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CHEMORADIOThERAPY OF NEWLY DIAGNOSED GLIOBLASTOMA WITH INTENSIFIED TEMOZOLOMIDE

MARKUS WEILER, M.D.,*† CHRISTIAN HARTMANN, M.D.,# DOROTHEE WIEWRODT, M.D.,**

ULRICH HERRLINGER, M.D.,†† THIERRY GORLIA, M.SC.,‡‡ OLIVER BÄHR, M.D.,*

RICHARD MEYERMANN, M.D.,‡ MICHAEL BAMBERG, M.D.,§ MARCOS TATAGIBA, M.D.,†

ANDREAS VON DEIMLING, M.D.,¶ MICHAEL WELLER, M.D.,*§§ AND

WOLFGANG WICK, M.D.*†

*Department of General Neurology, Hertie Institute for Clinical Brain Research, and Departments of
†Neurosurgery, ‡Neuropathology and §Radiation Oncology, University of Tübingen, Germany;
Departments of †Neurooncology and ¶Neuropathology, University of Heidelberg, Germany;
#Clinical Cooperation Unit Neuropathology, German Cancer Research Center Heidelberg, Germany;
*The Neurosurgery, University of Mainz, Germany;
††Clinical Neurooncology Unit, Department of Neurology, University of Bonn, Germany;
‡‡EORTC Data Centre, Brussels, Belgium;
§§Department of Neurology, University Hospital of Zurich, Switzerland.

Reprint requests to: Wolfgang Wick, M.D., Department of Neurooncology, University of Heidelberg, Im
Neuenheimer Feld 400, D-69120 Heidelberg, Germany. Tel: +49-6221-56-7075; Fax: +49-6221-56-
7554; E-mail: wolfgang.wick@med.uni-heidelberg.de

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CONFLICT OF INTEREST NOTIFICATION

Ulrich Herrlinger, Michael Weller and Wolfgang Wick have been consultants to and have received honoraria from Schering-Plough. The other authors declare no conflicts of interest.
ABSTRACT

Purpose: To evaluate toxicity and efficacy of chemoradiotherapy with temozolomide (TMZ) administered in an intensified one week on/one week off schedule plus indomethacin (INDO) in patients with newly diagnosed glioblastoma.

Patients and Methods: Forty-one adult patients (median Karnofsky performance status 90%; median age, 56 years) were treated with pre-irradiation TMZ at 150 mg/m² (one week on/one week off), involved-field radiotherapy combined with concomitant low-dose TMZ (50 mg/m²), maintenance TMZ starting at 150 mg/m² according to a one week on/one week off schedule, plus maintenance INDO (25 mg bid) treatment.

Results: The median follow-up interval was 21.7 months. Grade 4 hematotoxicity was observed in 15 patients (36.6%). Treatment-related non-hematologic grade 4-5 toxicities were reported for two patients (4.9%). The median progression-free survival (PFS) was 7.6 months [95% CI, 6.2 to 10.4 months]. The one year survival rate was 73.2% [95% CI, 56.8 to 84.2%]. O⁶-Methylguanine-DNA methyltransferase (MGMT) gene promoter methylation in the tumor tissue was associated with significantly superior PFS.

Conclusions: The dose-dense regimen of TMZ administered in a one week on/one week off schedule resulted in acceptable non-hematologic toxicity. Compared to data from the EORTC/NCIC trial 26981-22981/CE.3, patients with an unmethylated MGMT gene promoter appeared not to benefit from intensifying the TMZ schedule regarding median PFS and overall survival, whereas data are promising for patients with methylated MGMT promoter.

