HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 2 (2024)

Beyond The Tumor: A Review of Standard Procedures and Emerging Advancements in Treatments for Glioblastomas

Anargya Ramamoorthy[1], Prahlad Parajuli[#], Jothsna Kethar[#] and Virgel Torremocha[#]

[#]Advisor

ABSTRACT

Glioblastomas are a type of brain tumor, which–according to the Glioblastoma Research Organization–affects more than 241,000 people across the globe. While the causes of glioblastomas are largely unknown, research has shown that some glioblastomas arise likely due to genetic factors such as mutations in isocitrate dehydrogenase (IDH) gene or a 9p/13q deletion. Glioblastomas are extremely hard to treat because they rapidly proliferate, and spread into nearby brain tissues. Surgery has long been used to remove glioblastomas, in order to minimize the intracranial pressure due to the growth of tumor and to reduce tumor bulk for other therapies. However, surgical removal has been tough, since it is very hard to remove all the cancerous cells, and there is a high chance of tumor recurrence. In 2005, the FDA approved the drug temozolomide to be used in glioblastoma treatment following surgical removal. However, temozolomide is minimally effective and causes adverse effects in many patients. As of now, there is no real cure for glioblastoma. Therefore, there is a need to explore new treatments in order to increase the lifespan of glioblastoma patients, as well as to reduce unwarranted effects due to treatment. Newer treatments such as tumor treating fields and nanobots have shown to be promising for glioblastoma treatments. These new advancements have shown to be more precise, which reduces the concern of toxicity to healthy tissues. By researching various treatments for glioblastomas, this paper's main goal is to explore alternative ways to treat glioblastomas effectively.

Introduction

Cancer is defined as an uncontrolled proliferation of cells which spread to nearby tissues or other organs in the body. According to the World Cancer Research Fund, 18.1 million cancer cases were reported across the globe in 2023. The average survival rate for glioblastomas is roughly 8 months. Only 25% of patients survive more than one year, with only 5% of patients surviving more than five years. The poor prognosis of glioblastomas is due to their aggressive nature, which causes them to quickly spread to nearby brain tissue. The danger of glioblastomas is that they affect the central nervous system, which is composed of the brain and spinal cord. Glioblastomas often form in the frontal lobe of the brain, which helps with functions such as movement, judgment, and problem solving; additionally, it is essential for receiving, processing, and responding to information sent from sensory receptors. As of now, the most standard course of treatment is surgical removal, chemotherapy drugs such as temozolomide, radiation therapy, or a combination of these treatments. Surgery has long been used to remove glioblastomas, in order to minimize the intracranial pressure due to the growth of tumor, reduce the effects of the tumor on the brain's functions, and to reduce tumor bulk for other therapies. Surgical removal has shown to increase the median lifespan to about 15 months. A therapy often administered along with surgery is radiation therapy, which uses X-rays, gamma rays, or photons for targeted destruction of cancer cells. Additionally, gamma knife radiosurgery (GKRS)–a noninvasive surgical treatment–delivers high beams of focused

radiation which converge to target a specific area of the brain. GKRS has been shown to be an effective treatment for glioma, and has shown to improve survival rates in glioblastoma patients without significant adverse effects (Sadik et al., 2018). Temozolomide has been used for 18 years as a standard chemotherapy treatment for glioblastomas. As indicated by the National Cancer Institute, radiation and temozolomide had 1 year survival rates of 27.9% and 2 year survival rates of 10.4%, when compared to the 22.2% 1-year survival rate and 2.8% survival rate of radiation therapy alone. However, these therapies come with significant challenges. Although it is one of the most commonly used treatments, surgery is not a viable option for all patients, as many glioblastoma tumors lie in areas of the brain where operating would be too risky or inaccessible. Additionally, it is hard to remove all the cancerous cells during tumor resection, so there is a chance of the glioblastoma re-emerging. Radiation therapy is often given along with surgery, but it can pose significant harm as the power of the radiation can damage healthy cells as well, and may increase the risk for other cancers. Additionally, the commonly used chemotherapy drug temozolomide comes with its own challenges, as it has shown to cause nausea, fatigue, and hematologic toxicity, which can lead to a decrease in bone marrow and blood cells, leading to infection, bleeding, or anemia (Kamson, 2021).

The use of immunotherapy has also been looked into for glioblastoma treatment. Immunotherapy is described as a treatment which uses an individual's own immune system to fight cancer. The use of immunotherapy in cancer treatment opens up the possibility of treatments less toxic than chemotherapy, which helps preserve healthy surrounding brain tissue while removing the cancerous tumor. The body's immune system can be used to identify and destroy cancer cells, and immunotherapy utilizes this function by boosting the immune system to target cancer cells. Another way that the immune system is utilized for glioblastoma treatment is through the use of cancer vaccines, such as neoantigen vaccines or other immunomodulators to stop the proliferation of cancer cells. These treatments work by inducing a cytotoxic T-cell attack against the cancer cells. By delivering antigens of cancer cells to the patient, an effector/memory immune response will be induced that works to protect a person from further progression of the cancer cells. (Segura-Collar et al., 2023). The use of CAR-T Cell therapy has also been explored in glioblastomas. This therapy consists of genetically altered T cells by using Chimeric antigen receptor (CAR) T cells to fight cancer so that they can specifically target cancer cells. T cells work by recognizing antigens on the surface of cancer cells in the context of MHC class I molecules to destroy the cancer cells. These T cells are then altered by switching the antigen binding part of T cell receptor (TCR) with the antigen-binding fragment of a B cell receptor (BCR), which can recognize native antigen without depending on MHC expression, thus generating a chimeric antigen receptor CAR (Segura-Collar et al., 2023).

Due to limited efficacy and associated adverse effects of standard therapies, it is important to explore alternative treatments for glioblastoma. Many non-targeted therapies are cytotoxic and can perturb haematopoiesis and lead to vascular, lung and liver injuries (Baldo et al., 2013). The focus of this paper will be the standard treatments currently being used for glioblastoma treatment and the challenges that come with them, as well as currently developing treatments such as tumor treating fields and nanobots, which have shown to be promising options that alleviate some of the negative effects that come with standard treatments.

Methodology

With cancer, specifically glioblastomas, having a detrimental impact on individuals, there has been a surge in discovery and application of new treatments for its treatment. Databases such as the National Library of Medicine had a variety of journals and articles discussing upcoming treatments and studies across the globe, which were assessed in order to gather data and information to construct this review. Additionally, data from Clinicaltrials.gov was used to assess various clinical trials surrounding the treatments discussed. As a whole, the various treatments discussed have various approaches in which they were tested, to ensure that proper and precise results are collected. Surgery is a common component of treatment for glioblastomas, and there are

various surgical techniques that have been utilized over the years. Most of the findings regarding surgery and glioblastoma treatment are based on case studies, as unnecessary surgeries violate rules 6 and 10 of the Code of Ethics. Glioblastomas occur on a case by case basis, so surgical techniques can only be utilized in patients with glioblastomas who are in need of surgery. Additionally, various clinical trials have been run for drugs such as temozolomide. Usually, drug trials are run by starting with a small group of people, and slowly applying it to a larger population. In order for a drug, such as temozolomide, to be approved, a group must gather and review the drug to make sure that it is safe, and beneficial. All treatments require clinical trials and extensive testing, to ensure that they are safe and efficient for glioblastoma treatment.

The Causes of Glioblastomas

Cancer can arise in various forms, in various parts of the body. The rapid proliferation of cells is why cancer is dangerous, as it can destroy healthy tissue and deteriorate functioning systems and organs in the body, including the brain. There are two types of brain tumors: primary and metastatic tumors. Primary brain tumors are tumors that originate from the tissues of the brain, or the brain's surroundings. Some common primary brain tumors include meningiomas, gliomas, and medulloblastomas. On the other hand, secondary brain tumors–also known as metastatic tumors–are caused by cancer cells that spread from other organs to the brain. Some common types of cancer that can spread to the brain include cancers in the lungs, skin, kidneys, colon, or thyroid gland. One type of primary brain cancer is glioblastomas, which arise from glial cells, namely astrocytes (also called astrocytoma) or oligodendrocytes (also called oligodendrocytoma). Glioblastomas can arise from any glial cell tumor, and can be malignant–meaning that their tumor cells actively reproduce and spread to nearby tissues–or benign, meaning that their tumor cells do not rapidly grow or spread. The WHO classifies glioblastomas based on their histopathology and molecular patterns, which help place glioblastomas in a class of I to IV. Class I includes low-malignant tumors, and class IV consists of highly malignant tumors. Glioblastoma multiforme is considered a class IV tumor, due to its high malignancy and proliferation rate, making prognosis poor (Olar et al., 2014).

