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New trends in glioblastoma multiforme immunotherapy

Natalia Kurowska*, Marcel Madej, Barbara Strzałka-Mrozik

Department of Molecular Biology, Faculty of Pharmaceutical Sciences, Medical University of Silesia in Katowice, Jedności 8, 41-200 Sosnowiec, Poland

***Corresponding author: Natalia Kurowska, Department of Molecular Biology, Faculty of Pharmaceutical Sciences, Medical University of Silesia in Katowice, s74697@365.sum.edu.pl**

ABSTRACT

Glioblastoma multiforme is a malignant brain tumour characterized by extremely high mortality. For this reason, the attention of many scientists is focused on developing innovative therapies. Recent studies provide satisfactory data on the effectiveness of immunotherapy in some solid tumours, which raises hope also for glioblastoma treatment.

The aim of this review is to present the current state of knowledge in the field of glioblastoma immunotherapy.

The goal of immunotherapy is to change the immunosuppressive microenvironment of the tumour and to mobilize the patient's immune system, which leads to the eradication of tumour cells. One of the methods studied with glioblastoma is the use of cancer vaccines. It is based on direct exposure to antigens or stimulation of the patient's antigen-presenting cells. Another method that may prove effective is the use of oncolytic viruses. The positive effect of the therapy is not only due to the destruction of the cancer cell itself, but above all is the result of the release of antigens from it which mobilize the immune system. Another approach to overcoming tumour immunosuppression is the use of immune checkpoint inhibitors which have been shown to be effective in other cancers. CAR-T cell therapy may also be a new potential treatment for gliobla-stoma multiforme. It is based on genetic modification of T cells taken from the patient to produce glioma-specific surface antigens, which allows interaction with tumour cells leading to cell death. Apart from the use of monotherapy, the use of combined treatment methods is also of great interest. The possibilities of immunotherapy in glioblastoma are very limited, mainly due to its immunosuppressive microenvironment and the presence of multiple mechanisms of therapy resistance.

Undoubtedly, research on immunotherapy of glioblastoma contributes to the development of new treatment regimens that may prove most effective in the case of a therapy combined with other treatments.

INTRODUCTION

Glioblastoma multiforme (GBM) is rare and one of the most lethal among malignant solid tumours. The incidence of this disease increases rapidly after the age of 54 years, reaching a median of 64 years (Alexander, 2017). Current standard therapy consists of maximal tumour resection, radiotherapy and chemotherapy with temozolomide followed by adjuvant temozolomide therapy (Alexander, 2017; Chen, 2018). Unfortunately, among patients diagnosed with the disease, the average survival time is less than 15 months even with standard therapies (Chen, 2018). Given this grim prognosis, researchers around the world are attempting to develop effective therapies.

Early treatment failure has been attributed to many factors. The microenvironment of this cancer is incredibly diverse and also varies between patients (DeCordova, 2020). It also contains many non-cancerous cells, most notably immune cells. Much attention has been paid to tumour-associated macrophages which are associated with tumour progression, among other things (Chen, 2018). In the case of glioblastoma multiforme, a nonimmunogenic "cold" environment is observed (Young, 2020). This is associated with many features of the tumour, such as decreased expression of MHC class I molecules, impaired antigen presentation by APC cells, or an increase in immunosuppressive cells (Saha, 2018). All of these features prevent the recognition of the tumour by the immune system and, consequently, the body's effective fight against cancer. Moreover, tumours escaping immune surveillance cause the immune system to not only fail to fight the tumour, but may also act in its favour (Chen, 2018).

Immunotherapy is an innovative treatment method that involves modulating the immune system in a way that allows it to attack cancer cells. The regulation of the immune environment resulting from such therapy has been shown to be effective in the treatment of several cancers. Combining immunotherapy with other treatments to enhance its effect seems particularly promising (Tan, 2020). Numerous data on the effectiveness of immunotherapy in cancer treatment provide hope for patients with glioblastoma multiforme. Despite numerous clinical trials, to date no immunotherapy has been approved by the Food and Drug Administration (FDA) for the treatment of glioblastoma multiforme (McGranahan, 2019). There are several factors

that undoubtedly contribute to the low efficacy of this type of therapy. The first problem is the physical blood-brain and blood-tumour-brain barrier, which is an obstacle to therapeutic molecules (Young, 2020). Another problem is the effects of therapies usually used in glioblastoma, namely temozolomide and radiation, which lead to lymphopenia. Glioblastoma patients are also given dexamethasone, a synthetic corticosteroid which relieves brain swelling but also leads to suppression of the immune system (McGranahan, 2019; Young, 2020). Immunosuppression within the tumour, mentioned earlier, and its high resistance to treatment resulting from, among other things, the high heterogeneity of the tumour, as well as its low tumour mutational burden, are also not without significance (Medi-konda, 2021).

