Review

Current and future developments in the use of temozolomide for the treatment of brain tumours

Roger Stupp, Marc Gander, Serge Leyvraz, and Edward Newlands

Brain tumours comprise only 2% of all adult cancers, but they are among the most debilitating malignant diseases. Temozolomide, an alkylating agent that can be administered orally, has been approved for the treatment of recurrent malignant glioma on a daily schedule for 5-day cycles. Continuous administration schedules with a higher dose intensity are being explored, but an improvement in efficiency remains to be shown. The benefit from temozolomide given as a single agent in recurrent disease will be several weeks at best. This drug is therefore now undergoing clinical testing as neoadjuvant chemotherapy or with concomitant radiotherapy in patients with newly diagnosed glioma. Several phase I trials are investigating the combination of temozolomide with other agents active against brain tumours. This review briefly summarises the pharmacological background and clinical development of temozolomide and focuses on current and future clinical exploration of this drug for the treatment of brain tumours.

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Primary brain tumours comprise only about 2% of all adult cancers. Malignant gliomas are the most common type of brain tumours, and high-grade gliomas are among the most rapidly growing and devastating neoplasms. Despite surgery and radiotherapy, these tumours almost invariably recur, rapidly leading to death. The median survival of patients with glioblastoma (Figure 1) is less than 1 year from diagnosis. For anaplastic astrocytoma, median survival is about 18 months. Many of the commonly used chemotherapy agents have limited activity in these malignant diseases. The role of the blood–brain barrier as an obstacle to chemotherapy efficiency is debatable, because it is commonly disrupted in aggressive glioma.

Temozolomide was developed in the 1980s, through rational drug design, by the UK Cancer Research Campaign.1,2 It has some advantages over dacarbazine, with which it shares the active metabolite 5-(3-methyl)1-triazen-1-yl-imidazole-4-carboxamide (MTIC).3 It is rapidly and completely absorbed after oral administration and spontaneously converts into the active metabolite without the need for enzymatic demethylation in the liver. Temozolomide has excellent penetration into all body tissues, including the brain. One mechanism of resistance to this agent is mediated through the enzyme O6-alkylguanine transferase (AGT).

Pharmacology

Temozolomide is one of a series of imidazotetrazinone derivatives. The development of mitozolomide, the first compound of this class to show broad-spectrum antitumour activity in preclinical models,6,7 was abandoned because of the unpredictable and long-lasting haematological toxic effects seen in early clinical trials. Studies of the structure–activity relation suggested that 3-substituted derivatives of mitozolomide could cleave to form the linear triazene MTIC.8 Preclinical studies confirmed that temozolomide had a very different toxicity profile from mitozolomide in various tumour models, including astrocytomas, glioblastomas, and ependymomas.9,10 Whereas dacarbazine requires enzymatic demethylation in the liver to be converted into the reactive species, temozolomide is spontaneously activated into MTIC at physiological pH in aqueous solution (Figure 2). Preclinical studies revealed good bioavailability after oral administration, schedule-dependent antitumour activity, and good tissue penetration, including into the brain.4

Figure 1. Giant-cell glioblastoma (x 300). Most of the tumour-cell bodies express glial fibrillary acidic protein. Cellular and nuclear polymorphism is shown.

Many brain tumours express low concentrations of this enzyme.4,5

Correspondence: Dr Roger Stupp, Multidisciplinary Oncology Center, University Hospital CHUV, Lausanne, Switzerland. Tel: +41 21 314 0156. Fax: +41 21 314 0737. Email: Roger.Stupp@chuv.hospvd.ch
Pharmacokinetics
Temozolomide shows linear pharmacokinetics with the area under the concentration time curve (AUC) increasing in proportion to the dose. The excellent bioavailability allows oral administration of the drug. Temozolomide is rapidly absorbed after oral dosing,11 with moderate variability between individuals and some influence of the fasting state.12 Maximum plasma concentrations are measured 30–90 minutes after administration. In a daily schedule for 5 days, no accumulation of temozolomide was observed. When taken in conjunction with food, the drug’s absorption is delayed, leading to a lower peak concentration and a decrease in the AUC of about 10%.13 In most clinical trials temozolomide was given with fasting for at least 1 hour before administration and for 1 hour afterwards. On current data, the small change in the AUC with food is unlikely to be clinically relevant. Excretion of temozolomide is thought to occur mainly via the kidney, with a plasma half-life of 1.8 hours.14 There are, however, no recommendations for dose reduction in patients with renal failure. Published data on penetration into the cerebrospinal fluid in human beings are scarce. We prospectively measured cerebrospinal-fluid and plasma concentrations of temozolomide in 33 patients. The equilibrium was reached 4 hours after drug intake with a mean ratio of 40% (SD 11).15

