A Window of Opportunity to Overcome Therapeutic Failure in Neuro-Oncology

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overview

Glioblastoma is the most common primary malignant brain neoplasm and it remains one of the most difficult-to-treat human cancers despite decades of discovery and translational and clinical research. Many advances have been made in our understanding of the genetics and epigenetics of gliomas in general; yet, there remains an urgent need to develop novel agents that will improve the survival of patients with this deadly disease. What sets glioblastoma apart from all other cancers is that it develops and spreads within an organ that renders tumor cells inaccessible to most systemically administered agents because of the presence of the blood-brain barrier. Inadequate drug penetration into the central nervous system is often cited as the most common cause of trial failure in neuro-oncology, and even so-called brain-penetrant therapeutics may not reach biologically relevant concentrations in tumor cells. Evaluation of the pharmacokinetics and pharmacodynamics of a novel therapy is a cornerstone of drug development, but few trials for glioma therapeutics have incorporated these basic elements in an organ-specific manner. Window-of-opportunity clinical trial designs can provide early insight into the biological plausibility of a novel therapeutic strategy in the clinical setting. A variety of window-of-opportunity trial designs, which take into account the limited access to treated tissue and the challenges with obtaining pretreatment control tissues, have been used for the initial development of traditional and targeted small-molecule drugs and biologic therapies, including immunotherapies and oncolytic viral therapies. Early-stage development of glioma therapeutics should include a window-of-opportunity component whenever feasible.

Glioblastoma, the most common primary malignant brain neoplasm, remains one of the most difficult-to-treat human cancers despite decades of discovery and translational and clinical research. The median survival, after intensive therapy consisting of surgical resection of the tumor mass and treatment of the residual tumor cells that invade brain tissue with fractionated external beam radiotherapy, cytotoxic chemotherapy (most typically oral temozolomide), and externally applied electrical field therapy, remains stubbornly less than 2 years, and less than 10% of patients survive for more than 5 years after diagnosis with a glioblastoma.[1,2] Many advances have been made in our understanding of the genetics and epigenetics of gliomas in general, with the identification of a multitude of therapeutic targets, some of which overlap with other solid organ cancers that arise outside of the central nervous system.[3,4] It has also been recognized, however, that there is intratumoral heterogeneity in glioblastoma, that mutational burden in most glioblastoma tumors is relatively low, and that these tumors can promote a highly immunosuppressive tumor microenvironment that has contributed to the failure of all attempts to date to target these tumors with systemically administered immunomodulatory agents.[5,6] There remains an urgent need to develop novel glioblastoma-specific agents that will improve the survival of patients with this deadly disease.

What sets glioblastoma apart from all other cancers, however, is that it develops and spreads within an organ that renders tumor cells inaccessible to most systemically administered agents because of the presence of the blood-brain barrier (BBB). The BBB is constructed from a network of endothelial and glial cells that block most systemically administered agents from access to the central nervous system compartment (including the brain, spinal cord, and cerebrospinal fluid). Blood-brain barrier function is served by both active (e.g., drug transport pumps) and passive (e.g., tight intercellular junctions) components, and, even when impaired, these components still prevent most agents from reaching therapeutically effective concentrations in tumor and tumor-infiltrated tissues.[7] It is a common misconception that contrast enhancement within tumor tissue is evidence of an absent BBB, because this radiographic finding represents at least partial disruption of the passive elements of the BBB but not necessarily the active components, which are supplanted by the intrinsic active drug elimination properties of glioblastoma tumor cells themselves.[8] More to the point,

