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CLINICAL STUDY

Temozolomide and pasireotide treatment for aggressive pituitary adenoma: expertise at a tertiary care center

Filippo Ceccato · Giuseppe Lombardi · Renzo Manara · Enzo Emanuelli · Luca Denaro · Laura Milanese · Marina Paola Gardiman · Roberta Bertorelle · Massimo Scanarini · Domenico D'Avella · Gianluca Occhi · Marco Boscaro · Vittorina Zagonel · Carla Scaroni

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Abstract Aggressive pituitary adenomas (PAs) are clinically challenging for endocrinologists and neurosurgeons due to their locally invasive nature and resistance to standard treatment (surgery, medical or radiotherapy). Two pituitary-directed drugs have recently been proposed: temozolomide (TMZ) for aggressive PA, and pasireotide for ACTH-secreting PA. We describe the experience of our multidisciplinary team of endocrinologists, neurosurgeons, neuroradiologists, oncologists, otolaryngologists and pathologists with TMZ and pasireotide treatment for aggressive PAs in terms of their radiological shrinkage and genetic features. We considered five patients with aggressive PA, three of them non-secreting (two ACTH-silent and one becoming ACTH secreting), and two secreting (one GH and one ACTH). TMZ was administrated orally at 150–200 mg/m^2 daily for 5 days every 28 days to all 5 patients, and 2 of them also received pasireotide 600–900 μg bid sc. We assessed the MRI at the baseline and during TMZ or pasireotide treatment. We also checked for *MGMT* promoter methylation and *IDH*, *BRAF* and *kRAS* mutations. Considering TMZ, two patients showed PA progression, one stable disease and two achieved radiological and clinical response. Pasireotide was effective in reducing hypercortisolism and mass volume, combined with TMZ in one case. Both treatments were generally well tolerated; one patient developed a grade 2 TMZ-induced thrombocytopenia. None of patients developed hypopituitarism while taking TMZ or pasireotide treatment. No genetic anomalies were identified in the adenoma tissue. TMZ and pasireotide may be important therapies for aggressive PA, alone or in combination.

F. Ceccato (✉) · M. Boscaro · C. Scaroni
Endocrinology Unit, Department of Medicine DIMED, University-Hospital of Padova, Via Ospedale Civile, 105, 35128 Padua, Italy
e-mail: ceccato.filippo@gmail.com

G. Lombardi · V. Zagonel
Department of Experimental and Clinical Oncology, Medical Oncology 1, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy

R. Manara
Neuroradiology, University of Salerno, Salerno, Italy

E. Emanuelli
Department of Otorhinolaryngology and Otologic Surgery, University-Hospital of Padova, Padua, Italy

L. Denaro · L. Milanese · D. D'Avella
Neurosurgery, Department of Neurosciences DNS, University-Hospital of Padova, Padua, Italy

M. P. Gardiman
Surgical Pathology and Cytopathology Unit, Department of Medicine DIMED, University-Hospital of Padova, Padua, Italy

R. Bertorelle
Immunology and Molecular Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy

M. Scanarini
Neurosurgical Division, University-Hospital of Padova, Padua, Italy

G. Occhi
Department of Biology, University of Padova, Padua, Italy

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Introduction

Pituitary adenomas (PAs) are usually considered benign tumors, although some reveal an "aggressive" pattern characterized by an invasive local growth and/or resistance to conventional therapies. Such tumors may coincide only partially with the atypical adenomas described in the WHO classification (characterized by a MIB-1 above 3 %, p53 immunoreactivity and a high mitotic index [1]), which account for 3–15 % of cases in surgical series [2, 3], but they are not always clinically aggressive. Pituitary tumors that develop distant metastases are extremely rare, and considered as carcinomas [1]. Whether they are more or less aggressive, PA pose a serious therapeutic challenge for endocrinologists and neurosurgeons.

Temozolomide (TMZ), mainly used to treat glioblastoma multiforme (GBM), since 2006 has been proposed as a treatment for pituitary carcinomas and aggressive adenomas [4, 5], for which it has a place in the therapeutic algorithm [6, 7]. Response to TMZ was initially reported to depend on the adenoma's expression of 6 methylguanine DNA methyltransferase (MGMT), a DNA repair enzyme that has the potential to interfere with the action of TMZ [7]. TMZ treatment is not effective in all aggressive PAs, so other therapeutic options may be useful. Pasireotide is a new somatostatin (SST) receptor ligand with a broad affinity for SST receptors (type 1, 2, 3, and 5), that has attracted interest and recently been recommended for the treatment of ACTH- and GH-secreting adenomas [8–10].