Mechanism of action
Reaction of water with the electropositive C4 atom of temozolomide opens the heterocyclic ring, releasing MTIC, a reactive methylating compound. Like the chloroethylnitrosoureas, with which they have a common range of preclinical activity, imidazotetrazinones act as major groove-directed DNA-alkylating agents.17 They are base-selective and preferentially bind the middle guanine residue of a GGG sequence. The sites of methylation on DNA are the N7 atoms on guanine, O3 on adenine, and O6 on guanine. Although O6-methylguanine represents only a minority of adducts formed by temozolomide, it has a critical role in the cytotoxic action of the drug. The O6 atom of guanine is the initial site of attack on DNA of other active agents against malignant gliomas, such as the cross-linking chloroethylnitrosoureas. O6-methylguanine in itself is not lethal to cells; it does not inhibit processes such as DNA replication and transcription. However, the preferred base pairing during DNA replication results in incorporation of thymine instead of cytosine opposite O6-methylguanine. The mismatch repair pathway of the cell, which excises the aberrant thymine residue in the daughter strand, recognises this mismatch. However, unless the methyl adduct is removed from the guanine, thymine is likely to be reinserted opposite the lesion. The MutS branch of the mismatch repair pathway has a key role in signalling the initiation of apoptosis in response to O6-methylguanine.18 Repetitive futile rounds of mismatch repair are thought to result in a state of chronic strand breaks, which triggers an apoptotic response.19

Resistance mechanisms
Resistance to temozolomide is thought to be mediated through two independent repair mechanisms – AGT and the DNA mismatch repair proteins.

O6-methylguanine is subject to a single-step error-free reversal of damage reaction, in which the methyl adduct is transferred to a cysteine residue on AGT. The cellular concentration of this enzyme is a major determinant of temozolomide cytotoxicity in vitro.20,21 Interest in AGT cellular concentrations in malignant glioma arose from the enzyme’s role in the cytotoxicity of chloroethylnitrosoureas. Concentrations were higher in benign and in chemoresistant central-nervous-system tumours such as meningiomas and neurinomas and lower in more chemosensitive tumours such as oligodendrogliaomas and anaplastic oligodendrogliaomas.22 In a series of 187 samples of glioma and anaplastic astrocytoma, 26% had no measurable activity of AGT.23 In that study, patients with glioblastomas and anaplastic astrocytomas with low concentrations of AGT had significantly better survival than those with high concentrations of the enzyme. In a series of 38 patients with newly diagnosed glioblastoma and anaplastic astrocytoma treated with temozolomide after surgery and before radiotherapy, low expression of AGT (detection in less than 20% of cells) was found in 70% of tumours.24 The response rate to temozolomide was 60% in the low-AGT group and only 10% in the group with AGT expression in more than 20% of tumour cells. A strong association between AGT activity and response and survival was found with a functional assay measuring methylation of the AGT-gene promoter. Patients with glioma who had a methylated promoter, and thus inactivated AGT, had significantly longer survival when receiving carmustine chemotherapy than patients with a non-methylated AGT promoter (36 versus 22 months; p<0.001, univariate analysis).25 Prospective validation on a larger number of patients is now needed.

