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Original Research

Granulocyte-macrophage colony-stimulating factor for newly diagnosed glioblastoma

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ABSTRACT

Background: There is a clear need to improve the efficiency of therapeutic strategy for patients with newly diagnosed glioblastoma (GBM). The purpose of this study was to evaluate the feasibility of hypofractionated intensity-modulated radiation therapy (IMRT), temozolomide and granulocyte-macrophage colony-stimulating factor (GM-CSF) for patients with newly diagnosed GBM.

Methods: Patients were treated with hypofractionated IMRT (15 × 3.5Gy to the high-risk region and 15 × 3.0Gy to the low-risk region), temozolomide (75 mg per square meter of body-surface area per day, from 1 week before the beginning of radiotherapy to the last day of radiotherapy) and GM-CSF [200μg (equivalent to 125 μg/m^2 calculated dose) subcutaneously injected daily for 2 weeks, starting from the second week of radiotherapy]. The primary endpoint was 6-month progression free survival (PFS).

Results: Between June 2016 and Feburary 2020, 41 patients were enrolled. During concomitant chemo-radiotherapy, no grade 3 or 4 hematologic toxicities were observed and grade 3 non-hematologic toxicities were documented in 5 patients (12.2 %) due to GM-CSF. All patients completed both radiotherapy and concomitant temozolomide as planned. Only five patients (12.2 %) discontinued concomitant GM-CSF because of toxicity. At a median follow-up of 33.1 months (IQR 23.0-51.2), the 6-month PFS rate was 68.3 % (95 % CI: 54.0-82.6). The median overall survival of all patients was 16.7 months (95 % CI: 10.5-22.9). Compared with pre-GM-CSF, the concentrations of TNF-α (p = 1.9615E-10) and IL-18 (p = 6.8467E-8) were increased after GM-CSF, while the proportion of CD19 (p = 0.000015), the concentrations of IgG (p = 0.000015) and CXCL12 (p = 0.000257) were decreased.

Conclusions: The combination of hypofractionated IMRT, temozolomide and GM-CSF for GBM was feasible and safe.

Trial Registration: ClinicalTrials.gov Identifier: NCT02663440.

Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor, accounting for 48 % of all primary malignant central nervous system neoplasms [1]. Based on the first phase 3 trial firstly published in 2005, surgery followed by radiotherapy (60Gy over six weeks) plus concomitant and maintenance temozolomide (TMZ) was established as the standard treatment. Overall survival (OS) was 27.2 % at two years, and 9.8 % at five years with radiotherapy and TMZ, versus 10.9 %, and 1.9 % with radiotherapy alone. Progression-free survival (PFS) was 11.2 % at two years, and 4.1 % at five years with radiotherapy plus TMZ, versus 1.8 %, and 1.3 % with radiotherapy alone [2,3]. Tumor-treating fields (TTF) could also be given concurrently with maintenance TMZ for a median PFS benefit of 2.7 months [4]. Despite incremental advances in

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the therapeutic approach, the long-term survival of patients with GBM has not improved [5]. Hence, there is an urgent need to improve the efficacy of therapeutic strategy [1,5].

Immunotherapy has become a promising clinical strategy for treating many cancers [6]. For patients with recurrent or newly diagnosed GBM, the phase III trials of CheckMate-143, CheckMate-498 and CheckMate-548 failed to meet their endpoints [7–9]. The phase III, prospective, externally controlled nonrandomized trial indicated that adding autologous tumor lysate-loaded dendritic cell vaccine to standard of care extended survival among patients with recurrent or newly diagnosed GBM [10]. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a potent cytokine promoting the differentiation of myeloid cells, can also be used as an immunostimulatory adjuvant to elicit anti-tumor immunity [11]. For patients with unresectable or metastatic melanoma, the combination of ipilimumab (10 mg/kg, intravenously on day 1) and GM-CSF (250 μg subcutaneously, on days 1 to 14 of a 21-day cycle) can be more efficacious than ipilimumab alone [12]. However, monthly intradermal injections of rindopepimut (a vaccine targeting the EGFR deletion mutation EGFRvIII, 500 μg admixed with 150 μg GM-CSF) via concurrently with maintenance TMZ did not increase survival in patients with EGFRvIII-positive GBM [13].

