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Analysis of Chemopredictive Assay for Targeting Cancer Stem Cells in Glioblastoma Patients

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Abstract

Introduction: The prognosis of glioblastoma (GBM) treated with standard-of-care maximal surgical resection and concurrent adjuvant temozolomide (TMZ)/radiotherapy remains very poor (less than 15 months). GBMs have been found to contain a small population of cancer stem cells (CSCs) that contribute to tumor propagation, maintenance, and treatment resistance. The highly invasive nature of high-grade gliomas and their inherent resistance to therapy lead to very high rates of recurrence. For these reasons, not all patients with similar diagnoses respond to the same chemotherapy, schedule, or dose. Administration of ineffective anticancer therapy is not only costly but more importantly burdens the patient with unnecessary toxicity and selects for the development of resistant cancer cell clones. We have developed a drug response assay (ChemoID) that identifies the most effective chemotherapy against CSCs and bulk of tumor cells from of a panel of potential treatments, offering great promise for individualized cancer management. Providing the treating physician with drug response information on a panel of approved drugs will aid in personalized therapy selections of the most effective chemotherapy for individual patients, thereby improving outcomes. A prospective study was conducted evaluating the use of the ChemoID drug response assay in GBM patients treated with standard of care. *Methods:* Forty-one GBM patients (mean age 54 years, 59% male), all eligible for a surgical biopsy, were enrolled in an Institutional Review Board–approved protocol, and fresh tissue samples were collected for drug sensitivity testing. Patients were all treated with standard-of-care TMZ plus radiation with or without maximal surgery, depending on the status of the disease. Patients were prospectively monitored for tumor response, time to recurrence, progression-free survival (PFS), and overall survival (OS). Odds ratio (OR) associations of 12-month recurrence, PFS, and OS outcomes were estimated for CSC, bulk tumor, and combined assay responses for the standard-of-care TMZ treatment; sensitivities/specificities, areas under the curve (AUCs), and risk reclassification

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components were examined. *Results:* Median follow-up was 8 months (range 3-49 months). For every 5% increase in *in vitro* CSC cell kill by TMZ, 12-month patient response (nonrecurrence of cancer) increased two-fold, OR = 2.2 (P = .016). Similar but somewhat less supported associations with the bulk tumor test were seen, OR = 2.75 (P = .07) for each 5% bulk tumor cell kill by TMZ. Combining CSC and bulk tumor assay results in a single model yielded a statistically supported CSC association, OR = 2.36 (P = .036), but a much attenuated remaining bulk tumor association, OR = 1.46 (P = .472). AUCs and [sensitivity/specificity] at optimal outpoints (>40% CSC cell kill and >55% bulk tumor cell kill) were AUC = 0.989 [sensitivity = 100/specificity = 97], 0.972 [100/89], and 0.989 [100/97] for the CSC only, bulk tumor only, and combined models, respectively. Risk categorization of patients was improved by 11% when using the CSC test in conjunction with the bulk test (risk reclassification nonevent net reclassification improvement [NRI] and overall NRI = 0.111, P = .030). Median recurrence time was 20 months for patients with a positive (>40% cell kill) CSC test versus only 3 months for those with a negative CSC test, whereas median recurrence time was 13 months versus 4 months for patients with a positive (>55% cell kill) bulk test versus negative. Similar favorable results for the CSC test were observed for PFS and OS outcomes. Panel results across 14 potential other treatments indicated that 34/41 (83%) potentially more optimal alternative therapies may have been chosen using CSC results, whereas 27/41 (66%) alternative therapies may have been chosen using bulk tumor results. *Conclusions:* The ChemoID CSC drug response assay has the potential to increase the accuracy of bulk tumor assays to help guide individualized chemotherapy choices. GBM cancer recurrence may occur quickly if the CSC test has a low *in vitro* cell kill rate even if the bulk tumor test cell kill rate is high.

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Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor [1]. It is also the most aggressive brain tumor, exhibiting a very poor prognosis (median overall survival [OS] =14.2 months) even if treated with maximal therapy [2]. Currently, surgical resection (when possible) and radiotherapy with concomitant and adjuvant temozolomide (TMZ) are the gold standard for patients with newly diagnosed GBM[1]. However, the management of GBMs remains difficult in that no contemporary therapies are curative. In fact, despite maximal treatment, recurrence is nearly universal [3].

Open tumor resection is usually considered the first step within the management algorithm; however, the highly infiltrative growth pattern of GBMs into surrounding brain tissues makes the surgical approach almost invariably not radical [4]. It has been observed that complete resection is achieved in about 40% to 45% of patients, with a similar proportion receiving incomplete resection, whereas only about 10% to 20% are diagnosed by biopsy only. Although the use of TMZ has improved GBM outcome [2], almost all patients suffer from recurrent disease. Recurrent GBM has several treatment options depending on specific aspects of its presentation, including secondary cytoreductive surgery when possible, and numerous second-line chemotherapy treatment options [5]. Although most patients eventually succumb to progression of recurrent disease, a few will benefit from further therapy and experience variable remission and symptom-free survival [5].

Selection of effective chemotherapy is extremely important not only when therapy is first initiated but for recurrent disease as well. In fact, administration of ineffective anticancer therapy is associated with unnecessary toxicity and the development of more aggressive cancer cell clones that are resistant to subsequent therapies. The ability to initially choose the most effective chemotherapy may help to avoid the physical, emotional, and financial burden to patients of ineffective therapy, thereby improving their quality of life [6]. Each time patients are treated, they have a chance of relapse, and their cancer will likely become more resistant to therapy [7]. Presently used anticancer drugs have a high rate of failure, and cell culture chemotherapy testing has been used to identify which drugs are more likely to be effective against a particular tumor type. Measuring the response of the tumor cells to drug exposure is valuable in any situation in which there is a choice between two or more treatments. Many attempts have been made over the years to develop an *ex vivo* anticancer test that can provide clinically relevant treatment information. However, until now, this approach has been hampered by the chemotherapy testing only being performed on bulk of tumor cells derived from cancer biopsies [8–17]. GBMs contain a heterogeneous population of cells, among which is a population of self-renewing cancer stem cells (CSCs) that contribute to tumorigenesis, treatment resistance, and tumor recurrence [3,6].

Research on CSCs has failed thus far to discover universally informative biomarkers, mutations, or gene expression patterns [18]. CD133 is the best-studied CSC biomarker and is often used experimentally to identify and enrich tumor-propagating and -initiating cells. Also known as prominin-1, CD133 is associated with normal neural stem cells and is expressed during embryonic development [19]. In several experiments, tumor cells isolated from GBM that grew neurospheres in serum-free medium (indicating self-renewal capabilities) and grew tumors phenotypically similar to GBM were found to be CD133 positive, whereas tumor cells that lacked CD133 expression did not demonstrate self-renewal or tumorigenicity in xenotransplantation studies [20–23]. However, despite the evidence outlining its crucial relationship with CSCs, CD133 is not a universal marker for identifying CSCs. Additional biomarkers have been studied in GBM including CD24, CD44, CXCR4, CD34/CD38–, Oct3/4, and Nanog [24–30]. Given their critical role in tumor initiation, propagation, and maintenance, CSCs