Pituitary (2012) 15:37–43
DOI 10.1007/s11102-011-0341-0

New targeted therapies in pituitary carcinoma resistant to temozolomide

Emmanuel Jouanneau · Anne Wierinckx · François Ducray · Véronique Favrel · Françoise Borson-Chazot · Jérôme Honnorat · Jacqueline Trouillas · Gérald Raverot

Published online: 21 August 2011

Abstract To evaluate the antitumoral efficacy of everolimus in pituitary carcinoma resistant to temozolomide, the correlation with mammalian target of rapamycin (mTOR) signaling in the tumor and to present recent advances and future treatments of pituitary carcinomas. Pituitary carcinomas are rare and largely unresponsive to current treatment options. Recent reports on the antitumoral efficacy of temozolomide in some such patients are encouraging, yet most patients appear to show resistance to its actions. As a potential alternative, the mTOR inhibitor, everolimus, has been shown to potently inhibit pituitary cell proliferation highlighting mTOR inhibition as a promising therapeutic approach for pituitary carcinomas. We described the tumoral effects of a combination therapy with everolimus (5 mg/day) and octreotide (30 mg/month) and the mTOR signalling expression in a patient with pituitary ACTH carcinoma, compared to 17 other ACTH adenomas. Clinical and biochemical evaluation were performed every month, and imaging after 3 month of treatment. mTOR signaling was assessed by microarray expression analysis of each of the 18 adenoma tissues. Combined therapy failed to control pituitary tumor growth and ACTH secretion. Slight activation of mTOR signaling was found in all ACTH tumors alongside important variations between tumors. Low antitumor efficacy shown by everolimus might be explained by the weak activation of mTOR pathway in ACTH tumors. Everolimus treatment was inefficient at controlling secretion and tumor growth of one ACTH pituitary carcinoma. More clinical cases, with mTOR signalling expression analysis of the tumor, must be published before any conclusions can be drawn.

Keywords Pituitary carcinoma · mTOR · Everolimus · Temozolomide · Pituitary adenoma · Silent ACTH tumor

E. Jouanneau · A. Wierinckx · F. Ducray · J. Honnorat · J. Trouillas · G. Raverot
INSERM, U1028/CNRS, UMR5292. Lyon Neuroscience Research Center, Neuro-Oncology and Neuro-inflammation Team, 69000 Lyon, France

E. Jouanneau · F. Ducray · F. Borson-Chazot · J. Honnorat · J. Trouillas · G. Raverot
Fédération d'Endocrinologie (GR, FBC), Service de Neurologie (FD, JH), Service de Neurochirurgie (EJ), Centre de Pathologie Est (JT), Groupement Hospitalier Est, Hospices Civils de Lyon, 69003 Lyon, France

E. Jouanneau · A. Wierinckx · F. Ducray · F. Borson-Chazot · J. Honnorat · J. Trouillas · G. Raverot
Université de Lyon, Lyon1, 69372 Lyon, France

A. Wierinckx
Inserm U1052, Centre de Recherche en Cancérologie de Lyon, 69000 Lyon, France

V. Favrel
Service de radiothérapie, Centre Hospitalier Universitaire Lyon Sud, 69495 Pierre-Bénite, France

G. Raverot (✉)
Fédération d'Endocrinologie du Pole Est, Hospices Civils de Lyon, 59 Bd Pinel, 69677 Bron Cedex, France
e-mail: gerald.raverot@chu-lyon.fr

Introduction

Pituitary tumors account for around 15% of all intracranial neoplasms and are associated with macroscopically evident local invasion in 35–40% of cases. Some tumors are aggressive, with a high proliferative rate and short post-operative times before recurrence (10–15% in our personal experience or surgical series). Pituitary carcinoma, defined by the presence of subarachnoid, brain and/or systemic

metastases, is a rare disorder, accounting for about 0.2% of pituitary tumors [1]. Given the paucity of reported cases, information about the presentation, response to treatment and overall prognosis of these patients remains sparse [1]. These tumors grow rapidly, and are largely unresponsive to current combined treatment strategies associating surgery, radiation and systemic cytotoxic chemotherapy [2]. One recent case report detailed the successful use of temozolomide, an alkylating chemotherapeutic drug, in the management of aggressive pituitary tumors and pituitary carcinoma [3]. Since this first publication other case reports have followed ([4], for review) demonstrating the efficacy of this treatment in different pituitary aggressive adenomas or carcinomas and revealing a possible correlation between the anti-tumoral effect and expression of 06-methylguanine-DNA methyltransferase (MGMT). However, more recently, three independent studies, including ours, confirmed the efficacy of temozolomide treatment for some, but not all, aggressive pituitary tumors or carcinomas and revealed MGMT status as a poor predictor of treatment outcome [4–6].