There is significant interest in combining radiotherapy with immunotherapy for cancer treatment. However, how best to integrate these two modalities to maximize clinical responsiveness remains uncertain [14]. For metastatic solid tumors, abscopal responses were observed in 11 of 41 patients after hypofractionated radiotherapy (35 Gy in ten fractions) plus GM-CSF (125 μg/m^2 subcutaneously injected daily for two weeks, starting in second week of radiotherapy) for metastatic solid tumors [15]. A meta-analysis including 484 patients with newly diagnosed GBM indicated that hypofractionated radiotherapy seemed to improve OS and PFS, compared with the standard treatment [16]. In the phase I trial of hypofractionated intensity modulated radiotherapy (IMRT) for GBM, concurrent TMZ was given at 75 mg/m2/d for 28 consecutive days [17]. Furthermore, TMZ could be given in the early break of concomitant radiochemotherapy for a median OS benefit of 4.4 months [18]. With the renewed interest in cytokines, numerous clinical trials are evaluating the safety and efficacy of cytokine-based cancer treatment [19]. The purpose of this study was to evaluate the feasibility of hypofractionated IMRT, TMZ and GM-CSF treatment for newly diagnosed GBM.

Patients and methods

Study design and participants

This study was an open-label, single-arm, phase 2 trial. Eligible patients were aged 18-75 years with newly diagnosed and pathologically confirmed GBM, and a Karnofsky performance status ≥60. Also, all patients had to have adequate liver, kidney, and bone marrow function. Exclusion criteria were tumors involving the brainstem, allergies to GM-CSF, previous brain radiotherapy, receiving other investigational agents, or uncontrolled inter-current illnesses such as ongoing or active infection, myocardial infarction within the past six months, symptomatic congestive heart failure, unstable angina pectoris, or unstable cardiac arrhythmia. Pretreatment assessments included a complete medical history and physical examination, complete blood count, liver and renal biochemistry and magnetic resonance imaging (MRI) of brain with and without contrast. The pre- and post-GM-CSF peripheral blood samples were centrifuged at 2000 × *g* for 10 min at 4°C and stored at −80°C to identify biomarkers with possible prognostic value. The study protocol was approved by the institutional review board and registered with the *ClinicalTrials.gov*. All enrolled patients signed an informed consent form.

Procedures

The IMRT was started within eight weeks after surgery. Patients were immobilized using a perforated, thermoplastic head mask in a hyperextended position. Computed tomography (CT) images with a 3-mm slice thickness were obtained for treatment-planning. The target delineation was based on the fused images of simulation CT and pre-IMRT brain MRI. The target volumes were delineated using an institutional treatment protocol defined as follows: The gross tumor volume (GTV) included the entire surgical cavity and contrast-enhancing residual diseases based on the enhanced T1-weighted pre-IMRT brain MRI scan. The clinical tumor volume 1 (CTV1) was defined as the high-risk region that included GTV plus a 1-cm margin, and CTV2 was defined as the low-risk region that included GTV plus a 2-cm margin. The respective planning target volumes (PTVs) were generated with a minimum 3-mm margin in all directions. Using the simultaneous integrated boost technique, the total doses of the PTV1 and PTV2 were 52.5 Gy and 45 Gy delivered in 15 fractions, respectively. The goal of the prescribed dose distribution was to cover ≥ 95 % of each PTV. IMRT was delivered by 6-MV photons with one fraction daily, five days per week.

Concomitant TMZ was given daily at a dose of 75 mg/m2 from one week before the start of IMRT until the last day of IMRT. After a 4-week break, the patients received maintenance TMZ dose of 150 mg/m^2 for five days for the first cycle and then 200 mg/m^2 every four weeks, for a total of 12 cycles or until tumor progression. GM-CSF 200μg (equivalent to 125 μg/m^2 calculated dose) was given daily via subcutaneous injection for two weeks, starting one week after the start of IMRT (Fig S1).