The aim of this paper is to review recent studies on immunotherapy for glioblastoma multiforme, both as monotherapy but also in combination with other treatments.

SEARCH STRATEGY AND SELECTION CRITERIA

The following article was compiled from both review and original scientific publications, including data obtained from clinical trials. The PubMed database was used primarily to search for sources. The entering terms was: glioblastoma multiforme immunotherapy, glioblastoma therapy, CAR-T glioblastoma, glioblastoma vaccines, glioblastoma virotherapy, oncolytic viruses glioblastoma, immune checkpoint inhibitors glioblastoma and combined therapy glioblastoma. The cited articles were published between 2010 and 2021 and, except for one, are all in English. The ClinicalTrials.gov database was also used in compiling this review.

REVIEW

CAR-T THERAPY FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

With the progress and development of genetic engineering, it has become possible to model and reprogram cells, including the patient's own immune cells, to fight a specific disease entity. Such therapy has been called adoptive immunotherapy. Chief among such forms of therapy is CAR-T therapy, or Chimeric Antigen Receptor T cells whose appropriately engineered receptors are capable of interacting with antigens of cancer cells, including potentially glioblastoma multiforme (Marei, 2021). The process of immunotherapy with the use of genetically modified CAR-T cells takes place in seve-ral stages starting from the collection of leukocytes from the patient's blood through their genetic modification with the use of viral vectors, and then administering them back to the patient's body from which they were isolated (Giotta Lucifero, 2021). This method is currently approved by the FDA for the treatment of mainly B-cell lymphomas and leukaemias in paediatric patients, but the potential of this therapy is also being explored for other cancers, including GBM (Marei, 2021).

The structure of CAR molecules varies and, due to the number of costimulatory domains of the CAR receptor, a division into five generations has been made. Generation I contains a fragment of the CD3ζ chain, while II and III contain, in addition to the fixed CD3ζ fragment, successively one or two additional costimulatory domains, e.g. CD22, OX40 or 4- 1BB (Choi, 2019; Marei, 2021). In generation IV a cytokine domain is additionally present and in generation V, a cytokine receptor-binding domain is present (Feldman, 2021; Sterner, 2021; Yu, 2021). The overall structure of the CAR molecule consists of several elements, these are: the extracellular domain, whose function is to recognise and interact with the relevant antigen of tumour cells, connected by a hinge region to the second element, i.e. the endothelial domain which in turn is connected to several T-cell-derived intracellular chains. The function of the intracellular domain is to induce the T cell pathway. The only variable element in the structure of the CAR molecule is the antigen recognition domain, the appropriate selection of which influences the proper interactions with tumour cells (Choi, 2021; Giotta Lucifero, 2021; Marei, 2021; Sterner, 2021; Yu, 2021).

In clinical trials for the potential use of CAR-T therapy in the treatment of glioblastoma multiforme, specific receptors that are overexpressed in the majority of GBM patients have received much attention, these include, e.g. Interleukin-13 Receptor A2 (IL-13Rα2), Human Epidermal Growth Factor Receptor 2 (HER2) and Epidermal Growth Factor Receptor variant III (EGFRvIII). As most of these are activated in the tumourigenic process they are excellent

molecular targets for CAR-T treatment of GBM (Giotta Lucifero, 2021; Marei, 2021).

One of the most important receptors, with tyrosine kinase activity, associated with this type of cancer is HER2 (Feldman, 2021). This factor is responsible for cell proliferation, adhesion and survival (Feldman, 2021). It is expressed in both healthy tissues and in approximately 80% of cases in GBM, therefore targeting this receptor can induce an immune response against healthy body tissues, as observed in a 2010 study (Feldman, 2021; Morgan, 2010). Accordingly, in 2017, Ahmed et al. (Ahmed, 2017) conducted a study on a group of 17 individuals using CAR-T therapy containing a CD28 costimulatory domain and an extracellular domain directed against the HER2 receptor in GBM with overexpression of this receptor. The results of this study showed that the use of infusions of CAR-T cells with an anti-HER2 domain could induce a partial tumour response within 6 months of administration. In addition, CAR-T cells were shown to persist in the body for 12 months with no toxicity, which carries much hope for the future safe use of CAR-T in GBM therapy (Giotta Lucifero, 2021; Marei, 2021; Sterner, 2021).