Key words: Glioblastoma, Indomethacin, O⁶-methylguanine-DNA methyltransferase (MGMT), Radiotherapy, Temozolomide.
INTRODUCTION

The current standard of care in the treatment of newly diagnosed glioblastoma is combined chemoradiotherapy with concomitant and adjuvant temozolomide (TMZ) according to the phase III trial jointly conducted by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) (1). This trial also demonstrated that hypermethylation of the promoter region of the DNA repair gene $O^{6}$-methylguanine-DNA methyltransferase ($MGMT$) which partially mediates chemoresistance against alkylating agents such as TMZ or the nitrosoureas (2) correlates with improved survival in patients treated with combined radiotherapy and TMZ (3). TMZ dosing schedules allowing a more prolonged exposure may result in higher cumulative doses (4–6) than the standard 5-day regimen (7) and may deplete MGMT in the tumor, thus sensitizing tumor cells to the toxic effects of TMZ. However, depletion of MGMT by prolonged exposure to TMZ not only affects tumor cells but also normal cells, particularly hematopoietic precursor cells, potentially enhancing hematologic toxicity (5). Other dose-dense TMZ regimens did not show an unusual incidence of toxicities and also demonstrated promising efficacy data at recurrence (6).

Preclinical and clinical studies suggest anti-tumoral effects of cyclooxygenase (COX)-2 inhibitors (8, 9). COX-2 inhibition may result in a tumor cell-specific sensitization towards radiotherapy (10) and reduction of tumor neovascularization (11). Expression of COX-2 correlates with shorter survival times in patients with glioma (12). Though selective inhibition of COX-2 is regarded superior to non-selective COX inhibition, e.g. mediated by non-steroidal anti-inflammatory drugs (NSAID)s, in tumor therapy due to lacking adverse effects on gastrointestinal and platelet function, two available COX-2-selective inhibitors, rofecoxib and celecoxib,
were in discussion for lethal cardiovascular complications before this trial was initiated.

The University of Tübingen Medical Center (UKT)-05 phase II trial exploited the anti-angiogenic and radiosensitizing effects of the NSAID indomethacin (INDO) administered as a continuous maintenance therapy and the cytotoxic effects of an intensified concomitant and adjuvant (one week on/one week off) TMZ regimen in addition to radiotherapy in the first-line treatment of glioblastoma.
PATIENTS AND METHODS

Patients

This prospective phase II trial accrued between February 1, 2005 and October 31, 2006. The ethics committee at the University of Tübingen (Tübingen, Germany) approved the trial (253/2004). UKT-05 enrolled patients at the three German University Medical Centers of Tübingen (n = 27), Mainz (n = 13) and Bonn (n = 1). All patients gave written informed consent. The main inclusion criteria comprised histological diagnosis of supratentorial glioblastoma, age > 18 and \( \leq 65 \) years, Karnofsky performance status (KPS) of 60 or higher, no prior systemic chemotherapy or radiation therapy of the brain, no history of gastrointestinal ulceration, no anticoagulants, no orally treated diabetes mellitus, no HIV infection, and adequate bone marrow reserve, liver function, or renal function.

Treatment and surveillance

TMZ was administered orally before and after radiotherapy in a weekly alternating schedule starting at 150 mg/m\(^2\) on days 1 through 7 of 14-day cycles. Pre-irradiation TMZ differing from previous glioblastoma treatment schemes was intended to make therapeutic use of this otherwise treatment-free time interval. Radiotherapy was not postponed due to the administration of prior TMZ. If the beginning of radiotherapy coincided with days 1 through 7 of any cycle (week on TMZ), the entire cycle was completed first before low-dose TMZ at 50 mg/m\(^2\) was initiated. If the beginning of the radiotherapy coincided with days 8 through 14 (week off from TMZ), concomitant low-dose TMZ at 50 mg/m\(^2\) was started only after completion of the current week off from TMZ. According to a previously published trial (13), the dose of concomitant TMZ was chosen lower (50 mg/m\(^2\)) than the standard set by the EORTC/NCIC trial.
26981-22981/CE.3 (75 mg/m²) (1) in order to minimize the risk of adverse side effects on a combined treatment with TMZ, INDO and corticosteroids that are frequently required during radiotherapy. Four weeks after radiotherapy, the weekly alternating TMZ regimen was continued, starting at 150 mg/m². No maximum number of cycles was defined. Dose adjustments of adjuvant TMZ were done according to weekly hemograms after every second cycle (Table 1). Treatment was halted for at least two weeks at a neutrophil nadir below 1.0 x 10⁹/L and a platelet nadir below 50 x 10⁹/L or a neutrophil nadir below 0.75 x 10⁹/L or a platelet nadir below 30 x 10⁹/L. With stabilization of the neutrophil nadir > 1.5 x 10⁹/L and the platelet nadir > 100 x 10⁹/L for two weeks, dose-dense TMZ treatment was resumed at 50 mg/m². A treatment pause according to these guidelines was tolerated with clinical and MRI-documented stable disease. Adjuvant treatment with TMZ was discontinued at platelets below 10 x 10⁹/L, neutrophils below 0.5 x 10⁹/L or lymphocytes below 0.2 x 10⁹/L. INDO was orally administered at 25 mg bid without individual dose adjustments. Adverse reactions towards INDO led to continuation of the trial without INDO. Standard radiotherapy was delivered in daily single fractions of 1.8 to 2 Gy at 5 days per week. During radiotherapy and at any lymphopenia grade 3 or higher, a *Pneumocystis carinii* pneumonia prophylaxis was administered. Second-line therapy was at the discretion of the treating physician.

