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Incorporation of prognostic and predictive factors into glioma clinical trials

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Abstract

Treatment of brain tumors is increasingly informed by biomarkers which predict patient prognosis and response to therapy. While this progress represents a great opportunity for the field of neuro-oncology, it also presents significant challenges. Biomarkers are not straightforward to identify, and previously used clinical trial paradigms are poorly suited to the task of identifying treatments effective only in selected subsets of patients. Unless investigators adapt new tools and procedures which better account for the biological diversity of gliomas, future clinical trials run the dual risk of missing important treatment effects and exposing patients to interventions destined to prove ineffective for their tumors. In this article we will review the progress made in the past decade with respect to biomarkers in neuro oncology, address barriers to ongoing progress, and discuss clinical trial designs which may prove useful in moving neuro-oncology fully into the era of personalized medicine.

Keywords

brain tumor; glioma; glioblastoma; clinical trial; biomarker; prognostic; predictive

INTRODUCTION

Recent advances in biology and bioinformatics have revealed previously unrecognized heterogeneity within infiltrating gliomas. This wealth of new information has the potential to revolutionize the treatment of gliomas, but significant barriers must be overcome before these advances can readily be translated into action in the clinical setting. Nonetheless, a number of candidate prognostic (markers predicting patient outcome irrespective of treatment) and predictive (markers predicting response to specific treatment) biomarkers have already been identified within neurooncology. In order to build on this important direction, a systematic approach towards the development and use of biomarkers within the context of prospective clinical trials is necessary. The long-term goal of these efforts is to allow neuro-oncologists to identify patient-specific and tumor-specific factors which can be used to select maximally effective therapies and minimize treatment-related toxicity.

Personalized medicine has been recognized as an area of opportunity by the leaders of the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) [1]. While

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this initiative is important for medicine as a whole, it is especially vital in neuro-oncology, given the morbidity and mortality of brain tumors and the rarity of effective treatment options coupled with the potential toxicity of therapy. In a broad sense, brain tumor care is already highly customized, as neurooncologists, neurosurgeons, and radiation oncologists work together to create treatment plans based on the clinical circumstances of individual patients. Currently, however, differences in treatment plans between patients with the same histology are based primarily on tumor location or patient specific factors, rather than consideration of biological differences between the tumors themselves. When true personalized medicine in neuro-oncology becomes a reality, clinically similar patients with histologically identical tumors may be treated quite differently from the outset of therapy based on analyses of individual-level tumor biomarkers.

BIOMARKER EVALUATION

Biomarkers may be used diagnostically or they may provide information about expected patient outcome. It is the latter group, composed of prognostic and predictive biomarkers, that is of greatest relevance in the personalized medicine movement [2]. A prognostic factor is a patient-specific or tumor-specific trait that predicts outcome regardless of treatment. World Health Organization (WHO) tumor grade is the most well-established pathological prognostic factor in glioma, though many others have been proposed. Because prognostic biomarkers are independent of treatment, their application to therapy selection is not always straightforward, and their primary utility may rest in allowing potentially morbid treatments to be deferred in patients expected to have good long-term prognosis. Predictive factors, unlike prognostic factors, influence outcome within the context of specific treatment regimens; a classic example of a predictive marker in oncology is the responsiveness of human epidermal growth factor 2 (*HER2*) gene amplified breast cancer to trastuzumab, a monoclonal antibody that interferes with the *HER2/neu* receptor [3]. Predictive biomarkers have proven elusive in neuro-oncology until very recently, however.