All patients were evaluated weekly during concurrent chemoradiotherapy, then monthly during maintenance TMZ, and once every three months thereafter. The patient evaluation included history, physical examination, full blood counts and blood chemistry tests. Contrast-enhanced brain MRI was performed before study treatment, then one month after IMRT, and once every three months thereafter.

Immunophenotyping of the peripheral blood immune cells

The analysis of the peripheral blood immune cells was performed on whole blood samples collected into heparinized tubes. The following antibodies were purchased from Beckman Coulter Inc., USA: CD3-FITC (#A07746), CD4-PE (#A07751), CD8-PE (#A07757), CD56-PE (#55664), CD19-FITC (#555412), CD45RA-FITC (#A07786), CD45RO-PE (#A07787), CD38-FITC (#A07778), anti-HLA-DR-PC5 (#A07793), and FITC/RD1/ECD/PC5 isotype controls (#6607013; #6607073). The cells were analyzed by flow cytometry (FACSVia, BD Biosciences). The percentages of positively labeled lymphocytes were analyzed using Cellquest software (BD Biosciences).

Cytokine measurement in serum

Serum was separated for estimation of tumor necrosis factor-α (TNF-α), CCL5, CXCL12, IL-1β, IL-6, IL-18 (R&D Systems, Minneapolis, MN), monocyte chemotactic protein-1 (MCP-1), IL-10 and TGF-β1 [Hangzhou MultiSciences (Lianke) Biotech, China] using standard sandwich enzyme-linked immunosorbent assay (ELISA) kit specific for human cytokines according to the manufacturer's instructions.

Exosome measurement in serum

The exosomes were isolated according to the manufacturer's protocol. Briefly, the serum samples were thawed and centrifuged at 2000 × g for 20 min to remove cell debris, followed by addition of total exosome isolation (from serum, Thermo, California USA) reagent. Serum and reagent were mixed and incubated at 4°C for 30 min and centrifuged at 10,000 × g for 10 min. Finally, the exosome pellet was precipitated at the bottom and the supernatant was discarded. To define the molecular portrait of exosomes, the surface biomarkers CD9, CD63, and CD81 of exosomes were analyzed in the obtained samples by transmission electron microscopy (TEM) and Western blotting (Fig S2).

Total RNA was isolated from the prepared exosomes using an RNeasy

serum/plasma kit (CWBIO, China), as per the manufacturer's protocol. The quality and yield of each RNA sample was measured by BioDrop-DUO (Biochrom, Cambridge, UK). RT-qPCR was used to detect exosomal miRNAs. The primers for Mir-21, CD44, CD133, IDH-1, NESTIN and EGFR are listed in N

RT-PCR parameters were set according to the manufacturers' instructions. The detected exosomal miRNAs data were normalized to U6 and described as the ratios to the scramble control.

Endpoints

The primary endpoint was 6-month PFS. PFS was calculated from the initiation of treatment to symptomatic or radiographic progression. Response evaluation was conducted according to Response Assessment in Neuro-Oncology criteria [20]. The secondary endpoints were OS and safety. OS was calculated from the initiation of treatment to date of death. Treatment-induced toxicities were assessed and scored according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE version 3.0). GM-CSF was reduced by 50 % for documented white blood cell count >60, 000/mm3 and discontinued for ≥ grade 3 toxicity believed by the investigator to be caused by GM-CSF [12]. The exploratory objective was to assess peripheral blood biomarkers for prognosis.