The IL13Rα2 receptor, which is expressed only in tumour cells, is also promising for the treatment of glioblastoma multiforme (Marei, 2021). This factor is a major prognostic indicator and is associated with poor prognosis and the possibility of metastasis and tumour growth through activation of the PI3K/AKT/mTOR pathway (Feldman, 2021). It represents an accomplished molecular target as its expression is 75% correlated with glioblastoma multiforme. To date, two studies have been conducted using CAR-T cells directed against the IL13Rα2 antigen. The first study from 2015 by Brown et al. focused on the administration of first-generation CAR cells with an anti-IL13Rα2 extracellular domain to three patients by intravenous infusion. On completion of the CAR-T cell therapy, they observed significantly reduced IL13Rα2 expression in tumour cells, which contributed to the expansion of the study to include the use of second-generation CAR cells with a 4-1BB costimulatory domain. The results of the 2016 study thus demonstrated that this treatment method using higher-generation CAR cells allows local tumour cell death at the site of administration. In addition, the increased induction of an immune response as well as the safety of such therapy has been proven (Choi, 2019; Feldman, 2021; Giotta Lucifero, 2021; Marei, 2021).

In the case of cancer-related diseases, frequently depending on the location of the tumour, antigens that are specific to a particular type of cancer can be identified. In the case of glioblastoma multiforme, one such antigen is a mutated form of EGFRvIII (Choi, 2019). The mutation observed here relative to wild-type EGFR is the deletion of exons 2-7, resulting in the translocation of a glycine residue in the extracellular domain, making it impossible for the receptor to bind to its ligand. The hallmark of this form is its specific expression in glioblastoma multiforme cells and relatively low expression in normal body cells (Choi, 2019; Feldman, 2021). In 2017, O'Rourke et al. conducted a study on a group of 7 patients with glioblastoma multiforme with EGFRvIII over-expression diagnosed and confirmed by next-generation sequencing. Second-generation modified CAR cells containing a CDζ domain and a 4-1BB costimulatory domain along with an anti-EGFRvIII extracellular domain were used for treatment. CAR-T cells were administered by intravenous infusion. Following therapy, patients underwent tumour surgery to assess the therapeutic potential. The study found significantly reduced levels of mutant EGFRvIII receptors within the tumour tissue and increased levels of molecules responsible for immunosuppressive processes, i.e. PD-L1, IL-10 and Transforming Growth Factor β (TGF-β) (Choi, 2019; Feldman, 2021; Giotta Lucifero, 2021). Further clinical studies on the use of EGFRvIII-targeted CAR-T cells in GBM are currently underway.

With CAR-T therapy, it is possible to conduct a targeted immune response by selecting the appropriate external domain depending on the disease entity. It should be noted that the majority of studies have shown no toxicity associated with the administration of CAR-T cells to the human body, which indicates that this therapy may prove effective in the treatment of other diseases besides cancer. Clinical trials are currently being conducted on CAR-T therapies targeting other antigens associated with GBM, such as B7-H3, CD147 or MMP2 (Maggs, 2021).

CANCER VACCINES

One type of immunotherapy of great interest to brain tumour researchers is cancer vaccines. It is postulated that such vaccines would promote an anti-tumour involving the adaptive immune system (Medikonda, 2021). If we talk about a vaccine in the context of cancer therapy, it should be noted that its aim is to generate immunity against tumour antigens, which should ultimately lead to the destruction of tumour cells (Cuoco, 2018). The aim of this action is therefore not to prevent the disease, as in the case of vaccines against infectious diseases, but to lead to the eradication of tumour cells (McGranahan, 2019). For the treatment of glioblastoma, a distinction is made between peptide or DNA vaccines, which result in direct exposure to tumour antigens. These antigens are used together with immune response stimulators. For glioblastoma, there are few tumour-specific antigens, so common tumour-associated antigens are often used in vaccines (McGranahan, 2019). Another type of vaccines are those "tailor-made" for individual patients, which are created by stimulating dendritic cells previously collected from the patient (McGranahan, 2019).

