The next generation of oncological hyperthermia involves the medical innovation of selectively heating up the malignant cells of the body in a controlled way. The easily-distinguishable biophysical and physiological characteristics of cancer cells and their immediate environment are the focus of the targeted energy delivery of this treatment. This heterogenic heating concept breaks with the homogeneous nature of conventional hyperthermia, where an isothermally equal temperature is applied to the large surface area of a solid tumor. Due to its selectivity, the new concept enables the usage of a significantly lower energy, making it safer, less toxic, and easier to use.

This book shows the challenges facing oncological hyperthermia, and highlights clinical results obtained in various countries. It also presents discussions about the theoretical basis of the method, adding some technical discussions and clarifying the most difficult points of its design. The contributions dealing with clinical results use state-of-art conventional therapies with complementary hyperthermia and show the advantages of such a combination.

Professor Andras Szasz is Head of the Department of Biotechnics at St. Istvan University, Hungary, and Chief Scientific Officer of Oncotherm Hungary and Oncotherm Germany. His scientific focus is biophysics, and he developed the anticancer treatment method of modulated electro-hyperthermia. He is the author of over 400 publications, including eight books.

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Cover image *Co-culture with normal human skin fibroblast: 1. Benign case - Human immortalized keratinocytes [non-tumorigenic], (HaCaT), 2. Malignant Case - Human epidermoid carcinoma cells [tumorigenic], (A431)*

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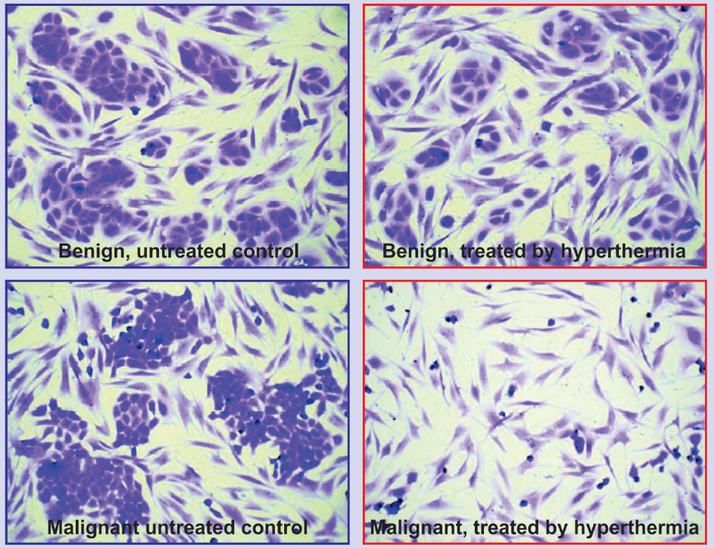
Challenges and Solutions of Oncological Hyperthermia

Andras Szasz



CHALLENGES AND SOLUTIONS OF ONCOLOGICAL HYPERTHERMIA

Edited by **Andras Szasz**

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TABLE OF CONTENTS

CHAPTER 7

PERSONALISED MEDICINE IN GLIOBLASTOMA MULTIFORME

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General overview

The prognosis of brain cancer remains poor, especially for patients with glioblastoma multiforme (GBM). Besides disease-related problems, the toxicity of neurosurgery, radiochemotherapy and maintenance chemotherapy is a burden for the patient and the community. However, paradigms for treatment are changing. For example, biological treatments and treatments based on physics have emerged as promising options for GBM patients. In addition, instead of designing treatment protocols based on pathology diagnosis and staging, targeted therapies built on the molecular machinery of the tumours are emerging, resulting in personalised medicine with different toxicity profiles that have fewer side effects. The concept of personalised medicine using all treatment modalities on the basis of daily experiences is developing strongly. A GBM interacts weakly or strongly with the immune system. Hence, the antigenic profile and surface molecules of GBM greatly differ in each patient. Specifically targeting these surface molecules forms a second dimension of personalised medicine. More knowledge is generated about the GBM tumour microenvironment and the

interaction of the immune system with GBM. Elucidating how the body's immune system fails to control the tumour and how interventions can be designed to re-establish immune control results in a third dimension of personalised medicine.

Furthermore, the immune system controls GBM, at best, in a situation of minimal residual disease. Accordingly, for each patient, an optimal treatment combination, including immunotherapeutic approaches, should be developed, resulting in a fourth dimension of personalised medicine. The response to treatment and the eventual necessary adaptation of the treatment represents the fifth dimension of personalised medicine. Ultimately, the installation of a permanent protective immune memory against GBM forms the equivalent of a roof and leads towards a permanent cure. Immunisation against GBM might be triggered by personalised combinations of anticancer treatment pillars, but immunisation against GBM might be strengthened by active specific immunotherapy aimed so as to directly stimulate the body's own immune system against the disease. The application of different individualised treatment concepts should be redefined regularly because the tumour itself and the patient are highly dynamic. Complementary medical interventions support and facilitate direct antitumor treatment combinations, immune interventions and modulations to obtain and maintain maximal control over tumour growth. It will be a challenge for medical doctors, responsible authorities and insurance companies to implement the complex concept of personalised medicine. Yet, personalised medicine seems the only way towards a possible GBM cure.

Glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most malignant form of glioma. According to the World Health Organisation (WHO), the tumour always qualifies as a grade IV malignancy. In the classification of 2016, three sub-entities were categorised: 1) secondary GBM coming out of low-grade gliomas which are mostly Isocitrate Dehydrogenase (IDH) mutated; 2) primary GBM which are mostly IDH wild type; 3) diffuse midline gliomas that harbour a histone H3K27M mutation [1]. In-depth molecular analyses, however, have categorised GBM into 6 different tumour entities with clearly different clinical profiles and molecular biology [2]. There is no known cause for the manifestation of GBM. However, a significant association has been found between increasing age, increasing immunosuppression and the incidence of GBM, suggesting that ageing

progressively suppresses normal immunosurveillance, thereby contributing to GBM cell initiation and/or outgrowth [3]. Although most GBMs are induced *de novo*, cancer predisposition syndromes include increased risk for GBM formation [4], [5], [6]. Irradiation is certainly a cause for GBM formation, and the prognosis of a second malignant GBM is extremely poor [7]. Viral infections like cytomegalovirus (CMV) (variants) have been mentioned as potential triggers for GBM formation, and some therapeutic approaches have been developed targeting CMV [8]. Finally, long-term exposure to higher doses of non-ionising irradiation has been associated with the formation of GBM, although the causality has yet to be proven [9]. The risk for GBM formation seems decreased in patients with atopic diseases [4].

GBM is the most common primary brain malignancy in adults. Nevertheless, its incidence is low, with about 4 new diagnoses per 100,000 adults per year [10]. Hence, GBM is considered an orphan disease, which makes the development of new treatments more challenging. Drug developmental programmes for orphan diseases are facilitated by regulatory authorities. Despite the low incidence of GBM, the severity of the consequences for patients and the community are extremely high. First, the acute appearance of symptoms has the potential to cause disabilities and affect the quality of life, not only for the patient but also for the surrounding family and friends.

Additionally, the chances for long-term overall survival (OS) are virtually absent [11], [12]. With the current standard therapies, the median OS of GBM is less than 15 months, and there is almost no chance of long-term survival [13]. This hard reality is reflected in two studies, demonstrating that, compared to other cancer entities, the highest number of years of life lost due to cancer is caused by GBM [14], [15]. This is due to the combination of relatively young age at the time of diagnosis and poor OS. The low investment in GBM research further contributes to the lack of improvement over the last decades. Still, the prognosis varies, not only based on the molecular subgrouping [2], but also the clinical characteristics of the patients. Counselling on the prognosis for patients should be based on recursive partitioning analysis (RPA), in which age, extent of resection, grading of tumour, Karnofsky performance index, Mini-mental state and quality of radiotherapy are taken into consideration [16], [17], [18].

GBM is a systemic brain disease. Its biology is very complex [19]. The tumour consists of residing glioma cancer stem cells, bulky growing tumour cells and infiltrating tumour cells. It contains several subclones with relative

variable presence during the disease. This is reflected in the so-called epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (TEM) [20]. The fast growth and lack of oxygen supply induce deficient neovascularisation [21]. The changed metabolism, with fast glycolysis outside the mitochondrial respiration, increases the oxidative burden in the tumour microenvironment (TME) [22]. The TME is further complicated by a high presence of microglia, tumour associated macrophages (TAM), myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) [23]. The combined tumour cell-bound and tumour cell-secreted immunosuppressive factors, together with the presence of immune-suppressive cells, contribute to a strong local immunosuppressive TME and even to systemic immunosuppression. Reflecting this biological reality in the light of the paradigm of tumour immune surveillance, consisting of elimination–equilibrium–escape [24], [25], [26], it becomes obvious that there is just a vanishingly small chance for the spontaneous development of anti-tumour immune responses in the course of the disease; this leads to the failure of the immune system to control tumour development and growth resulting in an almost immediate tumour escape. That is likely the reason why the monotherapy with checkpoint blockers currently being investigated in many clinical trials fails, even if the target is present on some tumour cells [27].

The procedures for accurate diagnosis in the context of GBM suspicion are quite simple. In the acute situation, a computed tomography (CT) scan can be performed in the short term for the diagnosis of urgent clinical problems that require immediate surgical or medical treatment. The standard diagnostic procedure is magnetic resonance imaging (MRI). Standard imaging protocols have only been developed recently throughout the radiology community (https://www.awmf.org/leitlinien/detail/ll/025-022.html). The MRI investigation can be further elaborated with perfusion MRI, diffusion MRI and/or spectroscopy. Functional MRI brain imaging may be required for neurosurgery planning. Nuclear medicine imaging is very rarely needed during diagnosis.

Treatment elements in the therapy for GBM: five pillars under one roof

In the last 100 years, the treatment of patients with GBM has greatly changed. Incredible developments were realised in all disciplines involved in the treatment. During the diagnostic phase, technical evolutions have been implemented systematically over time, resulting in improvements to

local treatments, precise staging, accurate treatment planning, and follow up. Both MRI technologies and nuclear medicine imaging technologies give information on the anatomical structures of cancer lesions in relation to the healthy environment [28], [29]. Modern imaging technologies, like advanced MRI technologies, Single Photon Emission Computed Tomography and Positron Emission Tomography technologies, provide additional insights into the molecular biology and behaviour of cancer lesions.