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PRACTICAL APPLICATIONS

- Treatment options for patients with malignant brain tumors—gliomas, in particular—are limited because of the presence of barriers that prevent therapeutics from reaching their targets in the brain.
- Most clinical trials of novel therapeutics do not account for the lack of drug access to tumor tissue and tumor-infiltrated brain and rely on clinical endpoints only.
- Phase 0 and window-of-opportunity clinical trial designs can be used in neuro-oncology to provide early-stage information about the biological plausibility of novel therapeutics.
- Window-of-opportunity trial designs have been used for traditional oncology drugs, targeted small-molecule drugs, immunotherapies, and other biological therapies (e.g., oncolytic viral therapies), and have provided valuable information for making go-no-go decisions for further development.
- A variety of window-of-opportunity trial design options are available to account for the presence or lack of pretreatment tissue for baseline comparison of pharmacodynamic effects.

glioblastomas develop and progress in a biphasic manner; they form a solid tumor that typically is contrast-enhancing, and they also infiltrate the surrounding brain; this portion of the disease is noncontrast-enhancing. All components of the BBB are intact in noncontrast-enhancing tumor–infiltrated brain. Contrast-enhancing glioblastoma most often can be treated surgically; noncontrast-enhancing glioblastoma, on the other hand, generally cannot be removed surgically and is the target of systemically administered therapies, which must overcome an intact BBB to be effective.

It is in this clinical context that one must critically evaluate the processes for development of novel therapeutics for glioblastoma and use this understanding of its unique biology to develop more-informative clinical trial strategies. Inadequate central nervous system penetration is often cited as the most common cause of trial failure in neuro-oncology.[9] Evaluation of the pharmacokinetics and pharmacodynamics associated with a novel therapy, molecularly targeted or otherwise, is a fundamental principle of therapeutic development. For non–central nervous system cancers, determination of blood pharmacokinetics properties is often a reliable indicator of drug exposure at the targeted site of disease, and pharmacodynamic markers in blood and/or tumor tissue provide verification of the expected pharmacological or biologic effects. In the central nervous system, however, knowledge of systemic pharmacokinetics values almost never provides a meaningful indication of brain exposure, because most systemically administered therapeutics are not brain-penetrant.[10] Furthermore, the free-drug hypothesis suggests that high levels of protein binding also prohibit on-target pharmacological effects, because circulating, protein-bound drug remains inactive.[11] Considering that most agents are highly protein-bound,[12] measuring total drug concentration in tumor tissue provides little insight into the pharmacologically active free fraction available capable of targeted inhibition. Even so-called brain-penetrant therapeutics may not have been evaluated for their ability to enter intact brain tissue, because their preclinical evaluation usually is limited to use of an experimental model of glioblastoma in which a tumor is implanted in the brain of a rodent. This orthotopic implant model recapitulates only contrast-enhancing glioblastoma; it does not provide information about drug exposure to the noncontrast enhancing–glioblastoma portion of the disease.

Evaluation of pharmacodynamic markers in glioblastoma is also more challenging than in other cancers. In general, an ideal pharmacodynamic biomarker is proximal to the target, pathway-specific, and quantifiable. For many studies, this is unattainable for the following reasons: (1) proximity can be challenging if the exact mechanism of drug action remains unclear[13]; (2) recursive pathways can complicate assumptions of pathway specificity[14]; and (3) biomarker quantification is only interpretable if there is a reliable baseline value for comparison.[15] Access to the site of disease usually requires a neurosurgical procedure under general anesthesia rather than a simple and repeatable blood sample collection or biopsy of tumor tissue via an office-based or image-guided outpatient interventional approach. Measurement of drug levels and/or pharmacodynamic markers in cerebrospinal fluid is not an adequate surrogate for tumors that arise in the brain parenchyma, because these sources of biological material behave physiologically and pharmacologically as separate compartments.[16] Another challenge to effective pharmacodynamic measurement is intratumoral heterogeneity, which may produce unreliable results.

