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Advances in the Treatment of Primary Brain Tumors: The Realm of Immunotherapy

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Abstract

Central nervous system (CNS) tumors, although rare, represent a group of neoplasms that have a disproportionate morbidity and mortality. Despite advances in our understanding of tumor pathogenesis coupled with improvements in therapeutic options, overall survival for primary brain tumors remains dismal. Although challenging, newer approaches such as brachytherapy, immunotherapy, and electric field generators are currently being evaluated in the clinical setting with promising results. The field of immunotherapy in neurooncology is still in its infancy, but several advances have already been made, including the development of tumor vaccines, utilization of immune checkpoint inhibitors, and activation of tumor dendritic cells to stimulate the host's immune system. Recent advances in noninvasive electric fields have been applied to the treatment of glioblastoma multiforme (GBM) with encouraging clinical outcome. In this chapter, we will review the latest advances in the treatment of glioblastoma multiforme with a focus on immunotherapy.

Keywords: glioblastomas, immunotherapy, tumor vaccines, immune checkpoint inhibitors, tumor treating fields

1. Introduction

Central nervous system (CNS) tumors comprise a relatively small portion of cancers, but they are among the most aggressive tumors and result in significant morbidity and mortality. It is estimated that approximately 77,670 cases of primary CNS tumors are expected to be diagnosed in the United States in 2016 [1]. Of these, roughly 40% will be malignant with the majority being glioblastoma multiforme (GBM). The median survival of newly diagnosed subjects with GBM is approximately 12–15 months [2]. Despite intense efforts into understanding disease mechanisms and advances in technology, overall survival has only improved by

3–6 months, and the 5-year survival rate ranks sixth lowest among all cancers after pancreatic, liver, intrahepatic bile duct, lung, stomach, and esophageal [3, 4].

Traditional treatment approaches for brain tumors have relied upon a combination of surgical resection, radiation, and chemotherapy. Newer approaches such as brachytherapy, immunotherapy, and electric field generators are currently being evaluated in the clinical setting. In this chapter, we review the latest advances in the treatment of GBM.

2. Gliomas

Gliomas are the most common primary malignant brain tumor, comprising more than 80% of all malignant brain neoplasms [5]. Gliomas can be further divided into astrocytomas, oligodendrogliomas, ependymomas, and mixed gliomas (i.e., oligoastrocytomas). These tumors can be further characterized based on grading. Astrocytomas are graded from I through IV and are represented as follows: grade I—pilocytic, grade II—diffuse, grade III—anaplastic, and grade IV—glioblastoma multiforme (GBM). Although we historically call all grade IV astrocytomas GBM and subsequently treat these tumors with the same treatment protocols, growing evidence suggests that even within GBM, there may be distinct disease processes that require a more specific targeting approach. Recently, GBM was re-classified into four subtypes based on unique molecular profiles and includes: classical, mesenchymal, proneural, and neural [6]. Further analysis of these subtypes identified subjects with classical GBMs lived the longest compared to those subjects with other GBM subtypes [6]. This observation may partly explain some subjects with GBM having lengthened overall survival compared to other GBM subjects.

Subjects with CNS tumors may present with any generalized or focal symptoms including a headache, seizure, or a specific neurological deficit. However, one of the most common complaints for CNS tumor subjects is a headache with roughly 77% of subjects reporting a dull tension-like headache [7]. Seizures are also very common in CNS tumor subjects with roughly 15–95% of subjects experiencing at least one seizure during the course of their disease process [8]. Interestingly, seizures are more common in subjects aged 30–50 years and are frequently associated with tumors involving the frontal, temporal, frontotemporal, and frontoparietal lobes [9].

Due to the relatively rapid natural progression of GBM, identification of prognostic factors is valuable in determining the most appropriate therapeutic approach for subjects. Traditional indicators used include subject's age, their Karnofsky performance score, tumor size and location, and finally grade of tumor. In addition to these indicators, tumor molecular features are now being incorporated into survival models for GBM subjects. Well-characterized molecular alterations include isocitrate dehydrogenase (IDH) mutation, 1p and 19q codeletion, epidermal growth factor receptor variant III (EGFRvIII) rearrangement, and MGMT promoter methylation (**Table 1**). Point mutations in isocitrate dehydrogenase (IDH) 1 and 2 have been associated with improved prognosis compared to patients with wild-type IDH [10]. The combined loss of chromosomal arms 1p and 19q has been shown to occur in

