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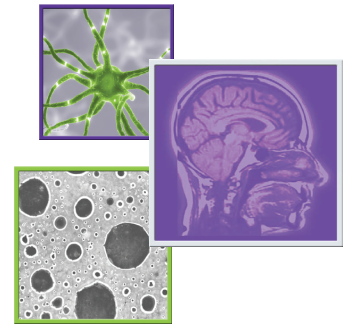

Immunotherapy in glioblastoma: emerging options in precision medicine

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Practice points

- Patients with glioblastoma (GBM), the most common primary malignant brain tumor of adulthood, have a median overall survival time of just 14–16 months despite optimized treatment including maximal safe resection followed by radiotherapy and chemotherapy.
- Precision medicine in GBM immunotherapy provides a unique opportunity for tumor-specific targeted therapies for each patient.
- Most therapeutic targets in GBM are only expressed in subsets of patients and, in many cases, rarely throughout the tumor.
- Comprehensive molecular profiling of large patient cohorts will likely be required to identify patients that may benefit from targeted approaches.
- In contrast to the implementation of precision medicine in other malignancies, GBM will require additional considerations for blood–brain barrier penetration for targeted agents and/or consideration of trafficking of antitumor immune responses to the CNS.
- There are obstacles, yet potential solutions, in precision medicine implementation in GBM immunotherapy.

Immunotherapy for glioblastoma (GBM) provides a unique opportunity for targeted therapies for each patient, addressing individual variability in genes, tumor biomarkers and clinical profile. As immunotherapy has the potential to specifically target tumor cells with minimal risk to normal tissue, several immunotherapeutic strategies are currently being evaluated in clinical trials in GBM. With the Precision Medicine Initiative being announced in the President's State of the Union Address in 2016, GBM immunotherapy provides a useful platform for changing the landscape in treating patients with difficult disease.

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Glioblastoma (GBM) is the most common primary malignant brain tumor in adults and has a median overall survival time of only 14–16 months despite optimal treatment including resection followed by radiotherapy and chemotherapy [1]. Unfortunately, GBM is an infiltrative tumor with vast heterogeneity, making a surgical cure impossible and treatment resistance frequent. The prognosis remains poor and recurrence is universal despite maximal treatment because the tumor carries mutations that may allow it to bypass drug-targeted pathways.

The overarching concept of precision medicine is personalized care that takes into account genetic variability, tumor biomarkers (including those that may correlate with immune therapeutic responses), and clinical profiles in order to provide targeted therapies for each individual patient. The ultimate goal of this strategy is to develop more specific therapeutics for effective and rational cancer treatment. Precision medicine is especially significant in cancer care where nonspecific, standardized chemotherapeutic treatments have the potential to induce significant toxicities. As such, this model has been implemented in the treatment of a variety of malignancies [2–6]. The USA has launched a Precision Medicine Initiative with an associated US$215 million investment, further indicating the importance of this treatment paradigm shift in human disease.

As our knowledge of glioma has advanced, including the designation of distinct molecular subtypes, identification of targetable molecular alterations and a better understanding of the tumor microenvironment, personalized GBM therapy based on specific tumor and patient factors is an increasingly viable therapeutic approach. As immunotherapy has the potential to specifically target tumor cells with minimal risk to normal tissue, several immunotherapeutic strategies are currently being evaluated in clinical trials. Immunotherapy is generally defined as therapy that centers on using the patient's own immune machinery to kill malignant cells. This treatment presents a unique opportunity for precision medicine in GBM, given that conventional therapy is nonspecific, leading to damage to surrounding normal brain tissue and systemic toxicity. There are certain components that are vital for an immunotherapeutic agent to be effective. First, there must be an appropriate therapeutic target. The ideal target would be specific to the tumor and have a high frequency of expression. Additionally, antigen expression would preferably be homogeneous so that potentially all cancer cells would be immunologically targeted and tied to the 'driver' activity of the tumor. Generation and maintenance of a robust immune response are also critical components of a successful immunotherapeutic. Agents should be able to activate the immune response, support infiltration of the tumor site and sustain immune effector function within the tumor microenvironment. In contrast to other malignancies, precision medicine in GBM requires additional considerations for blood–brain barrier penetration for targeted agents and/or consideration of trafficking of anti-tumor immune responses to the CNS. At this junction, there does not appear to be a monotherapeutic strategy that is capable of inducing all of these critical steps, and as such, ongoing efforts have been focused on the development of combining immune therapeutics with these various properties [7–10].

