Utilizing immunotherapy in the eradication of glioblastoma

Bachelor thesis - Pre-master biomedical sciences

Sylvanio Jacob Humberto Goedgedrag - S3027538
Supervisor: Prof. Frank Kuyt

Date: 01/3/2016
Groningen, The Netherlands

university of groningen

Author:
Sylvanio Jacob Humberto Goedgedrag – sylvaniogoed@gmail.com – s.j.h.goedgedrag@student.rug.nl

Studentnumber: S3027538
Address: Johan Winklerwei 123, 8915EM, Leeuwarden, The Netherlands
Tel: 0626325004

Institution: Rijksuniversiteit Groningen

Supervisor: Frank Kruyt – f.a.e.kruyt@umcg.nl

March 2016

Acknowledgements

I would like to thank everyone who helped and supported me during the writing of my thesis. I would like to thank Professor **Frank Kruyt** for helping and guiding me throughout the writing. I also want to thank my **mom**, **dad**, **brothers** and the rest of my **family** and **friends** for giving me their extra support to complete my thesis.

”Don’t worry about a thing

’Cause every little thing gonna be alright

Singing’ ”Don’t worry about a thing

’Cause every little thing gonna be alright!”

”Three Little Birds”
– by Bob Marley

For my family,

Sylvanio Jacob Humberto Goedgedrag

Leeuwarden, March 2016

Utilizing immunotherapy in the eradication of glioblastoma

Sylvanio Jacob Humberto Goedgedrag[1], Frank Kruyt[2]

[1,2]Rijksuniversiteit Groningen, The Netherlands

Abstract

Glioblastoma (GBM) is the most common primary central nervous system (CNS) malignancy. There is an estimate of 210.000 new cases each year worldwide. The overall chance of survival with current standard treatment options is very slim and recurrence is almost inevitable. The current standard treatment consists of safe resection of the tumor, followed by radiotherapy, chemotherapy (with temozolomide, bevacizumab, nitrosoureas) and electrical field treatment. In the majority of patients it is most unlikely to achieve therapeutic benefit from a single agent because glioblastoma are very heterogenous tumors. It is evident that there is a desperate need for new and more effective treatment strategies. Currently there is a novel therapy that has shown promising results, which is the immunotherapy approach. In the past it was thought that the brain was immune-privileged, due to the protective structure of the blood-brain barrier being the main reason of concern. In contrast, now it has been shown that the CNS including the brain is more immune competent than previously thought. The immune system is highly active in the brain and interacts with brain tumors. These findings have opened up the possibility to explore an important approach for treating malignant brain tumors, immunotherapy. Immunotherapies can be classified into 4 major categories, active immunotherapy, passive immunotherapy, adoptive strategies and immunomodulatory strategies. The most recent findings, progress and future prospects will be presented and discussed, including the underlying mechanisms to possibly eradicate glioblastoma using immunotherapy, such as active and passive immunotherapy, immunomodulatory- and adoptive strategies. Noticeable advancements in utilizing immunotherapy for GBM patients have been made and will most likely become the focus of future treatment strategies.

Inhoud

LIST OF ABBREVATIONS

APC - Antigen Presenting Cell
CAR – Chimeric Antigen Receptor
CSF - Cerebrospinal Fluid
CNS – Central Nervous System
CTLA-4 – Cytotoxic T-Lymphocyte-Associated antigen 4
CTLs – Cytotoxic T Lymphocytes
DC – Dendritic Cell
DCLNs – Deep Cervical Lymph Nodes
EGFR – Epidermal Growth Factor Receptor
HER2 – Human Epidermal growth factor Receptor 2
GBM – Glioblastoma (Multiforme)
IL-10 – Interleukin-10
MAb – Monoclonal Antibodies
MHC - Major Histocompatibility Complex
PD-1 – Programmed Death 1
SAS – Subrachnoid Space
TAA – Tumor-Associated Antigens
TAM – Tumor Associated Macrophages
TCR – T-Cell Receptor
TIGIT – T-cell immunoreceptor with Ig and ITIM domains
TIM3 – T-cell immunoglobulin and mucin domain-3
TGF-β – Transforming Growth Factor-β
Tregs – Regulatory T cells
LAG-3 – Lymphocyte Activation Gene 3
LLT-1 – Lectin-Like Transcript-1
VEGF – Vascular Endothelial Growth Factor

1. Introduction

1.1. Cancer in the brain – Glioblastoma

Glioblastoma (GBM), or astrocytoma WHO grade IV, is the most fatal and most common primary central nervous system (CNS) malignancy. Every year, there is an estimate of 5-6 cases out of 100,000 people diagnosed with primary malignant brain tumors, of which roughly 80% are malignant gliomas[1,2]. There is an estimate of 210.000 new cases each year worldwide. The overall chance of survival with current standard treatment options is very slim, which remains around 15 months and recurrence is almost inevitable[1,2,3]. Common characteristics of high grade malignant gliomas are their heterogeneity, genetic unstability and that they are highly infiltrative, thus making the glioma cells advantageous in resisting standard treatment[4].

1.2. Treatment possibilities – the current standard

The current standard treatment consists of safe resection of the tumor, followed by radiotherapy, chemotherapy (with temozolomide, bevacizumab, nitrosoureas) and electrical field treatment[3,5]. In the majority of patients it is most unlikely to achieve therapeutic benefit from a single agent because glioblastomas are very heterogenous tumors[6].

