Immunotherapy for glioblastoma: concepts and challenges

Weiss, T ; Weller, M ; Roth, P

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

Immunotherapy for glioblastoma: concepts and challenges

Tobias Weiss[1], Michael Weller[1], Patrick Roth[1]*

[1]Department of Neurology and Brain Tumor Center, University Hospital Zurich and University of Zurich, Switzerland

*Correspondence: Dr. Patrick Roth, Department of Neurology, University Hospital Zurich, Frauenklinikstrasse 26, 8091 Zurich, Switzerland, Tel.: +41 (0)44 255 5511, Fax: +41 (0)44 255 4380, E-mail: patrick.roth@usz.ch

Conflicts of interest: TW has no conflict of interest. MW has received research grants from Acceleron, Alpinia Institute, Bayer, Isarna, MSD, Merck Serono, PIQUR and Roche and honoraria for lectures or advisory board participation from Celldex, Isarna, Magforce, MSD, Merck Serono, Pfizer, Roche and Teva. PR has received honoraria for advisory boards and lectures from Roche, MSD, Novartis and Molecular Partners.

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 anti-tumor immune response. This review provides an overview of recent pre-clinical 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 pre-clinical work as well as phase I and phase II clinical trials revealed that immunotherapy against glioblastoma is overall safe and able to promote a potent anti-tumor immune response. Among the therapeutic approaches which are currently under investigation, vaccination, e.g. against EGFRvIII, as well as immune checkpoint inhibition targeting receptors such as CTLA-4 and PD-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: Glioma, brain tumor, vaccination, checkpoint inhibition, PD-1, nivolumab, pembrolizumab

Introduction

Glioblastoma is the most common primary malignant brain tumor in adults. Despite multi-modal 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 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 anti-tumor 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 (KLH) 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, 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 PFS and OS compared to 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 to 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 to 9.3 months in the placebo group ($p=0.0386$; HR 0.57 for the intention-to-treat (ITT) 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.

While rindopepimut targets a naturally occurring neoepitope, Hashimoto and colleagues performed a phase I study using a modified HLA-A*2402-restricted 9-mer peptide of the Wilms tumor peptide 1 (WT-1) in which an amino acid at the 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 (13). 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. 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 IDH1-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 which rely on multi-peptide 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-13Rα), survivin and WT-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, i.e. pan-HLA-DR tetanus toxoid peptide (TetA830) to enhance CD4 T helper cell responses and polyICLC 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 (IFN)-y Enzyme-Linked ImmunoSpot (ELISPOT) assays. Another multi-peptide formulation currently being tested is IMA-950 (NCT01920191). It contains 11 HLA-binding tumor-associated antigens (TAA) 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 anti-tumor 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 (DC)-based therapy

These approaches use DC to prime a tumor-specific immune response. DC 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- or donor-derived monocytes, followed by *ex vivo* amplification, maturation and subsequent exposure to a source of tumor-antigens, e.g. autologous tumor lysate or tumor-antigen-derived peptides. Alternatively, DC 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 DC and tumor cells (17). As a consequence, the MHC-binding epitopes of tumor antigens presented by DC 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 DC-based vaccines, also in glioblastoma trials (18). Various DC-based vaccination concepts have been examined within the last years, mostly within small, uncontrolled trials (19, 20). Accordingly, the anti-glioma activity of DC-based approaches has remained largely unclear. A clinically advanced concept of DC-based therapy is vaccination with ICT-107, an autologous vaccine of patient-derived DC pulsed with six synthetic, glioma-associated MHC class I peptides derived from absent in melanoma 2 protein **(**AIM-2), melanoma-associated antigen 1 (MAGE1), tyrosinase related protein-2 (TRP-2), glycoprotein 100 (gp100), human epidermal growth factor receptor 2 (HER2/neu), and IL-13Rα2. Treatment with ICT-107 was safe 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 DC-based vaccine which is generated using autologous DC 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 DC-based vaccination. Within this study, a vaccine site pre-conditioning strategy was applied by inducing inflammation at the vaccine site with tetanus/diphtheria (Td) toxoid prior to vaccination with CMV-specific DC pulsed with pp65 RNA (23). This approach resulted in a significant increase in PFS and OS in patients with newly diagnosed glioblastoma compared to subjects treated with DC alone.

Adoptive cell therapy

This strategy is a cell-based anti-tumor immunotherapy which involves the collection of circulating or tumor-infiltrating lymphocytes, their *ex vivo* expansion, activation, e.g. with cytokines and/or potential tumor antigens, and/or genetic modification as well as their re-administration 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 immunoglobuline 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, i.e. 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 anti-tumor effects mediated by CD4 T cells (25). Furthermore, when murine-based single-chain fragment variable domains are used, human anti-mouse 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 anti-glioma 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 auto-immune 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 IL13Rα2 which is selectively expressed in glioblastoma including glioma-initiating cells. The CAR targeting IL13Rα2 are based on ligands that are coupled to intracellular signaling elements for T cell stimulation. Administration of CAR T cells targeting IL13Rα2 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. Preclinical 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 gliobastoma are currently being planned.