Surgery

The first approach for the treatment of GBM is surgery. Oncologic neurosurgery has two main goals to fulfil. The first goal is to remove as much tumour tissue as safely possible and, also, to relieve space occupied by the expanding tumour. The extent of resection remains a major prognostic factor [17], [30]. Therefore, imaging techniques for use during neurosurgery have been developed, such as intraoperative MRI, and 5-Amino-Levulinic Acid (5-ALA) technology. The evolution of modern neurosurgical technologies has been partly supported by accompanying evolutions in electrophysiology, anaesthesiology support and intensive care medicine, as well as in nursing and supportive care. The second goal of surgery is to obtain tumour tissue for the appropriate diagnosis. Modern tumour diagnostics are aimed such as to categorise patients into correct diagnostic (sub)groups so that appropriate and risk-adapted treatment protocols can be initiated after surgery. The diagnostic procedures, which were almost exclusively by classical immunohistochemistry, have expanded nowadays with molecular biological test systems, including genetic and epigenetic tests. This allows a much more refined disease categorisation [2]. Since treatment is becoming more dependent on advanced molecular diagnostics, clinicians hold a plea for appropriate tumour tissue handling as a critical step during surgery [31]. Additionally, an accurate diagnosis of germline mutations at the time of tumour diagnostics greatly influences further treatment planning [5], [6].

Radiotherapy

Radiotherapy as local antitumour treatment has existed for more than 50 years [32]. Safety remained one of the most important issues in the development of radiotherapy over the years. Special value is put on the dosing, fraction and field of irradiation [33]. Attempts using hypofractionation and hyperfractionation usually did not result in major changes regarding the ultimate outcome of the patients [34], although hypofractionation is now recommended for elderly patients [35] and might induce some new

treatment avenues for younger patients [36]. The design of the most appropriate field was a great challenge, and technologies like Intensity-Modulated radiotherapy have been entered into clinical application [37]. Another approach to receive local radiotherapy was the conjugation of radioisotopes to antibodies or metabolites [38]. By changing photon radiotherapy towards proton radiotherapy, the field demarcation improved a great deal [39], [40]. However, other challenges appeared; for example, more stringent nursing care around the treatment sessions is usually needed. Additionally, it is not yet proven that proton therapy results in an improved OS as compared to photon therapy. However, it has been proven for distinct cancer entities that the sharp field demarcation allows a higher tumour dosage without affecting the important healthy tissues just beside the target field [41]. The toxicity of radiotherapy has remained an issue over the years, especially considering neural toxicity, endocrine toxicity and the induction of secondary malignancies [42].

Chemotherapy

Chemotherapy is the third pillar of anticancer treatment strategies. Although developed shortly after surgery and radiotherapy, a huge progression has been realised over time. Chemotherapy may even cure cancer patients, which is especially true for hematologic malignancies [43]. Different types of anticancer drugs have been developed over time. Amongst those are alkylating agents, antimetabolites, antitumour antibiotics, mitotic inhibitors, topoisomerase inhibitors and steroids. The specialisation of medical teams and nursing teams, and the development of supportive treatments to conduct chemotherapy allowed the evolution of combined chemotherapy schedules. These combined intense chemotherapy treatment protocols contributed to and increased the cancer cure rate, especially in children. In the context of GBM, routes of administration were adapted towards application into the cerebrospinal fluid to reach the intracranial space behind the blood-brain barrier (BBB). Intra-arterial chemotherapy allows the administration of higher doses to achieve better anticancer effects without increasing toxicity. The downside of the intense chemotherapeutic protocols is multiple side effects, both short-term (like bone marrow suppression, vomiting) and long-term (renal dysfunction, audiology, neural toxicity, second malignancy). For patients with GBM, surgery, subsequent radiochemotherapy using Temozolomide (TMZ) and radiation, followed by maintenance chemotherapy with Temozolomide (TMZ) for 6 cycles became the worldwide standard of care in the last 15 years [11], [12]. This combined treatment strategy also forms the backbone of further developments. TMZ during radiochemotherapy functions at multiple levels

and the effects of TMZ on tumour cells are rather complex [44]. TMZ has been described to have a radiosensitising effect [45] and acts as an alkylating agent resulting in direct anticancer activity [46]. Furthermore, TMZ has been shown to downregulate PD-L1 expression on tumour cells [47]. At the same time, TMZ induces hypermutation [48]. Finally, TMZ also has an effect on the TME by reducing the load of Treg [49]. The latter, together with a moderate T cell depletion in general, has been considered as a favourable starting point from which to initiate dendritic cell (DC) vaccination therapy as part of the primary treatment of GBM [50], [51], [52].

Targeted (immuno)therapy

The lack of further improvement in the cure rate – despite the modern advances in surgery, radiotherapy and chemotherapy – the increasing focus on the quality of life of longer-term surviving patients and the increased knowledge in molecular biology of tumours – plus the discovery of druggable pathways –all led to the creation of a fourth major pillar in anticancer treatment strategies, namely, targeted cancer therapy or precision medicine. This approach has been developed within the last 30 years. Targeted therapies contain many types of treatment approaches, deliberately designed to act on specific targets that are associated with cancer, and hence causing (at first glance) fewer side effects compared to classical chemotherapy [53], [54], [55], [56], [57], [58]. In general, targeted therapies include receptor-targeted therapies [38], gene expression modulators [59], apoptosis inducers [60], angiogenesis inhibitors [61], toxin delivery molecules [38], nanocarriers [62], [63], [64] and even several forms of immunotherapeutics. Examples of the last-named are antibodies against particular antigens inducing antibody-dependent, cell-mediated or complement-dependent cytotoxicity [65], [66], checkpoint inhibitors that unblock eventually existing anticancer effector immune cells [67], [68], [69], [70], Chimeric Antigen Receptor (CAR) T cells [71], [72], [73] or T cell receptor (TCR)-transduced T cells [74]. A critical review pointed to the overall disappointing results of targeted therapies for GBM [75].

However, the implementation of targeted therapies caused a major paradigm shift in oncology. For the first time, the GBM patient did not adapt to a certain treatment protocol anymore, but treatment became designed for the GBM patient. As already mentioned, personalised medicine is the new term to cover this concept. The big scientific challenge in the implementation of personalised medicine is a study design to prove the added value of personalised medicine versus classical protocol oncology using placebo-controlled, double-blind randomised clinical trials that result in evidence-

based medicine. In addition, clinical trials with combinations of targeted drugs have been hindered. First, there are economic reasons, as most drugs are developed by pharmaceutical companies. Next, the particular methodology in clinical trial research is focused far too much on a step-by-step approach. Even in modern clinical trial designs, immediate testing of combinational strategies is rarely performed. Hence, the debate is ongoing on how to cure brain cancer [76].

Biologic/physics therapy

Although GBM is a systemic brain disease, first attempts at medical care involved local treatment approaches. Because surgery could not be curative for GBM, local irradiation with broad margins was implemented into standard therapy first. Next, chemotherapy became part of standard treatment, significantly shifting the OS curve in the right direction. Nevertheless, chemotherapy has never provided any chance for long-term survival [11], [12]. Recently, the use of electromagnetic waves outside the spectrum of ionising irradiation has been implemented as an anticancer treatment strategy. The tumour-treating field (TTF, Optune), administering 200 kHz waves with an electric field of 1 to 3 V/cm over the entire brain was implemented, and the efficacy has been shown in a large phase III randomised clinical trial [77]. The aim of this technology is to disrupt the mitotic spindle and the membrane integrity of the tumour cell [78], [79]. Although the working mechanism of TMZ depends on cell division, TTF in combination with TMZ as maintenance therapy after radiochemotherapy has been implemented into daily routine. The continuous need for cell cycle arrest requires the use of TTF during at least 85% of the time, which raises concerns on the quality of life during treatment [80]. It is worthy of note that recent insights have pointed to the efficacy of TTF as an inducer of immunogenic cell death (ICD). Hence, its additive effect on tumour control is neutralised by a dexamethasone dose > 4 mg per day [81].

While TTFs have been applied to the entire brain over long periods of time, other technologies use electromagnetic waves at a higher frequency (13.56 MHz) and intensity (2 to 2.5 V/cm) focusing just on the tumour area for a shorter period (about one hour). The tumour cell selectivity of modulated electro-hyperthermia (mEHT) is linked to the increased fermentation pathway of glycolysis (Warburg effect), the changed ion concentration and the permittivity of tumours compared to adjacent normal tissues [82]. Clinical efficacy in patients with relapsed GBM has been published [83], [84]. Besides non-thermal effects like membrane disruption and the induction of mitotic catastrophe, mEHT causes dielectric thermal effects [85]. It improves

the immunological TME for subsequent DC therapy [86]. It is the experience of the authors to use mEHT at moderate intensity, thereby inducing ICD without inducing necrosis and inflammation [87], and thus avoiding the toxicities mentioned in earlier studies using much higher doses [88].

Another area for innovative treatments in the field of GBM is the use of oncolytic viruses (OVs) [89], [90], [91], [92]. Several viruses are under investigation: non-engineered or naturally oncolytic agents from which the host is non-human (reovirus, Newcastle Disease Virus [NDV], parvovirus) or attenuated strains of human viruses (mumps, polio, vaccinia), human pathogenic strains engineered to be onco-specific (adenovirus, herpes simplex virus) and engineered-armed viruses that produce cytokines like GM-CSF or IL-12. All these viruses have two major working mechanisms in common, albeit with different relative weights: they aim to kill tumour cells directly and they aim to induce an anticancer immune response. The mechanism for the onco-specificity of the viruses is different for each virus and is based on the lack of type I interferon production, tumour-specific entry receptors and/or pathways or tumour cell metabolic rate. Of particular importance, some viruses like NDV have the capacity to induce ICD in the context of GBM [93]. Combinations of OVs together with Checkpoint inhibitors are the focus of current research [94], [95].