Pathological prognostic factors are typically first identified via retrospective analyses of tumor samples collected through clinical trials or institutional tumor banks. Candidate prognostic markers are frequently replicated in other retrospective data sets and then prospectively validated before they are accepted by clinicians and researchers. Predictive biomarkers, like prognostic biomarkers, are typically first identified via retrospective studies. However, once a candidate predictive biomarker has been identified, it cannot be practically validated except in the context of a prospective randomized trial. Unfortunately, only an extremely small proportion of candidate prognostic or predictive markers are successfully validated, for reasons that range from biases inherent in retrospective analysis to poor statistical methods [4]. This problem is even more acute in neuro-oncology than in oncology as a whole, due in part the low incidence of primary brain tumors. Few single institutions see enough brain tumor cases to identify, much less validate, prognostic markers on their own. Thus, multi-institutional collaboration is absolutely necessary: even in this context, there are only a few completed or ongoing prospective randomized trials with the statistical power to unambiguously validate prognostic and predictive biomarkers.

PROGNOSTIC AND PREDICTIVE BIOMARKERS IN NEURO-ONCOLOGY

MGMT Methylation

Temozolomide chemotherapy in combination with radiation therapy has been the standard-of-care treatment regimen for glioblastoma since the results of the EORTC/NCIC CE3 randomized phase III trial were published in 2005 [5]. In a companion article to the original trial publication, the authors retrospectively analyzed the impact of methylation of O-6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme, on patient survival

in the subgroup of patients on whom methylation data was available [6]. At the time of the initial trial publication, there was a statistically significant difference in survival in favor of chemoradiotherapy in the trial subpopulation that was MGMT methylated, but only a non-significant trend towards a survival advantage in the unmethylated subpopulation. This finding, based on a retrospective unplanned subgroup analysis of patients in whom tissue was available (206 of 573 study patients), suggested that MGMT methylation was a predictive marker of response to temozolomide. However, by the time of the long-term analysis the survival difference between study arms had become statistically significant in both methylated and unmethylated subjects, though the magnitude of the benefit was larger in the methylated group [7]. Thus, MGMT methylation status is best considered a prognostic factor rather than as a predictive biomarker of sensitivity to temozolomide amongst patients with glioblastoma.

The Radiation Therapy Oncology Group (RTOG) 0525 trial prospectively evaluated the role of MGMT methylation in glioblastoma, and confirmed that it was a strong prognostic marker [8]. As both arms of the RTOG 0525 trial contained temozolomide, the hypothesis that MGMT methylation was predictive of response to temozolomide could not be evaluated, but MGMT methylation status was not predictive of response to dose-dense versus standard dose temozolomide [8]. The strongest evidence released to date suggesting that MGMT methylation status may in some circumstances be predictive of response to temozolomide comes from two recent studies which compared radiation therapy to temozolomide chemotherapy for treatment of malignant glioma, primarily glioblastoma, in elderly patients [9, 10]. In both of these trials, patients with methylated MGMT treated with temozolomide had better outcomes than unmethylated patients treated with temozolomide, while methylation was not associated with improved survival in patients treated with radiation. The ongoing NCIC CE.6 study is assessing the MGMT methylation status in frail elderly patients randomized to a short course RT/TMZ versus a short course of radiotherapy, and this study should provide insight as to whether MGMT methylation status can be used to identify elderly patients who may benefit from chemoradiotherapy.

1p/19q Co-deletion

Coexistent deletion of portions of the short arm of chromosome 1 and the long arm of chromosome 19, known as 1p/19q co-deletion, is a common finding in oligodendroglial tumors [11]. Two large randomized trials, RTOG 9402 and EORTC 26951, have been completed comparing chemotherapy with procarbazine, lomustine, and vincristine (PCV) in combination with radiation therapy to radiation therapy alone in patients with anaplastic oligodendroglial tumors [12, 13]. Tumor tissue from study patients was retrospectively analyzed for co-deletion status when possible. Initial trial results were released in 2006, and both studies suggested that 1p/19q co-deletion was prognostic of prolonged overall survival and predictive of superior progression-free but not overall survival in patients treated in the PCV chemotherapy-containing arm [12, 13]. Long-term follow-up results for both of these trials were announced at the American Society of Clinical Oncology Annual Meeting in 2012, and both trials demonstrated that survival was significantly longer in co-deleted patients treated with radiation and PCV than in co-deleted patients treated with RT alone, 14.7 vs. 7.3 years in RTOG 9402, and median survival not yet reached versus 9.3 years in EORTC 26951 [14, 15]. This finding had not been observed in the 2006 analysis, as the survival curves did not begin to notably diverge until approximately 6 years post-randomization. No significant survival difference by treatment arm was seen in patients who were not 1p/19q co-deleted. Though neither of these studies was powered to evaluate survival by co-deletion status, the near-identical results of these two trials, despite differences in chemotherapy/radiation sequencing and chemotherapy intensity, represent compelling evidence that 1p/19q co-deletion status is a predictive marker of chemotherapy

