



Immunotherapy for glioblastoma: concepts and challenges

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Abstract: PURPOSE OF REVIEW Immunotherapy is an emerging treatment strategy against various cancer types including glioblastoma. It comprises different strategies to induce, boost or restore an antitumor immune response. This review provides an overview of recent preclinical and clinical developments in the field of immunotherapy against glioblastoma. We elucidate the concepts and challenges and point out the strengths and weaknesses of the most promising immunotherapeutic approaches. RECENT FINDINGS Immunotherapy is one of the most active research areas in glioblastoma. Data from preclinical work as well as phase I and phase II clinical trials revealed that immunotherapy against glioblastoma is overall well tolerated and able to promote a potent antitumor immune response. Among the therapeutic approaches that are currently under investigation, vaccination, for example, against the variant III of epidermal growth factor receptor, as well as immune checkpoint inhibition targeting receptors such as cytotoxic T lymphocyte-associated antigen-4 and programmed cell death-1, are among the most promising and advanced treatment strategies. However, there are considerable challenges to overcome such as the identification of novel target molecules for vaccination, appropriate patient selection criteria, strategies to prevent or handle immune-related adverse events, and the implementation of immunotherapy in multimodal treatment regimens together with conventional treatment strategies. SUMMARY Key features of immunotherapy are target specificity, adaptability, and durability. Results from preclinical assessments and clinical trials applying immunotherapy alone or in combination with conventional treatment options are promising. However, intense research and stringent clinical development are required to optimize the available treatment options and to overcome potential pitfalls.

DOI: <https://doi.org/10.1097/WCO.0000000000000249>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-114462>

Journal Article

Published Version

Originally published at:

Weiss, T; Weller, M; Roth, P (2015). Immunotherapy for glioblastoma: concepts and challenges. *Current Opinion in Neurology*, 28(6):639-646.

DOI: <https://doi.org/10.1097/WCO.0000000000000249>



Immunotherapy for glioblastoma: concepts and challenges

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Purpose of review

Immunotherapy is an emerging treatment strategy against various cancer types including glioblastoma. It comprises different strategies to induce, boost or restore an antitumor immune response. This review provides an overview of recent preclinical and clinical developments in the field of immunotherapy against glioblastoma. We elucidate the concepts and challenges and point out the strengths and weaknesses of the most promising immunotherapeutic approaches.

Recent findings

Immunotherapy is one of the most active research areas in glioblastoma. Data from preclinical work as well as phase I and phase II clinical trials revealed that immunotherapy against glioblastoma is overall well tolerated and able to promote a potent antitumor immune response. Among the therapeutic approaches that are currently under investigation, vaccination, for example, against the variant III of epidermal growth factor receptor, as well as immune checkpoint inhibition targeting receptors such as cytotoxic T lymphocyte-associated antigen-4 and programmed cell death-1, are among the most promising and advanced treatment strategies. However, there are considerable challenges to overcome such as the identification of novel target molecules for vaccination, appropriate patient selection criteria, strategies to prevent or handle immune-related adverse events, and the implementation of immunotherapy in multimodal treatment regimens together with conventional treatment strategies.

Summary

Key features of immunotherapy are target specificity, adaptability, and durability. Results from preclinical assessments and clinical trials applying immunotherapy alone or in combination with conventional treatment options are promising. However, intense research and stringent clinical development are required to optimize the available treatment options and to overcome potential pitfalls.

Keywords

brain tumor, checkpoint inhibition, glioma, nivolumab, programmed cell death-1 (PD-1), pembrolizumab, vaccination

INTRODUCTION

Glioblastoma is the most common primary malignant brain tumor in adults. Despite multimodal treatment approaches including surgery, radiotherapy, and chemotherapy, the median survival is limited to, approximately, 16 months within clinical trial populations [1]. Accordingly, novel treatment modalities are urgently required. One of these is cancer immunotherapy. The recent advances achieved with immunotherapy in the treatment of patients affected by melanoma [2,3] and other tumor entities sparked widespread interest in the field and stimulated research exploring different immunotherapeutic strategies also against glioblastoma. In contrast to other tumors, glioblastoma is considered poorly immunogenic and its localization in the brain may further impede powerful immune

responses. Glioblastoma is characterized by defects in host cell-mediated immunity as well as various tumor-derived immune-inhibitory signals, which confer an immunosuppressive microenvironment [4,5]. However, the emergence of advanced immunotherapeutic approaches may now allow for an efficient targeting of glioblastoma by immunotherapy. The available concepts (Fig. 1) can be