Towards active specific immunisation

All the methods described above as the five pillars of anticancer therapy are directed against the tumour. A cure, however, is only possible through the generation of an active intrinsic immune protection, kept going long-term by the induction of an anticancer immune memory. All five of these treatment elements contribute to the generation of an active antitumour immunisation and can even trigger the body's own immune system to build up active immune protection. Simultaneous maximal neurosurgical debulking of the tumour removes a major source of immunosuppressive mechanisms, and hence changes the balance in favour of immunotherapeutic approaches, which are best performed with minimal residual disease [18], [96], [97]. A lot of research in the field of radiotherapy is now focusing on the induction of ICD, although for GBM there is not much data available, yet [98]. The immunologic effects of TMZ chemotherapy have already been mentioned [47], [48], [49]. HDAC inhibitors can exert divergent effects on tumour-host interactions [99], [100], [101]. The role of targeted immunotherapies like antibodies and target-specific T cells is obvious [65], [66], [67], [68], [69], [70], [71], [72], [73], [74]. The role of the immune system as part of the working mechanism of TTF has been recently suggested [81]. Moderate

modulated electro-hyperthermia has been demonstrated to build up an active immunisation against the tumour via ICD [82], [86]. Finally, the role of the immune system in the working mechanism of OVs has been previously described in detail in the former paragraph of this chapter [89], [90], [91], [92], [93], [94], [95]. Additionally, active vaccination technologies aiming to stimulate the immune system against tumour antigens directly might be necessary to help active specific immunisation against cancer, to reach ultimate anticancer immunisation, for long-term protection and for a cure. Finally, immunomodulatory strategies might be needed to reach this goal. Hence, within the context of the previously used metaphor, active specific immunotherapy to yield active specific anticancer immunisation may be considered as the roof, built up and supported by the five anticancer treatment pillars (figure 1). Several meta-analyses have described a significantly increased percentage of long-term surviving GBM patients when treated with active specific vaccination treatments [102], [103], [104], [105].

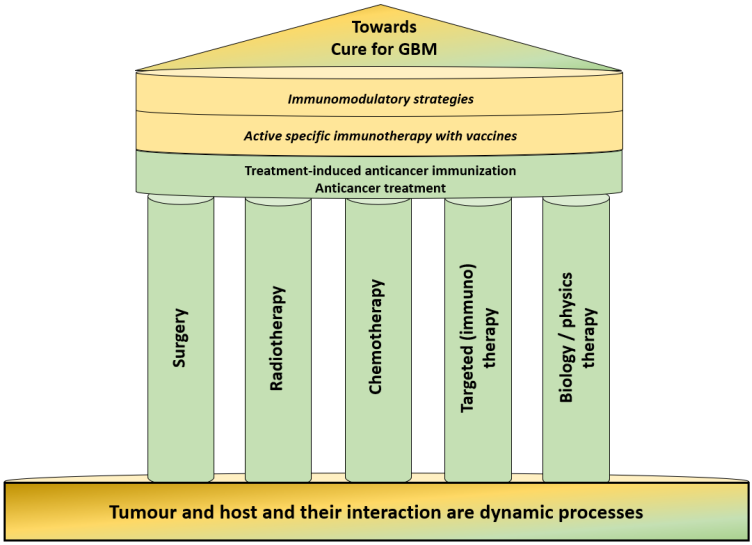

Figure 1. **Combined treatment approaches.** Treatment modalities in the vertical axis are targeted against glioblastoma multiforme (GBM). These treatment modalities can have indirect effects on active specific immunisation against GBM. The “active specific immunotherapy” treatment modality stimulates the immune system of the body against GBM. An integrated approach of all treatment modalities can ultimately result in a cure for GBM.

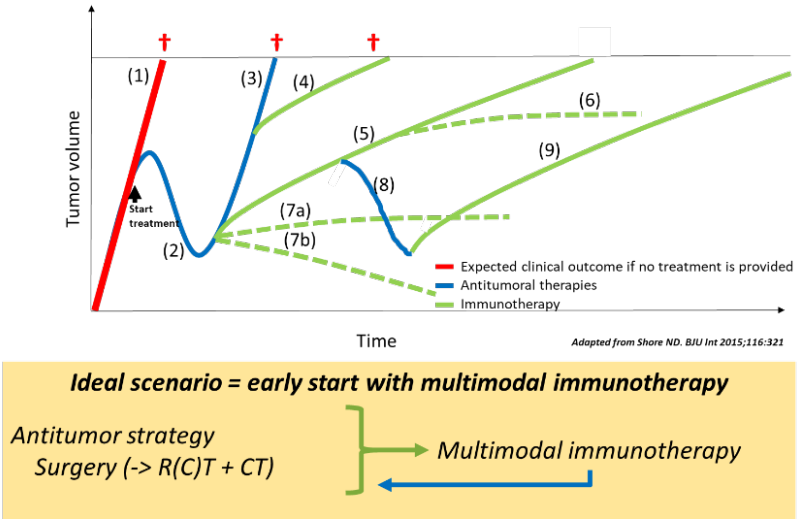

Figure 2. **The ideal scenario for the combining of anticancer treatment strategies with immunotherapy strategies.** This is a theoretical representation of the evolution of GBM tumour volume during time. Without diagnosis, a GBM tumour grows to a deadly tumour volume (1). However, at a certain point, the tumour is diagnosed, and the treatments to reduce the tumour volume and to keep a low tumour volume for as long as possible are initiated (2). However, early or late, the tumour might regrow (3). The immune system aims to reprogramme the body to fight cancer and slow down its growth. (4). When immunotherapy is started late, the gain in overall survival is less than when immunotherapy begins at a minimal residual tumour volume (5). The immune system might be able to stabilise the tumour volume at a certain level (6). When immunotherapy is started early during treatment, lower levels of tumour-mediated immune suppressive effects might result in better control of the tumour volume evolution (7a, 7b). When, however, the tumour is progressive, one can again use anticancer strategies to reduce the tumour volume (8) and maintain the slower tumour evolution due to the immune memory (9).

All treatments should be integrated together to create protection against cancer progression. The efficacy of active specific immunotherapy on long-term OS is strongest when placed early in treatment, as mentioned previously [106]. We have elaborated on this concept for GBM, including the multimodality of most anticancer treatments (figure 2). By introducing anticancer treatment strategies, one aims to reduce tumour volume. However, the fear is that a relapse might occur. Similarly to the figure published by Shore et al. [106], the gain in OS with vaccination late in the

treatment, at a stage of relapse or progress, is less than when immunotherapy is placed early in treatment when the tumour load is minimal. Moreover, the chance to get a stable tumour status or the chance to slow down or even reduce further residual tumour volume increases when immunotherapy is introduced at minimal residual disease. Finally, if the tumour progresses again, direct anticancer strategies can be used again, thereby maintaining the anticancer immune response. In this way, cancer becomes a chronic disease kept under control by the immune system and, eventually, a non-immunosuppressive intermittent anti-cancer treatment.

Combination treatment for GBM: from protocol medicine towards personalised medicine

Personalisation at the level of the molecular biology

Over the last ten years, insights into the molecular biology of GBM have increased tremendously. It has clearly been shown that O^6-methylguanine-DNA methyltransferase (MGMT) promoter-unmethylated GBMs, resulting in high MGMT metabolic activity, are much less sensitive to the tumour killing activity of TMZ [107]. Although the standard of care still includes TMZ as treatment, a lot of clinical trial protocols are stratified on the MGMT methylation status of the patient (for example, see reference [108]). The use of patient's MGMT status was the first introduction of personalised medicine within the domain of neuro-oncology.

Over time, many other druggable targets were discovered [53], [54]. These druggable targets included the overexpression and/or mutation of growth factor receptors and different types of signalling pathways. Many targeting drugs have been tested in the context of target-expressing GBM, yet there has been no breakthrough [55], [56], [57], [58]. The existence of the BBB, which hampers adequate bioavailability, remains one particular challenge. Another problem is the lack of the uniform expression of the target in all tumour cell clones. Hence, upon treatment, non-sensitive clones of tumour cells selectively expand [109]. Only recently has there been an increase in the number of strategies using more than one drug, and an increase in the number of drugs that can pass the BBB [110].

Personalisation at the level of surface antigens

Cell surface molecules have been used as targets via the immune system [38]. Along the same lines, CAR-T cells have been created against targets like IL-13Ra, EGFRvIII or GD2 [71], [72], [73]. The particular hurdle for

these approaches, again, is the lack of the uniform expression of the target on all tumour cells, thereby allowing clonal selection. Finally, checkpoint blockers like anti-PD-(L)1 have been studied in the context of GBM [68], [69], [70]. Although most studies did not result in any effect, a potential benefit has been demonstrated for hypermutant GBM resulting from germline biallelic mismatch repair deficiency. The chance for a spontaneously induced anticancer immune response in these tumours is much higher due to the high mutational burden resulting in increased antigenicity [111]. Additionally, the influence of TMZ treatment on the immune system should be considered [44], [47].

In the domain of active vaccination strategies, specific antigens have been used in the vaccine as a strategy of personalised medicine. The most elaborated example is the EGFRvIII-targeted vaccine [108]. It is worthy of note that targeting one single target moiety induced a tumour escape mechanism via downregulation of the target antigen [112]. This finding supports the idea that the use of multiple antigens in one vaccine might be advantageous. On this basis, mixtures of commonly spread glioma-associated antigens have been used [113], [114], [115]. Other authors have used patient-derived whole tumour lysate [52], [116], [117], [118], [119], [120], [121], [122], [123], acid-eluted peptides [124] or mRNA [125] from tumour cells as a source of antigen for loading DCs, thereby creating an entirely personalised vaccine. A further step in the personalisation of cancer vaccines is the creation of individualised mutanome vaccines using RNA-based poly-neo-epitope approaches [126] and HLA ligandome tumour antigen-based vaccines [127], though their use in GBM has not yet been published.

