Concurrent Temozolomide and Dose-Escalated Intensity-Modulated Radiation Therapy in Newly Diagnosed Glioblastoma

Christina I. Tsien, Doris Brown, Daniel Normolle, Matthew Schipper, Morand Piert, Larry Junck, Jason Heth, Diana Gomez-Hassan, Randall K. Ten Haken, Thomas Chenevert, Yue Cao, and Theodore Lawrence

Abstract

**Purpose:** To determine the maximum-tolerated dose (MTD) of radiation (RT) with concurrent temozolomide in patients with newly diagnosed glioblastoma (GBM), to estimate their progression-free (PFS) and overall survival (OS), and to assess the role of $^{11}$C methionine PET (MET-PET) imaging in predicting recurrence.

**Experimental Design:** Intensity-modulated RT (IMRT) doses of 66 to 81 Gy, assigned to patients by the time-to-event continual reassessment method, were delivered over 6 weeks with concurrent daily temozolomide (75 mg/m$^2$) followed by adjuvant cyclic temozolomide (200 mg/m$^2$ d1-5 q28d x6 cycles). Treatment was based on gadolinium-enhanced MRI. Pretreatment MET-PET scans were obtained for correlation with eventual sites of failure.

**Results:** A total of 38 patients were analyzed with a median follow-up of 54 months for patients who remain alive. Late CNS grade III toxicity was observed at 78 (2 of 7 patients) and 81 Gy (1 of 9 patients). None of 22 patients receiving 75 or less Gy developed RT necrosis. Median OS and PFS were 20.1 (14.0–32.5) and 9.0 (6.0–11.7) months, respectively. Twenty-two of 32 patients with pretreatment MET-PET uptake showed uptake beyond the contrast-enhanced MRI. Patients whose treatment did not include the region of increased MET-PET uptake showed an increased risk of noncentral failure ($P < 0.001$).

**Conclusions:** Patients with GBM can safely receive standard temozolomide with 75 Gy in 30 fractions, delivered using IMRT. The median OS of 20.1 months is promising. Furthermore, MET-PET appears to predict regions of high risk of recurrence not defined by MRI, suggesting that further improvements may be possible by targeting metabolically active regions. *Clin Cancer Res;* 18(1); 273–9. ©2011 AACR.

Introduction

Patients with glioblastoma (GBM) treated with a standard RT dose of 60 Gy, typically progress in the high dose region (1–3). Although the addition of concurrent and adjuvant temozolomide has improved overall survival (OS), a majority of tumors continue to progress locally (4, 5). One possible explanation for this lack of local control is that the current standard dose of 60 Gy is insufficient.

Recent advances in RT delivery, such as intensity-modulated radiation (IMRT; ref. 6) might permit us to safely escalate RT doses by limiting the RT dose to normal tissues (7) which, when combined with effective chemotherapy, may improve outcome.

An important obstacle to the effectiveness of dose escalated RT is the inability to precisely target the tumor. Evidence now suggests that amino acid positron emission tomography (PET) using $^{11}$C-MET-PET may identify glioma beyond the region identified by conventional magnetic resonance imaging (MRI; refs. 8–11). However, no large prospective studies have yet correlated MET-PET uptake with patterns of failure after treatment, although such information could be very useful in optimizing the regions requiring high-dose RT for primary GBM (12).

We hypothesized that the use of IMRT would permit us to escalate the dose of RT with concurrent temozolomide substantially above the 60 Gy currently used. Thus, the primary objective of this study was to determine the maximum-tolerated dose (MTD) of IMRT delivered over 6 weeks with concurrent temozolomide in primary GBM, as well as to make a preliminary estimate of the OS and the
Translational Relevance

Recent advancements in RT treatment planning and imaging have improved our ability to deliver highly conformal radiotherapy. Here, we sought to translate these new developments to facilitate a new approach to treating GBM. We show the safety and tolerability of delivering higher radiation (RT) doses with concurrent temozolomide. We evaluate the ability of molecular imaging such as $^{11}$C methionine positron emission tomography (MET-PET) to identify tumor beyond regions identified on standard MRI. We show that suboptimal coverage of the MET-PET target volume can occur when using only standard MRI imaging and is associated with increased noncentral failures. Therefore, MET-PET may identify tumor subvolumes that are at highest risk of recurrence. From this, we hypothesize that a new strategy that identifies these subregions and then targets these regions in conjunction with effective chemotherapy and other novel targeted agents will achieve higher, uncomplicated tumor control probabilities than currently obtainable.