Colon cancer cell lines with mismatch repair mutations...
are resistant to temozolomide, and the resistance cannot be overcome by AGT depletion. In a panel of xenograft tumours from children, a defect in MLH1, a key mismatch repair protein, was an important determinant of resistance to temozolomide. The DNA mismatch repair proteins MSH2 and MLH1 were detected by immunohistochemistry in at least 60% of tumour cells in more than 80% of glioblastomas and anaplastic astrocytomas. However, in that series, the rates of response to temozolomide of tumours with high and low expression of these proteins were similar. A combination of low concentrations of AGT and high concentrations of mismatch repair proteins might be expected to identify the majority of responders to temozolomide therapy. However, in the study by Friedman and colleagues, previous knowledge of these variables predicted only 66% of responses. These negative results do not disprove the role of AGT or mismatch proteins, but rather suggest that immunohistochemical determination may not reflect the functionality of these pathways. Further investigation with functional assays is required.

Another cell target that can potentiate the cytotoxicity of temozolomide is the nuclear enzyme poly-ADP-ribose polymerase. This enzyme recognises DNA strand breaks and is involved in their repair. An inhibitor of poly-ADP-ribose polymerase, 3-aminobenzamide, potentiates temozolomide in vitro. More recent work with two novel inhibitors of this enzyme (NU-1025 and NU-1005) showed that they can potentiate both temozolomide and topotecan in vitro. Further clinical development of these compounds is planned.

**Approved and investigational schedules of temozolomide administration**

On the basis of preclinical models suggesting increased activity with repeated exposure to temozolomide, the schedule of daily treatment for 5 days was developed. A daily dose of 200 mg/m² (150 mg/m² for the first cycle in patients who have received chemotherapy previously) was established as the maximally tolerated dose and recommended for phase II testing. The dose-limiting toxic effect with the intermittent schedule has been thrombocytopenia. At the recommended dose of 200 mg/m² daily, grade 4 thrombocytopenia is observed in less than 10% of patients, and grade 3/4 neutropenia in less than 5%. Nausea can easily be prevented by standard antiemetics or paediatric doses of 5-HT₁ antagonists. Some patients complain of fatigue in the week after therapy.

Several other schedules of temozolomide administration have been explored. In a phase I trial, the feasibility of continuous daily administration of temozolomide over 6–7 weeks was assessed. Doses were increased gradually from 50 mg/m² to 100 mg/m² daily. Myelosuppression was identified as the dose-limiting toxic effect; other side-effects with continuous administration are minor. Antiemetic prophylaxis is not required in most cases. The recommended dose for further studies using continuous administration is 75 mg/m² given daily for 6–7 weeks. This schedule allows the administration of 2.1 g/m² for each 4 weeks, which is twice the dose that is administered when temozolomide is given in cycles of daily doses for 5 days. Whether continuous low-dose administration and higher dose intensity lead to improved efficacy remains unclear.

Other schedules of continuous daily administration are being investigated (Table 1). With administration of temozolomide for 7 days every 2 weeks (7 days on, 7 days off) myelosuppression and thrombocytopenia were again dose limiting at 175 mg/m², and a daily dose of 150 mg/m² is recommended for further studies. A schedule of daily temozolomide for 21 days in each 28 days was explored, and a dose of 100 mg/m² recommended for phase II studies. In all trials, myelosuppression with neutropenia and thrombocytopenia is the dose-limiting side-effect. Continuous administration of temozolomide is associated with a high frequency of lymphocytopenia.

To date, there has been no comparative trial of the different schedules, and it is unclear whether a continuous administration schedule confers meaningful clinical advantage.

**Temozolomide for recurrent malignant glioma**

The initial trials of temozolomide were carried out by the UK Cancer Research Campaign. In 1993, the drug was licensed to the Schering-Plough Research Institute. Three pivotal trials led to the licensing of temozolomide in glioblastoma and anaplastic astrocytoma (Table 2). Two large single-group phase II trials were done in parallel for anaplastic astrocytoma and glioblastoma. The third trial was a randomised phase II trial for patients with recurrent glioblastoma. For the first

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**Table 1. Schedules and dosing of temozolomide**

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Dose mg/m²</th>
<th>Dose intensity mg/m²/week</th>
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<tr>
<td>Daily x 5 days, every 28 days</td>
<td>200</td>
<td>250</td>
<td>Approved dosing</td>
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<tr>
<td>Daily x 7 days, every 14 days</td>
<td>150</td>
<td>525</td>
<td>31</td>
</tr>
<tr>
<td>Daily x 21 days, every 28 days</td>
<td>100</td>
<td>525</td>
<td>32</td>
</tr>
<tr>
<td>Daily x 42 days, every 70 days</td>
<td>75</td>
<td>315</td>
<td>30</td>
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**Table 2. Pivotal trials in recurrent malignant glioma**

