

Prediction of response to everolimus in neuroendocrine tumors: evaluation of clinical, biological and histological factors

Noura Benslama^{1,2,3} · Julien Bollard² · Cécile Vercherat² · Patrick Massoma² · Colette Roche² · Valérie Hervieu^{2,3,4} · Julien Peron⁵ · Catherine Lombard-Bohas¹ · Jean-Yves Scoazec² · Thomas Walter^{1,2,3}

Received: 19 April 2016 / Accepted: 18 May 2016 / Published online: 26 May 2016
© Springer Science+Business Media New York 2016

Summary Objectives Several targeted therapies are available for metastatic neuroendocrine tumours (NETs) but no predictive factor of response to these treatments has been identified yet. Our aim was to identify and evaluate clinical, biological, histological and functional markers of response to everolimus. **Methods** We retrospectively reviewed 53 patients with NETs treated with everolimus (68 % in clinical trials). Clinical, biological and histological data were analyzed. The functional marker p-p70S6K, a main effector of the mTOR pathway, was studied by immunohistochemistry in 43 cases. Prognostic factors of progression-free survival (PFS) were studied by Kaplan Meier analysis. **Results** All patients had metastatic and progressive disease before everolimus treatment. Objective response was 9 % and median PFS was 8.1 (4.7–11.5) months. Hypercholesterolemia (HR = 0.13, $p < 0.0001$) was associated with longer PFS, whereas presence of bone metastases (HR = 3.1, $p < 0.001$) and overexpression of p-p70S6K by tumor cells (HR = 2.5,

$p = 0.01$) were associated with shorter PFS under everolimus at multivariate analysis. **Conclusion** Clinical markers are not useful to predict response to everolimus. However, occurrence of hypercholesterolemia under treatment may be an early marker of response. Prospective studies are required to confirm these results and to assess whether p-p70S6K immunostaining is a prognostic or predictive marker of no-response to everolimus.

Keywords Neuroendocrine tumours · Everolimus · Markers · P-p70S6K · Hypercholesterolemia

Introduction

Neuroendocrine tumours (NETs) are rare, but their incidence is rising [1]. They are frequently metastatic at diagnosis and require systemic treatments. Several strategies are available, including targeted therapies as everolimus [2–4], but were never compared to each other. Three large phase III placebo-controlled randomized trials [2–4] recently reported the efficacy of everolimus in patients with advanced pancreatic NETs (RADIANT-3), gastrointestinal (GI)-NETs and pulmonary NETs (RADIANT-2 and RADIANT-4).

Only a subset of patients draws a clear benefit from mTOR inhibition; for instance, the progression free survival (PFS) at 18 months was 34 % under everolimus as compared with 9 % under placebo in pancreatic NETs [4]. However, no predictive factor of response to everolimus has been identified in NETs. In other solid tumours, several possible markers of response to everolimus or other mTOR inhibitors with the same mechanism of action, such as temsirolimus, have been proposed. They include clinical and biological factors, including the occurrence of side effects under everolimus treatment, such as metabolic disorders, lymphopenia or stomatitis, which have

✉ Thomas Walter
thomas.walter@chu-lyon.fr

¹ Service d'hépatogastroentérologie et d'oncologie digestive, Hospices Civils de Lyon, Hôpital Edouard Herriot, 69437 Lyon cedex 03, France

² INSERM, UMR 1052, Lyon Cancer Research Center, Faculté Laennec, 69372 Lyon cedex 08, France

³ Université de Lyon, Université Claude Bernard Lyon 1, 69622 Villeurbanne cedex, France

⁴ Service Central d'Anatomie et Cytologie Pathologiques, Hospices Civils de Lyon, Hôpital Edouard Herriot, 69437 Lyon cedex 03, France

⁵ Medical Oncology Department, Centre Hospitalier Lyon Sud, Institut de Cancérologie des Hospices Civils de Lyon, Pierre-Bénite, France

been associated with higher efficacy [5–7]. They also include molecular markers, selected on the concept that a high activation of the mTOR pathway would improve tumor response to mTOR inhibitors. Such markers include genetic alterations such as *PTEN* loss of function [8], activating mutations of *PIK3CA* [9] and loss-of-function mutations of *TSC1* or *TSC2* [10]. They also include **protein alterations such as** loss of PTEN protein expression [8] and **presence of** high amounts of phosphorylated forms of mTOR and its targets p70S6K and 4EBP1 [11–14].

