CLINICAL INVESTIGATION

Challenges in clinical trial design for recurrent glioblastoma

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Glioblastomas are rare tumors but aggressive and often incurable. Since clinical trial enrollment is limited by the relative rarity of the disease, clinical trials should be well designed so that advances are made efficiently. The field has been dominated by smaller Phase II studies since they provide the initial screening of drug efficacy. These studies are prone to issues of selection bias, inappropriate historic controls and confounding clinical variables. There are also issues specific to glioblastoma, including the difficulty in interpreting radiographic responses in the setting of treatment effects and agents that affect vascular permeability and the difficulty of performing pharmacokinetic and pharmacodynamic studies given the relative inaccessibility of the CNS compartment. These barriers have also hindered the development of radiographic and molecular biomarkers. As we move into the era of personalized medicine, it is increasingly important to address these issues in the design of clinical trials.

> Keywords: angiogenesis • bevacizumab • clinical trial • glioblastoma • immunotherapy • molecular targeted therapy • pseudoprogression

The Central Brain Tumor Registry estimates that approximately 11,000 people will be diagnosed with glioblastoma (GBM) in 2013 [1]. Median survival with standard treatment, including maximal safe resection, chemoradiation and adjuvant temozolomide is 14.6 months [2] though there is some evidence that outcomes have improved in the past decade [3]. Despite recent advances in the field, including the use of bevacizumab at disease progression, outcomes continue to be poor [4,5]. Better treatments are urgently needed, especially in the setting of disease recurrence where current treatments are not curative and improve survival by only a matter of months, if at all [6]. Understanding the challenges in clinical trial design is critically important to our efforts to identify the most promising drugs and to understand how best to use them.

General issues in GBM trial design

Historical controls

The demonstration of the superiority of temozolomide combined with radiation (the 'Stupp regimen') over radiation alone set a new benchmark for outcomes in patients with newly diagnosed GBM, and these outcomes are often used as a historical control for clinical trials [2]. There is evidence, however, that outcomes are improving over time independent of any new and consistent therapeutic intervention. In a cohort of newly diagnosed patients treated on consortia trials from 2005 to 2009, including one trial where patients were treated with the Stupp regimen along with monitoring of CD4⁺ counts, median survival was 19.6 months [3], which compared favorably with the 14.6 months seen in the Stupp trial [2]. It is important to note that the consortia trials used the date of diagnosis for determination of survival while the

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Stupp trial used the date of randomization on the study. It is unlikely that the use of bevacizumab at recurrence, which became more prevalent between 2005 and 2009, explains the differences in outcomes between these two cohorts. Although treatments at recurrence were not captured in all of the consortia trials, in one of the trials only 28% of patients received bevacizumab at recurrence, suggesting that other factors are also contributing [7]. In addition, outcomes in the consortia cohort were significantly better than the 14-16 months reported in both treatment arms of RTOG 0825 and AVAglio, where newly diagnosed patients were randomized to the Stupp protocol alone or in combination with bevacizumab (and many patients in the control arms crossed over to bevacizumab at progression), so it is likely that factors other than the use of bevacizumab are contributing factors [8,9]. The authors postulate that the use of experimental agents with some activity and improving the standard of care for patients with GBM are contributing. They point out that our understanding of pseudoprogression (PsPD) in particular, improved over this period, so that it is likely that more patients received the full six cycles of temozolomide. Since these issues are also relevant in the setting of recurrent GBM, this 'outcome drift' needs to be kept in mind when designing clinical trials for recurrent disease so that the effect of the therapeutic intervention is not overestimated.

In 2008, Lamborn et al. published survival outcomes of a series of 437 patients with recurrent GBM treated on clinical trials between 1998 and 2002 with drugs that were ultimately deemed ineffective, and this cohort is now often used as a historical control group for clinical trials in recurrent disease. Median overall survival (OS) in that group was 30 weeks, and median progression free survival (PFS) was 8 weeks [10]. The validity of that historical control, however, has recently been called into question with the increasing use of bevacizumab, given its profound effects on MRI scans that makes it difficult to assess tumor response and progression. Furthermore, bevacizumab may be prolonging PFS but not increasing OS compared with other agents by allowing more rapid tumor growth once patients do progress on bevacizumab. Several small case series have described outcomes on salvage therapy after progression on bevacizumab, with median PFS ranging from 4 to 8 weeks [4,11-13], and a larger retrospective series of 100 patients found a median OS of 4 months [14]. As more patients are treated prospectively on clinical trials after recurrence on bevacizumab, it will be useful to describe their outcomes in a large cohort to provide a more accurate historical control for this patient population.

Some groups have questioned the validity of using historical controls, and have favored the use of randomized Phase II trials to avoid the effects of outcome drift, as well as patient selection effects. Tang *et al.* simulated the error rate of single-arm, historically controlled trials compared with randomized concurrently controlled trials in a large cohort of colorectal cancer patients treated on Phase III trials and found that the false-positive rate was two- to four-times higher in single-arm trials [15]. It seems clear that randomized trial designs should be favored whenever possible.

