Preface

The First Annual Meeting of the Society of Biotherapeutic Approaches was held in 1997 with the aim to enable the feedback of results of basic biotherapeutic research to patients with incurable diseases. These meetings are unique in the point that both basic and clinical investigators meet together and discuss the direction of research needed to find the best therapeutic strategy for patients. We have now reached the stage where results of basic research can be applied to patients with cancer. For example, humanized monoclonal antibodies against HER2/neu, CD20, and VEGF are now clinically available. Tumor vaccine therapies utilizing synthetic peptides and/or monocyte-derived dendritic cells are now included in translational research.

This special issue of ANTICANCER RESEARCH presents a part of the proceedings of “The 12th Annual Meeting of the Society of Biotherapeutic Approaches” which was held in Fukuoka, Japan, December 2008.

In editing this issue we felt very fortunate to find that most of the papers contain original data and valuable concepts. For this reason, we are confident that this issue will be a milestone in the history of our meetings.

We are deeply indebted to invited speakers, Drs. H. Niiro, Kyushu University Hospital, and T. Morisaki, Fukuoka Cancer General Hospital. We thank our sponsors who contributed to the success of this meeting. We thank all the participants, and especially the contributors to this issue. We also wish to express our thanks to the publishers of ANTICANCER RESEARCH.

Motomichi Torisu
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Editors in Chief

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Tetsushi Kinugasa
Gen-Ichiro Soma
Local Editorial Board Members
Review

Anti-glioma Therapy with Temozolomide and Status of the DNA-Repair Gene MGMT

TSUYOSHI FUKUSHIMA¹, HIDEO TAKESHIMA² and HIROAKI KATAOKA¹

Section of ¹Oncopathology and Regenerative Biology, Department of Pathology, and ²Neurosurgery, Department of Clinical Neuroscience, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Abstract. The prognosis of patients with glioblastoma is extremely poor despite multimodal treatments including surgery, chemotherapy and radiotherapy. Recently, the alkylating agent, temozolomide (TMZ) has been shown to improve survival in patients with malignant gliomas, including those with glioblastoma in some clinical studies, and has become one of the standard modalities for treatment of newly diagnosed and recurrent malignant gliomas. The epigenetic silencing of the DNA repair enzyme O⁶-methylguanine-DNA-methyltransferase (MGMT) is the strongest predictive marker for favorable outcome in patients treated with TMZ. However, it remains to be determined how patients with tumors lacking MGMT promoter methylation should be treated. Moreover, even patients with TMZ-sensitive glioblastoma cannot avoid eventual recurrence. In this article, we review the mechanism of the effect of TMZ on tumor cells and resistance to TMZ, and provide an overview of the current management and trials for patients with glioblastoma.

Glioblastoma (glioblastoma multiforme, GBM) is the most frequent and malignant subtype of glioma, and is classified into grade IV of the World Health Organization (WHO) grading system (1). Anaplastic astrocytoma, anaplastic oligodendroglioma and anaplastic oligoastrocytoma are classified into grade III; grade III and IV gliomas are designated as high-grade gliomas or malignant gliomas. Despite innovations in neurosurgical techniques, developments of new anticancer drugs and molecular targeted drugs, and advances in radiotherapy over the past decades, malignant gliomas, especially GBM, remain fatal diseases. Although patients with malignant gliomas have been treated with combined radiotherapy and chemotherapy using regimens such as PCV-3 (procarbazine, lomustine, and vincristine) (2), no regimen has demonstrated a significant beneficial improvement of median survival relative to radiotherapy alone. Temozolomide (TMZ) is the only anticancer drug that has been shown in a phase III study to improve survival in GBM when administered with concomitant radiotherapy (3). TMZ is an oral alkylating agent that leads to cell death by alkylation of the O⁶ position of guanine and subsequent disturbance of DNA replication (4). The DNA repair protein O⁶-methylguanine-DNA methyltransferase (MGMT) has been implicated in the resistance of tumor cells to alkylating agents (5). MGMT is expressed in gliomas and its contribution to resistance to TMZ has been reported (6-12). To date, many clinical trials have aimed to reduce TMZ resistance. In this article, we review the mechanism of anticancer action and the clinical trials of TMZ, and summarize current concepts of chemotherapy in the context of a multidisciplinary approach to GBM.