Materials and Methods

Patient eligibility

This study was approved by the University of Michigan’s Institutional Review Board. Eligible patients were 18 years or older, with a Karnofsky performance status (KPS) of 70 or greater, newly diagnosed with histologically-confirmed supratentorial (World Health Organization) grade IV gliomas including GBM and gliosarcoma, and had adequate bone marrow reserve, liver, and renal function. Treatment was required to begin within 5 weeks of surgical resection. Exclusion criteria precluded multifocal, recurrent gliomas, infratentorial tumors, evidence of cerebrospinal fluid dissemination, severe concurrent disease, prior malignancy requiring cytotoxic chemotherapy within 1 year, prior RT therapy leading to overlap of RT fields, planned final boost exceeding one third of the brain, or inability to undergo MRI. All patients underwent baseline MRI [T1, post-gadolinium T1, and fluid attenuated inversion recovery (FLAIR) etc.], and MET-PET.

PET imaging parameters

MET-PET was obtained on a Siemens ECAT EXACT HR+ whole body tomography [axial resolution 4.1 mm full width at half maximum in the center of the field of view (13)]. Following intravenous injection of approximately 740 MBq of $^{11}$C MET in a dynamic acquisition, emission scans were obtained in a 3-dimensional mode. Summed image data obtained between 10 and 30 minutes postinjection were used for further analysis. PET uptake was defined by automatic segmentation using a threshold of 1.5 times mean activity and normalized to the mean activity of the normal brain, defined as the cerebellum as previously described (14).

Image registration

A treatment planning computed tomography (CT) scan was obtained with the patient immobilized in an individualized thermoplastic mask. Image registration of research scans was carried out using functional imaging analysis tools (FIAT), a software package developed at the University of Michigan (15). Registration of the accumulated RT dose plan was accomplished by applying the same transformation. These methods are able to detect discrepancies in registration with a magnitude of 1 voxel or approximately 2 to 3 mm.

Time-to-event continual reassessment method RT dose allocation

Individual RT dose levels were allocated according to the time-to-event continual reassessment method (TITE-CRM) algorithm (16). Per protocol, a dose-limiting toxicity (DLT) was defined as any grade III or IV irreversible CNS toxicity, nonhematologic, non-CNS grade IV toxicity, or any grade V toxicity. For each patient, the probability of DLT was estimated on the basis of the expected risk, as well as the incidence of DLT in patients already treated, weighted by the amount of time patients had been followed. At the time of study enrollment, each patient was assigned RT dose with estimated probability of DLT closest but less than the target rate of 25%. RT dose escalation was restricted to one level between sequential patients. Prior to assigning patients to the next higher RT dose level, at least one patient treated at the previous level completed 3 months of observation without any DLT.

RT volumes

Gross tumor volumes (GTV) were defined as the residual gross tumor or resection cavity, based on the contrast-enhancing T1-weighted MRI. GTVs were expanded within the skull uniformly by 1.5 cm to form the clinical target volume (CTV). CTV and GTV were expanded uniformly by 0.5 cm to generate planning target volumes (PTV1 and PTV2, respectively). IMRT plans were generated to deliver 60 Gy in 30 fractions to PTV1 and a simultaneous higher dose (range, 66–81 Gy) to the smaller target, PTV2. T2/FLAIR signal abnormality was not targeted. The maximum dose limits to normal tissue organs at risk were defined as 60 bioGy to the optic nerves and chiasm, and brainstem was limited to 65 bioGy using alpha/beta ratio of 2.5.
Chemotherapy

Patients received concomitant temozolomide 75 mg/m² daily for 6 weeks. Four weeks following completion of RT, patients without evidence of disease progression continued to receive adjuvant temozolomide 200 mg/m² days 1 to 5 every 28 days, for 6 to 12 cycles or until evidence of disease progression. Additional cycles were prescribed at the discretion of the treating neurooncologist. Pneumocystis jirovecii prophylaxis using aerosolized pentamidine was maintained monthly during daily temozolomide chemotherapy. During the later phase of the study, CD4 counts were closely monitored in patients who developed grade III lymphopenia. Pneumocystis prophylaxis was continued during adjuvant temozolomide in patients with low CD4 counts during the final 3 weeks of concomitant temozolomide. Anti-seizure medications and steroids were given as clinically indicated. Doses were recorded at each treatment evaluation.

