The applications, limitations, and future consideration of immune checkpoint inhibitors in glioblastoma

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Abstract. Glioblastoma multiforme is a grade IV malignant tumor found in the brain, formed by mutated astrocytes. Patients with glioblastoma may experience headaches and nausea, and several different symptoms depending on where the tumor is located. Surgery is the primary treatment used for glioblastoma to remove the tumor, but the diffusive nature of this tumor makes it hard to completely remove. The survival years after treatments had not shown improvement with surgeries along with chemo and radiotherapy. Immune checkpoint inhibitors, a type of immunotherapy, were seen to provide a foreground to the development of an effective treatment. Immune checkpoint inhibitors block pathways that create suppression of T cells to restore their function and allow them to attack cancerous cells again, stimulating an immune response. Even though immune checkpoint inhibitors had shown effectiveness and success in preclinical trials and in treating other cancers, clinical trials using these drugs failed to show improved effectiveness and prognosis of patients with immune checkpoint inhibitors. Some patients didn't generate a response to the checkpoint inhibitors, while for others the effect of checkpoint inhibitors lasted relatively shortly. Though, several proposals for solutions to the limitations of checkpoint inhibitors were raised by scientists. Immune checkpoint inhibitor shows the potential of being a hope to enhance survival years and prognosis of this currently uncurable disease. The research targets at discussing the mechanisms of ipilimumab, nivolumab, and bevacizumab (types of immune checkpoint inhibitors) and their limitations to provide suggestions of different methods that could be applied to potentially solve the problem and enhance their effectiveness.

Keywords: Glioblastoma; immune checkpoint inhibitors; limitations; effectiveness.

1. Introduction

Glioblastoma multiforme (also known as GBM) is a primary brain malignancy of the central nervous system, commonly found under the cortex in several parts of the supratentorial cerebral hemisphere [1]. It spreads to other parts of the brain such as lobes, commonly the frontal lobe, but generally stays within the brain. Glioblastoma is considered the most aggressive and malignant brain tumor, rated as a grade IV (most aggressive) astrocytoma [1]. Glioblastoma happens when astrocytes, a cell that controls chemical and electrical cell communication, undergoes mutation and starts replicating. It is the one of the most commonly found primary brain tumor in adults. Depending on where the tumor grows, different symptoms occur. For instance, trouble with vision may occur when the tumor grows in the occipital lobe. Behavioral and emotional changes may occur when the tumor grows in the frontal lobe. Though, other symptoms such as headache and nausea are caused by increasing brain pressure as the tumor continuous to grow and spread, taking up places inside the brain.

The immune system targets foreign cells such as germs and eliminates them. Cancerous cells, however, are derived from normal cells which then experience mutation. The immune system doesn't see cancerous cells differently enough from normal cells. Therefore, the ability of the immune system to fight cancer diminishes. Cancerous cells also release certain substances that prevent the immune system to target them. Immunotherapy is a type of therapy that can be used to fight cancer by regulating how the immune system functions. It helps to target and attack cancer cells. Immunotherapy aims at enhancing the existing functions in the immune system either through stimulating the immune system using several methods. Immunotherapy restores and boosts the natural defense of the immune system so the body could fight cancer. The application of immunotherapy is

assessed on the type and stage of cancer. Immunotherapy can be given through oral (pills and capsules), infusion (intravenous therapy that goes into the patient's veins), etc. [2]. Immunotherapy can be divided into different branches; the immune system modulator is one of them. An example of an immune system modulator is the immune checkpoint inhibitor, which helps boost overall immune system performance. These immune checkpoint inhibitors had proven successful in other types of cancer such as bladder cancer, non-small cell lung cancer, and melanoma [3]. Therefore, they were seen as a potential therapeutic strategy to treat glioblastoma. Many immune checkpoint inhibitors had been developed and applied to clinical trials. These include ipilimumab which targets CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) and PD-1 (Programmed cell death receptor 1), nivolumab which targets anti PD-1 receptor, and bevacizumab which targets VEFG-A (vascular endothelial growth factor A), all aiming to allow T cells to attack cancerous cells thus treating the disease. Several treatments seemed promising in early-stages of development and preclinical trials but failed to prove their effectiveness when moved to phase 3 clinical trials [4].