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Curr Opin Neurol 2015, 28:639–646

DOI:10.1097/WCO.0000000000000249

KEY POINTS

- The emerging principles of immunotherapy against glioblastoma can be categorized by their dependence on the host immune system and their antigen specificity. Promising approaches comprise peptide and dendritic cell-based vaccination, adoptive cell therapy, checkpoint inhibition, tumor-targeting antibodies, immunovirotherapy, and inhibitors of immunosuppressive metabolic molecules (Fig. 1).
- For appropriate patient selection and treatment guidance, predictive and prognostic markers are needed. Furthermore, advanced imaging techniques that allow a differentiation of immune-related pseudoprogression due to inflammation from true tumor progression would be highly appreciated.
- Challenges of clinical trials exploring immunotherapeutic strategies against glioblastoma are delayed curve separation due to deferred antitumor effects and the reasonable design of appropriate trials that allow for a development of synergistic multimodal immunotherapeutic approaches.

categorized by their dependence on the host immune system and their antigen specificity [6] despite many intersections resulting from the enormous complexity of the immune system. Within this review article, we illustrate the basic principles underlying the different immunotherapeutic strategies, their strengths and weaknesses and applications in the context of glioblastoma.

PEPTIDE VACCINATION

This approach pursues an antigen-specific antitumor immune response by vaccination with full-length tumor antigens or short antigenic peptide fragments that are administered intramuscularly, subcutaneously or intradermally together with adjuvants. One of the best studied single-target approaches used for vaccination against glioblastoma is the variant III of the epidermal growth factor receptor (EGFRvIII). This mutated EGFR variant results from an in-frame deletion of exons 2–7 of the gene. It owns many features of an ideal immunotherapeutic target, which are cell surface expression, tumor-tissue specificity including expression on cancer stem cells and an association with tumor-driving mechanism [7,8]. Rindopepimut (Rintega), a clinically advanced peptide vaccine targeting EGFRvIII, is a 14-mer peptide conjugated to keyhole limpet hemocyanin that is administered intradermally together with granulocyte-macrophage colony-stimulating factor (GM-CSF). Rindopepimut has been assessed within several clinical trials so

far. The so far largest phase II clinical trial, Adoptive Cell Therapy III (ACT III) [9^{*}], demonstrated good tolerability except for grade 1/2 injection side reactions when rindopepimut was administered together with maintenance temozolomide therapy in patients with newly diagnosed glioblastoma. This trial demonstrated a robust, specific and durable immune response as well as encouraging progression-free survival (PFS) and overall survival (OS) compared with historical controls and confirmed previous results obtained with rindopepimut in glioblastoma patients [10,11]. Anti-EGFRvIII antibody titers may be potentially useful to monitor the immune response mediated by rindopepimut. An international multicenter, phase III randomized trial assessing the efficacy of rindopepimut in patients with newly diagnosed EGFRvIII-positive glioblastoma has completed accrual and the results need to be awaited (ACT IV; NCT01480479). Furthermore, rindopepimut has been assessed in a randomized phase II study in patients with recurrent glioblastoma. Here, the vaccine was used in combination with bevacizumab and compared with bevacizumab in combination with placebo injection. Again, anti-EGFRvIII immune responses were induced and the median OS was 11.6 months in the rindopepimut arm compared with 9.3 months in the placebo group ($P=0.0386$; hazard ratio 0.57 for the intention-to-treat population) [12]. Limitations of EGFRvIII-based vaccination include the presence of EGFRvIII on only 20–30% of all glioblastomas as well as potential antigen loss in recurrent tumors following vaccination.

Whereas rindopepimut targets a naturally occurring neoepitope, Hashimoto *et al.* [13] performed a phase I study using a modified human leukocyte antigen type A (HLA-A)*2402-restricted 9-mer peptide of the Wilms tumor peptide 1 in which an amino acid at the major histocompatibility complex (MHC) anchor position was replaced to increase binding affinity to the MHC molecule. Intradermal vaccination of the peptide emulsified in Montanide ISA51 in combination to temozolomide chemotherapy resulted in only minor toxicity in patients with newly diagnosed glioblastoma. This modified peptide induced mainly a cellular immune response.