Toxicity

Chemotherapy and RT toxicities were graded using the Common Toxicity Criteria (CTC) version 3.0 (17). Acute toxicity was assessed during the first 90 days following RT. Late toxicity was assessed every 3 months during the first year and every 6 months thereafter. All patients were monitored for late CNS toxicity until death. Additional imaging studies including MR Spectroscopy, MET-PET, and if possible stereotactic biopsies were obtained at time of suspected tumor progression to differentiate it from RT necrosis.

Response criteria

Conventional MRI was obtained at 1 month, and every 2 to 3 months thereafter. Response was defined using standard Macdonald criteria (18). As published data regarding pseudo-progression became available, a finding of worsening enhancement noted within 3 months of treatment completion was followed closely for progression versus pseudo-progression. Second line therapy was given at the physician’s discretion following progression.

Pattern of failure

A pattern of failure was determined by registering the MRI at progression with the delivered RT dose distribution as previously described (19). The location of failure was classified according to the proportion of the volume of the rVOI contained within the 95% high-dose prescription isodose surface: central (>95%), in field (>80%–95%), marginal (20%–80%), or distant (<20%). MET-PET images were coregistered with the baseline MR post-gadolinium T1 as well as recurrence MRI.

Statistical analysis

The primary endpoint was to evaluate the rate of acute and late treatment-related toxicities. A standard 2-parameter logistic regression model was used at the end of the trial to obtain estimated probabilities of toxicity by dose. Secondary endpoints included PFS and OS. Kaplan–Meier estimates of median PFS and OS functions were determined with 95% confidence intervals. PFS was measured from the date of resection to progression, death, or last follow-up. Univariate proportional hazards regression models were used to assess the relation of dose and other clinical covariates to PFS and/or OS. The Fisher exact test was used to test for an association between coverage of PET GTV and subsequent noncentral failure.

Results

Patient characteristics

Between November 2003 and August 2007, 42 consecutive patients were enrolled. Three patients did not participate because of a subsequent determination of ineligibility (delay in RT > 5 weeks in 2 patients) or withdrawn consent (1 patient). One patient was nonevaluable for DLT per protocol because of clinical deterioration following the initial 5 RT fractions. MR scan showed tumor progression; all therapies were halted and the patient was transferred to hospice. This patient was included for survival analysis. The remaining 38 patients were analyzed for long-term toxicity, with a median follow-up of 54 months (range: 42–62) for patients who remain alive. Patient characteristics are listed in Table 1. The median patient age was 56 years (range: 23–75).

<table>
<thead>
<tr>
<th>Age, y, median (range)</th>
<th>56 (23–75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
</tr>
<tr>
<td>KPS</td>
<td></td>
</tr>
<tr>
<td>90–100</td>
<td>33</td>
</tr>
<tr>
<td>80</td>
<td>3</td>
</tr>
<tr>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>RPA classification</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>13</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
</tr>
<tr>
<td>Extent of surgery</td>
<td></td>
</tr>
<tr>
<td>Gross total resection</td>
<td>15</td>
</tr>
<tr>
<td>Subtotal resection</td>
<td>17</td>
</tr>
<tr>
<td>Biopsy only</td>
<td>6</td>
</tr>
<tr>
<td>Radiation prescription dose</td>
<td></td>
</tr>
<tr>
<td>66 Gy</td>
<td>1</td>
</tr>
<tr>
<td>72 Gy</td>
<td>12</td>
</tr>
<tr>
<td>75 Gy</td>
<td>9</td>
</tr>
<tr>
<td>78 Gy</td>
<td>7</td>
</tr>
<tr>
<td>81 Gy&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: KPS, Karnofsky performance score; RPA, recursive partitioning analysis.

<sup>a</sup>One patient assigned to 81 Gy received less than prescribed dose because of early termination of treatment.
Toxicities

Acute toxicities were primarily hematologic toxicities and infections (Table 2). Three grade V acute toxicities because of temozolomide were observed: (1 patient) sepsis, 75 Gy; (1 patient) prolonged and severe thrombocytopenia with pancytopenia, 75 Gy; (1 patient) aplastic anemia assigned to 81 Gy. Aplastic anemia is a rare but reported adverse event with temozolomide (20). Neither of the patients was receiving trimethoprim-sulfamethoxazole or other drugs known to suppress blood counts.

