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CASE REPORT

# Temozolomide in the treatment of an invasive prolactinoma resistant to dopamine agonists

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**Abstract** Prolactinomas are common tumors of the anterior pituitary gland. While conventional therapies, including dopamine agonists, transsphenoidal surgery and radiotherapy, are usually effective in controlling tumor growth, some patients develop treatment-resistant tumors. In this report, we describe a patient with an invasive prolactinoma resistant to conventional therapy that responded to the administration of the alkylating agent, temozolomide.

**Keywords** Prolactinoma · Temozolomide · Dopamine agonist resistance

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## Introduction

Prolactinomas account for 40–50% of all pituitary tumors. Most are small and do not enlarge significantly over time [1]. Dopamine agonist therapy results in a rapid normalization of serum prolactin concentration and a reduction in tumor size in 70–90% of patients, making this the initial treatment of choice for most patients, if therapy is indicated [1]. However, resistance to conventional doses of dopamine agonists can occur. In such cases, alternative treatment approaches, including high-dose dopamine agonist therapy, surgery, and radiotherapy, may be successful [2]. When those modalities fail to control tumor growth, however, experimental therapies may need to be considered. We treated a woman with an invasive macroprolactinoma that was refractory to a variety of conventional therapies, including dopamine agonist therapy, transsphenoidal surgery, and radiation therapy. After unsuccessful trials of several unconventional treatments, including estrogen antagonism and octreotide therapy, the patient's tumor responded well to the alkylating agent, temozolomide.

## Case history

A 52-year-old Asian woman was referred to the Neuroendocrine Clinic at Tufts-New England Medical Center in 2003 for treatment of a macroprolactinoma that was refractory to dopamine agonist therapy. Menarche occurred around age 12, and she had regular menstrual cycles until her late 20s, when amenorrhea ensued. Evaluation at that time revealed hyperprolactinemia and a pituitary microadenoma. She was treated with bromocriptine for approximately 10 years, but despite pharmacotherapy, her serum prolactin level increased to 600 ng/ml (normal lab range 3–23 ng/ml).

In the late 1980s, magnetic resonance imaging (MRI) showed tumor growth, and the patient developed hypopituitarism. Visual field testing was normal. The bromocriptine dose was increased to 20 mg daily, and the tumor size decreased. However, the patient's prolactin level remained elevated and was associated with amenorrhea and infertility. In 1990, transsphenoidal surgery revealed old hemorrhage and a small sellar tumor. There was an initial reduction in the serum prolactin level, but the patient was unable to conceive, even with assisted reproductive technology.

In 1991, estrogen replacement therapy was begun for amenorrhea and osteoporosis. Due to continued tumor growth and hyperprolactinemia despite bromocriptine therapy, external beam radiation therapy was given. Over the course of one month in 1991, the patient received 44 Gy in 22 fractions delivered to the mid-plane of the sella turcica and the suprasellar portion of the base of the brain via parallel opposed lateral portals, using 6 MV photons.

In 1998, she was switched from bromocriptine to cabergoline at a dose of 1 mg twice per week, estrogen therapy was discontinued, and a bisphosphonate was initiated for osteoporosis. Despite treatment with cabergoline, the prolactin level increased during the following year to approximately 800 ng/ml and the adenoma increased in size from 1.7 cm to 2 cm, with lateral extension into the left cavernous sinus. The cabergoline dose was increased to 2 mg twice a week without benefit. Accordingly, Gamma knife radiotherapy was administered in 2000 with 18 Gy delivered to the 50% isodose line. After an initial reduction in both the serum prolactin and the size of the tumor, the prolactin level again began to increase, and by late 2001, it exceeded 2,000 ng/ml. The cabergoline dose was increased to 4.5 mg per week in divided doses, but in 2003 she developed diplopia due to a right sixth nerve palsy. Imaging revealed growth of the tumor into the right cavernous sinus. The serum prolactin concentration rose quickly to over 4,800 ng/ml, and the patient was referred to the Neuroendocrine Clinic for further evaluation and treatment.