Since temozolomide treatment is not effective for all pituitary carcinomas or aggressive adenomas, the development of new therapeutic options is therefore necessary. The Raf/MEK/ERK and phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) cascades are key signaling pathways interacting with each other to regulate cell growth and progression of tumor cells. Recent data indicate that in pituitary adenomas both the Raf/MEK/ERK and PI3K/Akt/mTOR pathways are upregulated in their initial cascade, implicating a proproliferative signal derangement [7]. The mTOR inhibitor everolimus has been recently shown to have antineoplastic activity in several human cancers, in particular when combined with the long-acting repeatable (LAR) formulation of the somatostatin analogue, octreotide, in patients with neuroendocrine tumors [8]. In vitro, both in pituitary cell lines [9] and in primary cultures [10], everolimus potently inhibited pituitary cell proliferation, significantly reduced cell viability, and promoted apoptosis, suggesting that mTOR inhibition may be a promising antiproliferative therapeutic option for pituitary carcinomas. Moreover, adjuvant treatment with somatostatin analogue has been shown to sensitize pituitary tumor cells to the antiproliferative effects of rapamycin [11].

The objective of this study was to present the different options for treating a pituitary carcinomas from the experience of one patient presenting with a ACTH carcinoma resistant to temozolomie and treated with the combination of everolimus and octreotide, secondly to correlate the therapeutic response to the microarray analysis of the mTOR signaling performed on the patient's tumor and on a further 17 ACTH pituitary tumors and finally to discuss the perspectives for the treatment of pituitary carcinomas using new targeted therapies.

Patient and methods

Patient

The patient with silent ACTH carcinoma was diagnosed and treated at the Hôpital Neurologique, Lyon, France. Initial presentation has been published independently [12] and this patient was included in a study on silent ACTH adenomas (patient n° 11; [13]).

Pituitary tumor cohort

To compare the mTOR signaling of the patient's tumor with that of other ACTH adenomas, we selected 17 patients who had undergone pituitary surgery between 1990 and 2005 for ACTH tumor at Hôpital Neurologique, Lyon, and had a frozen sample available in the Neurobiotec bank. All 18 tumor samples underwent histopathological analysis and were classified into 3 groups: micro (mCA) ($n = 4$), macro (MCA) ($n = 5$) and silent ACTH adenomas (SCA) ($n = 9$) depending on the tumor size and initial clinical presentation according to Raverot et al. [13].

This work is part of a collaborative multicenter study on prognostic factors of pituitary tumors in France (HYPOPRONOS). Informed consent was obtained from each patient before surgery and the study was approved by our local ethic committee.

RNA amplification

Total RNA (2 μg) was amplified and biotin-labeled by a round of in vitro transcription (dIVT) with a Message Amp aRNA kit (Ambion, Austin, Texas, USA) following the manufacturer's protocol. Before amplification, spikes of synthetic mRNA at different concentrations were added to all samples; these positive controls were used to ascertain the quality of the process. aRNA yield was measured with an UV spectrophotometer and the quality on nanochips with the Agilent 2100 Bioanalyzer (Agilent).

Array hybridization and processing

Ten micrograms of biotin-labeled aRNA was fragmented using 5 μl of fragmentation buffer in a final volume of 20 μl, before being mixed with 240 μl of Amersham hybridization solution (GE Healthcare Europe GmbH, Freiburg, Germany) and injected onto CodeLink Uniset Human Whole Genome bioarrays containing 55,000 human oligonucleotide gene probes (GE Healthcare

Europe GmbH, Freiburg, Germany). Arrays were hybridized overnight at 37°C shaking at 300 rpm in an incubator. The slides were then washed in stringent TNT buffer at 46°C for 1 h before a streptavidin-cy5 (GE Healthcare) detection step. Each slide was incubated for 30 min in 3.4 ml of streptavidin-cy5 solution as described previously (19), before being washed 4 times in 240 ml of TNT buffer, rinsed twice in 240 ml of water containing 0.2% Triton X-100, and dried by centrifugation at 600 rpm.

The slides were scanned using a Genepix 4000B scanner (Axon, Union City, USA) and Genepix software, with the laser set at 635 mm, the laser power at 100%, and the photomultiplier tube voltage at 60%. The scanned image files were analyzed using CodeLink expression software, version 4.0 (GE Healthcare), which produces both a raw and normalized hybridization signal for each spot on the array.

Microarray data analysis

The relative intensity of the raw hybridization signal on arrays varies between different experiments. CodeLink software was therefore used to normalize the raw hybridization signal on each array to the median of the array (median intensity is 1 after normalization) for better cross-array comparison. The threshold of detection was calculated using the normalized signal intensity of the 100 negative control samples in the array; spots with signal intensities below this threshold are referred to as "absent".