Of the 38 patients evaluated for DLT, one patient did not complete the prescribed RT course. This patient was assigned to 81 Gy but received only 75 Gy because of development of aplastic anemia. Late CNS grade III toxicity was reported at 78 Gy (2 patients) and 81 Gy (1 patient). Additional late RT toxicities included 1 patient with a grade III otitis with conductive hearing loss.

The number of dose-limiting toxicities noted was: 0 of 1 patient at 66 Gy, 0 of 12 at 72 Gy, 2 of 9 at 75 Gy, 3 of 7 at 78 Gy, and 2 of 9 at 81 Gy. The estimated probabilities of DLT with 90% confidence intervals (CI) are: 66 Gy, 0.02 (0.00–0.23); 72 Gy, 0.08 (0.02–0.25); 75 Gy, 0.14 (0.06–0.28); 78 Gy, 0.23 (0.13–0.38), and 81 Gy, 0.36 (0.17–0.61).

Adjuvant and salvage therapy

Thirty-three patients received adjuvant temozolomide, whereas 5 patients did not receive any adjuvant treatment because of clinical deterioration or death. Seventeen patients completed at least 6 cycles. Sixteen patients were discontinued earlier because of tumor progression, toxicity, or patient choice. Twenty-four patients (60%) of the 33 patients received salvage chemotherapy at time of progression; 9 of whom received salvage bevacizumab.

Fifteen patients underwent a second resection because of either new imaging findings to confirm tumor progression (9) or progressive clinical symptoms (6). Pathology review showed recurrent GBM in 5 patients; RT dose delivered was 66, 72 (2 patients), 75, and 78 Gy. Multifocal RT necrosis with no evidence of residual glioma was noted in 2 patients treated to 78 Gy. Both recurrent GBM and RT changes with areas of vascular hyalinization, gliosis, and coagulative necrosis were noted in 8 patients RT dose was delivered to 72 Gy (3 patients), 78 Gy (2 patients), and 81 Gy (3 patients).

Survival

Median PFS was 9.0 months (95% CI, 6.0–11.7) and median OS was 20.1 months (95% CI, 14.0–32.5; Fig. 1). With a median follow-up of 54 months, 7 patients remain alive, 3 patients without evidence of disease progression. Two patients developed other cancers: (1 patient) primary hepatocellular carcinoma 2 years after completing RT, succumbing shortly thereafter; and (1 patient) stage IB non-small cell lung cancer 14 months posttreatment eventually succumbing to diffuse metastases. An additional patient died from cardiopulmonary arrest shortly after re-resection.

We carried out exploratory analyses to determine if the escalated RT dose affected PFS, OS, or the risk of central failure. There was no statistically significant relationship between RT dose and PFS or OS (P > 0.5). We did note a change in the pattern of failure with decreased probability of central failures with increased RT dose (P = 0.05). Younger age (P < 0.03), resection (P < 0.03), and radiation

---

**Table 2. Chemotherapy and radiation toxicities (N = 38 patients)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Grade III</th>
<th>Grade IV</th>
<th>Grade V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood/bone marrow</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neurologic</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Auditory (hearing)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Acute toxicities**

**Late toxicities**

---

**Figure 1.** Kaplan–Meier overall (solid) and PFS curves (dotted line) of all patients (n = 39) receiving study treatment are shown.
therapy oncology group recursive partitioning analysis (RTOG RPA) class 3 ($P < 0.0003$) were associated with improved survival. Both smaller PET ($P < 0.005$) and MRI volumes ($P < 0.02$) were associated with improved PFS but not OS.

**MET-PET GTV and patterns of failure**

We then explored the potential utility of MET-PET in predicting eventual recurrence to determine if there were regions of PET uptake beyond conventional MRI that may benefit from additional boosting with RT.

