CLINICAL STUDY

Salvage therapy with temozolomide in patients with aggressive or metastatic pituitary adenomas: experience in six cases

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Abstract

Objective: The prognosis of either pituitary carcinoma or aggressive pituitary adenoma resistant to standard therapies is poor. We assessed the efficacy of treatment with temozolomide, an oral second-generation alkylating agent, in a consecutive series of six patients with aggressive pituitary adenomas.

Design: This was a 1-year prospective study of temozolomide therapy in six consecutive patients with pituitary carcinoma (one case) or atypical pituitary adenoma (five cases) resistant to standard therapies. There were three males and three females. Age at enrollment ranged between 52 and 64 years. Temozolomide was given orally at a dose of 150–200 mg/m² per day for 5 days every 4 weeks for a maximum of 12 cycles.

Methods: Response assessment was based on measurable change in tumor size, as assessed on magnetic resonance imaging, and hormone levels. Response was defined as reduction of at least 50% of tumor size and hormone levels.

Results: Four patients completed the 12 cycles of temozolomide treatment, as planned. Two patients stopped the drug after 3 and 6 months respectively because of the progression of disease. Two patients responded to temozolomide, while the remaining two patients had stable disease. Immunohistochemistry for O⁶-methylguanine-DNA methyltransferase (MGMT) in tumor sample showed a partial association with treatment response.

Conclusions: Temozolomide treatment has a wide range of efficacy in patients with pituitary carcinoma or locally aggressive pituitary adenoma. Positive staining for MGMT seems likely to predict a lower chance of response.

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Introduction

Pituitary adenomas comprise nearly 15% of intracranial neoplasm, and clinically evident local invasion is present in about one-third of cases (1). In most cases, pituitary adenomas are benign slow-growing tumors. However, some tumors are characterized by a fast rate of growth and are resistant to standard medical, surgical, and radiation treatments. According to the WHO 2004 Classification of Endocrine Tumors (2), a pituitary adenoma characterized by the presence of invasive growth and combinations of increased mitotic activity, a Ki-67 labeling index > 3%, and p53 immunoreactivity may be classified as atypical. Using WHO 2004 terminology, the term 'pituitary carcinoma' is restricted to tumors with proven cerebrospinal and/or systemic metastases (2). The incidence of pituitary carcinomas is <0.5% of symptomatic and 0.2% of operated pituitary tumors (2, 3). The prognosis of pituitary carcinoma

is poor, the mean survival being <4 years (4), and accelerated progression of the disease has been described (5). The response to various treatments is usually poor, and no consistent therapeutic scheme has been developed (4, 5).

Temozolomide is an oral second-generation alkylating agent that spontaneously undergoes chemical conversion to the cytotoxic metabolite 5-(3-methyl-1-triazeno)imidazole-4-carboxamide at physiological pH, without metabolic conversion. Temozolomide depletes the DNA repair enzyme O⁶-methylguanine-DNA methyltransferase (MGMT) in various cell types. The drug penetrates the blood–brain barrier effectively (6) and has antineoplastic activity against high-grade gliomas and cerebral metastases of malignant melanoma (7, 8). Moreover, the drug has been shown to possess activity against malignant neuroendocrine tumors (9).

Preliminary reports demonstrated a good response to temozolomide treatment in patients with either

pituitary carcinoma (10–12) or aggressive pituitary adenomas resistant to standard therapies (12–14). Herewith, we report our experience with temozolomide treatment and its relationship to MGMT expression in a series of six patients with aggressive pituitary tumors.

Subjects and methods

Patients

Between September 2006 and November 2008, six consecutive patients with either pituitary carcinoma (one case) or atypical pituitary adenoma that showed local growth despite previous surgery, radiation and standard medical therapies (five cases) were offered salvage treatment with temozolomide, based on case reports showing a good response in similar cases (10, 11, 13, 14).