Quality of processing was evaluated by generating scatter plots of positive signal distribution. Signal intensities were then converted to log base 2 values. Ingenuity Pathway Analysis (Mountain View, CA) was used for PI3/AKT/mTOR signaling.

Results

Clinical data

Therapeutic strategies, biochemical and, MRI follow-up of the patient are summarized in Fig. 1.

A 45 year-old man with a history of spina bifida aperta associated with Chiari malformation, presented in August 1999 with headaches and visual defects. Magnetic resonance imaging (MRI) revealed a pituitary macroadenoma with suprasellar extension. Clinical examination revealed no signs of hypersecretion, particularly hypercortisolism, however, preoperative hormonal evaluation revealed elevated ACTH (192 ng/l; normal <25 ng/L) and 8a.m. cortisol (970 nmol/l, normal 350–540 nmol/l) levels. Surgery through a transrhinoseptal approach (11/10/99) allowed a complete removal of the tumor. After surgery, ACTH concentration and MRI were normal. Pathological diagnosis was silent ACTH adenoma based on ACTH, β-LPH and β-endorphin positive immunostaining. The adenoma was p53 negative with low Ki-67 index; however mitoses were observed (4/10 fields x 400). One year after this first surgery, an intra, para and suprasellar tumor recurrence with an extension into the right cavernous sinus, but without clinical signs of hypercortisolism, was diagnosed. The patient underwent transcranial surgery in September 2001, which was completed with stereotactic radiotherapy. Pathological analysis revealed similar tumor characteristics to those at initial examination. Postoperative follow-up up demonstrated a normalization of ACTH secretion and a stable residual tumor in the right cavernous sinus until August 2005 when clinical and biochemical signs of hypercortisolism became evident, associated with tumor progression and subarachnoid metastasis located at the craniocervical junction on MRI. Considering the severity of the hypercortisolism, bilateral adrenalectomy was performed associated with fractionated radiotherapy focused on the metastasis, which disappeared. In October 2009 new tumor progression was noted and in view of the aggressiveness of this tumor, oral temozolomide treatment was initiated at 200 mg/day for 5 days every 28 days which failed to control the progression of the tumor. Multidisciplinary discussion led us to propose a salvage therapy with everolimus given orally (5 mg daily), in association with octreotide i.m. (30 mg every 28 days). Poor tolerance with invaliding diarrhea led to us stopping the octreotide treatment after 1 month. Unfortunately, the biochemical and MRI evaluation performed after 3 month demonstrated the absence of effect of this combination and treatment was stopped. The patient died 5 months after the end of the treatment.

PI3K/AKT/mTOR signaling (Fig. 2)

The transcriptomic analysis of the 18 ACTH adenomas demonstrated important variations between the three tumor groups (mCA, MCA and SCA groups). Only slight activation of the PI3/AKT/mTOR signaling was noted without any statistically significant difference between the groups (Table 1). AKT1 was activated in all three groups, PI3K expression was decreased in MCA and SCA but not in mCA. None of the tumors expressed *insulin receptor substrate 1* (IRS-1). While *rapamycin-insensitive companion of mTOR* (*RICTOR*) mRNA was upregulated in all tumors, *regulatory associated protein of mTOR* (*RAPTOR*) mRNA, coding for the target of the immunosuppressive drug rapamycin, was undetectable.

The transcriptomic analysis of the tumor removed from our patient showed similar levels of activation of AKT1 and inactivation of PI3K. However *RICTOR* was more