As previously mentioned for glioblastoma, not many tumour-specific antigens are known, but one of them is the EGFRvIII variant, a constitutively activated epidermal growth factor mutation found in about 25-30% of patients (McGranahan, 2019; Medikonda, 2021). The use of EGFRvIII as a vaccine antigen results in its low toxicity, as this variant is not expressed outside the tumour environment. However, on the other hand, the fact that it is only present in the tumours of some patients makes this therapy likely to be effective only in a selected, limited group of patients (Medikonda, 2021). Furthermore, in the case of tumour-specific antigens, their uneven expression inside the tumour is observed, which, if treated, results in the death of only a part of the tumour cells. This problem is even more serious as tumour cells may be able to promote the growth of cells not expressing a given antigen, which contributes to disease recur-rence (Wilcox, 2018). An example of EGFRvIII antigen-based therapy is rindopepimut, a vaccine consisting of a 14 amino acid peptide combined with the immunogenic carrier protein keyhole limpet hemocyanin (KLH) (Wilcox, 2018). Patients undergoing total resection and chemotherapy were eligible for phase II clinical trials and an increase in median overall survival (mOS) to 24 months was noted (Medikonda, 2021). Unfortunately, despite the promising results of phase II clinical trials, phase III trials were suspended because they did not yield the expected results (Daubon, 2020; Medikonda, 2021; Wilcox, 2018). In contrast, the results of a phase II study of the efficacy of the combination of rindopepimut with bevacizumab, in which an increase in patients' mOS was observed, appear promising (McGranahan, 2019; Medikonda, 2021; Muir, 2020). It is clear that rindopepimut has some pharmacological activity against GBM in a specific group of patients, but further studies are needed to develop specific treatment regimens, including but not limited to the addition of anti-angiogenic therapy and patient selection.

The low number of tumour-specific antigens creates the need to develop vaccines based on tumour-associated antigens. An example of such a vaccine is SurVaxM containing a survivin mimic peptide conjugated to KLH (McGranahan, 2019). Survivin belongs to the family of inhibitors of apoptosis and its overexpression is observed in many types of cancer, including GBM (Cuoco, 2018; Winograd, 2016). Furthermore, its levels correlate with disease progression (Cuoco, 2018). Studies of this vaccine have shown no serious side effects, and patients have been observed to develop humoral and cell-mediated immune responses. SurVaxM is currently being studied in combination with temozolomide treatment (Cuoco, 2018).

Another approach uses multipeptide vaccines which reduces the risk of immune tolerance and recurrence (Cuoco, 2018). An example is the IMA950 vaccine containing multiple tumour-associated peptides that are present in glioblastoma tissues. This includes the survivin discussed earlier, but also other antigens such as brevican (BCAN); chondroitin sulfate proteoglycan 4 (CSPG4); fatty acid-binding protein 7 (FABP7); IGF-2 mRNA-binding protein 3 (IGF2BP3); neuroligin 4, X-linked (NLGN4X); neuronal cell adhesion molecule (NRCAM); protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1); tenascin C (TNC); Met protooncogene (MET) (Cuoco, 2018; Winograd, 2016). Phase I trials of this vaccine provided satisfactory results, with 90% of patients observed to produce a single immune response, and 50% observed to respond to multiple, or at least two, antigens (Cuoco, 2018; Winograd, 2016).

Another multipeptide vaccine being tested in glioma is SL701. It is based on short synthetic peptides targeting IL-13Rα2, ephrin A2 and survivin. The study observed a CD8 response, which was associated with prolonged patient survival (McGranahan, 2019). Analysis of SL701 in combination with bevacizumab showed that the vaccine is well tolerated and has anti-tumour activity (Cuoco, 2018).

Vaccines using dendritic cells (DC) are also of interest among researchers. Dendritic cells, which are involved in the natural anti-tumour response, are stimulated with anti-gens from the patient's tumour and then delivered to the patient (Desland, 2020). Such vaccines can be derived using synthetic or tumour-derived peptides, RNA or crude tumour lysate. DC cell-based vaccines have advantages over cell-free peptide vaccines in that they can contain a much larger number of antigens (Winograd, 2016). Results from several preclinical studies suggest that such therapies have potential in the treatment of glioblastoma, as they increase CD8+ T-cell infiltration deep into the tumour, which is associated with increased survival (Desland, 2020). In the case of GBM, the production of individual vaccines for patients is possible if the tumour is located such that surgical intervention is possible, as vaccine production requires taking a section of the tumour. This unfortunately limits the group of patients for whom production of such a vaccine is likely (McGranahan, 2019). Clinical trials of DC vaccines have provided data supporting their excellent safety profile. In addition, they have also demonstrated mobilisation of the immune system of patients and initiation of humoral and cellular immunity to some extent (Desland, 2020). Among the DC vaccines that have been evaluated in clinical trials, the DC-Vax-L deserves special attention. It is produced by pulsing autologous patient dendritic cells using whole tumour lysate. It seems highly promising that some patients who received DC-Vax-L in phase I and phase III clinical trials have a survival of more than 10 and 7 years after administration, correspondingly (McGranahan, 2019).