Cellular transformation in PA may involve a number of genetic anomalies, such as those reported in aryl hydrocarbon receptor interacting protein (AIP) for familial acromegaly [11], or sporadic GH-secreting PAs (in which it may regulate response to medical therapy [12]), or those reported in *CDKN1B* gene, mutations of which have been associated with multiple endocrine neoplasia, including PAs [13, 14]. Point mutations in the *isocitrate dehydrogenase* (*IDH*) gene have recently been found in GBM [15], but they have yet to be studied in PA. Other genetic pathways investigated in PAs and endocrine tumors, such as the tyrosine kinase (*BRAF* or *RAS* genes) mutations recently described in acromegalic patients with thyroid cancer [16], have not been found in PA to date [17, 18].

The aim of this report is to describe our experience with TMZ and pasireotide in the management of five aggressive PAs, focusing on radiological shrinkage and hormonal control. We also report our findings after checking for *MGMT* promoter methylation and somatic mutations of *IDH*, *BRAF* and *KRAS* genes as possible markers of response to the therapies.

Materials and methods

Patients, TMZ treatment and safety

We retrospectively analyzed the clinical and radiological features of 5 patients being routinely followed up at our Endocrinology Unit as at May 2014. All 5 patients had aggressive PAs characterized by resistance to the usual medical therapy (monthly octreotide doses >40 mg) and/or rapid tumor growth despite several surgical procedures or radiotherapy. After obtaining patients' written consent, TMZ was administered orally at 150–200 mg/m^2 daily for 5 days every 28 days, until disease progression or unacceptable toxicity occurred, or a maximum 24 cycles had been completed (see Table 1). TMZ toxicity was scored on the National Cancer Institute–Common Toxicity Criteria scale, version 4.0 (CTCAE v4.0). Physical examination, full blood counts and blood chemistry, including hepatic and renal function tests, were performed before each dose of TMZ was administered.

The study was performed in accordance with the guidelines in the Declaration of Helsinki and the local Ethics Committee approved the protocol.

Radiological examination

All patients had serial magnetic resonance imaging (MRI). For our purposes, we considered the images obtained after the latest surgical procedure, at the time of starting TMZ therapy, and at the last follow-up. The volume of the PA was measured on contrast-enhanced T1 sequences: the area of the lesion was drawn manually on each slice, then the sum of all the areas was multiplied by the thickness of the slice according to the formula: ΣArea $\times$ (slice thickness + interslice gap). MRI scans were performed with a 1.5 T (Achieva, Philips Medical Systems, Best, Netherlands) with a standard quadrature head coil.

Genetic analyses

Molecular analyses were performed on DNA extracted by a QIAmp DNA kit (Qiagen, Milan, Italy) from surgical tumor tissue before starting TMZ therapy. To analyze *MGMT* promoter methylation status, DNA from 10 μm paraffin-embedded sections of brain lesions was modified with sodium bisulfite—which converts unmethylated but not methylated cytosine into uracil—according to the procedure in the EpiTect bisulfite kit (Qiagen, Courtaboeuf

Table 1 Clinical picture and TMZ therapy

Case	Sex, age (years)	PA	MIB-1 (%)	p53	Prior surgery (n)	RT (months)	Age at start of TMZ (years)	TLS to T0 (months)	No. of TMZ cycles	HR	Response to TMZ	Tumor volume change with TMZ (%)
1	F, 67	NFPA	<3	Neg	1	No	69	26	6	n.a.	PD	+42
2	F, 39	GH	<3	Pos	2	No	48	28	3	no	PD	+45
3	M, 40	NFPA[a]	<3	Pos	4	Yes (1)	43	29	12	n.a.	PR	−49
4	M, 32	ACTH	>3	Pos	4	Yes (126)	47	26	24	yes	PR	−63
5	M, 47	NFPA[a] → ACTH	>3	Pos	2	Yes (86)	60	93	12	n.a.	SD	−6, −21[b]