Patients receiving immune checkpoint inhibitors may experience certain adverse effects. Immune checkpoint inhibitors allow T cells to attack cancerous cells, but instead, they might also attack healthy cells in the body. These results in skin, gastrointestinal tract, liver, and endocrine system toxicities. Skin toxicities are the most commonly found toxicity, including rash, alopecia, and itching. Diarrhea and colitis are reported to be more commonly found in patients using CTLA-4 immune checkpoint inhibitors. Inflammation in the liver is caused by elevated levels of liver enzymes resulting from immune checkpoint inhibitors. Approximately 80% of the patients receiving immune checkpoint inhibitors experience these adverse effects [5]. The overall survival (OS) time of glioblastoma patients remains relatively low accompanied by a recurrence of the disease. This phenomenon can be due to the resistance of cells to immune checkpoint inhibitors. The resistance includes both primary and acquired resistance. Where primary refers to those who don't respond to the drug when first used, acquired resistance refers to those that had a successful treatment using immune checkpoint inhibitors but no longer work after some time. Resistance may happen due to several factors: genomic factors, immune system cells, tumor heterogeneity, cancer microenvironment, and cancerous cell interactions.

The investigation into possible enhanced treatments including combinational treatments between checkpoint inhibitors and combination therapy of checkpoint inhibitors with other treatments such as chemotherapy and radiation is important to the development of glioblastoma therapy, seeking chances to improve long-term survival rate and morbidity. This research compared and contrasted the mechanism and problems of three immune checkpoint inhibitors (ipilimumab, nivolumab, bevacizumab) and discussed about its limitations and the foreground of immune checkpoint inhibitors, including possible new methods of therapy that would enhance the effect. This research was completed through systematic review of published literature and the latest clinical studies.

2. Immune checkpoint inhibitors as treatment

2.1. History of the development of treatment

Glioblastoma is an infiltrative tumor with rapid cell division, which often spreads to different parts of the brain. Growth factors are produced which stimulate the growth of blood vessels and thus stimulate the growth of the tumor. Treatment for glioblastoma multiforme focuses primarily on diminishing the size of the tumor to minimize symptoms. Surgeries were being performed to remove the tumor. Though, it is challenging to remove the tumor without damaging other tissues in the brain as the tumor spreads in a mesh-like structure [6]. Due to its diffusive nature, surgeons are unable to completely take off the tumor. Tumor cells could spread in different regions of the brain that is crucial to survival over long distances [6]. The glioma cells metastasize away from the main tumor migrating through parenchyma and both grey and white matter tracks, encompassing vessels and neurons [7]. Surgeries are performed to remove as much of the tumor as possible, though complete elimination is unrealistic. Patients usually require other treatments after surgery.

Microscopic infiltrating tumor cells, also known as micrometastasis, are cancer cells that can't be seen clearly on an imaging test. Therefore, these microscopic infiltrating tumor cells escape from the main tumor, and even after treatment, these cancerous cells may be metastasized to originally healthy brain tissues thus causing reformation of tumor [8]. Radiotherapy aims to kill cancerous cells and diminish tumor size through using radiation. Under high doses of radiation, the DNA of cancerous cells is damaged to an extent that it cannot be repaired. With DNA being damaged, the cells won't replicate and eventually will die. Often used together with radiotherapy, chemotherapy is a therapy that requires the patients to take drugs that kills cancerous cells. These drugs target cells at the stage where they divide into two cells. Though, chemo drugs can't distinguish between normal and cancerous cells, which may cause damage to normal cells also [9].

The prognosis of glioblastoma is poor. Only a few of the patient population can survive up to 2.5 years post-operative, and merely 5% can survive up to 5 years [10]. Even with the combination of radio and chemotherapy, the OS isn't greatly improved. Therefore, scientists are seeking new treatments and due to the success of immune checkpoint inhibitors in other types of cancer, they are considered as another potential treatment for glioblastoma.