responsiveness in anaplastic oligodendroglial tumors. To date, this is the only predictive marker in all of neuro-oncology that has been demonstrated in multiple phase III trials, as well as the only biomarker to be used as an eligibility criterion for enrollment in large international clinical trials, the CODEL and CATNON studies [16, 17].

The role of 1p/19q co-deletion status in WHO grade II glioma, and WHO grade III glioma of astrocytic morphology, is less clear. It has been shown to be prognostic of survival in both of these tumor types [18-21]. Future clinical trials, or future analyses of now-completed clinical trials, will be necessary to determine if co-deletion is predictive of chemotherapy response in these tumors as well. For example, post-hoc analysis of the RTOG 9802 trial, in which patients with WHO grade II glioma were randomized to radiation and PCV chemotherapy or radiation alone, demonstrated that amongst 2-year survivors the addition of PCV to RT conferred a survival advantage [22]. Analysis of patient subgroups defined by molecular markers has not been released yet, and information on the impact of 1p/19q codeletion as predictor of benefit from PCV chemotherapy in high-risk low-grade glioma is eagerly awaited.

IDH1 Mutation Status

The 2008 report that somatic mutations on codon 132 of the isocitrate dehydrogenase 1 (*IDH1*) gene and corresponding mutations within the isocitrate dehydrogenase 2 (*IDH2*) gene are frequent findings within low grade glioma and secondary high-grade glioma has led to a great deal of interest in IDH mutation as a glioma biomarker [23, 24]. While IDH mutation has been shown to be associated with survival amongst patients with glioma [25-31], the exact nature of this relationship remains unclear, as IDH mutation is also associated with other biomarkers of prognostic significance such as 1p/19q codeletion, and MGMT methylation [32, 33]. Ongoing analysis of tissue deriving from RTOG 9402 will assess its possible independent prognostic and predictive value in 1p/19q codeleted patients. IDH mutation analysis has also been incorporated in the international CATNON and CODEL trials, as well as in the intergroup low grade glioma study E3F05. IDH mutation analysis is expected to become increasingly more clinically relevant in view of the ongoing development of IDH 1 inhibitors.

Gene Expression Profiling

The pattern of overexpression or underexpression of genes within a glioblastoma may provide insight into predicting tumor behavior. Gene expression profiling utilizes microarray-based mRNA panels to simultaneously evaluate the expression of hundreds or thousands of genes within a single tumor. Tumors can then be computationally “clustered” into groups with similar characteristics [34, 35]. For example, a recent publication by The Cancer Genome Atlas (TCGA) group identified four distinct types of glioblastoma, the Proneural, Neural, Classical, and Mesenchymal subtypes, and suggested that the survival advantage conferred by treatment varied between subtypes [35]. To date, gene expression has primarily been suggested to be prognostic of patient outcome. While it is plausible to suggest that expression profiling may have predictive power, for example by identifying tumors in which genes associated with angiogenesis are overexpressed, such as the mesenchymal subtype of glioblastoma, so that they may be treated with anti-angiogenic agents, prospective data in support of this approach are pending. RTOG 0525, assessing the role of dose dense temozolomide in adjuvant glioblastoma treatment, was the first large randomized trial to evaluate the impact of gene expression profiles on survival in glioblastoma[36] using a 9-gene predictor signature [37]; the recently completed RTOG 0825 trial will test the hypothesis that molecular signatures deriving from glioblastoma gene expression profiling can be used to predict benefit from the addition of bevacizumab to the

standard radiation and temozolomide based regimen in newly diagnosed glioblastoma patients.

