

# Temozolomide in the Treatment of Aggressive Pituitary Tumours – An Overview of Existing Knowledge and Future Perspectives

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### Abstract

The management of aggressive pituitary tumours remains a challenge, however, the recent identification of temozolomide as a chemotherapeutic agent with significant efficacy against these tumours has heralded a new therapeutic era. There has been an exponential growth in the international experience with temozolomide over the past five years, now totalling 50 published cases. Overall, 67 % of cases demonstrated a response to temozolomide. Prolactin- and adrenocorticotrophic hormone (ACTH)-secreting tumours respond more frequently than non-functioning tumours. Response is typically evident in the first three months of treatment. Adverse effects occur in almost half of patients, although the majority are mild. The expression of a DNA repair enzyme, O6-methylguanine-DNA methyltransferase (MGMT), as determined by immunohistochemistry, appears to be the primary determinant of response to temozolomide in pituitary tumours. There is suggestion that MGMT may also play a role in pituitary tumorigenesis. Over the next few years we will see temozolomide used earlier in the treatment algorithm of aggressive pituitary tumours, making it imperative to collect global long-term data on its use.

### Keywords

Pituitary tumour, pituitary carcinoma, temozolomide, MGMT

**Disclosure:** The author has no conflicts of interest to declare.

**Received:** 2 July 2012 **Accepted:** 21 August 2012 **Citation:** *European Endocrinology*, 2012;8(2):116–21 DOI:10.17925/EE.2012.08.02.116

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Pituitary tumours are common, with an estimated prevalence of 16.7 % in the general population based on imaging and autopsy studies.<sup>1</sup> Clinically significant pituitary tumours are more prevalent than previously recognised, with one case per 1,000–1,300 people.<sup>2,3</sup> The majority of pituitary tumours are indolent, slow-growing neoplasms. However, 40–50 % of pituitary tumours are locally invasive and commonly unable to be completely surgically excised.<sup>4,5</sup> An 'aggressive pituitary tumour' typically refers to an invasive pituitary tumour that demonstrates progressive growth despite multimodal therapy, including surgery and radiotherapy. Whilst these tumours have malignant potential, the term pituitary carcinoma is strictly reserved for those tumours with demonstrated craniospinal or systemic metastases.<sup>6</sup> As there is a lack of formal criterion used to define an aggressive pituitary tumour, epidemiological data with respect to this group is lacking. The World Health Organization pathological classification of an 'atypical pituitary adenoma' (Ki67 >3 %, excessive p53 immunoreactivity and increased mitotic activity) was coined in an attempt to identify a tumour with the potential for more aggressive behaviour.<sup>7</sup> A recent study, conducted in a tertiary referral centre, identified 15 % of atypical adenomas amongst their surgical cohort.<sup>8</sup> Pituitary carcinoma is rare, accounting for 0.2 % of pituitary tumours.<sup>9</sup>

