Temozolomide Treatment in Aggressive Pituitary Tumors and Pituitary Carcinomas: A French Multicenter Experience

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Context: To date only 18 patients with aggressive pituitary tumors or carcinomas treated with temozolomide have been reported. Increased expression of *O*6-methylguanine-DNA-methyltranferase (MGMT) has been suggested to predict resistance to temozolomide.

Objectives: The objective of the study was to describe the antitumoral efficacy and toxicity of temozolomide in patients with aggressive pituitary tumors or carcinomas and evaluate the possible prognostic value of MGMT promoter methylation and protein expression.

Patients: Eight patients, five with pituitary carcinomas (three prolactin (PRL) and two ACTH) and three with aggressive pituitary tumors (one PRL and two ACTH), all treated with temozolomide administered orally for four to 24 cycles, were included in our French multicenter study.

Design: MGMT expression was assessed by immunohistochemistry and MGMT promoter methylation by pyrosequencing.

Results: Three of the eight patients (two ACTH adenomas and one PRL carcinoma) responded to temozolomide as demonstrated by significant tumor shrinkage and reduced hormone secretion. Three cycles of temozolomide were sufficient to identify treatment-responsive patients. Additional cycles did not improve treatment efficacy in those not responding, even when associated with carboplatin and vepeside. MGMT expression did not predict tumoral response to temozolomide because it was positive in one responder and negative in two nonresponders. Similarly, MGMT promoter methylation (three of seven tumors) did not predict clinical response. Toxicity remained mild in all patients.

Conclusion: Temozolomide treatment may be an effective option for some aggressive pituitary tumors or carcinomas. Response to a trial of three cycles of treatment seems sufficient to identify responders and more reliable than patient MGMT status. **(*J Clin Endocrinol Metab* 95: 4592–4599, 2010)**

Pituitary tumors account for approximately 15% of all intracranial neoplasms and are associated with macroscopically evident local invasion in 35–40% of cases. Pituitary carcinoma is a rare disorder (accounting for approximately 0.2% of pituitary tumors) defined by the presence of craniospinal and/or systemic metastases. These tumors grow rapidly and are largely unresponsive to current combined treatment strategies associating surgery, radiation, and systemic chemotherapy. The average survival time is then less than 4 yr (1). A subset of invasive or atypical adenomas display aggressive behavior and are also resistant to medical therapy causing substantial morbidity (2).

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Abbreviations: MGMT, *O*6-methylguanine-DNA-methyltranferase; PRL, prolactin.

Temozolomide, an alkylating chemotherapeutic drug, is widely used in the management of glioblastoma (3) and is effective against neuroendocrine tumors (4). It is not cell cycle specific, thus increasing its usefulness in treating relatively slow-growing pituitary tumors. Recent case reports have detailed the successful use of temozolomide in the management of aggressive pituitary tumors and pituitary carcinoma. Temozolomide efficacy was reported to depend on the expression of O6-methylguanine-DNA-methyltranferase (MGMT), a DNA repair protein (5–16). Indeed, low tumor MGMT expression was shown in some studies to correlate with temozolomide response and increased survival in patients with brain tumors (17–19). MGMT activity is frequently lost in the presence of CpG island hypermethylation in the promoter region of certain types of human primary neoplasm (3). Therefore, the methylation status of the *MGMT* promoter was considered to be indicative of a good outcome in patients with malignant gliomas treated with an alkylating agent.

Data on the use of temozolomide in treating pituitary carcinoma and invasive, aggressive pituitary adenomas are sparse because only 18 cases have been published to date with a successful outcome in 16 patients. These included seven prolactin (PRL)-secreting tumors (5–8, 10, 11, 14, 20) with four carcinomas (5, 7, 8, 20), six ACTH-secreting tumors (11, 12, 13, 15, 16) with three carcinomas (13, 15, 16), two LH-secreting carcinomas (7, 14), one GH-secreting tumor (5), one mixed GH and PRL carcinoma (14), and one nonfunctional incidentaloma (15). Only two cases of a pituitary tumor nonresponsive to temozolomide have been published, one aggressive GH-secreting adenoma (5) and one silent ACTH adenoma (11).