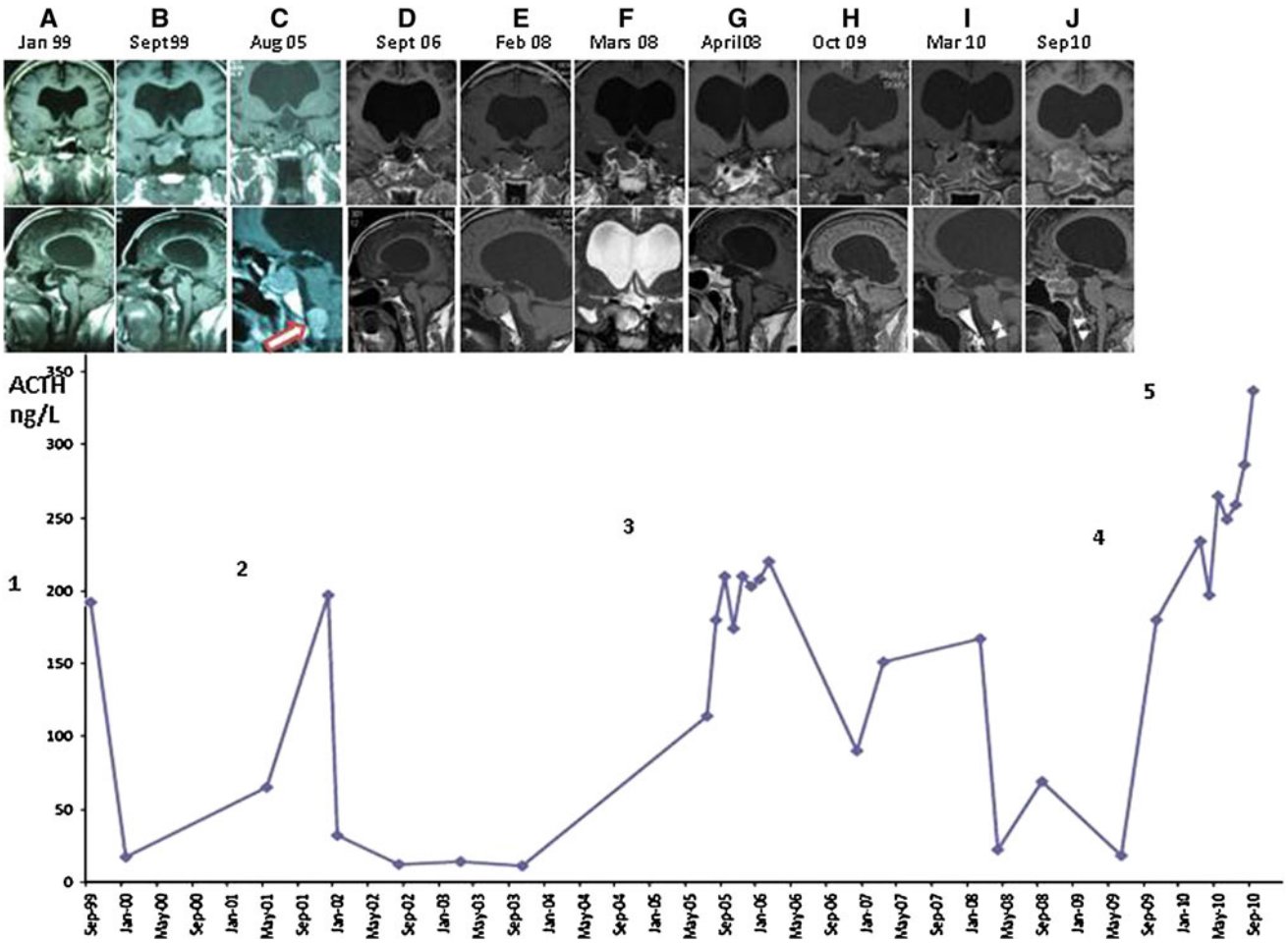

Fig. 1 Therapeutic strategies, biochemical and MRI follow-up in the patient with ACTH carcinoma. *Upper panel* pituitary MRI at diagnosis and during the follow-up. **a** MRI performed during the follow-up of spina bifida with congenital hydrocephalus showing partially empty sella turcica in conjunction with invagination of optic structures. Pituitary was considered as normal, **b** preoperative MRI with a partially cystic pituitary macroadenoma showing invasion of the right cavernous sinus (maximum diameter 30 mm), **c** clinical (hypercortisolism) and tumor recurrence with subarachnoid metastasis (*arrow*), **d** MRI showing the disappearance of the metastasis and the decrease in tumor size after the second stereotactic radiotherapy, **e** pseudo-tumor recurrence corresponding to necrosis secondary to radiotherapy (**f**). **g** Follow-up suggesting tumor control, **h** tumor recurrence, MRI before temozolomide treatment, **i** MRI after 3 cycles of temozolomide indicating the failure of this treatment and the appearance of new metastasis (arrowheads) **j** MRI showing tumor progression after 3 cycles of everolimus and octreotide combined therapy. *Lower panel* graph representing the ACTH secretion during follow-up and after the different treatments. *1* transphenoidal tumorectomy, *2* transcranial tumorectomy and stereotactic radiotherapy, *3* bilateral adrenalectomy and brain stem radiotherapy focused on the metastasis, *4* temozolomide : 3 cycles 200 mg/m^2/day for 5 days every 28 days *5* three cycles of everolimus 5 mg/day combined to octreotide 30 mg during the first month

strongly upregulated compared to the other tumors and the *CCND1* gene was highly overexpressed in the tumor of our patient in contrast to undetectable levels of mRNA in the majority of the other tumors.