Molecular marker	Description	Prognostic role
IDH mutation	Increases production of 2-hydroxyglutarate also IDH1 mutation associated with CpG island methylator phenotype in gliomas	Favorable
1p/19q co-deletion	Currently unclear	Favorable, better treatment response to chemotherapy and radiation therapy
EGFRvIII	Ligand-independent receptor activation leading to increased proliferation and reduced apoptosis	Reduced long-term survival
MGMT hypermethylation	Reduced DNA repair	MGMT promoter methylation associated with prolonged progression-free and overall survival with treatment of alkylating chemotherapeutic agents

Table 1. Molecular prognostic factors associated with gliomas.

oligodendrogliomas and oligoastrocytomas [11], but it is associated with better response to chemotherapy and radiation therapy leading to prolonged progression-free and overall survival [12, 13]. Epidermal growth factor receptor (EGFR) is a cell surface receptor involved in cell proliferation. A common alteration of EGFR is a truncated version called EGFRvIII, which is constitutively active leading to increased cell proliferation and reduced apoptosis [14]. Overexpression of EGFRvIII is observed in 24–67% of GBM [15]. Since EGFRvIII is a unique surface receptor, strategies to target this epitope have been explored; additional details will be discussed in the tumor vaccine section. Finally, O⁶-methylguanine methyltransferase (MGMT) is involved in the DNA repair pathway. Therefore, promoter methylation will lead to decreased protein levels and inability to repair the DNA. As such, promoter hypermethylation of MGMT has been observed in 20–40% of GBM [16]. The results from clinical trials and cohort studies have demonstrated that MGMT promoter methylation status is associated with prolonged progression-free and overall survival in patients with GBM treated with an alkylating chemotherapeutic agent [17–19].

3. Standard treatment regimen

The approach to GBM treatment has largely remained unchanged since 2005 with the publication of the Stupp et al. [20]. In this study, Stupp et al. [20] showed that giving temozolomide (TMZ) concurrently with radiation therapy after debulking surgery and then again following radiation therapy improved median survival in patients with newly diagnosed GBM. Each component of the Stupp protocol is important in the management of GBM. Surgery plays an important role as it allows for cytoreduction and histological confirmation of diagnosis. Achieving a gross total resection of >98% results in median survival of 12–15 months survival [21]. Approaches have been developed to aid surgeons in achieving a gross total resection while preserving baseline cognitive function. These include intraoperative MRI and neuronavigation, use of fluoride dye and imaging, and use of intraoperative brain mapping. Advances in imaging technology have allowed surgeons to incorporate functional MRI (fMRI)

and Diffusion tensor imaging (DTI) images into neuronavigation systems in order to improve achieving maximum safe resection [22]. Radiation therapy is also important in treating GBM with an improvement in median survival from 3–4 months to 9–12 months [20, 23]. Finally, as mentioned previously, TMZ, an alkylating agent, has shown to improve median survival [20]. Several chemotherapeutic agents targeting different cellular pathways have been studied with various results, including inhibitors of epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGFR), platelet-derived growth factor receptor (PDGFR), protein kinase C (PKC), mammalian target of rapamycin (mTOR), RAF-MEK-ERK pathway, and integrins [24]. Of note, an anti-VEGF monoclonal antibody, bevacizumab, which demonstrated improved progression-free survival in two randomized phase 3 clinical trials, failed to improve overall survival [25, 26]. Therefore, advancing the realm of neurochemotherapeutic agents hinges on our understanding of disease mechanism and may benefit from a combined multimodality approach utilizing various targets and approaches.

4. Immunotherapy

The concept of immunotherapy for cancer treatment is based on stimulating the body's own immune system, predominately cytotoxic T lymphocytes (CTL), to target and eliminate tumor cells. This concept is based on the body's own defense mechanism to eliminate cells that have undergone malignant transformation in a process called immune surveillance [27]. Theoretically, if the host immune system is stimulated with expansion of sufficient numbers of tumor-specific CTLs or non-functioning T cells are rescued within the tumor microenvironment, cell-mediated lysis of tumor cells could lead to tumor regression [28]. These concepts have been applied to several non-CNS malignancies with promising results [29]. However, because the CNS was originally considered to be an immune-privileged site, immunotherapy approaches for CNS malignancies were deemed futile. The notion of the CNS being immune-privileged stems from studies in which rat osteosarcoma cells injected intracranially grew significantly better than cells injected subcutaneously or intramuscularly [30]. Additional evidence has historically been that since there is an intact blood brain barrier (BBB), the CNS and specifically the brain are presumed to be immune privileged.