In light of the unique physiology of the central nervous system, it is not surprising that the traditional path for therapeutic development has not served patients with brain tumors well. Traditional early-stage trials (e.g., phases I and II) largely have incorporated only systemic evaluations of pharmacokinetics and few tumor tissue–based pharmacodynamic assessments in addition to their traditional clinical endpoints. In only a small percentage of early-

stage trials have researchers incorporated alternative design strategies that would permit some evaluation of treated tumor tissue; the various potential strategies were summarized in a 2002 publication.[17] In general, these types of alternative strategies can be lumped together into the phrase "window-of-opportunity" (WoO) clinical trials. Window-of-opportunity studies are designed to evaluate drug penetration and the biological effects of novel therapies on tumor targets and the tumor microenvironment. A typical WoO clinical trial consists of confirming the diagnosis of tumor, treatment with a therapeutic agent, collection of tumor tissue at surgery, and biological assessment of treated and untreated tumor tissues. It is called a WoO because after-treatment tissue acquisition by surgery provides an opportunity to evaluate the molecular, cellular, and immunologic effects of the agent on the tumor. In contrast to a classic phase 0 trial,[18] the patient's treatment may be continued after the resection. Correlations can be made with subsequent response to a biomarker elucidated in the tissues. Unlike traditional early-stage clinical trials, in which drug toxicity and efficacy are evaluated, in WoO trials, tumor tissues can be assessed directly after novel treatments, and researchers can determine if the tested agent exerted an expected biological effect on brain tumors and microenvironment, thus providing critical information to make go-no-go decisions before embarking on expensive and lengthy late-stage clinical trials.

For those agents in which direct tumor microenvironment modulation is desired or needed for target engagement, drug concentrations could also be measured in various regions, such as the infiltrating edge and tumor. Increasingly, this type of strategy is being used to assess immunotherapies for central nervous system tumors, because systemic immune assessments have not correlated with therapeutic response, likely because of the profound tumor-mediated immune suppression, which may not be fully accounted for during systemic blood monitoring and the heterogeneity of immune-cell distribution and migration throughout the tumor microenvironment.

Depending on the selection of controls, there are several designs for WoO trials, including the use of (1) a pretreatment biopsy specimen as the control, (2) surgical specimens from untreated patients, or (3) control specimens from a tumor tissue bank (Fig. 1). In the first design, all patients undergo a pretreatment biopsy, a treatment, and posttreatment surgery. Biopsy and surgery specimens are assessed and compared using established assays. Limitations for this type of strategy are that the biopsy sample is small and may not fully or accurately characterize a heterogenous tumor microenvironment. Furthermore, this process may increase the costs of the clinical trial because only one procedure may be covered by a third-party payer and considered as a standard of care. In the second design, patients do not undergo biopsy but are randomly assigned to one of two groups. One group receives the treatment before surgery, and the other does not. Both groups then undergo posttreatment surgery to resect tumors. Tumor specimens from untreated and treated patients are assessed and compared. This strategy typically has been used in the recurrent patient setting, although, theoretically, it could be