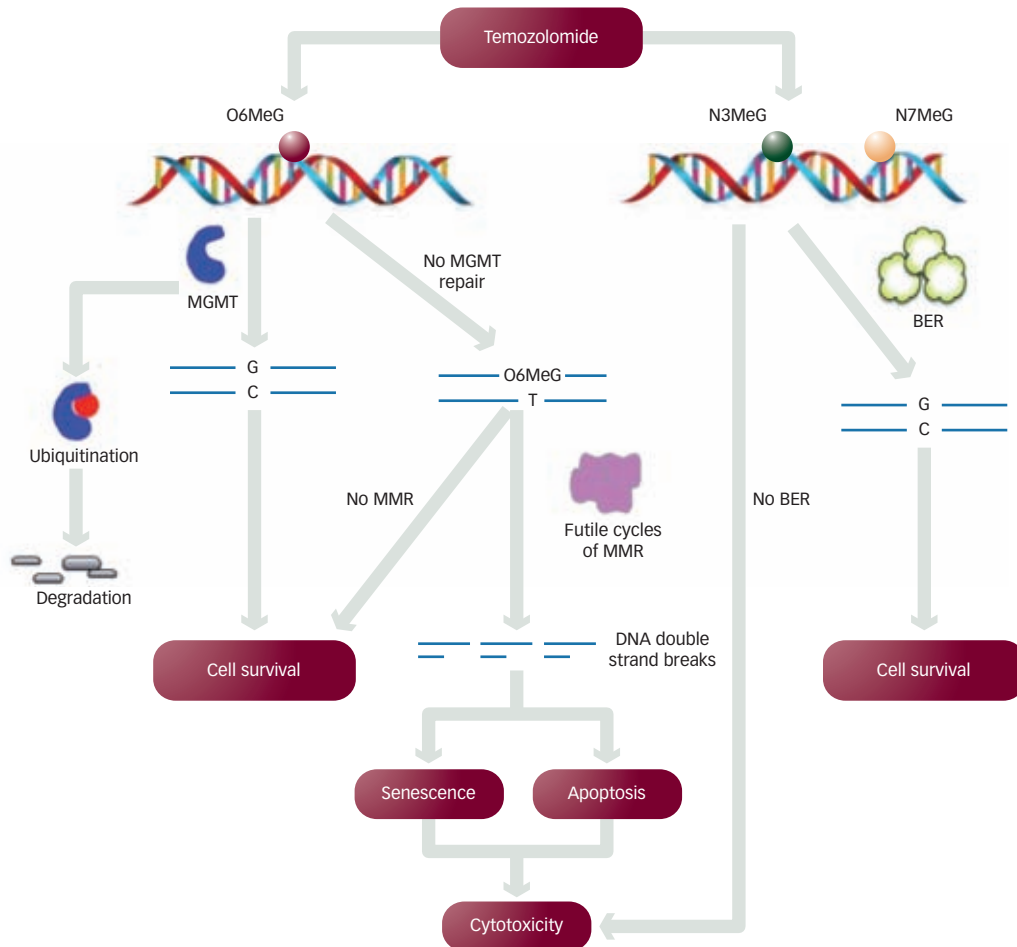
The management of aggressive pituitary tumours is challenging, and there is substantial morbidity and mortality associated with both the tumour and treatment. Patients often undergo multiple surgeries and radiotherapy in an attempt to control tumour growth.

These tumours are often also resistant to medical therapies, such as dopamine agonists. Historically, systemic chemotherapy was reserved as a 'last resort' therapy, principally due to the lack of identification of a consistently effective chemotherapeutic agent. However, over the past five years temozolomide, an oral alkylating agent commonly used in the management of glioblastoma, has emerged as the first chemotherapeutic with substantial efficacy in the treatment of aggressive pituitary tumours. International experience with temozolomide, as used in the management of an aggressive pituitary tumour, has grown exponentially, with 50 published cases now reported. This review will summarise and examine the existing knowledge base, but also speculate on the future possibilities regarding the use of temozolomide in pituitary tumours. The role of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, as a biomarker of response to temozolomide and its possible role in pituitary tumour biology will be discussed.

### Temozolomide and DNA Repair Systems

Temozolomide is an oral second-generation alkylating agent of the imidotetrazine class.<sup>10</sup> The lipophilic nature of the temozolomide molecule promotes efficient passage across the blood-brain barrier, advantageous when treating pituitary tumours. Conversion of temozolomide to a highly reactive methyl diazonium ion is responsible for the formation of methyl adducts with DNA purinic bases.<sup>11</sup> *Figure 1* illustrates the mechanism of temozolomide cytotoxicity.

**Figure 1: Mechanism of Temozolomide Cytotoxicity**



O6MeG = O6-methylated guanine; BER = base excision repair; MGMT = O6-methylguanine-DNA methyltransferase; MMR = mismatch repair; N3MeG = N3-methylguanine; N7MeG = N7-methylguanine.