Given the changing subclone profile of GBM in response to ongoing anticancer treatments, one should consider that the antigenic profile at the time of vaccination is not like the antigenic profile at the time of diagnostic tissue sampling. We, therefore, have been looking for strategies to obtain contemporary tumour antigens at the time of DC vaccination. GBM EVs have clearly been demonstrated and are the focus of study as the serially accessible biomarkers for diagnosis and treatment response [128]. Based on their scale, EVs are subdivided into exosomes, extracellular microvesicles and apoptotic bodies [129]. With the exception of exosomes, EVs include MHC molecules from tumour cell membranes, including the presented peptides. We obtained evidence that several days of ICD induction with injections of NDV and treatments with mEHT could induce an increase of antigenic extracellular microvesicles in the blood, which can then be used for DC loading [130]. By doing so, the antigenic profile of the vaccine

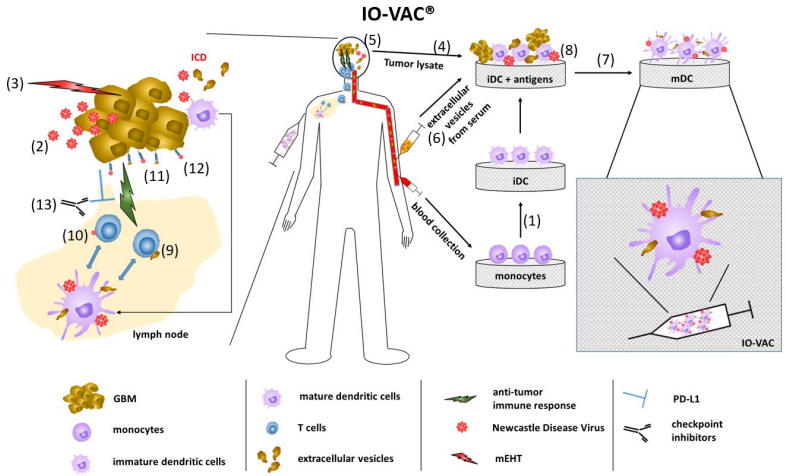

Figure 3. **The production process behind IO-VAC®.** Adherent monocytes are taken out of peripheral blood and cultured for 5 days in the presence of IL-4 and GM-CSF towards immature dendritic cells (1). During these 5 days, daily injections of Newcastle Disease Virus (NDV) (2) and daily treatments with modulated electro-hyperthermia (mEHT) (3) are given. They are loaded with antigens derived from tumour lysate (4), obtained from resected tumour tissue (5). They are also loaded with NDV/mEHT-induced serum-derived antigenic extracellular microvesicles and apoptotic bodies obtained from the serum (6). Loaded dendritic cells (DCs) are further matured with IL-1-beta, IL-6 and TNF-α (7) and with NDV (8) as described [87]. IO-VAC® is injected with the aim to stimulate the T cells in the lymph nodes that recognise the antigens (9) and other T cells that recognise NDV (10). The former T cells must act against the tumour cells (11), and the latter T cells must react against virally infected cells; hence, the tumour cells (12). Finally, as the tumour possesses immune suppressive mechanisms, personalised blockage of these should be added into the immunotherapy concept. For example, the interaction of PD-L1 on tumour cells with PD-1 on activated T cells can be blocked with anti-PD-(L)1 antibodies (13).

GBM: glioblastoma multiforme; ICD: immunogenic cell death; iDC: immature dendritic cells; mDC: mature dendritic cells; mEHT: modulated electro-hyperthermia; PD-L1: programmed death ligand 1.

eventually comes to include tumour antigens of existing (residual) tumour cells at the time of ICD induction (figure 3). The vaccine can stimulate T cells against tumour antigens. To maximise the potency of the vaccine, NDV is used as part of the maturation cocktail acting as a single strand RNA virus in synergy with a cytokine cocktail consisting of IL-1-beta, IL-6 and

TNF-α for DC maturation. At the same time, NDV antigens are included in the antigenic profile, thereby obtaining the potency to stimulate NDV-reacting T cells that target NDV-infected tumour cells. This complex, personalised and potent vaccine, called IO-VAC®, is an approved advanced therapy medicinal product in Germany (DE-NW-04-GMP-2015-0030). Experiences using this vaccine for GBM patients have been published previously [87].

Personalisation at the level of tumour-host interaction

The passive or active immunotherapeutic approaches described above are explicit examples of personalised medicine that affect tumour-host interactions. The effect of highly personalised DC-based vaccines on the prolongation of OS in patients with GBM has been demonstrated in meta-analyses and extensive reviews [102], [103], [104], [105]. However, as of today, no single placebo-controlled double-blind randomised clinical trial has proven this outcome, although the initial data are quite encouraging [131]. The lack of these types of trials lies in the combination of several hurdles that affect trial design: the disease rarity, the heterogeneity of the disease with different clinical and laboratory prognostic factors, the personalisation of the treatment, the deadly character of the disease, the search for escape treatments and the long-term OS as the single important primary end-point.

Also, within the tumour itself, personalised treatment approaches can be designed to improve tumour control. The TME of GBM is a very complex phenomenon in which cells from different origins meet each other: tumour cells from neuro-ectodermal tissue, microglia derived from primitive macrophages of the yolk sac and hematopoietic myeloid cells, and different inflammatory cells from the hematopoietic system [23]. The relative amount of these populations depends on the molecular subtype of GBM [119], [132], [133]. The TME is strongly immune-suppressive, based on a particular mixture of immunosuppressive stromal elements and cells like tumour-associated macrophages, MDSC and Tregs. These stromal elements and immunosuppressive cells contribute to the “cold” TME, pointing to the fact that there is only a low influx of anticancer immune cells in GBM. The presence of cytotoxic effector T cells in the TME has been shown to correlate with a good prognosis [134]. Therefore, targeting these negative elements in a personalised setting might facilitate immune control. Several examples exist in current practice: Tregs are targeted with low dose metronomic cyclophosphamide [135] or an anti-IL-2Ra blockade [136], MDSC can be targeted with short pulses of high dose all-trans-retinoic-acid

[137], [138], [139], Cox2 inhibitors are used to lower prostaglandin production [140] and bevacizumab [61] and an antisense oligodeoxynucleotide [141] target VEGF and TGF-b, respectively. Here, it needs to be mentioned that the administration of ICD-inducing OVs also might have a strong impact in improving the transition of TME from "cold" to "hot" [93].

GBM not only has an immunosuppressive TME but also causes systemic immunosuppression [142]. In a recent study, strong correlations between immune profiles of circulating lymphocytes prior to and after radiochemotherapy versus OS were found when the extent of the resection and the DC vaccination schedule were used as stratification variables [143]. This indicates that a strong interaction between a given functioning systemic immune system and the tumour stage at diagnosis, as well as during treatment, determines OS.

Personalisation at the level of combined treatment strategies

Till recently, cancer treatment had been mostly expounded in predetermined treatment protocols. For GBM, combination approaches like surgery followed by radiochemotherapy and maintenance chemotherapy were implemented. The addition of novel treatment strategies, exemplified by the addition of TTF [81] or immunotherapy [50], [51], [52], [108] to the Stupp-based standard treatment, is the focus of research. In the design of combination approaches, the working mechanism of the particular treatment element, mechanisms causing side effects and the kinetics of the effects on the tumour level and the host level should be considered. Two examples are illustrated: 1) Treatments that stimulate T cell proliferation in combination with TMZ, that kill proliferating T cells, are futile; 2) It is known that TMZ reduces PD-L1 expression on primary GBM tumour cells [47], and that radiochemotherapy might induce deep lymphodepletion [144]. It is not logical to add PD-1 – PD-L1 checkpoint blockers at the start of radiochemotherapy because of the loss of target molecules and the loss of T cells.

We believe that the mode of killing cells by the alkylating agent TMZ can be combined with ICD provided by NDV injections and mEHT. TMZ disrupts the genetic structure of tumour cells and can thereby kill dividing tumour cells, while NDV and mEHT can kill all tumour cells, including the non-dividing tumour cells and glioma cancer stem cells. Moreover, the TME will change, and DCs will be alerted by danger-associated molecular patterns. If ICD treatment is scheduled shortly after the 5 days of TMZ treatment, there are still about 2 weeks left to allow immune reactivity

before the next TMZ course. We now schedule the DC vaccinations after TMZ maintenance chemotherapy, so that dividing T cells are not affected by TMZ. Although there were arguments to insert DC vaccination immediately after radiochemotherapy in former clinical trials for primary GBM patients [52], [51], [52], the change to postpone DC vaccination until after TMZ maintenance chemotherapy is supported by data from the randomised HGG-2010 trial showing a trend to a higher 2-year OS in the late (37%, CI 95% = 13) versus the early vaccination arm (31%, CI 95% = 13%) for both completely resected and less than completely resected subgroups of patients [143].

For the next step, immunomodulatory approaches should be inserted into the global combination treatment strategy. One clinical trial for relapsed paediatric GBM includes low dose cyclophosphamide to deplete Tregs (NCT03879512). Anti-PD-1 monoclonal antibody therapies can be implemented in the case of MMR syndromes and a high mutational load of tumour cells [111]. We demonstrated the fluctuation of PD-L1 expression on circulating cancer cells during immunotherapy [87]. Hence, it can be useful to reconsider treatment with checkpoint inhibitors at a later time, even if the original tumour was PD-L1 negative. Finally, we implemented the use of short pulses of high dose oral All-Trans-Retinoic Acid (ATRA) in combination with Acetazolamide diuretics to avoid a headache resulting from pseudotumour cerebri. The chief dominant role of MDSC in GBM is well known in preclinical and clinical research [145], [146]. The potency of short pulses of high dose ATRA to deplete MDSC has been described [137], [138], [139].

The current multimodal treatment strategy, including multimodal immunotherapy, was implemented in clinical practice (figure 4), and the first cohort of patients treated according to this personalised medicine approach has been published [87]. The updated (01/04/2019) OS curve of this patient population is shown in figure 5. The 2-year OS is 52% (CI 95% = 29). With a median follow up of 22 months (range 10 to 43), the median OS had not yet been reached.

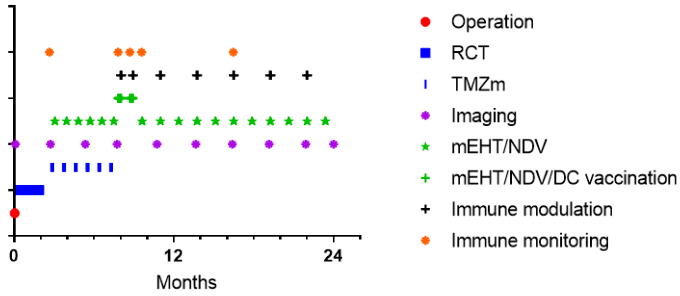

Figure 4. **Current combined treatment strategy for patients with a primary diagnosis of GBM.** After neurosurgery and radiochemotherapy, an immunodiagnostic analysis is performed prior to the first 5-day maintenance chemotherapy. At days 8 to 12 of each 28-day cycle, injections with Newcastle Disease Virus (NDV) and treatments with modulated electro-hyperthermia (mEHT) are included. After the maintenance chemotherapy cycles, two vaccination cycles are added. Each of these consists of 6 doses of NDV injections, 6 treatments with mEHT and one autologous dendritic cell (DC) vaccine injection loaded with autologous tumour antigens. Finally, immunomodulatory treatments are initiated. After the DC vaccination cycles, maintenance immunotherapy is continued, consisting of mEHT, NDV and immunomodulatory treatments.