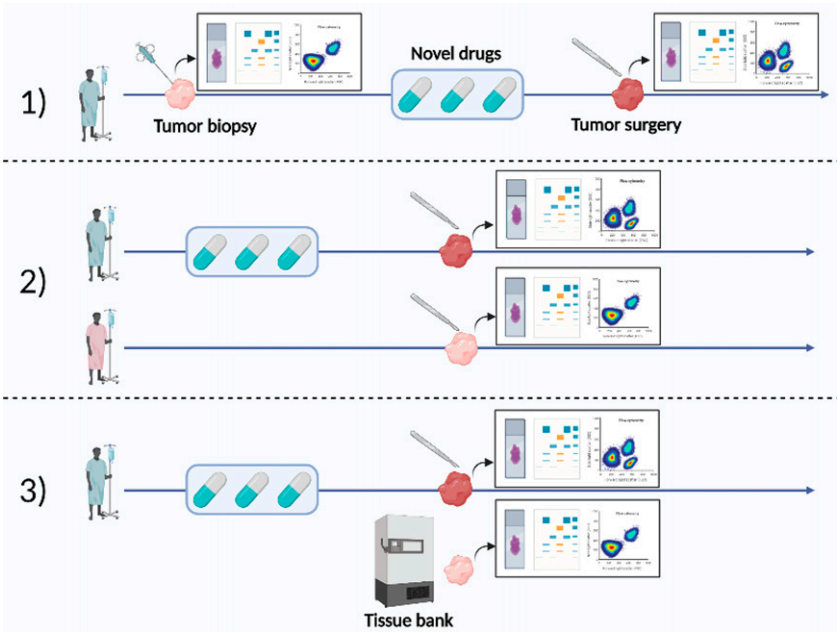

FIGURE 1. Designs for Window-of-Opportunity Trials With Different Controls
(1) Pretreatment biopsy specimens, (2) surgical specimens from untreated patients, and (3) specimens from tissue bank.

conducted in newly diagnosed patients. In the last design, all patients receive treatment and undergo surgery; tissue-bank specimens serve as a control. In the context of immunotherapies, there is marked immune-cell frequency heterogeneity across patients and within the tumor microenvironment, so a sufficient number of patients must be enrolled to obtain an appropriately powered analysis.

Additional critical features that must be considered for this type of trial design include the timing of the tissue acquisition, molecular assays, and dose schedules of the treatment.[19] Tissue acquisition is an important hurdle for brain tumors because of the anatomic location of the brain. Stereotactic biopsy is minimally invasive and serial biopsies can be done before and after drug treatment. However, the caveat is that biopsy specimens are usually small and may not truly represent the tumor. Thus, open craniotomy and surgical resection are incorporated in trials to obtain after-treatment specimens to more fully interrogate the tumor microenvironment and provide sufficient tissue for a variety of assays.

To conduct ex vivo immune functional assays and comprehensive immune phenotyping and characterization, usually at least 1 cm^3 of tumor tissue is needed. A major goal of WoO trials is to show therapeutic levels of the drug in the tumor after delivery or that an immune therapeutic is inducing the desired effector response. This is especially important for brain tumors because of the BBB. Resecting or sampling the adjacent, infiltrating, noncontrast-enhancing region of the brain can provide critical determinations of sufficient drug concentration or immune effector responses that, reasonably, may have a meaningful clinical impact. Although most preclinical assays can be used for WoO trials, such as Western blotting, immunohistochemistry, in situ hybridization, cytometry by time of flight, and single-cell RNA sequencing, it is critical that these assays be validated for clinical specimens. The last critical element is dose schedule. Unlike phase 0 trials in which a subtherapeutic dose is used, WoO trials deliver a therapeutic dose that is potentially effective on the tumor. Length of treatment and the time gap between the last dose and tissue acquisition must be carefully considered to maximize the potential biologic effects that can be detected.

Despite the logistical complexity inherent to WoO clinical trial designs, when successful, WoO trials can greatly facilitate go-no-go decisions, can define a clinically effective dose of the agent that may or may not be below the maximum tolerated dose in phase I trials, and may be used as a measure of efficacy in phase II trials.[17,19,20] Indeed, one of the first clinical trials in brain tumors to formally incorporate a biologic endpoint was a WoO trial assessing an adenoviral vector that expressed the p53 gene (Ad-p53).[21] Through analysis of the distribution of the p53 gene in posttreatment specimens, the investigators concluded that Ad-p53 was unlikely to be a successful therapy and that a vector capable of greater distribution in the tumor was necessary, prompting the investigators to make a no-go decision about gene therapy strategies in general. Since then, the application of WoO trials has increased in brain tumor trials but remains challenging.