The critical methyl adduct produced by temozolomide is the lesion at O6-guanine (O6MeG). O6MeG adducts account for only 5 % of alkylating lesions induced by temozolomide but deliver the greatest cytotoxicity. Other alkylating lesions induced by temozolomide are N3-methylguanine (N3MeG) and N7-methylguanine (N7MeG). In the presence of the base excision repair (BER) pathway, N3MeG and N7MeG lesions are repaired. MGMT is responsible for repairing O6MeG lesions. In the absence of MGMT, methylated guanine lesions incorrectly pair with thymine, triggering the mismatch repair (MMR) pathway. In the presence of MMR activation there is incorrect reinsertion of thymine opposite the O6MeG lesion resulting in futile cycles of repair and ultimately DNA strand breaks. This is followed by either activation of the apoptotic cascade or a senescence-like state.<sup>11,12</sup>

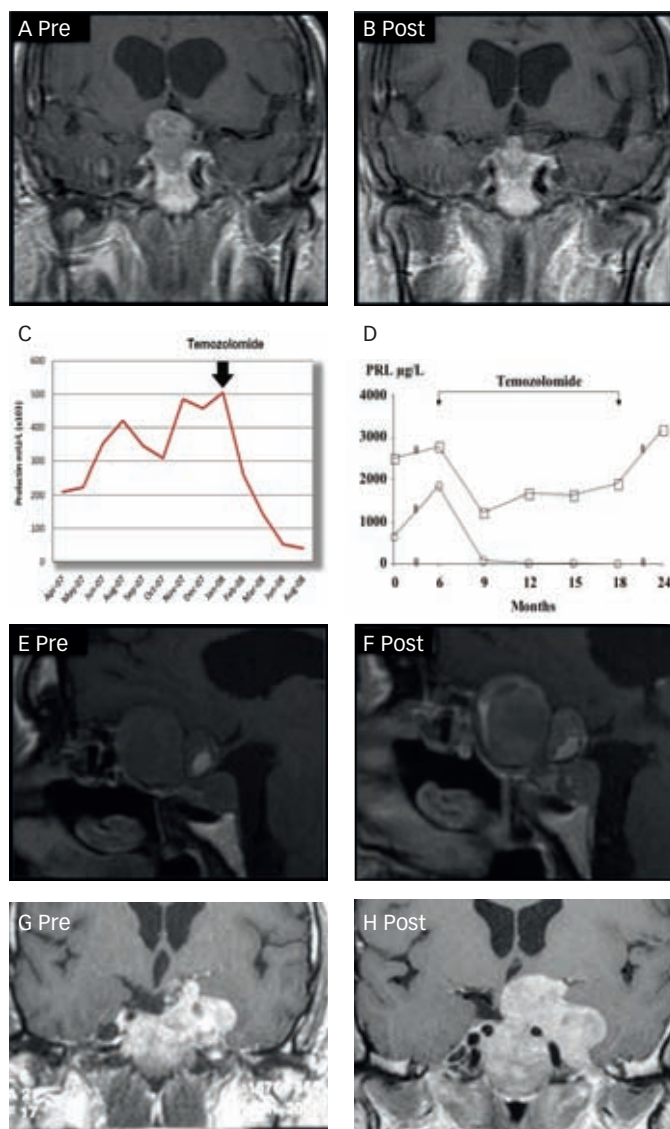
The standard temozolomide dosing regime is 200 mg/m<sup>2</sup> given daily for five days every 28 days.<sup>13</sup> Common alternative regimes include ‘dose-dense’ protocols, such as 150 mg/m<sup>2</sup> days 1–7 and days 14–21 of a 28 day cycle, and ‘metronomic’ protocols using continuous daily low-dose (50–75 mg/m<sup>2</sup>) temozolomide. These alternative dosing schedules have been reported to be effective in patients with progressive or recurrent gliomas, although this has been recently disputed.<sup>10,14</sup> Temozolomide is standard of care in the management of glioblastoma and advanced melanoma, and has demonstrated efficacy for malignant neuroendocrine tumours.<sup>15,16</sup> In glioblastoma, temozolomide monotherapy is typically administered for six months

following six weeks of concurrent radiotherapy and low-dose temozolomide.<sup>15</sup> However, improved survival rates have been reported with use of temozolomide for 12–24 months, and there are cases of sustained remissions with continuous treatment out to eight years.<sup>17,18</sup> Temozolomide is generally well tolerated as compared with most chemotherapeutic agents. Adverse effects are commonly mild and include nausea, vomiting and fatigue. Myelosuppression is also common and dose-limiting, particularly lymphopenia and thrombocytopenia, however, severe myelosuppression leading to discontinuation of temozolomide is rare. Dose-dense regimes have been associated with an increased myelotoxicity and risk of opportunistic infection.<sup>10</sup> Rare reports of myelodysplastic syndrome, aplastic anaemia and lymphoma have been described following temozolomide therapy.<sup>19–21</sup> Other rare adverse events reported include hypersensitivity pneumonitis and hearing loss.<sup>22,23</sup>