Despite this antiquated line of thinking, more recent observations indicate that the CNS is actually immunospecialized. This is based on the considerable interaction observed with the peripheral nervous system and the non-parenchymal ventricles, meninges, and subarachnoid space [31]. For example, antigen presenting cells (APCs) are found in many areas of the brain, including leptomeninges, ventricles, and perivascular spaces [32, 33]. Additionally, recent evidence has emerged indicating that the CNS possesses a functional lymphatic system, which is located within the walls of dural sinuses and actually communicates with deep cervical lymph nodes [34–36]. This network is able to transport immune cells and macromolecules and serves as a mechanism for antigens to pass through the walls of cerebral arteries and be carried to the cervical lymph nodes through the Virchow-Robin perivascular spaces [37]. Interestingly, dendritic cells (DC) have been shown to travel outside the brain and present antigens to T cells located in the cervical lymph nodes [38]. This presentation of CNS antigens

primes T cells for homing and infiltration to the tumor parenchyma [30]. Inflammatory stimuli, such as those induced by brain tumors, also increase CNS immunogenicity by provoking microglial activation and blood-brain barrier (BBB) disruption [39]. BBB disruption occurs secondary to glioma cell invasion of the basement membrane. This disruption also enables immune cells to migrate past the BBB, which normally would be intact, preventing such migration. As our understanding of immune function expands in the CNS, the field of immunotherapy as it pertains to CNS disease has emerged as a frontier player in the fight for CNS cancer. As a result, there are several immunotherapies currently being investigated in clinical trials with many producing promising results [30].

4.1. Tumor vaccines

The idea behind tumor vaccinations is to present tumor-associated antigens (TAAs) to the host immune system in order to evoke a pro-inflammatory antitumor response elicited by CD4⁺ and CD8⁺ T cells interacting with major histocompatibility complexes (MHC) I and MHC II, respectively [40]. Naturally, the success of tumor vaccinations and elegance of using this approach are that it is both tumor specific and subject specific, thereby, reducing inadvertent toxic side effects [40, 41]. Although there is great specificity in using tumor vaccinations, the challenge remains in optimizing the selection of targeted peptides since many TAAs are identified as “self” by the immune system [42]. Tumor vaccinations can be categorized according to their delivery method and includes peptide, dendritic cells (DCs), and heat shock protein (HSP).

Although several TAAs specific to GBM have been described in the literature including HER-2, gp100 [43], MAGE-1 [43], ATIA [44], and AIM-2 [45], peptide vaccination development using epidermal growth factor receptor variant III (EGFRvIII) has received the most attention [43]. First described by Heimberger et al. in 2003, the EGFRvIII vaccine, rindopepimut has been studied in several clinical trials with promising results [30, 46]. In a multicenter phase II trial, subjects with EGFRvIII-expressing GBM that received rindopepimut had a median progression-free survival from time of histological diagnosis of 14.2 months and an overall survival of 26.0 months [47]. In another multicenter phase II clinical trial (ACT III), the median overall survival was 21.8 months, which further confirms the results from the aforementioned phase II trial [48].

While these results are encouraging, a recent phase III clinical trial (ACT IV) evaluating rindopepimut was discontinued on the recommendations of the independent Data Safety and Monitoring Board based on observations that the treatment arm and control arms of the study were performing on *par* with each other and unlikely to meet its primary overall survival endpoint [49]. Another issue complicating the use of tumor peptide vaccinations is the notion that tumor recurrence post-peptide vaccination leads to altered tumor protein expression, which makes treatment approaches for tumor recurrences more challenging. Specifically, Sampson et al. analyzed those patients who received rindopepimut and subsequently experienced a recurrence. They demonstrated that in those tumors that recurred, 82% demonstrated loss of EGFRvIII expression. These results suggest that the peptide vaccine is able to successfully target EGFRvIII-expressing tumor cells. At the same time, these results indicate that the peptide

vaccine preferentially led to the selection of EGFRvIII-negative tumor cells, resulting in tumor regrowth [47]. Despite this obstacle, one proposed strategy to overcoming this tumor event is to target multiple TAAs in an attempt to overcome the inherent heterogeneity of GBMs [40].

