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Biomarkers usher in era of personalized care for malignant glioma patients

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“...biomarkers hold the potential to identify which patients may benefit, or be harmed by, the addition of targeted therapies to conventional treatment regimens.”

The median survival times of glioblastoma (GBM) patients remain in the 15-month range despite aggressive surgery, radiation and chemotherapy. The dismal prognosis of patients with GBM has been, in part, predicated upon the inherent resistance of these tumors to both chemotherapy and radiation. Over the past several decades of active clinical investigation, no curative therapies have been developed for malignant glioma patients. A recent landmark Phase III study from the European Organisation for Research and Treatment of Cancer (EORTC) has established concurrent and adjuvant temozolomide (TMZ) as the new standard-of-care for newly diagnosed GBM patients and has stirred much excitement in the neuro-oncology community [1]. However, this excitement has been tempered by the reality that not all GBM patients benefit from TMZ and the observation that some patients may, in fact, suffer the ill effects of TMZ without necessarily acquiring any of the tangible benefits.

Accordingly, there is a great and urgent need in the field of neuro-oncology to personalize care for malignant glioma patients, as these tumors tend to demonstrate heterogeneous behavior even among

histopathologically identical tumors. Biomarkers may provide a valuable means to this end. There is accumulating evidence that molecular and genetic biomarkers can be utilized to personalize care for malignant glioma patients by refining the diagnostic and prognostic classification of malignant gliomas and by serving as predictive markers to tailor treatment specifically for an individual's tumor as opposed to a broad histopathologic subclass.

Diagnostic biomarkers

Loss of heterozygosity of chromosomes 1p and 19q is the most common genetic alteration in oligodendroglioma tumors [2] and represents one of the biomarkers that can be classified as diagnostic, prognostic and predictive (see below). This hallmark alteration is detected in 40–90% of oligodendroglioma [2–5]; codeletion of 1p/19q is also found in 20–30% of mixed glial tumors [6] and less than 10% of diffuse astrocytic gliomas (including GBMs) [5]. It has become apparent that chromosome 1p and 19q codeletion status can distinguish anaplastic oligodendrogliomas that behave in a clinically aggressive (noncodeleted tumors) versus less aggressive manner (codeleted tumors). In fact, codeletion

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status appears to identify two distinct diagnostic groups of oligodendrogliomas that are otherwise indistinguishable by traditional histopathological means. This observation is manifested by the fact that there are distinct clinical trials for anaplastic oligodendroglioma patients based upon codeletion status of chromosomes 1p and 19q (e.g., CATNON vs NCCTG Codel Trial, respectively).

Although it could be argued that there are no established standards of care for anaplastic glioma patients, at many institutions treatment decisions are being rendered in a manner that is contingent upon 1p,19q status for grade III patients. As an example, certain codeleted anaplastic oligodendroglioma patients are being managed with upfront chemotherapy, with radiation delayed until the time of progression at some institutions, whereas patients without codeletion are being managed similarly to grade IV patients with radiation and concurrent and adjuvant TMZ chemotherapy. Ultimately, the results of ongoing Phase III trials will determine the best therapies for anaplastic glioma patients in a more definitive, less empirical manner.

Prognostic biomarkers

Prior to the era of molecular medicine, malignant glioma patients were often stratified based upon clinical variables, such as the Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis (RPA) model. The clinical RTOG RPA model includes clinical/demographic variables such as age, performance status and histopathology (among others), and groups malignant glioma patients into one of six different prognostic classes. For the better part of two decades, malignant glioma patients have been stratified based upon the clinical RTOG RPA model and, in fact, the clinical RTOG RPA model has been used as eligibility criteria for many malignant glioma clinical trials worldwide.

As we have ventured into the era of molecular medicine, one of the pressing questions has been to what degree the prognostic classification of malignant gliomas can be refined through the incorporation of molecular and genetic biomarkers. The companion study to the recent Phase III EORTC trial suggested that MGMT could prove to be such a powerful prognostic biomarker [7]. MGMT is located at chromosome 10q26 and encodes a DNA repair protein that removes the alkyl groups from the

O⁶ position of guanine, which are commonly produced by chemotherapeutic alkylating agents.