Toxicity monitoring was performed monthly according to the Common Terminology Criteria for Adverse Events (CTCAE; version 3.0). Hematologic toxicity was evaluated by weekly hemograms and monthly differential hemograms. Patients were to undergo contrast-enhanced MRI within 72 h after neurosurgery, four weeks after completion of radiotherapy, and every three months thereafter. Tumor progression was defined according to Macdonald criteria (14).
End points and statistical analysis

The primary end point was median progression-free survival (PFS). Secondary end points were PFS rate at six months (PFS-6), overall survival (OS), OS rate at two years, and tumor remission rates. Assessment on an intention-to-treat basis required initiation of trial treatment. Survival data were calculated according to the Kaplan-Meier method (15) starting with the day of surgery. Further comparisons were evaluated using the log-rank test, Fisher and Wilcoxon rank sum and the Cox Proportional Hazard model. In a sample size of 40 intention-to-treat subjects, the target was to obtain a median PFS of 10.4 months. The median PFS of 10.4 months was chosen because a 50% increase in PFS compared to the median PFS of 6.9 months obtained in the experimental treatment arm of the EORTC/NCIC trial 26981-22981/CE.3 (1) was regarded clinically relevant. The 95% confidence interval (CI) for the median was calculated using the exact binomial method. For acceptable toxicity, no more than 10% of patients should have experienced CTCAE grade 4 or 5 non-hematologic toxicity. Early stopping rules were implemented and toxicity data were screened regularly. The trial would have been closed prematurely if in five of the first ten included patients therapy had to be discontinued within six months due to tumor progression, toxicity or any other reason, or if 8 of the first 20 included patients experienced progressive disease within ten months. Data presented here are based on the censoring of March 1, 2008. Risk factor assessment was done by comparing the prognosis of included patients with the survival predictions of a recently published model based on EORTC/NCIC 26981-22981/CE.3 trial data (16). Allowing a historical comparison with the survival data obtained in the EORTC/NCIC trial 26981-22981/CE.3 (Table 4) (1, 16), individual survival data in UKT-05 were
corrected for the time interval between the day of surgery and the day of trial inclusion (median interval: 11 days). Treatment hazard ratios in this comparative analysis were computed without and with adjustment for the confounding effect of extent of surgery, age, performance status, and administration of corticosteroids at baseline which had been identified as major prognostic factors in the EORTC/NCIC trial 26981-22981/CE.3 (1, 16).

**MGMT analysis**

A tumor cell content of at least 80% was histologically determined. From each sample and the reference samples 200 to 500 ng of genomic DNA were treated with sodium bisulfite using the Qiagen EpiTect kit (Qiagen, Hilden, Germany) with the provided FFPE tissue protocol. The primer sequences used to detect methylated *MGMT* promoter sequences were 5′-tttcgacgttcgtaggttttcgc-3′ and 5′-gcacctttcgaaaaacgaacg-3′. This primer combination amplifies an 81-base pair (bp) fragment from methylated DNA. The primer sequences used to detect unmethylated *MGMT* promoter sequences were 5′-aactccacactctttttaaaaac-3′ and 5′-tttgtttttttgttaggtttttt-3′. This primer combination amplifies a 93-bp fragment from unmethylated DNA (17). The PCR products were separated on 2.5% agarose gels. As a positive control for methylation, we used genomic DNA from the glioma cell line U87 with known *MGMT* hypermethylation. CpGenome universal unmethylated DNA Vial A (Chemicon International, Temecula, CA) served as a negative control for methylation. In addition, a control reaction without any template DNA was performed for both PCR experiments.
RESULTS