According to the American Cancer Society, the chance that a person will develop brain cancer is less than 1%. However, for the people who are diagnosed with brain cancers, they can have detrimental effects, such as headaches, personality changes, memory loss, and seizures (Mayo Clinic, 2022). Brain cancers are classified by the uncontrollable division of cells, which spreads into surrounding tissues. However, what actually causes this rapid proliferation of cancerous cells is not clearly understood. Cancer does not arise from simply one mutation in a cell. Rather, it takes anywhere from 6 to 38 mutations for a normal cell to turn into a cancer cell (Liu et al., 2016). These mutations can be hereditary, or they can develop over time due to exposures to factors such as UV radiation. Some proteins can bind to the DNA, and cause mutations that can lead to cancer (Asadi et al., 2016). Glioblastomas, in particular, arise from genetic alterations of glial cells, which are cells that aid in protection and support for neurons. A majority of brain tumors are found to develop in the cerebral cortex, and spread to other regions of the brain. Tumors formed in the cerebral region have shown to be significantly hard to remove. In glioblastoma patients, genetic deviations occur in 80-85% of adults. Research has found that the formation of glioblastomas includes the formation of extrachromosomal DNA molecules (ecDNA), which help with signal amplification and mutations (Testa et al., 2018). One study of 38 children with brain tumors in Japan found that 7 of the patients had pathogenic variants, 2 had nonsense mutations, 2 had frameshift deletions, and 3 had missense mutations. Additionally, it was found that 3 out of 7 patients had a family member with cancer, which shows that there is a hereditary basis to the acquisition of cancer as well (Fukushima et al., 2021).

The Standard Treatment for Glioblastomas

For the past several years, the standard treatment for glioblastomas includes a combination of surgical removal, radiation therapy, and chemotherapy. As of now, surgical removal is used to remove the bulk of glioblastomas. A variety of surgical techniques have been developed to increase the precision of surgery: intraoperative magnetic resonance imaging (iMRI), brain mapping strategies, intraoperative ultrasound (IOUS), confocal intraoperative microscope (CIM), and intraoperative mass spectrometry (IMS). Intraoperative resonance imaging (iMRI) utilizes 5-aminolevulinic acid (5-ALA), a forerunner of hemoglobin, is a dye that is fluorescent, making the identification of glioblastomas more precise. It was found that the use of 5-ALA and iMRI technologies together resulted in a higher gross total resection (GTR). It was found in a study that iMRI technology alone led to 82% GTR, but the combination of iMRI and 5-ALA led to a 100% GTR. 5-ALA, along with Fluorescein, are useful tools as they can mark where the tumor is beyond its borders. Intraoperative ultrasounds have also proved to be an inexpensive and accessible resource that can heighten the success of surgical removal of glioblastomas (Sales et al., 2022). Walter Dandy, one of the founding fathers of modern neuroscience, stated that a number of glioblastomas spread to the contralateral side through the corpus callosum. Dandy found that surgical resection lengthened the life spans of many, and the rising use of computed tomography (CT) scans and magnetic resonance imaging (MRI) have increased the precision of this surgery (Wang et al., 2019). However, surgery is not a viable option for everyone with glioblastomas since glioblastomas can grow in central regions of the brain, making surgery a risky option. Additionally, Glioblastomas tend to relapse since they grow rapidly and are often resistant to chemotherapy (Xu et al., 2021). Another core reason that glioblastoma is extremely hard to remove through surgery is due to its tendency to infiltrate other parts of the brain. glioblastoma grows rapidly, but the growth rate can vary largely from individual to individual. By comparing the MRI scans of 106 patients, a study was able to determine that the average growth rate of tumors was close to 1.4% daily, and the average time it took for a tumor to double in size was 49.6 days (Stensjøen, 2015). glioblastoma has the tendency to recur after surgery, due to the difficulty of removing the entirety of the tumor during surgery, leaving behind cancer cells to proliferate. However, studies have used functional magnetic resonance imaging (fMRI) and diffusion MR tractography to aid in surgical management of gliomas. This can improve tumor resection and help to preserve brain functions. Diffusion MR tractography and fMRI technologies work by giving insight on the organization of cortical areas and subcortical connections near a tumor, which can help map the brain prior to surgery, to increase accuracy and avoid excessive damage (Castellano et al., 2017).

Radiation therapy has also played a significant role in glioblastoma treatment. Radiation therapy, commonly used along with surgery and chemotherapy, has improved progression free and overall survival for patients. Radiation therapy includes utilizing high energy particles or waves, such as protons or electron beams, in order to destroy cancer cells and slow their division. In studies on elderly patients, radiation therapy has shown to improve overall survival, compared to patients who received only supportive care. Radiation therapy did not have great effects on cognition or quality of life in these patients. Radiation therapy can be given in standard fractionation–which lasts about 6 weeks–or in shorter courses, known as hypofractionation. In hypofractionation, patients are given larger doses daily, which leads to a shorter duration of treatment. Trials showed that there were no significant differences in survival or quality of life between standard and hypofractionated courses of radiation therapy, which shows that radiation therapy can be beneficial for patients who require a more immediate course of treatment. One type of radiation therapy is salvage reirradiation, which is a form of treatment that utilizes external beam therapy; this treatment has been studied in the case of glioblastoma. Advancements in radiation therapy and imaging technology have helped researchers analyze more accurate delineation of treatment volume. One treatment technique is intensity-modulated radiotherapy (IMRT), which allows for conformality by using beams of different intensities at various angles. Another type of technique is stereotactic radiosurgery (SRS), which is more precise than IMRT due to utilizing multiple beams with

a dose gradient at the edge of the target. SRS is usually limited to smaller treatment volumes, due to risks of toxicity. Some specific technologies that utilize SRS are Cyberknife, Novalis, and Gamma Knife. Fractionated stereotactic RT is another option, which allows treatment to be divided up over several days into multiple fractions. Image guidance is used prior to– or in some cases, during each fraction–to reproduce the patient positioning. This treatment option is unique, as it has the radiobiological advantage of allowing for normal tissue repair between fractions, which makes it a viable option for larger volumes of glioblastoma. In one study of over 300 glioblastoma patients who received various forms of salvage reirradiation, the 6-month progression-free survival was found to be 28-39%, with the 1 year overall survival being 18-48%. Clinical improvement was seen in 25-45% of patients, making stabilization of performance an aim in this treatment (Mann et al., 2018).

Chemotherapy is also a prevalent treatment for glioblastoma. The drug temozolomide is the most commonly used chemotherapy treatment since it was approved by the FDA for glioblastoma in 2005. External beam radiation treatment along with adjuvant temozolomide has become the standard treatment for those aged less than 65. Temozolomide functions by methylating DNA, which is when a methyl group is added to DNA to change the DNA segment without changing the sequence. This works to prevent the proliferation of tumorous cells. Temozolomide works to damage and deplete O6-methylguanine-DNA methyl-transferase (MGMT), which is a repair enzyme; temozolomide can make this enzyme ineffective (Chua et al., 2019). Temozolomide adds its methyl group to the purine and pyrimidine in DNA to halt DNA replication, and initiate apoptosis in glioblastoma cells. However, it has been found that the oral consumption of temozolomide can lead to lymphopenia– the lack of lymphocytes in the blood. It has been shown that CD4 T-cells–cells that control the immune response– and B cells–the main producer of antibodies–decrease after temozolomide consumption. Additionally, temozolomide has not shown to be significantly helpful with the overall length of survival. However, temozolomide consumption still showed considerable anti-tumor activity. Temozolomide has also shown to increase cytokines and proliferation factors for lymphocytes. IL-7 and IL-15 are cytokines that promote the growth of CD8 T-Cells and increase effector functions. In environments with depleted lymphocytes, the remaining lymphocytes don't have to compete for IL-5 and IL-7, which ultimately elicits an antitumor immune response (Karachi et al., 2018).

The Use of Stem Cell Therapy and Immunotherapy in Glioblastoma Treatment

Stem Cell Therapy

Stem cells are defined as cells with the ability to self-renew and retain their stemness, and differentiate into many different cell types . Stem cells have also proved to be a useful tool in the treatment of glioblastomas. Glioblastomas contain self-renewing cancer stem cells that contribute to tumor initiations and resistance to treatments. Organs that renew frequently, such as the skin and intestine, have a high concentration of stem cells because they help replace dying cells. The marker CD133 (Prominin-1), and CD146–a glycoprotein on the cell surface on neural stem cells– have shown to enrich cells with increased self renewal and proliferation (Lathia et al., 2015). Engineered stem cell therapies have brought some promising results for glioblastomas. Many studies have used autologous cell-based therapies, but allogeneic cell based therapies such as mesenchymal stem cells (MSC) and neural stem cells (NSC) have been successful in treatment of glioblastomas. The MSCs in bone marrow are weakly immunogenic, which makes it a good source of cells to use in allogeneic cell therapy, without having to compromise the immune system. Engineered MSC cells can release tumor specific cytotoxic agents that can diminish the size of tumors, as well as prolong the patient's survival. However, the administration of engineered MSC cells has proven to be tough, but local delivery has shown great potential.

By using engineered MSC cells to target epidermal growth factor receptors (EGFRs), tumor protein 53 (TP53), Phosphatase and Tensin Homolog (PTEN), and Tumor necrosis factor receptor superfamily genes 10A and 10B (TNFRSF10A/B), the specific targeting of these receptors has proven to be a promising treatment. An optimal cell for stem cell therapy would be one that offers stable secretion of the therapeutic agent. Using EnMSC in models of glioblastoma resection, it was found that migration of MSC led to a decrease in tumor burden, similar to when the EnMSC kill switch is activated (Bhere et al., 2022).