Approximately 2 decades after the 2002 publication that summarized various alternative clinical trial strategies,[17] the Response Assessment in NeuroOncology working group undertook a review of how often and effectively these alternative clinical trial strategies were used in early-stage clinical trials of drugs for glioblastoma.[22] The Response Assessment in NeuroOncology review, which was limited to systemically administered small-molecule drugs (not biologic therapies), noted that over the course of more than 3 decades of drug development work for glioblastoma, only 22 publications included some form of pharmacokinetics or pharmacodynamic determination at the site of disease. Of those, only 50% involved use of the maximum tolerated dose or usual clinical dose; almost all of the others used a lower dose, which, in the setting of the BBB, raises questions about the value of any negative result. Tissue samples from noncontrast-enhancing tumors were obtained in only approximately a quarter of the studies, whereas contrast-enhancing tumors were sampled in all but one study. Pharmacodynamics studies were performed in only 68% of studies. In the end, most studies did not provide information that was useful for interpreting the clinical results, largely because of trial design failures (e.g., incorrect dosing, measurement of drug levels only). There were notable exceptions, however, in which analysis of the treated tumor and/or noncontrast-enhancing tumor–infiltrated brain provided mechanistically relevant information that indicated the potential, or lack thereof, of the therapeutic approach.[14,15,23–28] These studies demonstrated that, when properly designed and executed, trials that incorporate pretreatment with the study agent prior to surgery can provide relevant biological data that can support further therapeutic development or bring it to an early end (so-called fail fast) and, thereby, not subject additional patients to futile therapies. The Response Assessment in NeuroOncology review focused on studies with cytotoxic or targeted drug therapies only, and a summary of the studies can be found in that publication.[22] Next, we summarize a number of similar WoO strategies that have been used to evaluate the potential utility of biologic therapies for glioblastoma.

USE OF WOO TRIALS TO EVALUATE IMMUNOTHERAPEUTICS

Many immunotherapeutics are large molecules that are not expected to be able to cross the BBB. However, they may have access to contrast-enhancing tumor, and their systemic effects on the cellular compartment of the immune system potentially can alter the tumor-immune microenvironment within the central nervous system. de Groot et al[29] conducted an open-label, single-center, single-arm phase II

WoO trial with 15 patients with recurrent glioblastoma to ascertain the immune effector function of pembrolizumab on the glioblastoma microenvironment. In this study, patients received up to two doses of pembrolizumab before surgery and every 3 weeks afterward until disease progression or unacceptable toxicities occurred. de Groot et al[29] found that the after-treatment tumor microenvironment was enriched for $CD68^+$ macrophages but poorly infiltrated with effector T cells. Although the study provided detailed phenotypic information of tumor microenvironment in patients with glioblastoma treated with pembrolizumab, this study lacked a matched control group.

Cloughesy et al[30] conducted a trial evaluating neoadjuvant anti–PD-1 immunotherapy for recurrent glioblastoma. A total of 32 patients received either neoadjuvant pembrolizumab (16 patients) or adjuvant alone (16 patients) followed by tumor resection. Fifteen patients from each group were evaluated for tumor tissue analysis. Compared with adjuvant-alone specimens, neoadjuvant PD-1 blockade specimens showed upregulation of T-cell and interferon-γ–related gene expression, but downregulation of cell cycle–related gene expression, suggesting that neoadjuvant anti–PD-1 immunotherapy promoted intratumoral immune responses in recurrent glioblastoma and may represent an efficacious therapeutic strategy.

Weathers et al[31] conducted a phase I/II trial evaluating treatment with cytomegalovirus-specific T cells on glioblastoma. Of 20 patients who completed at least one cycle of T-cell infusion after dose-dense temozolomide, one patient with recurrent glioblastoma underwent tumor resection. In this recurrent-glioblastoma specimen, cytomegalovirus-specific T cells were found mostly confined to the perivascular space. The $CD8^+$ T cells isolated from the tumor microenvironment were found to be more refractory to immune stimulation and unreactive to cytomegalovirus-peptide stimulation indicating profound glioblastoma-mediated immune dysfunction on these T cells. These analyses, in addition to outcome data, ultimately informed the decision not to proceed to later-stage clinical trials without further refinements to the strategy.