The main clinical and demographic characteristics of the patients are depicted in Table 1. There were three males and three females. Age at enrollment ranged between 52 and 64 years. The median time at first diagnosis of pituitary adenoma was 7 years (range 4–25 years) before starting temozolomide therapy. Three patients had Cushing's disease, two patients had prolactin (PRL)-secreting adenoma, and one had Nelson's syndrome. All the patients had already received several treatments at our hospital or other hospitals (Table 1), but the pituitary tumor showed either liquora dissemination (one case) or rapid local growth after recurrence of disease. The two patients with PRL-secreting adenoma had resistance to high doses of cabergoline (3.5 mg/week). Octreotide-LAR (30 mg) was attempted without success in one patient with Cushing's disease and one with Nelson's syndrome. All the patients had previously undergone at least one radiation treatment, either fractionated radiotherapy (three patients), stereotactic radiotherapy with a linear accelerator (one patient), or gamma knife radiosurgery (three patients). Four patients received more than one radiation treatment (Table 1). In all the patients, radiation was performed after surgical debulking of

the tumor and initially led to reduction in the residual tumor in four patients, whereas initial stabilization of the tumor was obtained in the other two cases.

Before treatment with temozolomide, there was a clear radiological evidence of tumor regrowth in each patient. In Patient #1, the lesion extended into the left cavernous sinus and destroyed the sellar floor growing into both the nasal fossa and the left orbit. This patient complained of severe headache, nasal obstruction, and complete left ophthalmoplegia since 4 months. Transsphenoidal biopsy of the mass showed a poorly differentiated pituitary tumor characterized by area of necrosis and high mitotic activity. Patient #2 developed signs and symptoms of hypercortisolism, and a scheduled magnetic resonance imaging (MRI) showed several contrast-enhancing lesions disseminated along the liquora pathway (Fig. 1). Transcranial biopsy of a left frontal nodule confirmed metastasis from a pituitary adenoma that stained positive for ACTH. The largest metastasis, which was located just in front of the pons, was treated with gamma knife radiosurgery. Patient #3 had worsening hyperpigmentation, rising ACTH levels, and demonstration of progressive growth of the pituitary lesion that extended into both cavernous sinuses and had eroded the sellar floor growing within the sphenoid sinus. Patient #4 had clinical and biochemical recurrence of hypercortisolism 1 year after the second course of fractionated radiotherapy and a mild growth of residual tumor in both cavernous sinuses. Patient #5 had rising PRL levels and further increase in the pituitary tumor that occupied the sella turcica with upward and anterior extension. Another part of the tumor had eroded the skull base and occupied the sphenoid sinus and part of the ethmoid space (Fig. 1). Except for slight headache, the patient was still asymptomatic. Patient #6 presented with rapid rise of PRL levels, regrowth of the tumor within the left cavernous sinus, severe headache not responding to common analgesics and gabapentin 2 years after a second gamma knife treatment. She also had paralysis of the sixth left cranial nerve causing diplopia since 4 months.

Table 1 Demographic and clinical characteristics of six patients treated with temozolomide because of pituitary carcinoma or atypical pituitary adenoma.

Patient number	Sex	Age	Age at initial treatment	Clinical diagnosis	Presence of metastasis	Previous treatments
1	Male	64	57	Cushing	No	2 TNS, 2 SR, SSA, DA
2	Male	52	46	Cushing	Yes	1 TC, 2 TNS, 1 Rx, 1 GK
3	Female	55	40	Nelson	No	2 GK, SSA ^a
4	Female	53	28	Cushing	No	2 TNS, 2 Rx
5	Male	62	55	PRL-oma	No	DA, 1 TNS, 1 Rx
6	Female	57	53	PRL-oma	No	DA, 2 TNS, 1 GK

DA, dopamine agonists; GK, gamma knife radiosurgery; PRL-oma, PRL-secreting adenoma; Rx, fractionated radiotherapy; SR, stereotactic radiotherapy with a linear accelerator; SSA, somatostatin analogs; TNS, transsphenoidal surgery; TC, transcranial surgery.

^aThis patient had already performed two transsphenoidal surgical procedures for Cushing's disease without success before performing bilateral adrenalectomy.

Figure 1 Coronal contrast-enhanced T1-weighted MRI of the brain of Patient #2 obtained before (upper left panel) and 28 months after stopping 1-year treatment with temozolomide (upper right panel). Brain metastatic lesions (indicated by the arrows in the upper left panel) are no more evident on the post-treatment exam. Coronal contrast-enhanced T1-weighted MRI of the brain of Patient #5 obtained before (lower left panel) and 12 months after treatment with temozolomide (lower right panel). No significant change in the tumor mass can be appreciated.