MGMT immunostaining was studied in only 11 cases, nine responders and two nonresponders, with absent or low MGMT expression in eight of the nine responders, suggesting a correlation between tumor response and MGMT expression. However, reported cases of pituitary tumors treated by temozolomide are too rare to draw any definitive conclusions on treatment efficacy in this particular setting or on the possible correlation between MGMT expression and treatment response. Underreporting of poorly responsive cases is also likely to bias the assessment of the overall efficacy of this therapy.

The objective of this French multicenter study was therefore to report all cases of aggressive pituitary adenoma or pituitary carcinoma treated by temozolomide in participating centers and to try to correlate tumor response with MGMT immunohistochemistry and *MGMT* promoter methylation.

Patients and Methods

Patients

Eight patients (six males and two females), including four with PRL-secreting tumors (three carcinomas and one aggressive tumor) and four with ACTH tumors (two carcinomas and two aggressive tumors), treated with temozolomide were identified from all centers taking part in the French Pituitary Club [clinical network of tertiary referral centers for pituitary diseases, a work group of the French Endocrine Society (Société Française d'Endocrinologie)]. All patients treated for this indication in the participating centers were included in the present study, regardless of treatment outcome. All patients received detailed information on the side effects and potential benefits of temozolomide used in a condition different from its marketed indication and had signed an informed consent form allowing retrospective studies to be performed on their medical records for scientific purposes, as approved by our local ethics committee.

Individual clinical characteristics are summarized in Table 1. Magnetic resonance imaging revealed an invasive macroadenoma in all cases.

PRL tumors

All four tumors were invasive macroprolactinomas diagnosed because of headaches and/or visual field defects. Clinically significant hypogonadism was present in one man and amenorrhea and galactorrhea in one female. Age at diagnosis was 42.0 ± 12.8 yr (mean ± SD). Surgery was performed within the first few years of diagnosis before any medical treatment in two cases (no. 1 and no. 3) or because of the lack of efficacy of bromocriptine treatment in the other two (no. 2 and no. 4). During follow-up, high doses of dopamine agonist treatment (bromocriptine and/or cabergoline up to 1 mg/d) were administered in an attempt to control PRL secretion and tumor progression but proved insufficient. Fractionated stereotactic radiotherapy was performed in all cases and multiple surgeries in two cases.

ACTH tumors

Cushing's syndrome was present at diagnosis in only two patients; the two other tumors were diagnosed on tumoral signs with severe headaches and ocular palsy. Elevated ACTH levels were noted for all four ACTH adenomas, even in the absence of

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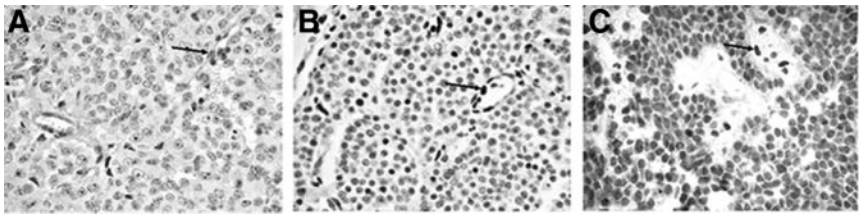

FIG. 1. MGMT immunohistochemistry in pituitary tumors. A, Case no. 3 with negative nuclear staining for MGMT. The endothelial cells (*arrow*) act as internal positive controls (original magnification ×400). B, Case no. 7 with focal nuclear staining for MGMT in 30% of tumorous cells, with strong positive staining in endothelial cells (*arrow*) (original magnification ×400). C, Case no. 4 with diffuse and strong nuclear staining for MGMT, with positive staining in endothelial cells (*arrow*) (original magnification ×400).

typical Cushing's syndrome. Age at diagnosis was 42.5 ± 8.3 yr (mean ± SD). Transsphenoidal surgery was performed in each case as primary treatment of invasive macroadenomas. Postoperative exploration revealed residual tumor with elevated plasma ACTH levels in each cases leading to fractionated stereotactic radiotherapy within 2 yr after surgery. Additional radiotherapy was performed in two cases (stereotactic radiotherapy in no. 5 or γ-knife radiosurgery in no. 8). Additional transsphenoidal debulking was required in three cases because of tumor progression and metastasis. The need for rapid control of hypercortisolism led to bilateral adrenalectomy 10 months after initial transsphenoidal surgery in one case (no. 5) and to the use of anticortisolic drugs (ketoconazole or mitotane) in two others (no. 7 and no. 8).