Statistical analysis

This prospective phase 2 study was designed to test whether hypofractionated IMRT, TMZ and GM-CSF improved PFS. The calculation of sample size was performed by using the Flemings single stage phase II design [21] and based on data from Stupp et al's study [2] with a 6-month PFS rate of 55 % in the radiotherapy plus TMZ arm. This protocol would be considered worthy of future study and consistent with a 75 % 6-month PFS if ≥28 of 41 patients do not progress by six months. If fewer than 28 patients do not progress by six months, then the 6-month PFS is consistent with <55 %, and the treatment will not be considered worthy of future study. This design has an alpha (probability of concluding PFS is consistent with 75 % when it is truly 55 %) of 0.06 and a beta (probability of concluding PFS is consistent with 55 % when it is truly 75 %) of 0.12 [22]. The recruitment period was initially planned for 12 months (from January, 2016, to December, 2016). However, the enrollment was extended to February, 2020 (last patient enrolled) due to the slow accrual of patients. PFS and OS were estimated using the Kaplan-Meier method. The segmented rates for PFS and OS at 6-month, 1-year and 2-year were also calculated, and the 95 % confidential intervals (CIs) were calculated by using the binomial distribution for a percentage. Biomarker changes were detected by paired t-tests between pre-GM-CSF and post-GM-CSF, and differences among groups were evaluated by t-test or the Mann-Whitney U test after accounting for a false positivity rate. The Statistical Package for Social Sciences (version 22.0; IBM Corp., Armonk, NY, USA) software was used for statistical analysis, and the sample size calculations were performed with PASS (version 11.0.10).

Results

Patient characteristics

Between June 6, 2016, and February 4, 2020, 42 patients were recruited, and one patient was ineligible before starting treatment due to disease progression (Fig. 1). The median age was 56 years, and 80.5 % of patients had undergone gross total resection. The baseline characteristics of the enrolled patients are listed in Table 1.

Treatment modalities

The median time from diagnosis to the start of therapy was three weeks (range, 2-8). All patients completed the planned radiotherapy and concomitant TMZ. Only five patients (12.2 %) discontinued concomitant GM-CSF because of toxicity. After radiotherapy, 33 patients (80.5 %) started adjuvant TMZ and 16 patients (39.0 %) completed ≥ 6 cycles. Only four patients (9.8 %) completed 12 cycles of maintenance TMZ. The median counts of the maximum of white blood cells (WBCmax) and the maximum of platelets (PLTmax) during

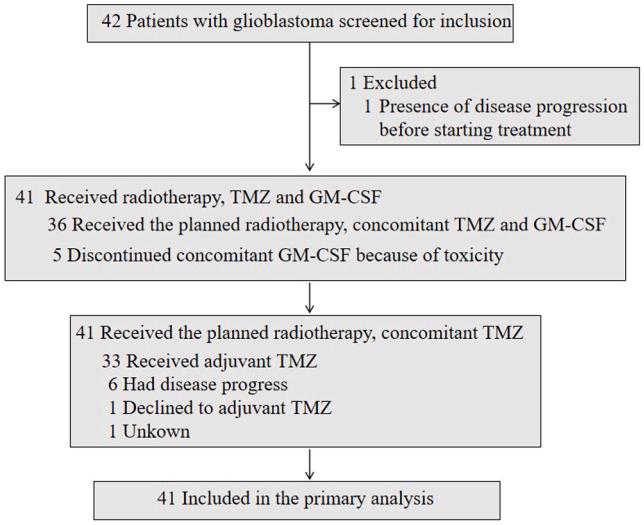

Fig. 1. Screening and patient flow in a trial of granulocyte-macrophage colony-stimulating factor for newly diagnosed glioblastoma. TMZ, temozolomide; GM-CSF, Granulocyte-macrophage colony-stimulating factor.

Table 1
Patient characteristics ($N = 41$).

