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Current Trends in Glioblastoma Multiforme Treatment: Radiation Therapy and Immune Checkpoint Inhibitors

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Glioblastoma multiforme (GBM) is the most common primary brain cancer. Even with aggressive combination therapy, the median life expectancy for patients with GBM remains approximately 14 months. In order to improve the outcomes of patients with GBM, the development of newer treatments is critical. The concept of using the immune system as a therapeutic option has been suggested for several decades; by harnessing the body's adaptive immune mechanisms, immunotherapy could provide a durable and targeted treatment against cancer. However, many cancers, including GBM, have developed mechanisms that protect tumor cells from being recognized and eliminated by the immune system. For new immunotherapeutic regimens to be successful, overcoming immunosuppression via immune checkpoint signaling should be taken into consideration.

Key Words Glioblastoma multiforme; Stereotactic radiosurgery; Immunotherapy; Anti-CTLA-4; Anti-PD-1; Temozolomide.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. It also has the highest malignancy grade (WHO grade IV) and median survival of 14.6 months with surgery, radiation, and chemotherapy [1]. The most significant prognostic factors for patients with malignant glioma are performance status and age [2]. The paradigm of treatment for newly diagnosed patients includes aggressive surgery followed by radiation therapy and concomitant temozolomide chemotherapy. This treatment prolongs median survival to 14.6 months versus 12.1 months with radiation alone, but essentially all patients recur [1,3]. Despite major aggressive treatment, GBM inevitably recurs. GBM also persists due to the heterogeneity of the tumor itself [4-6]. The tumor is comprised of different cell types including endothelial cells, fibroblasts, inflammatory cells, and neurons [7].

One of the challenges of implementing a robust anti-tumor immune response is the protective immune microenvironment of GBM. Specifically, GBM has established mechanisms of dampening the immune response by down regulation of HLA molecules, expression of immunosuppressive cytokines, increasing the activation of T-regulatory (T_{reg}) cells, and increasing the T helper cell phenotype [7-9]. A review of the immunosuppressive milieu of GBM is presented elsewhere [90]. This suppressive immune microenvironment created by the tumor is aided by the expression of immune checkpoint molecules, which transmit a negative signal to immune cells and decrease their antitumor response. Two checkpoint molecules that are currently under investigation in clinical and preclinical studies include Cytotoxic T lymphocyte Associated Antigen 4 (CTLA-4) and Programmed Death-1 (PD-1). Anti-CTLA-4 was recently FDA approved for treatment of metastatic melanoma, and there are clinical trials underway investigating other agents such as anti-PD-1, which may hold promise as treatment for other cancers, including GBM [78,79].

STANDARD OF CARE

Initial treatment

Standard treatment for newly diagnosed GBM includes surgical resection with adjuvant radiation and temozolomide. In both prospective and retrospective studies, complete surgical resection leads to increased survival compared to subtotal resection or biopsy [10,11]. Of 413 patients with newly diagnosed GBM, the median survival for patients who underwent biopsy compared to craniotomy was 21 weeks and 45.3 weeks respectively [10].

Older trials of patients with Grade III-IV gliomas showed an increase in survival when radiation was added postoperatively, establishing this as standard of care [12,13]. The Brain Tumor Study Group performed a prospective trial comparing best supportive care with carmustine (BCNU) and radiation treatment, alone or in combination [13]. In this study, radiotherapy alone or radiotherapy in combination with BCNU conferred a significant survival1 [3]. Similarly, Kristiansen et al. [12] reported in a trial of 118 patients, treatment with postoperative radiation with or without bleomycin had a median survival of 10.8 months versus 5.2 months in patients receiving supportive care only. Adjuvant fractionated external beam radiation therapy (EBRT) is typically administered by IMRT in 2 Gy fractions to a total dose of 60 Gy. Use of higher doses has not been found to increase survival [14].

1, 3-bis (chloro-ethyl)-1-nitrosourea (BCNU) remained the first line chemotherapy for malignant gliomas for several years [15]. A small prospective study found that implanting BCNU impregnated wafers (Gliadel) after surgery increased survival to 53.3 weeks from 39.9 weeks in the placebo arm [16]. A larger Phase III trial of 240 patients with malignant glioma confirmed this result with a median survival of 13.9 months in the Gliadel group vs. 11.6 months in the Gliadel group and placebo group respectively [17]. Stupp et al. [1] prospectively investigated the outcome of adding temozolomide (TMZ) to adjuvant treatment of GBM. The study found an increase in median survival from 12.1 months in the radiation alone group to 14.6 months with concomitant and adjuvant TMZ. This regimen provided a significant survival advantage without increased toxicity, and has become the new standard of care for treatment of newly diagnosed GBM for those patients not receiving a BCNU wafer.