<table>
<thead>
<tr>
<th>Histology</th>
<th>Number of patients</th>
<th>Therapy</th>
<th>Response rate (%)</th>
<th>Progression rate (%)</th>
<th>Median survival (months)</th>
<th>PFS at 6 months (%)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBM</td>
<td>138</td>
<td>TMZ</td>
<td>8</td>
<td>47</td>
<td>5.4</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td>AA (ITT)</td>
<td>162</td>
<td>TMZ</td>
<td>35</td>
<td>38</td>
<td>13.6</td>
<td>46</td>
<td>33</td>
</tr>
<tr>
<td>GBM</td>
<td>112</td>
<td>TMZ</td>
<td>5</td>
<td>54</td>
<td>7.3</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>versus</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBM</td>
<td>113</td>
<td>PCZ</td>
<td>67</td>
<td>5.8</td>
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</tr>
</tbody>
</table>

GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; PFS, progression-free survival; ITT, intention-to-treat; TMZ, temozolomide; PCZ, procarazine
time, patients with malignant glioma were enrolled into separate trials for anaplastic astrocytoma (WHO grade III) or glioblastoma multiforme (WHO grade IV). Previous trials included both patients with grade III and grade IV tumours in a single trial, and results were commonly reported for both histological variants combined. Among patients with malignant glioma, 70–85% have grade IV tumours at diagnosis or at recurrence. All trials included a specifically developed quality-of-life module and the EORTC QOL30 questionnaire. Histology and magnetic resonance images were centrally reviewed. A minimum Karnofsky performance status of 70% was required for inclusion in these studies. The primary endpoint in all trials was 6-month progression-free survival. In brain tumours responses are rare and delayed, so progression-free survival may better reflect a meaningful clinical benefit than the usual response criteria.36 This unusual endpoint now requires prospective validation in randomised trials.

A total of 162 patients were included in the phase II trial for anaplastic astrocytoma.33 However, on central pathology review the histology could be confirmed in only 111 patients, indicating the difficulties and uncertainties of histopathological classification of lower-grade gliomas. Nevertheless, there was no significant difference in outcome between the eligible patients and an intention-to-treat analysis. Objective responses with independent radiological confirmation were observed in 35% of patients, with disease stabilisation in another 27%. At 6 months, 46% of patients were progression free, and the 1-year survival was 56%. Patients without progression at 6 months showed improved quality-of-life scores, which were maintained until about 4 weeks before disease progression.37

In glioblastoma, a phase II study showed an objective response rate of only 8%, but temporary disease stabilisation was achieved in 53% of patients, suggesting a potential clinical benefit in 61%. A second trial was designed as a randomised phase II trial conducted mainly in the USA. The 225 patients were assigned either procarbazine (125–150 mg/m² daily by mouth for 28 days, in cycles of 56 days) or temozolomide (150–200 mg/m² daily for 5 days, in cycles of 28 days). All patients had had recurrences after previous surgery, radiotherapy, or both. Substantial proportions of the groups (65% and 68%, respectively) had received chemotherapy previously. At 6 months, 21% of the temozolomide group were progression free, compared with 8% of patients assigned procarbazine; however, overall survival did not differ significantly between the groups. Subgroup analysis suggested a potential benefit mainly in chemotherapy-naive patients. Temozolomide was better tolerated than procarbazine, with a lower rate of serious adverse events. This benefit translated into improved quality-of-life scores for the responding or non-progressing patients.38