BIOMARKER USE IN CLINICAL TRIALS

Biomarker Measurement

Measurement methods must be carefully considered when biomarkers are used in clinical trials. Even binary biomarkers, such as the presence or absence of a DNA point mutation, can be measured in a number of different ways. The situation becomes far more problematic with non-binary biomarkers such as DNA methylation analysis and reaches its zenith with gene expression profiling, which requires highly technical bioinformatics analysis in addition to sophisticated laboratory methods. We will use the example of MGMT methylation analysis to illustrate biomarker measurement issues that must be considered in clinical trial design.

A study designed to assess whether MGMT methylation is prognostic of survival in a given clinical trial population can use many different methods of ascertaining methylation status including methylation-specific PCR, methylight, pyrosequencing, methylation-specific high-resolution melting, and immunohistochemistry [38]. In an early phase trial, an investigator may wish to choose the method with the maximum reported predictive value for survival in previous retrospective studies. In a large phase 3 trial, it may be more important to choose a widely available CLIA certified method, enabling trial findings to be more easily applied to clinical practice. If novel methods of assessing MGMT methylation are chosen, they should be used to supplement rather than to supplant older more accepted tests, and agreement between the tests should be explicitly evaluated and reported.

Biomarker measurement issues are even more important when the biomarker in question is expected to be predictive of response to treatment. If MGMT methylation is proven to be predictive of response to drug, as for example has been recently hypothesized for PARP inhibitors [39], then MGMT methylation status will need to be assessed on every potential treatment candidate, and selection of a suboptimal test may lead to patients that could derive benefit from that drug not receiving it, or conversely patients receiving the therapeutic agent when it is unlikely to be successful. Incorporation of these tests for patient selection will require a CLIA certified laboratory. If a novel targeted therapeutic agent is submitted for consideration of approval by the Food and Drug Administration (FDA), it is increasingly likely that a companion diagnostic test will need to be approved simultaneously; if an FDA-approved test does not already exist. The drug omacetaxine mepesuccinate recently failed to win approval as a treatment for chronic myeloid leukemia (CML) due to lack of an FDA approved companion diagnostic test for the predictive T315I mutation [40], while crizotinib for lung cancer and vemurafenib for melanoma were approved simultaneously with their associated predictive tests [41]. Given the financial pressures of new drug development, it is increasingly incumbent on researchers to choose and/or develop FDA-ready tests so as not to allow biomarker test approval to interfere with or slow the approval of the associated therapeutic agent.

Clinical Trial Designs for Identification and Evaluation of Predictive Biomarkers

Until very recently, no tumor characterization other than grade and morphology were used to select patients for brain tumor clinical trials. If biomarkers were to be evaluated, they were done so either retrospectively or via pre-planned subgroup analyses. While this approach is readily understood and statistically straightforward, it does not make optimum use of biomarker data and may result in insufficient power to identify interactions between biomarkers and outcome. A number of clinical trial design schemas have been proposed to

overcome this limitation, and several large glioma trials have been launched using these designs.