It is evident that there is a desperate need for new and more effective treatment strategies. Thus, making it important to find new novel ways to treat this common malignant brain tumor. One of the newly and promising methods is using immunotherapy for cancer patients. In 2010 there were two US FDA approved immunotherapeutic options released. First being sipuleucel-T, a dendritic cell-based vaccine, for metastatic, hormone-resistant prostate cancer[7]. Secondly ipilimumab was released, a humanized monoclonal antibodies (MAb) targeting the immunomodulatory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4) for metastatic melanoma[8]. Interestingly, both methods improved the overall survival of the cancer patients. There is a growing interest to utilize immunotherapy for newly diagnosed glioblastoma patients and is still in its early development phase.

1.3. Immunotherapy

In the past it was thought that the brain was an “immune-privileged site”, due to the protective structure of blood-brain barrier and the absence of conventional lymphatic vessels the main reasons of concern[9]. In contrast, now it has been shown that the central nervous system including the brain is much more immune competent than previously thought. The immune system is highly active in the brain and interacts with brain tumors. One reason being that recent data have shown that memory T cells are present in the CNS, and these cells are suspected to be crucial for immune surveillance of the CNS[10]. These findings have opened up the possibility to explore an important approach for treating malignant brain tumors, immunotherapy[5]. The goal of immunotherapy is to seek to improve the body’s immune response against a tumor.

Immunotherapies can be classified into four major categories, active immunotherapy, passive immunotherapy, adoptive strategies and immunomodulatory strategies[6].

The most recent findings and progress in this area will be presented and discussed, including the underlying mechanisms to possibly eradicate glioblastoma using immunotherapy, such as “the immune system in the brain, immune-checkpoint inhibition, vaccines and T cell engineering”.

2. The immune system surrounding glioblastoma

The immune system is a biological system within organisms that consists of many structures and processes. Its main function is to sense and protect the organism from injury, caused by trauma or harmful foreign molecules, pathogens and viruses. The human immune system is divided between the innate and the adaptive immune systems. The innate immune system is the first line of defense against invading pathogens. It is mediated by the body's own phagocytes, such as macrophages and dendritic cells. The acquired immunity is an evolved system that focuses on eliminating the pathogens in the late phase of an infection and creating an immunological memory[11].

2.1. The immune system in the CNS and the blood-brain barrier

As it was previously noted, the immune system is not only involved in defending the body from foreign pathogens, but is also engaged in the eradication of cells that underwent malignant transformation. This process is also known as "immune surveillance"[12].

The immune system of the CNS has several distinct characteristics that make it different compared to the peripheral immune system, such as the absence of MHC expression by CNS parenchymal cells, the blood-brain barrier (BBB) and the anti-inflammatory attribute of the CNS tissue environment, which elicits the specialized CNS inflammatory responses that are essential for the conservation of the fragile non-regenerating tissue aspect in the CNS[10].
Moreover, another important distinction are the several different immune cell populations in the brain. For example the microglial cells, which are the first line of defense in the brain. Their key role is to migrate towards inflammatory zones and, once activated, they posses phagocytic properties and produce cytokines and chemokines that in turn recruit other immune cells[13,14].

The adaptive immune response towards antigens derived from the CNS is initiated in the periphery and in turn spread to the CNS by the available memory T cells. Furthermore, the memory T cells are re-initiated by antigens within the CNS. Regardless the absence of lympathic vessels, the CNS does acquire a soluble route for antigen transportation. In the CNS interstitial fluid culverts through perivascular channels into the cerebrospinal fluid (CSF). This connection allows macrophages and other APCs in the subrachnoid space (SAS) to obtain the antigens in the CNS. Interestingly, the antigen derived from the CNS are transported in the CFS to the nasal mucosa. Here lies the afferent lymphatics connection to the deep cervical lymph nodes (DCLNs). At this site antigens accumulate. It still remains unclear how this will lead to an immune response within the periphery[10,13].

The BBB is compost of complex cellular brain capillary vessels that in turn consist of endothelial cells with tight junctions associated pericytic and astrocytic cells. The BBB is known to be selective for the entry of immune cells from periphery into the brain parenchyma[13,16]. There is evidence of the arrival of T cells into the brain[10]. Unfortunately, most GBM patients have a disordered BBB, compromising the control of effector T cell trafficking into the tumor site.

2.2. The immune response to glioblastoma – Tumor immunosuppression

An adaptive immune response indicates the presence of antigen recognition. Several papers noted that there are several tumor antigens that reoccur frequently in GBM patients, including IL13Rα2,
EGFRvIII, gp100, and TRP2[17]. These antigens are known to induce immune reactions. Other described antigens are EphA2, survivin,WT1, SOX2, SOX11, MAGE1, AIM2, and SART1[13].

Unfortunately, it is evident that the body's own immune reactions that occur are mostly ineffective due to the constant recurrence of the disease. In contrast, it is important to note that the situation is not completely black or white. Primarily, tumor development and growth is indeed affected by the immune system. Secondly, the most immunogenic tumor cells will be recognized and eradicated by the immune system, when in fact, the lesser immunogenic tumor cells will be undetectable and will develop further, due to their low MHC expression and/or poor antigen presentation and/or no expression of immunogenic TAA. Consequently, the immune system will control the developing tumors according to its capability[6].