Still another approach to generate tumor vaccines while addressing the limitations of using one antigen is the use of heat shock protein (HSP) peptide complexes. HSP vaccines are generated from TAAs bound to HSP peptide complexes derived from GBM tissue. Two HSP peptide complexes that are currently being evaluated in clinical trials include HSP 70 and 96 [30]. In a phase II clinical trial, which evaluated a HSP peptide complex 96 vaccine, the authors demonstrated an increase in median overall survival of 42.6 weeks compared to historical controls [50]. Other HSPs, including HSP47, have been found to play a role in GBM pathogenesis specifically glioma angiogenesis and may serve as additional therapeutic targets [51, 52].

Several dendritic cell (DC) vaccines are currently being evaluated in various stages of clinical trials [30]. The mechanism of action for the majority of dendritic cell vaccines involves extracting autologous DC from the subject. Then *in vitro*, the DCs are stimulated or pulsed with tumor peptides or tumor lysate and subsequently re-introduced into the subject. The results of a phase I trial demonstrated a median progression-free survival of 16.9 months and median overall survival of 38.4 months after administration of a multi-epitope-pulsed DC vaccine [53]. In another phase I trial, median overall survival was 31.4 months after treatment with pulsed DCs followed by adjuvant treatment with either imiquimod or poly-ICLC [54]. In the latter study, the authors observed that subjects with GBMs with a mesenchymal gene expression profile were more susceptible to the DC treatment approach [54]. This observation underscores the importance of molecular characterization and developing a personal treatment approach.

Interestingly, as technologies advance, we now have the capability to develop computational modeling to identify potential tumor antigens through next-generation sequencing to identify mutations and peptide affinity algorithms to find peptides with high peptide-MHC affinity [30, 55, 56]. This approach has been validated in preclinical studies using melanoma cell lines [55]. It is currently unclear whether this approach can have similar efficacy against CNS tumors.

4.2. Immune checkpoint molecules

Many clinical studies are focusing on how to rescue the function of immune cells against non-immunogenic tumors and their immune suppressive microenvironments. It is well established that inhibitory receptors on T cells play a vital role in suppressing T cell-mediated antitumor responses [30, 57]. These inhibitory receptors, referred to as immune checkpoints, serve to prevent inappropriate or prolonged activation of the host immune system. There are several immune checkpoint protein inhibitors that have been developed and are demonstrating promising antitumor responses clinically—CTLA-4 and PD-L1 [30]. CTLA-4 has been shown to modulate T cell activation, thereby preventing unabated activation and proliferation [58]. A humanized CTLA-4 antibody, ipilimumab, has been FDA-approved and shown to have promising results in treating metastatic melanoma with an approximately 10.9% overall response rate that remains durable [59]. In the setting of GBM, administration of ipilimumab

has been limited to small cohorts [30]. PD-L1 is modulated by the PI(3)K-Akt-mTOR pathway [60] and its function is to suppress the proliferation and function of CTLs and also promote regulatory T cells (Tregs) activity through the binding of programmed cell death—1 (PD-1) [61]. PD-L1 is also found on the surface of GBM tumor cells, and expression is correlated with tumor grade and prognosis [62, 63].

Not surprisingly, the most promising outcomes regarding immune checkpoint therapy have been achieved through dual CTLA-4 and PD-L1 blockade. In a recent randomized controlled trial, blocking both CTLA-4 and PD-L1 in patients with advanced untreated melanoma resulted in a median progression-free survival of 11.5 months compared to CTLA-4 monotherapy with 2.9 months and PD-L1 monotherapy with 6.9 months [64]. Additionally, other checkpoint molecules (*e.g.*, LAG-3 and TIM-3) are currently being investigated in combination with PD-1 blockage in preclinical studies treating non-CNS tumors [65, 66]. With success in non-CNS tumor models, this strategy may also be effective in treating GBM and other CNS malignancies.