The clinical trial design which most closely resembles clinical trials conducted without regard to biomarker data, but still allows prospective biomarker evaluation, is the marker by treatment interaction design, also referred to as the biomarker-stratified design [42-44]. In this design, patients are randomized in different treatment groups. Although their biomarker status is prospectively determined, it does not impact on treatment decision. The sample size is, however, calculated to provide adequate power to test for a different treatment effect in the different marker groups. For example, in a hypothetical trial of 6 versus 12 cycles of adjuvant temozolomide in newly diagnosed glioblastoma, an investigator may reasonably hypothesize that the effect of prolonged temozolomide therapy, if present, would be more readily apparent in MGMT methylated patients. A trial using the marker by treatment interaction design could evaluate the hypothesis in the two groups using different hypothesized effect sizes to calculate the required sample sizes. If 12 months of temozolomide was found to be more effective than 6 months of therapy in methylated but not unmethylated patients, this would support the idea that MGMT methylation was predictive of outcome. If 12 months of temozolomide was found to be superior to 6 months of therapy in both groups, or neither of the groups, MGMT methylation would not be supported as a predictive marker, though it could still be prognostic. A variation on the marker by treatment interaction design allows for its use in trials in which each arm does not need to be individually powered to evaluate the primary hypothesis, but instead the trial as a whole is powered to assess for interaction between treatment effect and biomarker subgroup [44]. This trial design is best supported when the difference in response between biomarker groups is expected to be a matter of degree, rather than clear benefit in one group and total lack of benefit or harm in another.

The marker-based strategy design is a trial schema in which patients are randomized to either receive therapy based on their marker status or independent of marker status [44, 45]. For example, in a trial of an agent purported to be effective in tumors lacking MGMT methylation, patients randomized to marker-based treatment may receive the experimental agent if they are MGMT unmethylated and temozolomide if they are MGMT methylated, and patients randomized to be treated without regard to MGMT status would all receive temozolomide. The predictive utility of the marker-based treatment strategy would be assessed by comparing the outcome of all patients in the marker-based group to all patients in the standard therapy group. The marker-based therapy group need not be limited to two treatments; in principle a marker-based strategy involving any number of arms could be compared to standard therapy. This design has the potential flaw of suggesting that a marker-based strategy is effective if the experimental intervention is simply more effective than standard therapy, regardless of patient biomarker status. In order to deal with this issue, the marker-based strategy design can be modified to include a second randomization to the same interventions in the group treated without regard to biomarker status [45]. Rather than a simple 1:1 randomization, this second randomization should be informed by the prevalence of the marker in question in the population as a whole, to ensure balance between the study arms.

Biomarker-enrichment design trials are useful if patients are expected to have such different responses to therapy based on the presence or absence of a biomarker that it would be undesirable or even unethical to conduct a trial in which they are treated the same. In this design, patients are screened for the biomarker of interest before randomization, and only randomized if they have the desired biomarker profile [42, 46]. 1p/19q codeletion, as previously discussed, is prognostic and recently proved to be predictive of response to PCV chemotherapy in patients with anaplastic glial tumors. In recognition of this fact, two

companion clinical trials using enrichment designs were launched to evaluate different treatment regimens for anaplastic glioma based on 1p/19q codeletion status. The ongoing CATNON trial (EORTC26053/22054, RTOG 0834) randomizes patients without codeletion to either radiation therapy or chemoradiotherapy with temozolomide, followed by a second randomization to adjuvant temozolomide [17]. Amongst other benefits, the use of patient population defined by lack of codeletion allows this study to be powered based on more specific prognostic assumptions. CODEL (NCCTG N0577) is the companion trial to CATNON, for patients with codeleted anaplastic glioma [16]. Originally conceived as a radiotherapy versus chemoradiotherapy trial with a small exploratory chemotherapy-only arm, it is currently being redesigned in light of data demonstrating that radiation therapy in combination with PCV chemotherapy is superior to radiation alone for codeleted anaplastic glioma. A smaller scale example of an enrichment design in neuro-oncology was the evaluation of epidermal growth factor receptor variant III (EGFRvIII) peptide vaccination in patients known to carry EGFRvIII-expressing glioblastoma, now undergoing evaluation is an international phase III trial [47, 48].