Temozolomide treatment and follow-up visits

After having obtained informed consent, temozolomide treatment was initiated according to the following scheme. Temozolomide was given orally at a dose of 150 mg/m² per day for 5 days every 4 weeks for a maximum of 12 cycles. The dose was increased to 200 mg/m² per day beginning with the second cycle, so long as there were no hematological toxic effects. Doses of the drug were rounded up to the nearest 5 mg to accommodate capsule strength. The patients were instructed to fast 2 h before and 1 h after ingestion of the drug. In case of hematological toxicity, i.e. neutrophil and platelet counts lower than 1500 and 100 000/ml respectively, or other non-hematological severe side effects, the start of the next cycle could be delayed for a maximum of 2 weeks, and the dose was reduced to 150 mg/m² per day. Compliance with the treatment was ascertained by pill counts at each scheduled follow-up visit. The two patients with prolactinoma continued therapy with cabergoline at the same dose as done before temozolomide therapy.

Clinical examination and neurological evaluation were performed immediately before the administration of the next cycle of temozolomide. Blood counts and biochemical determination of hepatic and renal function were repeated on days 1 and 21 of each cycle, or as clinically indicated. MRI of the brain and of the spinal cord (in the patient with liquor dissemination of the tumor) was repeated every three cycles of temozolomide or when clinically indicated. Measurement of the hormone directly secreted by the tumor (ACTH in four

cases and PRL in the other two) was also repeated every 3 months. Basal pituitary function was assessed before and after 6 and 12 months of temozolomide treatment or when clinically indicated.

Response criteria

Response assessment was based on the measurable change in tumor size and hormone levels. Response was defined as reduction of at least 50% of tumor size, measured by the product of the greatest length and maximum width of all lesions, and normalization of hormone hypersecretion. Stable disease was defined as <50% reduction in tumor size and hormone hypersecretion. Progressive disease was defined as an increase >25% of tumor size, independent of hormone levels.

Histological examination

All the patients, except Patient #6, underwent at least one neurosurgical procedure at our center. Surgical material was fixed in 10% buffered formalin and embedded in paraffin for light microscopy. Sections were stained with hematoxylin–eosin, the periodic acid–Schiff (PAS), PAS–diastase, and Alcian technique. Immunohistochemical studies for anterior pituitary hormones and Ki-67 antigen were performed on 5 µm thick paraffin sections using the streptavidin–biotin–peroxidase-conjugated detection system, as described previously (15).

Methylation analysis and immunohistochemistry for MGMT

Methylation-specific PCR was performed to determine the presence of methylation in the region of the MGMT promoter, as described previously (12). Amplification of the methylated DNA from a tumor was considered a positive result, while amplification of unmethylated DNA was scored as negative. MGMT promoter and protein expression were assessed by immunohistochemistry on formalin-fixed paraffin embedded tumor tissue, as described previously (12). Briefly, a mouse MAB (Clone MT23.2, Cat: MA3-16537, Affinity Bioreagents, Rockford, IL, USA) was used at a concentration of 1 in 8000 using a biotin-free detection system (DS9713 Leica Microsystems, Mount Waverley, Victoria, Australia) on an automated staining platform (BondMax auto-stainer Leica Microsystems). Only nuclear staining was examined, as cytoplasmic staining was considered non-specific. Negative staining was defined as completely absent staining of more than 90% of tumor cells in the presence of a positive internal control (such as endothelial cells or lymphocytes) scattered throughout the slide. Staining of 10% of more tumor cells was considered positive. We emphasize that our interpretation required a positive internal control before

considering a staining as informative. This is analogous to the approach used for interpreting immunohistochemistry data for DNA mismatch repair proteins in microsatellite unstable colon carcinoma (16). If a case stained repeatedly negative in the absence of a positive internal control, it was classified as non-informative. All the samples were examined by a single observer (A J G) who was blinded to the molecular and clinical data of the patients.

