazard as the methylation percentage increased among patients, however the result was not statistically significant. The survival benefit observed with increased promoter methylation is consistent with existing literature, which identifies MGMT methylation as a prognostic and predictive biomarker [11, 72]. Accordingly, when evaluating PFS, the subgroup of studies where 20%−40% of patients had methylated MGMT showed a 46% reduction in PFS risk compared to the control group (HR = 0.54, $p < 0.01$).

However, it should be noted that subgroup cut-offs, for each group of patient percentage with methylated MGMT, were decided upon discussion among the investigators and are not described elsewhere in previous literature.

Grade of tumor resection

The extent of tumor resection is reported in 16 out of the 24 included studies, with gross total or maximal tumor resection documented in all patients from eight of these studies. Five studies constituted of a mixed population were both total and subtotal resection was achieved in different patients. At the same time, it is well-established that a greater extent of resection is associated with favorable outcomes [13]. However, the meta-regression analysis that was conducted demonstrated that the grade of tumor resection did not interfere with the effect size across studies. Future primary and secondary research, particularly clinical trials, should focus on evaluating vaccine regimens in homogeneous populations where a similar degree of tumor resection has been achieved. It is also crucial to establish standardized definitions for

the degree of resection, as well as consistent methods for evaluating it, to allow for reliable conclusions to be drawn from meta-analyses of individual studies.

Strengths

To our knowledge, this study is the first meta-analysis to gather and analyze data on glioblastoma vaccine immunotherapy across all vaccine types for both newly diagnosed and recurrent disease. Data extracted from included studies notably generated a statistically significant increase in overall survival and progression-free survival of patients treated with vaccines on top of their standard treatment compared to controls, indicating potential favorable patient outcomes. We also stratified our results by comparison groups, enabling us to discern the varying effects of vaccination therapy based on different clinical and treatment parameters. Another strength of our study is that heterogeneity, publication bias and the overall quality of the studies included were rigorously evaluated. Advanced statistical tests like Boujat and GOSH plots allowed for thorough inspection of the heterogeneity, enabling us to draw reliable conclusions for its source.

Limitations

As for the limitations of the current meta-analysis, we observed high discrepancies in the follow-up periods across studies, varying from 7,8 to 72 months. It is important to note that the current study primarily consists of phase II trials that were available at the time of the search. Therefore, it will be essential to further analyze the pooled effect estimates from the larger phase III studies that are currently being conducted, once their results are published. Furthermore, variations in adverse events reporting prevented the investigators from statistically analyzing the differences among studies and no definitive conclusions could be made regarding the potential complications of the vaccines. However, as aforementioned, adverse effects are collectively presented in the supplementary material. Additionally, each clinical trial, depending on the year it was designed, was based on each own glioblastoma WHO classification. Thus, we included studies with different definitions of glioblastoma, which constitute a source of potential inherent bias. Last, high heterogeneity was observed for which meta-regression analysis was conducted. As stated, vaccine type and year of publication variables were the factors to significantly influence the effect size across studies.

Future directions

It is crucial to gain a deeper understanding of the underlying pathophysiology of vaccines that contribute to potentially favorable outcomes. Research should focus on unraveling their molecular mechanisms and thoroughly investigating cancer resistance to these vaccines. To achieve this, emphasis should also be placed on preclinical and animal models. These steps are essential for advancing vaccine personalization and precise antigen targeting. To achieve feasibility for a clinical setting, resources should be allocated to automate and standardize the process of antigen mapping for each patient and integrate it into clinical practice, minimizing delays. Moreover, it has been shown that time variable may pose a major determent of vaccine efficacy and should be explored in all types of vaccines [73]. Additionally, combined therapies could be the solution for non-responders and should be further explored. Employing multimodal targeting strategies that address simultaneously different types of antigenic targets, personalized according to individual tumor characteristics [76], may enhance treatment efficacy and overcome tumor drug resistance [77].

As medicine progresses, new technologies emerge with the potential of benefiting patients. As such, artificial intelligence could serve as a powerful tool in patient stratification and prognostication. Machine and Deep Learning algorithms have been utilized to create predictive models and scores of treatment response [78]. Hence, research could be conducted to assess the potential response of patient to vaccines, integrating patient demographical, clinical and oncological characteristics into combined models.

Accordingly, future research should emphasize more on better patient stratification and identification of those high-risk patients who are going to benefit more from the rigorous administration of a vaccine. When identified, these patients should participate in the appropriate clinical trial. It is estimated that only 10% of glioblastoma patients eventually are included in clinical trials, leading to insufficient data that is difficult to generalize across different populations [79]. A plethora of suggestions has been made regarding the matter, such as including high grade glioma patients in phase I clinical trials, a strategy which was traditionally prevented [80]. Therefore, better representation of glioblastoma patients in these trials is crucial to produce more reliable and reproducible results and better understand these immunotherapeutic compounds.

Regarding study design, a transition from single-center and single-arm studies to randomized, controlled, and sufficiently powered clinical trials is essential. This will improve the generalization and reproducibility of results thereby optimizing the use of current financial resources.

Conclusions

Our study attempted to assess the efficacy of vaccination therapy in newly diagnosed and recurrent glioblastoma based on the existing published literature. Our

results suggest that vaccination therapy may enhance survival and progression free survival of patients diagnosed with both manifestations of the disease. As of vaccine types, dendritic cell vaccines generated the most beneficial results, in accordance with previous studies. Vaccines were not associated with serious adverse events; however, various confounding factors prevented causal conclusions. Nevertheless, it is important to consider the limitations of our study when evaluating our findings. Future research, evaluating larger, phase III studies, is needed to generate conclusive and concrete results. We encourage future researchers to improve patient stratification, explore different combinations of vaccines and immunotherapeutic drugs, and publish consensus papers that define standard characteristics of relevant trials. This includes setting minimum follow-up periods for patients and standardize vaccine administration protocols.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-14397-1.

Supplementary Material 1.

Acknowledgements
Not applicable.

Authors' contributions
IK, KK applied the searching strategy, screened the generated results and selected the studies that met the inclusion criteria. The same authors (IK and KK) along with PL and IK conducted the statistical analysis and assessed the heterogeneity of the studies. KD, ERK and VKK did the quality assessment of the studies. IP, ET and DK assisted in the writing of the main text and prepared the figures. KG gave the initial idea for the study and supervised it throughout every step of its writing process. TZ advised throughtout the writing process and edited the final version of the study. Mainly contributed to the conclusions of the study. All authors reviewed the final versions of the manuscript and made adjustions and revisions as needed.

Funding
We declare that no source of funding was obtained at any stage of the writing process of the present work.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 16 January 2025 Accepted: 27 May 2025
Published online: 01 July 2025

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