Treatment protocol

Temozolomide treatment was given following the standard regimen of 150–200 mg/m^2 · d; 5 d of a 28-d cycle for three to 24 cycles. Treatment efficacy was evaluated after three completed cycles.

Pathological methods

All the following analyses were performed on tissues sample obtained before temozolomide treatment.

Proliferative markers

Proliferative markers were detected by immunocytochemistry automatically with Benchmark XT (Ventana Medical System, Tucson, AZ). The antibodies Mib1 (1:50; Dako, Glostrup, Denmark) and anti-p53clone DO-7 (1:200; Novocastra Laboratories, Newcastle upon Tyne, UK) (21) were used. To determine Ki-67, p53 and mitotic indexes, we counted positive cells for 10 representative fields per tumor at ×400 magnification, with an average count of 5000 nuclei. Ki-67 and p53 labelings were expressed as a maximum percentage and the mitoses by their absolute number.

MGMT expression

Paraffin blocks were obtained for MGMT immunocytochemistry and DNA extraction for methylation analysis in all cases except one due to tumor apoplexy (no. 1). MGMT protein expression was studied by immunocytochemistry performed on 4-μm-thick sections of Bouin- or formalin-fixed paraffin-embedded tumor specimens. Sections were deparaffinized in xylene and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked by 0.6% H_2O_2 in methanol for 10 min. Antigen retrieval was performed by heating slides in a pressure cooker for 2 min in citrate buffer 0.01 M (pH 6). Nonspecific binding was blocked using protein-blocking solution for 5 min (Histostain Plus detection kit; Invitrogen reference 85-9043, Camarillo, CA).

Slides were washed in PBS and incubated with primary mouse monoclonal anti-MGMT antibody (clone MT3.1, 1:100; Dako reference M3610, Carpinteria, CA) for 1 h at room temperature. They were then incubated with biotinylated secondary antibody (mixture of antimouse and antirabbit antibodies) for 10 min followed by streptavidin-horseradish peroxidase complex for 10 min (all included in Histostain Plus kit; Invitrogen). NovaRed (Vector reference SK-4800, Burlingame, CA) was used as a chromogene. Counterstaining was performed with Mayer's hematoxylin. As a negative control, the primary antibody was omitted. Endothelial or lymphocyte staining was used as internal positive control.

Immunoreactivity was analyzed semiquantitatively by estimating the percentage of positive tumor cell nuclei. According to McCormack *et al.* (5), low MGMT expression was defined as absent or focal (<10%) positive nuclei, intermediate expression as 10–90% of positive nuclei, and high expression as diffuse positive staining of more than 90% of tumor nuclei, regardless of intensity (Fig. 1). All immunocytochemical analyses were centralized, performed by only one pathologist (N.S.) and carried out blind to clinical data and methylation status of the MGMT promoter.

Methylation analysis

MGMT methylation was analyzed by pyrosequencing. DNA was extracted from tumor samples using the QIAmp DNA minikit (QIAGEN, Valencia, CA) after the removal of paraffin by xylene extraction and 48 h of proteinase K digestion at 56 C. Methylation analysis was performed after bisulfite conversion of 2 μg of DNA with EZ DNA methylation-gold kit (Zymco Research, Orange, CA). DNA was then eluted in 20 μl of M-elution buffer and 5 μl then amplified and analyzed using the PyroMark MGMT kit (QIAGEN) as described by the manufacturer on the Pyromark ID sequencing system. DNA from peripheral blood lymphocytes, treated or not with Sss1 methyltransferase (New England Biolabs, Beverly, MA) was used as positive and negative control.