On the basis of these three phase II trials, temozolomide was approved for the treatment of malignant glioma. However, approval in the USA was granted for recurrent anaplastic astrocytoma only. The European Medicinal Evaluation Agency approved temozolomide for recurrent glioblastoma and anaplastic astrocytoma, whereas in Switzerland temozolomide is registered only for recurrent glioblastoma. This difference in interpretation of the data on temozolomide therapy reflects the remaining doubt on the validity of the progression-free endpoint and the absence of a true phase III trial. Temozolomide has never been compared with standard nitrosourea-based chemotherapy, such as procarbazine, lomustine, and vincristine. Nevertheless, in patients with recurrent high-grade glioma, the benefit in time to progression and possibly survival will be a few months at best. Efforts should therefore concentrate on integrating this agent into a multimodality treatment approach for malignant glioma. Another unresolved issue is treatment duration. In recurrent disease the protocol foresaw treatment until progression or for a maximum of 12 cycles. Subsequently, patients were allowed to continue therapy for up to 24 months. No cumulative toxic effects or late effects of exposure to the alkylating agent were observed, but the number of patients exposed to long-term therapy is very small.

**Temozolomide in newly diagnosed high-grade glioma**

The true value of new treatments or modalities is best explored in previously untreated patients. The activity of temozolomide shown in patients with recurrent anaplastic astrocytoma and glioblastoma together with the strong in vitro activity against several glioma cell lines and central-
nervous-system tumour xenografts are the basis for evaluating this chemotherapy in newly diagnosed patients. Temozolomide chemotherapy may be given as neoadjuvant treatment before definitive radiotherapy, concomitantly with radiotherapy, or as adjuvant therapy after completion of radiotherapy (Figure 3).

Neoadjuvant therapy was explored by Friedman and colleagues in 33 patients with glioblastomas and five with anaplastic astrocytomas (Table 3). All patients had histologically confirmed high-grade glioma, with the last surgical intervention 14–28 days before the start of therapy. All patients had to have residual disease measurable on magnetic resonance imaging to be eligible for the trial. About two-thirds of patients had undergone subtotal surgical resection, and the others had undergone biopsy only. Treatment consisted of up to four cycles of temozolomide (200 mg/m² daily for 5 days) every 4 weeks. Magnetic resonance imaging was repeated before each cycle. Treatment continued in the absence of progressive disease. An objective response was shown in 17 of 33 patients with glioblastomas, including three complete remissions. The response lasted for at least 4 months in 13 patients, and four patients showed progression during the neoadjuvant treatment. Disease stabilisation was achieved in four patients. Primary tumour progression occurred in 12 patients. The median survival for the responding patients was 12 months, and six patients showed stable disease for 12 months.

Courses of neoadjuvant therapy. Response to neoadjuvant chemotherapy may identify patients who might benefit from more intensive treatment with concomitant chemotherapy and radiotherapy or additional adjuvant chemotherapy. Only a randomised trial will be able to show the real impact and benefit from such a strategy.

**Temozolomide and radiation**

Synergy may be achieved when temozolomide chemotherapy and radiotherapy are given concomitantly. Additive growth inhibition was shown in vitro when the glioblastoma cell line U87MG, with low AGT expression, was incubated with temozolomide and treated with a single fraction of radiotherapy. By contrast, the colorectal Mawi cell line, which has high AGT expression, showed additive growth inhibition only after inhibition of AGT with O6-benzylguanine. Fractionated radiotherapy and concomitant treatment with temozolomide was explored by van Rijn and colleagues in two glioma cell lines. Increased cytotoxicity was shown in the D384 glioma cell line, with reduced cell survival and disappearance of the usual dose-effect relation. In the similar U251 cell line, however, no increase in cytotoxicity could be shown. There is no apparent explanation for this difference and further investigations are required.

The suggestion of increased cytotoxicity together with the known activity of temozolomide in recurrent and newly diagnosed high-grade glioma are the basis for introducing this agent in newly diagnosed patients at the same time as radiotherapy. All criteria for combining chemotherapy and radiation, as previously established, are met: temozolomide may eliminate microscopic disease outside the radiation field (spatial cooperation); temozolomide increases the radiation effects (radiosensitisation); the modalities have different toxicity profiles (toxicity independence); and temozolomide may be selectively active on glioma cells with

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**Table 3. Novel treatments with temozolomide for primary brain tumours**