Over the last several years the Stupp regimen has been utilized as the standard-of-care in randomized trials of patients with newly diagnosed GBM with the experimental agent added to radiation and/or postradiation temozolomide. With the growing recognition of the minimal benefit of temozolomide in GBMs with hypomethylated MGMT promoters; however, an increasing number of trials are being designed where the experimental arm does not utilize temozolomide at all and only the investigational agent(s). The issue is more complicated in recurrent GBM, where there is significant debate about the most appropriate standard treatment. Since bevacizumab is approved and commonly used for recurrent GBM, there is an interest in building on the success of this agent. This has led to several randomized Phase II trials comparing bevacizumab alone or combined with an agent of interest [16]. Some groups have randomized patients to experimental treatment or 'best standard of care' though the heterogeneity in the control group makes it difficult to compare outcomes [17]. Since bevacizumab has been proven to be active in recurrent GBM, the greatest clinical need going forward will be the treatment of patients with bevacizumab-resistant disease. At the very least, a better description of outcomes after bevacizumab-failure in a larger cohort will be necessary to give researchers a better benchmark for assessing new therapies. Randomized trials should be considered for bevacizumab-refractory patients, though it is less clear what the control group for those trials should be.

Pseudoprogression

Since the use of concomitant temozolomide has become standard in the initial management of GBM, a number of groups have reported an increased incidence of PsPD, defined as an increase in contrast enhancement postradiation that subsequently stabilizes or improves without a change in tumor treatment [18]. The incidence of PsPD ranges from 21 to 37% [18–21] compared with approximately 9% with radiation alone [20]. PsPD is more common in patients with a methylated MGMT promoter. In the largest published case series, 31% of all patients treated with radiation and temozolomide were diagnosed with PsPD, but the rate was significantly higher among methylated patients (58%), than amongst unmethylated patients (16%) [18]. Since 34% of patients diagnosed

with PsPD in that series were unmethylated, MGMT status cannot be used to definitively diagnose PsPD [18]. Recent response assessment in neurooncology (RANO) criteria addressed this issue by narrowing the definition of progression in the first 12 weeks after radiation to patients with progression outside of the radiation field or unequivocal pathologic evidence of viable tumor in a new biopsy sample [22]. Although these guidelines have certainly helped guide clinicians so that patients are not inappropriately enrolled onto clinical trials unless a diagnosis of recurrence is confirmed, there is some evidence that PsPD may occur substantially later than 12 weeks in some patients (Figure 1) [23]. PsPD should be considered even after 12 weeks, especially in patients with methylated MGMT and whose symptoms are stable or improving. This has to be weighed carefully against the risk of true early tumor progression, since 18-21% of patients had confirmed early tumor progression in these series [18,21] and in some cases biopsy is required for definitive diagnosis. This also means that patients with the most refractory GBMs, those that progress through concurrent radiation and temozolomide, might never be enrolled onto clinical trials since they are likely to be too ill to be appropriate for a trial by the time 12 weeks of postradiation has passed.

Prognostic factors

Age, performance status and extent of resection are all well-established prognostic factors for patient survival in GBM [24-26]. The differences in outcomes stratified by these variables are striking. For instance, in a large cohort of patients treated on Radiation Therapy Oncology Group trials, patients who were ≤30 years old survived almost three-times as long as patients who were >50 years old (18–20 vs 6–9 months) [27]. In another study, patients who received a complete resection lived 50% longer than those who received an incomplete resection (17 vs 12 months) [28]. These variables have such a profound effect on patient outcomes that they must be carefully considered in trial design, or they can confound results.

Although the median age for GBM patients is 61 years in population-based cohorts, and 25-30% of patients are ≥ 70 years at diagnosis [29,30], many clinical trials, including the landmark Stupp trial [2], restrict enrollment to patients younger than 70 years of age. Even in trials where enrollment is not restricted by age, patients enrolled in clinical trials tend to be younger, with the median age ranging from 45 to 58 years [3,31]. This not only makes it difficult to extrapolate results to unselected patient populations treated outside of clinical trials but also makes it difficult to interpret the results of such trials. It can be difficult to discern a true therapeutic effect of a drug when so much of the variability in outcomes can be explained by the differences in age.



Figure 1. Late pseudoprogression. T1-postcontrast MRI of the brain of a patient with glioblastoma with a methylated MGMT promoter (**A**) before radiation, (**B**) 1 month after completing radiation, (**C**) at the end of cycle 3 of adjuvant temozolomide and (**D**) at the end of cycle 6 of temozolomide. New foci of enhancement were seen on the postradiation scan and increased in size and coalesced during the six cycles of temozolomide, though the patient remained clinically stable. (**D**) Necrosis developed in the center of the enhancing lesion during treatment. Temozolomide was then stopped, and (**E**) 3 months and (**F**) 5 months after stopping treatment, the enhancing lesion decreased in size. The patient is currently progression free, 18 months after the initial diagnosis.