At presentation in August 2003, she complained of increasing fatigue and double vision for 5 months. Physical examination revealed normal visual fields but limited motion of her right eye on rightward gaze. There was no galactorrhea. The dose of cabergoline was increased gradually in divided doses to 8 mg per week (the maximal dose tolerated by the patient), but there was no resulting decrease in prolactin concentration. In the hope that estrogen receptor antagonism might have a beneficial effect, the patient's osteoporosis therapy was changed from a bisphosphonate to the selective estrogen receptor modulator (SERM) raloxifene, and the prolactin level declined modestly to 4,000 ng/ml. A nuclear medicine study revealed abnormal localization of the somatostatin analog OctreoScan® (Indium In-111 Pentetriotide) in the area corresponding to the tumor, so a trial of octreotide was initiated and high-doses of cabergoline were continued along with raloxifene. Therapy was initially begun with Octreotide 100 mcg subcutaneously three times per day and later replaced by Octreotide LAR, 30 mg intramuscularly once a month. Initially, there was a 1,400 ng/ml decline in the prolactin concentration without a concomitant decrease in the tumor size. After a few months, however, the prolactin level again began to rise rapidly and the tumor expanded further, compressing the optic chiasm. The patient reported increased severity of diplopia despite the use of prism lenses, along with fatigue, headaches, and anorexia, which led to a 13% weight loss. She was unable to continue working and went on disability leave. Her neurosurgeon felt additional surgery would involve excessive risk given the location of the tumor in the cavernous sinus and the amount of residual scarring from surgery and radiation. Raloxifene was replaced by the aromatase inhibitor, letrozole (2.5 mg daily), and a bisphosphonate was restarted for osteoporosis. The patient became progressively more debilitated, and her prolactin level increased to approximately 7,000 ng/ml within several months. At this point, there was concern about the possibility of malignant transformation. A computed tomography (CT) scan of the chest, abdomen, and pelvis revealed no evidence of extracranial metastasis, and while an MRI of the sella revealed slight extension of the tumor into the left side of the sphenoid sinus, there was no evidence of intracranial metastasis.

The histopathology of the tumor resected in 1990 was reviewed and immunohistochemical studies were performed to search for features that might have been predictive of the tumor's aggressive behavior. Paraffin

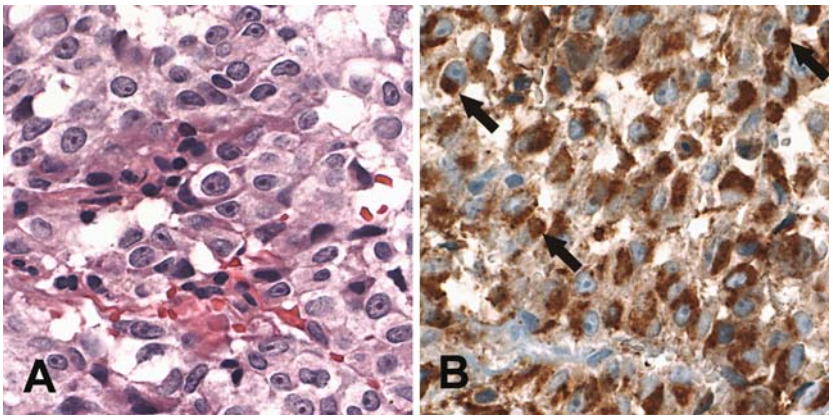


**Fig. 1** Histological appearance of the tumor resected in 1990. (**A**) Hematoxylin and eosin stain; (**B**) Immunohistochemical stain for prolactin. The tumor cells have somewhat prominent nucleoli, which may also be seen in non-aggressive pituitary adenomas, but no other distinctive features. Arrows in **B** indicate prominent dot-like or crescentic distribution of prolactin staining, corresponding to location of the cells' Golgi apparatus (original magnifications 40×)

sections were stained for prolactin, growth hormone, Ki67, p53, HER2 and galectin 3. The tumor showed no unusual histological features (Fig. 1A). All of the tumor cells stained for prolactin, mostly in a ''Golgi'' pattern (Fig. 1B) typical of sparsely granulated prolactin cell adenomas [3], while staining for growth hormone was negative. There was no identifiable mitotic activity. Labeling index for the proliferation marker, Ki67, was less than 5% and within the range for benign adenomas [3]. Staining was negative for p53, HER2 and galectin 3, which have been correlated with malignant or aggressive behavior of pituitary tumors in some studies [3, 4].