There are multiple reviews on GBM immunotherapy in the literature [11–15]; however, the goal of this particular review is to evaluate precision medicine strategies of selecting an appropriate immunotherapy based on a biomarker, thereby optimizing treatment regimens while minimizing ineffectual approaches for the patient.

Precision medicine in cancer

Precision medicine has played an increasingly significant role in the treatment of several malignancies via targeted therapies. A classic example is in the treatment of chronic myeloid leukemia (CML). The Philadelphia chromosome (translocation between the long arms of chromosomes 9 and 22) results in expression of a BCR–ABL fusion oncoprotein and is found in over 90% of CML cases [16]. This oncoprotein has constitutive tyrosine kinase activity promoting tumorigenesis [17]. Imatinib (Gleevec), a tyrosine kinase inhibitor, selectively targets this key oncogenic event, resulting in a complete and durable response in 69% of CML patients [18].

Precision medicine has also had success in the treatment of multiple solid tumors. A well-known example is the establishment of HER2-neu and ER as effective therapeutic targets in breast cancer. HER2/neu is specifically overexpressed in the tumors of approximately 20–25% of breast cancer patients and plays an oncogenic role in cell proliferation, conferring a poorer prognosis [19–21]. Trastuzumab is a humanized

monoclonal antibody that targets the protein encoded by the *HER2/neu* gene. A large study including 2091 patients with metastatic breast cancer showed that women with HER2/neu-positive disease who received trastuzumab had a 44% reduction in the risk of death compared with women with HER2/neu-negative disease ($p < 0.0001$) [21]. However, given the reported high incidences (over 30%) of trastuzumab-induced cardiotoxicity [22–25], patients must be closely monitored for cardiac effects of the drug.

Amplification of *EGFR* is a common genetic alternation in several malignancies, making it an attractive target for personalized therapy. Cetuximab, a monoclonal antibody against EGFR has proved effective in patients specifically with wild-type *KRAS* colon cancer, significantly increasing median overall (9.5 vs 4.8 months; $p < 0.001$) and progression-free survival times (3.7 vs 1.9 months; $p < 0.001$) in a study of 394 patients evaluated for *KRAS* tumor mutations [26]. Malignant melanoma is a devastating disease in which 60% of patients have a *BRAF* mutation that causes a decreased response to chemotherapy and increased disease severity [27,28]. A Phase III randomized clinical trial of 675 patients with previously untreated metastatic melanoma showed that vemurafenib (potent, selective inhibitor of mutated *BRAF*) resulted in a 63% reduction in the relative risk of death and a 74% reduction in the risk of either death or disease progression in patients with a *BRAF* mutation ($p < 0.001$) [29].

Cumulatively, these studies have demonstrated that the unique genetic features of a tumor can be exploited for therapeutic vulnerability. However, to date, no such therapeutic strategy has been successful in GBM, owing to a variety of factors including marked genetic diversity and heterogeneity and therapeutic delivery limitations produced by the blood–brain barrier.

GBM immunotherapy & precision medicine

Perhaps the most prototypical example of using immune therapy in the context of precision medicine in GBM has been the EGFRvIII peptide vaccine [30], which consists of a 14-mer peptide spanning the splice mutation site, GM-CSF and KLH. EGFRvIII is expressed in 30% of GBMs, and this mutant is ligand-independent and constitutively active, contributing to amplified cell proliferation [31–35]. Studied extensively in Phase II clinical trials in EGFRvIII-positive GBMs, the vaccine demonstrated a progression-free survival of 8.5 months from diagnosis, a median overall survival (OS) of 21.8 months and a 36-month overall survival of 26% [36]. Although not a randomized trial, these results fared favorably when compared with standard of care in which PFS is 6.8 months and OS is 14.6 months [1]. It is important to note that EGFRvIII expression does not impact median survival [34,37], as almost no GBM patients with EGFRvIII expression have historically survived more than 24 months. Further advancement of the EGFRvIII peptide approach has been halted due to recent Phase III results. Specifically, the control group, which included treatment with KLH, exceeded expectation (hazard ratio = 0.99; median overall survival: 20.4 months vs control 21.1 months) [38]. Since the GBM patients were selected based on tumor expression of EGFRvIII, an immunological target already exists for the immune system to be directed. Thus, additional systemic administration of an EGFRvIII peptide may not have been necessary. Viewed from this perspective, the Phase III clinical trial may have utilized immunological bio-equivalent strategies (i.e., a lymphodepleting temozolomide regimen to allow for expansion of clonotypic antigen-specific T cells with an immune activating agent such as KLH) in both arms. Since an antigenic target was already present in both cohorts, at least two criterion necessary for immunological clearance of a tumor were met. However, to date, there have been no published preclinical studies of therapeutic activity of KLH against EGFRvIII positive tumors within the CNS. Although restriction of EGFRvIII to GBM has made it an excellent target for immunotherapy from a safety and specificity perspective, treatment failure corresponds to the loss of the antigenic target [35]. This limitation of precision medicine is being increasingly recognized as a mechanism of treatment failure in other approaches that have targeted specific antigens.