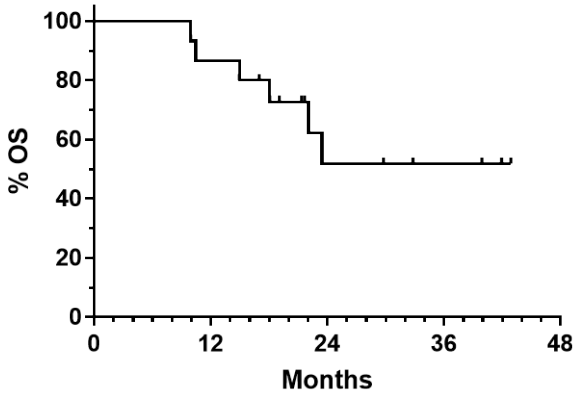

Figure 5. **Overall survival curve of patients with the first diagnosis of a primary GBM, treated with Stupp-based treatment combined with multimodal immunotherapy.** The updated survival (01/04/2019) of a previously published series of patients [87] is shown. The two-year OS is 52% (CI 95% = 29). With a median follow up of 22 months (range 10 to 43), the median OS was not yet reached.

What are the possibilities for combined treatment approaches at the time of relapse? Current experiences have been built up to combine lomustine or even Procarbazine, CCNU and vincristine (PCV) treatment with ICD induction consisting of NDV injections and mEHT. Again, after chemotherapy, full multimodal immunotherapy, including NDV injections, mEHT treatments, DC vaccines and immunomodulatory strategies are provided.

Another interesting approach is the combination of perillyl-alcohol (POH) intranasal inhalation in combination with multimodal immunotherapy. POH is a chemical product with multiple antitumoral activities and is the first anticancer treatment for brain tumours using the intranasal route. Its effect against malignant glioma has been demonstrated in several clinical trials [147], [148], [149], [150], [151]. Because of its local anticancer activity within the brain, the systemic immunity is not affected, and hence can be stimulated via multimodal immunotherapy in parallel with the POH inhalations.

Personalisation at the level of response to treatment

Response to treatment, and the eventual adaptation to new treatment strategies in case of no response, is a key factor in the determination of the ultimate prognosis for an individual patient. Response to treatment can be located both at the tumour level and at the level of the immune system. TMZ-induced radiosensitisation might induce radionecrosis and inflammatory response with an increased leakage of the BBB and, hence, increased contrast enhancement at the tumour location. This phenomenon of pseudoprogression is well known in literature, and MRI criteria for assessing tumour response have changed from the MacDonald [152] to the RANO criteria [153], additionally keeping clinical findings and the need for treatments in the assessment of response to treatment. It has been recognised that an immune attack on the tumour, similarly, can result in transient changes in MRI contrast, reflecting pseudoprogression. Therefore, adapted iRANO criteria have been released, including work-flows on how to deal with each individual patient under such conditions [154]. A whole panel of non-invasive diagnostic tests, including advanced MRI, PET, liquid biopsy, radiogenomics and radiomics, are now under development for predicting and monitoring treatment response in GBM [155].

The response to the treatment is visible via the immune system as well. The previously published results depicted the sometimes-dramatic change in immune profiles due to radiochemotherapy [50]. Significantly more data should be collected by which to assess to what extent these changes have an

influence on the ultimate outcome of the patient [143]. The efficacy of active immunisation strategies is usually assessed via immunomonitoring tests like Elispot, tetramer staining and FACS analyses, among others, on (eventually ex vivo expanded) immune cells. It remains difficult to correlate immunomonitoring data with the ultimate outcome of the patient [116]. Other tests focus on the relative change of immune cell populations [156] or Th1/Th2 shifts [87] during treatment. PanTum tests, which detect TKTL1 and Apo10 as tumour-related markers via intracellular staining in circulating CD14+CD16+ monocytes, have been used to monitor patients [157], and first experiences in the context of GBM treatment monitoring have been published [87]. Moreover, these tests even seem to reflect ICD efficacy day-by-day, and preliminary results have been released [130].

Anticancer medicine and complementary medicine

Reviewing all potential treatment strategies, it becomes evident that two major treatment axes meet each other at the tumour site in an attempt to control tumour growth (figure 6). One axis comprises all direct anticancer treatment strategies (surgery, radiotherapy, chemotherapy, targeted therapy), with the potential side effect that they can also systemically affect the body. The second axis comprises strategies to reposition the body's own defence mechanisms against the tumour by strengthening immune control (active specific immunotherapy, facilitated eventually by targeted immunotherapies, immunomodulatory strategies, OVs and electromagnetic waves). Immunomodulatory strategies have the potential side effect of inducing immune-related adverse effects. These two treatment axes can be designed to promote each other via the concept of ICD. Both treatment axes should start early in the course of the disease (figure 2). The induction of an active specific anticancer immunisation with the induction of immune memory response allows long-term control over cancer. Besides these important treatment axes, a whole area of complementary approaches has been developed and is in current clinical use, not only as prescribed by medical doctors but also directly purchased and taken by the patients themselves. It is beyond the scope of this manuscript to review all these complementary medical strategies. It should still be emphasised that all these treatments may eventually facilitate the alleviation of the patient's burden, but not result in a cure. Potentially useful complementary treatments in the context of GBM might be the metabolic cocktail including Atorvastatin, Doxycycline, Mebendazole and Metformin (NCT02201381). Melatonin, CBD/THC and low dose naltrexone belong to the psychoneuroendocrine-immunotherapy of cancer [158]. The role of the ketogenic diet has been demonstrated, at the preclinical level [159], and also

in clinical practice [151]. It is less clear whether the whole area of repurposing drugs belongs to the complementary medicine or might have direct antitumour effects as well [20], [160].

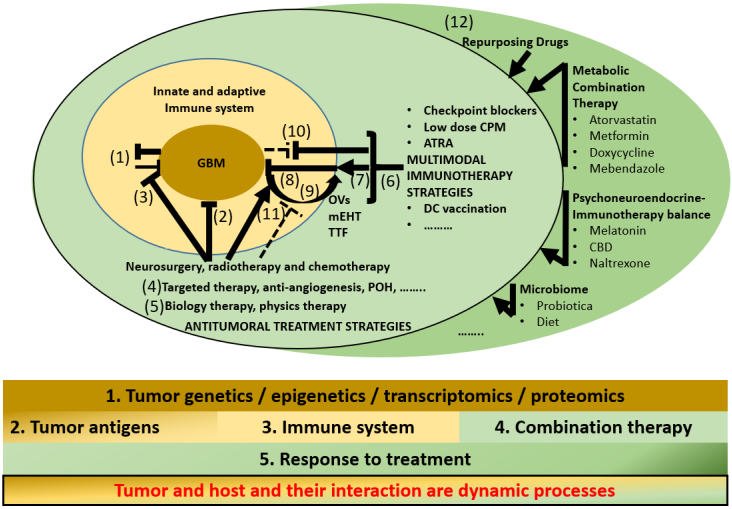

Figure 6. **A personalized, integrated combination treatment for patients with GBM.** Glioblastoma multiforme (GBM) is located within an immune environment, and after immunoediting, displays strong immunosuppressive mechanisms (1). Upon diagnosis, physicians start anticancer treatments, such as neurosurgery, radiochemotherapy and maintenance chemotherapy (2), which might even strengthen the imbalance as depicted in (3). With the goal to be more tumour-selective, anticancer targeted therapies, anti-angiogenesis strategies and chemical strategies like perillyl alcohol (POH) inhalations have been developed (4). Novel strategies against GBM include biological therapy with oncolytic viruses (OVs), electromagnetic tumour-treating field therapy (TTF), and modulated electro-hyperthermia (mEHT) (5). The immunotherapy approaches the tumour on another axis (6). Active immunotherapy aims to stimulate the immune system (7) with the goal to act against the antigen-expressing GBM cells (8). OVs, mEHT and TTF therapy target the tumour cells, induce immunogenic cell death and further stimulate the anticancer immune response (9). Cancer-mediated immunosuppressive effects are counteracted by patient-specific immunomodulatory treatments (10). Anticancer treatments should be designed in such a way that they can themselves stimulate the immune system, via immunogenic cell death mechanisms, and at least not block it (11). Alongside the anticancer treatments and the immunotherapy, complementary medicine (12) can support the body in the fight against GBM. Examples from four widely used categories are shown. Optimal anti-GBM treatment requires a personalised approach: 1) at the genetics/epigenetics/transcriptomics/proteomics

level; 2) at the level of surface antigens and target structures; 3) at the level of the immune reaction and tumour-host interaction; 4) at the level of the appropriate combination therapy; 5) at the level of response to treatment. In all aspects, one should consider that the tumour, the immune system and the interaction between the tumour and the immune system are dynamic processes; they change over time. Thus, the personalised approach should also change over time during the course of the disease and treatment.

ATRA: All-trans-retinoic-acid; CPM: cyclophosphamide

Summary

In this manuscript, we set out a personalised treatment approach for patients with GBM. We pointed out several important treatment concepts:

1. GBMs are heterogeneous tumours, which change dynamically over time; the host and the tumour-host interactions also change dynamically over time.
2. A combination of different anticancer treatment strategies is needed to control GBM on two different axes: a direct anticancer axis and an immune stimulation axis.
3. Both the anticancer treatments and the immunotherapies should start together, in well-designed combinations, early during the course of the disease.
4. IO-VAC® is the first approved advanced therapy anticancer vaccine medicinal product that includes contemporary personalised tumour antigen profiles, together with NDV antigens, and has a high potency to stimulate T cells against both tumour antigen-expressing and/or NDV-infected tumour cells.