Mechanism of Anticancer Action of TMZ

TMZ is an analog of mitozolomide, one of the antitumor imidazotetrazines which were synthesized by Stevens et al. in the 1980s (13). Although mitozolomide showed severe myelosuppression in the phase I study (14), a 3-methyl derivative, TMZ showed less toxic effect and broad-spectrum activity in mouse tumors (15). A phase I trial also showed good tolerances and responses in patients with melanoma and patients with recurrent malignant gliomas (16). Orally administered TMZ is converted to 5-(3-methyltriazen-1-
Methylation and expression of MGMT, and correlation between DNA methylation-mediated silencing of MGMT have been recognized as the essential mechanism for sensitivity to the alkylating agent, temozolomide (TMZ) (9). In the NCSC trial, TMZ improved progression-free survival compared with those treated with radiotherapy alone (47), and patients who were treated with a higher dose radiotherapy showed longer survival than those treated with lower dose radiotherapy (48). Although many trials of boost fractionation or radiosurgery following conventional radiotherapy were performed, no significant survival improvement was achieved compared with conventional radiotherapy alone (49-52).

Chemotherapy is expected not only to be a cytotoxic modality for malignant gliomas but also to sensitize the tumor cells to radiation effects. Before TMZ, hydroxyurea, nitrosoureas such as carmustine (BCNU), lomustine (CCNU), and nimustine (ACNU), and procarbazine were used for adjuvant chemotherapy for patients with malignant glioma (43). Nitrosoureas (alkylating agents like TMZ) and procarbazine were commonly used. The Brain Tumor Study Group revealed that intra-arterial administration of BCNU does not show beneficial effects, and the response rate is around 10% (12). Therefore, the standard treatment for malignant gliomas is surgery followed by chemotherapy with radiotherapy (19).}

**Treatment before TMZ**

The standard treatment for malignant glioma is surgery followed by chemotherapy with radiotherapy. Optionally, boost radiotherapy or chemotherapy is added, and stereotactic radiotherapy or convection-enhanced delivery (CED) is performed as a salvage therapy at recurrence/regrowth (42, 43). Regarding surgery, gross total resection is directly associated with longer survival, compared to subtotal resection (44), and novel modalities such as contrast-enhancing agents (45), navigation systems, and intraoperative monitoring systems (46) improve the performance of surgery. However, quite a few patients with malignant gliomas will have residual tumors because the brain is a vital organ and many tumors are located in eloquent regions of the brain, consequently, complete resection is often unattainable. No matter how the surgical technique develops, patients with malignant gliomas cannot be cured by surgery alone. In the late 1970s, it was reported that the addition of radiotherapy to surgery was more beneficial than surgery alone for patients with malignant gliomas (47), and patients who were treated with 60 Gy-dose radiotherapy showed longer survival than those with lower dose radiotherapy (48). Although many trials of boost fractionation or radiosurgery following conventional radiotherapy were performed, no significant survival improvement was achieved compared with conventional radiotherapy alone (49-52).