Legend: *PA* Pituitary adenoma, *NFPA* non-functioning PA, *TMZ* temozolomide, *TLS to T0* time from latest surgery to starting TMZ in months, *HR* hormonal response, *n.a.* not applicable, *RT* radiotherapy (in brackets time from radiotherapy to TMZ treatment start), *PD* progression of disease, *PR* partial response, *SD* stable disease

[a] NFPA with positive ACTH immunohistochemistry

[b] Adenoma reduction after 6 months of combination treatment Pasireotide + TMZ

Cedex, France). Modified DNA was used as s template for a nested methylation-specific polymerase (MSP) chain reaction protocol. The first round was performed using a pair of external primers that does not discriminate between methylated and unmethylated sequences. The PCR product thus obtained was submitted to a second round of PCR using two primer pairs specific for methylated and unmethylated *MGMT* promoter sequences [19, 20]. The final PCR products were then run on 8 % polyacrylamide gel and the results were compared with methylated and unmethylated controls, i.e. the SW48 cell line and normal peripheral blood, respectively.

Moreover, DNA isolated from formalin-fixed tumor tissues underwent PCR using primer pairs specific for exon 4 of the *IDH-1*, for exon 15 of *BRAF* and for exon 2 of *KRAS* genes. The amplified products were submitted to sequencing analysis by fluorescent capillary electrophoresis (ABI PRISM 310 genetic analyzer, Applied Biosystems). All primer sequences and polymerase chain reaction (PCR) conditions are available on request.

Statistical analyses

We analyzed TMZ efficacy in terms of the disease control rate (DCR) and median progression-free survival (PFS). PFS was calculated from starting TMZ treatment to disease progression, death due to any cause, or the last day of follow-up if patients were still alive. Progression of disease (PD) was defined as an increase in volume ≥25 % and/or an unequivocal increase in hormone levels produced by the tumor. Complete response (CR) was defined as a complete disappearance of the PA and stable or unequivocally decreasing hormone levels; partial response (PR) was defined as a volume shrinkage ≥50 % and stable or unequivocally decreasing hormone levels; stable disease (SD) was defined as any case not qualifying as CR, PR or PD. The DCR was obtained from cases of CR + PR + SD, and the overall response rate (ORR) from CR + PR.

Results

Clinical picture

In our sample of five patients, three had non-functioning PA (NFPA; immunohistochemistry revealed ACTH-positive cells in two cases, which were consequently considered ACTH-silent PAs), and two had secreting adenomas (one GH and one ACTH). Even though all of our described patients were affected with clinically aggressive PAs, not all PAs would be defined as atypical adenoma considering WHO criteria [1] based upon MIB-1 index and p53 immunoreactivity (#1, see Table 1).

The secretion pattern changed in two of the PAs: patient #2 with immunohistochemical evidence of a GH-positive NFPA developed acromegaly a year before starting to take TMZ, while on cabergoline treatment (to control prolactin excess due to pituitary stalk deviation); and patient #5 with an ACTH-silent NFPA developed full-blown Cushing's disease (CD) while taking TMZ, with an impaired salivary cortisol rhythm and high urinary free cortisol levels, for which pasireotide (600 μg sc. bid) was associated with the TMZ treatment. In Patient #4 ACTH secretion returned to normal after 3 months of TMZ therapy, and remained so for the 24 months of the therapy and for a further year after TMZ was discontinued; then there was a biochemical and radiological recurrence, which was treated effectively with pasireotide alone (first 600 μg bid, then 900 μg bid).