On the basis of a preliminary report describing a beneficial response of a treatment-refractory malignant prolactinoma to the alkylating agent, temozolomide (Temodar®) [5, 6], the patient was referred to a neuro-oncologist for consideration of temozolomide therapy. Octreotide LAR and letrozole were discontinued, and in August 2004, the patient started temozolomide 250 mg (150 mg/m$^2$) by mouth daily for 5 days out of each 28-day cycle, together with oral ondansetron and dexamethasone for nausea prophylaxis. High-dose cabergoline therapy was continued. Within several weeks, the patient noticed improvement in her appetite and physical stamina, less frequent diplopia, and a decrease in the frequency and severity of headaches. At her follow-up visit in clinic one month after treatment initiation, she had gained eight pounds and had noticeable improvement of abduction of her right eye. In addition, serum prolactin fell from approximately 7,000 ng/ml to 2,500 ng/ml. The cyclic treatments were continued and one month later, an MRI of the sella revealed a substantial decrease in the size of the tumor. A follow-up MRI in February 2005 showed further reduction in tumor size. Serum prolactin continued to decrease until July 2005, after which time it stabilized at 150–260 ng/ml. Throughout temozolomide treatment, the patient reported mild to moderate fatigue, a common side effect of temozolomide therapy. Otherwise, she tolerated the medication well, without significant nausea, vomiting, myelosuppression, or other adverse events. She regained all of her lost weight. She has been taking temozolomide for 26 months, and the tumor size and prolactin level continue to be stable. The right sixth cranial nerve palsy is present but stable, and strabismus surgery is scheduled. Figure 2 shows the patient's serum prolactin concentration over the course of treatment since 1990, and Fig. 3A and B are selected images from the patient's MRI studies before and after temozolomide therapy, respectively.