Results

Tumor and hormone responses

Four patients completed the 12 cycles of temozolomide treatment, as planned. Patient #1 and Patient #4 stopped the drug after 3 and 6 months respectively because of progression of disease. Two patients (Patient #2 and Patient #6) responded to temozolomide, while the remaining two patients (Patient #3 and Patient #5) had stable disease.

MRI of Patient #1 showed further tumor growth 3 months after the start of temozolomide therapy. The patient complained of episodic minor nasal bleeding and impaired nasal respiration. Temozolomide treatment was stopped, and salvage radiotherapy and chemotherapy were initiated, as described below. Patient #2 had a mild reduction in metastatic tissue already 3 months after starting the temozolomide therapy. At 12 months, MRI showed disappearance of all metastatic tissue. Similarly, ACTH and cortisol levels dropped from a baseline of 237 pg/ml and 159 ng/ml to 10 pg/ml and 13 ng/ml respectively requiring institution of glucocorticoid replacement therapy (Fig. 2). Patient #3 had radiologically stable disease with initial, transitory hormonal response to temozolomide treatment: at 6 months, there was a slight reduction in the inferior part of the tumor, but at 12 months, this portion had re-expanded, and there was a questionable increase in the suprasellar portion of the tumor. Corresponding ACTH levels decreased from 6144 to 3580 pg/ml and then rose again to 6750 pg/ml (Fig. 2). Patient #4 did not have any improvement of symptoms after temozolomide. At 6 months, MRI showed a mild increase in residual tumor in both cavernous sinuses and ACTH levels rising from 247 to 329 pg/ml. Urinary free cortisol levels remained elevated, fluctuating between 1548 and 608 $\mu\text{g}/24\text{ h}$ (normal values, 10–100 $\mu\text{g}/24\text{ h}$). The patient was offered other treatments as detailed below. Patient #5, who previously had progressive growth of his prolactinoma, showed an arrest of tumor growth. MRI after 12 cycles of temozolomide was unchanged in comparison with the pretreatment with MRI (Fig. 1). Moreover, PRL levels, which had previously shown an almost exponential rise, decreased from 2800 ng/ml to a nadir of 1232 ng/ml after the sixth cycle of temozolomide

Figure 2 Hormone levels before, during, and after temozolomide treatment in four patients with pituitary carcinoma or atypical adenoma not responding to standard treatments. The upper panel shows the ACTH levels in Patient #2 (solid circles) who had Cushing's disease and in Patient #3 (solid squares) who had Nelson's syndrome. Note the logarithmic scale. The lower panel shows the PRL levels in Patients #5 (open squares) and #6 (open circles) who had a prolactinoma.

(Fig. 2). However, there was a trend toward rising PRL levels during the successive six cycles of temozolomide (Fig. 2). Patient #6 had a prompt and dramatic response to temozolomide. The first MRI at 3 months already showed a marked reduction in tumor size, while PRL levels decreased from 1845 to 88 ng/ml (Fig. 2). Severe headache improved within 1 month, and the left sixth nerve palsy completely reversed after 6 months. At the end of the treatment, the tumor had further reduced in size, and the PRL levels had decreased to 8 ng/ml (Fig. 2).

Further follow-up and treatments

Follow-up treatments and disease status at last follow-up are summarized in Table 2. Patient #1 was treated with a combination of fractionated radiotherapy, limited to the anterior nasal spaces to avoid summation with the two previous courses of radiation and chemotherapy with cisplatin and capecitabine. This new regimen induced a marked reduction in the tumor and improvement of nasal obstructive symptoms. However, progressive growth of residual disease was observed after 1 year of this regimen, and the patient was

Table 2 Further treatments, control of tumor growth, and disease status at last follow-up in six patients who received temozolomide for a pituitary carcinoma or a locally aggressive pituitary adenoma.

Patient number	Tumor response to temozolomide	Further treatments	Tumor control at last FU	Death	Months to last FU or death
1	Progression	Rx, Chemotherapy ^a	No	Yes	30
2	Response	None	Yes	No	28
3	Stable	TNS ^b	No	No	24
4	Progression	None	No	Yes	11
5	Stable	TNS ^b	No	No	21
6	Response	None	Yes	No	12

FU, follow-up; Rx, fractionated radiotherapy; TNS, transsphenoidal surgery.