Stem cells can play a role in replacing cells lost due to tissue turnover or injury, as well as maintaining homeostasis in tissues. Several types of stem cells have been researched in glioblastoma treatment, including hematopoietic stem cells. However, among all the stem cells, NSCs have been the most successful, as they are mainly detected in the hippocampus and subventricular zone of the brain. NSCs have the ability to migrate deep into glioblastoma tumor tissue, which allows them to deliver therapeutics, such as drugs, to the tumor mass. NSCs have been used in cytokine gene transduction therapies, but also help with the expression of enzymes, pro apoptotic molecules, nanoparticles, and oncolytic viral therapies. In one NIH trial by members of City of Hope Medical Center (NCT01172964), 15 patients with recurring high-grade glioblastoma underwent intracranial administration of a NSC line with regulated expression of cytosine deaminase (CD-NSCs) which could convert the prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU).The goal of this trial was to determine the safety and efficiency of administering NSCs in combination with oral 5-fluorocytosine (5-FC). On day 0, patients underwent a debulking craniotomy, and received injections of neural stem cells directly into brain tissues. On days 4-10, patients were orally given 5-fluorocytosine every 6 hours. The study focused on the safety of one injection and the ability of NSCs to create an enzyme-prodrug system rather than the effects of NSCs on patient outcomes. However, the study showed that genetically modified NSCs could be used to target the tumor, and the strategy was deemed safe; no dose-limiting toxicity was found, and the CD-NSCs migrated to tumor sites and were non-tumorigenic. Additional trials are being conducted with NSCs; for example, two trials are researching stem cells loaded with oncolytic virus, with potential results that could help personalize glioblastoma therapy (Benmelouka et al., 2021).

Immunotherapy

The use of immunotherapy has also been looked into for glioblastoma treatment. Immunotherapy is described as a treatment which uses an individual's own immune system to fight cancer. The use of immune checkpoint inhibitors has been tested in various types of cancer, but it has not shown great outcome for patients with glioblastoma. However, the use of vaccines has shown to improve progression-free survival and overall survival in glioblastoma patients. There are three antigens that are commonly used in glioblastoma vaccines: tumor-associated antigens (TAAs), tumor-specific antigens (TSAs), and tumor lysate. Specifically, neoantigens are TSAs that are the result of somatic DNA alterations such as point mutations, insertions, deletions, and frameshift mutations. Trials have been conducted using neoantigen based peptide vaccines, which have shown high infiltration of CD8+ and CD4+ T cells in the tumor. One vaccine strategy called actively personalized vaccine (APVAC) included a pool (warehouse) of 59 HLA-binding peptides, which were then matched with individual patients for pre vaccine T cell reactivity. Thus, the APVAC1, comprising 7 best-matching unmutated HLA-binding peptides was administered to 13 patients. These peptides tended to induce CD4+ and CD8+ T cell responses. Another vaccine, APVAC2, containing mutated HLA-binding peptides, was administered to 10 patients. Eight of the 10 patients showed neo epitope-specific immune responses, mainly CD4+ T cell responses. APVAC2 peptides showed an 84.7% immunogenicity. These studies showed that CD8+ and CD4+ T cells tumor infiltrated following administration of neoantigen vaccines. One patient in this study had tumor resection at 26.8 months after diagnosis, showing the high infiltration of T cells due to the administration of the

vaccine (Hilf et al, Nature 2019). However, immunotherapy has its drawbacks; many patients reported injection site disorders, rashes, chills, and influenza-like illnesses. Nevertheless, the trials showed that neoantigen vaccines are relatively safe and efficient. Neoantigens are a more personalized cancer treatment, and have shown higher immunogenicity and efficacy in glioblastoma patients. However, these trials used a combination of neoantigens along with HLA-restricted personalized peptides. When only neoantigen based vaccines were administered, only two out of eight patients showed immune response. The sample size of these trials was very small, so further testing will be necessary to determine the efficacy of neoantigen vaccines (Khan et al., 2021).

Another type of vaccine that has been explored for glioblastoma is dendritic cell vaccines. Dendritic cell vaccination (DCV) is an active immunotherapy that induces an antitumoral immune response. Dendritic cells (DC) are key to the initiation of T-cell responses. As resting cells, they are found in most tissues; when they are activated by changes in the tissue, they take up pathogen- or cancer-associated proteins/peptides, travel to the lymph nodes and activate the T cells. In DCV, patients are vaccinated with dendritic cells loaded with tumor associated antigens (TAA). The DCs travel to lymph nodes to initiate an anti-tumoral T-cell response, which in turn can kill tumor cells and prevent the recurrence of tumors due to immunological memory. When mice were injected with DC pulsed with tumor lysates, the vaccination had helped mice from intracranial tumor challenges. Their survival was longer than mice who received unpulsed DC (Liau, 1999). In humans, one study administered DCV with DC pulsed with peptides of a HLA class I matched GBM cell culture (Liau, 2000). The efficacy of DCV may depend on prior treatments, such as cytoreductive surgery, as the minimal residual disease has helped with vaccination therapy. This can be due to the decreased immunosuppression, which correlates with tumor size. DCV showed to be tolerated well, with severe side effects only shown in 1 patient who suffered from an edema. Typically, DCV uses monocyte-derived DC, which come from peripheral blood. The higher the purity of the monocyte, the more stable the culture conditions, and therefore the more pure the DC preparations. Usually, mature DC are used for DCV, so that they can have the intended antitumoral immune response. However, there is the challenge of DC vaccines breaking through the tumor heterogeneity as well as immunosuppressiveness of glioma cells, which can affect their efficacy in treatment (Datsi et al., 2021).

CAR-T Cell Therapy

Glioblastomas are considered to be an immunologically cold tumor due to having high intratumoral heterogeneity, low mutation burden, high invasiveness, and systemic immunosuppression. Adoptive cellular therapy (ACT) is an area of immunotherapy that has been growing in the past few years. A developed modified cell therapy is chimeric antigen receptor (CAR)-T cell therapy, which is when T cells are genetically modified to express CAR.

The CAR has an antigen recognition domain of a specific antibody and allows T cells to activate regardless of the MHC restricted TCR signaling. Modifying the T cells of glioblastoma patients with CAR helps the patients recognize tumor antigens that are overexpressed in glioblastoma. This method is helpful, as it does not affect healthy brain tissue. Some antigens that might be used in this process include interleukin-13 receptor alpha 2 (IL13Rα2), epidermal growth factor receptor variant III (EGFRvIII), human epidermal growth factor receptor 2 (HER2) and erythropoietin-producing hepatocellular carcinoma A2 (EphA2). Usually, treatment consists of 3-6 of these infusions. This method has shown to be safe, as it has not shown off-tumor toxicity or cytokine release syndrome. However, it has been shown to demonstrate a low level of anti-tumor response. Since glioblastomas have a high immunosuppressive environment, it is hard to elicit a high antitumor response. However, engineering CAR-T cells to be multi specific, by engineering bi- and tri- antigen responding CARs, can make them target multiple glioblastoma surface receptors at a time, which can prevent tumor antigen escape (Wang, 2022). CAR-T cell therapy can be beneficial as they can directly kill tumor cells once bound to the receptor. CAR-T cell therapy targets the EGFR antigen. The expression of EGFR is found in over 50% of glioblastomas, making it a viable target. Another promising target is the interleukin-13 receptor α chain variant

2. In glioblastomas, cells express variant 1 of this receptor, which binds to IL-13 to activate JAK-STAT signaling , encouraging the growth of the tumor cell. Human epidermal growth factor receptor 2 (HER2) is also a promising target as it is overexpressed in many patients with glioblastomas. However, T-cell therapy has not proven to be effective in glioblastoma treatment, mainly due to antigen escape. Recurrences in glioblastoma often harbor CD19 negative tumor cells, which researchers have theorized is due to T-cells selectively targeting cells with CD19 mutations, which leads to a population of cells not detected by T-cells (Luksik et al., 2023).

Researchers have tested immune checkpoint inhibitors (ICBs) on tumors with low tumor mutation burden (TMB)s, to see if the results stay the same. Researchers have tested two types of T-cells— [CD4.sup.+] and [CD8.sup.+]—in order to see which type works best to eradicate tumors. Researchers found that rather than using one type of T cell, using both types worked best to target neoantigens and reduce the size of tumors. This is because a combination of [CD4.sup.+] and [CD8.sup.+] generated a modified tumor microenvironment (TME), which created an increased count of T cells that were specific to attacking neoantigens (Dolina et al., 2023). As a whole, CAR-T Cell therapy, and other stem cell treatments, are useful in the sense that they can stay in the body for a significant amount of time, which helps in defending against cancer cells if they re-emerge. However, it has been found that CAR-T cells are not the most efficient in glioblastoma patients, as the T cells cannot activate bystander immune cells.Further research that focuses on targeting the issue of antigen escape can work to make CAR-T Cell therapy a promising option for glioblastoma treatment.