Recurrence

Most recurrences occur within 1-1.5 years of initial therapy and occur within 2 cm of the surgical margins [1]. When patients develop disease recurrence, repeat surgery should be considered in all patients and is found to have limited complications [18]. The value of repeat surgery has been extensively studied in retrospective and prospective studies, with various factors found to be significantly associated with increased survival. These prognostic factors include an increased time interval between surgeries [19], age [20], and preoperative Karnofsky Performance Scale (KPS) [20,21]. Park et al. [22] created a preoperative scale using KPS, tumor volume and tumor location to predict patient outcome after repeat surgery for recurrence and can be helpful in evaluating a patient for surgery. In patients with diffuse disease or multiple foci, treatment should be palliative and supportive.

There is no consensus on the best adjuvant treatment in the setting of recurrent disease, although additional chemotherapy and radiation can be considered as well as enrollment in a clinical trial. Data from the following clinical trials are used to inform clinicians regarding treatment. BCNU wafers were first investigated for management of patients with recurrent malignant glioma and the results from phase III studies reported a 50% increase in 6 months survival in patients treated with BCNU polymers vs. placebo [23]. Bevacizumab, an angiogenesis inhibitor, was approved in 2009 for use in recurrent GBM. Data from Phase II trials in which patients were treated with bevacizumab with or without irinotecan reported median survival between 7 and 9.2 months [24,25]. Repeat radiation can be performed if patients responded well to initial radiation. RTOG 90-05 defined the maximum tolerated single dose that can be given for previously irradiated tumors as 24 Gy, 18 Gy, and 15 Gy for tumors ≤20 mm, 21-30 mm, and 31-40 mm respectively [26].

RADIATION

The standard dose of radiation for GBM remains 60 Gy. Attempts to increase the dose beyond this limit have not demonstrated a survival advantage and may increase the risk of radionecrosis [14]. The Radiation Therapy Oncology Group [27] studied the effect of adding a stereotactic radiosurgery (SRS) boost to conventional EBRT for patients with newly diagnosed GBM but found no survival difference between those patients receiving SRS compared to those that did not.

Stereotactic boost

Tsao et al. [28] reviewed the literature and concluded that the use of SRS followed by EBRT and BCNU does not show any survival benefit. Einstein et al. [29] believed these prior studies were limited by the imaging techniques used, therefore they devised a Phase II trial utilizing magnetic resonance spectroscopy to locate residual tumor prior to administering the SRS boost. Median survival for RTOG recursive partitioning analysis class IV patients was 18.7 months compared to 11.1 months for patients in the RTOG 93-05 study, showing the poten-

tial benefit of adding an SRS boost [29]. Additional data suggests that giving the SRS boost after EBRT leads to increase in survival with a median overall survival of 15.1 months for patients with newly diagnoses GBM [30]. Several studies have been conducted looking at outcome following SRS for recurrent GBM and found median overall survival between 8-10 months following treatment without increased toxicities [30-33].

Brachytherapy

Brachytherapy allows for delivery of higher doses of radiation for initial treatment of GBM. Data from a randomized clinical trial comparing EBRT alone vs. EBRT plus a brachytherapy boost of I-125 implants to a dose of 60 Gy, found no difference in survival [34]. A different brachytherapy delivery system called Gliasite, is a balloon catheter filled with an aqueous iodinated radiation source. The outcomes of patients with newly diagnosed GBM receiving Gliasite brachytherapy to a median dose of 50 Gy and EBRT to a median dose of 60 Gy was retrospectively reviewed and found a median overall survival of 11.4 months, which is similar to historical controls [35].