Personalised peptide vaccines may also be the future of therapy in GBM. The "Glioma Actively Personalised Vaccine Consortium" (GAPVAC) study evaluated the impact of treatment with two personalised vaccines with co-administration of temozolomide and radiotherapy (Dunn, 2020). The antigenic composition of the first vaccine was based on expression, physical HLA binding and immunogenicity, resulting in a vaccine composed of seven antigens. Subsequently, patients received a second vaccine containing two neoantigenic peptides that were identified by sequencing and HLA binding affinity (Dunn, 2020). In 11 of the 15 patients tested, both CD8+ and CD4+ cell responses were observed in reaction to these two vaccines. Moreover, the vaccines were well tolerated. The median progression-free survival and overall survival of 14.5 and 29.0 months, respectively, seems extremely optimistic. (Dunn, 2020). The results of these studies are encouraging and provide hope that the future of glioblastoma multiforme treatment may rely heavily on personalisation of therapy.

The use of vaccination in the treatment of glioblastoma multiforme may prove effective in the future. The data on increasing mOS are very promising. Unfortunately, more research is needed and, perhaps, to tailor the vaccines being tested to the molecular subtype of glioblastoma multiforme.

VIROTHERAPY

Interest in viruses as agents for anticancer therapy dates back over a century ago. Years of research in oncology and virology have led to the identification of two types of anticancer virotherapy. The first, using replication-competent viruses that are oncolytic, i.e. capable of infecting a cancer cell, which then undergoes lysis and releases viral progeny capable of infecting subsequent cancer cells (Foreman, 2017). Initially, it was thought that their positive impact lay solely in the destruction of cancer cells, but this is only one side of the coin. Oncolytic viruses exert an anti-tumour effect primarily because when they damage a cell, they cause the release of tumour-associated antigens and damage associated molecular patterns from the cell which are then recognised by the body and mobilise the immune system to fight the tumour (Desland, 2020; Fecci, 2019; Martikainen, 2019). In addition, oncolytic viruses themselves contain pathogen associated molecular patterns, the release of which is also associated with tumour cell destruction, as these patterns are recognised by the innate immune system (Fecci, 2019). Certain features of glioblastoma multiforme favour the use of oncolytic

virus therapy for its treatment. This is related, among other things, to the fact that glioblastomas do not give rise to distant metastases, so the disease is restricted to one organ only. Moreover, these tumours grow surrounded by nondividing cells, which favours the retention of oncolytic viruses in a restricted area, as they multiply exclusively in dividing cells (Wollmann, 2012). The second type of anticancer virotherapy relies on viruses incapable of replication as a vector to deliver therapeutic genes (Foreman, 2017). An example of the use of virotherapy in the treatment of GBM is the use of modified herpes simplex viruses with an embedded transgene encoding interleukin-12, a cytokine whose anticancer activity is inter alia due to its ability to induce interferon gamma (IFNγ) expression (Nguyen, 2020).

Several clinical trials are evaluating the effect of oncolytic viruses in the treatment of glioblastoma multiforme. Preliminary results from clinical trials of adenoviruses, herpes simplex virus, and replicating retroviruses have shown increased patient survival (Khansur, 2019). In addition, other viruses such as polio virus, cowpox virus and measles virus, among others, are also being studied. Of particular interest are the results of studies on DNX-2401, PVS-RIPO and Toca 511, against which an immune response was observed in 20% of GBM patients (Martikainen, 2019).

DNX-2401 is a viral vector based on oncolytic adenovirus 5. This virus has been modified to increase infectivity, it replicates in GBM cells and is specific for this cancer (Stepanenko, 2018). Specificity towards GBM results from the introduction of two rearrangements: modification of the E1A gene and addition of an arginine-glycine-aspartic acid motif. It is also associated with restriction of its replication in normal cells (Philbrick, 2019). Among patients to whom the virus was administered either by direct single injection into the tumour or using a catheter, survival of more than 3 years was observed in 20% of the patients studied. Furthermore, survival beyond 3 years with progression-free survival after treatment was observed in three patients (Stepanenko, 2018). DNX-2401 is also being studied in combination with other therapies, including temozolomide or pembrolizumab. It is worth mentioning that the therapeutic effect of DNX-2401 is due to both its ability to destroy tumour cells and generate an anti-tumour immune response (Philbrick, 2019).