Natural Killer Cells

Adoptive therapy with Natural killer (NK) cells is also a form of immunotherapy that is a unique potential treatment strategy for glioblastomas. NK cells can recognize and eliminate cancer cells without priming or prior stimulation. It has been shown in other types of cancer that NK cells can regress solid cancerous tumors. NK cells have a set of activation receptors that recognize stress-induced ligands on virus-infected and neoplastic cells. Once activated, NK cells can release interferon gamma (IFN-γ), perforin and granzymes, and upregulate death ligandssuch as FAS ligand and tumor necrosis factor–related apoptosis-inducing ligands (TRAIL). NK cells can also trigger apoptosis, through the caspase pathway (Wang, 2022). NK cells are a viable source of glioblastoma therapy as they are able to penetrate across the blood-brain barrier into the tumor microenvironment. NK cells can be altered into chimeric antigen receptor (CAR)-NK cells, dual antigen targeting CAR-NK cells, and adapter CAR-NK cells which can aid in glioblastoma immunotherapy. Some common problems with other types of immunotherapies are an immunosuppressive environment, low immunogenicity, immune heterogeneity, and escape from immune surveillance. NK cells mitigate some of these challenges, due to their MHC-independence. CAR modified NK cells have shown anti-glioma properties. In contrast to CAR-T cells, NK cells have the ability to thrive in allogeneic conditions, which expands the possibility of donors giving their NK cells to modify and transfer into glioblastoma patients. In contrast to CAR-T cells, NK cells can act free from antigen-MHC/TCR reactions, and they do not release the cytotoxic effects observed in CAR-T cells due to cytokine release syndrome. CAR-NK cells can briefly recognize CAR-targeted antigens, and stimulate NK cells, their proliferation, and secretion of cytokines and chemokines. CAR-NK cells can form a lytic gap between themselves and cancer cells to enable guided delivery of lytic granules against cancer cells. CAR-NK cells not only eliminate cancer cells with a CAR-dependent mechanism, but also eliminate cancer cells that do not express CAR antigens. CARs incorporate single-chain variable fragments (FRP5 and scFv) of an antibody as an antigen-binding domain that can identify tumor antigens that are overexpressed or distinct to cancer cells. Glioblastoma patients often overexpress EGFR and EGFRvIII, which are epidermal growth factor receptors. Researchers have added a specific CAR to the surface of NK cells, which has shown to increase glioma cell destruction. The CAR added EGFRvIII specific antibodies as a single-chain variable fragment to increase cytotoxicity to cancer cells (Hosseinalizadeh et al., 2022).

The use of natural killer cells in glioblastomas is a fairly novel idea, and only one major trial (Clincaltrials.gov ID NCT03383978) has been researching its use. At the Johann Wolfgang Goethe University Hospital. Michael Burger and his team have been studying the safety and tolerability of NK-92 /5.28z, as well as the maximum dosage that can be administered . The study has been monitoring doses for intraoperative injections, as well as repetitive injections. The group has been looking for potential signs of anti-tumor activity of NK-92 /5.28z, as well as the prognosis of patients who are administered NK-92 /5.28z along with the drug, Ezabenlimab. The trial currently consists of 42 patients, who had to meet specific criteria in order to be part of this trial. The researchers chose individuals who had recurrent or refractory HER2-positive glioblastoma, who required a relapse surgery or biopsy as part of the treatment process. In this trial, NK-92 /5.28z cells were administered through an intracranial injection. Currently, there are no results posted for this trial, but it plans to culminate soon: this will provide valuable insight on the efficacy of NK cells for glioblastoma treatment.

Challenges to Immunotherapy in Gliomas

Various immunotherapies, such as immune checkpoint inhibition and T cell therapies have been tested in glioblastoma, but trials did not show promising results. Immunotherapy has shown to be effective in extracranial tumors such as melanomas. Immune checkpoint inhibitors block inhibitory receptors and their ligands to elicit an antitumor CD8 T cell response. Trials have researched the efficacy of checkpoint inhibitors such as nivolumab and ipilimumab in glioblastoma treatment. Results showed that neither nivolumab or ipilimumab showed significant overall survival. Tumor vaccines have also been used; they typically utilize peptides, dendritic cells loaded with lysates or gene engineered to express certain antigens. In early phase trials of a peptide vaccine targeting EGFRvIII, it showed that there was evidence for immunogenicity and efficacy in glioblastoma treatment. However, a randomized ACT IV study did not confirm this result. EGFRvIII and other peptide vaccines have shown immune responses in some cases, but they have not shown significant benefits in clinical treatments. These challenges can be explained by various factors, one of them being the low-immunogenicity of glioblastoma. Compared to other immunogenic tumors, such as melanomas, the mutational burden and neoantigen content of glioblastomas are comparatively low. Only a few low expressed glioma neoantigens are made by mutated genes; other non-mutated targets such as Cancer Germline Antigens are expressed, but the levels of expression are low, or highly variable.

More antigens must pass through antigen processing, and presenting machinery, to be recognized by T cells. However, studies have shown that there were no human leukocyte antigen (HLA) mutated peptides in glioblastoma patient serum or tumor tissue. Another trial showed that HLA class I and II molecules are not found in close to 50% of the tissues extracted from glioblastoma patients. Additionally, the glioblastoma microenvironment makes it hard for an effective anti-tumor immune response, which decreases the efficacy of immunotherapy. Tumor associated macrophages are found in over half of glioblastoma microenvironments. These cells have the ability to suppress CD8 T cell activity in glioblastoma due to the expression of IL-4Rα and production of arginase and inducible nitric oxide synthase (iNOS). Regulatory T cells are CD4 T cells that secrete TGF-β and IL-10, which limit the function of CD8 T cells. Glioblastomas attract regulatory T cells through soluble factors, and become activated through tumor associated macrophages. Thus, immunotherapies have not worked well in glioblastomas, due to reasons such as the low-immunogenicity of glioblastoma, the suppression of CD8 T cell activity, and the immunosuppressive microenvironment (Weenink et al., 2020).

However, this does not mean that immunotherapy is not a viable treatment option for any glioblastoma patient. Professor Raul Rabadan at Columbia University has led a study that shows why some patients respond to immunotherapy. Less than 1 out of 10 patients respond to immunotherapy, but there is no way to know who will respond. By inhibiting proteins called PD-1, cancers are able to stop the activity of the immune system. Rabadan and his team administered PD-1 inhibitors–nivolumab and pembrolizumab–in 66 glioblastoma patients. 17 of these patients had a response to the drugs. The researchers discovered that the tumors which did

not respond to the drugs had a mutation in a gene called PTEN, which led to high levels of macrophages, which promote the spread of cancer cells. Patients who responded to the drugs had mutations in the signaling pathway, MAPK, which helps regulate cellular functions (Zhao et al., 2019). By studying the genes of patients, mutations can be found which can lead to ways to predict the efficacy of immunotherapy in a patient. This opens up the avenue of genomics for glioblastoma treatment, especially through genomic analysis.

The Emerging Use of Nanobots in Glioblastoma Treatment

The use of nanorobots in glioblastoma treatment has emerged recently, and they have shown to be a very precise treatment for glioblastomas. Nanobots are described as machines or robots that range from 1-1000 nm, and are made using biomaterials such as lipids, polymers, metals, and crystals. The field of neuromedicine has been striving to create a small, efficient, and cost effective treatment that can cross the BBB and target cancerous cells such as those of glioblastomas. Nanoparticles can be programmed to thermostatic delivery across the BBB, and they can carry out specific tasks due to their sensing, controlled maneuvering, and targeting. Nanorobots can be controlled through a magnetic field using magnetic resonance imaging (MRI) to locate specific areas for delivery in the brain. The magnetic torque directs the movement of nanorobots in a very specific manner, which gives a high level of control. Nanomaterials can be formed in different sizes and physical properties, making it very flexible and feasible to target specific sites depending on the context.

Nanobots can cross the BBB and facilitate the transport of nutrients to endothelial cells. Researchers are leaning towards using lipophilic molecules, as the membrane of the membrane has a high lipophilicity, and lower hydrogen bonding which reduces the rate of diffusion. Nanobots can work through a variety of power sources, including chemical fuels (such as hydrogen peroxide and acid), ultrasound, light, magnetic fields, electric fields, and heat. Swimming nanobots have the ability to convert chemical and physical energy into energy used for their movement, which aids in the delivery of drugs. Swimming nanobots can be autonomously navigated to target the cell membrane, which can help increase the efficiency of drug delivery. When nanobots are being developed, it is important to consider factors such as disruption, strain, fluidity, permeabilization, and repair. It was found that a small contact area leads to a smaller critical condition and strain rate. Researchers have found that nanobots typically have an applied force of 6000 pN, but this force is often insufficient enough to open the cell membrane.

Magnetically controlled swimming nanobots have been filled with green fluorescent protein expression plasmids to approach U87 glioblastoma cells. Due to the fluorescence, it was found that it was internalized into the glioblastoma cell after 24 hours of incubation. However, it was found that the force applied by the nanobot was still not enough to open the cell membrane mechanically, regardless of researchers' effort to reduce the contact area. Nanobots have also been navigated through electric fields, by utilizing two parallel electrodes which control nanowires. When nanowires were coated with the cytokine tumor-necrosis factor-alpha (TNF-α), swimming nanobots were able to translocate nuclear factor-kappaB, which was found through the change in fluorescence. This suggests that TNF-α was delivered through the nanobots (Wang et al., 2020).