Patient characteristics

Of 49 patients with newly diagnosed glioblastoma screened, seven patients were enrolled into a competing phase I/IIa trial of cilengitide, TMZ and radiotherapy (18) and one patient was excluded from the trial assessment retrospectively because he died from a *Legionella* pneumonia before the trial therapy was started. Along with the remaining 41 patients (Table 2), one more patient than originally planned was accrued because at the end of the accrual period, two eligible patients consented at the same time.

Treatment

At the cutoff date (March 1, 2008), the median follow-up interval for PFS was 18.7 months (95% CI, 17.8 to 34.8 months). A total of 443 cycles of intensified TMZ therapy were administered before and after radiotherapy. The median interval between surgery and the start of trial treatment was 18 days (range, 10 to 59). Twenty-six of 41 patients (63.4%) received a median of one treatment week TMZ prior to radiotherapy (range, 0 to 2). The median number of overall cycles per patient was 6.5 (range, 0 to 68). Thirty-nine of 41 patients (95.1%) received at least one cycle of TMZ during the course of their treatment. The median total dose of TMZ administered per patient during the entire course of the trial therapy was censored at 17.04 g (range, 1.40 to 81.31 g). The trial treatment had to be discontinued due to grade 4 hematologic toxicity in 3 of 41 patients (7.3%) and due to grade 4 non-hematologic toxicity in 1 of 41 patients (2.4%). In five of 41 patients (12.2%), the general health condition did not allow continuing chemotherapy. Thirty-six of 41 patients (87.8%) completed concomitant TMZ without interruption. Twenty-eight of
41 patients (68.3%) received INDO either until tumor progression or censoring. The reasons for premature discontinuation of INDO were as follows: thrombocytopenia (n = 3), neutropenia (n = 1), drug-induced hepatitis (n = 1), postoperative hygroma (n = 1), bleeding into the tumor during radiotherapy (n = 1), clinically asymptomatic elevation of liver enzymes (n = 1), gastric pain (n = 1), or other (n = 4). Radiotherapy was interrupted in three of 41 patients (7.3%) due to bleeding into the tumor during radiotherapy (n = 1), seizure (n = 1), and febrile gastroenteritis with ileus, septic disease and acute renal failure (n = 1).

Toxicity

Grade 4 hematologic toxicity per cycles was observed during adjuvant, not concomitant, therapy with TMZ and reported as follows: leukopenia 0.5%, thrombocytopenia 1.4%, neutropenia 1.4%, and lymphopenia 10.3%. Acute treatment-related toxicity per patient is summarized in Table 3. One grade 3 and one grade 5 opportunistic infection in a patient who had already been diagnosed with multifocal progressive disease were reported. This patient died from respiratory failure due to Pneumocystis carinii and cytomegalovirus lung infections ten days after completion of the preceding treatment week with TMZ. Overall, 17 of 41 patients (41.5%) experienced hematologic (15 of 41, 36.6%) or non-hematologic (2 of 41, 4.9%) grade 4/5 toxicity.