Another promising target for peptide vaccination is a neoepitope derived from isocitrate dehydrogenase (IDH)-1, a key metabolic enzyme in the tricarboxylic acid cycle. The most common IDH-1 mutation, that is R132H, is found in the majority of low-grade and anaplastic gliomas as well as secondary glioblastomas. This mutation contains an immunogenic epitope suitable for mutation-specific vaccination in the context of MHC class II.

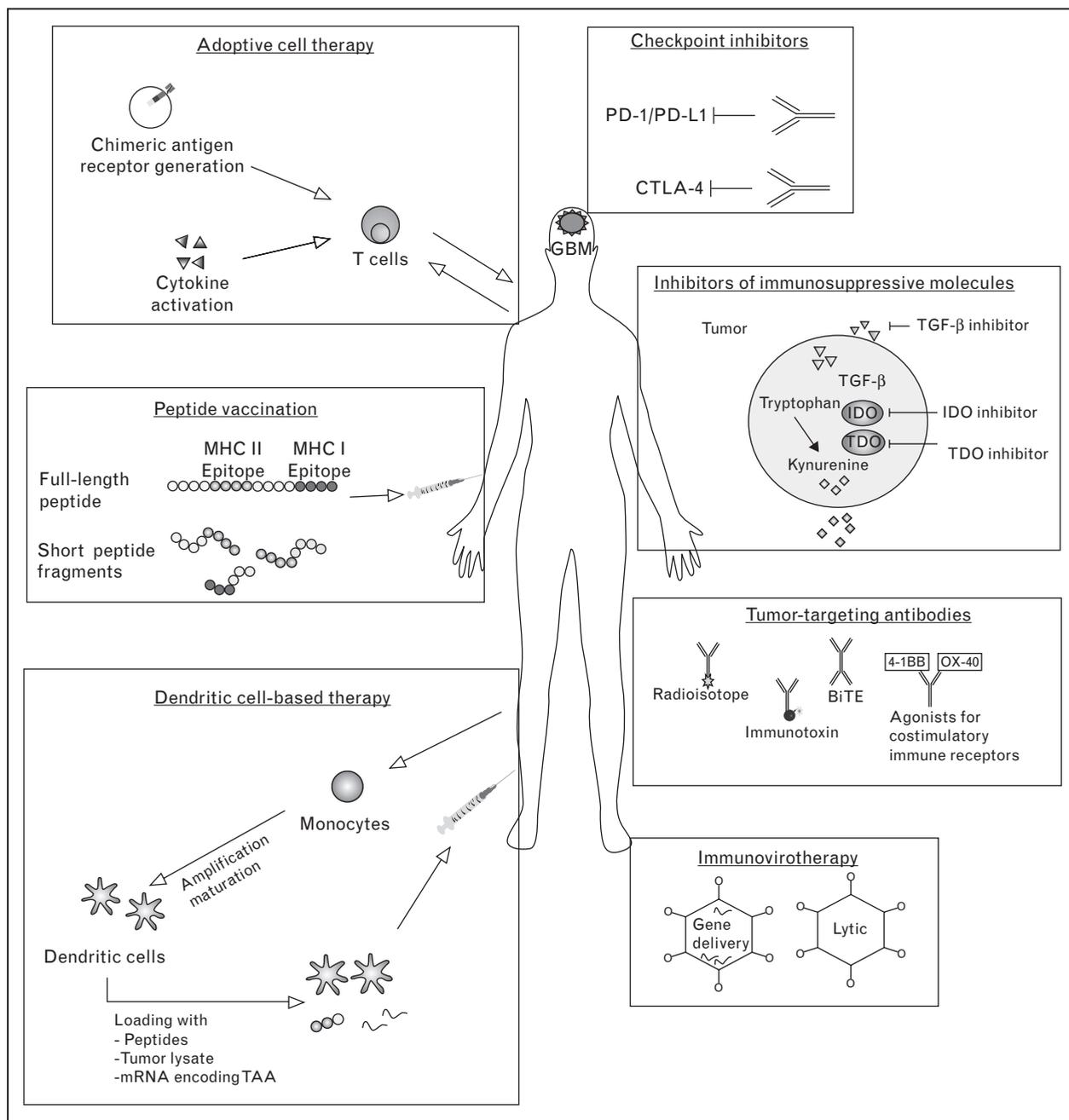


FIGURE 1. Immunotherapeutic approaches against glioblastoma. Illustration of emerging principles of immunotherapy against glioblastoma. The different categories are underlined, separated by squares and comprise peptide and dendritic cell-based vaccination, adoptive cell therapy, checkpoint inhibition, tumor-targeting antibodies, immunovirotherapy, and inhibitors of immunosuppressive metabolic molecules. Detailed mechanisms and abbreviations are explained in the text.

Vaccination with a peptide containing the IDH-1 R132H mutation induced a mutation-specific CD4 T-cell and antibody response in humanized mice and reduced the growth of subcutaneous mouse sarcoma cells that were engineered to express the IDH1 R132H mutation [14[¶]]. So far, it remains unknown whether this vaccine is also active in orthotopic brain tumor models. Several clinical studies using an IDH-1-specific peptide vaccine

are currently being planned to assess this approach in patients with IDH-1 mutant recurrent grade II glioma such as the RESIST (NCT02193347) trial and in patients affected by IDH-1-mutant grade III or IV gliomas such as the NOA-16 trial (EudraCT no. 2014-000503-27).

In addition to vaccination approaches that use single peptides as immunogens, there are also concepts that rely on multipptide vaccination. Such

multi-peptide strategies may potentially be more effective in boosting immune responses. Two phase I studies demonstrated the safety and immunogenicity of subcutaneous vaccinations with a cocktail of synthetic peptides derived from HLA-A2 restricted glioma-associated antigens of ephrin type-A receptor 2 (Epha2), interleukin-13 receptor subunit alpha-2 (IL-13R α), survivin and Wilms tumor peptide 