Disease control rate, progression-free survival and safety

Patient #1 and #2 discontinued TMZ due to the PA progression. Patients #5 showed no radiological shrinkage after 6 months of therapy, while patients #3 and #4 continued the treatment, the former achieving a radiological response, the latter a radiological and clinical response (see Table 1). In terms of TMZ treatment's activity (see Table 1), two patients obtained a PR, two had PD and one had SD. Thus, for all five patients, the DCR was 60 % and the ORR 40 %. Median PFS was 18 months. As regards safety, the treatment was generally very well tolerated, with only one patient (20 %) developing grade 2 thrombocytopenia. Two patients developed full-blown CD due to ACTH secretion. In one (#4), we started administering pasireotide 12 months after the patient had stopped taking TMZ; in the other (#5) we associated pasireotide with the TMZ treatment underway. In both cases, pasireotide normalized 24-h urinary free cortisol and late night salivary cortisol levels, moreover in one case pasireotide induced adenoma shrinkage. Pasireotide treatment was well tolerated: patients did not manifest gastrointestinal symptoms, gallstones or significant worsening of glucose control: patient #4 fasting blood glucose levels have raised from 6.8 to 7.5 mmol/L (normal value 3.7–5.6) and glycated hemoglobin from 39 to 43 mmol/mol (normal value 20–38) after pasireotide treatment, and patient #5 fasting blood glucose levels have raised from 5.4 to 6 mmol/L and glycated hemoglobin from 43 to 46 mmol/mol.

Radiological features

All patients showed an increase in tumor volume before starting chemotherapy (see Fig. 1). After taking TMZ, in three of them (all males) the tumor volume shrank or remained stable (with volume reductions of 49 % in Patient

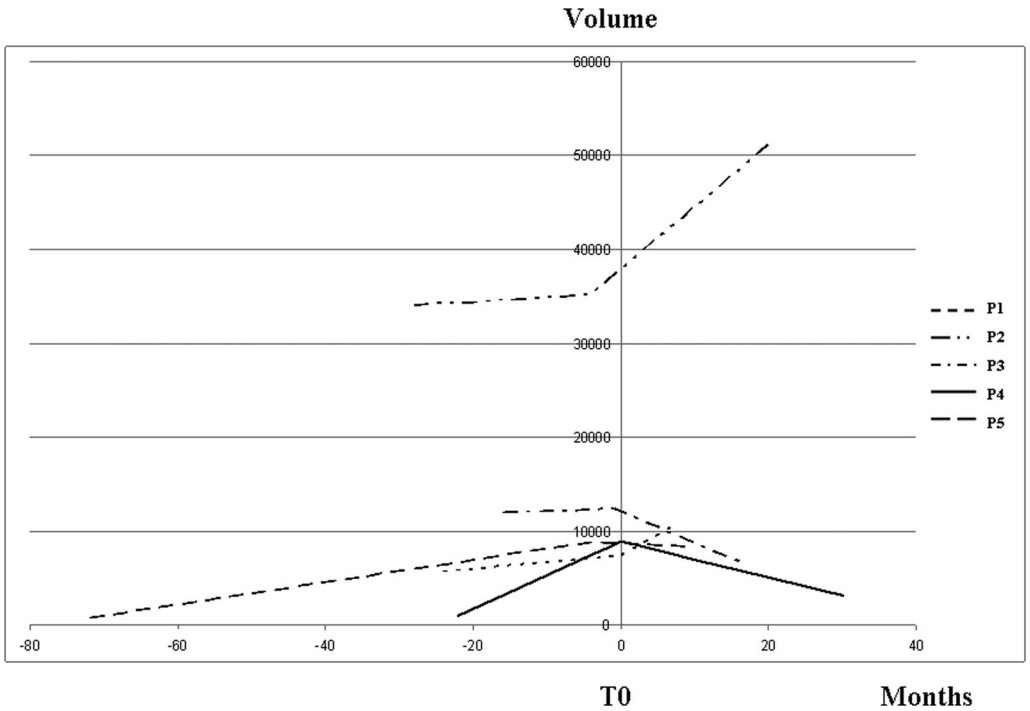

Fig. 1 Trend of adenoma volume in five patients treated with temozolomide, as measured on contrast-enhanced T1 sequences after latest surgery, before TMZ treatment and at latest follow-up (related to TMZ use). Months calculated from T0 (when TMZ treatment started) on the abscissa