Discussion

Pituitary carcinoma is a rare disorder associated with poor prognosis because of the lack of efficient chemotherapeutic drugs. Preliminary reports demonstrated a good response to temozolomide treatment in patients with either pituitary carcinoma or aggressive pituitary adenomas resistant to standard therapies. Low level of O(6)-methylguanine-DNA methyltransferase (MGMT) immunoexpression was correlated with a favorable response. However, underreporting of poorly responsive cases is also likely to bias the assessment of the overall efficacy of this therapy. So, following these first results, more recent publications reporting not only positive results [4–6] confirmed the efficacy of temozolomide treatment for some but not all aggressive pituitary tumors or carcinomas [4–6], and argued against MGMT status as a reliable predictor of treatment outcome. It has since been proposed that patients with aggressive

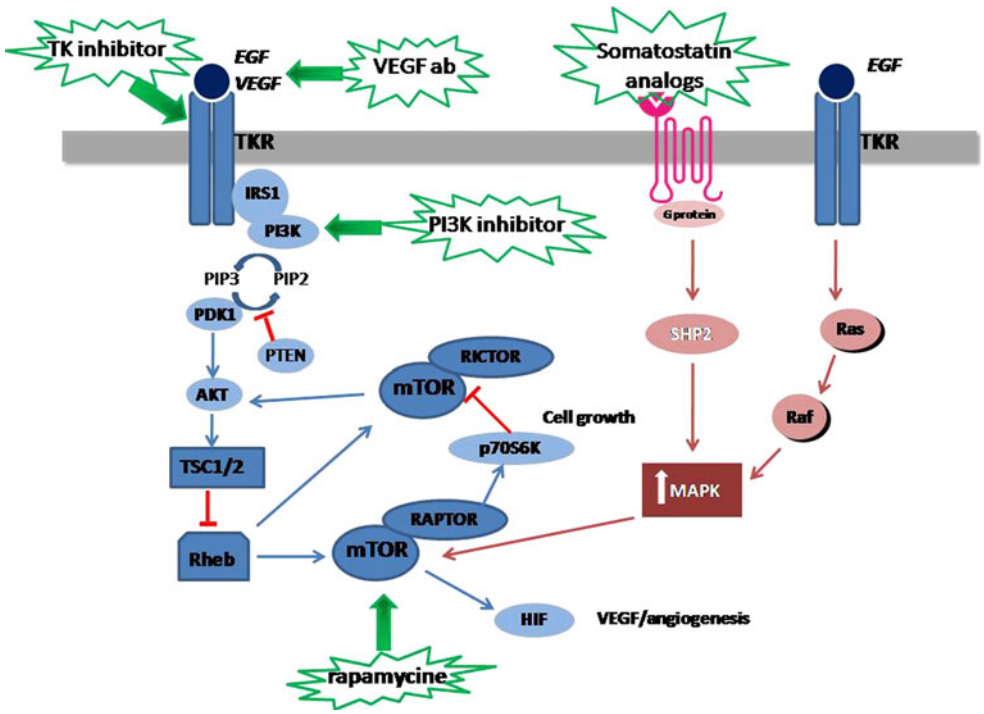

Fig. 2 PI3K/AKT/mTOR pathway and targeted therapies. Epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), tyrosine kinase (TK), tyrosine kinase receptor (TKR), antibody (ab), insulin receptor substrate 1 (IRS1), phosphatidylinositol 3-kinases (PI3K), phosphatase and tensin homologue (PTEN), pyruvate dehydrogenase kinase isozyme 1 (PDK1), thymoma in AKR mouse/protein kinase B (AKT1), tuberous sclerosis protein 1 and 2 (TSC1/2), ras homolog enriched in brain (Rheb), mammalian target of rapamycin (mTOR), rapamycin-insensitive companion of mTOR (RICTOR), regulatory associated protein of mTOR (RAPTOR), serine/threonine kinase (p70S6 K), tyrosine phosphatase SHP2 (SHP2), RAt sarcoma (ras), RAF proto-oncogene serine/threonine-protein kinase (raf), mitogene-activated protein kinase (MAPK)

Table 1 PI3K/AKT/mTOR expression by microarrays in 18 ACTH pituitary tumors

mRNA expression	mCA mean ± SD	MCA mean ± SD	SCA mean ± SD	Patient
PTEN	1.19 ± 0.49	–1.08 ± 0.2	1.09 ± 0.81	–2.09
IRS1	ub*	ub*	ub*	ub*
PI3K	1.14 ± 0.37	–1.18 ± 0.19	–1.13 ± 0.31	–1.88
AKT1	1.41 ± 0.69	1.33 ± 0.5	1.50 ± 0.4	1.35
RAPTOR	ub*	ub*	ub*	ub*
RICTOR	1.28 ± 0.58	ub*	1.52 ± 0.87	1.91
p53	1.14 ± 0.07	1.05 ± 0.2	1.05 ± 0.27	1.14
CCND1	ub*	ub*	ub*	2.18
MYC	3.85 ± 2.32	ub*	3.84 ± 2.45	–1.12