1. Within the first study, children with brain stem or high-grade gliomas were enrolled and treated with the vaccine in combination with radiotherapy or chemoradiotherapy [15[¶]]. In the second study, vaccination with the multi-peptide formulation was applied to adults with high-risk diffuse cerebral gliomas [16[¶]]. In both trials, a combination of potent adjuvants was administered, that is, pan-HLA-DR tetanus toxoid peptide (TetA830) to enhance CD4 T helper cell responses and polyI:CLC to promote type I polarization of T-cell responses and Montanide-ISA-51, which promotes antigen-specific cytotoxic T cells. The application of this multi-peptide mixture led to antigen-specific T-cell responses as quantified by interferon- γ Enzyme-Linked ImmunoSpot assays. Another multi-peptide formulation currently being tested is IMA-950 (NCT01920191). It contains 11 HLA-binding tumor-associated antigens and is administered in combination with GM-CSF and imiquimod following a single dose of cyclophosphamide. The latter is supposed to deplete regulatory T cells, which may interfere with a potent antitumor immune response. Two of the epitopes are CD4 T-cell epitopes following the principle to recruit both CD4 and CD8 T cells.

Fundamental challenges specific for peptide vaccinations are the identification of appropriate target antigens as well as immune escape of the tumor resulting from antigen loss following treatment.

DENDRITIC CELL-BASED THERAPY

These approaches use dendritic cells to prime a tumor-specific immune response. Dendritic cells are professional antigen-presenting cells, placed at the interface between the innate and the adaptive immune system, orchestrating various immunological functions including humoral as well as cellular immune responses. This treatment usually requires the isolation of patient-derived or donor-derived monocytes, followed by ex-vivo amplification, maturation, and subsequent exposure to a source of tumor-antigens, for example, autologous tumor lysate or tumor-antigen-derived peptides. Alternatively, dendritic cells can be pulsed with mRNA derived from the tumor bulk or specifically encoding for tumor antigens. Challenges specific to these