Extent of resection is more difficult to quantify in large patient cohorts. Several groups have used 3D volumetric imaging to quantify resections and have found a survival benefit when resecting anywhere from 78 to 98% of the enhancing tumor [28,32,33]. Since volumetric assessment of residual tumor volume is not feasible for many clinical trials, most groups report the percentage of patients receiving gross total resection (GTR), subtotal resection, or biopsy alone. The group of patients receiving subtotal resection in particular is quite heterogeneous and some in that category may receive a resection extensive enough to potentially affect their survival, although it is difficult to identify that subgroup and it probably varies widely between institutions and trials. Additionally, there are significant differences in the percentage of patients receiving GTR between trials and thus results need to be interpreted with caution in trials where a majority of patients receive a GTR or, conversely, where a majority receive only a biopsy.

Data from the Cancer Genome Atlas has recently demonstrated several distinct molecular subtypes of GBM characterized by different patterns of somatic mutations, DNA copy number alterations, gene expression changes and DNA methylation changes [34,35]. It has become increasingly clear that at least one of these subtypes is an important prognostic factor for survival. The Proneural subtype is characterized by *PDGFRA* amplification, *IDH-1* and *TP53* mutation [34]. The G-CIMP group is a subgroup of proneural tumors characterized by a distinct DNA methylation pattern [35], and defines a subset of patients who tend to be younger and have improved survival compared with other subtypes. Patients enrolled in clinical trials are, for the most part, not currently stratified by their molecular subtype since the technology is costly and not widely available. Reporting of the molecular phenotype/genotype in trial patients would allow for a more accurate understanding of a cohort's prognosis *a priori*. It might also identify subgroups of patients more likely to respond to particular treatments since these subtypes appear to have a different biology.

As discussed above, the best way to control for the impact these prognostic factors have on outcomes is to design randomized trials stratified for the most important of these prognostic factors. When this is not possible, these factors should be described fully for the cohort in trial reporting so that results can be interpreted in the context of the cohorts' expected prognosis.

Drug–drug interactions

Approximately 25% of patients with GBM have seizures as part of their initial presentation [36] and many are treated with enzyme-inducing anti-epileptic drugs (EIAEDs). EIAEDs cause significant drug-drug interactions through induction of hepatic CYP3A4 with variable metabolism of other concomitantly administered hepatically metabolized drugs. The degree of induction can vary greatly, both between different agents in this class and between patients [37]. In an early Phase I trial of enzastaurin in patients on EIAEDs, for example, where most patients were treated with potent enzyme-inducers such as phenytoin, a clear induction effect was seen [38]. This was not observed, however, in a subsequent Phase I trial using twice-daily dosing of the drug in patients on EIAEDs, where a large percentage of patients were treated with less potent inducers such as oxcarbazepine [39]. Accordingly, doses of agents established in Phase I trials in solid tumors may not be applicable to the GBM patient population and separate Phase I trials and pharmacokinetic (PK) studies in patients on EIAEDs are sometimes required. When such trials are performed, close attention should be paid to the specific EIAEDs used and the degree of induction expected when interpreting PK data. Many groups restrict enrollment in Phase I trials to patients who are not taking EIAEDs to avoid these issues thereby expediting Phase I and II testing. Only drugs that look promising in Phase II studies would then require separate Phase I studies in patients on EIAEDs.

Inaccessibility of the CNS compartment

The compartmentalization and relative inaccessibility of the brain compared with other organs represent major challenges in tissue acquisition and the design

and implementation of novel trial designs in GBM. The blood-brain barrier (BBB) prevents delivery of many drugs to the brain parenchyma and cerebrospinal fluid (CSF) through a variety of mechanisms. Tight junctions formed by endothelial cells, astrocytic end-feet processes and pericytes prevent the diffusion of molecules >400 Da into the brain parenchyma [40,41]. Although GBM can cause disruption of these tight junctions [42,43], areas of infiltrative tumor often exist behind an intact BBB. Active efflux mechanisms, including p-glycoprotein, multidrug resistance proteins (MRP1-6) and BCRP also inhibit drug penetration in many cases [44,45]. Ideally, Phase I trials should include measurement of drug concentrations within the tumor and the CSF (which themselves are two distinct PK compartments) since PKs can differ greatly between the CNS compartment and the systemic circulation. Nevertheless, both the cost and safety issues involved in biopsying these tumors often preclude such studies. These same issues often make pharmacodynamic (PD) studies prohibitive. Therefore, in cases where a drug is ineffective it can be difficult to differentiate insufficient drug delivery from incomplete target inhibition or adaptive resistance.

Since surgical resection is in some cases clinically indicated at the time of tumor progression for palliation of symptoms, a number of groups have addressed this issue by treating a subset of patients with a short course of experimental treatment before a clinically indicated surgical procedure, allowing for appropriate PK/PD studies to be performed on the resected tissue (Table 1). Due to the importance of these data in evaluating efficacy, especially for molecular targeted agents, Phase I studies should include such a cohort whenever possible.