Gliomas often possess decreased MGMT expression levels, which are thought to be primarily due to increased *MGMT* promoter methylation. A correlation between *MGMT* promoter methylation and response of malignant gliomas to alkylating chemotherapy has been observed [5]. MGMT hypermethylation has been identified in 11% of grade II astrocytoma, 27% of oligoastrocytoma and 62% of oligodendroglioma cases [8]. In addition, DNA hypermethylation was found in 36% of primary GBM and 75% of secondary GBM cases [2]. The heretofore reported frequencies of MGMT promoter methylation vary widely. In the EORTC/National Cancer Institute of Canada cohort, 45% of assessable cases had MGMT promoter methylation. In a series of 102 patients with various grades and gliomas subtypes, Jha and colleagues found the presence of MGMT promoter methylation in 67.6% cases (79, 71 and 57% in grade II, III and IV gliomas, respectively), suggesting an inverse relationship between methylation status and tumor grade [9]. Purely oligodendroglial tumors showed the highest percentage of cases with MGMT promoter methylation (84%), compared with 63.5% in astrocytic tumors. The methylation status of *MGMT* promoter was not shown to be significantly associated with 1p/19q loss of heterozygosity, nor was there significant association with EGFR amplification or TP53 mutation.

In the landmark EORTC/National Cancer Institute of Canada study by Stupp and colleagues, 573 patients with newly diagnosed GBM – 84% of which were surgically debulked – were randomized to receive radiation therapy (RT) alone (60 Gy in 30 fractions) or RT plus continuous daily TMZ (75 mg/m²), followed by six cycles of adjuvant TMZ (150–200 mg/m²). At a median follow-up of 28 months, the median survival was 14.6 months with RT plus TMZ versus 12.1 months with RT alone, rendering the hazard ratio for death 0.63 in the combined modality group. Moreover, 2-year overall survival was 26.5% with RT plus TMZ and 10.4% with RT alone [10]. The 5-year analysis showed that 5-year overall survival was 9.8% with RT and TMZ, versus 1.9% with RT alone [1]. In this trial, patients whose tumor had a methylated *MGMT* gene promoter had improved survival (median 21.7 vs 12.7 months, overall survival at 2 years 46 vs 13.8%) relative to those with an

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unmethylated MGMT promoter, and the methylation status was the strongest predictor for outcome and benefit from TMZ chemotherapy [7]. Another study analyzing 125 patients with GBM showed that MGMT promoter methylation was associated with improved median overall survival (61 vs 42 weeks) [11]. These studies suggest that MGMT promoter methylation status could possibly be used as a strong predictive marker of response to chemotherapeutic alkylating agents. Furthermore, it is thought that the benefit of MGMT promoter hypermethylation only applies to chemotherapy. However, one study suggested that decreased MGMT confers sensitivity to radiation alone [12]. However, this remains controversial as it is thought that this observation is owing to the overall prognostic value of MGMT and is independent of the treatment type [5].

In addition to MGMT, there appear to be a host of additional molecular and genetic biomarkers that, when combined with clinical variables, can result in a powerful classification model for GBM patients. The RTOG RPA classification model was recently updated to include molecular and genetic variables of significance [13]. The data set utilized was from RTOG 0525, a Phase III study examining radiation (RT) plus concurrent and adjuvant TMZ versus the same with the addition of dose-dense TMZ for the adjuvant component for newly diagnosed GBM. A total of 162 patients from RTOG 0525 had available tissues for profiling of key signaling molecules using the AQUA platform.

It was determined that pAKT, c-met and MGMT protein were significantly associated with adverse outcome on multivariate analysis. These variables were combined with clinical and genetic biomarkers (e.g., MGMT promoter methylation, IDH1 mutation and mRNA profiling) previously found to be of significance [14] to generate an even more robust, discriminatory RTOG RPA model. The explained variation for these three classification models was found to be 41.7 (current RPA) versus 14.9% (clinical RPA), respectively, with higher values indicating better separation of prognostic groups. Although promising, further validation of the current RTOG RPA classification model is planned to permit a more accurate head-to-head comparison with the clinical RPA model.