^aChemotherapy consisted of a course with cisplatin and capecitabine and a second course with gemcitabine.

^bBoth patients are now going to receive a trial with the somatostatin analog SOM 230.

switched to gemcitabine for 4 months, followed by etoposide for 8 months. The tumor was stable for 12 months but then progressed, and the patient died 4 months after the last chemotherapy, 30 months after entering the trial with temozolomide. Patient #2 still needs replacement therapies for complete anterior pituitary deficiency 28 months after stopping temozolomide. His ACTH and cortisol levels of medication are 6.3 pg/ml and 20 ng/ml respectively. MRI does not show any residual metastatic disease (Fig. 1). Patient #3 showed a mild increase in tumor volume on MRI 6 months after stopping temozolomide and developed new visual symptoms. She underwent another transsphenoidal surgery with the aim to relieve the optic pathway. This patient is now scheduled to receive a trial with SOM 230. After failure of temozolomide treatment, Patient #4 was scheduled to undergo gamma knife radiosurgery to both cavernous sinuses and bilateral adrenalectomy because of accelerated progression of Cushing's disease. However, the patient had a spontaneous vertebral fracture before receiving the planned treatment. The successive clinical course was complicated by hypertension, hypokalemia, and infectious complications, leading to death 5 months after stopping temozolomide. MRI of Patient #5 showed a slight increase in tumor size 6 months after stopping temozolomide. Sudden palsy of the right oculomotor nerve occurred, and the patient was admitted to our hospital to undergo another surgical debulking via a transsphenoidal approach, without improvement of the neurological defect. He is now undergoing a trial with SOM 230. Patient #6 has concluded temozolomide treatment, as scheduled and is now beginning the follow-up period (Fig. 3).

Response to temozolomide and MGMT status

Patient #1 had no sufficient viable tumoral tissue for immunohistochemistry or PCR analyses. PCR analysis of *MGMT* promoter methylation could be performed in only four samples (from Patients #3, 4, 5, and 6) because of paucity of tumor tissue from Patient #2, while immunohistochemistry for MGMT could be performed in five patients. In Patients #2, 4, and 6,

tumor tissue analyzed was prior to the commencement of temozolomide. However, for Patients #3 and 5, the only tumor tissue available for study was following the completion of temozolomide treatment.

Methylation analysis of the *MGMT* promoter was negative in all the studied samples (Table 3), including Patient #4 who showed no response to temozolomide. Immunohistochemistry for MGMT was negative in two samples (Patient #2 who responded to the drug, and Patient #5 who had arrest of tumor growth while on treatment). The immunohistochemistry result for Patient #6, who also exhibited a complete response to temozolomide, showed negative tumor MGMT staining, but lacked positive staining of internal controls,

Figure 3 Immunohistochemistry for MGMT. Upper left panel: diffuse strong positive nuclear staining in tumor from Patient #4 (original magnification 400×). Upper right panel: there is negative staining in more than 90% of tumor cells from Patient #5 (original magnification 100×). It is therefore classified as negative. Note the positive staining in endothelial cells (arrows), which act as an internal positive control in areas where the tumor shows negative staining. Lower left panel: completely negative staining of tumor cells from Patient #2 for MGMT. The presence of endothelial cells (arrows), which act as an internal positive control, is emphasized (original magnification 600×). Lower right panel: negative staining for MGMT in tumor from Patient #6. However, no internal positive control is evident, and it is therefore classified as non-informative by our classification (original magnification 600×).

Table 3 Histological characteristics of six patients treated with temozolomide because of pituitary carcinoma or atypical pituitary adenoma.

Patient number	IHC for pituitary hormones	Ki-67 (%)	Methylation of MGMT promoter	IHC for MGMT	Disease response to temozolomide
1	ND	ND	ND	ND	Progression
2 ^a	ACTH	1	ND	Negative	Response
3 ^b	ACTH	5	Negative	Positive	Stable
4	ACTH	2.5	Negative	Positive	Progression
5 ^b	PRL	9	Negative	Negative	Stable
6	PRL	ND	Negative	Non-informative ^c	Response

PRL, prolactin; IHC, immunohistochemistry; MGMT, methylguanine-DNA methyltransferase; ND, not done.