<table>
<thead>
<tr>
<th>Investigator Year</th>
<th>Ref</th>
<th>Histology</th>
<th>N</th>
<th>Treatment</th>
<th>CR+PR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>PFS</th>
<th>OS</th>
<th>Remarks</th>
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</thead>
<tbody>
<tr>
<td>Friedman 1998</td>
<td>5</td>
<td>GBM</td>
<td>33</td>
<td>Neoadjuvant TMZ x 5</td>
<td>52</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>12 months for responding patients</td>
</tr>
<tr>
<td>Gilbert 2000</td>
<td>39</td>
<td>GBM</td>
<td>33</td>
<td>Neoadjuvant TMZ x 5</td>
<td>39</td>
<td>3</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>Continuous daily administration during radiotherapy</td>
</tr>
<tr>
<td>Stupp 2000</td>
<td>43</td>
<td>GBM</td>
<td>18</td>
<td>Newly diagnosed: XRT + TMZ</td>
<td>39</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>1 year: 66%</td>
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<tr>
<td>Newlands Unpubl</td>
<td></td>
<td>GBM</td>
<td>45</td>
<td>Newly diagnosed: XRT + TMZ</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Newlands Unpubl</td>
<td></td>
<td>Oligo II</td>
<td>7</td>
<td>Recurrent: TMZ + PCZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chinot 2001</td>
<td>59</td>
<td>AOD</td>
<td>48</td>
<td>Recurrent: TMZ x 5</td>
<td>443</td>
<td>8</td>
<td>13</td>
<td>19</td>
<td>12 months: 25%</td>
<td>46%</td>
<td>Recurrent after prior XRT and PCV</td>
</tr>
<tr>
<td>Brada 2000</td>
<td>65</td>
<td>Astro II</td>
<td>13</td>
<td>Neoadjuvant: TMZ x 5</td>
<td>MR 85</td>
<td></td>
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</table>

GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; AOD, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; astro II, astrocytoma WHO grade II; oligo II, oligodendroglioma WHO grade II; TMZ, temozolomide; PCZ, procarbazine; XRT, radiotherapy; MR, minor response; CR, complete response; PR, partial response; PFS, progression-free survival; OS, overall survival; SD, stable disease; PVC, procarbazine, lomustine and vincristine.
low amounts of AGT (protection of normal tissues).

We have investigated the concomitant administration of temozolomide and standard fractionated radiotherapy in a prospective phase II trial. Temozolomide (75 mg/m²) was given daily (7 days per week) for 6–7 weeks during the entire period of radiotherapy, which was administered once daily (Monday to Friday) at 2 Gy per fraction for a total dose of 60 Gy. After completion of therapy, patients were scheduled to receive six additional cycles of adjuvant temozolomide (200 mg/m² daily for 5 days). Results on the first 45 patients treated showed that standard radiotherapy can be added to daily temozolomide at the previously established dose of 75 mg/m². All but four patients were able to complete the prescribed dose of radiation, and temozolomide was discontinued early in two patients owing to thrombocytopenia. However, two patients developed Pneumocystis carinii pneumonia, perhaps due to the frequent use of corticosteroids in these patients and the lymphocytopenia caused by continuous temozolomide administration. This treatment is being tested in a randomised European Organisation for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) trial comparing the combination of temozolomide and radiotherapy with radiotherapy alone in newly diagnosed patients with glioblastomas (Figure 4).

Temozolomide in combination

**Temozolomide and nitrosoureas**

Chloroethylnitrosoureas and temozolomide alkylate DNA at the O6 position of guanine. AGT, which can reverse the alkylation, is inactivated in this process, and its depletion can reverse the drug resistance. Similarly, O6-benzylguanine, a non-cytotoxic substrate of AGT, will deplete the AGT pool. Previous therapy with O6-benzylguanine sensitises tumour cells to alkylation by temozolomide or chloroethyl-nitrosoureas. However, AGT depletion will occur in all tissues, and systemic toxicity may necessitate dose reduction of nitrosourea or temozolomide.