Therapeutic efficacy

Forty-one patients were assessable for outcome. Of 22 patients with measurable residual tumor, two patients had a partial remission. The median PFS was 7.6 months (range, 1.4 to 34.8+ months; 95% CI, 6.2 to 10.4 months; Figure A). At the
censoring date, five patients (12.2%) were stable, and two of these (4.9%) were still on trial therapy. One patient was stable after more than 34 months on trial treatment. PFS-6 was 70.7% (95% CI, 54.3 to 82.2%). The one year PFS rate was 31.7% (95% CI, 18.3 to 46.0%). Thirty-six of 41 patients (87.8%) experienced tumor progression as documented by magnetic resonance imaging (MRI) or computed tomography (CT) scan. Twenty-five of the progressive patients were assessable for salvage therapy regimens, including a second resection plus nitrosourea-based chemotherapy in seven patients. Three patients had surgery and one patient had radiation therapy without adjuvant treatment. Most of the non-reoperated patients were also exposed to nitrosourea-based therapy ($n = 11$), three combined with reirradiation, TMZ rechallenge ($n = 2$) or enzastaurin ($n = 1$). Upon further tumor progression, 12 patients received third-line salvage therapy including reresection ($n = 3$), one combined with TMZ, reirradiation ($n = 4$), and chemotherapy with imatinib ($n = 3$) or lomustine ($n = 2$), either alone ($n = 1$) or combined with bevacizumab ($n = 1$). One patient experienced two reresections, reirradiation and four anti-neoplastic salvage therapies. OS from the diagnosis of glioblastoma is depicted in Figure B. Fourteen patients were alive at the end of the trial assessment. The median OS was 15.9 months (range, 3.3 to 34.8+ months; 95% CI, 15.0 to 23.8 months). The one year OS rate was 73.2% (95% CI, 56.8 to 84.2%). The administration of pre-irradiation TMZ had no significant impact on PFS ($p = 0.6212$, log-rank test) or on OS ($p = 0.2080$).

**MGMT methylation status and survival**

Tumor specimens of 39 patients (95.1%) were assessable for MGMT promoter methylation analysis. Twenty-three of 39 tumors (59.0%) were diagnosed with an
unmethylated *MGMT* promoter, and 16 tumors (41.0%) displayed methylation of the *MGMT* promoter. PFS was significantly longer in patients with a methylated *MGMT* promoter ($p = 0.0002^*$) whereas OS did not differ significantly between the two subgroups of patients ($p = 0.1008$). Moreover, the comparison with the EORTC/NCIC trial 26981-22981/CE.3 (1) indicated a trend in favor of UKT-05 for patients with methylated *MGMT* gene promoter with regard to median PFS (adjusted HR = 0.65) but not to median OS (adjusted HR = 0.91) (Table 4). Of note, these patients were not selected for favorable prognostic factors in comparison with the respective treatment arm of the EORTC/NCIC trial 26981-22981/CE.3. However, in patients with unmethylated MGMT gene promoter, no favorable trend was observed for median PFS or OS. At the cutoff date, 16 of 23 patients (69.6%) without *MGMT* promoter methylation vs. 10 of 16 patients (62.5%) with *MGMT* promoter methylation had died. The one year survival rate was 65.2% (95% CI, 42.4 to 80.8%) in patients without *MGMT* promoter methylation and 87.5% (95% CI, 58.6 to 96.7%) in patients with a methylated *MGMT* promoter.
DISCUSSION

First-line therapy with intensified TMZ combined with maintained daily INDO and involved-field radiotherapy plus concomitant daily low-dose TMZ was shown to be feasible and effective in patients with glioblastoma. In line with previous studies following other approaches (3, 18, 19), MGMT gene promoter methylation was highly predictive for survival in glioblastoma patients receiving alkylating chemotherapy. The PFS-6 of 70.7% in UKT-05 compares well with the results of the combined RT/TMZ treatment arm in the phase III randomized EORTC/NCIC trial that currently sets the benchmark for first-line therapy of glioblastoma (PFS-6: 53.9%) (1). However, the median PFS of 7.6 months obtained in the UKT-05 trial is not relevantly superior to the historical control data of 6.9 months. To exclude the possibility that data were biased in the UKT-05 patient sample by selection for favorable prognostic factors, we compared the baseline patient characteristics in UKT-05 with the EORTC/NCIC trial (1, 20). This comparison revealed no significant imbalance between the two trials.