Further immunotherapeutic strategies under development

Tumor-targeting antibodies

Beside 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 which 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 anti-microtubule 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 anti-tumor activity of ABT-414 in glioblastoma

patients are required. Bispecific T cell engagers (BiTEs) 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 non-pathogenic 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 immuno-competent 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 (MTD) 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 which interferes with TGF-β signaling, was safe 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 neurooncology. 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. Since 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 anti-tumor 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.

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 DC-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 anti-tumor effects and the reasonable design of appropriate trials that allow for a development of synergistic multimodal immunotherapeutic approaches

References

1. Weller M, van den Bent M, Hopkins K, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. The Lancet Oncology. 2014;15(9):e395-403.

2. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. The New England journal of medicine. 2010;363(8):711-23.

3. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. The New England journal of medicine. 2015.

4. Frei K, Gramatzki D, Tritschler I, et al. Transforming growth factor-beta pathway activity in glioblastoma. Oncotarget. 2015;6(8):5963-77.

5. Roth P, Junker M, Tritschler I, et al. GDF-15 contributes to proliferation and immune escape of malignant gliomas. Clinical cancer research : an official journal of the American Association for Cancer Research. 2010;16(15):3851-9.

6. Galluzzi L, Vacchelli E, Bravo-San Pedro JM, et al. Classification of current anticancer immunotherapies. Oncotarget. 2014;5(24):12472-508.

7. Fan QW, Cheng CK, Gustafson WC, et al. EGFR phosphorylates tumor-derived EGFRvIII driving STAT3/5 and progression in glioblastoma. Cancer cell. 2013;24(4):438-49.

8. Ramnarain DB, Park S, Lee DY, et al. Differential gene expression analysis reveals generation of an autocrine loop by a mutant epidermal growth factor receptor in glioma cells. Cancer research. 2006;66(2):867-74.

9. Schuster J, Lai RK, Recht LD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro-oncology. 2015.

10. Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro-oncology. 2011;13(3):324-33.

11. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(31):4722-9.

12. Reardon DA, Schuster J, Tran DD, Fink KL. ReACT: Overall survival from a randomized phase II study of rindopepimut (CDX-110) plus bevacizumab in relapsed glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33((suppl; abstr 2009)).

13. Hashimoto N, Tsuboi A, Kagawa N, et al. Wilms tumor 1 peptide vaccination combined with temozolomide against newly diagnosed glioblastoma: safety and impact on immunological response. Cancer immunology, immunotherapy : CII. 2015.

14. Schumacher T, Bunse L, Pusch S, et al. A vaccine targeting mutant IDH1 induces antitumour immunity. Nature. 2014;512(7514):324-7.

15. Pollack IF, Jakacki RI, Butterfield LH, et al. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and nonbrainstem gliomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(19):2050-8.

16. Okada H, Butterfield LH, Hamilton RL, et al. Induction of robust type-I CD8+ T-cell responses in WHO grade 2 low-grade glioma patients receiving peptide-based vaccines in combination with poly-ICLC. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(2):286-94.

17. Leone P, Shin EC, Perosa F, MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. Journal of the National Cancer Institute. 2013;105(16):1172-87.

18. Nava S, Dossena M, Pogliani S, et al. An optimized method for manufacturing a clinical scale dendritic cell-based vaccine for the treatment of glioblastoma. PloS one. 2012;7(12):e52301.

19. Ecoli M, Pellegatta S, Frigerio S, Finocchiaro G. Association of increased progression-free survival in primary glioblastomas with lymphopenia at baseline and activation of NK and NKT cells after dendritic cell immunotherapy. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(5s):suppl; abstr 2087.

20. Yamanaka R, Homma J, Yajima N, et al. Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005;11(11):4160-7.

21. Phuphanich S, Wheeler CJ, Rudnick JD, et al. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer immunology, immunotherapy : CII. 2013;62(1):125-35.

22. Wen PY, Reardon DA, Phuphanich S, Aitken R. A randomized, double-blind, placebo-controlled phase 2 trial of dendritic cell (DC) vaccination with ICT-107 in newly diagnosed glioblastoma (GBM) patients. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32((suppl; abstr 2005)).

23. Mitchell DA, Batich KA, Gunn MD, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature. 2015;519(7543):366-9.

24. Redd JM, Lagarde AC, Kruse CA, Bellgrau D. Allogeneic tumor-specific cytotoxic T lymphocytes. Cancer immunology, immunotherapy : CII. 1992;34(5):349-54.

25. Kohn DB, Dotti G, Brentjens R, et al. CARs on track in the clinic. Molecular therapy : the journal of the American Society of Gene Therapy. 2011;19(3):432-8.

26. Maus MV, Haas AR, Beatty GL, et al. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer immunology research. 2013;1(1):26-31.

27. Johnson LA, Scholler J, Ohkuri T, et al. Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. Science translational medicine. 2015;7(275):275ra22.

28. Sampson JH, Choi BD, Sanchez-Perez L, et al. EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. Clinical cancer research : an official journal of the American Association for Cancer Research. 2014;20(4):972-84.