approaches are the differences of the antigen-processing machinery of dendritic cells and tumor cells [17]. As a consequence, the MHC-binding epitopes of tumor antigens presented by dendritic cell and the ones that are presented in the context of MHC on tumor cells may vary. Furthermore, there is the potential risk of peripheral tolerance induction due to inadequate dendritic cell maturation signals. Adjuvants such as GM-CSF and IL-4 are frequently added to dendritic cell-based vaccines, also in glioblastoma trials [18]. Various dendritic cell-based vaccination concepts have been examined within the last years, mostly within small, uncontrolled trials [19,20]. Accordingly, the antiglioma activity of dendritic cell-based approaches has remained largely unclear. A clinically advanced concept of dendritic cell-based therapy is vaccination with ICT-107, an autologous vaccine of patient-derived dendritic cells pulsed with six synthetic, glioma-associated MHC class I peptides derived from absent in melanoma 2 protein, melanoma-associated antigen 1, tyrosinase-related protein-2, glycoprotein 100, human epidermal growth factor receptor 2 (HER2/neu), and IL-13R α 2. Treatment with ICT-107 was well tolerated and immunogenic in a phase I trial [21] and resulted in promising survival times in a phase II trial in patients with newly diagnosed glioblastoma. The effect was particularly encouraging in HLA-A2-positive patients [22]. DCVax-L is another dendritic cell-based vaccine which is generated using autologous dendritic cells pulsed with autologous tumor cell lysate. Despite the lack of compelling data from phase I or II studies, the vaccine is currently being assessed in a phase III trial in patients with newly diagnosed glioblastoma (NCT00045968).

A recent phase I clinical trial assessed a novel strategy to improve the efficacy of dendritic cell-based vaccination. Within this study, a vaccine site preconditioning strategy was applied by inducing inflammation at the vaccine site with tetanus/diphtheria toxoid prior to vaccination with cytomegalovirus (CMV)-specific dendritic cells pulsed with pp65 RNA [23[¶]]. This approach resulted in a significant increase in PFS and OS in patients with newly diagnosed glioblastoma compared with patients treated with dendritic cells alone.

ADOPTIVE CELL THERAPY

This strategy is a cell-based antitumor immunotherapy, which involves the collection of circulating or tumor-infiltrating lymphocytes, their ex-vivo expansion, activation, for example, with cytokines and/or potential tumor antigens, and/or genetic modification as well as their readministration to patients.

In a preclinical model, alloreactive cytotoxic T cells, derived from an HLA-disparate donor animal, were active against experimental gliomas [24]. The intratumoral injection of alloreactive T cells is currently assessed in a phase I study (NCT01144247) in patients with recurrent malignant gliomas or meningiomas. A more specific strategy is the development and application of T cells with genetically modified antigen-recognizing receptors. These chimeric antigen receptors (CAR) are based on a transmembrane protein, which comprises the tumor-antigen-binding domain of an immunoglobulin linked to one or more immunostimulatory domains. This approach has the advantage that it works independently of the available T-cell repertoire of a patient, which has undergone selection in the thymus to maintain central tolerance. Furthermore, it circumvents the drawbacks of classical MHC restriction, that is, the need for antigen processing and presentation. Similar to other antigen-specific strategies, CAR-based therapies require the identification of appropriate target antigens, which are expressed in a tumor-specific manner. Only such antigens may allow for specific targeting as well as potent anti-tumor effects in the absence of off-target immune-related cytotoxicity. Furthermore, most available CAR only target a single epitope, which renders this strategy vulnerable to antigen loss. In addition, many protocols for isolation and expansion of T cells for transduction with a transgene encoding for the CAR select predominantly CD8 T cells, neglecting potent antitumor effects mediated by CD4 T cells [25]. Furthermore, when murine-based single-chain fragment variable domains are used, human antimouse antibodies may potentially lead to anaphylactic side-effects and neutralization of effector cells [26]. The most advanced CAR T-cell studies against glioblastoma use EGFRvIII as their target. Murine and humanized CAR against EGFRvIII displayed pronounced antiglioma activity in preclinical in-vitro and in-vivo models [27^{***}]. An inherent problem of such preclinical models involving xenogenic implantation of human tumor cells and CAR T cells is their inability to predict CAR-mediated autoimmune side-effects. In the context of EGFRvIII, this point was addressed by another preclinical study that tested an EGFRvIII-specific murine CAR in a syngeneic, fully immunocompetent mouse glioma model [28]. Here, the infusion with EGFRvIII CAR T cells cured all mice harboring orthotopic gliomas and induced immunological memory without apparent toxicity. A prerequisite for EGFRvIII CAR T-cell activity was the depletion of lymphocytes in the host prior to adoptive CAR T-cell transfer. Based on the promising results of these preclinical studies, a phase I clinical

trial (NCT02209376) was initiated assessing T cells transduced with a lentiviral vector to express a human EGFRvIII CAR in patients with EGFRvIII-positive glioblastoma.