Chemotherapy is expected not only to be a cytotoxic modality for malignant gliomas but also to sensitize the tumor cells to radiation effects. Before TMZ, hydroxyurea, nitrosoureas such as carmustine (BCNU), lomustine (CCNU), and nimustine (ACNU), and procarbazine were used for adjuvant chemotherapy for patients with malignant glioma (43). Nitrosoureas (alkylating agents like TMZ) and procarbazine were commonly used. The Brain Tumor Study Group revealed that intra-arterial administration of BCNU does not show beneficial effects, and the response rate is around 10% (12). Therefore, the standard treatment for malignant gliomas is surgery followed by chemotherapy with radiotherapy (19).
Figure 1. TMZ is converted to 5-(3-methyltriazen-1-yl)imidazole-4-carboximide (MTIC) in water/blood with little or no enzymatic component. MTIC is broken down to methylazanium cation and 5-aminimidazole-4-carboxamide (AIC). AIC is excreted via the kidneys and methylazanium cations deliver methyl groups to DNA. Methyl groups are transferred to the 6th position oxygen atoms of guanine and O\textsuperscript{6}-methylguanines are formed. O\textsuperscript{6}-Methylguanine mispairs with thymine instead of cytosine during DNA replication. The O\textsuperscript{6}-methylguanine causes DNA break and apoptosis. MGMT removes methyl groups from O\textsuperscript{6}-methylguanines to repair the genome. The expression of MGMT is epigenetically controlled by some kinds of transcription factors or hormones. If the promoter region is methylated, the expression of MGMT is kept at a low level. Abbreviations, MTIC, 5-(3-Methyltriazen-1-yl)imidazole-4-carboximide; AIC, 5-aminimidazole-4-carboxamide; MGMT, O\textsuperscript{6}-methylguanine-DNA methyltransferase; Me, methyl group; AP-1, activator protein-1; NF-\kappaB, nuclear factor kappa B.
not yield more favorable results than intravenous administration (53). PCV-3 was only a combination chemotherapy regimen, which showed more beneficial survival and time to progression in patients with anaplastic astrocytoma than single agent and radiotherapy alone (2, 54). The median survival of patients with anaplastic astrocytoma treated with PCV-3 was 157 weeks and that of those treated with BCNU was 82.1 weeks; time to progression was also doubled. On the other hand, there was no statistically significant difference in survival of patients with GBM (median survival duration, 50.4 weeks with PCV and 57.4 weeks with BCNU) (2). In spite of the results in GBM, PCV-3 has been used most extensively for treatment of malignant gliomas for a long time. As recent studies have demonstrated that loss of heterozygosity (LOH) on chromosome 1p and 19q in anaplastic oligodendroglioma predicts sensitivity to chemotherapy and better overall survival (55, 56), PCV-3 is preferably used for patients with malignant gliomas with oligodendrogial components. In Japan, some groups perform IAR (interferon-β, ACNU and radiation) therapy for malignant gliomas, which is designed as an antitumor therapy with biological modulation. Although no data of any randomized large-scale clinical study are available for this therapy at present, a certain degree of improvement appears to be observed compared with the previous outcome (57, 58). Until the phase III trial of radiotherapy plus TMZ showed the median survival benefit (14.6 months), the median survival of GBM had been less than one year (3).

Clinical Studies of TMZ and Chemoresistance to TMZ

The TMZ therapy according to the regimen of Stupp et al. (3) is a standard first-line chemotherapy of malignant gliomas. In addition, various new attempts have been performed to overcome TMZ chemoresistance. Recent phase II and III studies of first-line treatment for GBM (59-69) and of treatment for the recurrent GBM (70-83) are shown in Tables I and II, respectively.

Gliadel wafer is a polymer implant conjugated with BCNU, which is used for local delivery of BCNU to the resection surface intraoperatively. Surgery with Gliadel wafers followed by standard TMZ administration plus radiotherapy may show relatively favorable outcome, but the scale of the clinical trials is small and not double-blind studies (60, 62). *Epidermal growth factor receptor (EGFR)* amplification is observed 34% of GBM, and the mutation or amplification of *EGFR* plays important roles in progression of GBM (84). Erlotinib is a tyrosine kinase inhibitor and inhibits EGFR selectively. Although no adverse event occurred even with TMZ therapy (63), apparent benefit was not detected in overall survival (61, 63, 73). Erlotinib has insufficient effects on unselected GBM cases. Imatinib is also a tyrosine kinase inhibitor and shows a favorable effect only on subsets of patients (82). Bevacizumab is also a tyrosin kinase inhibitor which represses vascular endothelial growth factor selectively. A small-scale study showed favorable efficacy of bevacizumab on GBM when combined with TMZ and radiotherapy (64). Although thalidomide and celecoxib were also expected to have an antiangiogenesis effect on tumors, no beneficial effect on survival has been observed (66).