PVS-RIPO is a live attenuated polio virus type 1 (Sabin) vaccine containing heterologous IRES from human rhinovirus type 2 (Walton, 2018). Virus entry into the cell occurs through interaction with the CD155 receptor, which is upregulated on the cell surface of solid tumours and in the tumour microenvironment. Moreover, this receptor is also expressed on the surface of antigen-presenting cells (Desjardins, 2018; Fecci, 2019). *In vitro*, this virus induces neoplastic cell death and promotes the release of proinflammatory cytokines (Desjardins, 2018; Fecci, 2019). The phase I clinical trial provided interesting results. Firstly, at 24 and 36 months after administration of PVS-RIPO, the survival rate was 21%, which is significantly higher than in the historical control group. Secondlyp, the study confirmed the lack of neurovirulent potential, including paralysis associated with poliomyelitis (Desjardins, 2018; Iorgulescu, 2018).

Toca 511 is a modified retrovirus that expresses cytosine deaminase (McGranahan, 2019). It is a nonlytic virus, and its mechanism of action involves the conversion reaction of 5-fluorocytosine using cytosine deaminase to 5-fluorouracil, an antitumour drug of the antimetabolite group (McGranahan, 2019; Stepanenko, 2018). This is a replication-capable vector based on a mouse leukaemia virus-expressing enzyme of yeast origin (Stepanenko, 2018). A study by Mitschell et al. (Mitchell, 2017) carried out on a mouse model of glioma demonstrated that when Toca 511 was administered together with the prodrug 5-flourocytosine in animals, there was a change in the qualitative composition of the cells of the immune system present in the tumour environment. A loss of immunosuppressive cells and an expansion of T lymphocytes deep into the tumour were observed. Application of Toca 511 and 5-fluorocytosine resulted not only in tumour cell death, but also in the infiltration of immune cells deep into the tumour, altering the tumour microenvironment, which promotes the generation of anti-tumour immunity (Mitchell, 2017). Similarly, Yagiz et al. (Yagiz, 2016) tested in mouse and rat models the combination of Toca 511, 5-fluorouracil and another alkylating anticancer drug, lomustine, also obtaining satisfactory results in terms of survival (Yagiz, 2016).

Toca 511 has also been evaluated in clinical trials. In a phase I clinical trial, Toca 511 was administered into the cavity after tumour resection, followed by oral administration of 5-fluo-

rocytosine. 23 of 56 patients responded to the recommended dose. In this subgroup, the median overall survival was 14.4 months. Moreover, one-year survival rates were 65.2%, while the two-year survival rate was 34.8 %. Furthermore, as of 25 August 2017, five patients showed a complete response and were alive 33.9-52.2 months after administration (Cloughesy, 2018; Stepanenko, 2018). To gather more data, a clinical trial was conducted to compare the efficacy of treatment using Toca 511 with oral 5-fluorocytosine with standard treatments in patients who have glioblastoma multiforme or anaplastic astrocytoma and have undergone tumour resection for a first or second relapse. However, the results of these studies did not yield significant differences in survival between the patient groups studied (Cloughesy, 2020). Nevertheless, the results of the phase I clinical trial offer much hope for the development of effective treatments for glioblastoma multiforme.

Certainly, the results of studies on the use of virotherapy in the treatment of glioblastoma multiforme seem promising and may prove to be the future of therapy for this type of cancer. However, there is undoubtedly a need for more research in this area, both preclinical and clinical. Nevertheless, the innovation of the described therapies is also evidenced by the fact that the FDA has granted "Fast Track" status to both Toca 511 and PVS- RIPO and DNX-2401 (Martikainen, 2019).

CHECKPOINTS INHIBITORS

Another approach uses immune checkpoints inhibitors such as programmed death receptor 1 (PD-1), programmed death-ligand 1 (PD-L1), or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (McGranahan; 2019). The therapy using monoclonal antibodies directed at blocking immune checkpoints has proven effective in several types of cancer (Darvin, 2018). This raises hope also for use in the treatment of glioblastoma multiforme. Immune checkpoints cause inhibition of the antitumour immune response. Their blockade should therefore lead to an enhanced immune response and elimination of tumour cells (Darvin, 2018). PD-1 is a molecule that is expressed on immune system components such as T cells, B cells, bone marrow and NK cells. It has its biological effects by interacting with specific PD-L1 ligands in peripheral tissues. High levels of PD-1 and its ligands are associated with, among other things, impaired cytotoxic function of T cells and their secretion of cytokines (Maxwell, 2017). Several studies have demonstrated that PD-L1 is highly expressed in glioblastoma, which may prove to be a good target for therapy, in addition, studies show that increased PD-L1 levels correlate with increased susceptibility to treatment using immune checkpoint inhibition (McGranahan, 2019; Wang, 2019). One molecule registered to block PD-1 is nivolumab, a fully humanised monoclonal antibody. Currently, this drug is used in the treatment of melanoma, non-small cell lung cancer, and kidney cancer, among others (Koseła-Paterczyk, 2016). Other monoclonal antibodies directed against PD-1 or PD-L1 include pembrolizumab, durvalumab and atezolizumab (Yang, 2021). Immune check-point inhibitors have achieved good results in preclinical studies in GBM, but their potential in therapy is limited because of the risk of central nervous system (CNS) toxicity (McGranahan, 2019).