Nanoparticles are also being developed to undergo paracellular transport, to create targeted drug therapy. However, researchers are focusing on using nanobots to cross the BBB through transcytosis, which allows for specificity. Additionally, nanobots can target receptor mediated pathways by pushing extracellular vesicles to secrete the drugs carried by the nanobots. By using ligand specific pathways, nanobots are able to specifically target areas that need the drug, which allows for the preservation of other cells in the brain. Nanoparticles have faced the systemic, microenvironmental, and cellular barriers that limit treatment – but nanobots have helped overcome these limits. Nanobots can be used in nanocarrier-mediated combinations, to modify pathways and increase therapeutic effectiveness against certain macromolecules (Wang et al., 2020).

Additionally, nanobots can aid in targeting certain stages of the cell cycle, and overcoming drug resistance. They also work to increase safety by protecting medications from degrading, improving solubility,

extending the plasma half life, and increasing tumor accumulation. The nanocarrier's extravasation in the tumor site occurs due to the physicochemical properties of the tumor's vasculature and lacking lymphatic drainage. Wnt signaling affects apoptosis in a cell, specifically glioblastoma cells, by inhibiting other pathways in the cell. Researchers used Curcumin, nanomicellar curcumin, and nano micellar curcumin along with TMZ, which all showed to decrease the migration of U87 cells, but increase the levels of autophagy biomarkers and apoptosis-related proteins, such as Bcl-2 and caspase 8 (Barzegar et al., 2022). Researchers have looked into using IONPs, which are inorganic nanoparticles used to deliver therapeutic agents to tumor tissues, and its iron oxide core can be useful in imaging. IONPs can work as a contrast agent in MRIs, and the iron oxide core is biodegradable and can be recycled by cells using biochemical pathways for iron metabolism. A study by Keivet et al. used an IONP that provided a T2 contrast in MRI; it also delivered siRNA against apurinic endonuclease 1, an enzyme that helps with base excision repair. Keivet and his team used a nanoparticle with an iron oxide core, which was coated with chitosan, PEG, and polyethyleneimine, which allow the siRNA to not degrade and enter glioblastoma tissues, to decrease the expression of apurinic endonuclease 1, and radiosensitivity in glioblastoma cells (Michael et al., 2018). New discoveries are being made about glioblastomas on a regular basis, from new drugs to new techniques, but nanobots provide a unique role in making these treatments more efficient by helping overcome the challenges of these new treatments on their own (Singh et al., 2021).

Researchers have been working on determining the efficacy of nanobots, and how their mechanisms can be improved. A group of researchers at Virginia Tech have founded a mini optical fiber device which uses electrode-embedded optic fibers to deliver medications, keep track of the efficacy of the tumor, and monitor the growth of tumors, such as glioblastomas. It has been found that the use of antibody delivery with this device along with photodynamic therapy leads to resistance for the growth and formation of tumors. The electrical signals of tumors can be recorded through this device, which allows providers to track the tumor in real time. Additionally, this device can monitor the effects of the antibodies delivered, which can prevent toxicities before they occur. The antibodies delivered through this device can activate T-cells around tumor cells, which can increase the resistance to tumor formation (Murphy, 2021). As of now, few clinical trials have been run to assess the use of nanoparticles (Table 2).

Table 2. Clinical Trial Using NU-0129 with Gold Nanoparticles in Glioblastoma Patients

Number of Participants who Started	8
Number of Participants who Completed	8
Group Description	NU-0129 will be administered inpatient at ~ 0.04mg/kg IV 8-48 hours once prior to scheduled tumor resection. Dose corresponds to 1/50th of the no-observed-adverse-event level.
Average Age	55.5 (range 28-66)
Number of Female Participants	4
Number of Male Participants	4

Description for Testing Safety	To evaluate the safety of intravenous NU-0129 in patients with recurrent GBM or GS, the number of adverse events will be assessed and will be graded according to the NCI's Common Terminology Criteria in Adverse Events (CTCAE) version 4.03 where the grading is as follows: Grade 1: Mild Grade 2: Moderate Grade 3: Severe Grade 4: Life-threatening Grade 5: Fatal
Number of Participants who Experienced Adverse Effects due to NU-01289	4
Description for Testing Blood Concentration After Maximum Concentration is Administered	Blood samples will be collected post-infusion to analyze drug concentration at specific time points after drug administration. Median plasma concentrations of NU-0129 were derived from time profiles for both Seven different small interfering RNA (siRNA) and gold (Au) concentrations, with Au plasma concentration determined by inductively coupled plasma mass spectrometry (ICP-MS) and siRNA concentration assessed by liquid chromatography-high performance liquid chromatography (LC-HPLC) using an atto dye-labeled PNA probe.
Time Frame	At 1, 3, 5, 10, 30, and 60 minutes, and 4, 8, and 24 hours post infusion
Au maximum observed plasma concentration, in ng/ml	4290 (ranges from 3120 to 7140)
Biodistribution of NU-0129 in Tumor Tissue Description	Tissue will be collected during the scheduled surgery and assayed with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to analyze the concentration of particles in various parts of tumor tissue. To analyze spatial distribution of Au within tumor tissue, synchrotron XFM elemental maps of GBM tissue slices were acquired at micron and submicron resolution and matched to adjacent hematoxylin and eosin (H&E)- and Ki67-stained tumor sections. Approximate percentage of gold (Au) found in cancer cells is reported below.
Percentage of gold Au	8.15%
Feasibility of NU-0129 as a Standard Treatment Description	Feasibility will be calculated as the rate of successful production, delivery, and administration of the investigational product and subsequent resection.

Number of patients (out of 8) that had drug infused successfully	8
Number of patients who underwent subsequent resection	8

Table 2: Summary of the main results of a clinical trial (Clinicaltrials.gov ID NCT03020017) evaluating the safety and efficacy of a drug NU-0129. NU-0129 is based on a Spherical Nucleic Acid platform, which consists of nucleic acids on the surface of small spherical gold nanoparticles. NU-0129 is a drug that can cross the blood brain barrier, and is one of the first studies to assess the efficacy of nanoparticles in glioblastoma treatment. The gold nanoparticle cores are filled with surface-passivating polyethylene glycol or polyethylene glycol to improve colloidal stability, and the circulation half-life. The gold was found to be accumulated within perivascular Ki67-positive tumor cells, as well as other tumor samples, which were assessed by XFM-BNP. The results show that the nanoparticle of gold is able to cross the blood brain barrier, and accumulate in the tumor associated endothelium (Kumthekar et al., 2021).

However, one challenge with nanobots is the chemical fuel that is used, such as hydrogen peroxide, is toxic; therefore, the use of nanobots with biocompatible or no fuels, or nanobots that are biodegradable, are being sought after. Additionally, nanobots have faced the issue of biofouling and immune elimination. However, a relatively new option that has been explored is the material of nanodiamonds, as they are less toxic than carbon nanoparticles, and are highly biocompatible, versatile, and can increase the efficacy of drug delivery. There are also biological barriers, such as intravascular flow, the cell membrane, mucus, and vitreous humor as they can impede with the efficacy of treatments administered through nanobots. Additionally, swimming nanobots have shown to be more efficient in drug delivery due to their self navigation and precision. However, the swimming nanobots struggled to mechanically open cell membranes, due to their lack of driving force (Wang et al., 2020). However, nanorobots are useful, as they can pass the membrane of the brain and release molecules into neurons, without disrupting the central nervous system (CNS). Nanorobots have proved to be helpful in tasks such as imaging, and precision surgery. Nanobots still have a long way to go, and researchers are still working to see how they can be more biocompatible, efficient, and require less contact area. The properties of the cell membrane can be utilized to design nanobots to have efficient shapes as well as be made of materials that are permeable through the cell membrane. Nanobots are growing to become an efficient, and cost effective treatment, but their recent emergence calls for further research to make nanobots a viable treatment in glioblastoma patients (Wang et al., 2020). The field of nanorobotics has only recently started developing, but they are a vital tool in glioblastoma treatment as they can sense, make decisions, and have actuation properties.

The Use of Tumor Treating Fields in Glioblastoma Treatment

TMZ has been widely used as the standard treatment option for gliomas, but the long-term survival rates are low. With CNS tumors growing 17.3% from 1990 to 2016, new treatments have emerged. One of these treatments includes tumor treating fields (TTFields), a locoregional antineoplastic treatment modality that utilizes low intensity, intermediate frequency, and alternating electric fields. TTFields are administered through two pairs of transducer arrays, which are placed perpendicular to the scalp. This positioning can be altered to maximize the efficiency of treatment on a case by case basis. TTFields are effective in cancer, as they have selective antimitotic effects on proliferating cells, but have a minimal impact on non-proliferating cells. TTFields affect cells in mitosis, leading to plasma membrane contractions and the formation of plasma membrane blebbing– an irregular bulge in the plasma membrane of a cell caused by the decoupling of the cytoskeleton from the

plasma membrane. TTFields affect cells in metaphase, by affecting the assembly of macromolecules for spindle formation, which causes chromosomal breakage and cell death. TTFields also disrupt the polarity of cell structures in anaphase, telophase, and cytokinesis, leading to apoptosis through p-53 or p-53 independent processes (Figure 1). TTFields, along with maintenance TMZ, have been shown to greatly increase overall survival, compared to TMZ alone . Due to significant survival benefits, TTFields have been approved for adult patients with recurrent glioblastoma as a monotherapy, and a combination therapy along with TMZ for patients with glioblastoma (Rominiyi, 2021).