Gene expression in normal pituitary tissue was used as a standard and set to 1.Micro (mCA), macro (MCA) (n = 5) and silent corticotroph adenoma (SCA). Insulin Receptor Substrate 1 (IRS1), Phosphatidylinositol 3-kinases (PI3K), Phosphatase and Tensin homologue (PTEN), thymoma in AKR mouse/protein kinase B (AKT1), mammalian Target of Rapamycin (mTOR), rapamycin-insensitive companion of mTOR (RICTOR), regulatory associated protein of mTOR (RAPTOR), tumor protein p53 (p53), Cyclin D1 (CCND1), V-Myc avian myelocytomatosis viral oncogene (MYC), Underbackground (ub*)

adenomas or carcinomas who are resistant to conventional treatment be submitted to 3 cycles of temozolomide after which treatment should be stopped in the absence of hormonal or tumor response, since a delayed tumor response appears unlikely [4].

Resistance to cytotoxic and cytostatic treatments is a common limitation in cancer treatment. Since temozolomide treatment is not effective for all pituitary carcinomas or aggressive adenomas, the development of new therapeutic options is necessary. Recent in vitro studies on

primitive pituitary tumor cell cultures or pituitary cell lines demonstrating the common activation of the PI3K/AKT/mTOR pathway (Fig. 2) opened the door to targeted drugs like mTOR inhibitors in the treatment of pituitary carcinomas resistant to other therapies. Among the mTOR inhibitors, everolimus has demonstrated its efficacy in treating neuroendocrine tumors. It is also known, from neuroendocrine tumors that some tumors become resistant to rapamycine. Combination therapies may resolve some resistance. Indeed, rapamycin resistance, in part attributed to the elimination of the negative feedback loop of the mTOR target p70SP6K on the PI3K pathway [11], may be overpass by the association of octreotide or other somatostatin analogues including pasireotide [10, 11]. Everolimus has been extensively studied in combination with octreotide in the treatment of neuroendocrine tumors [8] and studies are ongoing using association with pasireotide. Recent results of in vitro studies on rat or mouse pituitary tumor cell lines (GH-3, At-T20) [9, 11] or on human pituitary primary cultures (GH tumors or nonfunctioning pituitary tumors) [9–11], support this hypothesis. However, in primary cultures only 40–70% of tumors presented reduction of cell viability and increase of apoptosis under everolimus treatment, even in association with octreotide or pasireotide.

Thus, in the absence of any alternative approved drug and based on these in vitro and in vivo studies on neuroendocrine tumors and pituitary cell lines, the only "evidence based" option is the use of everolimus, combined with octreotide for treating pituitary carcinoma. Unfortunately, our patient did not tolerate the combination of everolimus with octreotide due to digestive side effects, nor did the tumor respond to everolimus alone. Recent data demonstrated that the feedback of rapamycin on AKT phosphorylation was present in At-T20, an ACTH cell line, suggesting that ACTH cells may be resistant to rapamycin antiproliferative action [11]. This feedback was partially reversed by octreotide treatment; unfortunatly in our patient octreotide was stopped after one injection which could therefore explain, at least partially, the resistance to everolimus. This mechanism seems to be absent in GH/PRL cell lines (GH-3) and suggests that rapamycin alone could be effective in PRL or GH pituitary tumors.

PI3K/AKT/mTOR signaling analysis demonstrated that mTOR signaling was probably less activated in our patient's tumor compared to the other tested ACTH pituitary tumors. Interestingly, AKT1 activation seems independent of PI3K signaling, since PI3K and IRS1 expression levels were decreased whereas AKT1 was overexpressed in all tumors. Moreover a large heterogeneity has been shown between the different tumor samples. This heterogeneity was also demonstrated in an in vitro study performed on nonfunctioning pituitary adenoma primary cell cultures. Indeed, it has been demonstrated that less than 25% of nonfunctioning pituitary adenomas are sensitive to rapamycin treatment even in the presence of AKT overactivation [11]. Transposed to clinical use, these results suggest that 25% of patients should be sensitive to everolimus treatment which is similar to that expected with temozolomide treatment. Unfortunately, because of this heterogeneity, it is difficult to identify a predictive marker of tumor response to mTOR inhibitor.