Another target for CAR T-cell therapy against glioblastoma is IL13R α 2, which is selectively expressed in glioblastoma including glioma-initiating cells. The CAR targeting IL13R α 2 are based on ligands that are coupled to intracellular signaling elements for T-cell stimulation. Administration of CAR T cells targeting IL13R α 2 resulted in prolonged survival in an orthotopic mouse glioma xenograft model [29]. A phase I trial (NCT01109095) assesses the safety of cytotoxic T lymphocytes engineered to express a CAR targeting EGFR2 (HER2). HER2 is expressed by a subset of glioblastomas and may, therefore, be a suitable target for CAR-based treatment.

IMMUNE CHECKPOINT INHIBITORS

Within the last years, a new class of drugs has emerged, which has been called 'immune checkpoint inhibitors'. These agents block inhibitory immune cell receptors or their corresponding ligands with the ultimate goal to reverse peripheral T-cell tolerance. Established target molecules are cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and the receptor programmed cell death-1 (PD-1) as well as its ligand PD-L1. The CTLA-4 blocking antibody ipilimumab was the first immune checkpoint inhibitor that obtained approval for the treatment of patients with metastatic melanoma [2]. PD-1 blocking drugs such as nivolumab or pembrolizumab have shown unprecedented activity in patients with advanced melanoma and other tumor entities [30,31]. However, similar to other immunotherapeutic approaches, only some of the patients may benefit from treatment with immune checkpoint inhibitors. So far, it remains unclear whether the expression of PD-L1 by tumor cells is required for sustained activity of anti-PD-1 antibodies. A further concern is that these molecules do not induce tumor-specific immune responses but may cause off-target effects related to a general activation of the immune system. Pre-clinical data suggest that such agents may also work against tumors in the brain [32]. Currently, clinical trials assessing the activity of immune checkpoint inhibitors in glioblastoma patients are ongoing. A phase III study comparing the efficacy of the PD-1 antibody nivolumab with bevacizumab in patients with recurrent glioblastoma has completed accrual (NCT02017717). A small safety run-in cohort of patients within this trial was treated with nivolumab in combination with ipilimumab. Due to

considerable toxicity in this combination arm, only single agent nivolumab was used in the phase III part of the trial [33]. Clinical trials adding PD-1 inhibitors or ipilimumab to standard chemoradiotherapy in patients with newly diagnosed glioblastoma are currently being planned.

FURTHER IMMUNOTHERAPEUTIC STRATEGIES UNDER DEVELOPMENT

Tumor-targeting antibodies

Apart from checkpoint inhibitors, other strategies based on monoclonal antibodies comprise drugs acting as agonists for costimulatory immune cell receptors such as 4-1BB or OX40 aiming at more efficiently activating immune effector cells [34,35]. Immunoconjugates are antibodies directed against tumor-associated or tumor-specific antigens that are coupled to toxins or radioisotopes, which may promote the induction of cell death. A clinically advanced antibody conjugate is ABT-414, an antibody recognizing amplified or mutated EGFR on tumor cells that is coupled to the toxic antimicrotubule agent monomethylauristatin. ABT-414 alone or in combination with temozolomide was assessed in a phase I study in patients with recurrent glioblastoma [36]. Ocular adverse events were common and further studies assessing the antitumor activity of ABT-414 in glioblastoma patients are required. Bispecific T-cell engagers are recombinant proteins composed of two-linked single chain variable fragments, one specifically recognizing a molecule expressed on T cells and one specifically recognizing a tumor antigen. Bispecific antibodies were active against experimental gliomas in preclinical models but clinical data are lacking so far [37,38]. All antibody approaches share the short half-life with the necessity of repetitive applications. Furthermore, the activity of antibodies, which are directed against brain tumor-expressed antigens, may be limited due to their insufficient blood–brain barrier penetration.