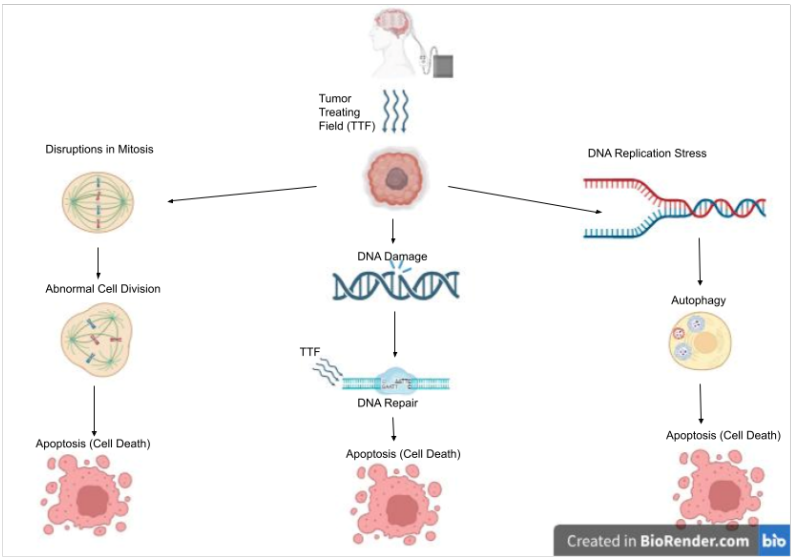

Figure 1.

Figure 1: The mechanism of how TTFields can lead to the death of glioblastoma cells. One way TTFields can affect glioblastoma cells is by disrupting the cell division of rapidly proliferating cancer cells. Tumor treating fields can lead to abnormal cell division in the proliferating cells, which can lead to programmed cell death, also known as apoptosis. Additionally, TTFields can lead to DNA replication stress. This in turn can lead to DNA damage response, which in turn leads to autophagy. Autophagy is the process in which cells try to recycle nutrients, which can lead to apoptosis. Additionally, DNA replication stress and disruptions in mitosis can lead to DNA damage. The DNA will try to repair itself through DNA repair, but TTFields will affect this repair, which will ultimately lead to cell death. Created with BioRender.com.

TTFields have been shown to interfere with DNA fork replications, which can lead to the repositioning of DNA fragments created during replication, which can affect the DNA damage repair, and in turn lead to apoptosis. TTFields may also lead to endoplasmic reticulum stress in the mitotic glioblastoma cells, which can trigger monophosphate-activated protein kinase-dependent autophagy, or cell death. TTFields have also shown to affect cell migration and invasion, which is done by inducing an adhesive cell phenotype, which is created through the dysregulation of cytoskeletal structures and proteins that are related to the epithelial-mesenchymal transition, which reduces the chance of invasion or recurrence. TTFields can also increase the permeability of the plasma membrane in glioblastoma cells by prompting the mislocalization of tight junction proteins, such as Claudin-5 and ZO-1. This increased permeability can help with the uptake of intraoperative agents such as 5-ALA, that are used to decrease the margins of tumors. Also, TTFields can aid in stopping angiogenesis–a mechanism that leads to tumor growth and progression. As mentioned previously, the TTField is beneficial in the aspect that it can be administered at differing frequencies, based on the patient's needs. The electrical in-

tensity determines the extent of cell death. Positioning of TTFields and the intensity of the impulses are individualized to ensure the maximum field intensity at the tumor bed. The frequency of the treatment is about twice a week, and the scalp of the patient is saved to ensure that maximum efficiency is reached (Ghiaseddin et al., 2020).

Table 3. Summary of Novocure EF-14 Clinical Trial

Category	Patients with TTFields + Temozolomide (n=466)	Patients with Only Temozolomide (n=229)
Age (median)	56 (range 19-83)	57 (range 19-80)
Median Karnofsky Performance Score	90 (range 60-100)	90 (range 70-100)
Men	316	157
Women	150	72
Tumor Location: Corpus Callosum	25	12
Tumor Location: Frontal Lobe	190	84
Tumor Location: Occipital Lobe	58	27
Tumor Location: Parietal Lobe	146	89
Tumor Location: Temporal Lobe	191	90
MGMT promoter region methylated	137	77
MGMT promoter region unmethylated	209	95
MGMT promoter region invalid	40	13
EGFR amplified	102	43
EGFR not amplified	147	68
EGFR invalid	3	1
Progression-free survival, primary end point in months	6.7 (range 6.1-8.1)	4.0 (range 3.8-4.4)
Overall Survival in months	20.9 (range 19.3-22.7)	16.0 (range 14-18.4)
Progression-free 6 month survival rate	56%	37%

Table 3: Summary of the main results of a clinical trial (Clinicaltrials.gov ID NCT00916409) done by Novocure to test the efficacy of the TTField technology on newly diagnosed glioblastoma, specifically in older populations. The researchers tested one group which received the Novo-TTF-100A device along with TMZ, and another group which received only TMZ. NovoTTF-100A treatment consisted of wearing 4 electrode arrays on the head. Each participant had to return to the clinic in order to be monitored. Out of all the participants, 54% had undergone gross total resection (over 95% of the tumor was removed), and 13% only had a biopsy taken. TTFields and TMZ were administered based on typical procedures, and the median number of TMZ

cycles until first tumor progression was 6 for TTFields and TMZ, and 5 for the TMZ only group. The results show that TTFields played a significant role in increasing progression-free survival, as well as overall survival in glioblastoma patients (Stupp et al., 2017)

TTFields have shown to have minimal adverse events, and its continuous use has improved the patients' quality of life. As of now, the most widely used system for TTField delivery is Optune (Novocure™), which has four transducer arrays, a field generator, and a power source. Simulation software is used to find the optimal array positioning for each patient. Each array has nine ceramic disks, each with a hydrogel coating to improve the conductivity on the skin. Many patients have reported contact dermatitis or irritation at the site of the arrays due to prolonged exposure to sweat, hydrogel, or the adhesive added. However, these irritations can be easily addressed through OTC treatments such as topical corticosteroids. TTFields have shown to have 0.34 life years gained when administered in addition to TMZ. Data suggests that TTFields may increase 5-year OS from 5% to 13%. The number of patients receiving TTField treatment is increasing, but many more could benefit from it. However, this can be tough due to the geographical distribution of Optune, with the majority of TTField treatment being administered in the United States. TTFields have shown to be highly efficient in terms of survival and the preservation of healthy cells, but it also has its drawbacks. People may be reluctant to use TTFields due to their high cost, making them inaccessible to the typical patient. TTFields can have a monthly cost of approximately $21,000, whereas standard therapies such as chemotherapy range from $1,000 to $12,000. Additionally, patient compliance may be low as many of them might not want to carry around the device. Also, TTFields are newer than many other treatments, and the lack of research and implementation could lead to people wanting to use standard treatments with known and broader results (Rominiyi et al., 2021).

Results and Discussion

Through various clinical studies throughout the years, it has been established that the current course of treatment for glioblastomas is not very efficient. Glioblastomas do not have a cure, but treatments can help mediate the adverse effects of the tumor, as well as expand one's life span. Currently, the common course is surgery jointly administered with chemotherapy–through drugs such as temozolomide– or radiation therapy. However, it has been found across various studies and trials across the years that surgical removal leads to a high tumor resection rate; radiation and chemotherapy have shown to aid in treating the glioblastoma itself, but have shown to have minimal effects on a patient's lifespan and have shown to have adverse side effects. Treatment modalities from other fields of medicine have shown to be hopeful in glioblastoma treatment. CAR-T Cell therapy is a type of immunotherapy which has been explored for glioblastoma treatment. T-Cells can be administered through neoantigen vaccines, which are highly specific and induce a cytotoxic T-Cell attack against cancer antigens in order to fight the cancer. CAR-T cell therapy has been very effective in some cancers and diseases such as leukemia and lymphoma, but it hasn't shown promising results in glioblastoma treatment. It is hard for the T-Cells to cross the blood brain barrier, making it hard at the time for it to be utilized as an efficient treatment modality.

However, one of the most promising avenues of research for glioblastoma treatment is tumor treating fields. TTFields work by placing transducer arrays on a person's scalp to administer electrical fields which affect proliferating glioblastoma cells. TTFields have shown to affect glioblastoma cells while they are in mitosis, and have shown to provoke cell death, and mislocate junction proteins in order to increase the absorption of various drugs. Additionally, TTFields have shown to increase the 5 year survival rate from 5% to 13%. Additionally, TTFields are a promising option as they are administered externally, and are not as invasive as treatments such as surgical removal. However, TTFields are still developing to be more efficient. Due to their recent forthcoming, TTFields cost around $21,000–a very high price, making this treatment unattainable for many patients. However, TTFields are not as invasive as other treatments, and have shown minimal adverse effects; patients typically reported having mild irritation on the administration site, but that was easily resolved

with over the counter medications. TTFields are noninvasive, and have still shown to greatly improve progression free and overall survival rates. Currently, they are mainly administered in the US, but hopefully through further testing, they will become an accessible option to patients across the globe. In addition to TTFields, nanobots have shown to be a promising avenue of research as well. Nanobots are robots sized 1-1000 nm, which can cross the blood brain barrier and target glioblastoma cells. These robots can be programmed and controlled, and can serve functions such as administering drugs to tumor cells, making nanobots a highly specific treatment. Nanobots can be made from a variety of biomaterials, and can be customized to serve specific functions. Nanobots are a highly specific treatment that can be tailored based on a patient's needs. Nanobots also mediate some of the concerns attached with the traditional course of treatment, as they are highly specific and do not affect healthy tissues and cells around the tumor. However, there is a risk of nanobots affecting the blood brain barrier, so researchers have been working to alleviate this issue by experimenting with different sizes, shapes, and materials for nanobots. Treatment through nanobots is fairly new, but it holds a promising future for the treatment of glioblastomas. Some researchers have proposed using emerging treatments such as TTFields and nanobots along with chemotherapy or surgical removal, to increase the efficiency of standard treatment.