On the basis of the observed progressive and cumulative depletion of AGT after a daily schedule of temozolomide, we have explored the sequential combination of temozolomide and the chloroethyl nitrosourea fotemustine in a phase I trial. Temozolomide was given in two daily doses, followed by fotemustine (100 mg/m²) 4 hours after the second dose. At doses of 400 mg/m² or more of temozolomide (200 mg/m² on days 1 and 2) myelosuppression with grade 4 leucocytopenia and thrombocytopenia was dose limiting. AGT depletion in peripheral-blood lymphocytes was not related to the dose of temozolomide. In our current protocol we give a low dose of temozolomide (100 mg/m² on days 1 and 2) before fotemustine (100 mg/m²) on day 2.

A dose of 100 mg/m² O6-benzylguanine has been shown to suppress AGT activity effectively in malignant glioma. In a phase I trial in malignant glioma O6-benzylguanine was combined with carmustine. As predicted, dose-limiting toxicity (myelosuppression) was reached at far lower doses of carmustine than after administration of that drug alone. Similarly, temozolomide has been combined with carmustine in a randomised phase I trial evaluating the sequence dependence of the administration of these two drugs. This trial suggested greater toxicity when temozolomide preceded carmustine. A similar trial established a dose of 150 mg/m² carmustine on day 1 followed by 100 mg/m² temozolomide on days 1–5, but only 80 mg/m² temozolomide on days 1–5, when followed by 100 mg/m² carmustine on day 5, suggesting greater AGT depletion with previous exposure to 5 days of temozolomide. Again, temozolomide doses that can be given in combination with carmustine are well below established single-agent doses. Temozolomide has been combined with thioguanine, and a dose of 40 mg...
Thioguanine 6 hours before and after temozolomide intake combined with 150 mg/m 2 temozolomide for 5 days is considered safe. However, no data on AGT depletion are available. Trials combining O6-benzylguanine and temozolomide are underway.

In all trials, the doses of the nitrosourea or temozolomide, or both, had to be substantially lower than those for single-agent therapy. No data on efficacy with these lower doses are available, and the possibility that such a combination will lead to clinically meaningful increased activity remains doubtful.

**Temozolomide and procarbazine**

A phase I trial combining temozolomide and procarbazine is in progress on the basis that both these drugs inhibit AGT and have activity against malignant gliomas. The trial is designed to use procarbazine as an AGT inhibitor given 1 hour before temozolomide, with both drugs given daily for 5 days in cycles of 28 days. Temozolomide has been given in the standard dose of 200 mg/m 2, then combined in subsequent courses with procarbazine and given in escalating doses (50, 75, then 100 mg/m 2). This trial is continuing and is near to the dose-limiting toxic effect of thrombocytopenia. The combination has been well tolerated, and responses have been seen in patients with both low-grade and high-grade gliomas (Newlands and colleagues, unpublished).

**Temozolomide and irinotecan**

Irinotecan is also active against malignant glioma. 58 Patients with recurrent glioma after radiotherapy (53) and/or chemotherapy (41) were treated with irinotecan (125 mg/m 2 weekly for 4 weeks repeated after 6 weeks). An overall response rate of 15% was reported, with a further 55% of patients having stable disease for longer than 12 weeks. Owing to enzyme induction by concomitant antiepileptic treatment, the AUC of irinotecan and its active metabolites was up to 40% lower than previously reported in patients with colorectal cancer. 59 60 In a subsequent phase I study, the dose of irinotecan could be increased up to 1200 mg/m 2 every 3 weeks, compared with the standard dose of 350 mg/m 2 every 3 weeks in patients with colorectal cancer. 52 Studies combining these two active agents with only partially overlapping toxicity need to be done. In vitro studies suggest a schedule-dependent synergy between the two agents with an increased effect of irinotecan after exposure to temozolomide. 61 Patients requiring antiepileptic therapy should be considered for treatment with one of the newer non-enzyme-inducing antiepileptic drugs. 64

**Anaplastic oligodendroglioma and oligoastrocytoma**

Anaplastic oligodendroglioma and mixed oligoastrocytoma are very chemoresponsive, with response rates of 60–75% to combination treatment with procarbazine, lomustine, and vincristine. 65 66 Patients who had previously received radiotherapy were as likely to respond as those who had not. Specific chromosomal aberrations on chromosomes 1p and 19q were found to be predictive of chemoresponsiveness. 67 Neoadjuvant therapy with this combination of drugs in anaplastic oligodendroglioma and oligoastrocytoma has been explored. 68