In the view of our molecular data in ACTH pituitary tumors and data from in vitro studies on mTor inhibitor efficacy, and the regulatory pathway of the pituitary cells summarized in Fig. 2, it seems necessary to look for other therapeutic options. From recent preclinical studies, different combination therapies could be suggested, in particular therapy targeting Vascular Endothelial Growth Factor (VEGF) pathway. Indeed, it has been demonstrated that VEGF expression was associated with pituitary tumorigenesis [14] and somatostatin analogues (octreotide or pasireotide) inhibit NFPA cell viability by inhibiting VEGF secretion [15]. Moreover, recent studies demonstrated that the antiangiogenic approach using anti-VEGF antibody [16] or a small-molecule tyrosine kinase inhibitor (lapatinib) [17] targeting EGF receptors Erb1 and Erb2 was effective, in inhibiting in vivo the growth of dopamine-resistant prolactinoma from dopamine receptor D2R mouse knockout [16] or in estrogen-induced Fischer344 rat prolactinomas [17]. More recently, Liu et al. identified a pharmacologic CDK2/cycline E inhibitor, R-roscovitine, and demonstrated that R-roscovitine suppressed ACTH expression and induced ACTH tumor cell senescence using a transgenic fish model and AtT20 ACTH tumor cells injected in nude mice [18].

The identification of new pathways associated with pituitary tumor aggressiveness such as Aurora B kinase or CENP-E [19, 20] may lead to the development of new specific therapies. However, to date no specific drug is available for the treatment of pituitary carcinomas and the rarity of this tumor will certainly limit a specific development. So, because of the similarities, hopefully drug developed for the treatment of neuroendocrine tumor may be effective in the treatment of pituitary tumors.

From our study, we cannot exclude the possibility of a beneficial action of everolimus in treating human pituitary tumors based on the clinical experience of one case. Despite this negative experience, in the absence of any other specific drug and in view of positive preclinical data, we believe that for the patients with pituitary carcinoma resistant to temozolomide, an everolimus treatment associated with octreotide should be proposed as the second line therapy. More clinical cases with mtor signaling study of the tumor, even with negative results, must be published before any conclusions can be drawn. The rarity of these

tumors and the lack of established treatment guidelines underline the necessity for collaborative studies on pituitary carcinomas or aggressive tumors.

Acknowledgments The authors thank Angloscribe for English language editing. This work was supported by a grant from the Ministère de la Santé (Programme Hospitalier de Recherche National no. 27-43).

Conflict of interest The authors have nothing to disclose.