2.2. Immune checkpoint inhibitors

Elimination of cancer cells relies on the state of balance between activation and inhibition. Cancerous cells may produce high levels of protein that disallow the attack of T cells. Proteins on T cells called immune checkpoint proteins identify and bind to partner cells, in this case, tumor cells, which signals the T cells to prevent destruction of cancerous cells. Immune checkpoint inhibitors are used to regulate immune response. They bind with the immune checkpoint protein on T cells to prevent it from binding to tumor cells. This signals the T cell to attack the cancerous cells. Some of the most effective immune checkpoint inhibitors for the treatment of cancer target PD-1 and CTLA-4, both of which exist on T cells and have a synergistic inhibitory effect, playing a negative regulatory role in preventing over activation of the immune response [11].

Immunostimulatory factors and immunosuppressive factors plays important role in the balance of the complex immune network. Often, immunosuppressive factors may surpass immunostimulatory factors by regulation the pathway between cytokines and immune cells. Exhaustion occurs to the immunostimulatory factors, such as CD4+ T cells, CD8+ T cells, M1-TAMs, because they are being suppressed by immunosuppressive cells such as T regulatory cells, myeloid cells, M2-TAMs [12]. Cytokines released by these immunosuppressive cells (IL-6, IL-10, VEGF) causes further suppression to the immunostimulatory cells. Tumor cells also may recruit T regulatory cells by releasing suppressive cytokines and through reduced MHCs and costimulatory molecules [12].

2.3. Types of Immune checkpoint inhibitors in application

2.3.1 Ipilimumab

Ipilimumab is a monoclonal antibody against CTLA-4. CTLA-4 is a regulatory checkpoint protein in the priming stage of immune activation, where initiation and activation of naïve T cells happen in the secondary lymphoid organs [13]. CTLA-4 interferes with a costimulatory signal. Typically, the CD28 in the surface of T cells binds with ligand B7 on APCs, eventually secreting IL-2 [13]. CTLA-4 is also on the surface of T cells, and it shares ligand B7 with CD28. CTLA-4 has a higher affinity for B7, so it outcompetes CD28 and binds with B7, thus inhibiting activation and reducing the immune response. Ipilimumab prevents CD80 and CD86 (subtypes of B7) from binding with CTLA-4 by blocking the pathway of signaling. This then activates T cells and allows for T cell-mediated immunity, in which cancerous cells could be attacked. Ipilimumab has shown success in treating malignant melanoma and brain metastases with extended OS time. Prolonged OS time, reduced tumor size, and effective T cell activated response of blockage of CTLA-4 are shown in mice models in preclinical trials [13]. CTLA-4 successfully restored CD4+ T cell proliferative capacity and increased the population of CD4+ T cells and CD25- cells.

Adverse effects of ipilimumab are generated due to the excess activation of T cells. The gastrointestinal and integumentary systems are the most commonly affected ones [14], resulting in diarrhea, colitis, etc. These complications can generally be solved through the application corticosteroids. The limited effectiveness of ipilimumab can be associated with the expression of CTLA-4 in the patient's body.

2.3.2 Nivolumab

Nivolumab is an IgG4 anti monoclonal antibody that targets PD-1. PD-1 exists in T cells, B cells, dendritic cells, natural killer cells, and T regulatory cells, and plays an important role in the effector stage [13]. PD-1 sends signals to decrease cytokine production and proliferation, which eventually may lead to the exhaustion of T cells. PD-1 is expressed on TILs (tumor infiltrating lymphocytes), and depending on which TIL, the outcome is different. If PD-1 is being expressed on CD4+ TIL, which closely correlates with T regulatory cells, then it reflects that the TEM (tumor microenvironment) is immunosuppressive; If PD-1 is being expressed on CD8+ TILs, then T cell exhaustion will be observed. Both lead to a decrease in the capability of phagocytic ability against the tumor [15]. Usually, the PD-1/PD-L1 pathway prevents inflammation and autoimmune disease, maintaining peripheral immune tolerance. However, in the case of cancer, PD-1/PD-L1 inhibits TIL activation, perforin production, and inflammatory cytokine production [15]. Nivolumab prevents the binding of PD-1 to its ligand PD-L1. Nivolumab had shown success in treating metastatic melanoma, non-small cell lung cancer, and other malignancies.