As unlimited dose escalations of adjuvant TMZ were allowed in the UKT-05 trial protocol, the grade 4 lymphotoxicity rate per cycle was higher (10.3%) than observed in the recurrent glioma trial conducted at the same institutions (0.7%) (6). Notably, grade 3/4 lymphopenia per patient did not exceed markedly grade 3/4 lymphopenia reported with a phase II trial that investigated the combined concomitant and adjuvant therapy with the integrin inhibitor cilengitide and six courses of conventionally-dosed TMZ in addition to radiotherapy in patients with newly diagnosed glioblastoma (55.8%) (18). Moreover, the incidence of grade 3 through 5 opportunistic infections in the UKT-05 trial (4.9%) is the lowest for all reported trials that tested intensified TMZ dosing schedules.
The most important observation of the UKT-05 trial comes from the MGMT methylation efficacy data: In the subgroup of patients suffering from an MGMT-inactive glioblastoma, there was a clinically relevant trend for a superior PFS. Yet, this trial did not indicate that this trend transformed into an overall survival benefit. Compared to data from the EORTC/NCIC trial (3), patients with an unmethylated MGMT gene promoter appeared not to benefit from intensifying the TMZ schedule. This profoundly affects the concept of enhanced MGMT depletion, as current efforts strive for an efficient reduction of MGMT activity in the tumor tissue to overcome chemoresistance by applying intensified TMZ dosing regimens. Several studies using TMZ at a three weeks on/one week off (21 of 28 days) schedule at recurrence in malignant glioma (21, 22) or at a one week on/one week off schedule in non-resectable glioblastoma (23) have investigated alternative dosing schedules for TMZ. Encouraging data on the beneficial effects of a first-line treatment with dose-intensified one week on/one week off TMZ were first obtained in the experimental arm of a randomized phase II trial (median PFS: 10.8 months; PFS-6: 67.1%; one year survival rate: 56.1%). However, that trial did not provide differential survival data regarding the methylation status of the MGMT gene promoter (24). Preliminary evidence indicates that the one week on/one week off TMZ schedule could also be active in patients with tumors lacking MGMT gene promoter methylation (6). The PFS-6 of 65.2% suggests that, in the first-line treatment situation, patients with MGMT-expressing tumors might benefit from the intensified TMZ schedule. In contrast, this translates not into a relevant overall improvement regarding median PFS and OS, similar to other trials for patients with MGMT-active glioblastoma (1, 3, 18, 19). Conversely, the comparative statistical analysis with the current standard therapy documented a possible effect on PFS but failed to show an effect on OS in
patients with a methylated MGMT gene promoter (Table 4) that needs to be assessed in randomized trials. In this regard, UKT-05 is the first trial to deliver data concerning the efficacy of first-line treatment with dose-intensified TMZ respecting the patient’s MGMT methylation status before large-scale results from the currently open two-armed phase III trial jointly conducted by the RTOG and the EORTC (RTOG trial 0525 and EORTC trial 26052-22053) comparing chemoradiotherapy with conventional adjuvant and dose-intensive three weeks on/one week off TMZ. Meanwhile, the UKT-05 experience points towards the necessity of assessing the MGMT methylation status prospectively and suggests treating patients with a methylated MGMT gene promoter according to a dose-dense TMZ protocol.
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APPENDIX
Wolfgang Wick was the principal investigator and designed the trial. Markus Weiler was the coordinator and co-investigator, recruited and assessed patients, did the overall data management and data analyses, and with Wolfgang Wick, prepared the statistics and wrote the draft of the article. Dorothee Wiewrodt, Ulrich Herrlinger and Oliver Bähr were co-investigators, recruited and assessed patients at different sites, and took part in the data collection. Thierry Gorlia did the comparisons with the EORTC/NCIC trial 26981-22981/CE.3. Richard Meyermann was the pathologist who reviewed all immunohistochemistries. Christian Hartmann and Andreas von Deimling provided the MGMT promoter methylation data. Michael Bamberg was the radiation oncologist and Marcos Tatagiba was the neurosurgeon in the scientific board and both were involved in most of the patients’ treatments. Michael Weller was the neurooncologist responsible for the design of the trial and treatment of most patients. All authors were involved in data analysis and writing the final report.
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activation in HeLa cells occur via a mechanism involving p38 MAP kinase. 


FIGURE LEGEND

Fig. 1. (A) Progression-free and (B) overall survival in the 41 patients of UKT-05.

Abbreviations: PFS-6 = progression-free survival rate at six months; MST = median survival time.
Cumulative Proportion Surviving

Median PFS: 7.6 months

PFS-6: 70.7%

MST: 15.9 months