Results

Temozolomide efficacy and tolerance

PRL tumors

Temozolomide treatment was initiated 15.5 ± 3.4 yr after diagnosis because of metastasis in one case (no. 1) and lack of tumor control despite medical treatment in three cases. Temozolomide treatment was poorly efficient (after three to eight cycles) in three cases with metastasis occurring in two (no. 3 and no. 4) and tumor size and PRL levels increasing in one (no. 2). However, the positive effect of temozolomide was remarkable for one case (no. 1)

TABLE 1. Clinical characteristics and treatment

Case no.	Sex	Age at diagnosis (yr)	Tumor type	Number of surgeries	Radio therapy	Ki67 (%)	p53 (%)	Mitoses nb	Temozolomide treatment					
									Age at treatment	Number of cycles	Hormonal response	Tumoral response	MGMT ICC (%)	Promoter methylation (%)
1	M	32	PRL Carcinoma	1	ST	ND	ND	ND	43	24	Yes	Yes	ND	ND
2	M	52	PRL Adenoma	1	ST (×2)	2	0.5	5	71	8	No	No	30	No
3	M	54	PRL Carcinoma	4	ST (×2) + GK	7	1	8	69	5	No	No	0	8.5
4	F	30	PRL Carcinoma	4	ST (×2)	30	10	4	47	3	No	No	100	No
5	M	31	ACTH Carcinoma	3	ST (×2)	20	10	3	37	14[a]	No	No	50	2.6
6	M	49	ACTH Adenoma	3	ST	20	60	8	54	7[b]	No	No	<1	No
7	M	38	ACTH Carcinoma	4	ST	10	1	7	52	6	Yes	Yes	30	No
8	F	42	ACTH Adenoma	2	ST + GK	0.5	0	1	50	4	Yes	Yes	0	9.8

M, Male; F, female; ST, fractioned stereotactic radiotherapy; GK, γ-knife radiotherapy; ND, not determined; ICC, immunocytochemistry; nb, number.

[a] After eight cycles on monotherapy, carmustine was associated with temozolomide for six additional cycles.

[b] After three cycles on monotherapy, carboplatin was associated with temozolomide for five additional cycles.

with the disappearance of the metastasis, 60% reduction in tumor size, and PRL level were noted after three cycles of temozolomide. Reduction of tumor size and normalization of the PRL level were confirmed during the additional cycles. After 24 cycles the treatment was stopped and 10 months after temozolomide discontinuation, the remnant tumor size (at magnetic resonance imaging scan) was unchanged and the PRL level remained controlled without treatment.

ACTH tumors

Temozolomide treatment was initiated 8.3 ± 4 yr after diagnosis because of metastasis in two cases (no. 5 and no. 7) and lack of tumor control in another two cases (no. 6 and no. 8). Despite seven to 14 cycles of temozolomide, even when associated with carboplatin (four cycles) or carmustine (six cycles), no tumoral or hormonal response was noted for cases no. 5 and no. 6. In contrast, a significant tumoral, hormonal, and clinical response was observed for the two other cases (no. 7 and no. 8) after only four cycles of temozolomide. These latter two cases are still receiving treatment, but a marked shrinkage of the pituitary tumor has already been noted alongside a markedly reduced extension of the vertebral metastasis in the perivertebral soft tissue (case no. 7). Plasma ACTH levels have decreased (from 135 and 114 ng/liter to 64 and 47 ng/liter, respectively) with clinical improvement allowing the cessation of mitotane treatment in both cases and insulin treatment cessation and a reduction in oral antidiabetic therapy in case no. 8.

Temozolomide tolerance

Treatment was well tolerated in two cases. Fatigue was noted in two cases but did not require any modification of treatment. Severe hematological toxicity with agranulocytosis after three cycles led to the cessation of temozolomide treatment in one case (no. 4). A thrombocytopenia in two cases (no. 2 and no. 8) led to a reduction in the temozolomide dose (150 mg/m^2 · d in case no. 2) or an increase in the interval between cycles (every 6 wk, no. 8).