Conclusion

Glioblastoma is a devastating cancer that affects more than 300,000 individuals every year. This brain cancer is detrimental, as glioblastomas rapidly proliferate and can infiltrate into other areas of the brain. When one is diagnosed with glioblastoma, they're often guided towards the treatment of chemotherapy, through drugs such as Temozolomide, and surgical removal. However, both these treatments have shown to have adverse effects, and have not shown to be effective in many glioblastoma patients. Glioblastomas are hard to treat, due to their aggressive nature and their location in the brain. Improvements have been attempted through the use of immunotherapy and stem cell therapy in glioblastoma treatment. A developed modified cell therapy is CAR T-Cell therapy, which is when T cells are genetically modified to express CAR. By utilizing the immune system and the cells within it, immunotherapy has been seen as a promising option, but it has its limitations such as a highly immunosuppressive environment. Additionally, the use of immunotherapy such as CAR T-Cell therapy has not been significantly researched in glioblastoma, which opens a new avenue of research for glioblastoma treatment. To mitigate this issue, treatments such as nanobots are used, as they are highly specific and have the ability to cross the blood brain barrier. The use of nanobots has shown to be of great promise, due to their selective traits that allow them to target specific cells in order to carry out tasks such as delivering drugs, while still minimizing the effects on other parts of the brain. Nanobots are still a fairly new field with ongoing development, but with further research and testing, nanobots may prove an effective treatment with great precision that can target glioblastoma cells. Another new therapy for glioblastoma is TTFields, which focuses on using transducer arrays which targets glioblastoma cells and suppresses their proliferation. TTFields are a promising option as they can be customizable in their placement, to ensure efficacy. Additionally, TTFields are noninvasive, as they are administered externally, and do not damage other internal systems. The side effects of TTFields include skin irritation and contact dermatitis, but these effects are minimal in comparison to other glioblastoma treatments. With the diagnoses of glioblastoma rising and survival statistics remaining unchanged, it is important to address the most effective form of treatment that focuses on targeting the glioblastoma while minimizing the effects on surrounding cells and tissues. Hopefully in the future, the mechanisms behind these emerging treatments can improve, along with the costs associated with them, which will broaden the types of treatments available for glioblastoma patients. Nanobots, TTFields, and other emerging technologies could potentially be used together, to create an efficient treatment option which will improve the therapeutic outcome in this deadly disease.

Acknowledgment

I'd like to thank Professor Virgel Torremocha for helping me along the process of writing this paper by providing valuable insight. I'd also like to thank Professor Prahlad Parajuli for helping me understand the concepts of this paper.

References

Asadi, M.H., Khalifeh, K. & Mowla, S.J. OCT4 spliced variants are highly expressed in brain cancer tissues and inhibition of OCT4B1 causes G2/M arrest in brain cancer cells. *J Neurooncol* 130, 455–463 (2016). https://doi.org/10.1007/s11060-016-2255-1

Balañá, C., & Vaz, Á. (2021). *Dose-dense temozolomide for newly diagnosed glioblastoma: A randomized ...*
ClinicalTrials.gov. https://ascopubs.org/doi/10.1200/JCO.2013.49.6968

Baldo, B. A., & Pham, N. H. (2013). Adverse reactions to targeted and non-targeted chemotherapeutic drugs with emphasis on hypersensitivity responses and the invasive metastatic switch. *Cancer metastasis reviews*, *32*(3-4), 723–761. https://doi.org/10.1007/s10555-013-9447-3

Barzegar Behrooz, A., Talaie, Z., & Syahir, A. (2022). Nanotechnology-Based Combinatorial Anti-Glioblastoma Therapies: Moving from Terminal to Treatable. *Pharmaceutics*, *14*(8), 1697. https://doi.org/10.3390/pharmaceutics14081697

Benmelouka, A. Y., Munir, M., Sayed, A., Attia, M. S., Ali, M. M., Negida, A., Alghamdi, B. S., Kamal, M. A., Barreto,
G. E., Ashraf, G. M., Meshref, M., & Bahbah, E. I. (2021). Neural Stem Cell-Based Therapies and Glioblastoma
Management: Current Evidence and Clinical Challenges. *International journal of molecular sciences*, *22*(5), 2258. https://doi.org/10.3390/ijms22052258

Bhere, D., Choi, S. H., van de Donk, P., Hope, D., Gortzak, K., Kunnummal, A., Khalsa, J., Revai Lechtich, E.,
Reinshagen, C., Leon, V., Nissar, N., Bi, W. L., Feng, C., Li, H., Zhang, Y. S., Liang, S. H., Vasdev, N., Essayed, W., Quevedo, P. V., Golby, A., … Shah, K. (2022). Target receptor identification and subsequent treatment of resected brain tumors with encapsulated and engineered allogeneic stem cells. *Nature communications*, *13*(1), 2810. https://doi.org/10.1038/s41467-022-30558-3

Castellano, A., Cirillo, S., Bello, L., Riva, M., & Falini, A. (2017). Functional MRI for Surgery of Gliomas. *Current treatment options in neurology*, *19*(10), 34. https://doi.org/10.1007/s11940-017-0469-y

Chua, J., Nafziger, E., & Leung, D. (2019). Evidence-Based Practice: Temozolomide Beyond Glioblastoma. Current Oncology Reports, 21(4), 1-9.

Datsi, A., & Sorg, R. V. (2021). Dendritic Cell Vaccination of Glioblastoma: Road to Success or Dead End. *Frontiers in immunology*, *12*, 770390. https://doi.org/10.3389/fimmu.2021.770390

Dolina, J. S., Lee, J., Brightman, S. E., McArdle, S., Hall, S. M., Thota, R. R., Zavala, K. S., Lanka, M., Premiai, A. L. R., Greenbaum, J. A., Cohen, E. E. W., Peters, B., & Schoenberger, S. P. (2023). Linked
[CD4.sup.+]/[CD8.sup.+] T cell neoantigen vaccination overcomes immune checkpoint blockade resistance and enables tumor regression. *Journal of Clinical Investigation*, *133*(17), NA.
https://link.gale.com/apps/doc/A763929738/HWRC?u=light025&sid=bookmark

Fukushima, H., Suzuki, R., Yamaki, Y., Hosaka, S., Inaba, M., Muroi, A., Tsurubuchi, T., Morii, W., Noguchi, E., & Takada, H. (2021). Cancer-predisposition genetic analysis in children with brain

tumors treated at a single institution in Japan. *Oncology*, *100*(3), 163–172. https://doi.org/10.1159/000521621

Ghiaseddin, A. P., Shin, D., Melnick, K., & Tran, D. D. (2020). Tumor treating fields in the management of patients with malignant gliomas. *Current Treatment Options in Oncology*, *21*(9). https://doi.org/10.1007/s11864-020-00773-5]

Hilf, N., Kuttruff-Coqui, S., Frenzel, K., Bukur, V., Stevanović, S., Gouttefangeas, C., Platten, M., Tabatabai, G., Dutoit,

V., van der Burg, S. H., Thor Straten, P., Martínez-Ricarte, F., Ponsati, B., Okada, H., Lassen, U., Admon, A., Ottensmeier, C. H., Ulges, A., Kreiter, S., von Deimling, A., … Wick, W. (2019). Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature*, *565*(7738), 240–245. https://doi.org/10.1038/s41586-018-0810-y

Hosseinalizadeh, H., Habibi Roudkenar, M., Mohammadi Roushandeh, A., Kuwahara, Y., Tomita, K., & Sato, T. (2022). Natural killer cell immunotherapy in glioblastoma. *Discover. Oncology*, *13*(1), 113. https://doi.org/10.1007/s12672-022-00567-1

Kamson, D. O., & Grossman, S. A. (2021). The role of temozolomide in patients with newly diagnosed wild-type *idh*, unmethylated MGMT*p* glioblastoma during the COVID-19 pandemic. *JAMA Oncology*, *7*(5), 675. https://doi.org/10.1001/jamaoncol.2020.6732

Kanderi, T., & Gupta, V. (2022). *Glioblastoma multiforme - statpearls - NCBI bookshelf*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK558954/

Karachi, A., Dastmalchi, F., Mitchell, D. A., & Rahman, M. (2018). Temozolomide for immunomodulation in the

treatment of glioblastoma. *Neuro-oncology*, *20*(12), 1566–1572. https://doi.org/10.1093/neuonc/noy072