Our aim was therefore to analyze whether the response to everolimus could be predicted by i) conventional clinical, biological and histological **tumour** characteristics, ii) occurrence of side effects such as hyperglycemia, hyperlipemia or haematological toxicity, or iii) functional in situ markers of mTOR activity, **such as phosphorylated p70S6K, which we selected** because of the availability of **a robust immunohistochemical test working in the archival formalin-fixed paraffin-embedded tissue material available for this retrospective study, and already validated within our laboratory in several clinical and preclinical studies [11].**

Subjects and methods

Patients

We retrospectively included all patients who i) had histologically proven advanced well differentiated NET, ii) received everolimus for more than 14 days in our institution (ENETs center of excellence), between 2006 and 2013, iii) gave informed consent to clinical research studies, and iv) adhered to complete follow-up in our institution. Patients could be treated with everolimus during a clinical trial (RADIANT-1, -2, -3, or COOPERATE-2) or according to clinical practice, with or without concurrent somatostatin analogs.

The following clinical, biological and histological data were collected: age at diagnosis, gender, MEN1 syndrome, primary tumour location, functional status, Ki67 index, World Health Organization 2010 classification, TNM stage, location and number of metastatic sites, prior antitumour treatments and progressive disease status at beginning of everolimus.

Efficacy assessment

The efficacy of everolimus was assessed from clinical, biological and morphological parameters. When tumours were functioning, clinical response was defined as a > 50 % decrease in their clinical syndrome (daily numbers of flushes and of stools for carcinoid syndrome, number of stools for VIPoma, number of hypoglycemic episodes for insulinoma, disappearance

of cutaneous lesions for glucagonoma). Biological response in plasma chromogranin A (CgA) levels was evaluated among patients with baseline CgA above twice the upper limit of normal and was defined as a > 30 % decrease in plasma CgA as compared to pre-treatment value. We analyzed the best tumour response obtained in accordance with Response Evaluation Criteria In Solid Tumors (RECIST) criteria v1.1, stable disease being defined as a tumour stable after at least 2 months of treatment. Patients were assessed at 2–3 month intervals of treatment according to clinical trial protocols or to clinicians' appreciation, or earlier if clinically indicated, using clinical examination and spiral computed tomography scan of the thorax, abdomen and pelvis and/or magnetic resonance imaging of the liver when appropriate. Patients who stopped everolimus within 2 months for another reason than disease progression were considered unavailable for best tumor response, but were censored for calculation of progression free survival (PFS).

Safety assessment

All grade 3 and 4 adverse events according to the National Cancer Institute's Common Terminology Criteria (NCI-CTC) for Adverse Events version 4.0 were collected. We reported dyslipidemia and hyperglycemia when they required the introduction or the increase of a medical treatment according to the protocol for patients in clinical trial or according to our current recommendations for other patients [15, 16]. Briefly, Grade 2 or higher hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or Grade 2 or higher hypertriglyceridemia (>2.5 x ULN) were treated with a statin (HMG-CoA reductase inhibitor), fibrate, or appropriate lipid-lowering medication. We recorded the time to initiation or increase of hypolipidemic treatment, calculated from the start of everolimus. For hyperglycemia, metformin was used in first intent.

