Glioblastoma multiforme: etiology, progression, and treatment

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Thesis

GLIOBLASTOMA MULTIFORME:
ETIOLOGY, PROGRESSION, AND TREATMENT

by

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GLIOBLASTOMA MULTIFORME:
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RAJIKA JINDANI

ABSTRACT

Glioblastoma multiforme is the most common and malignant brain tumor, accounting for more than 52% of all primary brain tumors. The molecular heterogeneity of the tumor has made it difficult to treat, and even more difficult to cure. Due to the high mortality rate associated with the current treatments used, new innovative medical techniques are being explored. Prominent treatments that are currently being investigated are immune based therapies, focused on checkpoint inhibitors and biomarkers, the use of 2-deoxy-D-glucose to initiate tumor cell apoptosis, and the induction of alterations in DNA and miRNA to inhibit glioblastoma stem cell accelerated growth and tumorigenesis. Throughout the paper, various ongoing studies are summarized and discussed to compare the outcomes of different treatments. The goal of this paper is to present the different therapies and analyze which one of them is the most effective in treating and prolonging survival for patients with glioblastoma multiforme.

This thesis reviewed the large collection of publications about glioblastoma multiforme and treatments for the disease. The use of immune based therapies, such as
checkpoint inhibitors and biomarkers, are increasingly delivering promising results as an immunotherapy approach, but it is necessary to complete the phase III trials in order to truly know if these products are successful as anticancer agents or if further research into the matter is required. More research must be done to find the best route of treatment. In addition, the use of 2-deoxy-D-glucose has been successful in treating other types of cancer, such as breast cancer, and now studies look promising in GBM patients. This treatment is still in its initial stages of testing, so more work will need to be done to determine how efficacious this treatment is.

By comparing the results of the different therapeutic agents, it was determined that genetic alterations seemed to be the most promising avenue of treatment with current information. The data showed that the greatest increase in survival time and least recurrence was achieved by MGMT promoter methylation and gene modifications of the tumor. Though these treatments have varying results in their efficacy and there are many different combinations of medications that have yet to be assessed, research in the area is greatly advancing and increasing the lives of GBM patients. By allocating resources in the best possible treatment, researchers can change the fatal prognosis of this illness.
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<td>--------------</td>
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<tr>
<td>2-DG</td>
<td>2-deoxy-D-glucose</td>
<td></td>
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<tr>
<td>2-HG</td>
<td>2-hydroxyglutarate</td>
<td></td>
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<tr>
<td>AKT</td>
<td>Protein Kinase B</td>
<td></td>
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<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
<td></td>
</tr>
<tr>
<td>BU</td>
<td>Boston University</td>
<td></td>
</tr>
<tr>
<td>CAMK2A</td>
<td>Calcium/ Calmodulin-Dependent Protein Kinase II Alpha Subunit</td>
<td></td>
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<tr>
<td>CAMK2B</td>
<td>Calcium/ Calmodulin-Dependent Protein Kinase II Beta Subunit</td>
<td></td>
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<tr>
<td>CD4+</td>
<td>Cluster of Differentiation 4</td>
<td></td>
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<td>CD44</td>
<td>Cluster of Differentiation 44</td>
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<td>CD8+</td>
<td>Cluster of Differentiation 8</td>
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<tr>
<td>CDC42</td>
<td>Cell Division Cycle 42</td>
<td></td>
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<tr>
<td>CDKN2A</td>
<td>Cyclin-Dependent Kinase Inhibitor 2A</td>
<td></td>
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<tr>
<td>COL1A1</td>
<td>Collagen Type I, Alpha 1</td>
<td></td>
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<tr>
<td>COL1A2</td>
<td>Collagen Type I, Alpha 2</td>
<td></td>
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<tr>
<td>CTLA-4</td>
<td>Cytotoxic T-lymphocyte-associated antigen 4</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
<td></td>
</tr>
<tr>
<td>eEF-2</td>
<td>Eukaryotic Elongation Factor 2</td>
<td></td>
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<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
<td></td>
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<tr>
<td>G1</td>
<td>Gap 1 Phase</td>
<td></td>
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<tr>
<td>G2</td>
<td>Gap 2 Phase</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>GBM</td>
<td>Glioblastoma Multiforme</td>
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<tr>
<td>IDH</td>
<td>Isocitrate dehydrogenase</td>
<td></td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
<td></td>
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<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
<td></td>
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<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
<td></td>
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<tr>
<td>MGMT</td>
<td>Methyl Guanine Methyl Transferase</td>
<td></td>
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<tr>
<td>MMP9</td>
<td>Matrix Metalloproteinase 9</td>
<td></td>
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<tr>
<td>MSP</td>
<td>Methylation Specific PCR</td>
<td></td>
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<tr>
<td>mTOR</td>
<td>Mammalian Target of Rapamycin (a protein)</td>
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<tr>
<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate Hydrogen</td>
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<tr>
<td>OS</td>
<td>Overall survival</td>
<td></td>
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<tr>
<td>PD-L1</td>
<td>Programmed Death Ligand 1</td>
<td></td>
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<tr>
<td>PET</td>
<td>Positron Emitted Tomography</td>
<td></td>
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<tr>
<td>PFS</td>
<td>Progression-free survival</td>
<td></td>
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<tr>
<td>PI3K</td>
<td>Phosphatidylinositol-3-OH kinase</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and Tensin</td>
<td></td>
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<tr>
<td>RAF</td>
<td>Rapidly Accelerated Fibrosarcoma</td>
<td></td>
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<tr>
<td>RAS</td>
<td>Renin-Angiotensin System</td>
<td></td>
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<tr>
<td>RTK</td>
<td>Receptor tyrosine kinase</td>
<td></td>
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<tr>
<td>TGF-β</td>
<td>Transforming growth factor-β</td>
<td></td>
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<tr>
<td>TMZ</td>
<td>Temozolomide</td>
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<td>TP53</td>
<td>Tumor Protein p53</td>
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</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<td>WHO</td>
<td>World Health Organization</td>
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INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor, with a grade IV World Health Organization classification. Patients with GBM have a poor prognosis, with the life expectancy being less than 15 months. 10% survive within three years, and only 3-5% survive for more than 5 years (Alifieris et al., 2015). Patients that survive beyond 36 months are deemed “long-term survivors.” Glioblastomas are highly invasive tumors. Despite treatment of surgical resection of greater than 95% of the tumor, followed by radiotherapy or chemotherapy, most patients suffer recurrence of the tumor because of the molecular heterogeneity of the tumor and the invasion of therapeutics agents across the blood brain barrier (Szopa et al., 2017). These factors affect the treatment time, as well as the prognosis of the illness and the acquisition of tumor resistance.