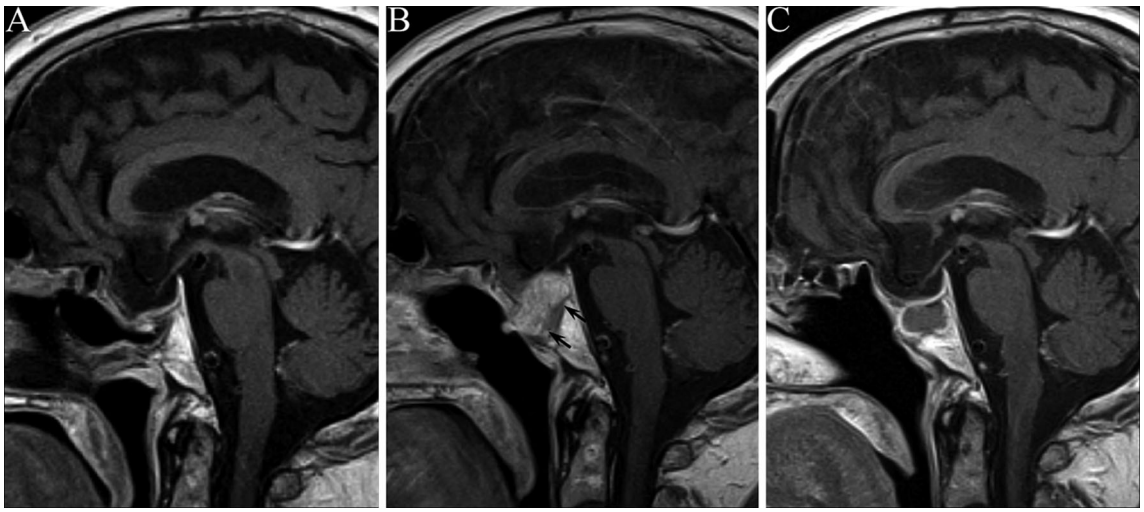

Fig. 2 MRI of Patient #4 showing evolution of pituitary adenoma volume: **a** after latest surgery (1,034 mm^3), **b** before TMZ (8,887 mm^3; *arrows* indicate the contrast enhancing mass appeared in the pituitary sella); and **c** after TMZ (3,224 mm^3)

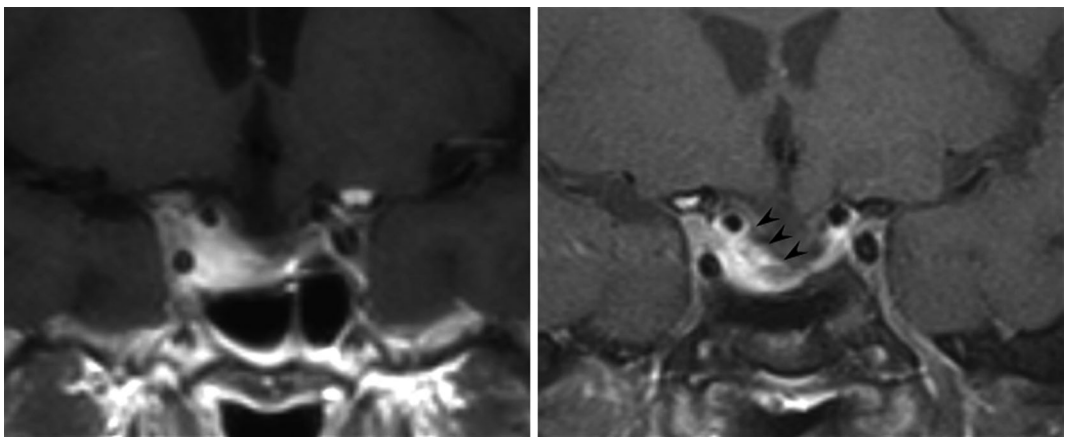

Fig. 3 Contrast enhanced T1-weighted MRI images of Patient #5 showing the pituitary adenoma shrinkage (*arrowheads*) from 3,632 mm^3 at baseline to 2,875 mm^3 after 6 months of Pasireotide + TMZ combined treatment (volume reduction of 21 %)

#3, 63 % in Patient #4, and 6 % in Patient #5) (Fig. 1); they had all previously been administered radiotherapy (two conventional and one stereotaxic). Patient #4 showed a marked reduction in tumor volume (Fig. 2). The lesion in Patient #3 revealed cystic necrosis, with a persistent suprasellar remnant.

In the other 2 patients (both females; aged 69 and 48 years), TMZ treatment did not prevent tumor growth (Fig. 1). The tumor's volume increased by 42 % in Patient #1, and by 45 % in Patient #2; neither of these patients had received any prior radiotherapy.

As for the pasireotide treatment, the adenoma shrank in Patient #5 who took it in association with TMZ (3,632 mm^3 at baseline, 2875 mm^3 after 6 months of Pasireotide + TMZ, volume reduction of 21 %, as depicted in Fig. 3), but not in Patient #4, who was given pasireotide alone.