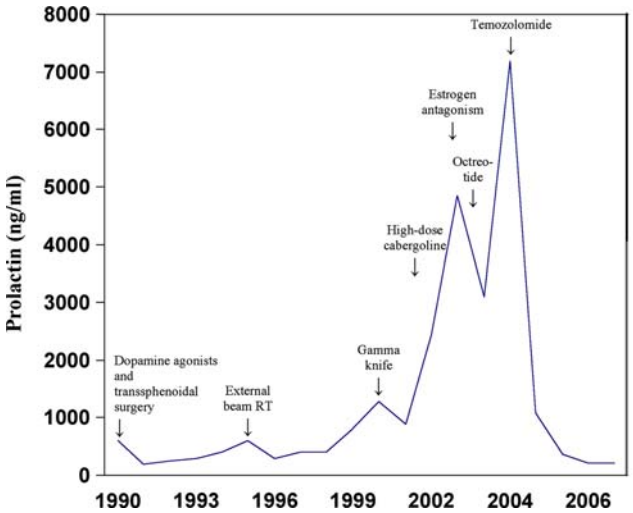


**Fig. 2** Serum prolactin level over the course of treatment since 1990

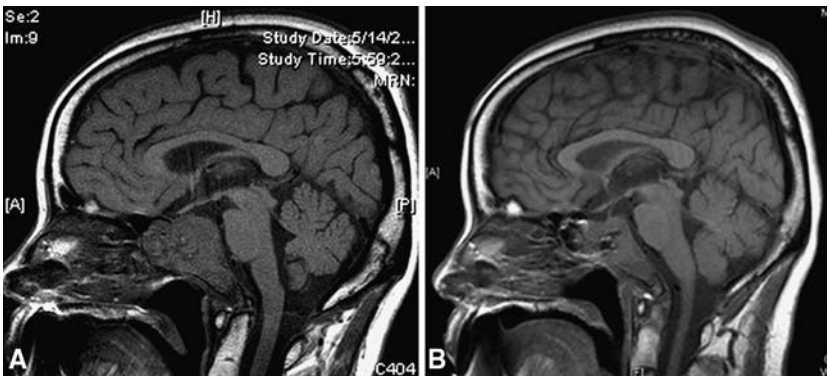


**Fig. 3** Magnetic resonance images from studies done before (**A**) and after (**B**) temozolomide therapy. Note the marked reduction in the size of the invasive prolactinoma following therapy in **B**

## Discussion

For patients with prolactinomas refractory to dopamine agonist therapy, transsphenoidal surgery is often recommended. With neurosurgery, long-term resolution of hyperprolactinemia is achieved in around half of patients with microadenomas and less than a quarter of those with macroadenomas [1]. However, many individuals who undergo tumor resection may continue to be symptomatic or have residual disease. These patients require additional treatment, either with continued dopamine agonist therapy or with other therapeutic modalities. One such modality, external beam radiation therapy, has been shown to control tumor growth and normalize serum prolactin concentration in 20–50% of patients [7, 8]. Stereotactic radiosurgery or gamma knife radiotherapy may also be an option, normalizing serum prolactin levels in around 25% of patients, achieving a partial hormonal response in over 60%, and reducing tumor size in over 50% [9].

Our patient had an inadequate response to high-dose dopamine agonist therapy, transsphenoidal excision, external beam radiation therapy, and gamma knife radiotherapy, and additional courses of radiation and neurosurgical intervention were not advised. As a result, estrogen antagonism, aromatase inhibition and octreotide therapy were begun, but without significant response.

The rationale for the use of an estrogen antagonist is based on evidence that prolactinomas may enlarge in association with hormone-replacement therapy or pregnancy and may regress during menopause [10–13]. In retrospect, the use of hormone replacement therapy in this patient may have contributed to the growth of the tumor between 1991 and 1998, although the most rapid and clinically significant tumor growth occurred after 2000, when the patient was no longer on estrogen. Estrogen receptors have been identified on prolactinoma cells, and estrogen stimulates the production and release of prolactin in vitro [14, 15]. In addition, estrogen appears to decrease the responsiveness of pituitary tumor cells to dopamine agonists, and in vitro studies suggest that tamoxifen, a SERM, can attenuate this effect [15]. Small studies suggest that up to 62% of patients with dopamine agonist-resistant prolactinomas may have a beneficial response to SERMs [16, 17].

Unlike SERMs, which act by binding to the estrogen receptor, aromatase inhibitors block the peripheral aromatization of androgens, thereby reducing blood and tissue levels of estrogen. In women with estrogen receptor-positive breast cancers, aromatase inhibitors have been shown to be more effective than SERMs at reducing recurrence rates [18, 19]. The use of aromatase inhibitors has not been studied in patients with prolactinomas, although an isolated case report suggested that it may have some therapeutic potential [20]. Unfortunately, estrogen antagonism with raloxifene and letrozole had no beneficial effect in our patient.

Somatostatin analogs are able to reduce the secretion of both growth hormone and prolactin in over 50% of patients with pituitary tumors that co-secrete these hormones [21, 22]. Prolactinomas have been shown to express all five subtypes of the somatostatin receptor (SSTR), with SSTR-5 predominating in 70% of tumors [23]. Currently available somatostatin analogs which have selective affinity for SSTR-2 and SSTR-5, however, have produced favorable results in only a small minority of prolactinomas [12, 24, 25]. Somatostatin receptor analogs that have broader affinity for somatostatin receptors (such as SOM-230) may have greater promise in the treatment of refractory prolactinomas [23, 25, 26]. In addition, given the potential for interaction between somatostatin and dopamine receptors to enhance postreceptor signaling, chimeric drugs such as the somatostatin-dopamine molecule, BIM-23A307, may also have utility in the treatment of resistant tumors [27, 28]. Nevertheless, attempts at control of our patient's invasive prolactinoma with a combination of octreotide and cabergoline were ineffective.