References

1. Kaltsas GA, Nomikos P, Kontogeorgos G, Buchfelder M, Grossman AB (2005) Diagnosis and management of pituitary carcinomas. J Clin Endocrinol Metab 90(5):3089–3099. doi:10.1210/jc.2004-2231
2. 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(12):4233–4238
3. Lim S, Shahinian H, Maya MM, Yong W, Heaney AP (2006) Temozolomide: a novel treatment for pituitary carcinoma. Lancet Oncol 7(6):518–520
4. Raverot G, Sturm N, de Fraipont F, Muller M, Salenave S, Caron P, Chabre O, Chanson P, Cortet-Rudelli C, Assaker R, Dufour H, Gaillard S, Francois P, Jouanneau E, Passagia J-G, Bernier M, Cornelius A, Figarella-Branger D, Trouillas J, Borson-Chazot F, Brue T (2010) Temozolomide treatment in aggressive pituitary tumors and pituitary carcinomas: a French multicenter experience. J Clin Endocrinol Metab 95(10):4592–4599
5. Bush ZM, Longtine JA, Cunningham T, Schiff D, Jane JA, Vance ML, Thorner MO, Laws ER, Lopes MBS (2010) Temozolomide treatment for aggressive pituitary tumors: correlation of clinical outcome with O6-methylguanine methyltransferase (MGMT) promoter methylation and expression. J Clin Endocrinol Metab 95(11):E280–E290. doi:10.1210/jc.2010-0441
6. Losa M, Mazza E, Terreni MR, McCormack A, Gill AJ, Motta M, Cangi MG, Talarico A, Mortini P, Reni M (2010) Salvage therapy with temozolomide in patients with aggressive or metastatic pituitary adenomas: experience in six cases. Eur J Endocrinol 163(6):843–851. doi:10.1530/eje-10-0629
7. Dworakowska D, Wlodek E, Leontiou CA, Igreja S, Cakir M, Teng M, Prodromou N, Goth MI, Grozinsky-Glasberg S, Gueorguiev M, Kola B, Korbonits M, Grossman AB (2009) Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. Endocr Relat Cancer 16(4):1329–1338. doi:ERC-09-0101[pii]10.1677/ERC-09-0101
8. Yao JC, Shah MH, Ito T, Bohas CL, Wolin EM, Van Cutsem E, Hobday TJ, Okusaka T, Capdevila J, de Vries EG, Tomassetti P, Pavel ME, Hoosen S, Haas T, Lincy J, Lebwohl D, Oberg K (2011) Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med 364(6):514–523. doi:10.1056/NEJMoa1009290
9. Gorshtein A, Rubinfeld H, Kendler E, Theodoropoulou M, Cerovac V, Stalla GK, Cohen ZR, Hadani M, Shimon I (2009) Mammalian target of rapamycin inhibitors rapamycin and RAD001 (everolimus) induce anti-proliferative effects in GH-secreting pituitary tumor cells in vitro. Endocr Relat Cancer 16(3):1017–1027. doi:ERC-08-0269[pii]10.1677/ERC-08-0269
10. Zatelli MC, Minoia M, Filieri C, Tagliati F, Buratto M, Ambrosio MR, Lapparelli M, Scanarini M, degli Uberti EC (2010) Effect of everolimus on cell viability in nonfunctioning pituitary adenomas. J Clin Endocrinol Metab 95(2):968–976. doi:10.1210/jc.2009-1641
11. Cerovac V, Monteserin-Garcia J, Rubinfeld H, Buchfelder M, Losa M, Florio T, Paez-Pereda M, Stalla GK, Theodoropoulou M (2010) The somatostatin analogue octreotide confers sensitivity to rapamycin treatment on pituitary tumor cells. Cancer Res 70(2):666–674. doi:0008-5472.CAN-09-2951[pii]10.1158/0008-5472.CAN-09-2951
12. Jouanneau E, Ducluzeau PH, Tilikete C, Borson-Chazot F, Trouillas J, Perrin G (2001) Should silent corticotroph-cell adenoma be classified as a non-functional pituitary adenoma? Neurochirurgie 47(2–3 Pt 1):128–132
13. Raverot G, Wierinckx A, Jouanneau E, Auger C, Borson-Chazot F, Lachuer J, Pugeat M, Trouillas J (2010) Clinical, hormonal and molecular characterization of pituitary ACTH adenomas without (silent corticotroph adenomas) and with Cushing's disease. Eur J Endocrinol doi:10.1530/EJE-10-0076
14. McCabe CJ, Boelaert K, Tannahill LA, Heaney AP, Stratford AL, Khaira JS, Hussain S, Sheppard MC, Franklyn JA, Gittoes NJ (2002) Vascular endothelial growth factor, its receptor KDR/Flk-1, and pituitary tumor transforming gene in pituitary tumors. J Clin Endocrinol Metab 87(9):4238–4244
15. Zatelli MC, Piccin D, Vignali C, Tagliati F, Ambrosio MR, Bondanelli M, Cimino V, Bianchi A, Schmid HA, Scanarini M, Pontecorvi A, De Marinis L, Maira G, degli Uberti EC (2007) Pasireotide, a multiple somatostatin receptor subtypes ligand, reduces cell viability in non-functioning pituitary adenomas by inhibiting vascular endothelial growth factor secretion. Endocr Relat Cancer 14(1):91–102. doi:14/1/91[pii]10.1677/ERC-06-0026
16. Luque GM, Perez-Millan MI, Ornstein AM, Cristina C, Becu-Villalobos D (2011) Inhibitory effects of antivascular endothelial growth factor strategies in experimental dopamine-resistant prolactinomas. J Pharmacol Exp Ther 337(3):766–774. doi:10.1124/jpet.110.177790
17. Fukuoka H, Cooper O, Mizutani J, Tong Y, Ren SG, Bannykh S, Melmed S (2011) HER2/ErbB2 receptor signaling in rat and human prolactinoma cells: strategy for targeted prolactinoma therapy. Mol Endocrinol doi:me.2010-0353 [pii]10.1210/me.2010-0353
18. Liu NA, Jiang H, Ben-Shlomo A, Wawrowsky K, Fan XM, Lin S, Melmed S (2011) Targeting zebrafish and murine pituitary corticotroph tumors with a cyclin-dependent kinase (CDK) inhibitor. Proc Natl Acad Sci USA 108(20):8414–8419. doi:10180911 08[pii]10.1073/pnas.1018091108
19. 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(4):1708–1716. doi:jc.2009-1191 [pii]10.1210/jc.2009-1191
20. Wierinckx A, Raverot G, Nazaret N, Jouanneau E, Auger C, Lachuer J, Trouillas J (2010) Proliferation markers of human pituitary tumors: contribution of a genome-wide transcriptome approach. Mol Cell Endocrinol doi:10.1016/j.mce.2010.02.043