Some groups have also attempted to overcome drugdelivery issues by delivering therapy directly to the tumor, either through diffusion-based or convectionenhanced drug delivery. Gliadel® (MGI Pharma, MN, USA) wafers are the most studied example of the former, and consist of biodegradable polymer matrix impregnated with carmustine, which is implanted within the surgical cavity at the time of tumor resection. A randomized Phase III trial showed a modest improvement in survival - 13.9 months compared with 11.6 months with placebo wafers [46]. The toxicity of these wafers, which includes CSF leak (5%), wound healing abnormalities (14–16%), intracranial hypertension (4–9%) and intracranial infection (4-5%) has limited their utility [47]. Furthermore, PK studies in animals suggest that drug penetration is typically only 1-6 mm, and the gradient is quite steep, due to capillary clearance of this highly lipophilic drug [48]. Glioma cells, by contrast, are known to infiltrate >1 cm beyond the enhancing margin [49]. There has therefore been more interest

Table 1. Examples of clinical trials in recurrent glioblastoma with a surgical cohort for pharmacokinetic/ pharmacodynamic studies.					
Author	Drug	Patients (n)	Pharmacokinetic data	Pharmacodynamic data	Ref.
Hegi <i>et al</i> .	Gefitinib	22	Median concentration 4.1 µg/g in tissue, on average 22-fold higher than plasma	EGFR was efficiently phosphorylated, but no significant effect on 12 downstream pathway constituents	[95]
Cloughesy <i>et al</i> .	Rapamycin	15	Rapamycin concentration in tumor tissue 0.36–36.6 nM (concentrations of ~1 nM are active <i>in vitro</i>)	No consistent effect on downstream signaling, as measured by ribosomal protein S6 phosphorylation	[108]
Gilbert <i>et al</i> .	Cilengitide	30	Cilengitide concentrations in tissue 224–4210 ng/g, three- to four-fold higher than concentrations in plasma	Tumor samples were either too small to measure both drug concentration and perform molecular analysis, or sample was inadequate after removal of necrosis and gliosis	[109]
Lassman et al.	Erlotinib and gefitinib	18	Trough erlotinib concentrations 6–8% of plasma Trough gefitinib concentrations 221–370% of plasma	No consistent effect on EGFR phosphorylation or downstream signaling through ERK and AKT No correlation between EGFR activity or downstream signaling and sensitivity to drug	[110]
EGFR: EGF receptor.					

in the past decade in convection-enhanced delivery, where drug delivery is powered by pressure gradients rather than diffusion, to allow larger volumes of distribution [50,51]. Clinical trials using chemotherapies [52], monoclonal antibodies [53] and targeted toxins [54], have demonstrated the feasibility of convection-enhanced drug, but have also highlighted issues to be addressed in future trials, including high rates of elimination and elevated interstitial fluid pressure within bulk tumor [50]. Toxicities including increased edema, neurologic deterioration, seizures, infection and bleeding have limited enthusiasm for this approach to some extent [55]. There is increasing interest in the use of nanoparticles to improve drug delivery and to maintain therapeutic levels in the tissue after the infusion, which could address some of the technical issues [50].

Response assessment

Traditional efficacy end points for Phase II trials in recurrent GBM include measurements of PFS and OS. Many trials also report the number of radiographic responses. The relative importance of each of these response criteria depends on the mechanism of action of the drug. For antiangiogenic agents, for instance, effects on vascular permeability complicate radiographic response assessment, and survival end points are a more reliable indicator of activity. For molecular targeted agents, in contrast, signaling pathways are so complex

and interconnected that significant improvements in PFS or OS with single agents are unlikely, and radiographic responses may be the best indicator of activity to warrant further study in combination trials. These issues will be discussed in further detail below but in general it is clear that the response criteria need to be tailored to the mechanism of action of the drug being studied.

There is increasing interest in looking at more functional outcomes, such as cognition and quality of life as secondary end points, in addition to traditional response criteria. Anti-VEGF agents, for instance, can have significant effects on decreasing GBM-induced cerebral edema, thereby improving neurological symptoms and signs and decreasing the amount of glucocorticoids a patient may require. A number of groups have documented a significant decrease in steroid doses in patients treated with these agents [4,56-58] as well as improvements in independent living [57], Karnofsky performance status [58], activities of daily living [59] and cognitive function [60]. Henricksson et al. published a review of health-related quality of life and cognitive function, and touched on some important issues in the use of these response criteria in clinical trials [61]. These include the availability of different instruments and questionnaires, lack of well-powered longitudinal studies of functional and neurocognitive outcomes to use as historical comparators, high rates of nonrandom missing data that can introduce bias and difficulty in collecting lengthy questionnaires, especially in patients with cognitive impairments and physical limitations. Nevertheless, these outcomes are important to measure, particularly since survival after recurrence is often short and many patients value these functional outcomes as much as improvements in survival. The identification of instruments that are easy to administer and are sensitive to changes in functional and neurocognitive outcomes will allow for better clinical trial design.