The use of a variety of chemotherapeutic agents, including 5-fluorouracil, lomustine, carboplatin, and etoposide, has been reported in the treatment of highly aggressive or malignant prolactinomas [29, 30]. No histologic features or immunohistochemical markers suggestive of aggressive clinical behavior were apparent in this patient's tumor at the time of surgery in 1990. Furthermore, there was no evidence of intra- or extracranial metastasis to suggest that a malignant transformation had occurred. However, in light of the aggressive and refractory nature of the tumor, it is possible that the tumor evolved over time and that a specimen obtained now might exhibit features suggestive of transformation. Nevertheless, response rates to chemotherapeutic agents are very poor, and clinical improvements, if they occur, are often fleeting.

The report by Zhu et al. of the successful use of the alkylating agent, temozolomide, in a patient with a refractory malignant prolactinoma led us to consider the use of this agent [5, 6]. Temozolomide is a prodrug, which is rapidly hydrolyzed into the active compound, 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC), under physiological conditions. MTIC is thought to exert a cytotoxic effect through the methylation (alkylation) of DNA. Temozolomide can be given orally and is currently approved in the U.S. for the treatment of patients with refractory anaplastic astrocytoma and for the treatment of newly diagnosed patients with glioblastoma multiforme, as an adjunct to radiotherapy. Trials have also been conducted to investigate the use of temozolomide alone or in combination with other agents in patients with other malignancies, including neuroendocrine tumors and metastatic melanoma [31–33]. In comparison to many other chemotherapeutic agents, temozolomide is well tolerated, although mild to moderate nausea, vomiting, and fatigue commonly occur. Myelosuppression occurs in a minority of patients and is more common in women and elderly patients. As with other alkylating agents, there is theoretical concern about the potential for development of myelodysplastic syndromes and secondary hematological malignancies, but these are rare.

The explanation for the response of prolactinomas to temozolomide is speculative, but it is conceivable that like temozolomide-responsive gliomas, responsive prolactinomas harbor a methylated methyl-guanine methyltransferase (MGMT) gene promoter [34]. In a variety of human tumors, MGMT gene promoter hypermethylation has been associated with epigenetic inactivation of the gene and the subsequent loss of the MGMT protein, which plays an important role in the repair of DNA damage, including that induced by alkylating agents such as temozolomide [35, 36]. In therapeutic trials of radiation and temozolomide in patients with malignant gliomas, those whose tumors contained a hypermethylated MGMT gene promoter had a significantly longer survival time than patients whose tumors had an unmethylated MGMT gene promoter [34, 37]. Analysis of the methylation status of the MGMT promoter in a series of patients with invasive prolactinomas treated with temozolomide would allow confirmation of this hypothesis.

We conclude that the alkylating agent, temozolomide, may be an effective and well-tolerated drug for the treatment of patients with invasive prolactinomas unresponsive to conventional therapy. This drug has the potential not only to reduce circulating prolactin levels, but also to substantially reduce tumor size. Further studies should be conducted in order to determine the usefulness of this medication in patients with prolactinomas that are refractory to standard therapies.