The effectiveness of Nivolumab is doubted. Checkmate 143, a phase 3 clinical trial, showed that compared to bevacizumab, rGB patients receiving chemotherapy and radiation did not improve their OS after receiving nivolumab treatment [4]. In the tumor microenvironment, nivolumab did show maintenance of the T cell population, however, T cell discrepancies are absent.

The reason that the OS time of the patients treated with Nivolumab doesn't show improvement might be associated with the low expression levels of PD-1 in the patient's body. The problem that emerged here is that doctors have to find ways to predict the checkpoint inhibitor response of the patients.

Nivolumab shares similar negative effects as other immune checkpoint inhibitors. Patients treated with Nivolumab may demonstrate adverse effects such as pneumonitis (lung inflammation), skin rash, and other autoimmune disorders. Though most of the side effects are manageable, severe conditions do happen which require close medical attention.

2.3.3 Bevacizumab

Bevacizumab is a humanized recombinant IgG1 monoclonal antibody against VEGF-A. VEGF-A plays a critical role in angiogenesis which induces the growth of blood vessels. VEGF-A is secreted by cancer cells, and it binds with its receptors, usually VEGFR-2 (vascular endothelial growth factor receptor-2) on endothelial cells that are responsible for lining blood and lymph vessels [16]. When VEGF-A binds to VEGFR-2, it activates the intracellular signaling pathway and thus leads to angiogenesis, causing the growth and metastasis of cancerous cells [17]. VEGF-A also contributes to the inhibition of apoptosis of endothelial cells so that it creates an environment for the newly formed blood vessel network [16]. Similar to other immune checkpoint inhibitors, bevacizumab treats the disease by producing blockers that block the binding of VEGF-A to VEGFR-2, thereby reducing the growth of tumor blood vessels.

Clinical trials examining the effect of inhibition of the VEGF protein showed relatively disappointing results in its long-term effectiveness and general OS years [18]. A population of patients in clinical trials doesn't show a response to the drug. Scientists suggest that the long-term effectiveness of using Bevacizumab might be limited as resistance may develop. Alongside, bevacizumab can also cause adverse effects including high blood pressure, blood clots, increased risk of bleeding (especially when the patient may already experience vascular damage), and gastrointestinal perforation [17].

3. Limitations

Glioblastoma is a malignancy in the brain. For the immune checkpoint inhibitors to reach the site of the tumor, they must cross the blood-brain barrier (BBB). The BBB is a specialized structure of a diffusive barrier that consists of endothelial cells, astrocytes, and pericytes [18]. The tight junctions between endothelial cells create the barrier, which keeps most compounds and substances from inflowing into the brain from outside. It separates the circulating blood from the brain and the rest of the central nervous system [18]. The barrier is designed to prevent harmful substances and molecules from entering the brain by limiting the entrance of large molecules and drugs. This makes immune checkpoint inhibitors, and also immune cells unable to cross the BBB. Additionally, the cells that immune checkpoint targets, such as T cells, aren't present in the BBB, thus making it difficult to penetrate through the barrier. The BBB can explain why a patient in clinical trials shows relative ineffectiveness. Ipilimumab, nivolumab, and bevacizumab have a high molecular weight, meaning that they can't passively cross the BBB. The presence of efflux transporters may also pump out the drugs [19].