This paper will first discuss relevant background information about glioblastoma multiforme, specifically the prognosis factors, the genetic components, the progression and diagnosis, and current treatments available for the disease. Next, proposed treatments for the cancer will be provided, along with studies that have been done to show the efficacy and safety of these treatments. Discussion about which of these treatments is most promising for the future of GBM therapeutics will follow. These new treatments are essential in paving the way to better quality of life in glioblastoma multiforme patients and longer survival times after diagnosis of this universally fatal disease.
A. Prognosis

Prognostic factors that are commonly used with glioblastoma multiforme are age, Karnofsky performance status, mini-mental status examination score, O6-methylguanine methyltransferase promoter methylation, and extent of surgery (Kanu et al., 2009). Favorable prognostic factors are younger age at diagnosis (<50 years), a Karnofsky Performance Status of at least 70 points, and the tumor location being in an area of the brain that does not impact sensory processing or linguistic ability (Szopa et al., 2017). The Central Brain Tumor Registry of the USA statistical report states that the age-adjusted incidence rate for the cancer is 3.19/100,000. This prevalence is highest at the ages of 75-84 years (14.93/100,000) and is more common in males (3.91/100,000) (Li et al., 2016). Moreover, glioblastomas cause severe hypoxia, which leads to pathological characteristics, such as necrosis and microvascular proliferation (Toyonaga et al., 2017).

B. Genetics

Glioblastoma multiforme is a molecularly heterogeneous disease. Based on decades of molecular studies on human GBM, the main genetic mutations reside in three different categories. The first would be the impairment of growth factor signaling pathways through amplification or mutational activation of receptor tyrosine kinase (RTK) genes (Dang et al., 2009). The second is the activation of the phosphatidylinositol-
3-OH kinase (PI3K) pathway. The third is the inactivation of the p53 and retinoblastoma tumor suppressor pathways (Li et al., 2016).

A number of potential diagnostic, prognostic, and predictive biomarkers related to GBM are being investigated (Szopa et al., 2017). These molecular signatures allow for a better understanding of the tumor and its pathogenesis. The biomarkers characterize an accurate tumor classification that can be used to predict a prognostic outcome for the cancer. In addition, these predictive biomarkers can enable better patient management, as a treatment can be created to best fit the patient’s illness, biology, and needs.

Some biomarkers are routinely tested in the clinic, such as isocitrate dehydrogenase mutations, 1p19q deletion, MGMT promoter methylation, and EGFRvIII amplification (Szopa et al., 2017). Bioinformatics studies have shown that glioblastoma is related to mutations in the pathways of cancer, MAPK signaling, focal adhesion, and calcium signaling. Key genes associated with this cancer are MMP9, CD44, CDC42, COL1A1, COL1A2, CAMK2A, and CAMK2B (Huse et al., 2010).

With recent advancements in genomic sequencing technology, personalized therapeutic approaches have been made possible with detailed distinctions of GBM molecular markers. Treatments can thus be created, such as molecular inhibitors targeting growth factor receptors, vaccines, antibody-based drug conjugates, and inhibitors blocking the immune checkpoints (Szopa et al., 2017).
Clinically, GBM can be characterized as primary and secondary. Primary glioblastomas are malignant tumors that do not present with a precursor lesion. Secondary glioblastomas slowly develop from low-grade diffuse astrocytomas (WHO grade II) or anaplastic astrocytomas (WHO grade III) (Ohgaki et al., 2004). The majority of patients present with primary GBM, a de novo grade IV lesion.

A smaller percentage, about 5-10%, present with secondary GBM, less aggressive grade II diffuse astrocytomas and grade III anaplastic astrocytomas. These two presentations have a distinct molecular basis. Primary GBMs contain amplifications or mutations of the EGFR gene (in 36-60% of primary versus 8% of secondary tumors), PTEN mutations (in 25% of primary versus 4% of secondary tumors), and CDKN2A-p16\textsuperscript{INK4a} deletion (in 31-78% of primary versus 8% of secondary tumors) (Ohgaki et al., 2004). The EGFR, or epidermal growth factor receptor, gene codes for receptor transmembrane proteins, which are activated by the epidermal growth factor family of ligands. Phosphatase and tensin homolog (PTEN) mutations are causal factors in many cancers. CDKN2A-p16\textsuperscript{INK4a} deletions are major targets in the pathogenesis of cancers and are used to detect different types of cancer.