Tumor cells can also invert the immune system to their advantage, by promoting angiogenesis, the release of angiogenic factor such as V-EGF (Fig.1). This factor is secreted during the "wound healing" phase of the inflammatory reaction and will indirectly encourage tumor development, by the transport increase of oxygen and nutrients. Furthermore, tumor vascularization will also promote metastasis. Thus, making inflammation another important component to consider. Glioblastoma is known for their ability to execute immunosuppression through systemic- and/or local environmental immunosuppression (Fig.1)[6].

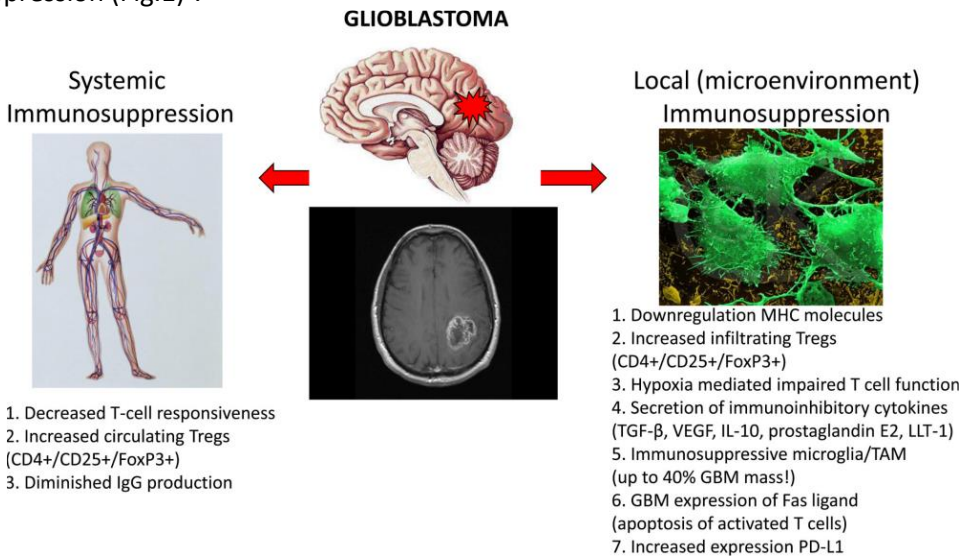

Figure 1 Summary of the immunosuppression opposed by glioblastoma. Immunosuppression by glioblastoma can arise from (left) systemic and/or (right) local (microenvironment) factors. Systemic inhibitory factors includes, decreased T-cell responsiveness, increased circulating regulatory T cells (Tregs) (CD4+/CD25+/FoxP3+) and dimished IgG production. Local immunosuppression surrounding GBM microenvironment consists of: MHC molecule downregulation; elevated infiltrating Tregs (CD4+/CD25+/FoxP3+), impaired T cell functioning caused by hypoxia, immunoinhibitory cytokine secretion (TGF-β, VEGF, IL-10, prostaglandin E2, LLT-1), immunosuppressive response of microglia/tumor associated macrophages (TAM), which can be the reason for 40% of the glioma mass and an increase of PD-L1 expression[6].

2.3. The use of immunotherapy – the major four

The goal of immunotherapy is to seek to improve the body's own immune response to a tumor. Using the benefit of having a multitude of glioma-associated antigens and several ways to improve an aspect of the immune response, it is theoretically possible to enhance the immune response in different angles to eradicate glioblastoma. This standpoint has been proven in several research papers. Throughout the past decade many immunotherapeutic advancements have been made. Some are already being explored in clinical trials, while others are still in development.

Currently there are four major categories in immunotherapy. Firstly, are the immunomodulatory strategies, which aim to enhance general immunoreactivity by "augmenting" co-stimulatory molecules or by blocking inhibitory molecules. An example of this strategy is the use of ipilimumab. Secondly, is the so-called active immunotherapy, which includes methods to directly sensitize the immune system to tumor-specific antigens. An example of this method is the use of cancer vaccines. Thirdly listed is the passive immunotherapy, which is in context the opposite of the previously listed approach. This method utilizes immune effector molecules, in the form of antibodies, to specifically target tumor antigens without directly activating the immune system. Lastly, is the adoptive strategy, which incorporates the use of adoptive T cell transfer or administration of T cells with chimeric antigen receptors (CARs). This strategy utilizes the patient's own immune cells. Moreover, these cells are manipulated ex vivo to react against tumor antigen before reinfusion to the patient[6].

It is important to note, that the listed major four immunotherapies all have different angles of approach to treat and/or suppress or even abolish glioblastoma. Unfortunately in many cases it is unlikely that a single agent or approach will be sufficient and/or as effective for all patients. The reason being the significant immunosuppression characteristics of glioblastoma[18].Thus, it has been stated before to the possibility of creating potentially "complimentary combinatorial immune-based approaches" for GBM patients[6].

3. Underlying mechanisms of immunotherapy – current status and future challenges

As it has been noted in the previous chapter that malignant gliomas are in most cases very effective in their resistance against treatment (Fig. 1), making them a great challenge to suppress and/or eradicate6,18. To further elucidate the "hopes and promises" of the major 4 immunotherapy approaches, it is essential to present the current understanding of each of their mechanisms behind counteracting glioblastoma and (future) challenges.