The tumor microenvironment created by the cancer cells creates difficulties for the immune checkpoint inhibitors to perform their mechanisms. The presence of immunosuppressive cells such as T regulatory cells and MDSCs recruited by the tumor inhibits the functions of immune cells that are responsible for attacking the cancer cells [20]. Cytokines and chemokines are also released by the tumor to recruit certain substances such as TGF-beta (transforming growth factor-beta) that inhibit the function of T cells [20]. This creates an environment where substances that suppress cytotoxic T cells are being constantly produced and those that stimulate T cell activation is being inhibited. This makes immune checkpoint inhibitor therapy difficult to target and activate T cells, therefore diminishing its effectiveness

Tumor heterogeneity refers to the diversity of genetic and phenotypic characteristics present in a single tumor where there are different clones. Metastasis heterogeneity is also part of tumor heterogeneity where the metastasized secondary tumor may express different genetic alterations [21]. The different genetic variants in the tumor results in the response in certain parts but inaction in the rest. Scientists also found out that genetic variants in the tumor may evolve, so that the tumor condition is never constant [21]. When the genetic variants in the tumor undergo evolution, the amount of certain immune checkpoint proteins will be expressed in fewer amounts for instance in the metastasized tumor, thus when immune checkpoint inhibitors are used, the tumor becomes insensitive to immune checkpoint inhibitor therapy.

Glioblastoma is a highly aggressive tumor that develops resistance to drugs and an immune checkpoint inhibitor. Upregulation of the MDR1 (multidrug resistance 1) gene which specifically codes for P-glycoprotein may cause resistance to the immune checkpoint inhibitors. P-glycoprotein is responsible for transporting certain drugs out of the brain [22]. When P-glycoprotein is upregulated, it prevents ATP hydrolysis, disrupts the lipid membrane, and blocks the binding site of the substances so that immune checkpoint inhibitors can't act upon its target [22]. Cancer cells in glioblastoma may also develop upregulation of alternative immune checkpoint proteins or pathways. For instance, when the PD-1/PD-L1 pathway is targeted by checkpoint inhibitors, then a new pathway TIM-3 pathway may be upregulated to maintain tumor growth.

4. Future developments

One of the problems that arose in clinical trials of nivolumab and are applicable to other immune checkpoint inhibitors is the lack of response from the patients because the of low expression levels of the immune checkpoint protein. To make treatments more effective, predictive biomarkers can be used to identify which patients would most likely benefit more from using the treatment. Glioblastoma can be divided into IDH (isocitrate dehydrogenase)-mutant and IDH-wildtype [23]. Mutations in IDH are detected in glioblastoma. Scientists have found out that people with the IDH-mutant are more likely to respond to checkpoint inhibitors than those with IDH-wildtype [23]. The

mutational status of IDH gene can be used as a biomarker that tells the doctor the IDH status of the patients to identify between IDH-wildtype and IDH-mutant. In the clinical trials performed for nivolumab, some patients remain unresponsive to immune checkpoint inhibitors. Through trials performed for melanoma, sensitivity or responsiveness to treatments can be determined by looking at the proportion of PD-1/PD-L1 and density of TILs. Based on these markers, tumors can be divided into different types. Type 1 tumors expressing TLs+, and PD-L1+ are more likely to be responsive to immune checkpoint inhibitors. Type 2 tumors expressing TLs-, PD-L1- are more likely to be unresponsive to the therapy [20]. Type 3 tumors expressing TLs-, PD-L1+ demonstrate intrinsic induction and patients with this type of tumor are generally insensitive when immune checkpoint inhibitors are used alone [20]. Although this is for melanoma, it still provides a foreground to application in glioblastoma. Certain biomarkers allow doctors to assess the risk of immune-related adverse events (irAE) in advance so that severe complications can get treated in the early stages [24]. When adverse events happen, it is usually treated with glucocorticoids.