In situ functional analysis of mTOR activation

We used one of the main downstream effector of the mTOR pathway, phospho-p70S6 kinase, as a marker of mTOR activation. The apparent expression levels of the phosphorylated protein were evaluated by immunohistochemistry applied to representative formalin-fixed, paraffin-embedded tumor tissue samples. Tissue samples from either primary tumor or metastatic lesions were available in 43 patients. Four- μ m-thick sections were prepared according to conventional procedures; after deparaffinization and dehydration, tissue sections were immersed in citrate buffer pH 6 using a water bath at 98 °C for 35 min; endogenous peroxidase activity was blocked by incubation in 3 % hydrogen peroxide in sterile water. Immunostaining was performed by using a polyclonal antibody directed to phospho-p70S6 kinase at 1/200 dilution

(Santa Cruz Biotechnology, Dallas, TX). The apparent intensity of expression of p-p70S6K was evaluated by comparison with internal controls, usually intra-tumoral endothelial cells. Expression levels were defined as high when they were higher than in internal controls. They were defined as low when they were comparable to, or lower than, in internal controls. Patients presenting tumours with high p-p70S6K expression levels were compared to patients presenting tumours with normal or low expression levels.

Statistical analysis

Categorical variables were expressed as percentages, and compared by the Chi-square test or with Fisher's exact test when appropriate. Continuous variables were expressed as median with range. PFS was calculated from initiation of everolimus to the date of disease progression according to RECIST criteria or death from any cause, whichever occurred first. Overall survival (OS) was calculated from initiation of everolimus to the date of death or last follow-up. PFS and OS were estimated by the Kaplan–Meier method and comparisons were performed using the log-rank test. Cox proportional hazard models were developed using relevant clinicopathological variables to determine the association of each parameter with OS or PFS. Relative risks were expressed as hazard ratios (HRs) with 95 % confidence intervals (CIs). All testing was two-tailed and $p < 0.05$ was considered to be statistically significant. Statistical analysis was performed with R software version 3.2.2.

Results

Patient and tumour characteristics

Fifty-three patients with advanced NETs treated with everolimus between 2006 and 2013 were included: 68 % of them were treated in clinical trials and 75 % had a pancreatic primary location (Table 1). All patients had metastatic and progressive disease at time of everolimus treatment, which was started 44 (3–237) months after diagnosis, after a median of 2 prior systemic treatments.

Histological data were obtained from either the primary tumour ($n = 34$) or from one metastasis ($n = 34$); in 8 patients, specimens from both primary and metastasis were available. All tumours were well-differentiated. Median Ki67 was 10 % (0–50). In primary tumours, histological grade was G1 in 5 (24 %), G2 in 10 (50 %), and G3 in 6 (26 %, but with a median Ki67 of 29 (22–50)), whereas, in metastases, histological grade was G1 in 6 (26 %), G2 in 13 (56 %), and G3 in 4 (18 %). In patients for whom both the primary and a metastasis were available, histological grades of metastasis were recorded.

Functional in situ study of mTOR activation

The results of phospho-p70S6K immunostaining were interpretable in 42 (20 primary tumours and 22 metastases) out of the 43 specimens studied. The staining was nuclear in all cases; it could be nuclear and cytoplasmic in the cases with the highest expression levels. The apparent expression levels were high in 50 % of cases studied, and low in the other 50 % (Fig. 1a–b). The two groups had similar clinical and histological characteristics (data not shown) except that high p-p70S6K immunostaining was more frequent when patients had two or more metastatic sites (57 % vs 19 %, $p = 0.01$) and in metastatic specimens (71 % vs 33 %, $p = 0.01$).

Response and tolerance to everolimus treatment

Clinical, biological and morphological responses are shown in Table 2. Objective response and stable disease were achieved in 9 % and 68 %, respectively. The median PFS and OS were 8.1 (4.7–11.5) and 27.5 (16.5–38.5) months.

Safety is shown in Table 3. Grade 3–4 toxicities occurred in 25 (47 %) patients. Grade 2 metabolic disorders, which were treated before becoming grade 3, occurred in 21 (40 %) patients: 23 % for hypercholesterolemia and 25 % for diabetes mellitus. The median time between everolimus start and cholesterol-lowering treatment was 3.7 (0.5–14.7) weeks.