3.1. Immune checkpoints – Immunomodulatory strategies

Over the past 5 years, immunotherapy utilizing immune checkpoint inhibitors has accounted for clinical breakthroughs in the treatment of other tumors (see #3 and #5 in Fig.2)[19]. These drugs promote effective anti-tumoral immune responses by inhibiting co-inhibitory receptors and pathways that are activated by tumors to counteract T-cell response against tumor cells. What is evident is that the findings indicate that immune checkpoint inhibitors can facilitate an effective and long-lasting alleviation that sometimes can last for years. However, treatment-related toxicities and negative outcome can be abundant, but they are feasible in most cases[19].

The communication that tumor cells have with the immune system is a significant determinant of cancer pathogenesis. In the case of immunomodulatory strategies, it is critical to understand the key steps that this approach accounts for. The immune system pursuits to eradicate tumor cells by a distinct response cycle that consists of several steps. Starting by attempting to release antigens from tumor cells at cell death and in turn presenting these antigens by APCs to T cells that are primed and activated against tumor-specific antigens in the lymph nodes[20] The cytotoxic T cells, in this case named CD8+ cells, journey themselves to the tumor-site where they infiltrate the tumors. The CD8+ cells will recognize the specific tumor cells, and initiate tumor-cell death, consequently causing the release of more tumor-associated antigens (TAA), as a result continuing the cycle[20]. During this process, several checkpoint pathways, or ligand-receptor interactions, between (1) APCs and T cells and between (2) tumor cell and T cells produce signals to stimulate or inhibit T-cell activation, and to coordinate the duration and magnitude of the immune response[20].

There are two signals that are interplay in the activation or inhibition of the T-cell response. First being, the primary signal that arises when antigens are presented through the MHC to the T cell receptor. And secondly, the secondary signal that is either co-inhibitory or co-stimulatory. The secondary signal also determines the T cell response[21,22,23]. Molecules that reflect these signals are the so-called "checkpoint molecules". These checkpoint molecules can be either co-stimulatory or co-inhibitory. Those that stimulate immune activation are: CD28, CD40L, CD58, CD80, CD86, CD137 and TNFRSF4 (also called OX40). The ones that suppress immune activation are: cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), PD1, lymphocyte activation gene 3 (LAG-3), TIGIT, and T cell immunoglobulin and mucin domain-3 (TIM3)[21,24,25,26]. Tumor cells use these immune checkpoint pathways to their own advantage to avoid immune detection, and can therefore be targets to consider for therapy use. However, the data derived from these molecules are obtained from models from other tumor types. Currently, the precise association of checkpoint pathways in brain tumors pathogenesis remains to be elucidated[19].

There are currently two checkpoint molecules for which clinical application have been developed, PD-1 and CTLA-4. Both are accountable for the down regulation of T cell activity[19,34]. CTLA-4 is found on cytotoxic and helper T cells. This protein inhibits the activity of the T cells. The inhibition of T cell activation is caused by the expression of its receptor and the generation of IL-2. Moreover, CTLA-4 causes a delay of lymphocytes in the G1 phase of the cell cycle[19]. The expression of PD1 is promoted upon activation of the T cell. PD1 functions to limit the possibility of detrimental activity of lymphocytes in peripheral tissues. PD1 is expressed on Tregs and activation of its receptor seems to play a role in their proliferation.

In an *in vivo* study it has been shown that the blockade of CLTA-4 alone had modest impact on the survival and did not cure any mice. However, the combination of early vaccination and CTLA-4 mAb administration significantly improved survival in mice. This concept is yet to be examined in patients[38].

Anti-CTLA-4 (Ipilimumab) is the first agent used for immune-checkpoints blockade that has been approved by the FDA. In a study of 77 patients with melanoma brain metastases were treated with ipilimumab, the average overall survival rate was increased from 4.9 up to 21.3 months[40]. Another study showed similar findings[41]. Furthermore, the latest data of a phase II study with recurrent GBM treated with either nivolumab (antibody against PD1) alone or combined with ipilimumab showed an overall survival rate of 75% at 6 months[42].