Issues specific to antiangiogenic therapies

The use of bevacizumab, and other antiangiogenic agents to a lesser degree [62-65], at the time of recurrence may be improving patient outcomes but has also presented new challenges to the field. Results in early Phase II trials of bevacizumab at recurrence were encouraging with radiographic responses seen in 57-71% of patients and median PFS and OS of 4-6 and 8-10 months, respectively [4,5]. The radiographic response rate in particular was significantly higher than that seen for other therapies, where radiographic response rates of 11-14% had been reported [66-70]. Unfortunately, the 6-month PFS of 29-46% was correspondingly not as high as one might have expected given the high radiographic response rate thereby suggesting that a transient radiographic response did not lead to a durable antitumor response in a significant number of patients. Interpreting radiographic responses to bevacizumab continues to be a significant challenge in the field.

It has long been understood that tumor-associated blood vessels are abnormally permeable and lack the typical tight junctions found in the intact BBB [71,72]. It is this permeability that allows extravasation of intravascular contents such as fluid and contrast dye into the brain parenchyma, resulting in the contrast enhancement that is typically found in GBM and other malignant tumors of the CNS. Bevacizumab and other VEGFtargeted therapies cause rapid normalization of vascular permeability [73], often visible on MRI within 24–96 h of the first infusion [4,74]. This early decrease in contrast enhancement is felt to represent a vascular effect since this is too rapid to represent true tumor regression. Nevertheless, some patients with recurrent GBM treated with bevacizumab remain progression free for many months, consistent with a true antitumor response. Thus, it is nearly impossible to determine when an early MRI response will be a transient 'pseudoresponse' (Figure 2) and when it will ultimately be a durable tumor response. These pseudoresponses are so common that it is unclear that standard MRI response criteria are an appropriate outcome measure in clinical trials of antiangiogenic therapies. The RANO criteria attempted to address this issue by emphasizing the evaluation of

nonenhancing FLAIR disease in addition to enhancing disease in patients treated with antiangiogenic agents [22]. This has been most useful in defining an important category of disease progression on antiangiogenic agents, characterized by significant increase in FLAIR/T2 disease in the setting of stable enhancing disease. Response is still defined by the disappearance (CR) or decrease (≥50%, PR) in enhancing disease by RANO criteria, however, and differentiation of pseudoresponse and durable enhancing response remains difficult. Accordingly, a number of groups have attempted to identify other imaging biomarkers that may predict more durable responses to bevacizumab. Increased perfusion on MRI [75], decreased uptake on flourodeoxyglucose PET [76,77], higher mean apparent diffusion coefficient (ADC) [78] and a decrease in (18)-fluorothymidine (FLT) uptake on (18)F-FLT PET [79] were predictive of response to bevacizumab and improved survival in small groups of patients. None of these biomarkers has been validated in larger, prospective studies or compared in randomized trials; therefore, it is unclear whether in fact these will hold up to being truly predictive of long-term response to bevacizumab. Furthermore, many of these newer imaging methodologies are expensive, labor intensive and not widely available. Nevertheless, validation of imaging biomarkers continues to be a priority, not only because it would be of clinical use, but also because clinical trials evaluating these agents would be enriched if radiographic response to these drugs could be more reliably measured.

For now, OS may be the only reliable and accurate outcome measure for true antitumor effect of any novel anti-VEGF drug.

Issues specific to molecular targeted therapies

As our understanding of the genetic and epigenetic alterations that drive GBM has improved, molecular targeted therapies have become increasingly attractive agents to study at the time of disease progression. The complexity of the signaling pathways involved, however, presents unique challenges in the design of clinical trials to study such agents. Research involving the EGFR pathway provides an illustrative example of a number of these issues.

EGFR is the most frequently amplified gene in GBM, occurring in 40–50% of GBMs [80,81]. It activates a signaling cascade via PI3K, which leads to downstream activation of AKT and mTOR and multiple downstream effects on cell proliferation and survival [82]. The pathway is associated with resistance to therapy and poor prognosis [83,84], making it an attractive therapeutic target. Despite this, outcomes with EGFR inhibitors such as erlotinib and gefitinib have been disappointing with radiographic response rates of 0–6% and minimal impact on PFS and OS [85–89].