### Collective Experience with Temozolomide in Pituitary Tumours

The first reports describing the successful use of temozolomide in pituitary tumours were published in 2006.<sup>24,25</sup> These early cases were pituitary carcinomas, but it was not long before significant responses were also reported for locally aggressive pituitary adenomas.<sup>26–30</sup> There are now 50 published reports, both carcinomas and aggressive adenomas, in which temozolomide has been used. More recent case series, each including 6–8 cases regardless of outcome, have

**Figure 2: Clinical Spectrum of Temozolomide Response**



(a) to (c) show a case example of an excellent and sustained response: 42 year old male with malignant prolactinoma.<sup>51</sup> (a) and (b) shows radiological images prior to and after 15 cycles of temozolomide. (c) shows a decline in prolactin levels after temozolomide therapy. (d) to (f) show a case example of a stable response: 62 year old male with aggressive prolactinoma;<sup>33</sup> (d) shows a square line graph demonstrating initial hormonal response with subsequent plateau as compared with another case illustrating complete hormonal response. (e) and (f) show radiological images prior to and after 12 cycles of temozolomide. (g) and (h) show a case example of progressive disease: 41 year old male with silent adenocorticotrophic hormone-secreting tumour;<sup>41</sup> radiological images prior to and after eight months of temozolomide therapy.

contributed to correcting the initial positive publication bias.<sup>31–33</sup> The clinical spectrum of response to temozolomide is now recognised; whilst the majority demonstrate an excellent and sustained response, stable or transient responses and occasional cases with clear progressive disease are also seen. *Figure 2* illustrates this clinical spectrum utilising cases drawn from the literature.

## Patient Characteristics

*Table 1* summarises the clinical and pathological characteristics of the published case cohort. Temozolomide has been most commonly used for prolactin (PRL)-secreting (19 cases) and ACTH-secreting (19 cases) carcinomas and aggressive adenomas, reflecting the predominance of these tumour types amongst cases of aggressive pituitary tumours. There are 10 non-functioning (NF) pituitary tumours in the cohort and

**Table 1: Published Cases – Clinical and Pathological Characteristics**

Tumour Subtype	PRL		ACTH		NF		GH		TOTAL	
Total	19	19	10	2	50					
Carcinoma/Adenoma	Ca	Ad	Ca	Ad	Ca	Ad	Ca	Ad	Ca	Ad
Ki67* (average)	8	11	10	9	3	7	0	2	21	29
Number of surgeries (average)	0–60 % (14.3 %)		0.5–31 % (10.7 %)		1–20 % (7.6 %)		4 %		0–60 % (11.2 %)	
	1–6 (2.7)		1–5 (2.6) <sup>#</sup>		0–6 (2.4) <sup>#</sup>		2–6 (4)		0–6 (2.7)	

ACTH = adrenocorticotrophic hormone-secreting; GH = growth hormone-secreting; NF = non-functioning; PRL = prolactin-secreting. \*Ki67 reported for: 14 PRL, 14 ACTH, 8 NF and 1 GH tumours. <sup>#</sup>Not reported in one case.

only two growth hormone (GH)-secreting tumours. Male patients with aggressive pituitary tumours form the majority of reported cases (29/42). The average patient age is 46.4 years, range 13–72. Whilst the range of measured Ki67 indices varies widely between cases, the aggressive nature of the tumours comprising this cohort is reflected in the high average Ki67 of 11.2 %. In the vast majority of cases, treatment with temozolomide is used as salvage therapy. Patients have typically undergone multimodal therapy, often including several surgeries and one or more courses of radiotherapy over several years. Tumours also display resistance to medical therapy with dopamine agonists and somatostatin analogues, although these agents are often continued whilst the patient is receiving temozolomide.