Another peptide vaccine strategy targets the *IDH1* mutation, specifically at the *R132H* site, which is found in the majority of WHO grade II and III gliomas and secondary GBM [39]. Although *IDH* mutations are drivers of tumor progression [40], patients with *IDH*-mutated gliomas exhibit improved prognosis compared with those with *IDH* wild-type [41,42].

Administration of this vaccine induces a specific antitumor immune response against *IDH1(R132H)*-mutated tumors [43], and significantly prolongs survival in an intracranial glioma murine model system [44]. The IDH1 peptide vaccine is currently being investigated in Phase I clinical trials in *IDH1(R132H)*-mutated grade III–IV gliomas (NCT02454634) and recurrent grade II gliomas (NCT02193347). Both trials are utilizing precision medicine in screening for the *IDH1R132H*-mutation to determine patient eligibility.

Considering the obstacle of antigenic loss, alternative approaches have included a multipeptide vaccine strategy, in which 10 to 15 tumor-associated peptides are combined in a single vaccine. This strategy has been studied in a Phase I trial in renal cell carcinoma [45], and is now being evaluated in GBM (NCT01920191). The development of a GBM multipeptide vaccine is based on a prior screen of 11 tumor-associated peptides found to be overexpressed in malignant glioma samples of 45–50 patients. In a Phase I trial of 45 GBM patients, 60% of the patients had an immunogenic response to one of the peptides and 35% had a response to two or more of the peptides [46]. Although this multipeptide vaccine is based on predetermined overexpressed antigens from a pooled cohort of GBM patients, this strategy provides the future possibility of screening an individual's primary tumor and creating a vaccine that specifically targets the patient's tumor based on the peptide screen.

Similarly, the dendritic cell (DC) strategy provides a means of targeting multiple GBM antigens by utilizing tumor lysates, total tumor RNA, tumor peptides or products from cancer stem cells [47–49]. Autologous DCs manipulated *ex vivo* can then be administered to the patient. DC vaccination is safe and well tolerated [7,50–53], and is currently being investigated in several GBM clinical trials (NCT01204684, NCT01204684, NCT0004596, NCT01280552). Interestingly, infiltration of intratumoral cytotoxic T cells [54] as well as $CD8^+$ immune responses [55] have been observed in some patients after vaccination with DCs. More importantly, DC vaccination has shown improved survival and tumor regression compared with historical or contemporary controls [7,51,54–56]. For example in a clinical study of 12 GBM patients, median overall survival was 23.4 months ($p = 0.006$) and median time to progression was 15.5 months ($p = 0.028$), compared with concurrent control patients who had an overall survival of 18.3 months. Two patients treated with DC vaccination were also long-term survivors (≥4 years) [54]. However, there are distinct limitations with DC strategies regarding the antigens (via tumor lysates, RNA, peptides, or cancer stem cell products) used to load them. Specifically, these antigens may induce nontumor-specific toxicities, fail to induce an immunological response, be limited by the immunosuppressive tumor microenvironment and target bystander cells that have no impact on the process of tumorigenesis, recurrence or resistance. Also, as approximately 65% of GBM patients are surgical candidates [57], a major limitation is that there must be sufficient tissue in order to implement this immune-based strategy.