There are a number of potential explanations for the lack of activity of these drugs and understanding them will help us to design better trials in the future, not only for EGFR-targeted drugs, but also for other targeted therapies. First, there is interconnectivity between signal transduction pathways and redundancy in the inputs that drive the PI3K signaling pathway so that downstream signaling can be maintained despite EGFR inhibition. PI3K signaling can be maintained by an AKT-independent pathway dependent on PKC [90], or through activation of the pathway through alternate receptor tyrosine kinases such as MET, PDG-FRa and ErbB3 [91]. Assaying the activity of the other dominant nodes in these pathway networks in clinical trials would allow a more nuanced understanding of how a drug is working in vivo and what mechanisms of resistance are present. Based on the experience in other tumor types, there may also be genetic alterations in these target pathways that confer resistance to EGFR inhibition that could explain the lack of response in a subgroup of patients. An increased effort needs to be made to identify genetic and other biomarkers that are predictive of response and resistance to targeted agents such as EGFR inhibitors.

The difficulty of identifying reproducible and robust biomarkers for responsiveness to targeted agents is exemplified by the findings of Mellinghoff et al. who published a retrospective study examining 49 patients with recurrent GBM treated with EGFR inhibitors [92]. They hypothesized that loss of PTEN, an inhibitor of PI3K and a tumor suppressor, would cause persistent PI3K signaling and dissociation from EGFR signaling, and thereby cause resistance to EGFR inhibitors. Conversely, they hypothesized that the EGFRvIII mutation, which causes constitutive activation of the EGFR receptor, would cause 'pathway addiction' and sensitize cells to EGFR inhibitors. They found that co-expression of EGFRvIII and PTEN was significantly associated with a response to EGFR inhibitors, and finding that they confirmed in another small cohort of 33 patients. These findings, however, were not reproducible in a number of larger prospective studies [87,89,93,94], perhaps due to some of the other mechanisms of resistance outlined above [95]. This highlights the idea that biomarkers need to be studied in large groups of patients in a prospective manner and validated in prospective cohorts before they can be used to predict response or resistance to therapy. Since most molecular targeted agents are expected to be effective in subsets of patients with relevant alterations in the pathway targeted by the agent, the development of robust biomarkers would aid in enriching for patients most likely to respond to a given agent in future clinical trials.



Figure 2. Avastin pseudoresponse. (A) T1-postcontrast and **(B)** FLAIR before treatment with avastin. **(C)** After two avastin infusions, there is a slight decrease in enhancement and **(D)** a more profound decrease in edema on FLAIR, after three more infusions; however, in **(E)** there is growth in the enhancing tumor, although, as shown in **(F)**, edema is still well controlled, suggesting that the initial response was a 'pseudoresponse'.

Clinical trials of molecular targeted agents should optimally provide a clear understanding of whether the tested agent is having a biological effect, which can be difficult to measure in standard Phase II clinical trial design because this does not always correlate with a clear clinical benefit. Trials that look only at traditional clinical end points such as radiographic response, PFS and OS may miss a biologically relevant effect of a drug that warrants further study, either in selected patient populations and/or potentially in combination with other agents. There are several important issues to clarify in trials of such agents. First, were intratumoral drug concentrations sufficiently achieved to cause target inhibition? Second, did significant inhibition of the target occur? Third, did this lead to a relevant alteration in downstream signaling? Fourth, if not, can mechanisms of resistance be identified? Fifth, were these mechanisms innate or adaptive? Finally, can factors predictive of response or resistance be identified? Well-designed clinical trials require a deep understanding of complex signaling networks, and reliable assays of different nodes in these networks to assess the effect of a drug and potential mechanisms of resistance. They require well-validated biomarkers to aid in patient selection, and appropriate PK/PD studies to evaluate drug delivery and target modulation.

A final issue worth considering in the design of Phase I trials of molecular targeted agents is that the biologically active dose may be different from the maximum tolerated dose (MTD). For instance, a retrospective review of 135 patients treated on Phase I trials of targeted agents demonstrated no significant difference in the rate of nonprogression, median duration of nonprogression, PFS or OS between patients treated with 0-33, 34-65 or >66% of the MTD, suggesting that doses well below the MTD were biologically active [96]. Although it is possible that there were no significant differences between these groups because all dose levels were inactive, the fact that there were objective radiographic responses in all three groups suggests that some of the drugs are indeed active. Accordingly, several groups have advocated defining a dose range with the upper limit defined by toxicity and a lower limit defined as a 'minimally effective dose' based on PK and PD data [97]. Definition of a minimally effective dose could be particularly relevant in trials combining targeted agents, where toxicity can be additive [98]. Appropriate PK and PD studies are crucial in clinical trials of targeted agents not only to measure drug penetration across the BBB and its biological effect on the tumor, but also to define a range of acceptable doses for future studies.