Characteristic	*N* (%)
Gender	
Male	24 (58.5)
Female	17 (41.5)
Age (yr)	
Median, range	56 (22-72)
Karnofsky performance status (%)	
Median, range	70 (60-80)
RTOG RPA class	
IV/V	15/26
MGMT promotor region methylation status	
Methylated	26 (63.4)
Unmethylated	9 (22.0)
Invalid	6 (14.6)
IDH1-R132H status	
Negative test results	34 (82.9)
Mutated	1 (2.4)
Invalid	6 (14.6)
Tumor location	
Parietal lobe	5 (12.2)
Occipital lobe	4 (9.8)
Frontal lobe	13 (31.7)
Temporal lobe	14 (34.1)
Thalamus	2 (4.9)
Cerebellum	2 (4.9)
Callosum	1 (2.4)
Surgical resection	
Gross total resection	33 (80.5)
Partial resection	7 (17.1)
Biopsy	1 (2.4)
Corticosteroid therapy	
Yes	18 (43.9)
No	23 (56.1)

RTOG RPA, Radiation Therapy Oncology Group recursive partitioning analysis; MGMT, O-6-methylguanine-DNA methyltransferase gene; IDH, isocitrate dehydrogenase.

chemoradiotherapy were 24.1 (25th-75th percentile 16.2-31..0) × 10^9/L and 262 (25th-75th percentile 207.5-329.5) × 10^9/L, respectively. Table 2 summarizes the details of treatment.

Toxicity

During concomitant chemoradiotherapy and GM-CSF, no grade 3 or 4 hematologic toxicities were observed, while grade 3 non-hematologic toxicities were documented in five patients (12.2 %) due to GM-CSF (Table 3). During maintenance TMZ, no patient had grade 3 or 4 toxicities, but grade 2 radiation necrosis was observed in two patients (4.9 %) (Fig S3).

Survival outcomes

The PFS data of all 41 eligible patients are shown in Fig 2A. The 6-month PFS was 68.3 % (95 % CI: 54.0-82.6), which exceeded the null hypothesis (28 of 41 patients do not progress by six months), confirming the study hypothesis. At a median follow-up of 33.1 months (25th-75th percentile 23.0-51.2), 31 patients (75.6 %) had died. The median OS of all patients was 16.7 months (95 % CI: 10.5-22.9). The 6-month, 1-year and 2-year OS rates were 87.8 % (95 % CI: 77.8-97.8), 70.7 % (56.8-84.6) and 35.6 % (19.9-51.3), respectively. The median PFS of all patients was 8.3 months (95 % CI: 6.3-10.3). The 1-year and 2-year PFS rates were 34.1 % (95 % CI: 19.6-48.6) and 9.8 % (0-19.6), respectively (Fig. 2). MGMT promotor region methylation status (Methylated vs. Unmethylated or Invalid), IDH1-R132H status (Negative test results vs. Mutated or Invalid) and corticosteroid therapy (Yes vs. No) had no statistical influence on PFS or OS ($p > 0.05$) (Fig. S4). According to the receiver operating characteristic curve of PFS, the area under the curve (AUC) of neutrophil to lymphocyte ratio (NLR) before adjuvant chemoradiotherapy was 0.763 (Fig. S5).

Biomarkers and prognosis

As shown in Fig S6A and Table S2, compared with pre-GM-CSF, the concentrations of TNF-α (p = 1.9615E-10) and IL-18 (p = 6.8467E-8) were increased after GM-CSF, while the proportion of CD19 (p = 0.000015), and the concentrations of IgG (p = 0.000015) and CXCL12 (p = 0.000257) were decreased. Heat maps were used to determine the associations between immunophenotyping of the peripheral blood immune cells, cytokines and exosomes. Before GM-CSF, CD19 was associated with IL-6 (p = 3E-6) and IgA was associated with NESTIN (p = 4.5049E-12) (Fig. S6B1-B6). The changes in biomarkers including immunophenotyping of the peripheral blood immune cells, cytokines and exosomes after GM-CSF had no statistical influence on PFS or OS (Table S3).

Table 2
Disposition of patients and intensity of treatment.