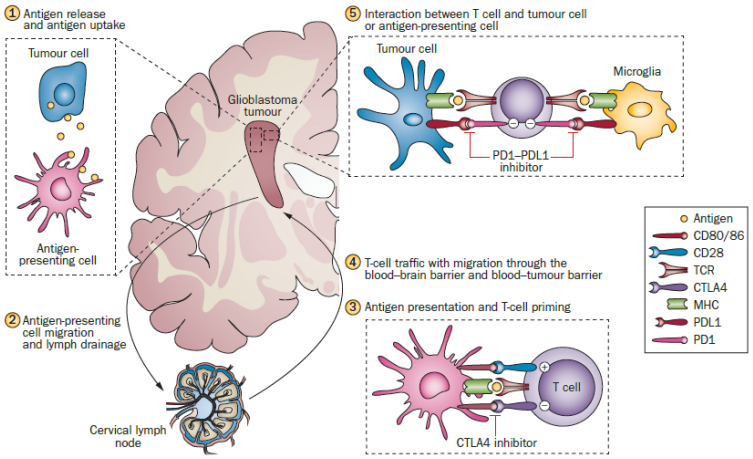

Figure 2 Summary of the immune response and the most fundamental immune checkpoint molecules in the immune cycle of glioblastoma. Once antigens are released from deteriorated tumor cells, they are taken up by anti-presenting cells, and through microglia and macrophages (#1). Antigens are supplied to lymph nodes through the migration of APCs, and by drainage through lymphatic vessels in the meningeal sinuses (#2). The arrival of antigens in the lymphatic tissues leads to antigen presentation and T-cell priming. This interaction is securely controlled by a multitude of co-inhibitory (CTLA4) and co-stimulatory (CD80, CD86, CD28) immune checkpoint molecules. This interaction can be manipulated by the use of specific antibodies (e.g., ipilimumab, an CTLA4 inhibitor) (#3). Furthermore, once the T cells are activated, they will travel to reach the tumor by the blood stream and migration via the blood-brain barrier (#4). Finally, tumor-associated immunosuppressive factors, along with immune checkpoint molecules, prevent tumor cell destruction by T cells. PDL-1 is expressed on tumor cells and microglia and inhibits T cells through binding to PD1. Inhibition of PD1-PDL1 (e.g., pembrolizumab or nivolumab) leads to the blockage of this immunosuppressive mechanism and induces an increase of tumor cell lysis by lymphocytes (#5)[19].

3.2. The use of Vaccines – Active and Passive immunotherapy

In the field of using vaccine as a immunotherapy approach there are two strategies, the "active " and "passive". Active vaccines aim to stimulate the patient's own immune response. Currently there are several known approaches, but mainly encompass "cell-based" or "non-cell based" strategies. There are several more strategies, but the most recent approach of each category will be presented.

An example of an active "cell-based" strategy is the use of dendritic cells for vaccination. Dendritic-cell-based vaccines are created by pulsing DCs with specific peptides from TAA or by fusing them with whole tumor cells[27,28]. The approach of pulsing DCs with specific TAA is based on using DCs as the APCs. The aim is to train the body's immune system to create an anti-tumoral response[29,30]. The APCs undergo stimulation with a specific TAA and is injected as a vaccine. Consequently, this will lead to the development of appropriate T-cells that will cause an antitumor response. Researchers have reported that vaccination of mature "booster DCs" have shown to increase a significant CD8+ T-cell response[31] and DC vaccines have shown to stimulate production of Th1 cytokines, which are associated with immunomodulatory responses against cancer[32]. Moreover, dendritic-cell-based vaccines are not only suitable for targeting the initial tumor, but it may also cause a memory immune response, which in turn offers defense to the body from future tumor recurrences[30].

In a review they examined the results of 171 studies of GBM patients treated with "dendritic cell-based vaccine" strategies. The outcome of this study strongly suggested that there was a significant increase in overall survival, ranging up to an estimate of 2 years compared to conventional therapy[29].

An example of an active "non-cell based" strategy is the application of peptide-based immunotherapy (Fig.3). This strategy encompasses peptide vaccination, but can also be suitable for loading dendritic cells or priming CTLs before an adoptive transfer (see also "3.3 T cell engineering – adoptive strategy") to a patient. This approach is still under development and has many questions to be answered regarding its strategy for possible future use[33].

Passive immunotherapy consists of immune effector molecules such as monoclonal antibodies, tumor associated antigens, cytokine stimulation using IL-12, or cell-based therapy. These approaches focus on targeting tumor antigens without direct initiation of the immune system[6,29,33].

In GBM patients the main challenge is overcoming not only the BBB, but also the "blood-tumor" barrier. These structures are often disorganized due to irregular angiogenesis caused by the tumor. This will result in immature pericytes, abnormal flow dynamics and thus making lymphocyte recruitment difficult. Experiments *in vivo* and clinical observations demonstrated that immunotherapy is less effective as the area becomes more vasculature and chaotic[34,45].

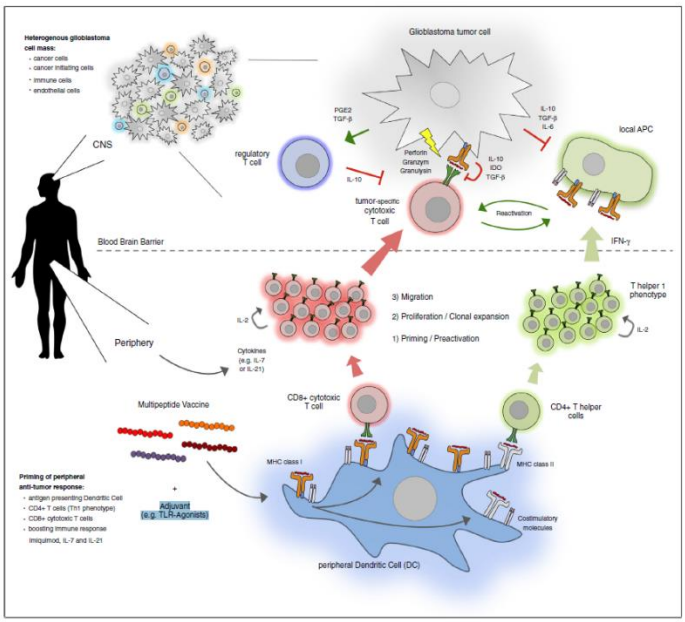

Figure 3 Summary depicting an overview of the idealized vaccination-induced peripheral priming of a tumor-specific immune response. Following peptide uptake and processing peripheral APCs (peripheral dendritic cells), they present the antigens through HLA-class I and –II molecules to tumor-specific T cells. Co-stimulatory and HLA molecule interactions will lead to an induced activation of the T cells. The T-cells will proliferate and perform their effector functions. CD8+ cytotoxic T-cells will lyse tumor cells via promoting apoptosis, by using granulysin, granzyme and perforin. CD4+ T helper cells encourages the continuation of ongoing immune response by the (local) APC. In turn, glioblastoma cells attempt to avoid immune-mediated lysis by recruiting regulatory T cells. The Tregs will promote the reaction to create and anti-inflammatory milieu and influence the local environment to the tumor's advantage[33].