In addition, there are genetic aberrations expressed in an increased amount in secondary GBMs, such as TP53 mutations with 28% of primary tumors containing this aberration versus 65% of secondary tumors (Ohgaki et al., 2004). Moreover, MGMT
promoter methylation is in 36% of primary tumors versus 75% of secondary tumors (Nakamura et al., 2001). Furthermore, isocitrate dehydrogenase 1 mutations are contained in 5% of primary tumors versus 75% of secondary tumors (Yan et al., 2009).

**Figure 1**: Genetic alterations that are significantly different in frequency between primary and secondary glioblastoma.

Source: American Association for Cancer Research
a. TP53 Pathway Mutations

The TP53 tumor suppressor gene encodes a protein that regulates genes involved in cell cycle arrest in the G1 or G2 phase, cell death and differentiation, DNA repair, and neovascularization (Gomez-Manzano et al., 1996). There are various ways TP53 can be altered, such as indirect inactivation, mutation, deletion, or damage to the genes (Ohgaki et al., 2009). These types of mutations are genetic hallmarks of secondary glioblastoma, as shown by the following study. In a population-based study done in the Canton of Zurich, Switzerland, the frequency of genetic alterations and the outcome of patient mortality was determined. Throughout the 14-year study, from 1980 to 1994, 715 glioblastoma cases were included in the study. TP53 mutations were very prevalent in secondary GBMs. Of the secondary GBM cases in this study, 57% of the TP53 mutations were in hotspot codons 248 and 273. In primary GBM, the TP53 mutations were more proportionately dispersed. Moreover, G:C to A:T mutations at the CpG sites were more prevalent in secondary GBM rather than primary GBM (56% versus 30%). These findings revealed that TP53 mutations in the two subtypes of glioblastoma arise through separate mechanisms (Ohgaki et al., 2009).

b. Receptor Tyrosine Kinase Mutations
Mutations and amplifications in RTK include EGFR, PDCFRA, basic fibroblast growth factor receptor 1, and insulin-like growth factor receptor (Ohgaki et al., 2007). These proteins act in a cascade to drive and regulate cellular processes throughout the cell. Gliomas utilize two main signaling pathways: the RAS/RAF/MAPK pathway, which controls cellular proliferation, differentiation, and migration, and the PI3K/AKT/mTOR pathway, which promotes cell proliferation and survival through the cell cycle and the inhibition of apoptosis (Ohgaki et al., 2007). The tumor suppressor gene PTEN, which negatively regulates the pathway, regulates PI3K (Mellinghoff et al., 2005). About 36% of gliomas lack PTEN, which upregulates the pathway and causes resistance to EGFR therapies (Padfield et al., 2015). EGFR mutations and amplifications are the most common genetic cause of GBM, occurring in about 57% of tumors (Furnari et al., 2015).

c. **Isocitrate Dehydrogenase Mutations**

IDH1 and IDH2 genes are responsible for two important metabolic enzymes: isocitrate dehydrogenase 1, which is present in peroxisomes and cytosol, and isocitrate dehydrogenase 2, which is present in the mitochondria. These enzymes are crucial in catalyzing the oxidative carboxylation of isocitrate to alpha-ketoglutarate, which forms NADPH in the citric acid cycle (Xu et al., 2004). When there are mutations in the IDH genes, reactions are catalyzed that generate an oncometabolite, 2-hydroxyglutarate (2-HG), which is a common feature in human brain cancers (Dang et al., 2009). These
mutations have been found in secondary GBMs, in 73%-85% of cases, as well as grade II and III astrocytic and oligodendroglial tumors, in 72%-100% of cases (Yan et al., 2009).

Although normal IDH and mutant IDH gliomas are histologically similar, gliomas that contain the IDH mutation occur in the presence of other abnormalities, such as a TP53 mutation (Huse et al., 2010). IDH-mutant tumors are connected to epigenetic changes, like DNA methylation disorders (Duncan et al., 2012). In order to further determine the best technique in treating IDH mutations, more work needs to be done. At this time, the 2-HG level is a very useful tool as a biomarker in measuring response to treatments in glioblastomas, as well as other tumors (Popovici-Muller et al., 2012).

<table>
<thead>
<tr>
<th>Common Molecular Causes of GBM</th>
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<tbody>
<tr>
<td>Deletion of chromosome 10</td>
<td>70%</td>
</tr>
<tr>
<td>EGFR amplification</td>
<td>40%-60%</td>
</tr>
<tr>
<td>p16INK4a deletion</td>
<td>30%</td>
</tr>
<tr>
<td>p14ARF mutation</td>
<td>30%</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>30%</td>
</tr>
<tr>
<td>PTEN mutation</td>
<td>25%</td>
</tr>
<tr>
<td>RB1 methylated</td>
<td>15%</td>
</tr>
<tr>
<td>MGMT methylated</td>
<td>36%</td>
</tr>
</tbody>
</table>

This table lists the major molecular causes of GBM along with the percentage of patients that display with a certain type of molecular alteration. The incidence of each mutation is important to consider when researching treatments.
Figure 2: Glioblastoma characterization
Common alterations in critical signaling pathways found in glioblastoma multiforme.

Source: BioMed Research International
Figure downloaded from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5337853/
Published online February 20, 2017
Copyright © 2017 Wojciech Szopa et al.
C. Progression and Diagnosis

The World Health Organization (WHO) classifies gliomas into four grades, from 1 being the lowest to 4 being the highest, based on increasing degrees of undifferentiation, anaplasia, and aggressiveness (Louis et al., 2007). Grade I tumors are lesions that have a low proliferative potential and can possibly be cured with surgical resection alone. Grade II tumors are infiltrative, can recur despite low proliferative activity, and can progress to more malignant forms. Patients who are diagnosed with these types of tumors typically have a prognosis of surviving more than 5 years. Grade III tumors are lesions that have histological evidence of malignancy and are treated with chemotherapy or adjuvant radiation. Patients diagnosed with these types of tumors have a prognosis of surviving 2-3 years. Grade IV tumors are functionally malignant, mitotically active, necrosis-prone neoplasms, which generally progress quickly and have a fatal outcome. The prognosis of patients with these types of tumors is dependent on the efficacy of treatment. Most elderly patients succumb to this disease within a year, while others could survive up to 5 years (Louis et al., 2007).