Perhaps reflecting the mounting international experience and encouraging evidence of efficacy is the recent report by Whitelaw et al. of use of temozolomide in a non-salvage setting. They successfully used temozolomide as a means of deferring radiotherapy in a paediatric patient and facilitated subsequent radiotherapy in another patient with chiasmal compression by effecting tumour shrinkage.<sup>34</sup>

## Temozolomide Treatment Regimes

Patients are typically chemotherapy-naïve when temozolomide treatment is commenced. On the whole, temozolomide has been given as monotherapy. Significant side effects were encountered when temozolomide was commenced in combination with interferon or thalidomide,<sup>25,32</sup> however, in another two cases it was well tolerated when used with capecitabine or pasireotide.<sup>35,36</sup> In two cases demonstrating progressive disease whilst on temozolomide therapy alone, alternative chemotherapeutic agents have been added with no additional benefit; carmustine in one case and carboplatin in another case.<sup>31</sup> The standard temozolomide-dosing regime of 150–200 mg/m<sup>2</sup> /day for five days over 28 days is most commonly used. In the case series reported by Bush et al., a dose-dense regime was employed (75 mg/m<sup>2</sup> /day for 21 days with seven days off). The most variable aspect in the temozolomide treatment regime between cases is therapy duration. More commonly, patients receive a prescribed course of 6–12 months of temozolomide. Early progressive disease has necessitated cessation as early as two or three months, whilst other cases demonstrating good response continue on temozolomide beyond 24 months and in one patient five years.<sup>27,31,33,37</sup>

## Adverse Effects

Temozolomide-related adverse effects have been reported in 22 of the 50 cases. Nausea, fatigue, headaches and thrombocytopenia are

most frequently described and typically mild. However, fatigue contributed to a decision to cease treatment in three cases. Dose reduction or extension in dosing interval has permitted continuation of temozolomide in patients developing mild myelosuppression. Severe adverse events necessitating temozolomide cessation have occurred in two cases: in one patient agranulocytosis and the other sensorineural hearing loss.

**Response to Temozolomide**

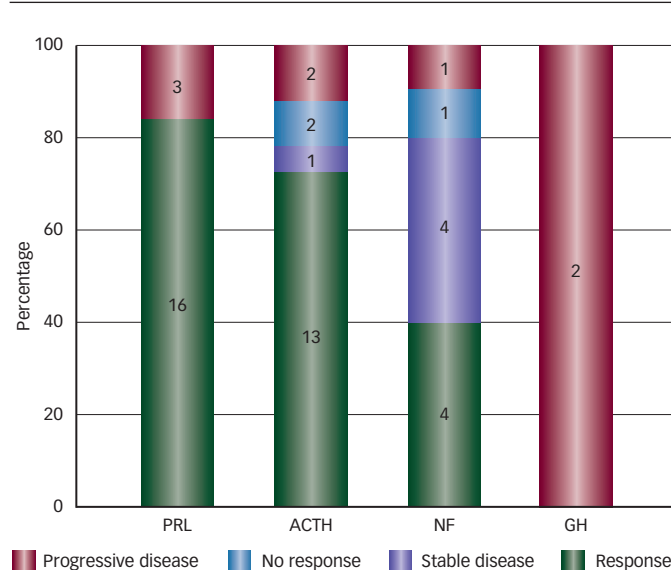
Overall, 33 of 49 patients (67 %) have demonstrated a response to temozolomide therapy. Both adenomas and carcinomas may show response. *Figure 3* illustrates temozolomide response by pituitary tumour subtype. PRL and ACTH tumours had a higher response rate (84 % and 72 %, respectively) than NF tumours (40 %), although a significant proportion of NF tumours demonstrated stable disease (40 %). Invariably a clinical response predicted a subsequent radiological tumoural response, and in functioning tumours, a hormonal response as well. In just one case, a hormonal response was not accompanied by any clinical improvement or tumour shrinkage.<sup>33</sup> When it occurs, response is typically seen within the first three months, leading some to propose a three-month treatment trial.<sup>31</sup> However, maximal response is often only seen after 10–12 cycles of treatment.<sup>30,33</sup> In addition, stable disease may be a valuable outcome; there is a low rate (16 %) of reported tumour progression during temozolomide treatment.