Another way to potentially overcome screening for antigens and their limited frequency of expression is to target CMV, a herpes virus that leads to asymptomatic infection followed by viral persistence and latency. Although the role of CMV in GBM is not fully elucidated with conflicting data regarding the presence [58–60] or absence [61,62] of CMV in GBM, the association of CMV antigens with GBM is well established [63]. Adoptive transfer of CMV-specific effector T cells that have been collected from the patient and expanded *ex vivo* has been shown to be safe and to confer a median survival time of approximately 14 months [64]. A recent pivotal study by Mitchell *et al.* had extreme responders (>40 months survival) who received autologous dendritic cells pulsed with CMV mRNA phosphoprotein 65 (pp65) and underwent preconditioning of the vaccine site with tetanus/diphtheria (Td) toxoid, a potent recall antigen [7]. Another method of adoptive transfer therapy is the administration of cytotoxic T lymphocytes that have been collected from the patient, activated and amplified *ex vivo*. The tumor antigen-specific T cells then traffic to the malignant tumor cells. Preclinical studies demonstrate that administration of tumor antigen-specific T lymphocytes leads to rejection of brain tumors [65]. The applicability and feasibility of this treatment strategy in GBM have been evaluated in small Phase I trials and pilot studies [66–75]. For example, in a study of ten patients with recurrent or progressive malignant glioma, 6-month radiographic regression was observed in two patients with recurrent tumors, one patient demonstrated stable disease

lasting more than 17 months and four patients remained alive more than 1 year after surgery for recurrent tumor [72]. Such adoptive cellular strategies are currently being evaluated in clinical trials (NCT02661282, NCT00693095, NCT00730613), but by strict definition cannot really be considered as precision medicine because the unique characteristics of the tumor are not, *per se*, being used to identify the applicable target patient population.

Another immunotherapeutic strategy that would lend itself to the precision medicine model is using chimeric antigen receptor (CAR) T cells, which are genetically modified to target surface tumor-associated antigens independently of major histocompatibility complex (MHC) presentation. CARs can be built with any tumor-specific or tumor-associated antigen of interest, and they can be fine-tuned to the level of antigen expression to distinguish tumor from nontumor cells [76]. Such fine-tuning is also seen in an EGFR-targeting probody, which remains inert in healthy tissues and active at the tumor site, minimizing on-target/off-tumor toxicities and improving the safety profile of antibody strategies [77]. An emerging treatment paradigm includes accessing the tumor, analyzing it for antigens and then selecting a CAR that is specific for that individual patient's tumor. Typically, it can take approximately 3–5 weeks to manufacture clinical-grade modified CAR T cells [78–80], depending on the genetic modification method used. The EGFRvIII and IL13Rα2 CAR T-cell therapies have shown efficacy in murine model systems of glioma and CNS melanoma [81–84], and these strategies, as well as HER2-CAR T cell therapy, are currently being investigated in GBM Phase I clinical trials (NCT02209376, NCT01454596, NCT02208362, NCT02442297, NCT01109095). However, this approach will require a portfolio of CARs and may also have treatment failures owing to antigenic loss/clonotypic selection. Upon recurrence, the tumor would require reprofiling (via surgical resection or biopsy) and would require alternative antigen-directed CAR therapeutics.

Approved by the US FDA in 2011, ipilimumab, an immune checkpoint inhibitor, became the first drug ever shown to extend survival of patients with metastatic melanoma in a large randomized Phase III trial [85]. The 25-year story of the development and implementation of ipilimumab, a CTLA-4 receptor blockade immunotherapeutic, has incited considerable efforts in cancer immunotherapy. CTLA-4 and PD-1 are immune checkpoint molecules that downregulate T-cell activation pathways, thereby hindering the immune response to cancer. Immune checkpoint inhibition, specifically by CTLA-4 and PD-1 blockade, has been implemented in cancers such as melanoma [86], renal cell carcinoma [87] and non-small-cell lung cancer [88], with significant clinical efficacy and survival benefit. In a Phase III study of 676 patients with unresectable stage III or IV melanoma, ipilimumab revealed improved overall median survival in patients with advanced melanoma (10 vs 6.4 months in controls; $p < 0.001$) [85]. Similarly, anti-PD1 therapy conferred 6-month disease stabilization in advanced melanoma, lung cancer and renal cancer [89,90]. Interestingly, patients shown by immunohistochemistry to have PD-L1-negative tumors did not have an objective response, implicating the need to further understand the influence of PD-1/PD-L1 expression on therapeutic response or failure and also the potential applicability of the immune checkpoints within the precision medicine initiative. In light of the clinical efficacy in the treatment of other cancers, there are an unprecedented number of clinical trials actively recruiting GBM patients for treatment with immune checkpoint blockade strategies (NCT02313272, NCT02530502, NCT02337686, NCT02658279, NCT02311582, NCT02529072, NCT02311920, NCT02017717, NCT02550249, NCT02526017, NCT02423343, NCT02327078). Moreover, overall mutational load, neoantigen load and expression of cytolytic markers in the immune microenvironment are all associated with clinical response to immune checkpoint inhibitors [91–93]. Thus, high mutational burden, as seen in other cancers, could also be considered as a 'target' in GBM. Such potential targets could be used to develop new immunotherapeutic agents and as selection biomarkers for patients who may benefit from this particular type of immune therapy. The field is rapidly heading toward moving many of the aforementioned approaches into combinatorial strategies; however, as we develop more precise approaches, the number of patients to whom they are applicable may become much more limited, as large-scale comprehensive profiling to identify those that will benefit is required.