Glioblastoma multiforme usually presents as a single tumor. The tumor is often placed on the corpus callosum, growing bilaterally into the occipital and temporal lobes, and is called a “butterfly glioma” (Zhang et al., 2016). The most common symptom of
this type of cancer is epilepsy and other symptoms include bloating, pelvic pain, difficulty eating, and frequent urination (Huse et al., 2010). In the early stages of cancer (I/II), it is difficult to diagnose because of the nonspecific symptoms.

GBM tumors begin to develop through infiltrative growth in nerve fiber pathways or in a metastatic spread via cerebrospinal fluid. This makes it easy for satellite lesions to form in adjacent parts of the brain (Yan et al., 2009). GBM progresses rapidly, doubling about every ten days. The presence of the tumor can be primarily confirmed with a magnetic resonance imaging test. After this first step, a definite diagnosis is given by a histopathological examination of tumors removed by surgery (Zhang et al., 2016).

Onset and progression of primary GBMs differ from those in secondary GBMs. Secondary GBMs are usually diagnosed at a younger age, around 45 years, and primary GBMs are diagnosed at an older age, around 62 years. In addition, secondary GBMs have a longer clinical history, around 16.8 months, and primary GBMs have a shorter clinical history, around 6.3 months. The two are histologically similar; however, secondary GBMs have a better prognosis, with a survival expectancy of 7.8 months versus 4.7 months in primary GBMs (Szopa et al., 2017).
**Figure 3: Magnetic resonance imaging performed following initial hospitalization.**

This figure shows different views of a glioblastoma multiforme tumor. (A) Sagittal and (B) axial T2-weighted views show round, long signals in the left temporal, parietal, and occipital lobes. There is a defined shape to the lesion, which is about 7 mm in diameter. The inferior horn of the lateral ventricles is compressed. (C) Axial diffusion-weighted views exhibit homogenous signals. (D) Enhanced axial T1-weighted images compare the enhancement of the cystic wall with that of the solid tumor.

**Source:** Oncology Letters

Figure downloaded from [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5228339/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5228339/)

Published online October 5, 2016.

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Figure 4: Magnetic resonance imaging performed 12 days after hospitalization. This figure shows different views of a glioblastoma multiforme tumor. (A) Sagittal and (B) axial T2-weighted views show the lesion size has increased and the diameter of the cyst-solid lesion is now about 13 mm, including an increase in the lesion cavity. The tumor’s wall is smooth.

Source: Oncology Letters
Figure downloaded from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5228339/
Published online October 5, 2016.
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Figure 5: Magnetic resonance imaging performed 23 days after hospitalization. This figure shows different views of a glioblastoma multiforme tumor. (A) Sagittal and (B) axial T2-weighted views show a lesion that is 17 mm in diameter. There is a defined shape to the tumor, as well as peritumoral edema. (C) Axial diffusion-weighted views exhibit homogenous signals. (D) Enhanced axial T1-weighted images compare the weak enhancement of the cystic wall with the definitive enhancement of the solid tumor.

Source: Oncology Letters
Figure downloaded from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5228339/
Published online October 5, 2016.
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CURRENT TREATMENTS

Glioblastoma multiforme is currently primarily treated with surgical resection of over 95% of the tumor, using state of the art preoperative and intraoperative neuroimaging. Complete resection of the tumor is very rare because of the extent of infiltration of the tumor (Szopa et al., 2017). After the operation, most patients elect to have concurrent chemotherapy and radiotherapy (Kanu et al., 2009). Radiotherapy, in addition to surgery, has shown an increase in survival time from 3-4 months to 7-12 months. Radiotherapy is often accompanied by concomitant and adjuvant therapies, such as temozolamide, an oral chemotherapy drug used to treat brain cancers. In older patients, generally over the age of 70, less aggressive treatments are used, with radiation or temozolomide alone. To treat circulating vascular endothelial growth factor (VEGF) during progression of cancer, bevacizumab is used, also in combination with lomustine (Szopa et al., 2017).

Even with these aggressive treatments, many patients are afflicted by recurrence of the tumor. Studies have been done to observe when radiotherapy should be administered. In the past, studies have claimed that the greatest benefit comes when the time between surgery and radiotherapy is minimized. Other studies state that starting radiotherapy immediately after surgery could be detrimental because patients need time to recover from the aggressive treatment (Randolph et al., 2016). The ideal time to begin radiotherapy is being explored to better treat glioblastoma multiforme patients and prolong survival rates.
PROPOSED TREATMENTS AND TRIALS

Despite current treatments for glioblastoma multiforme, the prognosis remains grim. New approaches need to be explored to increase survival rates for the disease. In recent years, alternative treatments have been developed to better the quality of life and care for glioblastoma multiforme patients. In this section of the paper, four different therapies will be discussed: two immune-based therapies, checkpoint inhibitors and biomarkers, the use of 2-deoxy-D-glucose analog, and the induction of alterations in DNA and miRNA to inhibit glioblastoma stem cell accelerated growth and tumorigenesis

A. Immune-based Therapies

Cancers, such as melanoma, lung cancer, and kidney cancer, have used immunotherapy in the past to increase efficacy of treatments, and now it is being used for glioblastoma. In the past 30 years, advances have been made in immunosuppression uses for glioblastoma. Two approaches will be discussed in this section: checkpoint inhibitors and viral based therapies.