The application of traditional RECIST (Response Evaluation Criteria In Solid Tumours) radiological criteria to report degree of tumour shrinkage (partial response >30 % decrease in tumour diameter; complete response disappearance of tumour) may significantly underestimate the degree of tumour shrinkage observed in many responders. Most pituitary tumours that respond to temozolomide show a >50 % tumour shrinkage, in some cases >80 %, and in three cases complete response was seen in metastatic lesions.<sup>30–33,35,37</sup> Similarly, hormonal responses can be remarkable, often occurring earlier and to a greater degree than radiological responses, and with more than half of cases demonstrating biochemical normalisation. Hormonal response may translate into significant clinical benefits most obviously for ACTH or GH tumours.

Another notable feature seen in a number of pituitary tumours is a prolonged and sustained response following temozolomide cessation, with no tumour regrowth for as long as three years.<sup>24,30,33</sup> Morphological change in the tumour following response has been described in one case, with evidence of increased differentiation, may contribute to this phenomenon.<sup>38</sup> However, eventual tumour regrowth is increasingly described.<sup>33,39,40</sup> Unfortunately, a second course of temozolomide in the setting of tumour regrowth has to date not been effective<sup>40</sup> (and personal communication with William Drake and Richard Ross). It is possible that a subset of tumour cells originally resistant to temozolomide explain eventual tumour regrowth. Alternatively, the development of additional acquired genetic alterations in the tumour may drive temozolomide resistance. Murakami et al. recently eloquently described transformation of an atypical prolactinoma into a pituitary carcinoma in association with acquired temozolomide resistance and the loss of MutS Homolog 6 (MSH6) (a MMR protein).

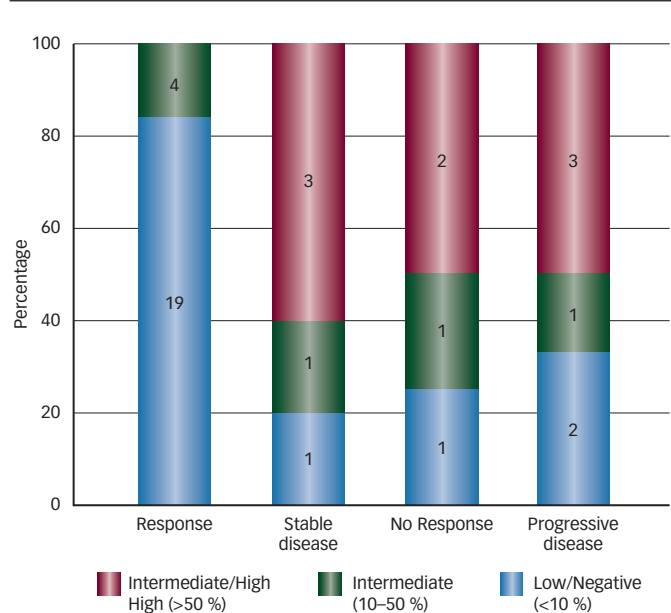
In patients with non-response or tumour progression, various alternative treatments have been tried. Trials of alternative chemotherapeutic agents have been largely unsuccessful.<sup>33,35,40</sup> Bevacizumab (vascular

**Figure 3: Temozolomide Response by Pituitary Tumour Subtype**



PRL = prolactin-secreting tumour; ACTH = adrenocorticotrophic hormone-secreting tumour; NF = non-functioning tumour; GH = growth hormone-secreting tumour.