Variables	*N* (%)
Concomitant temozolomide	41 (100.0)
Radiotherapy	41 (100.0)
Concomitant GM-CSF	
Received planned dose	36 (87.8)
Early discontinuation of concomitant GM-CSF	5 (12.2)
Reason for discontinuation of GM-CSF	
G3 Malaise	1 (2.4)
G3 Bronchospasm	1 (2.4)
G3 Abdominal pain	1 (2.4)
G3 Chest pain	2 (4.9)
Maintenance therapy period	
Maintenance temozolomide started	33 (80.5)
Median cycles of temozolomide (range)	5 (0-12)
Patients completing < 6 cycles	25 (61.0)
Reason for completing < 6 cycles	
Disease progression	21 (51.2)
Decision by patient	3 (7.3)
Missing data	1 (2.4)

GM-CSF, granulocyte-macrophage colony-stimulating factor.

Table 3
Non-hematologic toxicities during concomitant chemoradiotherapy.

Toxicity	G1	G2	G3	G4
Malaise	0	0	1	0
Bronchospasm	0	1	1	0
Chest pain	0	1	2	0
Abdominal pain	0	2	1	0
Diarrhoea	1	1	0	0
Nausea or vomiting	15	2	0	0
Fever	15	2	0	0
Rash	1	2	0	0
Eye hemorrhage	9	0	0	0

Discussion

This phase 2 study assessed the feasibility of hypofractionated IMRT, TMZ and GM-CSF for newly diagnosed GBM. We found that the combination of GM-CSF and adjuvant chemoradiotherapy was efficacious and safe when administered in a larger fractionation dose within a shorter period of time compared with the standard 6-week schedule. To the best of our knowledge, this is the first report based on modern concurrent chemoradiation with GM-CSF.

In 2006, the ASCO guidelines advised against GM-CSF with concurrent chemoradiotherapy for severe toxicities [23,24]. For patients with GBM, IMRT could improve target conformity and reduce neurological toxicities in contrast to three-dimensional conformal radiotherapy [25]. Patterns of failure in GBM patients who received concurrent TMZ-based chemoradiotherapy using contemporary, MRI-based planning indicated that ≥80-90 % of recurrences had a component of failure within the high-dose volume [26]. In a phase I trial of hypofractionated IMRT with TMZ for GBM, PTV1 was defined as the GTV (the entire surgical cavity and the contrast-enhancing residual diseases based on the enhanced T1-weighted pre-IMRT brain MRI scan) plus a 5-mm margin and 60 Gy in 6 Gy fractions within two weeks was feasible [17]. In the Stupp et al. study, the incidences of acute grade 3 or 4 hematologic toxicities, fatigue and infection during concomitant TMZ therapy were 7 %, 7 % and 3 %, respectively. The incidence of interruption or delay in radiotherapy was 32 % in the radiotherapy plus TMZ group [2]. In the present study, the total dose of the PTV1 was 52.5 Gy delivered in 15 fractions. Although 12.2 % patients discontinued concomitant GM-CSF because of toxicity, all patients completed concurrent chemoradiotherapy with the addition of GM-CSF and no grade 3 or 4 hematologic toxicities were observed during chemoradiotherapy.

Based on the study [15] of metastatic solid tumors treated by hypofractionated radiotherapy (35 Gy in ten fractions) plus GM-CSF (125 μg/m^2 subcutaneously injected daily for two weeks, starting in second week of radiotherapy), GM-CSF 200μg (equivalent to 125 μg/m^2 calculated dose) was given daily via subcutaneous injection for two weeks, starting one week after the start of hypofractionated IMRT in the present study. Although monthly intradermal injections of rindopepimut (a vaccine targeting the EGFR deletion mutation EGFRvIII, 500 μg admixed with 150 μg GM-CSF) did not improve survival [13], the combination of GM-CSF with checkpoint inhibitors may offer potential benefits for patients with GBM [27].