Pathological data

Proliferative markers

Pathological data were lacking for one case (no. 1) because of tumor apoplexy. The index of Ki-67 and the number of mitosis were consistently high in all tumors, respectively, 2–30 and 1–8%. P53 labeling was very high (10–60%) in three tumors including two carcinomas (Table 1).

MGMT immunostaining and correlation with MGMT promoter methylation

Of seven tumors tested, MGMT immunocytochemistry showed levels of MGMT expression that were low or ab-

sent in three (42.8%), intermediate in three (42.8%), and high in one (12.4%). No difference was found between PRL- and ACTH-secreting tumors or between carcinomas and aggressive pituitary tumors.

MGMT promoter methylation, as determined by pyrosequencing, was demonstrated in three cases (42.8%) (one PRL carcinoma and two ACTH tumors, one carcinoma, and one aggressive tumor). When present, MGMT promoter methylation was associated with low (n = 2) or intermediate MGMT expression (n = 1). However, the absence of *MGMT* promoter methylation was associated with high MGMT expression in only one case. In the three other cases, low (n = 1) or intermediate (n = 2) MGMT expression was observed. Finally, MGMT promoter methylation analysis predicted MGMT expression in four of the seven tumors (57.1%).

MGMT expression, MGMT promoter methylation, and response to temozolomide treatment

In the absence of a consensus for a cutoff level of MGMT expression by immunocytochemistry, it was impossible to perform any statistical analysis. However, MGMT expression was discordant between the two responding tumors because one had intermediate expression (30%) and the other had no MGMT expression. Moreover, among the nonresponding tumors, two showed almost no MGMT expression and three intermediate to high MGMT expression. The same dissociation was noted with *MGMT* methylation, which was present in three tumors, one responding and two nonresponding. Absence of methylation was present in three nonresponding and one responding tumor. MGMT expression or *MGMT* promoter methylation analysis thus predicted tumor response to temozolomide in only four of the seven tumors (57.1%).

Discussion

Pituitary carcinoma, defined by the presence of tumor tissue within the central nervous system not contiguous with the pituitary fossa or other extracranial systemic metastases, is a rare condition (1). However, more cases are being identified in recent years with the increasing sensitivity of imaging and enhanced knowledge. Indeed, in a recent review, Van der Klaauw *et al.* (22) described 59 cases published since 1937, 34 of which were identified after 1990 and 25 after 2000. Moreover, the new World Health Organization classification characterizes atypical adenoma as a new group of pituitary tumor (2). Whereas the definition of this new entity is not easy, it is now clear that some pituitary tumors are characterized by an aggressive behavior that requires specific treatment strategies similar to therapy used for pituitary carcinomas. Indeed, conventional treatment approaches such as surgery, radiotherapy, or pharmacological treatment (dopamine agonist or somatostatin analog) are often insufficient to control tumor growth and hormone secretion. For this reason different groups started to use cytotoxic chemotherapy with variable results (23).

The publication of the successful treatment of some cases with temozolomide is encouraging. To date, however, only 13 publications reporting a total of 18 cases are available, and almost all, except two, report only positive results (Table 2). Our multicenter study is the largest published to date, reporting the efficacy and tolerance of temozolomide treatment in patients, regardless of the result. The results of our eight cases increase the number of published cases to 26 with five new carcinomas giving a total number of 12 carcinomas.

The majority of currently published cases are PRL-secreting adenomas (n = 4) or carcinomas (n = 3) and ACTH-secreting adenomas (n = 3) or carcinomas (n = 3), whereas LH adenomas and GH carcinomas are rare. Only three of our eight patients were classified as responders to temozolomide with normalization of hormone secretion and a significant decrease in tumor size (pituitary and metastases). Tolerance was acceptable in all three and cessation of all other treatment (cabergoline, 1,1-dichlorodiphenildichloroethane, ketoconazole) was allowed. Whereas a longer follow-up is needed for two cases, these results are encouraging and support the use of temozolomide in responsive cases. However, five of the eight patients were classified as nonresponders and two died because of tumor progression.