29. Krebs S, Chow KK, Yi Z, et al. T cells redirected to interleukin-13Ralpha2 with interleukin-13 mutein--chimeric antigen receptors have anti-glioma activity but also recognize interleukin-13Ralpha1. Cytotherapy. 2014;16(8):1121-31.

30. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. The New England journal of medicine. 2015;372(4):320-30.

31. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014;384(9948):1109-17.

32. Belcaid Z, Phallen JA, Zeng J, et al. Focal radiation therapy combined with 4-1BB activation and CTLA-4 blockade yields long-term survival and a protective antigen-specific memory response in a murine glioma model. PloS one. 2014;9(7):e101764.

33. 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. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(no. 15_suppl 3010).

34. Curti BD, Kovacsovics-Bankowski M, Morris N, et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. Cancer research. 2013;73(24):7189-98.

35. Lin GH, Liu Y, Ambagala T. Evaluating the cellular targets of anti-4-1BB agonist antibody during immunotherapy of a pre-established tumor in mice. PloS one. 2010;5(6):e11003.

36. Gan Hk, Fichtel L, Lassmann AB, et al. A phase 1 study evaluating ABT-414 in combination with temozolomide (TMZ) for subjects with recurrent or unresectable glioblastoma (GBM). Journal of

clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32((suppl; abstr 2021)).

37. Wang X, Zhang FC, Zhao HY, et al. Human IP10-scFv and DC-induced CTL synergistically inhibit the growth of glioma in a xenograft model. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):7781-91.

38. Iwahori K, Kakarla S, Velasquez MP, et al. Engager T cells: a new class of antigen-specific T cells that redirect bystander T cells. Molecular therapy : the journal of the American Society of Gene Therapy. 2015;23(1):171-8.

39. Ning J, Wakimoto H, Rabkin SD. Immunovirotherapy for glioblastoma. Cell cycle. 2014;13(2):175-6.

40. Li M, Bolduc AR, Hoda MN, et al. The indoleamine 2,3-dioxygenase pathway controls complement-dependent enhancement of chemo-radiation therapy against murine glioblastoma. Journal for immunotherapy of cancer. 2014;2:21.

41. Zakharia Y, Johnson TS, Colman H, Vahanian NN. A phase I/II study of the combination of indoximod and temozolomide for adult patients with temozolomide-refractory primary malignant brain tumors. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32:5s((suppl; abstr TPS2107)).

42. Anderton MJ, Mellor HR, Bell A, et al. Induction of heart valve lesions by small-molecule ALK5 inhibitors. Toxicologic pathology. 2011;39(6):916-24.

43. Rodon J, Carducci MA, Sepulveda-Sanchez JM, et al. First-in-human dose study of the novel transforming growth factor-beta receptor I kinase inhibitor LY2157299 monohydrate in patients with advanced cancer and glioma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(3):553-60.

Figures “original”

Fig. 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 DC-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.

Selected Publications:

*9. Schuster J, Lai RK, Recht LD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro-oncology. 2015.

This is a report of a phase II trial demonstrating the safety of a combination therapy comprising rindopepimut and temozolomide in patients with newly diagnosed glioblastoma.

*23. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature. 2015;519(7543):366-9.

This is a phase I trial in newly diagnosed glioblastoma assessing the strategy of vaccine site pre-conditioning with tetanus/diphtheria (Td) toxoid prior to a vaccination with CMV-specific DC. Pre-conditioning promotes DC migration to the vaccine site and is associated with an increased progression-free- and overall survival compared to DC-only treated patients.

*14. Schumacher T, Bunse L, Pusch S, et al. A vaccine targeting mutant IDH1 induces antitumour immunity. Nature. 2014;512(7514):324-7.

This is the first preclinical study demonstrating the effectiveness of a vaccination with a peptide containing the IDH1 R132H mutation. The immune response is mediated by CD4 T cells and antibodies and reduced the growth of IDH1 R132H expressing subcutaneous mouse sarcomas.

* 15. Pollack IF, Jakacki RI, Butterfield LH, et al. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and nonbrainstem gliomas. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32(19):2050-8.

This phase I study is the first that demonstrates the safety and immunogenicity of a multipeptide vaccination with HLA-A2 restricted glioma-associated antigen-derived peptides in children with newly diagnosed high-grade and brainstem glioma. It also emphasizes pseudoprogression as an important immune-related adverse event.

*16. Okada H, Butterfield LH, Hamilton RL, et al. Induction of robust type-I CD8+ T-cell responses in WHO grade 2 low-grade glioma patients receiving peptide-based vaccines in combination with poly-ICLC. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015;21(2):286-94.

This phase I study is the first that demonstrates the safety and immunogenicity of a multipeptide vaccination with HLA-A2 restricted glioma-associated antigen-derived peptides in adults with low grade glioma.

**27. Johnson LA, Scholler J, Ohkuri T, et al. Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. Science translational medicine. 2015;7(275):275ra22.

This is a preclinical study assessing a CAR against EGFRvIII *in vitro* and *in vivo*. This study demonstrated a potent anti-tumor response mediated by EGFRvIII CAR transduced T cells. Furthermore, it was show that CD8 CAR cells infiltrate the brain tumor region.