The maintenance treatment regimen is initially designed arbitrarily with six cycles of maintenance TMZ, and the optimal number of maintenance cycles remains unclear. In 2017, a pooled analysis of patients with newly diagnosed GBM indicated that treatment with maintenance TMZ beyond six cycles does not improve OS, even for patients with MGMT promoter methylated tumors [28]. Moreover, a recent randomized phase II study including 159 patients found that continuing TMZ after six maintenance cycles was associated with greater toxicity but conferred no additional benefit in PFS and OS [29]. The present study

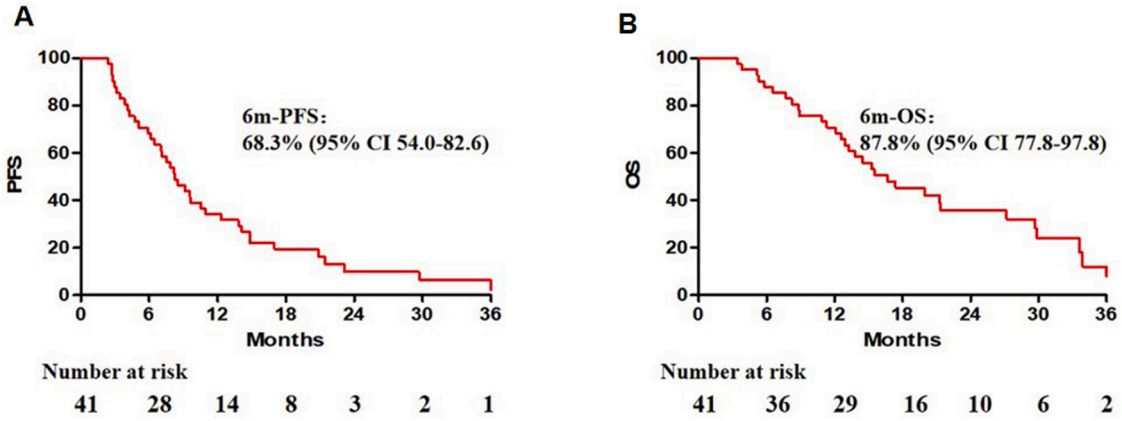

Fig. 2. Kaplan-Meier analysis of overall survival (OS) and progression-free survival (PFS).

was designed in 2015 and a total of 12 cycles or until tumor progression was adopted for maintenance treatment. During maintenance TMZ, 25 patients (61.0 %) completed <6 cycles and no patients had grade 3 or 4 toxicities.

In a meta-analysis of hypofractionated radiotherapy for GBM without age restrictions, the 1-year and 2-year OS rates were 71.3 % and 34.8 %, while the 6-month and 1-year PFS rates were 74.0 % and 40.8 % [16]. In the present study, the 1-year and 2-year OS rates were 70.7 % (95 % CI: 56.8-84.6) and 35.6 % (19.9-51.3), respectively. The 6-month and 1-year PFS rates were 68.3 % (95 % CI: 54.0-82.6) and 34.1 % (19.6-48.6), respectively, which was comparable with the results of the previous meta-analysis. In the framework for GBM of designing clinical trials associated with combination immunotherapy, OS was recommended as the primary endpoint for accurately evaluating immunotherapy-based approaches [30]. The efficacy of GM-CSF for GBM needs to be confirmed in studies with longer follow-up durations.

The NLR is a useful marker for assessment of the inflammatory response and related to prognosis in patients with solid tumours [31]. For solid tumors treated with radiotherapy and GM-CSF, responders presented with lower baseline median NLR than non-responders (p = 0.015) [15]. In a wide range of immune checkpoint inhibitors treated cancers, NLR <5 was associated with worse prognosis [32]. Similarly, the AUC of NLR before adjuvant chemoradiotherapy reached 0.763 for predicting PFS in the present study. For unresectable or metastatic biliary tract cancers treated with nivolumab plus gemcitabine and cisplatin, the baseline percentage of peripheral blood CD3+ cells in responders was higher than that in non-responders (p = 0.046) and on-therapy changes in serum soluble FasL (OS, $p < 0.001$), MCP-1 (PFS, p = 0.019) and interferon-γ (OS, p = 0.032; PFS, p = 0.033) were correlated with prognosis [33]. We also evaluated the associations among other biomarkers and their roles for predicting the prognosis of patients with GBM receiving GM-CSF and chemoradiotherapy, including immunophenotyping of the peripheral blood immune cells, cytokines and exosomes, which are the current research focuses for GBM, especially on-therapy biomarkers [34–37]. The changes in biomarkers including immunophenotyping of the peripheral blood immune cells, cytokines and exosomes after GM-CSF had no statistical influence on PFS or OS probably because of the limited number of patients in the present study.