It had been reported that combinations of alkylating agents such as TMZ and BCNU depleted MGMT activity and increased antitumor activity of each other (85). However, a phase II trial revealed that BCNU plus TMZ had only a modest effect, with significant toxicity, and appeared to be no more effective than single-agent TMZ (83). Anticancer drugs with different mechanisms of action from TMZ, such as cisplatinum, irinotecan, teniposide, procarbazine, and hydroxyurea, biomodulator (polyinosineic-polycytidylic acid stabilized with polykysine and carboxymethylcellulose and interferon), and matrix metalloprotease inhibitor (marimastat) were used as investigational agents, however, no satisfactory benefit was obtained. The enzyme activity of MGMT is the most important mechanism underlying the resistance to TMZ. However, other unknown mechanisms may exist because some cell lines with low MGMT expression still show significant resistance to TMZ. Disturbance of the mismatch repair system is one of the mechanisms of TMZ resistance (86-88). Moreover, nucleotide excision repair system may also be involved in TMZ resistance. Some human tumor cells treated by TMZ show increased expression of chromatin-associated gene poly(ADP-ribose) polymerase-1 (PARP), which is involved in nucleotide excision repair (89). PARP inhibitor enhanced sensitivity to TMZ both *in vitro* (90, 91) and *in vivo* (92, 93), thus the clinical usefulness of this compound should be evaluated. An ATP-competitive small-molecule inhibitor (94) and a gastrin-releasing peptide receptor antagonist (95) also showed antitumor effects in combination with TMZ *in vitro* and *in vivo* experiments. Interleukin-24 is reported to have the ability to inhibit MGMT in human melanoma cells (96).

Chemoresistance and Glioma Stem Cells

Recently, evidence supporting the cancer stem cell concept has been increasingly provided. A relationship between the chemoresistance of cancer cells and stemness has been suggested repeatedly. Cancer stem cells show multidrug-resistant phenotype by overexpressing drug transporters such as adenosine triphosphate-binding cassette (ABC) superfamily, vaults such as lung resistance-related protein/major vault protein (LRP/MVP), and anti-apoptotic protein such as B-cell lymphoma/leukemia-2 (bcl-2). Glioma stem cells are also believed to exist and show the multidrug-resistant phenotype, and thus would be important therapeutic targets (reviewed in 97). Analysis of neural stem cells and sorted cells using neural
stem cell marker CD133 revealed the existence and features of brain tumor stem cells (or glioma stem cells) (98). It is reported that one of the ABC superfamily, multidrug resistance 1 (MDR1), plays an important role in the chemoresistance of GBM independent of MGMT status, and a single nucleotide polymorphism status of the MDR1 gene dictates TMZ sensitivity (99). CD133 expression as a signature of stem cell phenotype is reported to be a candidate predictor for poor survival in patients with GBM treated with concomitant TMZ chemoradiotherapy (100). On the other hand, it has been reported that TMZ administration can reduce the number of glioma stem cells (101).

### TMZ Sensitivity and p53

P53 is a pleiotropic molecule and plays an important role in DNA repair and apoptosis with a different mechanism from MGMT. Wild-type p53 can reduce the level of MGMT in cells in vitro (102). Conversely, in another report, p53 directly induced MGMT expression in murine astrocytic glioma cells (103). Moreover, p53 inhibitor enhanced the effect of TMZ in a mouse intracranial tumor implantation model, suggesting that p53 may induce MGMT to regulate TMZ sensitivity negatively (104). Although p53 plays a protective role against cell death on treatment with chloroethylating agents, this is not

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**Table I. Recent phase II and III studies of first-line treatment of glioblastoma.**

<table>
<thead>
<tr>
<th>References</th>
<th>Regimen</th>
<th>Patients (n)</th>
<th>1-year (%)</th>
<th>2-year (%)</th>
<th>Overall (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stupp et al. (3)</td>
<td>TMZ + radiation</td>
<td>287</td>
<td>61</td>
<td>27</td>
<td>14.6</td>
</tr>
<tr>
<td>Buckner et al. (59)</td>
<td>Cisplatinum + BCNU + radiation</td>
<td>451</td>
<td>11</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Smith et al. (60)</td>
<td>Gliadel wafer implantation (BCNU) + γ-knife radiation + radiation</td>
<td>30</td>
<td>22</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Prados et al. (61)</td>
<td>Erlotinib + radiation</td>
<td>65</td>
<td>19.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGirt et al. (62)</td>
<td>Gliadel wafer implantation (BCNU) + TMZ + hyperfractionated radiation</td>
<td>33</td>
<td>36</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>Brown et al. (63)</td>
<td>Erlotinib + TMZ + radiation</td>
<td>97</td>
<td>15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narayama et al. (64)</td>
<td>Bevacizumab + TMZ + radiation</td>
<td>15</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minniti et al. (65)</td>
<td>Hyperfractionated radiation +TMZ</td>
<td>43</td>
<td>35</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Kesari et al. (66)</td>
<td>TMZ + thalidomide + cerecoxib + radiation</td>
<td>50</td>
<td>47</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Chinot et al. (67)</td>
<td>TMZ (alternate regimen) + radiation</td>
<td>29</td>
<td>28</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Fountzilas et al. (68)</td>
<td>Irinotecan + TMZ + radiation</td>
<td>22</td>
<td>12.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colman et al. (69)</td>
<td>Interferon-β + radiation</td>
<td>109</td>
<td>13.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BCNU, Carmustine; TMZ, temozolomide; γ-knife, gamma-knife.