It is important to underline that, as published elsewhere, any tumoral or hormonal response to temozolomide treatment is always observed soon after treatment initiation in responders. Indeed, among our patients, the absence of response after three cycles consistently predicted further resistance to this treatment. It should also be noted that the initial response to temozolomide treatment is not always associated with long-term control because two cases have presented a tumor relapse 5 and 6 months after initiation (15, 16).

So far, no clinical criteria have been proposed able to predict this resistance to temozolomide. Concerning glioblastomas, however, based on a limited number of cases, MGMT expression as analyzed by immunostaining has been suggested to predict the tumor response to temozolomide and that the absence of MGMT expression may be associated with a positive response. To date, MGMT expression has been analyzed in only 10 of the 16 patients published with low levels of expression in the eight responders and high levels in the two nonresponder patients.

TABLE 2. Temozolomide treatment of pituitary tumors: review of the literature

										Temozolomide treatment					
Reference	Sex	Age at diagnosis (yr)	Tumor type		Number of surgeries	RxT	Ki-67 (%)	p53 (%)	Mitoses nb	Age at treatment initiation	Number of cycles	Hormonal response	Tumoral response	MGMT	Methylation
7	M	38	LH	Carcinoma	3	Meta	1	ND	ND	47	12	ND	Yes	ND	ND
7	M	26	PRL	Carcinoma	2	Pit and Meta	10	ND	Numerous	ND	10	Yes	Partial	ND	ND
8	M	72	PRL	Carcinoma	5	No	0	ND	Rare	81	18	Yes	Yes	ND	ND
10	F	20	PRL	Adenoma	1	Pit	<5%	ND	ND	52	26	Yes	Yes	ND	ND
15	F	28	NFA	Adenoma	0	No	ND	ND	ND	28	10	No	Yes	ND	ND
6,11	M	46	PRL	Adenoma	5	Pit	40–60%	15%	ND	61	7	Yes	Yes	Negative	ND
15	F	43	ACTH	Adenoma	3	Pit	ND	ND	ND	45	16	ND	Yes	Negative	ND
14	F	48	GH-PRL	Carcinoma	1	Pit	5%	ND	ND	57	23	Yes	Yes	Negative	ND
14	M	60	PRL	Adenoma	1	No	2	ND	ND	79	12	Yes	Yes	Negative	ND
5	M	42	PRL	Carcinoma	6	Pit (×2)	ND	ND	Few	64	4	Yes	Yes	Negative	Yes
14	M	20	NFA	Adenoma	6	Pit	2	ND	ND	35	15	ND	Yes	Weak	ND
11	M	ND	ACTH	Adenoma	ND	ND	ND	ND	ND	41	ND	No	No	Positive	ND
15	M	60	ACTH	Carcinoma	2	Pit	ND	ND	ND	ND	12	Yes	Yes	Positive	ND
5	M	48	GH	Adenoma	4	Pit	ND	ND	ND	55	3	No	No	Strong	ND
13	F	46	ACTH	Carcinoma	4	Pit	3	Weak	ND	55	18	Yes	Yes	Low (<5%)	ND
12	F	64	ACTH	Adenoma	2	Pit	High	ND	ND	70	4	Yes	Yes	Negative	ND
16	M	50	ACTH	Carcinoma	1	Pit (×2)	31	ND	ND	52	4[a]	Yes	Yes	ND	ND
20	M	55	PRL	Carcinoma	5	Pit (×2)	ND	ND	ND	64	12	Yes	Yes	ND	ND

M, Male; F, female; Pit, pituitary; ND, not determined; NFA, nonfunctioning pituitary adenoma; Meta, metastasis; nb, number.

[a] In association with capecitabine1000 mg twice daily, 14 d.

Contrarily, our results demonstrated no such correlation between MGMT expression and tumor response. Indeed some responder patients showed intermediate MGMT expression, and conversely, the absence of MGMT expression was not always predictive of tumor response.

The difficulty in defining the cutoff of MGMT expression allowing the classification of these pituitary tumors as low- or high-expressing tumors could only partly explain this discordance (5, 13, 24). Moreover, little is known about MGMT expression during the tumor progression. Indeed, it is possible that initial MGMT expression is not representative of the MGMT expression at the time of the recurrence or in the metastasis. This hypothesis could explain a part of the discrepancy between MGMT expression and response to the treatment. For patient no. 3, MGMT expression was performed on tumor samples obtained at the time of the recurrence, but we have no information on the metastasis.