Issues specific to immunotherapy

Issues of patient selection have been particularly problematic in immunotherapy trials, and are exacerbated by the fact that most published trials are quite small, generally enrolling fewer than 30 patients. In a recent review of vaccine trials, for instance, the average age of trial participants ranged from 38-62 years of age and in the majority of trials the average age was <55 years old [99]. In nine of the 19 trials summarized, all patients received gross total resections and >50% received GTRs in three additional studies [99]. Both patient age and extent of resection (good prognostic factors) are likely to be highly significant confounding variables in interpreting the outcomes of these small trials. In the largest vaccine trial published to date, these clinical variables did indeed predict outcomes. A total of 56 patients were treated with autologous dendritic cells loaded with autologous tumor lysate in three different vaccination schedules. In a multivariate analysis only Karnofsky Performance Score ≤80 was a significant predictor of OS, though there was a trend towards significance for age >35 years as well (p = 0.062). Survival was not significantly different between the three vaccination schedules. Total resection was a significant predictor of improved PFS in a multivariate analysis, though not OS [100]. In this trial, clinical variables were more predictive of outcomes than the different therapeutic interventions and larger randomized trials will be required to determine if there is a therapeutic effect not explained by these confounders.

It is not clear that standard Phase I dose-escalation trial designs are appropriate for the initial evaluation of immunotherapies. Since most vaccinations are well tolerated, dose-limiting toxicities have not been defined in most Phase I studies and dose escalation has been limited practically by the number of cells that can be produced, or the 'maximum feasible dose' [101–103]. In addition, there has not been a clear relationship between dose and toxicity in Phase I trials to suggest that toxicity is dose dependant [101,104].

Appropriate outcomes measures for Phase II trials are even more difficult to determine. Many use traditional outcomes measures such as PFS and OS, though, as outlined above, clinical variables such as age and extent of resection are significant confounders and make results difficult to interpret. Many groups have attempted to measure immune responses as a primary outcome measure, though the variability in immune responses between patients and the small magnitude of these responses makes it difficult to appropriately power studies to detect significant differences in immune responses [102]. Another issue is that there is currently no standardized immune response measure that has been validated in GBM immunotherapy trials. The assays currently used in Phase I and II trials were summarized by Heimberger and Sampson and include measurement of delayed-type hypersensitivity reactions, binding of peptide MHC tetramers to antigen-specific T cells, lymphoproliferative assays, cytotoxicity assays and cytokine-specific ELISPOT. It is not at all clear, however, which (if any) of these is the most accurate reflection of the in vivo activity of an immunotherapy or is the most appropriate to use in response measurements [102].

A related and equally important issue is that many vaccine trials have not demonstrated a clear correlation between immune responses and clinical efficacy [99]. In the largest dendritic cell vaccine trial published to date, for instance, a number of patients had positive delayed-type hypersensitivity (DTH) skin tests prior to vaccination, making positive tests after vaccination hard to interpret, and there was no correlation between positive DTH test after vaccination and clinical response [100]. It may be that more sensitive assays of immune response are necessary, given that the confounding clinical variables are so prevalent in these trials, interpreting the meaning of improved clinical outcomes in these small studies in the absence of a clear immunological correlates is highly problematic. More recent trials have provided some evidence of correlation between immune responses and outcomes, including a significant increase in IFN-y production [105] and production of a PEP-vIII-specific antibody and a positive DTH response to PEP-vIII antigen in responders [106]. As measures of immune-responsiveness become more standardized and validated it will be easier to interpret these findings.

Randomized Phase III studies of CDX-110, an EGFRvIII-targeted vaccine, and DCVax[®]-L (Northwest Biotherapeutics, MD, USA), a dendritic cell vaccine using total tumor lysate, are currently accruing newly diagnosed patients. These studies will give us a better sense of the efficacy of these strategies and address the confounding factors that have hampered smaller Phase II studies. Immune responses will also be measured and may help clarify whether there are correlations between immune response and clinical outcome, as well as which immune response metrics are most appropriate. All of these data will be useful in designing future trials in recurrent disease.

Novel trial designs

Most clinical trials in recurrent GBM have been single-arm Phase I/II trials, which, as described above, are prone to outcome drift and patient selection bias. As better targeted therapies are developed, and especially as we move into studying combinations of these drugs, the number of potential clinical trials will grow exponentially and will outstrip our ability to accrue to standard Phase II studies. This underscores the need for clinical trials to be driven by a strong biological rationale so that only the most promising agents and combinations are studied. It also requires development of trials that provide the necessary data in the most efficient way possible. Bayesian adaptive trial designs, for instance, allow for dynamic allocation of patients to experimental arms based on the efficacy of treatments in early patients. Trippa et al. modeled such a Bayesian approach with patient data from 150 patients in four clinical trials for recurrent GBM, and found that a Bayesian adaptive design would have allowed 30 fewer patients to be enrolled in the trials and 12 more patients to be enrolled in the effective treatment, while retaining the same statistical power [107]. They modeled scenarios where none of the treatments was effective, where accrual differed significantly from assumptions or there was a significant delay in the availability of data, and in each scenario the Bayesian approach performed well. The authors point out that early stopping rules could also be employed in these trials so that treatment arms that are not efficacious are rejected earlier. Adaptive trial designs should be considered, especially when randomized trials are not feasible ethically, logistically or scientifically.