This general concept can be applied to other types of solid tumours. It is now a major challenge to implement it in the current static double-blind, placebo-controlled, randomised clinical trial design structures and to demonstrate superiority for patients in terms of overall survival, quality of life and health economics.

References

[1] Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol 2016;131:803-20.
[2] Sturm D, Witt H, Hovestadt V, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell 2012;22:425-37.
[3] Ladomersky E, Scholtens DM, Kocherginsky M, et al. The Coincidence Between Increasing Age, Immunosuppression, and the Incidence of Patients With Glioblastoma. Front Pharmacol 2019;10:200.
[4] Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a "state of the science" review. Neuro Oncol 2014;16:896-913.
[5] Ripperger T, Bielack SS, Borkhardt A, et al. Childhood cancer predisposition syndromes-A concise review and recommendations by the Cancer Predisposition Working Group of the Society for Pediatric Oncology and Hematology. Am J Med Genet A 2017.
[6] Grobner SN, Worst BC, Weischenfeldt J, et al. The landscape of genomic alterations across childhood cancers. Nature 2018.
[7] Izycka-Swieszewska E, Bien E, Stefanowicz J, et al. Malignant Gliomas as Second Neoplasms in Pediatric Cancer Survivors: Neuropathological Study. Biomed Res Int 2018;2018:4596812.
[8] Batich KA, Reap EA, Archer GE, et al. Long-term Survival in Glioblastoma with Cytomegalovirus pp65-Targeted Vaccination. Clin Cancer Res 2017;23:1898-909.
[9] Hardell L, Carlberg M, Hansson Mild K. Use of mobile phones and cordless phones is associated with increased risk for glioma and acoustic neuroma. Pathophysiology 2013;20:85-110.
[10] Thakkar JP, Dolecek TA, Horbinski C, et al. Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol Biomarkers Prev 2014;23:1985-96.
[11] Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Eng J Med 2005;352:987-96.
[12] Stupp R, Hegi ME, Mason WP, et al. 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. Lancet Oncol 2009;10:459-66.
[13] Gately L, McLachlan SA, Philip J, Ruben J, Dowling A. Long-term survivors of glioblastoma: a closer look. J Neurooncol 2018;136:155-62.
[14] Burnet NG, Jefferies SJ, Benson RJ, Hunt DP, Treasure FP. Years of life lost (YLL) from cancer is an important measure of population burden--and should be considered when allocating research funds. Br J Cancer 2005;92:241-5.
[15] Rouse C, Gittleman H, Ostrom QT, Kruchko C, Barnholtz-Sloan JS. Years of potential life lost for brain and CNS tumors relative to other cancers in adults in the United States, 2010. Neuro Oncol 2016;18:70-7.

[16] Mirimanoff RO, Gorlia T, Mason W, et al. Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. J Clin Oncol 2006;24:2563-9.
[17] Pichlmeier U, Bink A, Schackert G, Stummer W, Group ALAGS. Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. Neuro Oncol 2008;10:1025-34.
[18] De Vleeschouwer S, Ardon H, Van Calenbergh F, et al. Stratification according to HGG-IMMUNO RPA model predicts outcome in a large group of patients with relapsed malignant glioma treated by adjuvant postoperative dendritic cell vaccination. Cancer Immunol Immunother 2012;61:2105-12.
[19] Perrin SL, Samuel MS, Koszyca B, et al. Glioblastoma heterogeneity and the tumour microenvironment: implications for preclinical research and development of new treatments. Biochem Soc Trans 2019.
[20] Kast RE, Skuli N, Karpel-Massler G, Frosina G, Ryken T, Halatsch ME. Blocking epithelial-to-mesenchymal transition in glioblastoma with a sextet of repurposed drugs: the EIS regimen. Oncotarget 2017;8:60727-49.
[21] Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM, Breakefield XO. Multidimensional communication in the microenvirons of glioblastoma. Nat Rev Neurol 2018;14:482-95.
[22] Rizza S, Rasola A, Townsend DM, Filomeni G. Editorial: Redox and Metabolic Circuits in Cancer. Front Oncol 2018;8:403.
[23] Tomaszewski W, Sanchez-Perez L, Gajewski TF, Sampson JH. Brain Tumor Micro-environment and Host State - Implications for Immunotherapy. Clin Cancer Res 2019.
[24] Dunn GP, Fecci PE, Curry WT. Cancer immunoediting in malignant glioma. Neurosurgery 2012;71:201-22.
[25] Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991-8.
[26] Arrieta VA, Cacho-Diaz B, Zhao J, Rabadan R, Chen L, Sonabend AM. The possibility of cancer immune editing in gliomas. A critical review. Oncoimmunology 2018;7:e1445458.
[27] Garg AD, Vandenberk L, Van Woensel M, et al. Preclinical efficacy of immune-checkpoint monotherapy does not recapitulate corresponding biomarkers-based clinical predictions in glioblastoma. Oncoimmunology 2017;6:e1295903.
[28] Chiang GC, Kovanlikaya I, Choi C, Ramakrishna R, Magge R, Shungu DC. Magnetic Resonance Spectroscopy, Positron Emission Tomography and Radiogenomics-Relevance to Glioma. Front Neurol 2018;9:33.
[29] Nandu H, Wen PY, Huang RY. Imaging in neuro-oncology. Ther Adv Neurol Disord 2018;11:1756286418759865.
[30] Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. Lancet Oncol 2006;7:392-401.

[31] Rutkowski S, Modena P, Williamson D, et al. Biological material collection to advance translational research and treatment of children with CNS tumours: position paper from the SIOPE Brain Tumour Group. Lancet Oncol 2018;19:e419-e28.
[32] Gzell C, Back M, Wheeler H, Bailey D, Foote M. Radiotherapy in Glioblastoma: the Past, the Present and the Future. Clin Oncol (R Coll Radiol) 2017;29:15-25.
[33] Kazda T, Dziacky A, Burkon P, et al. Radiotherapy of Glioblastoma 15 Years after the Landmark Stupp's Trial: More Controversies than Standards? Radiol Oncol 2018;52:121-8.
[34] Khan L, Soliman H, Sahgal A, Perry J, Xu W, Tsao MN. External beam radiation dose escalation for high grade glioma. Cochrane Database Syst Rev 2016:CD011475.
[35] de Moraes FY, Laperriere N. Glioblastoma in the elderly: initial management. Chin Clin Oncol 2017;6:39.
[36] Azoulay M, Shah J, Pollom E, Soltys SG. New Hypofractionation Radiation Strategies for Glioblastoma. Curr Oncol Rep 2017;19:58.
[37] Amelio D, Lorentini S, Schwarz M, Amichetti M. Intensity-modulated radiation therapy in newly diagnosed glioblastoma: a systematic review on clinical and technical issues. Radiother Oncol 2010;97:361-9.
[38] Sharma P, Debinski W. Receptor-Targeted Glial Brain Tumor Therapies. Int J Mol Sci 2018;19.
[39] Adeberg S, Harrabi SB, Verma V, et al. Treatment of meningioma and glioma with protons and carbon ions. Radiat Oncol 2017;12:193.
[40] Scaringi C, Agolli L, Minniti G. Technical Advances in Radiation Therapy for Brain Tumors. Anticancer Res 2018;38:6041-5.
[41] Ahmed SK, Brown PD, Foote RL. Protons vs Photons for Brain and Skull Base Tumors. Semin Radiat Oncol 2018;28:97-107.
[42] New P. Radiation injury to the nervous system. Curr Opin Neurol 2001;14:725-34.
[43] Pui CH, Yang JJ, Hunger SP, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol 2015;33:2938-48.
[44] Karachi A, Dastmalchi F, Mitchell DA, Rahman M. Temozolomide for immunomodulation in the treatment of glioblastoma. Neuro Oncol 2018;20:1566-72.
[45] Chamberlain MC, Glantz MJ, Chalmers L, Van HA, Sloan AE. Early necrosis following concurrent Temodar and radiotherapy in patients with glioblastoma. J Neurooncol 2007;82:81-3.
[46] Danson SJ, Middleton MR. Temozolomide: a novel oral alkylating agent. Expert Rev Anticancer Ther 2001;1:13-9.
[47] Heynckes S, Daka K, Franco P, et al. Crosslink between Temozolomide and PD-L1 immune-checkpoint inhibition in glioblastoma multiforme. BMC Cancer 2019;19:117.
[48] Daniel P, Sabri S, Chaddad A, et al. Temozolomide Induced Hypermutation in Glioma: Evolutionary Mechanisms and Therapeutic Opportunities. Front Oncol 2019;9:41.

[49] Ridolfi L, Petrini M, Granato AM, et al. Low-dose temozolomide before dendritic-cell vaccination reduces (specifically) CD4+CD25++Foxp3+ regulatory T-cells in advanced melanoma patients. J Transl Med 2013;11:135.
[50] Ardon H, Van Gool SW, Verschuere T, et al. Integration of autologous dendritic cell-based immunotherapy in the standard of care treatment for patients with newly diagnosed glioblastoma: results of the HGG-2006 phase I/II trial. Cancer Immunol Immunother 2012;61:2033-44.
[51] Ardon H, Van Gool S, Lopes IS, et al. Integration of autologous dendritic cell-based immunotherapy in the primary treatment for patients with newly diagnosed glioblastoma multiforme: a pilot study. J Neurooncol 2010;99:261-72.
[52] Van Gool SW. Brain Tumor Immunotherapy: What have We Learned so Far? Front Oncol 2015;5:98.
[53] Sathornsumetee S, Reardon DA, Desjardins A, Quinn JA, Vredenburgh JJ, Rich JN. Molecularly targeted therapy for malignant glioma. Cancer 2007;110:13-24.
[54] Chen R, Cohen AL, Colman H. Targeted Therapeutics in Patients With High-Grade Gliomas: Past, Present, and Future. Curr Treat Options Oncol 2016;17:42.
[55] dos Santos MA, Pignon JP, Blanchard P, et al. Systematic review and meta-analysis of phase I/II targeted therapy combined with radiotherapy in patients with glioblastoma multiforme: quality of report, toxicity, and survival. J Neurooncol 2015;123:307-14.
[56] Su J, Cai M, Li W, et al. Molecularly Targeted Drugs Plus Radiotherapy and Temozolomide Treatment for Newly Diagnosed Glioblastoma: A Meta-Analysis and Systematic Review. Oncol Res 2016;24:117-28.
[57] Cihoric N, Tsikkinis A, Minniti G, et al. Current status and perspectives of interventional clinical trials for glioblastoma - analysis of ClinicalTrials.gov. Radiat Oncol 2017;12:1.
[58] Sim HW, Morgan ER, Mason WP. Contemporary management of high-grade gliomas. CNS Oncol 2018;7:51-65.
[59] Yelton CJ, Ray SK. Histone deacetylase enzymes and selective histone deacetylase inhibitors for antitumor effects and enhancement of antitumor immunity in glioblastoma. Neuroimmunol Neuroinflamm 2018;5.
[60] Arrillaga-Romany I, Chi AS, Allen JE, Oster W, Wen PY, Batchelor TT. A phase 2 study of the first imipridone ONC201, a selective DRD2 antagonist for oncology, administered every three weeks in recurrent glioblastoma. Oncotarget 2017.
[61] De Fazio S, Russo E, Ammendola M, Donato Di PE, De Sarro G. Efficacy and safety of bevacizumab in glioblastomas. Curr Med Chem 2012;19:972-81.
[62] Van't Root M, Lowik C, Mezzanotte L. Targeting Nanomedicine to Brain Tumors: Latest Progress and Achievements. Curr Pharm Des 2017;23:1953-62.
[63] Karim R, Palazzo C, Evrard B, Piel G. Nanocarriers for the treatment of glioblastoma multiforme: Current state-of-the-art. J Control Release 2016;227:23-37.