Immune checkpoint inhibitors might not be effective when used alone, but combinations may help enhance the effectiveness of immune checkpoint inhibitors. First, combinations between immune checkpoint inhibitors allow for different pathways to be targeted, thus leading to the maximization of the anti-tumor effect. Ipilimumab targets CTLA-4, whereas nivolumab targets the PD-1/PD-L1 pathway. The two immune checkpoint inhibitors target different pathways and cells, which creates synergistic effects on the tumor microenvironment. By applying both immune checkpoint inhibitors, the two microenvironments will all be targeted which enhances the attacking of cancer cells. Preclinical trials on mouse models showed efficacy when ipilimumab and anti-PD-1 therapy and anti-CTLA-4 therapy are applied. In clinical trials conducted on melanoma patients, approximately 60% of the patients experienced reactions and OS increased by 3 years [15]. Nevertheless, these patients do experience serious adverse events [15]. A combination of bevacizumab and other immune checkpoint inhibitors also demonstrates additive effects. Bevacizumab may regulate the tumor microenvironment by enhancing the activity of immune cells. This creates a better microenvironment for the other immune checkpoint inhibitors to perform their mechanisms. The combination between vaccines and anti-PD-1/PD-L1 drugs also shows improvement in their effectiveness. Vaccines could induce upregulation of PD-1 expression [25]. This creates an additive effect when anti-PD-1 drugs are used as their effectiveness can be fully applied. Despite combination therapies between drugs, immune checkpoint inhibitors are used with radio and chemotherapy. Radio and chemotherapy may help turn cold tumors into hot tumors. Hot tumors have a high mutational load, meaning that these tumors produce neoantigens that make the tumor more inclined to immune responses [26]. This, therefore, makes the immune checkpoint inhibitors more efficacious when applied because of the increased tendency of blocking the pathways.

The BBB doesn't allow large molecules, like the immune checkpoint inhibitors, to cross through. Though, smaller molecules, like nanoparticles, can penetrate through the BBB and reach into the brain to target the tumor. Disruption of tight junctions was being put forth to allow the drugs to cross the BBB; however, this resulted in the damage of the barrier which might instead allow the inflow of many unwanted toxins and substances [27]. Nanoparticles are small enough to cross the barrier, and their size and shape can be modified to fit specific receptors that facilitate their transport across the BBB [28]. Nanoparticles can be used for drug delivery to help target the immune checkpoint inhibitors to their destination, which increases concentration at the tumor site. The amyloidogenic proteins absorbed on the surface of nanoparticles can regulate the association and dissociation of the proteins, which contributes to target efficiency [27]. Neurodegenerative diseases might be a problem using nanoparticles as oligomers, which cause neurodegenerative disease, might be formed [27]. Though, there still are nanoparticles that don't induce neurodegenerative disease present and can be applied.

5. Conclusion

Glioblastoma multiforme is the most common primary brain tumor that has a poor prognosis and OS in adults. It is an incurable disease where astrocytes in the brain replicate uncontrollably, which may metastasize to other destinations and cause several complications. As traditional methods of treatment including surgery, chemo, and radiotherapy, don't show prolonged OS years and improvements in control of the disease post-treatment, immune checkpoint inhibitors are being tested in preclinical and clinical trials as a prospective more effective therapy. Though, the results of the research showed that immune checkpoint inhibitors didn't meet its anticipation. Patients using ipilimumab, nivolumab, and bevacizumab showed certain adverse events, development of resistance to the drugs, and unresponsiveness to checkpoint inhibitors. These are due to many factors and the complexity of the environment of the brain. Immune checkpoint inhibitors have to cross the BBB and overcome the resistance. Doctors also have to determine which patients will show a response to the immune checkpoint inhibitors to find adequate treatment. Possible solutions to these limitations could be the application of nanoparticles to cross the BBB, combination therapy to provide different pathways to target the tumor, and biomarkers to recognize how sensitive the patients are towards the therapy. Through the applications of these methods, the effectiveness of immune checkpoint inhibitors can be enhanced. Even though a couple of limitations and problems are still present with using immune checkpoint inhibitors for patients with glioblastoma, its future remains optimistic as certain solutions are being proposed and put into trial to see clinical reactions. These proposed methods of treatment or drugs have shown success in other cancers sharing some characteristics with glioblastoma, so the foreground of success for the fixation of arising problems is optimistic. Generally, with the high demanding need for an efficacious therapy, the advancement of immune checkpoint inhibitor therapy is under rapid development, thus showing huge prospects for becoming a successful treatment.

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