**Figure 4: Temozolomide Response and MGMT Expression by Immunohistochemistry**



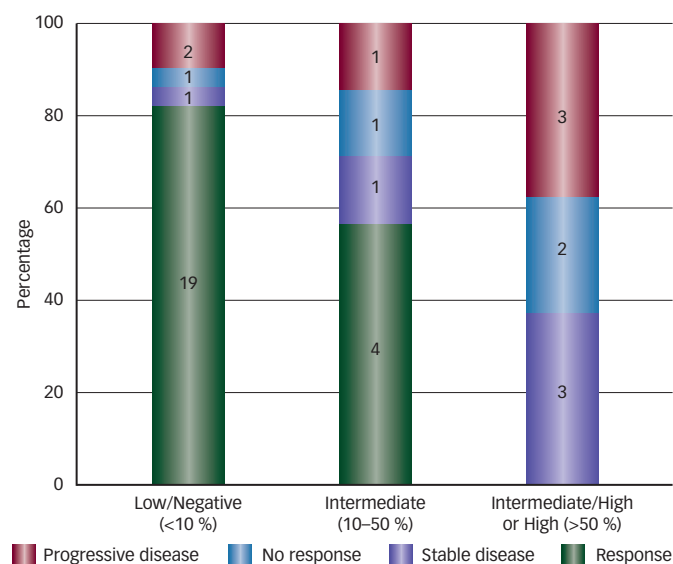
MGMT = 06-methylguanine-DNA methyltransferase.

endothelial growth factor [VEGF] inhibitor) has been used successfully in one case,<sup>41</sup> whilst pasireotide has also been trialed.<sup>33</sup>

**MGMT Status and Response to Temozolomide Therapy in Pituitary Tumours**

MGMT is an evolutionarily conserved gene, responsible for repairing 06MeG cytotoxic adducts that may arise as a result of endogenous DNA damage, or exogenously via ionising radiation or chemotherapeutic agents such as temozolomide.<sup>11,42</sup> Low MGMT expression and temozolomide efficacy has been repeatedly demonstrated in brain tumours, but also seen in melanoma and neuroendocrine tumours.<sup>43–47</sup> MGMT expression as assessed by immunohistochemistry has been studied in 38 of the published pituitary cases. Response to temozolomide has only been described in association with low or

**Figure 5: MGMT Expression by Immunohistochemistry and Temozolomide Response**



MGMT = 06-methylguanine-DNA methyltransferase.

negative MGMT expression (83 %) or intermediate expression (17 %) (Figure 4). However, stable or progressive disease occurred in association with a wide range of MGMT expression (Figure 4). As illustrated in Figure 5, low or negative MGMT expression predicts a high chance of response (83 %), although there were two cases with low expression demonstrating clearly progressive disease. In addition, response to temozolomide has not been described in association with >50 % MGMT expression. These results strongly suggest MGMT expression is the primary determinant of response, although in some instances other resistance mechanisms clearly operate. In their recent review, Whitelaw et al., found that pituitary tumours with low or negative MGMT expression were 9.35 times more likely to respond to temozolomide than tumours with intermediate or high MGMT expression.<sup>34</sup>

The presence of MGMT promoter methylation has been linked with response to temozolomide in glioblastomas.<sup>44,45,48</sup> In fact, MGMT immunohistochemistry may be unreliable in glioblastomas because of difficulty in distinguishing MGMT expression in neoplastic from non-neoplastic inflammatory cells, the latter extensively found in glioma specimens.<sup>49</sup> However, MGMT methylation status does not correlate with temozolomide response in pituitary tumours. MGMT methylation analysis has been reported in 20 cases: three of five methylated tumours and nine of 15 unmethylated tumours were associated with treatment response.<sup>50</sup> Progressive disease has also been seen in cases demonstrating promoter methylation. Furthermore, MGMT promoter methylation is not the predominant mechanism causing loss of MGMT expression in pituitary tumours: of 10 cases with low MGMT expression by immunohistochemistry only 4 were found to be methylated.<sup>50</sup> The mechanism responsible for loss of MGMT expression in pituitary tumours remains unknown. MGMT gene mutations have not been detected in pituitary tumours.<sup>51</sup> Loss of heterozygosity at 10q26, the region containing the MGMT gene, has also not been identified in a small number of low MGMT-expressing pituitary tumours.<sup>51</sup>