Prediction of the response to everolimus

Factors associated with progression-free survival in patients treated with everolimus at univariate analysis are presented in Table 4. Occurrence of hypercholesterolemia under everolimus was associated with longer PFS (HR = 0.13, $p < 0.001$) (Fig. 2a), whereas presence of bone metastasis, number of metastatic sites, high tumour grade and a high tumoral expression of p-p70S6K (HR = 2.51, $p = 0.013$) (Fig. 2b) were associated with shorter PFS under everolimus at multivariate analysis. Among other biological markers, plasma CgA levels ≥ 5 ULN, but not CgA ≥ 2 ULN, was associated with a worse PFS (2.03 (1.05–3.95), $p = 0.04$). A biological response at 3 months and need of treatment for grade 2 diabetes mellitus were not predictive of longer PFS in our study (Table 4). Results after multivariate analysis are similar (data not shown), but with wide ranges of CI due to the low number of patients by groups.

We also studied the association of these factors with overall survival (data not shown), showing similar results than on PFS: more than one metastatic site, bone metastasis, plasma CgA level ≥ 5 ULN, no surgery of primary tumour, no occurrence of hypercholesterolemia under everolimus and a high tumoral expression of p-p70S6K were associated with shorter overall survival from the beginning of everolimus treatment after univariate analysis.

Table 1 Patient and tumour characteristics at initiation of everolimus treatment

Patient characteristics	<i>n</i> = 53
Age in years, median (range)	56 (33–79)
Male/Female, <i>n</i> (%)	26 (49) / 27 (51)
Primary tumour location, <i>n</i> (%)	
Pancreas	40 (75)
Small bowell	9 (17)
Other ^a	4 (8)
Functioning tumours, <i>n</i> (%)	20 (38)
Serotonin-producing tumor with the carcinoid syndrome	8 (15)
Functioning pancreatic tumour ^b	12 (23)
Synchronous/metachronous metastases, <i>n</i> (%)	35 (66)/ 18 (34)
Number of metastatic sites, <i>n</i> (%)	
1	33 (62)
≥ 2	20 (38)
Metastatic sites, <i>n</i> (%)	
Liver	48 (91)
Bone	14 (26)
Peritoneal	10 (19)
Lung	3 (6)
Prior surgery of primary tumour, <i>n</i> (%)	42 (79)
Prior liver directed treatments (radiofrequency ablation, transarterial embolization...), <i>n</i> (%)	16 (30)
Number of prior systemic treatments, median (range)	2 (0–5)
Type of prior systemic treatments received, <i>n</i> (%)	
Somatostatin analogs (during everolimus for 15 patients)	29 (55)
Interferon	13 (25)
Cytotoxic chemotherapy	39 (74)
Sunitinib	1 (2)
Peptide receptor radionuclide therapy	1 (2)
Serum chromogranin A level, median (range) times ULN	7.4 (0.3–198)
Time between diagnosis and everolimus in months, median (range)	44 (3–237)
Tumor characteristic	
Well-differentiated tumours, <i>n</i> (%)	53 (100)
Grade, <i>n</i> (%)	
Grade 1	12 (24)
Grade 2	27 (55)
Grade 3	10 (21)
Not available	4
p-p70S6 kinase (<i>n</i> = 43), <i>n</i> (%)	
Normal or low intensity	21 (50)
High intensity	21 (50)
Not available	1

Abbreviations: *ULN* upper limit of normal, *VIP* Vasoactive intestinal peptide, *PTHrp* Parathyroid hormone-related protein