Conclusion

Some of the challenges in GBM clinical trial design are inherent to these tumors, such as the small number of eligible patients and thus the subsequent difficulty in carrying out large randomized trials, the cost and risk associated with biopsy of these tumors, significant clinical prognostic factors that dramatically impact on patient outcome and the profound genetic and molecular heterogeneity found among different GBMs. Since these issues will continue to be obstacles in the future, it is even more important that the trials that can be performed are well designed and provide meaningful results. As our understanding of the biology of these tumors increases over time, many of the hurdles outlined above can and will be overcome.

Future perspective

As our understanding of the genetic alterations and signaling pathway aberrations that drive GBM improves in the next 5-10 years, the design of clinical trials will be increasingly driven by glioma biology. Agents selected for study will be chosen based on their ability to modulate key pathways that drive glioma proliferation and invasion as identified by data from the Cancer Genome Atlas, Rembrandt and other similar genetic/molecular/clinical databases. Early Phase I testing of these compounds will include surgical cohorts so that critical PK/PD studies can be performed. As we learn more about the genetic and epigenetic changes that drive glioma behavior, we will develop reliable assays for these alterations so that they can be routinely tested in patients and studied as biomarkers of response to treatment. Testing of molecular and genetic biomarkers will be routinely incorporated into the eligibility criteria for clinical trials, so that trials are enriched for patients who are more likely to respond based on the biology of their tumors. Response or 'biological activity' will be measured at the molecular level in addition to standard clinical and imaging criteria using these biomarkers. As we improve our ability to evaluate response or resistance at the molecular level, treatment will become more adaptive. When mechanisms of resistance to treatment in a particular tumor are identified, therapy will be modified to address those mechanisms. We will focus on trial designs that maximize efficiency and minimize bias, including randomized trials when possible and adaptive trial designs when randomized trials are not feasible. As we do so, we hope to make the advances that are so critically needed in this field.

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Executive summary

General issues in glioblastoma trial design

- Outcomes are improving in glioblastoma (GBM), so comparison with historical controls may overestimate the therapeutic effect of new drugs.
- Treatment with bevacizumab may change the pattern or rate of progression so new historical controls need to be defined for this patient population.
- The incidence of pseudoprogression has increased with the routine use of temozolomide with radiation and the diagnosis of pseudoprogression must be considered when enrolling a patient onto a clinical trial for recurrent disease.
- Age, performance status and the extent of resection are strong prognostic factors for outcome in GBM and need to be carefully reported since their impact can outweigh any therapeutic effect of a drug.
- Distinct molecular subtypes of GBM are being defined. In the future, these will have to be described with other patient characteristics in the reporting of results since they may be predictive of therapeutic response to a given targeted therapy.
- Enzyme-inducing antiepileptics are commonly used in these brain tumor patients and separate Phase I testing is required for this cohort for drugs with some evidence of activity.
- The blood-brain barrier represents a major challenge in GBM and drug delivery to the tumor should be measured when evaluating new drugs if possible.

Issues specific to antiangiogenic therapies

- Agents that inhibit VEGF cause a rapid vascular normalization in most patients but many of these do not ultimately have a durable antitumor response.
- Traditional radiographic response criteria have failed to differentiate 'pseudoresponses' from true responses and better radiographic biomarkers of response are being studied, and will be necessary to evaluate response to these agents.
- At present, overall survival is the most reliable outcome measure for evaluating the effectiveness of this class of agents.
- Quality of life measures should be incorporated into trial designs for these agents, since they may cause a significant decrease in cerebral edema, thereby improving neurologic symptoms, or may have direct toxicity to the CNS.

Issues specific to molecular targeted agents

- Signal transduction pathways are complex, interconnected and redundant.
- It is critical to measure not only the direct effect on the molecular target but also the effect on downstream signaling and signaling along interconnected pathways.
- Given the complexity of these pathways, it is unlikely that inhibiting single targets will provide durable tumor control and rational combinations need to be explored.
- Since single targeted agents are unlikely to lead to durable tumor control, traditional clinical end points may miss a relevant biological effect. Targeted agents that have been proven ineffective based on these criteria may have an important role in combination therapy and measuring responses at the molecular level will be required to select drug combinations worthy of further study.
- For many targeted agents, doses well below the maximum-tolerated dose are biologically active so a minimally effective dose should be defined in Phase I studies. This requires appropriate pharmacokinetic and pharmacodynamic studies and is particularly important when combination studies are considered, since toxicities are additive.

Issues specific to immunotherapy

- Many vaccine trials require extensive resection and enroll younger patients so outcome data have to be interpreted cautiously.
- 'Maximum-feasible dose' may be the most appropriate target for Phase I trials.
- Since clinical responses are clearly affected by prognostic factors such as age and extent of resection, future trials will need to demonstrate a clear immune response to the immunotherapy, and a correlation between immune responses and clinical responses to prove that these treatments are efficacious.
- A standardized immune response measure will be necessary in order to compare results between trials.
- Randomized studies in newly diagnosed disease will control for the prognostic effects of age and extent of resection, and will provide a better sense of the efficacy of these treatments.

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