[64] Van Woensel M, Wauthoz N, Rosiere R, et al. Development of siRNA-loaded chitosan nanoparticles targeting Galectin-1 for the treatment of glioblastoma multiforme via intranasal administration. J Control Release 2016;227:71-81.
[65] van den Bent M, Gan HK, Lassman AB, et al. Efficacy of depatuxizumab mafodotin (ABT-414) monotherapy in patients with EGFR-amplified, recurrent glioblastoma: results from a multi-center, international study. Cancer Chemother Pharmacol 2017;80:1209-17.
[66] Babic I, Nurmemmedov E, Yenugonda VM, et al. Pritumumab, the first therapeutic antibody for glioma patients. Hum Antibodies 2017.
[67] Modak S, Kramer K, Gultekin SH, Guo HF, Cheung NK. Monoclonal antibody 8H9 targets a novel cell surface antigen expressed by a wide spectrum of human solid tumors. Cancer Res 2001;61:4048-54.
[68] Huang J, Liu F, Liu Z, et al. Immune Checkpoint in Glioblastoma: Promising and Challenging. Front Pharmacol 2017;8:242.
[69] Wang X, Guo G, Guan H, Yu Y, Lu J, Yu J. Challenges and potential of PD-1/PD-L1 checkpoint blockade immunotherapy for glioblastoma. J Exp Clin Cancer Res 2019;38:87.
[70] Ameratunga M, Coleman N, Welsh L, Saran F, Lopez J. CNS cancer immunity cycle and strategies to target this for glioblastoma. Oncotarget 2018;9:22802-16.
[71] Brown CE, Badie B, Barish ME, et al. Bioactivity and Safety of IL13Ralpha2-Redirected Chimeric Antigen Receptor CD8+ T Cells in Patients with Recurrent Glioblastoma. Clin Cancer Res 2015;21:4062-72.
[72] O'Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017;9.
[73] Golinelli G, Grisendi G, Prapa M, et al. Targeting GD2-positive glioblastoma by chimeric antigen receptor empowered mesenchymal progenitors. Cancer Gene Ther 2018.
[74] Chuntova P, Downey KM, Hegde B, Almeida ND, Okada H. Genetically Engineered T-Cells for Malignant Glioma: Overcoming the Barriers to Effective Immunotherapy. Front Immunol 2018;9:3062.
[75] Stepanenko AA, Chekhonin VP. Recent Advances in Oncolytic Virotherapy and Immunotherapy for Glioblastoma: A Glimmer of Hope in the Search for an Effective Therapy? Cancers (Basel) 2018;10.
[76] Aldape K, Brindle KM, Chesler L, et al. Challenges to curing primary brain tumours. Nat Rev Clin Oncol 2019.
[77] Stupp R, Taillibert S, Kanner AA, et al. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. JAMA 2015;314:2535-43.
[78] Burri SH, Gondi V, Brown PD, Mehta MP. The Evolving Role of Tumor Treating Fields in Managing Glioblastoma: Guide for Oncologists. Am J Clin Oncol 2018;41:191-6.
[79] Chang E, Patel CB, Pohling C, et al. Tumor treating fields increases membrane permeability in glioblastoma cells. Cell Death Discov 2018;4:113.

[80] Taphoorn MJB, Dirven L, Kanner AA, et al. Influence of Treatment With Tumor-Treating Fields on Health-Related Quality of Life of Patients With Newly Diagnosed Glioblastoma: A Secondary Analysis of a Randomized Clinical Trial. JAMA Oncol 2018.
[81] Branter J, Basu S, Smith S. Tumour treating fields in a combinational therapeutic approach. Oncotarget 2018;9:36631-44.
[82] Vancsik T, Kovago C, Kiss E, et al. Modulated electro-hyperthermia induced loco-regional and systemic tumor destruction in colorectal cancer allografts. J Cancer 2018;9:41-53.
[83] Roussakow SV. Clinical and economic evaluation of modulated electrohyperthermia concurrent to dose-dense temozolomide 21/28 days regimen in the treatment of recurrent glioblastoma: a retrospective analysis of a two-centre German cohort trial with systematic comparison and effect-to-treatment analysis. BMJ Open 2017;7:e017387.
[84] Fiorentini G, Sarti D, Milandri C, et al. Modulated Electrohyperthermia in Integrative Cancer Treatment for Relapsed Malignant Glioblastoma and Astrocytoma: Retrospective Multicenter Controlled Study. Integr Cancer Ther 2018:1534735418812691.
[85] Lee S, Son B, Park G, et al. Immunogenic Effect of Hyperthermia on Enhancing Radiotherapeutic Efficacy. Int J Mol Sci 2018;19.
[86] Tsang YW, Huang CC, Yang KL, et al. Improving immunological tumor microenvironment using electro-hyperthermia followed by dendritic cell immunotherapy. BMC Cancer 2015;15:708.
[87] Van Gool SW, Makalowski J, Feyen O, Prix L, Schirrmacher V, Stuecker W. The induction of immunogenic cell death (ICD) during maintenance chemotherapy and subsequent multimodal immunotherapy for glioblastoma (GBM). Austin Oncol Case Rep 2018;3:1010.
[88] Wismeth C, Dudel C, Pascher C, et al. Transcranial electro-hyperthermia combined with alkylating chemotherapy in patients with relapsed high-grade gliomas: phase I clinical results. J Neurooncol 2010;98:395-405.
[89] Lundstrom K. New frontiers in oncolytic viruses: optimizing and selecting for virus strains with improved efficacy. Biologics 2018;12:43-60.
[90] Samson A, Scott KJ, Taggart D, et al. Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. Sci Transl Med 2018;10.
[91] Bommareddy PK, Shettigar M, Kaufman HL. Integrating oncolytic viruses in combination cancer immunotherapy. Nat Rev Immunol 2018;18:498-513.
[92] Martikainen M, Essand M. Virus-Based Immunotherapy of Glioblastoma. Cancers (Basel) 2019;11.
[93] Koks CAE, Garg AD, Ehrhardt M, et al. Newcastle disease virotherapy induces long-term survival and tumor-specific immune memory in orthotopic glioma through the induction of immunogenic cell death. Int J Cancer 2014;136:e313-e25.
[94] Jiang H, Rivera-Molina Y, Gomez-Manzano C, et al. Oncolytic Adenovirus and Tumor-Targeting Immune Modulatory Therapy Improve Autologous Cancer Vaccination. Cancer Res 2017;77:3894-907.

[95] Saha D, Martuza RL, Rabkin SD. Oncolytic herpes simplex virus immunovirotherapy in combination with immune checkpoint blockade to treat glioblastoma. Immunotherapy 2018;10:779-86.
[96] Gray A, Raff AB, Chiriva-Internati M, Chen SY, Kast WM. A paradigm shift in therapeutic vaccination of cancer patients: the need to apply therapeutic vaccination strategies in the preventive setting. Immunol Rev 2008;222:316-27.
[97] De Vleeschouwer S, Fieuws S, Rutkowski S, et al. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. Clin Cancer Res 2008;14:3098-104.
[98] Vandenberk L, Belmans J, Van Woensel M, Riva M, Van Gool SW. Exploiting the Immunogenic Potential of Cancer Cells for Improved Dendritic Cell Vaccines. Front Immunol 2015;6:663.
[99] Woods DM, Sodre AL, Villagra A, Sarnaik A, Sotomayor EM, Weber J. HDAC Inhibition Upregulates PD-1 Ligands in Melanoma and Augments Immunotherapy with PD-1 Blockade. Cancer Immunol Res 2015;3:1375-85.
[100] Gameiro SR, Malamas AS, Tsang KY, Ferrone S, Hodge JW. Inhibitors of histone deacetylase 1 reverse the immune evasion phenotype to enhance T-cell mediated lysis of prostate and breast carcinoma cells. Oncotarget 2016;7:7390-402.
[101] Marchini A, Scott EM, Rommelaere J. Overcoming Barriers in Oncolytic Virotherapy with HDAC Inhibitors and Immune Checkpoint Blockade. Viruses 2016;8.
[102] Cao JX, Zhang XY, Liu JL, et al. Clinical efficacy of tumor antigen-pulsed DC treatment for high-grade glioma patients: evidence from a meta-analysis. PLoS ONE 2014;9:e107173.
[103] Wang X, Zhao HY, Zhang FC, Sun Y, Xiong ZY, Jiang XB. Dendritic cell-based vaccine for the treatment of malignant glioma: a systematic review. Cancer Invest 2014;32:451-7.
[104] Eagles ME, Nassiri F, Badhiwala JH, et al. Dendritic cell vaccines for high-grade gliomas. Ther Clin Risk Manag 2018;14:1299-313.
[105] Vatu BI, Artene SA, Staicu AG, et al. Assessment of efficacy of dendritic cell therapy and viral therapy in high grade glioma clinical trials. A meta-analytic review. J Immunoassay Immunochem 2019;40:70-80.
[106] Shore ND. Advances in the understanding of cancer immunotherapy. BJU Int 2015;116:321-9.
[107] Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005;352:997-1003.
[108] Weller M, Butowski N, Tran DD, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol 2017.
[109] Jain KK. A Critical Overview of Targeted Therapies for Glioblastoma. Front Oncol 2018;8:419.
[110] Guishard AF, Yakisich JS, Azad N, Iyer AKV. Translational gap in ongoing clinical trials for glioma. J Clin Neurosci 2018;47:28-42.