^a 1 lung, 1 kidney, 1 rectum and 1 unknown

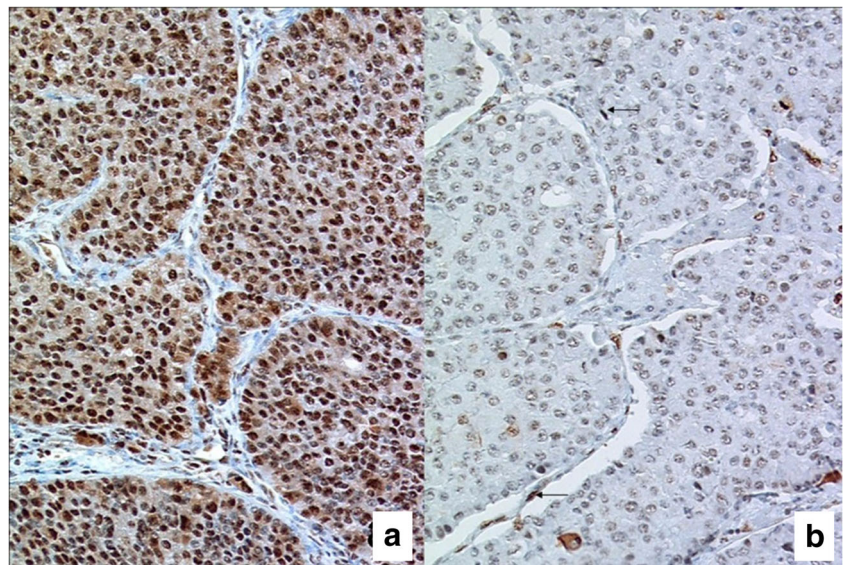
^b 5 gastrinomas, 3 glucagonomas, 1 insulinoma, 1 VIPoma, 2 PTHrp-producing tumors

Discussion

Everolimus is already approved for pancreatic NETs [4]. Data from Radiant-2 and Radiant-4 studies suggest that the drug

might be of interest also in intestinal and pulmonary NETs [2, 3]. We here report the outcome of 53 patients treated with everolimus for a metastatic NET, including pancreatic and non-pancreatic tumors. Our population also includes some

Fig. 1 Immunostaining for p-p70S6K: two pancreatic neuroendocrine tumours with high (**a**: $\times 20$) and low (**b**: $\times 20$) staining in comparison with endothelial cells (*arrow*)



patients with grade 3 tumors, but well-differentiated, not include in Radiant-2, -3, or -4 [2–4]. The median Ki67 index was 10 %. Efficacy of everolimus (9 % of objective response and 8.1 months of PFS) was similar **close** to that published in randomized phase III studies, **but with worse outcome**. Indeed, **in Radiant-2, -3, and -4**, response rate was less than 5 % and median PFS was **11–16.4** months [2–4]. **Median overall survival was also shorter in our study (27.5 months) than in published phase 3 studies (23.7–44 months). This can be explained by a selection of fit patients in phase 3 trials. For instance, in Radiant-3 study, patients had better**

performance status (PS) (97 % of PS 0), less prior lines of treatments (previous chemotherapy was reported in 50 % of patients in Radiant-3 vs 74 % in our patients), and no grade 3 tumor (vs 21 % in our study). Therefore, some patients do not benefit from everolimus, at least at the time of treatment initiation. Predictive factors of response to everolimus are clearly needed because other systemic treatments are available, and that adverse events and cost of everolimus are not negligible.

Table 2 Efficacy of everolimus ($n = 53$)

	$n = 53$
Patients treated in clinical trials ^a , n (%)	36 (68)
Duration of treatment in months, median (range)	6.3 (0.8–54.6)
Clinical response in 20 patients with functional tumour, n (%)	4 (20)
Biological response in 26 patients with high baseline chromogranin A levels, n (%)	14 (54)
Best radiological response, n (%)	
Objective response	5 (9)
Stable disease	36 (68)
Progressive disease	8 (15)
Not available ^b	4 (8)
Median Progression Free Survival in month (95 % confidence interval)	8.1 (4.7–11.5)
Median Overall Survival in months (95 % confidence interval)	27.5 (16.5–38.5)