^aThe tissue sample was from a frontal metastasis.

^bTissue sample was obtained from surgery performed 8 and 7 months after completing temozolomide treatment.

^cTissue sample stained completely negative but lacked positive internal control.

making this result non-informative using our criteria. Patient #3 (transient partial response) and Patient #4 (no response) had strongly positive MGMT expression on immunohistochemistry.

Discussion

Previous case reports suggested that the alkylating agent temozolomide was very effective in the treatment of patients with pituitary carcinoma or locally aggressive pituitary adenoma (10–14, 17–19). Three patients with metastatic and progressive disease due to prolactinoma resistant to dopamine agonists (two cases) and LH-secreting tumor (one case) were alive and well 42, 28, and 25 months after beginning temozolomide treatment (10, 11). Another patient with a mixed GH- and PRL-secreting carcinoma was treated for 23 months with temozolomide and was in remission of disease 3 years after terminating the treatment (17). Even though the clinical course of patients with pituitary carcinoma is not entirely predictable (4), the marked improvement observed in all four cases was attributable to temozolomide (10, 11, 17). These results were remarkable, as several different chemotherapeutic regimens tried in the past in similar cases were reported to cause short-lasting responses in sporadic cases only (20, 21). Encouraged by these early findings, temozolomide was used with success in three of the four patients with locally aggressive pituitary adenoma (12–14), whose prognosis is usually poor because of locally rapid growth of the tumor.

However, case reports may overestimate the success rate of proposed interventions because of reporting bias. On the other hand, pituitary carcinomas are very uncommon (3), and atypical adenomas truly resistant to standard surgical, medical, and radiation therapy are not frequent too. Therefore, implementation of a formal, controlled study on this issue seems unlikely.

Our results were obtained in an unselected series of six consecutive patients with pituitary carcinoma or locally aggressive adenoma, thus, allowing one to gauge some information about the frequency, degree, and duration of tumoral response to temozolomide.

Two of the six patients (Patients #2 and 6) had a long-lasting response to temozolomide as demonstrated by regression and normalization of ACTH and PRL hypersecretion respectively. The good response in the patient with dissemination of the pituitary tumor through the liquor pathway is similar to that observed in the three index cases initially reported in the literature (10, 11). Two other patients (Patients #1 and 4) had no benefit from therapy with temozolomide. At present, only few cases irresponsive to temozolomide have been reported in the literature (12, 22, 23). The response in the other two patients (Patients #3 and 5) is more difficult to interpret. Considering the rapid course of disease before and after temozolomide treatment, the arrest of tumor growth during therapy with the drug might be considered a worthy result. Both patients initially had a mild reduction in their hypersecreted hormone that later showed a trend to increase again despite continuing treatment with temozolomide. Supporting our results, two very recent studies of eight and seven patients reported good response to temozolomide treatment in three (38%) and two cases (29%) respectively (22, 23). In the latter study, four additional patients had stabilization of tumor growth during treatment with the drug, a response similar to that observed in Patients #3 and 5 of our study. Raverot *et al.* (22) found that a trial of three cycles of treatment was sufficient to identify the patients who will eventually respond to the drug. Our results support this suggestion, as both responders already showed a clear reduction in tumor volume and hormone hypersecretion 3 months after beginning temozolomide treatment.

Recent data in patients with gliomas suggest the possibility to resume treatment with temozolomide in patients who had relapse of the tumor after stopping upfront treatment (24). Alternative regimens of temozolomide treatment, such as 3 weeks on and 1 week off, have also been explored to overcome chemoresistance of brain tumors (25, 26). Bush *et al.* (23) chose the 3 weeks on and 1 week off protocol to treat seven patients with aggressive pituitary tumors (including two carcinomas) because the high dose of temozolomide per unit time might theoretically deplete tumor cells of their MGMT more efficiently than the standard regimen.