Temozolomide has shown activity as second-line chemotherapy in patients previously treated by radiotherapy and procarbazine, lomustine, and vincristine chemotherapy. 69 Patients (median age 42 years) with anaplastic oligodendroglioma (38) and oligoastrocytoma (nine) were treated with 150–200 mg temozolomide daily for 5 days. Complete responses were seen in seven, and partial regression in 13 patients, for an overall response rate of 43%. Only eight patients (17%) progressed initially. At 12 months, 34% of patients were progression free. These data suggest that there is no major cross-resistance between the combination regimen and temozolomide. A confirmatory phase II trial is continuing. The EORTC is also evaluating first-line chemotherapy with temozolomide in patients with recurrent oligoastrocytoma and anaplastic oligodendroglioma after radiotherapy.

**Temozolomide for low-grade glioma**

Treatment of low-grade glioma remains controversial. 70 71 Radiotherapy is commonly given and may prolong time to progression. Prospective and retrospective analyses have shown, however, that deferred radiotherapy does not compromise overall survival. 72 73 Radiotherapy is associated with a significant risk of late neurological sequelae. In a retrospective analysis, radiation necrosis was reported in nine of 62 patients, and delayed cognitive impairment (diagnosed without prospective neuropsychometric testing) in 13. 74 These findings suggest that at a median observation time of 6 years, over a third of the patients had clinically significant sequelae from the previous radiotherapy. Patients with low-grade astrocytoma, and in particular the more chemo-sensitive oligodendroglioma and mixed oligoastrocytoma, have median survival of longer than 10 years.

Primary chemotherapy with temozolomide may be an alternative to immediate radiotherapy. 75 In particular, the novel continuous exposure schedules have some theoretical advantages in the treatment of slowly proliferating diseases. Preliminary results of a continuing trial for newly diagnosed patients with low-grade glioma were recently reported. 76 Patients with grade II astrocytoma or oligodendroglioma were treated with the daily 5-day schedule for 6–12 months. Minor responses were seen in 11 of the 13 patients with astrocytoma, and three of the seven with oligodendroglioma had partial remissions and one a minor response. No patient progressed. Investigators at Duke University, NC, USA, reported on 16 evaluable patients with progressive, low-grade glioma treated with the intermittent schedule of temozolomide. 44 Disease stabilisation was observed in 13 patients, and three patients progressed. A large randomised trial is needed to assess the true value of this approach and to assess the price in terms of toxicity of primary chemotherapy in low-grade disease. Late myelodysplastic syndrome and second malignant disorders are of concern after chronic exposure to an alkylating agent. 87 88

**Temozolomide for other brain tumours and metastases**

Primary temozolomide chemotherapy or chemoradiotherapy for metastatic brain tumours is currently under investigation. This approach may be of particular interest in diseases for
which temozolomide or dacarbazine have shown activity outside the brain. Responses to temozolomide have been reported for patients with non-small-cell lung cancer and primary central-nervous-system lymphoma; disease stabilisation has been reported for metastatic melanoma in a small randomised trial of only 45 evaluable patients with brain metastases (mainly from lung cancer), an increased response rate was observed when standard-dose temozolomide was combined with radiotherapy (2 Gy per fraction, 40 Gy total) compared with radiotherapy alone (96% versus 66%), but no difference in overall survival. Time to local failure has not been reported.

Conclusions
Temozolomide has some, generally modest, activity against high-grade glioma and has shown activity in relapsed oligodendroglioma after nitrosourea chemotherapy. However, no formal comparative trial against nitrosourea-based chemotherapy has been done. To define the true role of the drug in the treatment of brain tumours, further exploration within well-designed and controlled clinical trials is necessary. Assessment of the activity of new drugs in brain tumours is a difficult task, because measurement of response rates according to conventional criteria may not reflect the real clinical benefit. New endpoints, such as progression-free survival at 6 months, require prospective validation and should be supported by quality-of-life analysis.

References
Temozolomide for brain tumours

Review

Alkyltransferase in conferring resistance to temozolomide but not to 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 1996; 56: 5375–79.


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