^a 12 in Radiant-1, 4 in Radiant-2, 12 in Radiant-3, and 8 in Cooperate-2

^b because everolimus was stopped within 2 months for another reason than disease progression

Table 3 Safety ($n = 53$)

Variables	n (%)
Grade 3 and 4 Adverse Events (CTCAE, version 3.0), n (%)	25 (47)
Hypophosphatemia	6 (11)
Diarrhea	6 (11)
Mucositis	3 (6)
Interstitial lung disease	3 (6)
Lymphopenia	3 (6)
Neutropenia	1 (2)
Anemia	1 (2)
Cerebral hemorrhage	1 (2)
Acute renal failure	1 (2)
Acute hepatitis	1 (2)
Vomiting	1 (2)
Hypokalemia	1 (2)
Hyperglycemia	1 (2)
Hypercholesterolemia	1 (2)
Grade 2 metabolic disorders, n (%)	21 (40)
Diabetes mellitus	13 (25)
Hypercholesterolemia	12 (23)

Abbreviations: *CTCAE* Common terminology criteria for adverse events

Table 4 Factors associated with progression-free survival in patients treated with everolimus after univariate analysis

	n	Cox proportional hazards regression	
		Unadjusted analysis	
		HR (95 % CI)	P
Female	27 (51 %)	0.85 (0.46–1.6)	0.62
Primary tumour location			
- Pancreas	40 (75 %)	REF	0.56
- Small Bowel	9 (17 %)	0.96 (0.40–2.3)	
- Other	4 (8 %)	1.8 (0.61–5.0)	
Functional tumour	20 (38 %)	0.6 (0.3–1.2)	0.12
Number of metastatic sites ≥ 2	20 (38 %)	2.3 (1.2–4.3)	0.009
Bone metastasis	14 (26 %)	3.1 (1.5–6.1)	<0.001
Concurrent somatostatin analogs	15 (28 %)	0.64 (0.32–1.3)	0.21
Surgery of primary tumour	42 (79 %)	0.6 (0.3–1.2)	0.13
Baseline chromogranin A ≥ 2 ULN	32 (64 %)	1.5 (0.7–3.0)	0.26
Biological response at 3 months*	11 (55 %)	1.4 (0.51–3.9)	0.50
Ki67**, \geq median (10 %)	26 (55 %)	1.3 (0.67–2.6)	0.43
Grade***			
- G1	12 (25 %)	REF	0.0026
- G2	26 (54 %)	0.75 (0.32–1.77)	
- G3	10 (21 %)	3.0 (1.2–8.0)	
p-p70S6 kinase, high vs normal/low staining****	21 (50 %)	2.5 (1.2–5.4)	0.013
Grade 3–4 adverse events			
- All	25 (47 %)	0.91 (0.48–1.7)	0.77
- Interstitial lung disease	3 (6 %)	0.37 (0.05–2.7)	0.31
- Diarrhea	6 (11 %)	1.1 (0.44–2.9)	0.78
- Hypophosphatemia	6 (11 %)	0.77 (0.30–2.0)	0.29
- Lymphopenia	3 (6 %)	0.56 (0.17–1.8)	0.33
Grade 2 diabetes mellitus	13 (24 %)	0.68 (0.31–1.5)	0.34
Grade 2 hypercholesterolemia	12 (23 %)	0.13 (0.04–0.43)	<0.001