Several clinical trials have investigated the efficacy of immune checkpoint inhibitors in the treatment of glioblastoma multiforme. One of these studies examined the efficacy of nivolumab or pembrolizumab in patients with recurrent highgrade glioma. In this study, some patients were also taking bevacizumab, an anti-angiogenic agent used in cancer therapy. Unfortunately, the results showed no survival benefit (Kurz, 2018). Subsequent studies have also failed to provide groundbreaking evidence of the efficacy of immune checkpoint inhibitors in the treatment of GBM. The CheckMate 143 trial compared the efficacy of nivolumab and bevacizumab in patients with first relapse who had previously received temozolomide and radiotherapy. Unfortunately, this study showed no improvement in overall survival in the group of patients treated with nivolumab relative to the group receiving bevacizumab (Reardon, 2017). In contrast, Lukas et al. (Lucas, 2018) studied the efficacy of atezolimumab in patients with glioblastoma multiforme at first or second relapse. The drug was well tolerated, but the 12-months overall survival time was comparable to that with chemotherapy or bevacizumab.

Although the development of immune checkpoint inhibitors has revolutionised the treatment of some cancers, the future of their use in the treatment of glioblastoma multi-forme is unclear. The results of clinical trials of the use of single immune checkpoint inhibitors have not provided breakthrough data. This may be due in part

to the immu-nosuppressive environment of this tumour, including but not limited to a reduced number of lymphocytes infiltrating the tumour. Therapy failures may also be related to PD-L1 expression in tumour cells, the number of mutations, or microsatellite instability (Yang, 2021). Consequently, combination therapies – which include the use of more inhibitors of different checkpoints – are being investigated. Combination therapies using immune checkpoint inhibitors are discussed later in this article.

COMBINATION THERAPIES

Currently, classical therapies for glioblastoma multiforme focus on surgical treatment or the use of chemotherapy or radiotherapy. All of these methods place a heavy burden on the patient, which is why new therapeutic options are increasingly being researched (Huang, 2021). An example of such a method is immunotherapy which includes a number of different technologies, i.e. the use of monoclonal antibodies, CAR-T therapy or the use of checkpoint inhibitors (Chan, 2021; Huang, 2021). Using only one of these methods is called monotherapy. Often this choice of treatment is insufficient, so current clinical trials will largely focus on the possibility of combining different therapies. This significantly increases therapeutic options, leading to a synergistic effect of two or more therapeutic substances. Of greatest interest among researchers have been combinations of immunotherapy together with CAR-T therapy or multiple checkpoint inhibitors, but also immunotherapy with radiotherapy (Chan, 2021; Huang, 2021).

The combination of radiotherapy and immunotherapy offers promising opportunities. It is assumed that adequate doses of radiation are able to lead to tumour necrosis by triggering the abscopal effect which has been described in detail by Mole et al. (De Martino, 2021; Medikonda, 2021). In addition, the presentation of antigens and neo-antigenes is increased, resulting in immunomodulation. Ongoing clinical trials are currently focused on evaluating the safety of using a combination of hypofractionated stereotactic radiotherapy (HFSRT) with immunotherapy, mainly with anti-PD-1 monoclonal antibodies (Medikonda, 2021; Sahebjam, 2021). The clinical trial comprised 32 patients who were treated with bevacizumab plus pembrolizumab with concomitant HFSRT (Sahebjam, 2021). The results confirmed the safety of immunotherapy combined with radiotherapy. Significant PD-1 expression ≥ 10% was observed in only one of the patients studied, suggesting the high efficacy of this treatment method (Sahebjam, 2021). In addition, researchers are focusing on combining laser ablation with the simultaneous use of anti- PD-1 monoclonal antibodies in the treatment of GBM. It is suggested that with this method it is possible to eliminate the main problem of treating brain tumours, i.e. the blood-brain barrier (Medikonda, 2021). As a result of the loss of this barrier, there may be an influx of immune cells into the tumour and there is also an increase in the availability of tumour antigens, but the safety of this method is still under investigation (Medikonda, 2021).