Molecular biology

We performed genetic analyses on the tumor tissue from four of the five patients to identify *MGMT* methylation status; and *IDH1*, *BRAF* and *KRAS* gene mutations. All four patients showed an unmethylated *MGMT* gene status and no mutations were found on the other genes analyzed (see Table 2).

Discussion

In recent years, the use of TMZ treatment has emerged as a possible new pituitary-directed therapy for patients with aggressive PAs if conventional medical, surgical, or radiotherapies fail [6, 7, 21]. In our series, the tumor type treated with TMZ was mainly ACTH- silent/secreting,

Table 2 Findings of genetic analyses

Case	*MGMT* methylation status	*IDH1* mutation	*BRAF* mutation	*KRAS* mutation
1	n.a.	n.a.	n.a.	n.a.
2	Not methylated	wt	wt	wt
3	Not methylated	wt	wt	wt
4	Not methylated	wt	wt	wt
5	Not methylated	wt	wt	wt

Legend: *n.a.* not available, *wt* wild-type

which is known to be aggressive, especially when it has the features of a macroadenoma. This type of tumor differs slightly from the types most often described in the literature, i.e. prolactin-secreting PA, followed by NFPA. We found TMZ capable of inducing a good radiological and clinical response only in some cases of aggressive PA (in our series the DCR was 60 % and the ORR 40 %). We found a better response in PAs with a positive ACTH immunohistochemistry (either silent or secreting with CD), and the positive response to TMZ was recorded within 3 months of starting the therapy, suggesting that an alternative therapeutic strategy should be considered if no response is apparent by then.

The two patients who experienced an "endocrine shift" in their secreting pattern (from GH- or ACTH-silent to acromegaly and full-blown CD, before starting TMZ in the former and while on TMZ treatment in the latter) showed some degree of resistance to TMZ: in the first, we recorded an increase in PA volume and hormonal progression; the second's hormonal status progressed from the silent to the ACTH-secreting form, while the volume of the tumor remained stable. This unusual finding points to a possible correlation between radiological and hormonal improvements in cases of hormone-secreting PA, in which case the secreted hormone may therefore be a sensitive marker of tumor response. On the other hand, combining pasireotide with TMZ in the second of the above-mentioned patients proved successful in improving both the secreting pattern and the radiological picture.

In our series pasireotide, the recently available SST receptor ligand with high affinity to SST 2 and 5 receptors [8, 9], was used in two patients with TMZ-resistant aggressive ACTH-secreting PA. In both cases pasireotide was effective in controlling cortisol secretion within 3 months, as reported in a large series of CD patients [9], thus offering a further chance of successful treatment, either in combination with or after TMZ. Moreover in one case the association of Pasireotide with TMZ induced a consistent adenoma shrinkage in 6 months (volume-21 %), whereas TMZ alone did not affect tumoral mass; the association of TMZ and pasireotide treatment has been already reported by Bode et al. in a pituitary ACTH-secreting carcinoma with intracranial, spinal and liver metastases, obtaining a stabilization of disease: we confirm that combined TMZ and pasireotide treatment may be a salvage therapy also in aggressive PAs [22]. Tumor regrowth after an initially positive response to TMZ, as in our Patient #4, has already been reported in the literature, and some such cases were retreated with TMZ, but with disappointing results [7].