3.3. T cell engineering – Adoptive strategy

Another immunotherapy approach that has been stated to hold great promises in treatment of GBM is, the "adoptive strategy", which incorporates adoptive T cell transfer or administration of T cells with chimeric antigen receptors (CAR)[34,35]. T cells that identify specific TAAs can be produced by combining an extracellular domain (usually derived from a TAA-specific monoclonal antibody) to the intra-cellular signaling domain of the T cell receptor to form a CAR[34]. CARs function is to recognize antigens expressed on the surface of tumor cells. CAR T cel activation is independent of MHC and thus results into resolving issues involving down regulation of HLA class I molecules and irregularities in antigen processing that tumors use to avoid T cell detection[36]. The modified T cells are possibly more practical than antibody-based immunotherapies considering their ability to penetrate solid tumor, migrate through blood vessel walls and recruit additional constituents of the immune response[37]. There are several CARs created for glioma-specific antigens, including HER2, IL-13Ra2, and EGFRvIII. More importantly, they have demonstrated promising antitumoral activity within *in vivo* models[34,36,44]. It is important to note, a research finding suggested that CARs generated against HER2 in GBM patients, recognizes a distinct stem cell population that is presumably associated with tumor recurrence[36]. There are several clinical trials set to explore the effectiveness and safety of CARs against HER2[34].

4. Conclusion

It is evident that the current standard strategies are not effective. The chance of overall survival is estimated to be around 15 months. Moreover, recurrence of the disease is almost inevitable, despite the aggressive surgery and cytotoxic approaches. Immune-oriented therapies have not been evaluated until recent, because of the dogma that stated that the CNS is immune privileged. This belief has been proven wrong and has shown that there is a capable interaction between the CNS and periphery immune system. In studies published it has been stated that immune-oriented therapies have proven to be relatively secure with low toxicities, in particularly compared to current therapies (i.e. traditional cytotoxic chemotherapy) (Wang[29], 2014; Agarwalla[38], 2012; Ampie[34], 2015).

The progress that have been made in understanding of underlying mechanisms of treating GBM has increased immensely, unfortunately it seems that immunotherapy is still in its preliminary stage. A better understanding of the immunotherapy challenges is still required involving various immunesuppression factors in a broad range of different cases of GBM patients, the capabilities of the CNS, the tumor microenvironment and being able to efficiently increase anti-tumoral immune response. However, several studies have shown evidence that the available preliminary overall survival estimates have been promising (Wang[29], 2014; Agarwalla[38], 2012; Ampie[34], 2015). Furthermore, while immunotherapy is progressing, diagnostic markers will be essential to determine which patients will benefit from which therapy. Given the broad range of immunotherapy options targeting different pathways, it is challenging to acquire well-designed systematic evaluations.

In terms of the clinic, it is clear that the main obstacle in the studying clinical trial results is the need for cooperation between groups and improvement in designing a well-standardized validation system. Furthermore, several other factors should be included, such as dosage control, and the possibility of increase of toxicity and/or side-effects. In other words, validation of the newest immunotherapeutic options take an unfortunate long time leading up to clinical trials.

In conclusion, noticeable advancements have been made in treating cancer using immunotherapy. Most of this progress have already been implemented into eradicating GBM in patients. The most promising immunotherapy approach until recent is the use of dendritic-cell-based vaccines. Significant increase of the overall survival in GBM patients are noticeable compared to the current standard treatments. This breakthrough may progress even more if combined with immunomodulatory strategies. The adoptive strategy is still in development and the effectiveness and safety are yet to be examined. As soon as the results from clinical trials are collected in a well-standardized system, the future prospect of treating GBM patients with immunotherapy looks to treat patients individually, offering personalized-medicine, and complementary combinatorial immunotherapy-based strategies will be explored and will most likely become the focus of future treatment strategies.

References

1. Schwartzbaum, J. a, Fisher, J. L., Aldape, K. D., & Wrensch, M. (2006). Epidemiology and molecular pathology of glioma. *Nature Clinical Practice. Neurology*, *2*(9), 494–503; quiz 1 p following 516. http://doi.org/10.1038/ncpneuro0289

2. Stupp, R., Hegi, M. E., Neyns, B., Goldbrunner, R., Schlegel, U., Clement, P. M. J., … Weller, M. (2010). Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. *Journal of Clinical Oncology*, *28*(16), 2712–2718. http://doi.org/10.1200/JCO.2009.26.6650

3. R. Stupp, W. P. Mason, M. J. van den B. et al. (2005). "Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma,." *The New England Journal of Medicine, Vol. 352, No. 10*, pp. 987–996.

4. Gomez, G. G., & Kruse, C. A. (2006). Mechanisms of malignant glioma immune resistance and sources of immunosuppression. *Gene Therapy and Molecular Biology*, *10*(1), 133–146.

5. Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J., Janzer, R. C., … Mirimanoff, R. O. (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *The Lancet Oncology*, *10*(5), 459–466. http://doi.org/10.1016/S1470-2045(09)70025-7

6. Reardon, D. A., Wucherpfennig, K. W., Freeman, G., Chiocca, E. A., Wen, P. Y., Jr, W. T. C., … Sampson, J. H. (2013). An Update of Vaccine Therapy and Other Immunotherapeutic Approaches for Glioblastoma. *NIH Public Access*, *12*(6), 597–615. http://doi.org/10.1586/erv.13.41

7. Pitt, B., Pfeffer, M. a., Assmann, S. F., Boineau, R., Anand, I. S., Claggett, B., … McKinlay, S. M. (2014). Spironolactone for heart failure with preserved ejection fraction. *The New England Journal of Medicine*, *370*(15), 1383–92. http://doi.org/10.1056/NEJMoa1313731

8. Hodi, F. S., Day, S. J. O., Mcdermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., … Quirt, I. (2010). NIH Public Access. *N Engl J Med*, *363*(8), 711–723. http://doi.org/10.1056/NEJMoa1003466.Improved

9. MEDEWAR Medewar P. Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue and to the anterior chamber of the eye. Br J Exp Pathol 1948;29:58–69.

10. Ransohoff, R. M., & Engelhardt, B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. *Nature Reviews Immunology*, *12*(9), 623–635. http://doi.org/10.1038/nri3265

11. Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen Recognition and Innate Immunity. *Cell*, *124*(4), 783–801. http://doi.org/10.1016/j.cell.2006.02.015

12. Vesely, M. D., Kershaw, M. H., Schreiber, R. D., & Smyth, M. J. (2011). Natural innate and adaptive immunity to cancer. *Annual Review of Immunology*, *29*, 235–71. http://doi.org/10.1146/annurev-immunol-031210-101324

13. Vauleon, E., Avril, T., Collet, B., Mosser, J., & Quillien, V. (2010). Overview of cellular immunotherapy for patients with glioblastoma. *Clinical and Developmental Immunology*, *2010*. http://doi.org/10.1155/2010/689171

14. Tambuyzer, B. R., Ponsaerts, P., & Nouwen, E. J. (2009). Microglia: gatekeepers of central nervous system immunology. *Journal of Leukocyte Biology*, *85*(3), 352–370. http://doi.org/10.1189/jlb.0608385

15. Schwartzbaum, J. a, Fisher, J. L., Aldape, K. D., & Wrensch, M. (2006). Epidemiology and molecular pathology of glioma. *Nature Clinical Practice. Neurology*, *2*(9), 494–503; quiz 1 p following 516. http://doi.org/10.1038/ncpneuro0289

16. Davies, D. C. (2002). Blood-brain barrier breakdown in septic encephalopathy and brain tumours. *Journal of Anatomy*, *200*(6), 639–646. http://doi.org/10.1046/j.1469-7580.2002.00065.x

17. Saikali, S., Avril, T., Collet, B., Hamlat, A., Bansard, J. Y., Drenou, B., … Quillien, V. (2007). Expression of nine tumour antigens in a series of human glioblastoma multiforme: Interest of EGFRvIII, IL-13Rα2, gp100 and TRP-2 for immunotherapy. *Journal of Neuro-Oncology*, *81*(2), 139–148. http://doi.org/10.1007/s11060-006-9220-3

18. Gomez, G. G., & Kruse, C. A. (2006). Mechanisms of malignant glioma immune resistance and sources of immunosuppression. *Gene Therapy and Molecular Biology*, *10*(1), 133–146.

19. Preusser, M., Lim, M., Hafler, D. A., Reardon, D. A., & Sampson, J. H. (2015). Prospects of immune checkpoint modulators in the treatment of glioblastoma. *Nature Reviews Neurology*, *11*(9), 504–514. http://doi.org/10.1038/nrneurol.2015.139

20. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 39, 1–10 (2013).

21. Driessens, G., Kline, J. & Gajewski, T. F. Costimulatory and coinhibitory receptors in anti-tumor immunity. Immunol. Rev. 229, 126–144 (2009).

22. Jackson, C., Ruzevick, J., Phallen, J., Belcaid, Z. & Lim, M. Challenges in immunotherapy presented by the glioblastoma multiforme microenvironment. Clin. Dev. Immunol. 2011, 1–21 (2011).

23. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer. 12, 252–264 (2012).

24. Bakdash, G., Sittig, S. P., van Dijk, T., Figdor, C. G. & de Vries, I. J. The nature of activatory and tolerogenic dendritic cell-derived signal II. Front. Immunol. 4, 53 (2013).

25. Joller, N. et al. Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. J. Immunol. 186, 1338–1342 (2011).

26. Hastings, W. D. et al. TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. Eur. J. Immunol. 39, 2492–2501 (2009).

27. Celluzzi CM1, Mayordomo JI, Storkus WJ, Lotze MT, Falo LD Jr. Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. J Exp Med 1996;183(1):283–287.

28. Koido S, Homma S, Okamoto M, Namiki Y, Takakura K, Uchiyama K, Kajihara M, Arihiro S, Imazu H, Arakawa H, Kan S, KomitaH, Ito M, Ohkusa T, Gong J, Tajiri H. Fusions between dendritic cells and whole tumor cells as anticancer vaccines. Oncoimmunology 2013;2(5):e24437.