MET-PET was particularly helpful in distinguishing residual tumor after resection from postsurgical changes. The tumor volume estimated by MET-PET was generally smaller than the contrast-enhancing volume on MRI in patients that underwent resection. The median MRI GTV (defined as areas of peripheral enhancement excluding regions of central necrosis) was 16.4 cm$^3$ (range: 0.8–57.9), whereas the median MET-PET tumor volume was 5.7 cm$^3$ (range: 0.5–43.8 cm$^3$). Of the 32 patients who had appreciable pretreatment MET-PET uptake (region of uptake >1cm$^3$), 22 showed MET-PET uptake that extended beyond the gadolinium-enhanced MRI target volume. Among these 22 cases, the mean distance that the MET-PET uptake extended beyond the gadolinium-enhancing tumor volume was 1 cm (range: 0.8–3.5 cm). In the vast majority of cases, the pretreatment MET-PET uptake volume fell within the MR FLAIR volume because the MR FLAIR volumes are considerably larger because of peritumoral edema (Fig. 2A). In 4 cases, the PET GTV extended beyond the MR FLAIR volume by a maximum distance of 1.5 cm.

Of the 28 CNS recurrences noted, 16 were central, 2 were in-field, 8 were marginal, and 2 were distant. Suboptimal coverage of the tumor defined by MET-PET (defined as less than 95% isodose coverage of MET-PET tumor) resulted in a higher risk of subsequent noncentral failure ($P < 0.001$). Seven of 8 patients with suboptimal PET GTV coverage recurred with noncentral failures, whereas 5 of 20 patients with adequate PET GTV coverage developed noncentral failures. Furthermore, all of the noncentral failures showed overlap with the initial PET GTV. Overlap between the areas of initial increased PET uptake and the eventual area of recurrence on MRI is shown in Fig. 2B.

**Discussion**

In this study, we have shown that the use of highly conformal RT techniques permits the safe administration of substantially higher RT doses than the standard 60 Gy. The observed median survival of 20.1 months and the change in pattern of failure with higher RT doses suggest improved efficacy. Late CNS toxicity was not observed with RT doses at or below 75 Gy with concurrent temozolomide. Furthermore, we found that patients tended to progress in regions of inadequate coverage of the tumor defined by MET-PET uptake. This suggests that MET-PET is useful in determining tumor extent, and encourages further...
exploration of using functional imaging to define target volumes as a method of decreasing recurrence in primary GBM.

Prior RT dose escalation trials using RT alone or less effective chemotherapy, including UM 90 Gy dose escalation study (21), RTOG phase III stereotactic radio-surgery boost trial (22), RTOG phase 1 3D dose escalation study (23), and Brain Tumor Cooperative Group (BTCG) randomized phase III interstitial brachytherapy boost trial (24), all failed to show improved survival. Most likely, these studies failed because the RT doses required for tumor cell kill exceeded normal brain tolerance limits. The EORTC/NCIC phase III trial of concurrent temozolomide with standard 60 Gy RT followed by adjuvant temozolomide confirmed modest gains in long-term outcome (5). The combination of highly conformal, dose-escalated RT, and effective chemotherapy that radiosensitizes may improve the effectiveness of RT at dose levels tolerable to the normal brain.

A key finding of our study is that MET-PET appears to define regions of active tumor not apparent using T1 gadolinium-enhanced MRI. 11C MET-PET imaging identifies metabolic activity through increased transport mediated by type I amino acid carriers at the level of the blood–brain barrier, which are highly expressed in malignant tumors than in normal brain (25). Biopsy studies have confirmed that increased MET-PET uptake correlates with Ki-67 staining, proliferating cell nuclear antigen expression, and microvessel density (26–28). These studies suggest that MET-PET may improve delineation of tumor extent in gliomas compared with conventional MRI imaging (11). These findings are consistent with our observation that inadequate coverage of the region of MET-PET uptake, which can occur when using only standard targeting of the T1-gadolinium enhanced region was associated with area of eventual recurrence.

Our median OS compares favorably with other recently reported GBM trials. However, our study was conducted in an era prior to the routine use of salvage bevacizumab. A number of patients underwent stereotactic biopsies, and a number of patients older than 70 years of age were included. Methylguanine-DNA methyltransferase (MGMT) analysis was carried out in a limited number of patients (26%). MGMT analysis is currently underway (MGMT) analysis was carried out in a limited number of patients predicted to have poor outcome, that is, non-methylated MGMT tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported by NIH PO1 CA59827, PO1 CA87634, and PO1 CA85878. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 17, 2011; revised October 10, 2011; accepted October 12, 2011; published OnlineFirst November 7, 2011.

References

Radiation Dose Escalation and Concurrent Temozolomide in Primary GB