Initially, the immune response of peripheral blood cells from glioblastoma patients were harvested and used. Patients with intracranial tumors, such as GBM, exhibit a decrease in immunity. In studies done by Thomas Roszman, Lucinda Elliott, and William Brooks, results were obtained that showed defects in interleukin 2 secretion, as
well as in the expression of the high affinity IL-2 receptor. This was thought to play a role in the immunosuppressive properties of gliomas (Roszman et al., 1991). In order to explore this effect, more work was done to search for soluble factors released by the tumor for induction of immunosuppression, as shown in Figure 6.

**Figure 6: Immunosuppressive Properties of Glioma Stem Cells**

Source: ASCO University
Publication: 2016 Educational Book
Modified from Hatiboglu et al., 2016.

In the search for soluble factors released by cultured glioblastoma cells, various factors were identified, such as transforming growth factor (TGF)-β, a key
immunosuppressive cytokine. TGF-β is a part of a family of cytokines, which interact with heterodimeric receptors. There are three isoforms that have been identified in the biology of glioblastoma. Therapeutic targets related to this cytokine were limited in their efficacy to treat the tumor effects (Frei et al., 2015). Prostaglandin E2 and interleukin 10 were also identified as having a role in the immunosuppression of glioblastoma.

Other theories about the origin of the immunosuppressive environment is that contact of immune cells with the glioblastoma microenvironment causes them to develop into an immunosuppressive population that indirectly acts systematically. Glioblastoma cells have the ability to alter the phenotype of the host cells to maintain the immunosuppressive environment and facilitate tumor growth (Nduom et al., 2015).

Recently, research has been focused on altered expression of PD-L1 in glioblastoma cells. PD-L1, the ligand for PD-1, and CTLA-4, cytotoxic T-lymphocyte-associated antigen 4, are T-cell surface molecules that are immune checkpoints that regulate the inactivation of T-cells (Preusser et al., 2015). Inhibition of immune checkpoints creates a general, nonspecific immune response that is effective against cancers that have a high mutation rate.

In order to assess the radiological changes that the immunotherapy is causing, a board of neuro-oncology immunotherapy experts has created the immunotherapy Response Assessment for Neuro-Oncology (iRANO). These criteria help determine the
progression of the tumor based on the immune-related response criteria within 6 months of starting immunotherapy. In order to screen for progress, the development of new lesions, and the radiographic progression from follow-up images are commonly used. This allows patients to monitor the success of their therapy and avoid failures that would be detrimental to their health. These new advances are beneficial in immunotherapy for patients with neuro-oncological malignancies (Okada et al., 2015).

**Checkpoint Inhibitors**

The interaction of a T-cell with an antigen-presenting cell primes the cell for an immune response. However, in order to create this response, a second interaction of a checkpoint molecule with the T-cell is needed to either suppress or activate the T-cell. Checkpoints are pathways that are a part of the immune system that are crucial for maintaining the balance of the system and self-tolerance, as well as regulating the duration and intensity of the physiological immune response in peripheral tissues (Padfield et al., 2015).

Checkpoint inhibitors are antibodies, which are designed to either activate or inhibit these pathways. Studies are underway for assessing how effective the treatment of checkpoint inhibitors is for solid tumors. In Leach et al., CTLA-4 was used to conclude an anti-CTLA-4 antibody could induce an antitumor immune response. The inhibition of the effects of CTLA-4 could create and potentiate potent immune responses against
tumor cells (Leach et al., 1996). Similarly, anti-PD-1 checkpoint inhibitors have shown an antitumor immune response in solid tumors, one of which was glioblastoma (Reardon et al., 2014). In light of successful checkpoint inhibition treatment for other cancers, including melanoma and renal cell carcinoma, momentum for its use with glioblastoma is high (Topalian et al., 2012).

Preclinical data is available that postulates checkpoint inhibitors can initiate an antitumor immune response. In Fecci et al., a murine glioma model was treated with anti-CTLA-4, which resulted in improved survival regulated by a CD4+ T-cell immune response (Fecci et al., 2007). In Zeng et al., anti-PD-1 monotherapy used on murine models for glioblastoma exhibited improved survival. In the case of anti-PD-1, CD8+ T cells created the antitumor immune response (Zeng et al., 2013). There are several trials currently ongoing to further explore the impact of checkpoint inhibitors on glioblastoma.

A Bristol Myers Squibb sponsored trial, NCT02017717, is a large, randomized, phase III open-label trial for patients with glioblastoma with first recurrence. The treatment that is being tested is anti-PD-1 for safety and efficacy. Initially, patients were treated with either only anti-PD-1 or anti-PD-1 along with anti-CTLA-4. Following these treatments, data was produced that showed worse outcomes in patients who received both anti-PD-1 and anti-CTLA-4. Of this group, around forty percent of patients discontinued their involvement in the study. The anti-PD-1 cohort was expanded and bevacizumab was
added to the treatment in an additional arm of the study. Outcomes were that anti-PD-1 was the best route of treatment in this study (Lim et al., 2017).