An interesting idea with promising preliminary results from clinical and preclinical studies is the possibility of using a combination of multiple checkpoint inhibitors, i.e. PD-1, CTLA-4, indoleamine 2,3-dioxygenase 1 (IDO1) and a combination of lympho-cyte-activation gene 3 (LAG-3) and T-cell immunoglobulin and mucin-domain con-taining-3 (TIM-3) with anti-PD-1. The concept of combination therapy has currently been approved by the FDA for the treatment of melanoma thanks to the use of PD-1 and CTLA-4 inhibitors. Researchers suppose that such a combination is also worth considering for the treatment of glioblastoma multiforme. Thus, preclinical phase studies are currently being conducted on the utility of these inhibitors against GBM (Chan, 2021). One of the characteristic checkpoint molecules for glioblastoma multiforme is the IDO1 molecule. Its function described to date is to convert the amino acid tryptophan to kynurenine which has an immunosuppressive role and additionally increases salience to metastasis (Chan, 2021; Zhai, 2021). Increased expression of the IDO1 molecule is also correlated with higher mortality by increasing the influx of immunosuppressive Treg cells. Therefore, a clinical trial was conducted using triple checkpoint blockade, i.e. PD-1, CTLA-4 and IDO1. The results showed that under the application of this antibody combination, there was a significant reduction of Treg cells within the tumour (Chan, 2021). Similar effects in preclinical studies were obtained with the combination of anti-PD-1 inhibitors with LAG-3 and anti-PD-1 with TIM-3 (Chan, 2021).

In the treatment strategy for glioblastoma multiforme, combinations of modern cancer therapy

regimens are also being considered. An example of such a combination is the use of the previously described CAR-T system with checkpoint inhibitors (Chan, 2021; Maggs, 2021). The use of these inhibitors as a monotherapy is often limited due to the fact that a T-cell response is required and also through the presentation of neoantigens by class I tissue compatibility system antigens (Maggs, 2021). In ongoing preclinical studies on glioblastoma multiforme models, anti-PD-1 and anti-CTLA-4 checkpoint inhibitors were used in combination with CAR-T cells. In the first case, CAR-T cells containing the extracellular domain of anti- IL13Rα2 were used in combination with ipilimumab and nivolumab to assess the safety and the lack of toxicity on body cells excluding tumour cells. The postulated synergistic effect of this therapy is to help immune cells fight the tumour and also lead to the inhibition of tumour cell growth and metastasis (Maggs, 2021). The combination of anti-EGFRvIII CAR-T cells with an anti-PD-1 monoclonal antibody is also one of the similar studies currently underway (Maggs, 2021).

The possibility of combining several therapies offers great opportunities for the treat-ment of glioblastoma multiforme. The main problem, however, continues to be the blood-brain barrier, through which only selected molecules can pass. Researchers are therefore attempting to combine the therapeutic methods available to date for different types of cancer in order to effectively bypass this obstacle. In addition to the described combinations of different methods, clinical or preclinical studies are conducted combining immunotherapy with protein vaccines, chemo-therapy or epigenetic drugs (Chan, 2021; Maggs, 2021) as well as with nanotechnology in its broad sense, thanks to which it is possible to deliver a therapeutic substance to a precisely targeted place within a given tumour (Maggs, 2021).

SHORT CONCLUSION

Immunotherapy has changed the lives of many cancer patients. Research into its use in glio-blastoma multiforme is extremely important, as standard treatments do not extend the life span of patients very much. Due to the complex biology of glioblastoma multiforme, it is difficult to find an effective therapy, as this is related not only to the high heterogeneity within the tumour, but also between individual patients. The low mutational burden of the tumour is also unfa-vourable. Furthermore, the immunosuppressive microenvironment of GBM is also responsible for immunotherapy failures. However, data coming from an increasing number of clinical trials raise hope. The use of different types of immunotherapy, such as cancer vaccines, CAR-T therapy, virotherapy or immune checkpoint inhibitors has proven effective in some patients. Even a slight prolongation of survival among patients is a significant achievement. Moreover, immunotherapy seems to be much more effec-tive when not one, but more strategies are used. In addition, the results of trials of combination therapies using both immunotherapy and other treatments seem very promising. Undoubtedly, more research is needed, both on the biology of glioblastoma multiforme itself, which will allow the development of patient-specific personalised therapies, and on the development of new combination therapy regimens.

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