In an ideal clinical scenario, a patient would have surgical resection/biopsy for a definitive diagnosis of GBM. Individual tumor and blood

samples then undergo personalized characterization, including genomic sequencing, immune evaluation, metabolic profiling and pathway analysis. Tumors would also be evaluated for expression of distinct immunoregulatory ligands and receptors. With the results of such testing and the selection of targeted therapies, patients would then receive biomarker-directed immunotherapy determined by the analysis of personalized tumor characterization. Bayesian statistical approaches can be used at this juncture to streamline and facilitate building complicated but maximally informative trials [94]. As the size and expense of current Phase II clinical trials in oncology continue to escalate, their success remains dismally low at 29% [95]. The use of adaptive clinical trial design has the distinct advantages of: identifying the appropriate patient population and therapeutic combinations; shortening the duration of drug development; and modeling longitudinal information, including immune monitoring assays. Such flexible clinical trials allow for stopping early if there is either superiority or futility, assigning doses to more efficiently assess the dose–outcome relationship, dropping arms or doses, allowing for seamless phases of drug development within the same trial, changing the proportion of patients randomized to each arm, homing in on an indication for a responder population, adding arms or doses, and changing accrual rate. Treatment response and side effects can then be monitored using imaging, tumor genome evolution, and immune monitoring to evaluate early progression and intervention **(Figure 1)**. This algorithm provides an opportunity for combinatorial treatment strategies, in which T-cell-enhancing therapies and antigen-targeted approaches are tailored according to the patient's tumor profile. This strategy also provides an opportunity to treat patients with resistance to targeted therapies, which could be due to selective therapy pressure or activation of compensatory tumorigenic pathways.

Obstacles/solutions

Although the clinical potential of immunotherapy is clear, the delivery of personalized GBM therapy has many challenges. The heterogeneity of this disease due to accumulation of diverse genetic changes, redundant signaling pathways and the complex interaction between the tumor and the microenvironment make generating a global suitable therapeutic candidate difficult [96–98]. Administrative execution, cost and feasibility are all major obstacles. It is assumed that ultimately, personalized therapy will result in health/economic gains at the population level by streamlining treatment, and hence costs, by focusing on the most patient-specific, effective therapies [26]. However, developing a comprehensive precision medicine strategy for GBM will require a global effort, a large and diverse patient enrollment, an expansive database to maintain a robust portfolio of clinical data, the ability to do comprehensive and universal genomic screening and a way to systematically match patients with targeted treatment strategies. This requires the collaboration of multiple centers, a portal for storing clinical data and a large clinical and research team for data entry and maintenance. Additionally, genomic testing and sequencing of tumor blocks can be cost prohibitive, and only a limited number of centers have the ability to implement these tests. Moreover, the development of patient-specific therapies (i.e., adoptive cellular therapies) are more costly to produce than other treatment modalities (i.e., antibody approaches), due to complex cellular processing, labor intensive processes, availability of materials and technical demands.

The second major obstacle is time. From drug development, to preclinical testing, to clinical trial evaluation, to FDA approval, the path of getting a therapeutic to a patient can take several years. One reason for such an extensive time frame is that the traditional clinical trial framework has not changed since the early 20th century. However, innovative clinical trial designs (basket trials, adaptive Bayesian clinical trials, etc.) such as those seen in NCI-MATCH [99], FOCUS4 [100], I-SPY 2 [101] and the forthcoming GBM AGILE [102], represent the progress of biomarker, multiagent collaborative trials. The GBM AGILE trial, for example, will include multiple research arms and allocate patients based on Bayesian probability of treatment efficacy, thereby dropping treatments that are ineffective and accruing treatment arms that are successful. Such adaptive trial designs save time, cost and resources, with the goal of rapidly and dramatically reducing mortality in cancer.