Immunovirotherapy

This therapeutic approach is based on nonpathogenic virus strains that specifically infect tumor cells and damage them either directly or by delivery of therapeutic/immune modulatory genes such as IL-12 [39]. Currently, several phase I clinical trials in newly diagnosed (NCT01811992) and recurrent (NCT02026271) glioblastoma are ongoing. The challenge with virus-based immunotherapies is that viruses may be cleared by the host immune system before exerting an effect.

Inhibitors of immunosuppressive metabolic pathways

Tryptophan depletion and kynurenine metabolites that are generated by tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) inhibit effector T cells and enhance the function of regulatory T cells. IDO is expressed by glioma cells. The preclinical assessment of IDO inhibitors demonstrated a survival benefit in an immunocompetent mouse glioblastoma model and a synergistic effect when applied together with chemoradiotherapy [40]. Furthermore, a phase I clinical trial has been conducted, applying the IDO inhibitor indoximod in combination with temozolomide in patients with recurrent glioblastoma to define the maximal tolerated dose of the drug [41]. A subsequent phase II study is planned. In addition, novel dual TDO and IDO inhibitors for tumor immunotherapy are under development. Another major immunosuppressive molecule that maintains the malignant phenotype of glioblastoma is transforming growth factor (TGF)- β . Although systemic TGF- β inhibition was associated with significant cardiac toxicity in rats [42], a recent phase I study demonstrated that LY2157299, a pharmacological inhibitor that interferes with TGF- β signaling, was well tolerated in patients with advanced malignancies including gliomas [43]. Clinical trials assessing this approach in more detail are ongoing.

CONCLUSION

Immunotherapy is one of the most active research fields in neuro-oncology. The key features of glioblastoma, that are, its immunosuppressive phenotype and the localization in an immune-privileged organ such as the brain with the presence of the blood–brain barrier, add another layer of complexity to the challenges of the currently explored immunotherapeutic approaches. So far, there are no broadly accepted criteria that can be applied for the selection of patients who may be ideal candidates for immunotherapy. Patients with low tumor burden, which allows higher effector to target ratios and patients with newly diagnosed tumors with good performance status without prior myelosuppressive treatment that may impair the immune system are considered the best candidates to benefit from immunotherapy. However, further research is required to develop predictive markers for appropriate patient selection.

Another goal that would strengthen many immunotherapeutic strategies is the identification of suitable target molecules. Ideally, such molecular targets are expressed in a tumor-specific manner,

which minimizes the risk for off-target side-effects. The combination of genomic and proteomic large-scale screenings as well as functional assessments will help to identify suitable candidates.

Another focus of research must aim at better understanding the side-effects, which occur in the context of immunotherapy against glioblastoma as well as the subsequent development of strategies to prevent or manage these immune-related adverse events. Due to the feature of immunological memory, immune-related adverse events can manifest even after the cessation of treatment and the need for a close monitoring of all patients should be emphasized. Another challenge is the differentiation of radiological pseudoprogression due to inflammation from true tumor progression. To tackle this problem, advanced imaging techniques or novel biomarker might be helpful for treatment guidance and to prevent premature withdrawal of patients from immunotherapeutic treatments. As immunological effects following immunotherapy may occur only with a delay, appropriate endpoints must be defined for clinical trials. Accordingly, not PFS but rather OS might be the most adequate endpoint to determine the antitumor activity of these novel drugs.

Finally, the complexity of the immune system with its innate and adaptive arms on a cellular level, cytokines and antibodies as secretory molecules as well as stimulating and inhibitory receptors on a molecular level allow for synergistic multimodal immunotherapeutic strategies. These may add a strong therapeutic weapon to the conventional treatment options such as surgery, radiotherapy, and chemotherapy, hopefully pushing some boundaries of glioblastoma research and ultimately improving prognosis and quality of life of the affected patients.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

T.W. has no conflicts of interest. M.W. has received research grants from Acceleron, Alpinia Institute, Bayer, Isarna, MSD, Merck Serono, PIQR and Roche and honoraria for lectures or advisory board participation from Celldex, Isarna, Magforce, MSD, Merck Serono, Pfizer, Roche and Teva. P.R. has received honoraria for advisory boards and lectures from Roche, MSD, Novartis and Molecular Partners.

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