While the biomarker-enrichment design evaluates patients with tumors containing binary biomarkers (e.g. the presence or absence of IDH 1 or 2 mutations) or biomarkers in which a binary cutpoint has already been established (e.g. the threshold of chromosomal loss within a tumor necessary to consider it deleted of 1p and 19q), the biomarker analysis design is useful for studying continuous biomarkers [42]. This trial design consist of two parts, an initial randomized trial with measurement of a continuous biomarker in all participants and a subsequent analysis aimed at identifying subgroups with different responses to treatment by plotting treatment benefit versus biomarker level. A number of different plotting and analysis procedures have been described, allowing for the identification of optimal binary cutpoints for continuous data, as is desirable in clinical medicine [49-53].

The clinical trial designs reviewed thus far are alike in that the hypothesized associations between biomarker signature and treatment response are pre-specified, and once launched the trials are not responsive to real-time results other than through the use of interim analyses and early stopping rules. Adaptive clinical trial paradigms, designed using Bayesian approaches, allow for the identification and evaluation of biomarker/treatment response associations and respond to patient-level data as it becomes available [54]. For example, in a hypothetical adaptive trial of radiation plus temozolomide versus radiation plus temozolomide plus bevacizumab versus radiation plus temozolomide plus erlotinib for newly diagnosed glioblastoma, early results may suggest that patients with a certain tumor gene expression profile do well when assigned to the bevacizumab-containing arm. The randomization scheme could then be altered so that these patients are more likely to be assigned to the bevacizumab containing arm, and accrual to this arm could be expanded to allow for the evaluation of an association between gene expression profile and outcome when treated with bevacizumab. If the predicted probability of success of the erlotinib-containing treatment arm drops below a specified threshold for all gene expression profiles of interest, that arm can be dropped from the trial or replaced with a new treatment arm. The feasibility of this approach has been demonstrated in trials such as the “Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging And moLecular Analysis 2” (I-SPY 2 TRIAL) trial in breast cancer [55], the “Biomarker-based Approaches of targeted Therapy for Lung Cancer Elimination” (BATTLE) trial in non-small cell lung cancer and the first multicenter adaptive trial in neuro-oncology, evaluating the efficacy of bevacizumab with or without vorinostat in recurrent malignant glioma, which is now enrolling patients [56]. One barrier that must be overcome before the full benefit of Bayesian trial designs can be realized in neuro-oncology is the identification of a reliable early marker of treatment efficacy, to avoid the need to await survival data before adapting the design of a study. These adaptive studies are not designed to produce definitive results,

rather they efficiently identify combinations of biomarkers and interventions for evaluation in phase III trials. A number of other study designs have been proposed which also permit the identification of patient biomarker or risk subgroups and the evaluation of treatment effect in the subgroups as part of a single trial [42, 57], and additional discussion of the role of different biomarker-driven trial designs in neuro-oncology is provided in a recent review from the Response Assessment in Neuro-Oncology (RANO) group [58].

CONCLUSIONS

Less than a decade ago, there were no proven prognostic or predictive biomarkers in neurooncology other than tumor grade and morphology. Currently, there are multiple prospectively validated prognostic markers and one predictive marker, 1p/19q codeletion in anaplastic oligodendroglial tumors. Advances in molecular characterization of brain tumors will undoubtedly accelerate the rate of biomarker identification in the future, but if these biomarkers cannot be properly evaluated then their potential contributions to scientific understanding and clinical care will be lost. Moving forward, biomarkers must be considered as a major factor when designing clinical trials, with rapid identification of potentially important biomarker/outcome interactions in early phase clinical trials and statistical designs sufficient to definitively prove or disprove predictive associations in phase III trials. Although this review does not incorporate a discussion on peripheral blood biomarkers (i.e., SNP, humoral and cellular factors of angiogenesis) or imaging biomarkers, their role will also need to be further defined. While the clinical implications of these discoveries are just starting to be realized, they represent the beginning of an inexorable movement towards personalized medicine in neuro-oncology, with the promise of offering patients customized therapies to maximize efficacy and minimize toxicity.

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