**Table II. Recent phase II studies of recurrent glioblastoma.**

<table>
<thead>
<tr>
<th>References</th>
<th>Regimen</th>
<th>Patients (n)</th>
<th>6-Month PFS (%)</th>
<th>6-Month overall survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinn et al. (70)</td>
<td>O6-Benzylguanine</td>
<td>34</td>
<td>17</td>
<td>26</td>
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<tr>
<td>Wick et al. (71)</td>
<td>Rechallenge with TMZ</td>
<td>80</td>
<td>29</td>
<td></td>
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<tr>
<td>Glas et al. (72)</td>
<td>ACNU + teniposide (VM26)</td>
<td>35</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td>van den Bent et al. (73)</td>
<td>Erlotinib</td>
<td>110</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Brandes et al. (74)</td>
<td>Fotemustine</td>
<td>43</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Fabrini et al. (75)</td>
<td>Fotemustine</td>
<td>50</td>
<td>52</td>
<td>25</td>
</tr>
<tr>
<td>Chamberlain and Johnston (76)</td>
<td>Bevacizumab</td>
<td>25</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Butowski et al. (77)</td>
<td>Poly-ICLC</td>
<td>45</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Balmaceda et al. (78)</td>
<td>TMZ (alternate regimen)</td>
<td>120</td>
<td>43</td>
<td>75</td>
</tr>
<tr>
<td>Silvani et al. (79)</td>
<td>Procarbazine + fotemustine</td>
<td>54</td>
<td>27</td>
<td></td>
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<tr>
<td>Brandes et al. (80)</td>
<td>TMZ</td>
<td>33</td>
<td>30</td>
<td></td>
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<tr>
<td>Groves et al. (81)</td>
<td>Marimastat + TMZ</td>
<td>49</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Reardon et al. (82)</td>
<td>Imatinib mesylate + hydroxyurea</td>
<td>33</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Prados et al. (83)</td>
<td>BCNU + TMZ</td>
<td>41</td>
<td>21</td>
<td>66</td>
</tr>
</tbody>
</table>

PFS, Progression-free survival; TMZ, temozolomide; ACNU, nimustine; BCNU, carmustine; Poly-ICLC, polynosinic-polycytidylic acid stabilized with polysylane and carboxymethylcellulose.

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the case in the treatment with methylating agent (105). Collectively, the roles of p53 are complicated and diverse depending on cell type, status of p53 (wild or mutant) and the kind of antitumor agent used.

Conclusion

Although the improvement of outcome of GBM patients by the new alkylating agent TMZ has an impact on treatment of malignant gliomas, GBM is still an incurable disease. Breakthrough is required for therapeutic modalities. Much remains to be clarified; the mechanism of chemoresistance and the roles of related molecules including MGMT, mismatch repair enzymes, DNA excision repair enzymes, PARP, p53, ABC superfamily, and apoptosis-related factors. Not only approaches to increase sensitivity to TMZ but also understanding the cellular biology underlying chemoresistance and the stem cell phenotype will lead us to a complete cure of GBM.

Acknowledgements

This work was supported by Grant-in-Aid for Young Scientists (B) No. 20390114 from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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Fukushima et al: MGMT and Temozolomide Treatment of Glioblastoma (Review)


Received April 28, 2009
Revised July 20, 2009
Accepted August 13, 2009