In order for a proposed therapy to progress from the bench to the clinic, decisive clinical trials are required. Logistically, implementing an immunotherapy-based clinical trial is a feat. Patient selection based on individual genetic alterations is difficult, limiting the power of many immunotherapy clinical trials. An

acceptable clinical protocol requires a standardized sample collection methodology, preparation and biomarker testing. Because GBMs are rare and sample numbers may be small due to limited tissue availability (needle biopsies, tumors in eloquent cortex decreasing the extent of resection), this issue becomes critical. As the availability and methods of testing vary from center to center, obtaining large-scale results focused on rare genomic alterations is difficult. Also, the current histopathological interpretation of GBM diagnosis can vary from pathologist to pathologist; thus, a tumor sample that is read as an anaplastic astrocytoma at one center may be classified as a GBM at another center. Indeed, as the field is moving toward genetic and molecular characterization of these tumors (e.g., the advent of microarray analysis, discovery of the *IDH1* mutation, *TCGA* database, etc.), the classification system is bound to change as we continue to understand more about the biology of gliomas.

Another major challenge is utilizing proper, and preferably noninvasive, methods to monitor treatment response in patients who receive immunotherapeutic drugs for GBM. Immune monitoring of blood and tumor tissue is a method that has been used to predict clinical efficacy of immunotherapeutics and confirm immune responses [55,103–106]. For example, measuring tumor-specific immune responses via various assays for T-cell proliferation, CD4/CD8 cell phenotype, secretion of IFN-γ, cytokine responses of $CD8^+$ T cells and downstream transcription markers have been used in GBM clinical trials [55,103–106]. Interpretation of immune-monitoring is primarily restricted to biomarkers that may be surrogate measures [107]. Also, the use of 'liquid biopsies', in which analysis of blood components can provide a real-time comprehensive picture of tumor-associated biomarkers, may have unique applications in tumor diagnostics and monitoring treatment responses [108]. Evaluated in a proof-of-principle study of various tumor types with a reported 96% accuracy, tumor-educated platelet RNA profiling appears as a unique platform for cancer diagnostics [109]. However, the clinical relevance, validation and applicability of these parameters have yet to be determined, as it is unclear if these assays truly recapitulate the genetic and immune composition of the tumor and the tumor microenvironment. The administration of steroids to suppress brain tumor symptoms from mass effect also