The phase II NCT02337491 trial is testing the efficacy of anti-VEGF therapy with immunotherapy. In the past, bevacizumab in combination with ipilimumab blocks VEGF influences on inflammation, lymphocyte trafficking, and immune regulation in metastatic melanoma patients. These findings show that immune checkpoint blockade has a positive effective in controlling the angiogenic factors in blood vessel formation, as well as immune regulation (Hodi et al., 2014). Merck has sponsored this study of its anti-PD-1 drug to measure the survival of patients at 6 months free of progression. The trial is testing both only anti-PD-1 and anti-PD-1 with bevacizumab (Lim et al., 2017).

The Ludwig Foundation has sponsored a phase II trial to assess AstraZeneca’s anti-PD-1 therapy. There are various cohorts in this trial to study multiple objectives. The first is studying the effects of anti-PD-L1 and radiation in patients with recently diagnosed glioblastoma containing unmethylated O(6)-methylguanine DNA methyltransferase. Temozolomide (TMZ) is withheld in this arm of the study. In the next cohort, anti-PD-L1 therapy is being assessed alone in patients with recurring glioblastoma. The last cohort will assess the efficacy of anti-PD-L1 along with bevacizumab treatment in patients with recurrent glioblastoma (Lim et al., 2017).
Merck’s phase I trial, NCT02313272, is observing the safety of its anti-PD-1 drug with bevacizumab and hypofractionated stereotactic irradiation in patients with recurrent high-grade gliomas (Lim et al., 2017). Another phase I trial by Merck is testing the anti-PD-1 drug, MK-3475, in an open-label, randomized safety study. The cohort will contain patients with recurrent glioblastoma who will be treated with laser ablation (Lim et al., 2017).

Bristol Myers Squibb’s phase III trial, NCT02617589, is measuring the efficacy of anti-PD-1 with radiation in patients with newly diagnosed glioblastoma. An additional arm of the study will take patients in for a treatment with radiation and temozolomide (Lim et al., 2017).

Finally, Merck is also conducting a phase I/II trial for patients recently diagnosed with glioblastoma. The first phase of the study will assess the safety of anti-PD-1 drugs along with radiation and temozolomide. The phase II compares the efficacy of anti-PD-1 and radiation or temozolomide with patients that are being treated with the latter (Lim et al., 2017). These studies are just a few of the many that are being undertaken to assess the effect of checkpoint inhibitors on glioblastoma.

Biomarkers
Therapies involving biomarkers concentrate on protein expression, immune cell characterization, and mutational burden. One approach has been to observe the activation status of immune cells in order to predict the response they would generate. In the past, this approach has been used with other types of cancer, for example, melanoma. In the case of melanoma, the activation status of CD8+ T cells can be measured by assessing the eomesodermin status. This measure revealed that eomesodermin levels could foretell relapse free survival. Additional markers that could be used are interferon gamma expression, Helios expression, and more (Wang et al., 2012).

Other investigators have been looking into whether mutational burden could predict the response. Trials have been done with lung cancer and colon cancer, such as Le et al., that have yielded results that say patients who have defective DNA repair genes have a higher number of mutations, which correlates with survival (Le et al., 2015). The measured improvement in antitumor immune responses in those with increased mutations in their tumors have a greater amount of target antigens within their immune system (Diaz et al., 2015).

B. 2-deoxy-D-glucose

In this part of the thesis, 2-deoxy-D-glucose (2-DG) treatment will be explored, including the available therapies for tumor control, the combination of 2-DG with radiation, the contributions to genetic mutations, and the mechanisms of how this
treatment aids in poor prognosis of malignant tumors. Next, the extent to which 2-DG is effective in treating glioblastoma can be explained using models of tumor progression and assessed using data from literature. In this section, the biochemistry of 2-DG will be discussed and the background of its influence on glioblastoma will be given.

**The Importance of 2-deoxy-D-glucose in Enhancing Apoptosis**

2-deoxy-D-glucose (2-DG), a glucose analog and glycolytic inhibitor, has been used in treatment of malignant tumors. Metabolism of tumor cells differs from that of normal cells. Instead of gaining energy from respiration like normal cells do, malignant cells utilize glycolysis, even when oxygen is available (Wu et al., 2009). 2-DG usage prior to radiation has been seen to inhibit DNA repair, which increases the effectiveness of radiation treatment as well (Venkataramana et al., 2013). The physiological effects of 2-DG are a result of the central nervous system decreasing the use of glucose.

In glioma cells, different pathways, such as the histone acetylation pathway, can be targeted to induce cell death by increasing the amount of cell death inducers in tumor cells (Egler et al., 2008). Histone acetyltransferase and histone deacetylase regulate the affinity between protein complexes and DNA. Therefore, this is a key step in turning off pathways that up-regulate tumor growth, invasion, and resistance to apoptotic stimuli (Egler et al., 2008). In addition, usage of 2-DG activates eEF-2 kinase and inhibits protein synthesis in glioma cells (Wu et al., 2009). A depletion of cellular ATP contents
followed the activation of eEF-2 kinase by 2-DG, as well as inactivation of key proteins in the mTOR pathway, activation of AMPK, and phosphorylation of intracellular components (Wu et al., 2009). All of these reactions suggest that the use of 2-DG elicits an energy stress response, as can be seen in Figure 1 (Wu et al., 2009).

Figure 7. Effects of 2-DG on the activity of eEF-2 kinase (A) and protein synthesis (B) in glioma cells.

The Combination of 2-deoxy-D-glucose with Radiation

Many different therapies have been attempted to treat gliomas in the past. 2-DG has been successful in the past with treating human breast cancer cells where it was
investigated first. In order to visualize the cancer tumor, the glucose analog was tagged with a fluorine atom that could be observed through a PET scan. The analog goes on to block the first step of glycolysis so that metabolism cannot continue and the cell will die (Aft et al., 2002).