USE OF WOO TRIALS TO EVALUATE ONCOLYTIC VIRAL THERAPIES

Oncolytic viruses are a good example of novel agents that have been effectively assessed in early-phase trials using WoO designs. Oncolytic viruses are genetically engineered viruses that can selectively infect and replicate within cancer cells and cause subsequent lysis of the cells.[32] Several types of oncolytic viruses have been developed as cancer therapeutics, including adenovirus, herpesvirus, measles virus, coxsackievirus, reovirus, retrovirus, and vesicular stomatitis virus.[33]

One oncolytic virus that has been extensively evaluated using the WoO design is Delta-24-RGD (DNX2401), an oncolytic adenovirus. This agent has been evaluated in a phase I, dose-escalation, WoO clinical trial of intratumorally injected Delta-24-RGD in 37 patients with recurrent malignant glioma.[34] The study included two arms, a standard arm and a WoO arm, with the goal of assessing safety, efficacy, and the mechanism of action of Delta-24-RGD (Fig. 2). Patients in arm A received a single intratumoral injection of Delta-24-RGD into biopsy-confirmed high-grade gliomas. These patients were monitored for toxicity according to standard phase I criteria and for response based on serial MRI. Patients in arm B received an intratumoral injection through an implanted catheter, followed 14 days later by en bloc resection of the tumor along with the catheter (to mark the injection site), providing a posttreatment specimen for analysis of biologic endpoints. After resection, Delta-24-RGD was injected into the postresection tumor bed and patients were followed for tumor recurrence.[34] By incorporating both traditional assessments and WoO assessments, the investigators were able to define the maximum tolerated dose, demonstrate

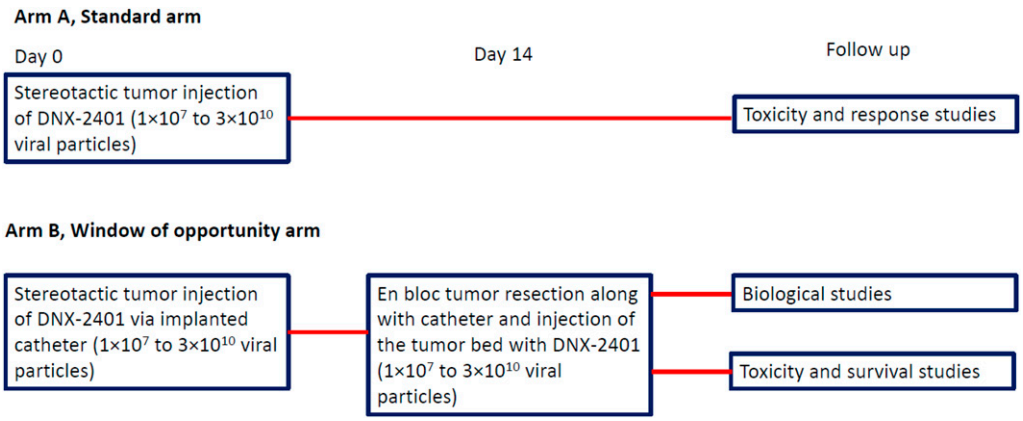

FIGURE 2. Delta -24-RGD Clinical Trial Schema

potential efficacy, and document that these effects were a result of the action of the virus. Specifically, assessments of patients in arm A enabled determination of the maximum tolerated dose and that this dose appeared to provide clinical benefit in nearly half of patients. Also, immunostaining for viral hexon and E1A proteins of posttreatment specimens from the WoO arm (arm B) demonstrated that Delta-24-RGD could infect tumor cells and then replicate, lyse, and spread to nearby cells in human glioblastoma specimens, providing a biological basis for the efficacy seen in arm A and thereby documenting that the virus could do what it was developed to do.[34] Additional assessment of posttreatment specimens showed $CD8^+$ and T-bet^+ T-cell infiltration after treatment. Compared with pretreatment specimens, there was an increase of CD4 and CD8 cells and a decrease in TIM-3 expression after treatment, providing seminal evidence for an immune-mediated effect of this virus. Given the signals of direct infection and replication along with positive immune modulation within the tumor microenvironment, this strategy has been advanced to later-stage clinical trials.