29. Wang, X., Zhao, H.-Y., Zhang, F.-C., Sun, Y., Xiong, Z.-Y., & Jiang, X.-B. (2014). Dendritic Cell-Based Vaccine for the Treatment of Malignant Glioma: A Systematic Review. *Cancer Investigation*, *7907*(August), 1–7. http://doi.org/10.3109/07357907.2014.958234

30. Bregy, A., Wong, T. M., Shah, A. H., Goldberg, J. M., & Komotar, R. J. (2013). Active immunotherapy using dendritic cells in the treatment of glioblastoma multiforme. *Cancer Treatment Reviews*, *39*(8), 891–907. http://doi.org/10.1016/j.ctrv.2013.05.007

31. Dhodapkar, M. V., Krasovsky, J., Steinman, R. M., & Bhardwaj, N. (2000). Mature dendritic cells boost functionally superior CD8+ T-cell in humans without foreign helper epitopes. *Journal of Clinical Investigation*, *105*(6), 9–14. http://doi.org/10.1172/JCI9051

32. Matias, B. F., de Oliveira, T. M., Rodrigues, C. M., Abdalla, D. R., Montes, L., Murta, E. F. C., & Michelin, M. A. (2013). Influence of immunotherapy with autologous dendritic cells on innate and adaptive immune response in cancer. *Clinical Medicine Insights: Oncology*, *7*, 165–172. http://doi.org/10.4137/CMO.S12268

33. Mohme, M., Neidert, M. C., Regli, L., Weller, M., & Martin, R. (2014). Immunological challenges for peptide-based immunotherapy in glioblastoma. *Cancer Treatment Reviews*, *40*(2), 248–258. http://doi.org/10.1016/j.ctrv.2013.08.008

34. Ampie, L., Woolf, E. C., & Dardis, C. (2015). Immunotherapeutic advancements for glioblastoma. *Frontiers in Oncology*, *5*(January), 12. http://doi.org/10.3389/fonc.2015.00012

35. Choi, B. D., Suryadevara, C. M., Gedeon, P. C., Herndon, J. E., Sanchez-Perez, L., Bigner, D. D., & Sampson, J. H. (2014). Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma. *Journal of Clinical Neuroscience*, *21*(1), 189–190. http://doi.org/10.1016/j.jocn.2013.03.012

36. Ahmed, N., Salsman, V. S., Kew, Y., Shaffer, D., Powell, S., Zhang, Y. J., … Gottschalk, S. (2010). HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors. *Clinical Cancer Research*, *16*(2), 474–485. http://doi.org/10.1158/1078-0432.CCR-09-1322

37. Miao, H., Choi, B. D., Suryadevara, C. M., Sanchez-Perez, L., Yang, S., De Leon, G., … Sampson, J. H. (2014). EGFRvIII-specific chimeric antigen receptor T cells migrate to and kill tumor deposits infiltrating the brain parenchyma in an invasive xenograft model of glioblastoma. *PLoS ONE*, *9*(4). http://doi.org/10.1371/journal.pone.0094281

38. Pankaj Agarwalla, Zachary Barnard, Peter Fecci, Glenn Dranoff, and W. T. C., Jr., Cells, E. G., (2013). Sequential Immunotherapy by Vaccination with GM-CSF Expressing Glioma Cells and CTLA-4 Blockade Effectively Treats Established Murine Intracranial Tumors. *NIH Public Access*, *35*(5), 385–389. http://doi.org/10.1097/CJI.0b013e3182562d59.Sequential

39. Seystahl, K., Wick, W., & Weller, M. (2016). Therapeutic options in recurrent glioblastoma – an update. *Critical Reviews in Oncology/Hematology*. http://doi.org/10.1016/j.critrevonc.2016.01.018

40. Knisely, J. P. S., Yu, J. B., Flanigan, J., Sznol, M., Kluger, H. M., & Chiang, V. L. S. (2012). Radiosurgery for melanoma brain metastases in the ipilimumab era and the possibility of longer survival. *Journal of Neurosurgery*, *117*(2), 227–233. http://doi.org/10.3171/2012.5.JNS111929

41. Margolin K, Ernstoff MS, Hamid O, Lawrence D, McDermott D, Puzanov I, et al: Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. Lancet Oncol 13:459–465, 2012

42. Sampson JH, Vlahovic G, Sahebjam S, et al. Preliminary safety and activity of nivolumab and its combination with ipilimumab in recurrent glioblastoma (GBM): CHECKMATE-143. J Clin Oncol. 2015:33;(suppl; abstr 3010).

43. Camacho, L. H. (2015). CTLA-4 blockade with ipilimumab: biology, safety, efficacy, and future considerations. *Cancer Medicine*, *4*(5), 661–72. http://doi.org/10.1002/cam4.371

44. Miao, H., Choi, B. D., Suryadevara, C. M., Sanchez-Perez, L., Yang, S., De Leon, G., … Sampson, J. H. (2014). EGFRvIII-specific chimeric antigen receptor T cells migrate to and kill tumor deposits infiltrating the brain parenchyma in an invasive xenograft model of glioblastoma. *PLoS ONE*, *9*(4). http://doi.org/10.1371/journal.pone.0094281

45. Manzur, M., Hamzah, J., & Ganss, R. (2008). Modulation of the “blood-tumor” barrier improves immunotherapy. *Cell Cycle*, *7*(16), 2452–2455. http://doi.org/10.4161/cc.7.16.6451