There have recently been reports of different outcomes between males and females with ACTH-secreting PA [23]. Even in our small series, we too found a gender-related difference, with males responding better to TMZ than females (though we have to bear in mind the likely effect of radiotherapy, which was only administered to our male patients, and the combination of radiotherapy and TMZ may have been beneficial). It is worth noting that TMZ was administered a month after radiotherapy in our patient #3, and 6 months later the adenoma showed signs of cystic degeneration and necrosis, with a persistent suprasellar remnant. The effect of combining radiotherapy and TMZ could not be clearly evaluated in our patients. Considering all the 12 patients with PA who underwent both radiotherapy and TMZ treatment (9 reported in the literature and 3 described in the present series), 2 did not respond and showed tumour growth, 8 presented tumour size reduction and 2 manifested initial stabilization and subsequent progression after several months [23–29]. Even though these results are encouraging, further studies are needed to establish the increased efficacy of combined therapies and the correct timing to combine TMZ and pituitary radiation. Previous publications on gliomas reported that TMZ treatment administered concurrently with radiotherapy could lead to radiation-induced necrosis being mistaken for tumor progression or recurrence: this so-called "pseudo-progression" might lead to follow-up neuroimages being misinterpreted, and consequent inappropriate treatment planning [30]. Patient #4 experienced adenoma shrinkage and a reduction in hormonal secretion already after three cycles of TMZ, and right up until last dose. Other studies had indicated that an early response to TMZ treatment predicted a good further response [31]. TMZ might induce metabolic and structural changes in the adenoma, inducing shrinkage, hemorrhage or necrosis [32], but response to treatment varies among patients, in terms of both tumor volume reduction and timing of the response. Our case series confirmed this variety of responses to TMZ

treatment, with 2/5 patients experiencing a significant reduction in tumor volume—consistent with a PR to TMZ therapy [7]—while in one the therapy only prevented the adenoma from growing further.

A correlation between low levels of MGMT protein, attributed to epigenetic silencing by gene promoter methylation, and TMZ efficacy has been shown in several tumours. In aggressive PAs or carcinomas, on the basis of the inconsistency of published data Raverot et al. in a recent review indicated that MGMT status should not be taken into account to select patients who might benefit from TMZ treatment [7]. Assessment of MGMT status should be performed by immunohistochemistry, which evaluates the level of protein expression, or by MSP, that assesses gene epigenetic silencing at DNA level; however both analyses showed a variable positive predictive value of TMZ response (respectively mean 67 and 53 %) [7]. Furthermore, Salehi et al. reported a lack of relationship between methylation status and MGMT protein expression in aggressive pituitary tumours [33], probably because other mechanisms of silencing may also play a role. In our surgical specimens we studied *MGMT* promoter methylation by MSP as routine analyses (since this is the only validated test to obtain prognostic or predictive information for patients with GBM [34]), but we treated patients with TMZ independently to MGMT status (all the patients in our study had unmethylated *MGMT* gene promoter). Our results confirm previous data about the lack of correlation between *MGMT* promoter methylation and clinical response to TMZ treatment in pituitary tumors, suggesting that other cellular mechanisms may be involved.

The molecular mechanisms of pituitary cell transformation are far from being fully discovered. Recently, point mutations in the *IDH* gene have been found as an early genetic alteration in most GBM, and other genetic pathways (as tyrosine kinase) might be involved also in PAs [15, 16]. We checked this gene and looked for any *KRAS* and *BRAF* mutations to see if they had a role in pituitary tumors too. All these genes were wild-type in our patients, however. It is likely that studies on larger samples patients are needed to identify any role of these genes in pituitary tumors.

When considering antineoplastic drugs, it is important to assess both their efficacy and their safety. Our series of patients had aggressive PAs that had already been treated with multiple surgical procedures (mean 2.6 per patient) or radiotherapy (in three out of five). None of the patients developed new pituitary deficiencies while taking TMZ, confirming that any hypo-pituitarism could be related more to surgery or radiotherapy than to medical treatments [35, 36]. Although TMZ therapy is well-tolerated by most patients, it may carry side effects, the most important of which is hematological toxicity [37]. In our series, only one patient developed mild thrombocytopenia (grade 2), indicating that TMZ is generally safe. In two patients, pasireotide induced none of the well-known side effects typical of SST analogs or worsening of glucose control [9].

Conclusions

New pituitary-directed drugs, such as TMZ and pasireotide, are available at tertiary care centers nowadays for the management of aggressive PA. TMZ is a therapeutic option recommended for patients who suffer from multiple recurrences or tumor growth despite maximal standard therapy (i.e. multiple surgery or radiotherapy): it prompts a positive clinical and/or radiological response in some patients, usually without any severe adverse events. Pasireotide is another novel pituitary-directed drug that has been approved for use in adult patients with CD recurring after surgery has failed; we tested its efficacy in two patients with aggressive PA. Close cooperation between members of a multidisciplinary team of endocrinologists, neurosurgeons, otolaryngologists, neuroradiologists, pathologists and oncologists is of the utmost importance in the management of aggressive PAs.

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