Brachytherapy has also been investigated in patients with recurrent GBM. A retrospective cohort study of 111 patients previously treated with surgery, radiation and chemotherapy for their primary tumors compared outcomes of patients treated with brachytherapy, surgery or temozolomide for recurrence [36]. This study found a significant increase in median survival for patients receiving brachytherapy to 37 weeks, compared to patients who underwent reoperation (30 weeks) or dose dense temozolomide (26 weeks) [36]. Additional prospective data showed a median survival of 52 weeks and 64 weeks respectively, following treatment with repeat resection and permanent low activity Iodine 125 brachytherapy [37,38]. This approach offers an alternative to temporary brachytherapy, which needs to be anchored to the tumor [37,38]. Multiple studies reported a median survival between 35.9-36.4 weeks after use of GliaSite (to a median dose of 53-60 Gy) following re-resection [39,40].

New radiation techniques

New techniques such as thermotherapy, and pulsed reduced dose rate radiotherapy (PRDR) were recently studied in patients with recurrent glioma. Maier-Hauff et al. [41] published a study of 59 patients with recurrent GBM receiving iron-oxide nanoparticles combined with fractionated stereotactic radiosurgery and reported a median survival of 13.4 months. PRDR is a technique that reduces the dose rate of radiation allowing normal tissues to repair while cancer cells remain radiosensitive. 86 patients with Grade 4 recurrent glioma were treated with PRDR to a total median dose of 50 Gy. The median overall survival of these patients from the start of PRDR treatment was 5.8 months [42].

IMMUNE CHECKPOINT INHIBITORS

Immune checkpoint proteins consist of a group of surface proteins and secreted molecules that inhibit over-activation of the immune system upon challenge. They are therefore used as an immune break to prevent a detrimental effect of the immune response against healthy tissue. The list of immune checkpoints is long [43-53] with the most well known being CTLA-4 and PD-1 with its ligands (PDL1 and PDL2) [54,55]. CTLA-4 is expressed exclusively in T cells and antagonizes with CD28 for binding to their common ligands CD80 and CD86 attenuating the immune response against antigens [55-63]. PD-1 has a broader distribution than CTLA-4, being expressed on the surface of T, B and natural killer cells and is upregulated upon their activation attenuating the immune response in situ [55]. Cancer in general utilizes immune inhibitory molecules to escape elimination by the immune system to further promote an immunosuppressive microenvironment [55,64-76]. A comprehensive review of the available antibodies and the ongoing clinical trials is provided elsewhere [55].

The anti-CTLA-4 antibody, Ipilimumab, was approved in 2011 for the treatment of metastatic melanoma and became the first checkpoint inhibitor available on the market [91]. In a phase III trial, 676 patients were randomized to receive either glycoprotein 100 (gp100) vaccine with ipilimumab, gp100 alone, or ipilimumab monotherapy [69]. Patients receiving ipilimumab monotherapy or in combination with gp100 experienced a significantly longer median overall survival compared to those who received gp100 alone (10 months vs. 6.4 months respectively) [69].

Extrapolation of these results to patients with brain tumors is difficult as many of these trials excluded patients with brain metastases. However, blockade of CTLA-4 is currently being investigated in patients with central nervous system tumors (Table 1). The efficacy of Ipilimumab in patients with brain metastases has been evaluated in prospective studies in patients with symptomatic and asymptomatic disease [77]. Median overall survival in the asymptomatic cohort was 7 months (95% CI 4.1-10.8), and 3.7 (95% CI 1.6-7.3) months in the symptomatic brain metastases group. These patients did not have increased toxicity as compared to previous reported patients with melanoma without brain metastases [77]. The efficacy of anti-CTLA-4 therapy in patients with brain metastases lays the groundwork for exploring its efficacy in GBM. A phase I clinical trial is recruiting patients to assess the side effects and the maximum tolerated dose of the combination of ipilimumab and imatinib mesylate in patients with advanced cancer, including patients with intracranial glioblastoma, gliosarco-

Table 1. Ongoing clinical trials in patients with metastatic melanoma in the brain treated with ipilimumab alone or in combination with radiotherapy

Study	Phase	Primary outcome	Secondary outcomes	Reference
Stereotactic or whole brain radiotherapy in combination with ipilimumab in metastatic melanoma in the brain (NCT10703507)	I	Provide the maximum tolerate dose	1. Rate of developing new brain metastases 2. Response of systemic disease 3. Overall survival rate 4. Progression free survival	87
Palliative radiation therapy in combination with ipilimumab in patients with melanoma Grade IV including brain metastases (NCT01449279)	I	Safety of the combination therapy	1. Response rate 2. Duration of response 3. Overall survival	88
Use of ipilimumab for the treatment of metastatic melanoma in the brain (NCT00623766)	II	Safety and use in combination with corticosteroids	1. Response to treatment and 2. Progression free survival	89

ma and anaplastic astrocytoma [83].