Some studies have suggested that *MGMT* promoter methylation analysis could predict MGMT loss of expression in glioblastomas. Only a few studies have analyzed *MGMT* promoter methylation in pituitary tumors (5, 13, 25), which seems to be a rare event (about 25% of tumors). In our study *MGMT* promoter methylation was evident in three of the seven tumors analyzed (42.8%); however, methylation levels were very low without perhaps any effect on protein expression. *MGMT* promoter methylation may not therefore be the principal event for loss of MGMT expression in pituitary tumors. Because the cutoff level for MGMT expression has yet to be determined, it seems too early to conclude on any correlation between MGMT expression and promoter methylation or between the presence of methylation and response to temozolomide.

In conclusion, our study confirms the efficacy of temozolomide treatment for some aggressive pituitary tumors or carcinomas; however, the study reveals MGMT status as a poor predictor of treatment outcome that should not be used to select patients who may benefit from this treatment. Larger prospective studies are necessary to determine predictive factors of response to temozolomide. Until results from such studies are available, we propose that patients with aggressive adenomas or carcinomas who are resistant to conventional treatment be submitted to three cycles of temozolomide. We propose stopping treatment in the absence of hormonal or tumoral response after three cycles because a delayed tumor response appears unlikely.

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References

1. **Kaltsas GA, Nomikos P, Kontogeorgos G, Buchfelder M, Grossman AB** 2005 Diagnosis and management of pituitary carcinomas. J Clin Endocrinol Metab 90:3089–3099
2. **Lloyd RV, Kovacs K, Young Jr WF, Farrell WE, Asa SL, Trouillas J, Kontogeorgos G, Sano T, Scheithauer BW, Horvath E** 2004 Pituitary tumours: introduction. Lyon, France: IARC Press
3. **Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG** 2000 Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 343:1350–1354
4. **Ekeblad S, Sundin A, Janson ET, Welin S, Granberg D, Kindmark H, Dunder K, Kozlovacki G, Orlefors H, Sigurd M, Oberg K, Eriksson B, Skogseid B** 2007 Temozolomide as monotherapy is effective in treatment of advanced malignant neuroendocrine tumors. Clin Cancer Res 13:2986–2991
5. **McCormack AI, McDonald KL, Gill AJ, Clark SJ, Burt MG, Campbell KA, Braund WJ, Little NS, Cook RJ, Grossman AB, Robinson BG, Clifton-Bligh RJ** 2009 Low O6-methylguanine-DNA methyltransferase (MGMT) expression and response to temozolomide in aggressive pituitary tumours. Clin Endocrinol (Oxf) 71:226–233
6. **Syro LV, Uribe H, Penagos LC, Ortiz LD, Fadul CE, Horvath E, Kovacs K** 2006 Antitumour effects of temozolomide in a man with a large, invasive prolactin-producing pituitary neoplasm. Clin Endocrinol (Oxf) 65:552–553
7. **Fadul CE, Kominsky AL, Meyer LP, Kingman LS, Kinlaw WB, Rhodes CH, Eskey CJ, Simmons NE** 2006 Long-term response of pituitary carcinoma to temozolomide: report of two cases. J Neurosurg 105:621–626
8. **Lim S, Shahinian H, Maya MM, Yong W, Heaney AP** 2006 Temozolomide: a novel treatment for pituitary carcinoma. Lancet Oncol 7:518–520
9. **Kovacs K, Horvath E, Syro LV, Uribe H, Penagos LC, Ortiz LD, Fadul CE** 2007 Temozolomide therapy in a man with an aggressive prolactin-secreting pituitary neoplasm: morphological findings. Hum Pathol 38:185–189
10. **Neff LM, Weil M, Cole A, Hedges TR, Shucart W, Lawrence D, Zhu JJ, Tischler AS, Lechan RM** 2007 Temozolomide in the treatment of an invasive prolactinoma resistant to dopamine agonists. Pituitary 10:81–86
11. **Kovacs K, Scheithauer BW, Lombardero M, McLendon RE, Syro LV, Uribe H, Ortiz LD, Penagos LC** 2008 MGMT immunoexpression predicts responsiveness of pituitary tumors to temozolomide therapy. Acta Neuropathol 115:261–262
12. **Moyes VJ, Alusi G, Sabin HI, Evanson J, Berney DM, Kovacs K, Monson JP, Plowman PN, Drake WM** 2009 Treatment of Nelson's syndrome with temozolomide. Eur J Endocrinol 160:115–119
13. **Takeshita A, Inoshita N, Taguchi M, Okuda C, Fukuhara N, Oyama K, Ohashi K, Sano T, Takeuchi Y, Yamada S** 2009 High incidence of low O6-methylguanine DNA methyltransferase expression in invasive macroadenomas of Cushing's disease. Eur J Endocrinol 161:553–559
14. **Hagen C, Schroeder HD, Hansen S, Hagen C, Andersen M** 2009 Temozolomide treatment of a pituitary carcinoma and two pituitary