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## References

1. Gillam M, Molitch M, Lombardi G, Colao A (2006) Advances in the treatment of prolactinomas. Endocrine Rev 27:485–534
2. Molitch M (2005) Pharmacologic resistance in prolactinoma patients. Pituitary 8:43–52
3. Saeger W, Farrell WE, Horvath E, Lloyd RV, Kovacs K, Watson RE Jr, Lindell EP (2004) Proloctin secreting adenoma. In: DeLellis R, Lloyd R, Heitz P (eds) WHO classification of tumours, pathology & genetics, tumours of endocrine organs. IARC Press, Lyon, pp 20–23
4. Riss D, Jin L, Qian X, Bayliss J, Scheithauer B, Young WJ, Vidal S, Kovacs K, Raz A, Lloyd R (2003) Differential expression of galectin-3 in pituitary tumors. Cancer Res 63:2251–2255
5. Zhu Y, Shahinian H, Bonert V, Lim S, Heaney A (2004) OR 43–5 Temodar: novel treatment for pituitary carcinoma. In The Endocrine Society Annual Meeting
6. Lim S, Shahinian H, Maya M, Yong W, Heaney A (2006) Temodar: novel treatment for pituitary carcinoma. Lancet Oncol 7:518–520
7. Tsagarakis S, Grossman A, Plowman P, Jones A, Touzel R, Rees L, Wass J, Besser G (1991) Megavoltage pituitary irradiation in the management of prolactinomas: long-term follow-up. Clin Endocrinol 34:399–406
8. Tsang R, Brierley J, Panzarella T, Gospodarowicz M, Sutcliffe S, Simpson W (1996) Role of radiation therapy in clinical hormonally-active pituitary adenomas. Radiother Oncol 41:45–53
9. Brada M, Ajithkumar T, Minniti G (2004) Radiosurgery for pituitary adenomas. Clin Endocrinol 61:531–543
10. Garcia M, Kapcala L (1995) Growth of a microprolactinoma to a macroprolactinoma during estrogen therapy. J Endocrinol Invest 18:450–455