Another type of therapy, radiotherapy, has generally increased the survival expectancy from 4 to 10 months (Venkataramana et al., 2013). The use of therapies, such as surgery, radiotherapy, and chemotherapy, allow for local regrowth of the tumor, which means that it is not as effective in successfully treating the cancer. Administration of 2-DG affects the tumor cells and normal cells differently, while radiation affects all the same way (Egler et al., 2008). Thus, it is more understandable to be using the 2-DG treatment.

In order to form a better treatment regimen, a different approach was taken. The combination of 2-DG and radiotherapy causes necrosis of the tumor (Venkataramana et al., 2013). Positron Emission Tomography studies have revealed that the degree of malignancy of cerebral gliomas can be estimated by the amount of accumulated 2-DG (Venkataramana et al., 2013). In studies done post treatment, re-exploratory surgery after the combined treatment showed great amounts of tissue necrosis of the tumor, while the normal brain tissue surrounding the tumor was healthy and undamaged (Dwarakanath et al., 2009). In the past, with other types of treatment, the results have not been so beneficial.
In Singh et al., patients that were previously untreated were given seven weekly fractions of gamma rays directed at the tumor. During this time, increasing levels of 2-deoxy-D-glucose was administered orally before the irradiation. In addition to the effects on the tumor, the toxicity levels, radiation damage, and treatment responses were also monitored and studied in surviving patients (Wu et al., 2009). This study found that oral administration of 2-deoxy-D-glucose along with radiation was well tolerated by patients and considered safe, without acute toxicity or damage to the brain. Further work is being done in this area to evaluate the efficacy of the treatments.

C. Genetic alterations

A plethora of molecular modifications have been identified in GBM; however, because of the dismal prognosis, there must be further work into the molecular changes that occur during treatment to elucidate therapeutically resistant recurrences. Intrinsic or acquired resistance to the alkylating agents of chemotherapy used in treatment of patients in a common cause of treatment failure. The alkylating agents are used to irreversibly damage DNA and cause apoptosis, which will cause cytotoxic activity because of the DNA repair pathways (Sarkaria et al., 2008). O6-methylguanine DNA adducts result in double-strand breaks, but it is necessary for the cell to also contain a functional mismatch repair pathway in order for this to be successful. Tumor cell lines that have mutations in mismatch repair are therefore resistant to alkylating agents. There are two mechanisms of
resistance to alkylating agents. One is DNA repair enzyme O6-methylguanine methyltransferase, which minimizes the harm caused by the cytotoxic O6-methylguanine DNA adduct by taking it out. Another mechanism is using the base excision repair (BER) pathway (Sarkaria et al., 2008).

MGMT, a DNA-repair enzyme, works by removing the alkyl groups at the O6 part of guanine, which is added on by alkylating drugs like temozolomide (Liu et al., 2006). New therapeutics have been explored through the promoter methylation induced epigenetic silencing of MGMT, which has increased the response to chemotherapy and has longer survival in GBM patients (Hegi et al., 2005). However, even though there has been an increase in the survival time, it has only been to about 21 months for GBM with a methylated MGMT promoter (Hegi et al., 2005). Various studies have been done to assess the impact of MGMT profiles in patients and make a correlation with the length of survival or susceptibility to alterations in recurrent GBM.

In Parkinson et al., the methylation status of the MGMT promoter was studied in 22 samples from 10 patients (Parkinson et al., 2008). It is known that the methylation of the MGMT gene promoter region indicates a response to TMZ in GBM patients. However, prior to this study, little was known about the variations in the MGMT promoter when treatment was finished or throughout the entire tumor. The samples were analyzed by assessing the promoter sequencing. Additionally, 20 of the 22 samples were analyzed using Methylation Specific PCR (MSP). Results showed that the methylation
status of the MGMT promoter differed in 2 of 9 samples studied with MSP and 7 of 10 patients studied with promoter sequencing. Other findings showed that the MGMT promoter was unmethylated before surgery in 4 patients, but had some methylation post-treatment. MSP findings correlated with these findings, observing 2 of the 4 patients had an alteration in status from unmethylated to methylated. Three to four samples were taken from each tumor to ensure that there was no intra-tumor variability that could skew the results. The results indicated that variation in MGMT promoter methylation was possible within the same tumor after treatment, which is important for clinicians to know when making important treatment decisions (Parkinson et al., 2008).

In Wiewrodt et al., MGMT activity in GBM patients who had received radiation therapy or radiation therapy plus chemotherapy with alkylating agents, such as temozolomide, was studied (Wiewrodt et al., 2008). MGMT activity increased from untreated GBM to the first, second, and third recurrence. There was a significant increase in the MGMT in the first recurrence in patients who received only radiation therapy versus those with radiation therapy plus chemotherapy. This means that alkylating agents tend to either select MGMT expressing cells or induce the MGMT gene. The findings of this study showed that MGMT activity in the primary tumor was useful in predicting how effective GBM therapy would be (Wiewrodt et al., 2008).

In Metellus et al., 22 patients who had recurrent GBM and surgery with carmustine wafer implantation were enrolled in the study. The purpose of the study was
to assess the correlation between MGMT silencing in the tumor during recurrence and how it impacted survival. The status of MGMT was ascertained with methylation-specific polymerase chain reaction analysis, immunohistochemistry, and high-throughput quantitative methylation assay. The progression-free survival and overall survival rates were 3.6 months and 9.9 months. The 6-month PFS rate was 27.2%. MGMT promoter hypermethylation, as well as age, during recurrence correlated with a better PFS. Only MGMT promoter hypermethylation during recurrence was associated with a better OS. The findings of this study showed that MGMT methylation status was useful in determining the outcome of GBM therapy at recurrence (Metellus et al., 2009).
DISCUSSION AND CONCLUSION

There are several proposed glioblastoma multiforme treatments that could provide benefits to patients and their families. This paper has looked into four major researched treatments: Two immune based therapies, checkpoint inhibitors and biomarkers, 2-deoxy-D-glucose analogs, and genetic alteration techniques. Throughout this review of the current glioblastoma multiforme literature, it has been concluded which of the above treatment plans could be the most beneficial for patients and that promise the best results. Genetic alterations, specifically MGMT promoter methylation, have the strongest data supporting it as a successful treatment. This section will further analyze the results of the above papers and provide insight into why this area is best for upcoming research.