## MGMT and Pituitary Tumour Biology

The observation of a pituitary tumour subtype difference in response to temozolomide, as well as differential expression of MGMT across pituitary

tumours, have led to examination of MGMT expression in larger archived cohorts of unselected pituitary tumours. Overall in one such cohort, 13 % of pituitary tumours were found to have low MGMT expression (defined as <10 %), and 28 % and 59 % with intermediate (10–90 %) and high (>90 %) MGMT expression, respectively.<sup>51</sup> There is a clear greater propensity for prolactin-producing tumours towards low MGMT expression, which is likely to account for the higher response rate of prolactinomas to temozolomide therapy.<sup>34,51,52</sup> Some studies have not found significantly increased differences in MGMT expression between invasive and non-invasive pituitary tumours.<sup>51,52</sup> However, other studies have reported a higher incidence of low MGMT expression amongst more aggressive pituitary tumour subtypes, including invasive Crooke's cell adenoma and silent subtype 3 pituitary adenomas.<sup>37,53</sup> A higher occurrence of low MGMT expression has been reported amongst primary surgical specimens of NF pituitary adenomas showing subsequent tumour regrowth. Furthermore, those primary NF pituitary adenomas with low MGMT expression had a shorter interval until re-operation.<sup>54</sup> These limited studies do suggest a role for MGMT in pituitary tumour biology.

## Future Directions

The identification of temozolomide as the first chemotherapeutic agent with significant efficacy in aggressive pituitary tumours represents a major advance in management. It is hoped the next few years will see further clarity develop in delineating the role of temozolomide in the treatment of pituitary tumours. There is excitement about the potential for its use earlier in the treatment algorithm, but also justifiable concern about the lack of long-term safety data in particular with regard to secondary malignancies.

One of the more pressing questions relates to the optimal length of temozolomide therapy. Response is invariably seen within three months, and this appears a good time frame for a therapeutic trial. In responding tumours, maximal benefit is most commonly seen within the first 12 months, but whether longer treatment would affect more prolonged responses is unknown. Various combination therapies may offer the possibility of enhanced clinical benefit, particularly in non-responding cases or tumours with high MGMT expression. MGMT inhibitors, such as 06-benzylguanine, in combination with temozolomide, have had limited success in resistant gliomas.<sup>55</sup> Synergistic chemotherapeutic effect has been described for temozolomide in combination with bevacizumab, and there is interest in inhibition of poly (ADP-ribose) polymerase (PARP) (another DNA repair enzyme) and potentiation of temozolomide efficacy.<sup>56,57</sup>

The use of temozolomide as salvage therapy for aggressive pituitary tumours is generally well accepted, including recommendation in recent Endocrine Society Clinical Guidelines for consideration of its use for malignant prolactinomas.<sup>58</sup> The possibility of utilising temozolomide earlier in the treatment paradigm has recently been raised. Whitelaw et al. have successfully used temozolomide in order to achieve tumour shrinkage in a tumour compressing the optic chiasm prior to administering radiotherapy. They also described temozolomide treatment in a paediatric patient as a means of deferring radiotherapy.<sup>34</sup> Temozolomide has demonstrated radiosensitising properties, and potential concurrent administration of temozolomide and radiotherapy may provide increased benefit.<sup>11</sup>

Whilst MGMT appears to play a primary role in determining response to temozolomide in pituitary tumours, a better understanding of additional resistance mechanisms is needed. Reports in glioblastoma

have implicated high epidermal growth factor receptor expression and upregulation of stem cell gene pathways in MGMT-independent temozolomide resistance.<sup>59,60</sup> In the author's opinion, MGMT expression as determined by immunohistochemistry does provide guidance for more effective use of temozolomide in pituitary tumours. However, the wider application of MGMT immunohistochemistry into clinical practice requires the development of standardised scoring methods. Finally, the role of MGMT in pituitary tumourigenesis and

the mechanism(s) responsible for loss of MGMT expression in pituitary tumours remain to be elucidated.

Prospective clinical trials would provide the ideal platforms to properly address many of the uncertainties surrounding the use of temozolomide in aggressive pituitary tumours. International collaborations will become immensely important in driving forward advancements in this challenging area of clinical practice. ■

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