Khan, M., Li, X., Yan, M., Li, Z., Yang, H., & Liao, G. (2021). Efficacy and Safety of Actively Personalized Neoantigen Vaccination in the Management of Newly Diagnosed Glioblastoma: A Systematic Review. *International journal of general medicine*, *14*, 5209–5220. https://doi.org/10.2147/IJGM.S323576

Kumthekar, P., Ko, C. H., Paunesku, T., Dixit, K., Sonabend, A. M., Bloch, O., Tate, M., Schwartz, M., Zuckerman, L.,

Lezon, R., Lukas, R. V., Jovanovic, B., McCortney, K., Colman, H., Chen, S., Lai, B., Antipova, O., Deng, J., Li,

L., Tommasini-Ghelfi, S., … Stegh, A. H. (2021). A first-in-human phase 0 clinical study of RNA interference-based spherical nucleic acids in patients with recurrent glioblastoma. *Science translational medicine*, *13*(584), eabb3945. https://doi.org/10.1126/scitranslmed.abb3945

Lathia, J. D., Mack, S. C., Mulkearns-Hubert, E. E., Valentim, C. L., & Rich, J. N. (2015). Cancer stem cells in glioblastoma. *Genes & development*, *29*(12), 1203–1217. https://doi.org/10.1101/gad.261982.115

Liau LM, Black KL, Prins RM, Sykes SN, DiPatre PL, Cloughesy TF, Becker DP, Bronstein JM. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. J Neurosurg. 1999 Jun;90(6):1115-24. doi: 10.3171/jns.1999.90.6.1115. PMID: 10350260.

Liau LM, Black KL, Martin NA, Sykes SN, Bronstein JM, Jouben-Steele L, et al. Treatment of a Patient by Vaccination With Autologous Dendritic Cells Pulsed With Allogeneic Major Histocompatibility Complex Class I-Matched

Tumor Peptides. *Case Rep Neurosurg Focus* (2000) 9:e8. doi: 10.3171/foc.2000.9.6.9

Liu, A., Hou, C., Chen, H., Zong, X., & Zong, P. (2016). Genetics and Epigenetics of Glioblastoma: Applications and Overall Incidence of IDH1 Mutation. *Frontiers in oncology*, *6*, 16. https://doi.org/10.3389/fonc.2016.00016

Luksik, A. S., Yazigi, E., Shah, P., & Jackson, C. M. (2023). CAR T Cell Therapy in Glioblastoma: Overcoming

Challenges Related to Antigen Expression. *Cancers*, *15*(5), 1414. https://doi.org/10.3390/cancers15051414

Mann, J., Ramakrishna, R., Magge, R., & Wernicke, A. G. (2018). Advances in Radiotherapy for Glioblastoma. *Frontiers in neurology*, *8*, 748. https://doi.org/10.3389/fneur.2017.00748

Mayo Foundation for Medical Education and Research. (2022, October 25). *Brain metastases*. Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/brain-metastases/symptoms-causes/syc-20350136#:~:text=Brain %20 metastases%20 may%20from%20one,changes%2C%20memory%20 loss%20 and%20 seizures.

Michael, J. S., Lee, B. S., Zhang, M., & Yu, J. S. (2018). Nanotechnology for Treatment of Glioblastoma Multiforme.

Journal of translational internal medicine, *6*(3), 128–133. https://doi.org/10.2478/jtim-2018-0025

Murphy, J. (2021, November 24). *Mini Optical Fiber Device may boost cancer treatment success*. Laser Focus World. https://www.laserfocusworld.com/fiberoptics/article/14214166/mini-optical-fiber-device-may-boost-can cer-treatment-success

Olar, A., & Aldape, K. D. (2014). Using the molecular classification of glioblastoma to inform personalized treatment. *The Journal of pathology*, *232*(2), 165–177. https://doi.org/10.1002/path.4282

Rominiyi, O., Vanderlinden, A., Clenton, S. J., Bridgewater, C., Al-Tamimi, Y., & Collis, S. J. (2021). Tumour treating fields therapy for glioblastoma: current advances and future directions. *British journal of cancer*, *124*(4), 697–709. https://doi.org/10.1038/s41416-020-01136-5

Sadik, Z. H. A., Hanssens, P. E. J., Verheul, J. B., Beute, G. N., Te Lie, S., Leenstra, S., & Ardon, H. (2018). Gamma knife radiosurgery for recurrent gliomas. *Journal of neuro-oncology*, *140*(3), 615–622. https://doi.org/10.1007/s11060-018-2988-0

Sales, A. H. A., Beck, J., Schnell, O., Fung, C., Meyer, B., & Gempt, J. (2022). Surgical Treatment of Glioblastoma: State-of-the-Art and Future Trends. *Journal of clinical medicine*, *11*(18), 5354. https://doi.org/10.3390/jcm11185354

Scheck, A. C., Perry, K., Hank, N. C., & Clark, W. D. (2006). Anticancer activity of extracts derived from the mature roots of scutellaria baicalensis on human malignant brain tumor cells. *BMC Complementary and Alternative Medicine*,

6(1). https://doi.org/10.1186/1472-6882-6-27 https://link.gale.com/apps/doc/A748826985/HWRC?u=light025&sid=bookmark HWRC&xid=278c083f\

Segura-Collar, B., Hiller-Vallina, S., de Dios, O., Caamaéo-Moreno, M., Mondejar-Ruescas, L., Sepulveda-Sanchez, J. M., & Gargini, R. (2023). Advanced immunotherapies for glioblastoma: tumor neoantigen vaccines in combination with immunomodulators. *Acta Neuropathologica Communications*, *11*(1), NA.

Singh, A. V., Chandrasekar, V., Janapareddy, P., Mathews, D. E., Laux, P., Luch, A., Yang, Y., Garcia-Canibano, B., Balakrishnan, S., Abinahed, J., Al Ansari, A., & Dakua, S. P. (2021). Emerging application of nanorobotics and artificial intelligence to cross the BBB: Advances in design, controlled maneuvering, and targeting of the barriers. *ACS Chemical Neuroscience*, *12*(11), 1835–1853. https://doi.org/10.1021/acschemneuro.1c00087

Stensjøen, A. L., Solheim, O., Kvistad, K. A., Håberg, A. K., Salvesen, Ø., & Berntsen, E. M. (2015). Growth dynamics of untreated glioblastomas in vivo. *Neuro-oncology*, *17*(10), 1402–1411. https://doi.org/10.1093/neuonc/nov029

Stupp, R., Taillibert, S., Kanner, A., Read, W., Steinberg, D., Lhermitte, B., Toms, S., Idbaih, A., Ahluwalia, M. S., Fink,

K., Di Meco, F., Lieberman, F., Zhu, J. J., Stragliotto, G., Tran, D., Brem, S., Hottinger, A., Kirson, E. D.,
Lavy-Shahaf, G., Weinberg, U., … Ram, Z. (2017). Effect of Tumor-Treating Fields Plus Maintenance
Temozolomide vs Maintenance Temozolomide Alone on Survival in Patients With Glioblastoma: A Randomized Clinical Trial. *JAMA*, *318*(23), 2306–2316. https://doi.org/10.1001/jama.2017.18718

Testa, U., Castelli, G., & Pelosi, E. (2018). Genetic Abnormalities, Clonal Evolution, and Cancer Stem Cells of Brain Tumors. *Medical Sciences*, *6*(4), 85. MDPI AG. Retrieved from http://dx.doi.org/10.3390/medsci6040085

Wang, L., Liang, B., Li, Y. I., Liu, X., Huang, J., & Li, Y. M. (2019). What is the advance of extent of resection in glioblastoma surgical treatment-a systematic review. *Chinese neurosurgical journal*, *5*, 2. https://doi.org/10.1186/s41016-018-0150

Wang, G., & Wang, W. (2022). Advanced Cell Therapies for Glioblastoma. *Frontiers in immunology*, *13*, 904133. https://doi.org/10.3389/fimmu.2022.904133

Wang, W., Wu, Z., & He, Q. (2020). Swimming nanorobots for opening a cell membrane mechanically. *VIEW*, *1*(3). https://doi.org/10.1002/viw.20200005

Weenink, B., French, P. J., Sillevis Smitt, P. A. E., Debets, R., & Geurts, M. (2020). Immunotherapy in Glioblastoma:
Current Shortcomings and Future Perspectives. *Cancers*, *12*(3), 751. https://doi.org/10.3390/cancers12030751

Xu, X., Li, L., Luo, L., Shu, L., Si, X., Chen, Z., Xia, W., Huang, J., Liu, Y., Shao, A., & Ke, Y. (2021). Opportunities and challenges of glioma organoids. *Cell communication and signaling : CCS*, *19*(1), 102. https://doi.org/10.1186/s12964-021-00777-0

Zhao, J., Chen, A. X., Gartrell, R. D., Silverman, A. M., Aparicio, L., Chu, T., Bordbar, D., Shan, D., Samanamud, J., Mahajan, A., Filip, I., Orenbuch, R., Goetz, M., Yamaguchi, J. T., Cloney, M., Horbinski, C., Lukas, R. V., Raizer, J., Rae, A. I., Yuan, J., … Rabadan, R. (2019). Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma. *Nature medicine*, *25*(3), 462–469. https://doi.org/10.1038/s41591-019-0349-y