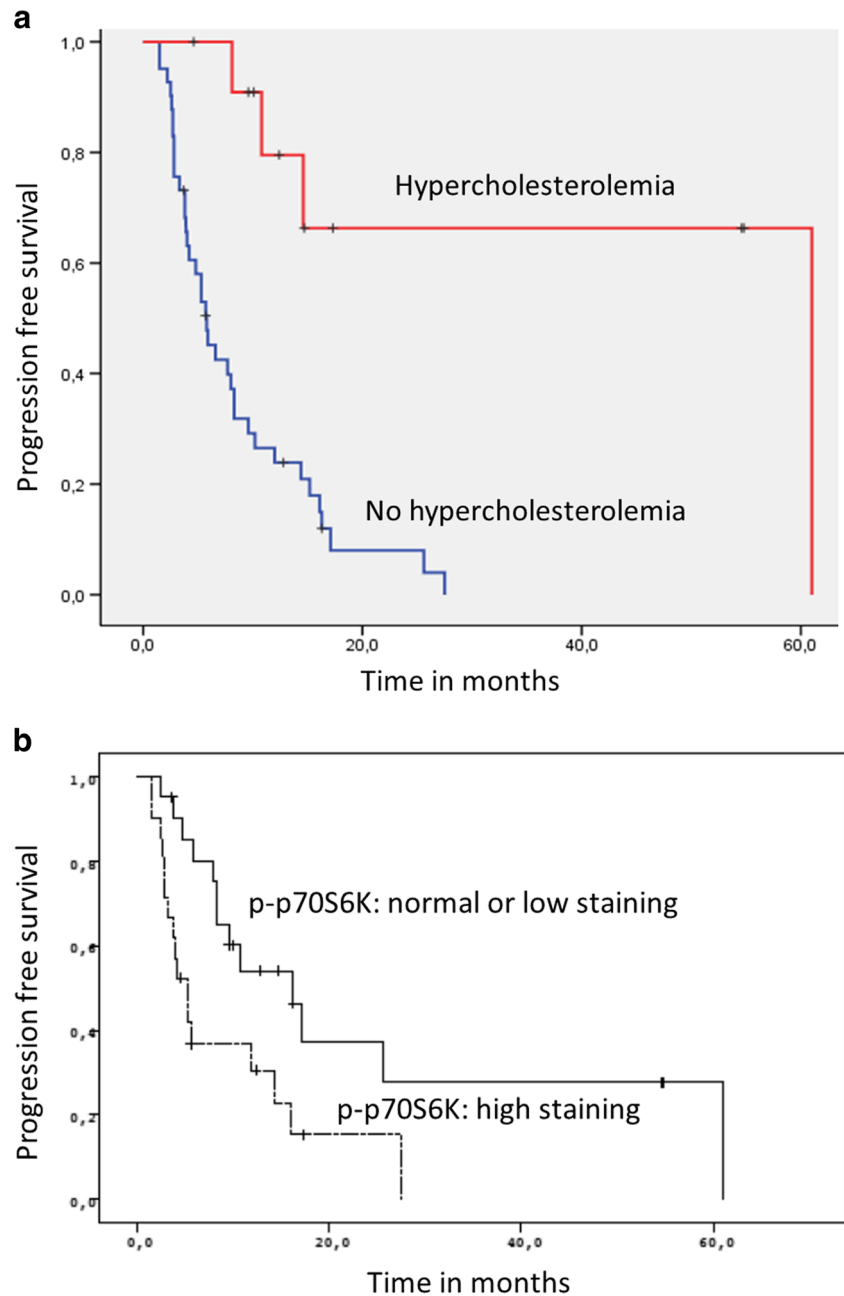
*20 patients evaluated; **47 patients evaluated; ***48 patients evaluated; ****42 patients evaluated

We first aimed at studying the role of conventional clinical, biological or histologic markers to predict the response to everolimus. We found several factors associated with a better PFS under everolimus treatment, such as only one metastatic site or the absence of bone metastases, but these variables have been identified previously as independent prognostic factors [2, 17]; as we have no control group, we cannot conclude that the corresponding patients get a higher benefit from everolimus treatment. Similarly, a high histological grade was associated with shorter PFS under everolimus, but this factor is known to be associated with worse outcome independently of everolimus treatment [2, 18]. The present results are thus in agreement with those of phase III studies vs placebo, where no clinico-biological parameters clearly predicted response to everolimus [2–4].