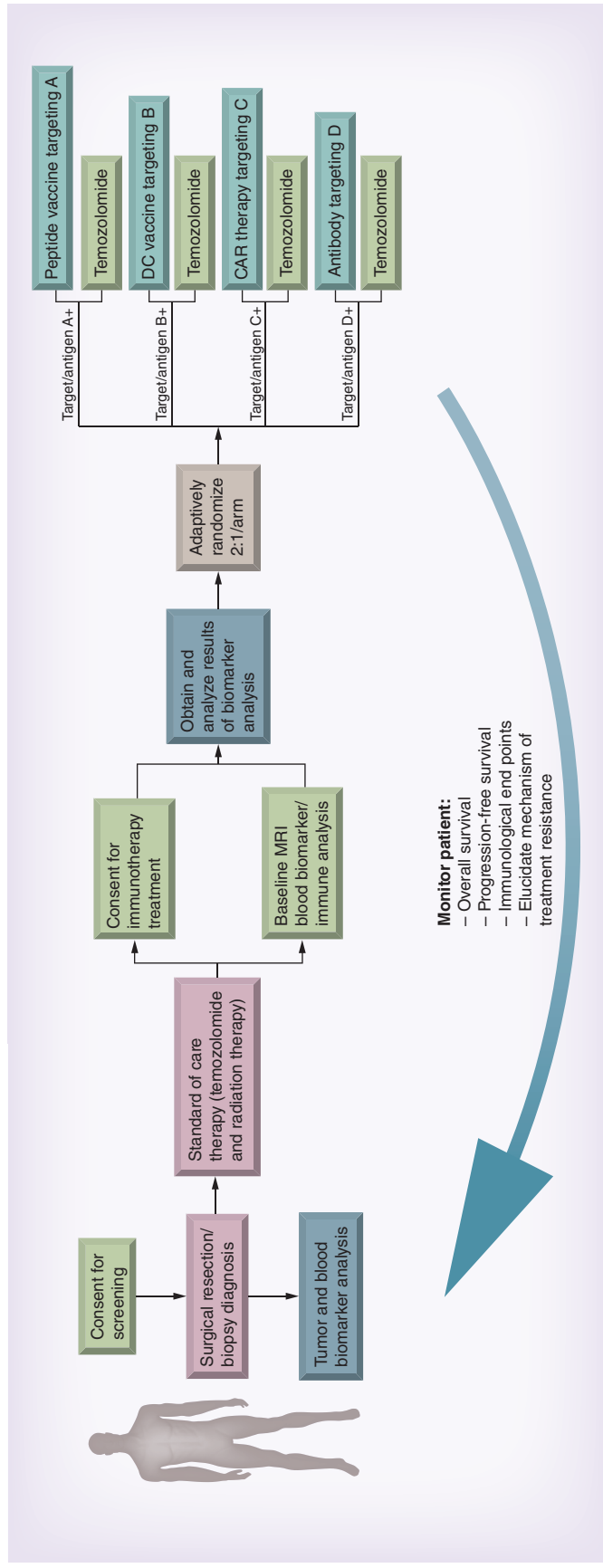

Figure 1. Precision immunotherapy schema for newly diagnosed glioblastoma patients.

suppresses the immune system, which may distort results. This is possibly due to several reasons, including reduced T-cell proliferation, disruption of the TCR complex after glucocorticoid-receptor-ligand binding [110] and suppression of immunomodulators resulting in fewer IFN-γ-producing T cells and increased IL-4-producing T cells [111].

Moreover, how does the field resolve the problem of distinguishing tumor progression from therapeutic immune response/inflammation as it pertains to clinical trial end points and current standard of care? The modified Response Assessment in Neuro-Oncology (RANO) criteria are now being considered for use in immunotherapeutic clinical trials to evaluate response and progression in malignant glioma [112] and to guide decision-making, preventing premature termination of immunotherapy [98]. The multimodal use of advanced brain tumor imaging, molecular imaging and magnetic resonance (MR) spectroscopy is potentially advantageous for noninvasively monitoring malignant glioma patients. For example, MR imaging inflammatory textural analysis, where volumetric and heterogeneity features are extracted from T1-post contrast MR and fluid-attenuated inversion recovery (FLAIR) images, can be used to build a classifier capable of discriminating inflammation status. Quantitative imaging tumor metrics and texture maps can then be used to assess the gene signatures of tumor cell apoptosis, tumor invasion and immune cell infiltration [113,114]. Advanced imaging not only provides potential in clinical trial design, correlating histological and immune functional data obtained directly from the tumor after surgical resection, but may also help with immunotherapeutic dose modification and treatment optimization.

Moreover, as the field is on the cusp of understanding GBM tumor biology and exploring effective therapeutic targets in this disease, we have yet to elucidate which combinatorial treatment strategies are actually beneficial with limited toxicity. For example, understanding chemoimmune interactions over time may shed some light on which patients may truly benefit from combinatorial approaches. Indeed, MGMT-methylated GBMs respond more favorably to temozolomide, a chemotherapy that has mutagenic properties [115–117] and can potentiate antitumor immune responses [103]. Therefore, theoretically, should an immunotherapy that targets increased 'antigen load' be used in combination with temozolomide in MGMT-positive GBM patients? Moreover, what is the best timing strategy for combinatorial therapy? How multiple immunotherapies can be safely combined and also be combined with other therapeutic modalities, such as small molecule inhibitors, tyrosine kinase inhibitors, viral-based strategies, antiangiogenic therapies and more, has yet to be determined. Additionally several preclinical studies are examining the best ways to combine therapeutic treatments for GBM [8,118,119]. Certainly there are ongoing and planned immunotherapy combinatorial clinical trials underway in GBM (NCT02423343, NCT02311920, NCT02529072, NCT02337491, NCT02017717, NCT02526017, NCT02423343, NCT02327078, NCT02327078, NCT02017717).

Conclusion & future perspective

A new era is emerging in precision medicine, as the field of GBM immunotherapy is rapidly progressing toward providing tumor-specific targeted therapies. However, there are challenges that must be resolved in order to address this unmet need in the field. With the development of immune-targeted drugs, progress in clinical trial design and a paradigm shift in the genetic and molecular characterization of gliomas, precision medicine in GBM immunotherapy provides a unique opportunity to change the landscape of how we treat cancer patients.

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