This study had several limitations, including the slow accrual of patients, limited number of patients, more than 80 % patients receiving gross total resection, one patients with IDH1-R132H mutation, more than 60 % patients with MGMT promoter-methylated GBM and the inclusion of six (14.6 %) patients with glioblastoma, not otherwise specified, which could affect the outcomes. In addition, the trial did not include prospective cost of care analysis, neurocognitive assessment and quality of life analysis. With impacts of a daily injection for 2 weeks, the current experimental treatment may result in unintended quality of life detriment on patients. Nevertheless, this trial attempted to evaluate the feasibility of combining modern chemoradiotherapy with GM-CSF in newly diagnosed GBM. Prospective, multicenter, randomized phase III trials are needed to further verify our results. With the development of immunotherapy, trials combining GM-CSF, radiotherapy and immune checkpoint inhibitors are ongoing for various cancers [38].

Conclusions

The combination of hypofractionated IMRT, TMZ and GM-CSF was efficacious and safe in patients with newly diagnosed GBM.

Consent for publication

All patients have written informed consent.

Data sharing

Individual participant data that underlie the results and the study protocol will be shared with researchers who provide a methodologically sound proposal for individual participant data meta-analysis. Proposals should be directed to chenxz@zjcc.org.cn; to gain access, data requestors will need to sign a data access agreement. Data will be made available beginning 3 months and ending 5 years following article publication.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Zhejiang Cancer Hospital and performed in accordance with the principles of the Declaration of Helsinki (No. IRB-2016-6).

Funding

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Fig. S1: Treatment schema for the study group. GM-CSF was administered concurrently with RT. No maintenance GM-CSF was given. RT = radiotherapy; TMZ = temozolomide; GM-CSF = Granulocyte-macrophage colony-stimulating factor

Fig. S2: Characterization of the extracellular vesicles. A. Transmission electronic microscopy of the preparation from patients'serum. Scale bars, 500 nm. B. Western blotting detection for exosomal markers (CD63, CD81, CD9).

Fig. S3: Radiation necrosis in one patient with glioblastoma. Axial contrast-enhanced T1-weighted magnetic resonance images in a 22-year-old man show radiation necrosis 10 months after completing radiotherapy.

Fig. S4: Kaplan-Meier curves of progression-free survival and overall survival of patients with MGMT promotor region methylation status (Methylated vs. Unmethylated or Invalid), IDH1-R132H status (Negative test results vs. Mutated or Invalid) and corticosteroid therapy (Yes vs. No). MGMT = O-6-methylguanine-DNA methyltransferase gene; IDH =isocitrate dehydrogenase.

Fig. S5: Receiver operating characteristic curves predicting overall survival by NLR are represented.

AUC = area under the curve; NLR = neutrophil to lymphocyte ratio.

Fig. S6: Biomarkers for prognosis. (A) Biomarkers in peripheral blood pre- and post-GM-CSF in glioblastoma patients. *$p < 0.0015625$ (0.05/32) was considered to be statistically significant. (B) Heat maps of associations among immunophenotyping of the peripheral blood immune cells, cytokines and exosomes pre-GM-CSF (B1-B3) and post-GM-CSF (B4-B6). **$p < 0.000080906$ (0.05/618) was considered to be statistically significant.

GM-CSF = Granulocyte-macrophage colony-stimulating factor

CRediT authorship contribution statement

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Declaration of competing interest

All authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2025.101156.

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