4.3. Human cytomegalovirus

Human cytomegalovirus (HCMV) was first reported to be associated with GBM in 2002 by Cobbs et al. [67]. Since that time, there has been much controversy surround this topic with a high degree of variability in the literature regarding the detection of HCMV in CNS tumors [67–92]. To help resolve some of this controversy, a consensus paper was published in 2012 [93]. Despite this, a consensus paper stating the existence of HCMV in gliomas and their potential role in tumorigenesis, recent studies using next-generation sequencing have not been able to identify any HCMV in CNS tumor tissue [73, 81, 85–87, 92, 93]. Furthermore, anti-CMV therapy has been relatively unremarkable in the clinical setting with results being unclear and several clinical trials currently underway. For example, results from the Sweden (VIGAS) study, a randomized, double-blinded, placebo-controlled trial published in 2013, demonstrated trends but no significant differences in tumor volumes between the valganciclovir (an anti-CMV drug) and placebo groups at 3 and 6 months [94]. However, when the authors performed a retrospective analysis of the same cohort adding in additional patients taking valganciclovir for compassionate reasons, the rate of survival of treated patients at 2 years was 62%, as compared with 18% of contemporary matched controls [95]. The conclusion as to whether HCMV is associated with GBM remains unclear and warrants additional studies to completely resolve this ongoing issue.

5. Advancing treatment products

In a concerted effort to combat CNS malignancies, the Brain Tumor Biotech Summit was created as a way to bring the private sector and researchers together to discuss and exchange novel ideas that would ultimately lead to advances in CNS malignancy therapy [96]. From this summit, several products were highlighted, all of which demonstrate promising results. ONC201/TIC10 is a small molecule drug that can cross the BBB [97] and effectively target the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway in both cancer

stem cells and tumor cells [96]. Preclinical studies in GBM and colorectal tumors have shown promising results with regression of tumors without adverse side effects [98, 99]. Several vaccines are currently being developed including the Prophage Series G-100 and G-200 vaccines, which utilize the HSP complex 96 purified from tumor tissue [96], synthetic immune-stimulant multi-peptide SL-701 DC vaccine [96], and EGFRvIII vaccine [47, 48, 100, 101]. SL-701 is derived from several unregulated factors in GBM, including IL-13Ralpha2, EphA2, and surviving [96].

ANG1005 is an angiopep-2-paclitaxel chemotherapeutic agent conjugated to cellular receptor ligand, LRP-1 [102, 103]. LRP-1 is highly expressed on the surface of the BBB and allows for entry into the brain parenchyma since LRP-1 is also highly expressed in GBM [103, 104]. Another cellular receptor ligand being investigated is HER2 receptor, which may be useful in targeting breast cancer brain metastases since HER2 receptor has been shown to be overexpressed in roughly 25–30% of breast cancers [105, 106]. Toca 511 is a replicating amphotropic murine leukemia virus that preferentially infects malignant cells and delivers cytosine deaminase (CD) protein. Inside malignant cells, the CD enzyme converts the antifungal drug 5-FC (5-fluorocytosine) to the anticancer drug 5-FU (5-fluorouracil) [107]. A new form of brachytherapy seed has also been developed, ^{131}Cs , which has a higher mean energy and a shorter half-life, allowing for fewer radioactive seeds and reduced exposure to family members and medical staff [108].

The most recent FDA-approved treatment for GBM is Novocure's Optune device, which uses a noninvasive tumor treating field generator that results in the slowing and ultimate reversal of tumor growth [109, 110]. The concept of the device is that it creates low intensity, alternating electric fields within the tumor site that act on the electrically charged cellular components, thereby preventing normal cellular functions such as mitosis, which ultimately leads to tumor cell death [109]. In a prospective, randomized, multi-institutional control trial designed to compare the effectiveness and safety of newly diagnosed GBM subjects treated with Optune in combination with temozolomide (TMZ) ($n = 210$) to those treated with TMZ alone ($n = 105$), progression-free survival in the treatment arm was 7.1 months compared to 4.0 months in the TMZ only group [111]. In addition, overall survival was 20.5 months in the Optune and TMZ group compared to 15.6 months in the TMZ only group [111]. The median follow-up for the study was 38 months (range 18–60 months) [111]. The authors concluded that adding Optune to maintenance TMZ can significantly prolong progression-free and overall survival in patients with newly diagnosed GBM [111].

6. Conclusion

GBM is a highly heterogeneous disease requiring a meticulous treatment approach. Despite advances in treatment options over the past decades, overall survival has remained relatively unchanged. As our understanding of GBM tumorigenesis increases, our treatment efforts have become more targeted. With tremendous strides in immunotherapy and biotechnology, the field of neurooncology holds promise for improving survival in those patients with CNS cancer. The notion of highly specific therapy with minimal side effects is

the benchmark for all cancer therapies striving to accomplish. As we usher in this new era in treating CNS tumors, our approach to fighting CNS disease will change with the ultimate goal of improved survivorship.

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