Predictive biomarkers

In addition to representing a diagnostic marker, chromosome 1p and 19q status also appears to

represent a promising predictive marker based on long-term follow-up data from RTOG 9402 for both pure and mixed anaplastic oligodendrogliomas. In RTOG 9402, patients with pure anaplastic oligodendrogliomas/mixed anaplastic oligodendrogliomas were randomly assigned to procarbazine, lomustine and vincristine (PCV) followed by RT versus RT alone. Early analysis showed no overall survival benefit for the PCV plus RT group despite significantly longer progression-free survival in the combined arm. It also showed that the finding of 1p/19q codeletion was associated with a longer overall survival independent of treatment. In the 2012 American Society of Clinical Oncology update of RTOG 9402 [15], PCV plus RT was associated with longer progression-free survival (2.5 vs 1.7 years; $p = 0.003$) and the 1p/19q codeletion with a longer median survival time (8.7 vs 2.7 years; $p < 0.001$). For the entire cohort, there was no difference in median survival time by treatment (4.6 years for PCV plus RT vs 4.7 years for RT; $p = 0.1$). However, patients with 1p/19q codeleted tumors lived significantly longer after PCV plus RT ($n = 59$) than after RT ($n = 67$; 14.7 vs 7.3 years; $p = 0.03$). There was no difference in median survival time by treatment in patients without the 1p/19q codeletion ($n = 137$; 2.6 vs 2.7 years; $p = 0.39$). In this setting, 1p/19q codeletion was both prognostic and predictive, indicating a survival advantage for pure anaplastic oligodendrogliomas/mixed anaplastic oligodendrogliomas patients with 1p/19q codeletion when treated with RT plus PCV versus RT alone.

Predictive markers are also emerging in the realm of targeted therapies for GBM. RTOG 0211 was a Phase I/II study of gefitinib, an EGFR tyrosine kinase inhibitor, in combination with radiation for newly diagnosed GBM patients [16]. Tissue microarrays (70 cases from RTOG 0211 and 112 from historical controls) were used for clinical molecular correlative analysis. There was no median overall survival benefit for patients treated with gefitinib plus RT versus RT alone. Younger age was significantly associated with better outcome. Higher levels of Src and IGFR1 were associated with adverse overall survival in patients treated by gefitinib plus RT. In patients treated with RT alone, low/absent PTEN and elevated ratios of cytoplasmic/nuclear pAKT were significantly associated with adverse overall survival, hence significant prognostic markers in this setting.

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Investigations were then undertaken to identify GBM patients who may benefit from the addition of gefitinib to RT (e.g., significant predictive markers). Patients with low PTEN levels demonstrated a trend towards improved clinical outcome when treated with RT plus gefitinib versus RT alone. Patients with high Src or PTEN appeared to have significantly worse overall survival when treated by RT plus gefitinib versus RT alone.

Therefore, these data indicate that the addition of gefitinib to RT does not appear to benefit the general population of GBM patients. Src and PTEN appeared to be of significant negative predictive value in identifying GBM patients who may be adversely impacted from the addition of gefitinib to RT.

Conclusion & future perspective

These data indicate that molecular and genetic biomarkers can play instrumental roles in personalizing care for malignant glioma patients. Biomarkers can be useful diagnostically and prognostically by better characterizing behavior of individual tumors and by predicting clinical outcomes of individual patients with malignant gliomas. The chromosome 1p/19q story and the revised RTOG RPA classification model

epitomize how biomarkers can be used for such purposes in cases where there may be considerable variation in behavior among individual tumors that are otherwise look identical under the microscope. Beyond predicting natural history of disease, biomarkers hold the potential to identify which patients may benefit, or be harmed by, the addition of targeted therapies to conventional treatment regimens. Ultimately, biomarkers will enable clinicians to tailor therapies towards an individual's tumor rather than a broad histopathological group of tumors.

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