WoO trials have been used to demonstrate the activity of other oncolytic viruses. A genetically engineered herpes simplex virus (G207) that replicates conditionally in replicating cells including glioblastoma was developed and tested in preclinical models with promising results.[35–37] A phase I study of G207 for treatment of patients with glioblastoma demonstrated antitumor activity of G207 and presence of the virus in the tumors, based on analysis of biopsy samples from some of the patients in the study.[38] In subsequent studies in which posttreatment tumor specimens were analyzed, researchers have demonstrated viral replication of G207 within tumors and immune-cell activation,[39] immune-cell infiltration of the tumors after injection of the virus,[40] and activation of an interferon-driven adaptive immune response, which correlated with survival outcomes.[41] Other herpes simplex-1 viruses that are being evaluated for glioblastoma therapy include rQNestin34.5v.2 and C134.[42,43]

An oncolytic parvovirus (ParvOryx01) has been evaluated in glioblastoma via intratumoral injection of the virus prior to tumor resection, followed by tumor resection and injection of the virus into the tumor bed postresection.[44] Examination of the tumor tissue after viral injection demonstrated viral replication, induction of cathepsin B, and tumor infiltration by activated immune cells; clinical outcomes were similar to those of historical controls.[44] These results suggest that ParOryx01 may be an effective therapy when combined with immunotherapies.

An oncolytic human orthoreovirus has also been evaluated in a WoO trial. In this trial, researchers investigated the effects of intravenous administration of this oncolytic virus on the brain tumor microenvironment.[45] Nine patients were recruited to a phase Ib trial in which they were treated with an oncolytic virus before surgical resection of the brain tumor. Patient tumors outside this trial were used as controls. The researchers detected oncolytic viruses in resected brain tumors, using immunohistochemistry and in situ hybridization. Immunohistochemical analysis demonstrated $CD3^+$ T-cell infiltration in virus-treated tumors but not in untreated control tumors. More $CD68^+$ microglia or infiltrating macrophages were found in tumors from patients treated with oncolytic virus than in control tumors. Expression of genes associated with apoptosis and PD-L1 expression was higher in tumors resected from patients treated with oncolytic virus than in matched control tumors, suggesting that an immune response had been induced. On the basis of these results, this virus may eventually be used in combination with PD-1/PD-L1–axis inhibitors for the treatment of brain tumors.[45]

CONCLUSION

Window-of-opportunity clinical trials offer a unique opportunity to assess biological changes induced by novel therapeutic agents in the tumor and its microenvironment. For pharmacological therapies, given the multiplicity of implicated signaling pathways,[46] as well as the ever-shifting intratumoral landscape,[47] monotherapy clinical trials are unlikely to succeed. Arriving at intelligent drug combinations, however, requires detailed understanding of tumor pharmacodynamics, as well as logical pairing of complementary targets. Tissue-based trialing is an essential first step in evaluating any putative clinical therapeutic combination in glioblastoma. Devising study protocols that not only evaluate clinical effects but, more importantly, elucidate the tumor's molecular responses to combined inhibition will be increasingly important as the field evolves beyond monotherapy studies. For immunotherapy and/or oncolytic viral therapy of brain tumors, trials can tell us if the therapeutic engaged the target, induced tumor-cell death, and/or modulated the tumor microenvironment (e.g., increased T-cell infiltration). Fundamental components of WoO trials include (1) posttreatment tumor acquisition, (2) predetermined assays to detect biological effects, and (3) baseline controls. Window-of-opportunity trials routinely should be incorporated into future early-stage clinical trials for glioblastoma and other brain tumors.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT
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