11. Molitch M (2002) Medical management of prolactin-secreting pituitary adenomas. Pituitary 5:55–65
12. Bronstein MD (2005) Prolactinomas and pregnancy. Pituitary 8:31–38
13. Karumakaran S, Page R, Wass J (2001) The effect of the menopause on prolactin levels in patients with hyperprolactinaemia. Clin Endocrinol 54:295–300
14. Kaptain G, Simmons N, Alden T, Lopes M, Vance M, Laws E (1999) Estrogen receptors in prolactinomas: a clinicopathological study. Pituitary 1:91–98
15. Lamberts S, Verleun T, Hofland L, Oosterom R (1986) Differences in the interaction between dopamine and estradiol on prolactin release by cultured normal and tumorous human pituitary cells. J Clin Endocrinol Metab 63:1342–1347
16. Volker W, Gehring W, Berning R, Schmidt R, Schneider J, von zur Muhlen A (1982) Impaired pituitary response to bromocriptine suppression: reversal after bromocriptine plus tamoxifen. Acta Endocrinol 101:491–500
17. Lamberts S, Verleun T, Oosterom R (1982) Effect of tamoxifen administration on prolactin release by invasive prolactin-secreting pituitary adenomas. Neuroendocrinol 34:339–342
18. Mouridsen H, Gershanovich M, Sun Y, Perez-Carrion R, Boni C, Monnier A, Apffelstaedt J, Smith R, Sleeboom H, Jaenicke F, Pluzanska A, Dank M, Becquart D, Bapsy P, Salminen E, Snyder R, Chaudri-Ross H, Lang R, Wyld P, Bhatnagar A (2004) Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. J Clin Oncol 21:2101–2109
19. Thurlimann B, Keshaviah A, Coates A, Mouridsen H, Mauriac L, Forbes J, Paridaens R, Castiglione-Gertsch M, Gelber R, Rabaglio M, Smith I, Wardly A, Price K, Goldhirsch A (2005) A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. New Engl J Med 353:2747–2757
20. Gilliam M, Middler S, Freed D, Molitch M (2002) The novel use of very high doses of cabergoline and a combination of testosterone and an aromatase inhibitor in the treatment of a giant prolactinoma. J Clin Endocrinol Metab 87:4447–4451
21. Freda P (2002) Somatostatin analogs in acromegaly. J Clin Endocrinol Metab 87:3013–3018
22. Lamberts S, Zweens M, Klijn J, van Vroonhoven C, Stefanko S, Del Pozo E (1986) The sensitivity of growth hormone and prolactin secretion to the somatostatin analogue SMS 201–995 in patients with prolactinomas and acromegaly. Clin Endocrinol 25:201–212
23. Jaquet P, Ouafik L, Saveanu A, Gunz G, Fina F, Dufour H, Culler M, Moreau J (1999) Quantitative and functional expression of somatostatin receptor subtypes in human prolactinomas. J Clin Endocrinol Metab 84:3268–3276
24. Shimon I, Yan X, Taylor J, Weiss M, Culler M, Melmed S (1997) Somatostatin receptor (SSTR) subtype-selective analogues differentially suppress *in vitro* growth hormone and prolactin in human pituitary adenomas. Novel potential therapy for functional pituitary tumors. J Clin Invest 100:2386–2392
25. Hofland L, van der Hoek J, van Koetsveld P, de Herder W, Waaijers M, Sprij-Mooij D, Bruns C, Weckbecker G, Feelders R, van der Lely A, Beckers A, Lamberts S (2004) The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. J Clin Endocrinol Metab 89:1577–1585
26. Grottoli S, Gasco V, Broglio F, Baldelli R, Ragazzoni F, Gallenca F, Mainolfi A, Prodam F, Muccioli G, Ghigo E (2006) Cortistatin-17 and somatostatin-14 display the same effects on growth hormone, prolactin, and insulin secretion in patients with acromegaly or prolactinoma. J Clin Endocrinol Metab 91:1595–1599
27. Rocheville M, Lange D, Kumar U, Patel S, Patel R, Patel Y (2000) Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 288:154–157
28. Seveanu A, Lavaque E, Gunz G, Barlier A, Kim S, Taylor J, Culler M, Enjalbert A, Jaquet P (2002) Demonstration of enhanced potency of a chimeric somatostatin-dopamine molecule, BIM-23A387, in suppressing growth hormone and prolactin secretion from human pituitary somatotroph adenoma cells. J Clin Endocrinol Metab 87:5545–5552
29. Kaltsas G, Mukherjee J, Plowman P, Monson J, Grossman A, Besser G (1998) The role of cytotoxic chemotherapy in the management of aggressive and malignant pituitary tumors. J Clin Endocrinol Metab 83:4233–4238
30. Hurel S, Harris P, McNicol A, Foster S, Kelly W, Baylis P (1997) Metastatic prolactinoma: effect of octreotide, cabergoline, carboplatin, and etoposide: immunocytochemical analysis of proto-oncogene expression. J Clin Endocrinol Metab 82:2962–2965
31. Kulke M, Stuart K, Enzinger P, Ryan D, Clark J, Muzikansky A, Vincitore M, Michelini A, Fuchs C (2006) Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. J Clin Oncol 24:401–406
32. Bafaloukos D, Tsoutsos D, Kalofonos H, Chalkidou S, Panagiotou P, Linardou E, Briassoulis E, Efstathiou E, Polyzos A, Fountzilas G, Christodoulou C, Kouroussis C, Iconomou T, Gogas H (2005) Temozolomide and cisplatin versus temozolomide in patients with advanced melanoma: a randomized phase II study of the Hellenic Cooperative Oncology Group. Ann Oncol 16:950–957
33. Nagasubramanian R, Dolan M (2003) Temozolomide: realizing the promise and potential. Curr Opin Oncol 15:412–418
34. Hegi M, Diserens A, Godard S, Dietrich P, Regli L, Ostermann S, Otten P, Van Melle G, de Tribolet N, Stupp R (2004) Clinical trial substantiates the predictive value of *O*-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. Clin Cancer Res 10:1871–1874
35. Roos W, Batista L, Naumann S, Wick W, Weller M, Menck C, Kaina B (2006) Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion $O^6$-methylguanine. Oncogene (advance online publication). doi:10.1038/sj.onc.1209785
36. Esteller M, Hamilton S, Burger P, Baylin S, Herman J (1999) Inactivation of the DNA repair gene $O^6$-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 59:793–797
37. Hegi M, Diserens A, Gorlia T, Hamou M, de Tribolet N, Weller M, Kros J, Hainfellner J, Mason W, Mariani L, Bromberg J, Hau P, Mirimanoff R, Cairncross J, Janzer R, Stupp R (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352:997–1003