In this study, two of the seven patients had improvement of disease, four had stable disease, and the remaining one had progression of disease (23). However, at present, it remains unknown whether alternative regimens of temozolomide would work better than the standard regimen in patients with pituitary tumors.

The mechanism(s) by which temozolomide may act in pituitary tumors is still unknown. The patient with atypical PRL-secreting adenoma reported by Syro *et al.* (13) was subjected to another surgical procedure after successful treatment with temozolomide for 7 months. Histological analysis showed the presence of hemorrhages, necrosis, and fibrosis in comparison with the same tumor before temozolomide treatment (27), suggesting a potent antitumoral effect of the drug. This interpretation was confirmed by measuring the Ki-67 nuclear labeling index: it decreased from 40 to 60% in the pre-treatment specimen to 5% in the post-treatment sample (27). Neuronal transformation was also detected in the post-treatment specimen. It was supposed to indicate differentiation of the tumor, an event that may slow neoplastic growth (27). Recently, Sheehan *et al.* (28) reported the 'in vitro' effect of temozolomide on three pituitary adenoma cell lines. Temozolomide caused significant inhibition of cell proliferation at a concentration of 50 μ M in one cell line (AtT20) and 250 μ M in the other two cell lines (MMQ and GH3). Moreover, apoptosis significantly increased in all the cell lines after few hours of incubation (28).

More relevant is the search for prognostic factors able to predict which patient will benefit the most from this novel therapy. High levels of the DNA enzyme repair MGMT in tumor cells counteract the effects of alkylating agent (29). In patients with glioblastoma, epigenetic silencing of the MGMT gene by methylation of the MGMT promoter leads to low expression of MGMT in tumor cells and has been suggested as a predictive marker for a positive response to temozolomide (30, 31). A similar correlation with MGMT expression has been suggested for aggressive pituitary tumors (12, 17, 32). Indeed, absence or low levels of MGMT immunoreactivity predicted a positive response to temozolomide, while MGMT immunoreactivity in tumor cells was associated with a negative response to treatment (12, 17, 32). Our data on the correlation between MGMT status and clinical response to temozolomide are incomplete because the results of MGMT staining were not available in one case, whereas in the other two cases the tested samples were those obtained after temozolomide treatment. It is unclear, at present, whether previous exposure to temozolomide can affect MGMT expression in tissue samples. Despite these drawbacks, MGMT status, as assessed by immunohistochemistry, provided some useful information on the clinical response to temozolomide. Indeed, both patients with a positive response to temozolomide had a negative tumor staining for MGMT (however, one lacked a positive internal control), while one patient with no response to

temozolomide showed a strong positive staining for MGMT (no tissue was available for the other non-responsive patient). It is more difficult to interpret the data in the remaining patients who had stabilization but not regression of disease during temozolomide treatment. The tissue samples from both the patients have been harvested after drug treatment: one had a negative and the other a positive staining. Heterogeneity of tumor MGMT expression is well described in glioblastoma (33), and we have also previously observed heterogeneous tumor staining in the same pituitary tumors (12). It is possible that the positive MGMT immunohistochemistry result in this latter patient indicates resistance to temozolomide acquired by selection of an MGMT expressing clone during temozolomide treatment. However, no correlation between MGMT staining and the response to temozolomide was detected in the other two largest series (22, 23). Further studies, possibly through a multicenter collaboration, would be necessary to clarify this issue. Methylation of the MGMT promoter is less likely to possess any predictive value, as it was negative all the four patients tested in our series and in all the seven patients in the series of Bush *et al.* (23), irrespective of the clinical response to temozolomide.

In conclusion, temozolomide treatment has a wide range of efficacy in patients with pituitary carcinoma or locally aggressive pituitary adenoma. Some patients have a dramatic response, as demonstrated by shrinkage or disappearance of the tumor and normalization of hormone hypersecretion. The response may persist even after termination of a 12-month treatment. Arrest of tumor growth may be achieved in other patients, but the effect may not be sustained. Continuous treatment with temozolomide in such cases should be explored in the future. Lastly, some patients do not show any response to temozolomide. Study of the expression of MGMT in tumor cells may aid in selecting those patients with the highest probability to benefit from this drug, as positive staining for MGMT seems to predict a lower chance of response.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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