Another checkpoint inhibitor being investigated clinically is anti-PD-1 and its ligand anti-PDL1. Anti-PD1 was first clinically tested in 39 patients with recurrent solid tumors and demonstrated an appropriate safety profile with evidence of antitumor effect [78]. Another recent study by Topalian et al. [79] investigated the use of anti-PD-1 in patients with a variety of cancer types, including melanoma, non-small cell lung cancer (NSCLC), prostate cancer, renal cell carcinoma (RCC) and colorectal cancer. Durable responses of greater than 1 year were seen in a percentage of patients with NSCLC, melanoma, and RCC. Patients received either 1, 3, or 10 mg/kg of anti-PD-1 therapy. No dose limiting toxicity was found. The drug was found to have an acceptable adverse event profile with 14% of patients experiencing Grade 3 or 4 adverse events, most commonly fatigue, GI disorders, decreased appetite, and skin disorders [79].

A multicenter phase I clinical trial including patients with different types of advanced cancer (75 patients with non small cell lung cancer, 55 with melanoma, 18 with colorectal cancer, 17 with renal cell carcinoma, 17 with ovarian cancer, 14 with pancreatic cancer, 7 with gastric cancer and 4 with breast cancer) assessed the efficacy and the toxicity of anti-PDL1 antibody. Grade 3 or Grade 4 toxicities were observed in only 9% of the patients; partial or complete response was observed in 6-17% of the patients; 8 out of 16 patients with more than a year of follow-up had durable response of at least a year [80].

COMBINATION THERAPY

Traditionally, radiotherapy has served as adjuvant therapy in cancer treatment to eliminate the residual disease or as an alternative of surgery in inoperable cases. High doses of radiation are used to deplete any radioresistant subpopulations of cancer cells in the tumor mass. A recent review describes radiation as an "*in situ* vaccine" based on the observed abscopal effect in various case reports, where systemic disease virtually disappears after treatment with radiosurgery [84]. Although not described in GBM, case reports in patients with metastatic melanoma, report the abscopal effect in patients receiving both radiation and anti-CTLA-4 therapy [85,86]. One of the patients had developed brain metastases, which had completely resolved at the last follow-up, and developed new antibodies to melanoma specific antigens demonstrating a systemic immune response [85].

For GBM specifically, this approach has not yet reached clinical practice, although the previous case reports show the potential of this combination for patients with central nervous system disease. Recent preclinical data showed that combination of radiosurgery with immunotherapy can produce long term survivors in GBM challenged mice [81]. Zeng et al. [81] characterized the immune profile of long term survivors after treatment with combined stereotactic radiosurgery with anti-PD1 blockade, showing an increased $T_{effector}/T_{reg}$ infiltrating population in the tumor. Furthermore, surviving mice retained systemic immunity when re-challenged with flank tumors 90 days later, suggesting that this approach generates strong immunologic memory [81].

Preliminary evidence suggests that this approach of combining radiation with immune checkpoint inhibitors may be translated effectively and safely to the clinic. Retrospective data of patients with intracranial melanoma metastases who received SRS with or without anti-CTLA-4, showed a significant improvement in overall survival (21.3 vs. 4.9 months) vs. SRS alone, with 47% of patients who received anti-CTLA-4 still living at 2 years [82].

CONCLUSION

Glioblastoma multiforme continues to be a difficult disease to treat despite multiple clinical trials testing the efficacy of various chemotherapeutic approaches, survival in patients with GBM remains dismal. Immunotherapy suggests that it may improve the outcomes of patients with various malignancies,

including GBM. Immune checkpoint inhibitors are currently being extensively tested in clinical trials against many cancer types in advanced stages. These inhibitors have the potential to be a very attractive therapeutic modality used in combination with other chemotherapy, radiation, or immunomodulatory treatments. A multi-modal approach involving these new drugs and procedures has the potential to effectively implement a new paradigm in cancer treatment.

Conflicts of Interest

The authors have no financial conflicts of interest.

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