Immunotherapy has been very effective in the past with solid tumors, thus research has begun to determine its effects on glioblastoma. Many trials have been conducted throughout the past two decades to try different combinations of approaches; however, many checkpoint inhibitors have yet to be investigated. Checkpoint inhibition activates the immune system in a nonspecific manner, which could be a good approach for glioblastoma, as it has been with melanoma, lung cancer, and renal cancer. The brain has a different set of checkpoint inhibitors that must be further looked into so that the technology that has been developed for solid tumors can be used with GBM patients. Furthermore, biomarkers are crucial in helping identify the correct patients for treatment and ensuring that the treatment will be effective rather than toxic.
Immune toxicity is an important factor to take into consideration when looking into new types of immunotherapies. The most prevalent toxicity is caused by autoimmune reactions. Such toxicities include colitis, pneumonitis, hepatitis, pancreatitis, dermatitis, hypophysitis, and thyroiditis. These toxicities must be identified and treatment must be stopped (Weber et al., 2012). An antidote to this type of toxicity would be high-dose corticosteroids and administration of infliximab (Weber et al., 2012).

Though trials have had promising results in certain arms of studies with immunotherapy, much work needs to be done before this can be a viable solution for patients. In addition, because different people have different biomarkers, this solution is not sustainable for the population at large. This is an individualized approach, which would be very costly and time consuming. The trials that are being done have shown that certain biomarkers can predict whether or not a treatment will be effective. As these trials are still in preliminary stages, the efficacy of the approach has not been fully determined.

Moreover, the use of 2-deoxy-D-glucose has made much progress recently. Greater rates of glycolysis and use of glucose indicates a poor prognosis in malignant tumors. 2-deoxy-D-glucose is a nonmetabolizable glucose analog, which was tested to increase the efficacy of radiotherapy. In Singh et al., the method used was to target specific sensitive cancer cells in a dose-dependent manner of 2-DG, while at the same time protecting noncancerous cells (Toyonaga et al., 2017). During this treatment,
patients experienced side effects similar to hypoglycemia. The patient tolerance to the medication, as well as compliance was effective up to about 250 mg/kg BW of 2-DG (Toyonaga et al., 2017). At higher doses, two patients expressed discomfort and refused to finish treatment; however, the outcomes were the same. Of the ten patients that followed up, seven had no significant damage to normal brain tissue, and they survived from 11 to 46 months after the treatment (Toyonaga et al., 2017).

This treatment is showing to be effective and is increasing the average life expectancy in patients. However, at this early stage, results cannot be confirmed that would apply to the greater population. In the study done by Singh, the population size was very small, with only about ten patients (Singh et al., 2005). In addition, the results varied greatly, from 11 to 46 months. Though the initial testing looks promising, more experimentation must be done to determine whether this treatment really is the best approach for GBM patients.

Finally, looking at the data produced by MGMT promoter methylation, the approach of genetic alterations has had the most promising results. In Hegi et al., two hundred and six patients participated in the study by the National Cancer Institute of Canada and the European Organization for the Research and Treatment of Cancer (Hegi et al., 2005). These trials showed a median survival of 21.7 months rather than 12.7 months in GBM patients with unmethylated MGMT that were treated with TMZ and radiotherapy (Hegi et al., 2005). This demonstrated that epigenetic silencing by promoter
methylation of the MGMT gene is successful in increasing survival times. In Metellus et al., twenty-two cases were studied, and of those, 3 yielded an increase in MGMT protein expression by IHC (Metellus et al., 2009). MGMT methylation at recurrence was determined to be an important indicator of improved survival in GBM patients in this study.

In Wiewrodt et al., MGMT activity was measured in 40 cases of primary GBM using a radioactive assay (Wiewrodt et al., 2008). Tumors from first, second, and third recurrence were also studied. In these trials, it was determined there was an increase in the MGMT activity at each subsequent recurrence. In the treatment group of radiotherapy plus chemotherapy, there was a significantly higher increase in activity than the treatment group of just radiotherapy patients (Wiewrodt et al., 2008). In addition, increased MGMT activity at second and third recurrences was related to an increase in chemoresistance.

Out of the studies that have been analyzed, a major drawback has been the small sample size. This makes it difficult to make conclusive determinations that would be applied to the greater GBM population. Also, many studies that have been done seem to have contradictory results (Sarkaria et al., 2008). This could be due to the sample size, as well as the methods that were undertaken during the studies. Other factors, such as intra-tumor variability, contamination of the tumor tissue, and therapy-induced tumor cell death could be responsible for differing results.
Since GBM is a condition that invariably leads to death, in order to increase the efficacy of therapeutics, the molecular changes during disease progression must be studied. Understanding how MGMT methylation affects the tumor response to treatment is crucial to developing therapy that is targeted and less toxic for the patient. Progress is being made in finding a better treatment for GBM, and by looking at the current literature and identifying promising treatments, the most effective therapeutics can be found.
REFERENCES