[111] Bouffet E, Larouche V, Campbell BB, et al. Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency. J Clin Oncol 2016;34:2206-11.
[112] 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. J Clin Oncol 2010;28:4722-9.
[113] Okada H, Kalinski P, Ueda R, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol 2011;29:330-6.
[114] 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 Immunol Immunother 2013;62:125-35.
[115] Akiyama Y, Oshita C, Kume A, et al. alpha-type-1 polarized dendritic cell-based vaccination in recurrent high-grade glioma: a phase I clinical trial. BMC Cancer 2012;12:623.
[116] Wheeler CJ, Black KL, Liu G, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res 2008;68:5955-64.
[117] Chang AE, Redman BG, Whitfield JR, et al. A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. Clin Cancer Res 2002;8:1021-32.
[118] Fadul CE, Fisher JL, Hampton TH, et al. Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy. J Immunother 2011;34:382-9.
[119] Prins RM, Soto H, Konkankit V, et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin Cancer Res 2011;17:1603-15.
[120] Cho DY, Yang WK, Lee HC, et al. Adjuvant immunotherapy with whole-cell lysate dendritic cells vaccine for glioblastoma multiforme: a phase II clinical trial. World Neurosurg 2012;77:736-44.
[121] Lasky JL, III, Panosyan EH, Plant A, et al. Autologous Tumor Lysate-pulsed Dendritic Cell Immunotherapy for Pediatric Patients with Newly Diagnosed or Recurrent High-grade Gliomas. Anticancer Res 2013;33:2047-56.
[122] Hunn MK, Bauer E, Wood CE, et al. Dendritic cell vaccination combined with temozolomide retreatment: results of a phase I trial in patients with recurrent glioblastoma multiforme. J Neurooncol 2015;121:319-29.
[123] Inoges S, Tejada S, de Cerio AL, et al. A phase II trial of autologous dendritic cell vaccination and radiochemotherapy following fluorescence-guided surgery in newly diagnosed glioblastoma patients. J Transl Med 2017;15:104.
[124] Liau LM, Prins RM, Kiertscher SM, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses

modulated by the local central nervous system tumor microenvironment. Clin Cancer Res 2005;11:5515-25.
[125] Vik-Mo EO, Nyakas M, Mikkelsen BV, et al. Therapeutic vaccination against autologous cancer stem cells with mRNA-transfected dendritic cells in patients with glioblastoma. Cancer Immunol Immunother 2013;62:1499-509.
[126] Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 2017.
[127] Rammensee HG, Singh-Jasuja H. HLA ligandome tumor antigen discovery for personalized vaccine approach. Expert Rev Vaccines 2013;12:1211-7.
[128] Redzic JS, Ung TH, Graner MW. Glioblastoma extracellular vesicles: reservoirs of potential biomarkers. Pharmgenomics Pers Med 2014;7:65-77.
[129] Consortium E-T, Van Deun J, Mestdagh P, et al. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. Nat Methods 2017;14:228-32.
[130] Van Gool SW, Feyen O, Makalowski J, Neinhuis A, Schirrmacher V, Stuecker W. How to monitor immunogenic cell death in patients with glioblastoma. Neuro Oncol 2018;20:vi7-vi8.
[131] Liau LM, Ashkan K, Tran DD, et al. First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. J Transl Med 2018;16:142.
[132] Rutledge WC, Kong J, Gao J, et al. Tumor-infiltrating lymphocytes in glioblastoma are associated with specific genomic alterations and related to transcriptional class. Clin Cancer Res 2013;19:4951-60.
[133] Berghoff AS, Kiesel B, Widhalm G, et al. Correlation of immune phenotype with IDH mutation in diffuse glioma. Neuro Oncol 2017;19:1460-8.
[134] Kmiecik J, Poli A, Brons NH, et al. Elevated CD3+ and CD8+ tumor-infiltrating immune cells correlate with prolonged survival in glioblastoma patients despite integrated immunosuppressive mechanisms in the tumor microenvironment and at the systemic level. J Neuroimmunol 2013;264:71-83.
[135] Veltman JD, Lambers ME, van Nimwegen M, et al. Low-dose cyclophosphamide synergizes with dendritic cell-based immunotherapy in antitumor activity. J Biomed Biotechnol 2010;2010:798467.
[136] Sampson JH, Schmittling RJ, Archer GE, et al. A Pilot Study of IL-2Ralpha Blockade during Lymphopenia Depletes Regulatory T-cells and Correlates with Enhanced Immunity in Patients with Glioblastoma. PLoS ONE 2012;7:e31046.
[137] Iclozan C, Antonia S, Chiappori A, Chen DT, Gabrilovich D. Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer. Cancer Immunol Immunother 2013;62:909-18.
[138] Long AH, Highfill SL, Cui Y, et al. Reduction of MDSCs with All-trans Retinoic Acid Improves CAR Therapy Efficacy for Sarcomas. Cancer Immunol Res 2016;4:869-80.

[139] Tobin RP, Jordan KR, Robinson WA, et al. Targeting myeloid-derived suppressor cells using all-trans retinoic acid in melanoma patients treated with Ipilimumab. Int Immunopharmacol 2018;63:282-91.
[140] Qiu J, Shi Z, Jiang J. Cyclooxygenase-2 in glioblastoma multiforme. Drug Discov Today 2017;22:148-56.
[141] Hau P, Jachimczak P, Schlingensiepen R, et al. Inhibition of TGF-beta2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. Oligonucleotides 2007;17:201-12.
[142] Dix AR, Brooks WH, Roszman TL, Morford LA. Immune defects observed in patients with primary malignant brain tumors. J Neuroimmunol 1999;100:216-32.
[143] Antonopoulos M, Van gool SW, Dionysiou D, Graf N, Stamatakos G. Immune phenotype correlates with survival in patients with GBM treated with standard temozolomide-based therapy and immunotherapy. Anticancer Res 2019;39: 2043-51.
[144] Grossman SA, Ye X, Lesser G, et al. Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. Clin Cancer Res 2011;17:5473-80.
[145] Otvos B, Silver DJ, Mulkearns-Hubert EE, et al. Cancer Stem Cell-Secreted Macrophage Migration Inhibitory Factor Stimulates Myeloid Derived Suppressor Cell Function and Facilitates Glioblastoma Immune Evasion. Stem Cells 2016;34:2026-39.
[146] Gielen PR, Schulte BM, Kers-Rebel ED, et al. Elevated levels of polymorphonuclear myeloid-derived suppressor cells in patients with glioblastoma highly express S100A8/9 and arginase and suppress T cell function. Neuro Oncol 2016;18:1253-64.
[147] da Fonseca CO, Simao M, Lins IR, Caetano RO, Futuro D, Quirico-Santos T. Efficacy of monoterpene perillyl alcohol upon survival rate of patients with recurrent glioblastoma. J Cancer Res Clin Oncol 2011;137:287-93.
[148] da Fonseca CO, Teixeira RM, Silva JC, et al. Long-term outcome in patients with recurrent malignant glioma treated with Perillyl alcohol inhalation. Anticancer Res 2013;33:5625-31.
[149] da Fonseca CO, Soares IP, Clemencon DS, Rochlin S, Cardeman L, Quirico-Santos T. Perillyl alcohol inhalation concomitant with oral temozolomide halts progression of recurrent inoperable glioblastoma: a case report. Journal of Histology Histopathology 2015;2:Article 12.
[150] da Fonseca CO, Khandelia H, Salazar MD, Schonthal AH, Meireles OC, Quirico-Santos T. Perillyl alcohol: Dynamic interactions with the lipid bilayer and implications for long-term inhalational chemotherapy for gliomas. Surg Neurol Int 2016;7:1.
[151] Santos JG, Da Cruz WMS, Schonthal AH, et al. Efficacy of a ketogenic diet with concomitant intranasal perillyl alcohol as a novel strategy for the therapy of recurrent glioblastoma. Oncol Lett 2018;15:1263-70.
[152] Macdonald DR, Cascino TL, Schold SC, Jr., Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 1990;8:1277-80.

[153] Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol 2010;28:1963-72.
[154] Okada H, Weller M, Huang R, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. Lancet Oncol 2015;16:e534-42.
[155] Verduin M, Compter I, Steijvers D, et al. Noninvasive Glioblastoma Testing: Multimodal Approach to Monitoring and Predicting Treatment Response. Dis Markers 2018;2018:2908609.
[156] Prins RM, Wang X, Soto H, et al. Comparison of glioma-associated antigen peptide-loaded versus autologous tumor lysate-loaded dendritic cell vaccination in malignant glioma patients. J Immunother 2013;36:152-7.
[157] Grimm M, Kraut W, Hoefert S, et al. Evaluation of a biomarker based blood test for monitoring surgical resection of oral squamous cell carcinomas. Clin Oral Investig 2016;20:329-38.
[158] Lissoni P, Messina G, Lissoni A, Franco R. The psychoneuroendocrine-immunotherapy of cancer: Historical evolution and clinical results. J Res Med Sci 2017;22:45.
[159] Khodadadi S, Sobhani N, Mirshekar S, et al. Tumor Cells Growth and Survival Time with the Ketogenic Diet in Animal Models: A Systematic Review. Int J Prev Med 2017;8:35.
[160] Kast RE, Karpel-Massler G, Halatsch ME. CUSP9* treatment protocol for recurrent glioblastoma: aprepitant, artesunate, auranofin, captopril, celecoxib, disulfiram, itraconazole, ritonavir, sertraline augmenting continuous low dose temozolomide. Oncotarget 2014;5:8052-82.