macroadenomas resistant to conventional therapy. Eur J Endocrinol 161:631–637
15. **Mohammed S, Kovacs K, Mason W, Smyth H, Cusimano MD** 2009 Use of temozolomide in aggressive pituitary tumors: case report. Neurosurgery 64:E773–E774; discussion E774
16. **Thearle M, Freda P, Bruce J, Isaacson S, Lee Y, Fine R** 4 December 2009 Temozolomide (Temodar®) and capecitabine (Xeloda®) treatment of an aggressive corticotroph pituitary tumor. Pituitary 10.1007/s11102-009-0211-1
17. **Karayan-Tapon L, Quillien V, Guilhot J, Wager M, Fromont G, Saikali S, Etcheverry A, Hamlat A, Loussouarn D, Campion L, Campone M, Vallette FM, Gratas-Rabbia-Re C** 2010 Prognostic value of O(6)-methylguanine-DNA methyltransferase status in glioblastoma patients, assessed by five different methods. J Neurooncol 97:311–322
18. **Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R** 2005 MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003
19. **Cao VT, Jung TY, Jung S, Jin SG, Moon KS, Kim IY, Kang SS, Park CS, Lee KH, Chae HJ** 2009 The correlation and prognostic significance of MGMT promoter methylation and MGMT protein in glioblastomas. Neurosurgery 65:866–875; discussion 875
20. **Byrne S, Karapetis C, Vrodos N** 2009 A novel use of temozolomide in a patient with malignant prolactinoma. J Clin Neurosci 16:1694–1696
21. **Raverot G, Wierinckx A, Dantony E, Auger C, Chapas G, Villeneuve L, Brue T, Figarella-Branger D, Roy P, Jouanneau E, Jan M, Lachuer J, Trouillas J** 2010 Prognostic factors in prolactin pituitary tumors: clinical, histological, and molecular data from a series of 94 patients with a long postoperative follow-up. J Clin Endocrinol Metab 95:1708–1716
22. **van der Klaauw AA, Kienitz T, Strasburger CJ, Smit JW, Romijn JA** 2009 Malignant pituitary corticotroph adenomas: report of two cases and a comprehensive review of the literature. Pituitary 12:57–69
23. **Kaltsas GA, Mukherjee JJ, Plowman PN, Monson JP, Grossman AB, Besser GM** 1998 The role of cytotoxic chemotherapy in the management of aggressive and malignant pituitary tumors. J Clin Endocrinol Metab 83:4233–4238
24. **Widhalm G, Wolfsberger S, Preusser M, Woehrer A, Kotter MR, Czech T, Marosi C, Knosp E** 2009 O(6)-methylguanine DNA methyltransferase immunoexpression in nonfunctioning pituitary adenomas: are progressive tumors potential candidates for temozolomide treatment? Cancer 115:1070–1080
25. **Bello MJ, De Campos JM, Isla A, Casartelli C, Rey JA** 2006 Promoter CpG methylation of multiple genes in pituitary adenomas: frequent involvement of caspase-8. Oncol Rep 15:443–448