High levels of phosphorylated p70S6 kinase (**p-p70S6K**) in tumor samples were proposed to identify patients with glioblastoma and NETs more likely to derive benefit from

tensirolium, another mTOR inhibitor [5, 13]. **p-p70S6K is a direct effector of the activated mTOR complex and targets the S6 ribosomal protein to induce protein synthesis. The main location of the protein is nuclear. p-p70S6K can be demonstrated by a robust immunohistochemical test which might be applied to routinely processed and archival tissues, making this test particularly suitable for our retrospective study.** We explored whether this mTOR pathway effector could help to predict tumor response under everolimus. In contrast with prior results, our patients with high p-p70S6K have an even worse outcome under everolimus than patients with low p-p70S6K. These results are in keeping with some recent studies suggesting that high expression levels of mTOR pathway proteins are associated with worse overall survival in NETs [19–21]. Therefore, again because we have no control arm, we cannot demonstrate from our study whether high activation of mTOR pathway would be a predictive factor of response to everolimus; its predictive

Fig. 2 Progression-free survival according to occurrence of hyperlipidemia (a) and expression of p-p70S6K (b)



weight may actually be much lower than its poor prognostic value.

The occurrence of metabolic disorders induced by everolimus treatment looks more promising. Metabolic effects have been reported to yield early markers of response under everolimus in other solid cancers [5, 7]. Metabolic disorders are well-known adverse events of everolimus, but are often underestimated in prospective studies (5 % grade 3 and 4 hyperglycemia, no severe dyslipidemia) because grade-2 metabolic disorders required treatment that prevented them from reaching grade 3 [16]. In our study, 40 % of patients developed grade-2 metabolic disorders, 23 % were treated for

hypercholesterolemia within 4 weeks of treatment by everolimus and 25 % of patients needed the initiation or the increase of an anti-diabetic treatment. **The occurrence of hypercholesterolemia under everolimus is explained by the decrease of the expression of LDL-receptors by mTOR inhibitors, inhibiting cholesterol endocytosis and increasing its serum level [22]. Therefore, hypercholesterolemia could be a reliable surrogate evidence of everolimus exposure, in the absence, for most of the patients, of direct measurements of drug levels in the serum.** We here show that occurrence of hypercholesterolemia was associated with much higher PFS under everolimus. In glioblastoma, Galanis et al. also reported

that development of grade-2 or higher hyperlipidemia in the first two treatment cycles of temsirolimus was associated with a higher percentage of radiological response (71 % v 31 %; $p = 0.04$) [5]. Similar results were reported in renal cell carcinoma treated by temsirolimus [6]. At the 2015 ENETS meeting, Custodios et al. reported on the predictive value of everolimus-induced hyperglycemia in patients with NETs [23]. The risk of confounding predictive versus prognostic factors does not exist here, because we cannot speculate that metabolic disorders could be a good prognostic factor independently of everolimus treatment. Ravaud et al. reported the relationship between everolimus exposure, safety and efficacy [7]. In patients with pancreatic NETs treated by everolimus, the probability of 12-months PFS was higher in patients with average minimum concentration (Cmin) between 10 and 35 ng/mL than in those with an average Cmin between 0 and 10 ng/mL (54 % and 26 %, respectively). In a meta-analysis on more than 900 patients treated by everolimus, they demonstrated that pulmonary, stomatological and metabolic events were associated with increase in everolimus Cmin and efficacy. We did not study serum everolimus concentration in our patients. However, we can hypothesize that stomatological [24] and metabolic disorders, as already reported for lymphopenia [25], could be indirect markers of everolimus exposure.

In conclusion, this retrospective study of 53 patients with NETs treated with everolimus, shows that conventional clinical and pathological markers are not useful to predict response to everolimus. However, occurrence of hypercholesterolemia under treatment may be an early marker of response. Prospective studies are required to confirm these results.

Author contributions Study design: NB, CV, CR, JYS, TW
Collection of data: NB, VH, JYS, JB, PM, TW
Writing the manuscript: NB, JYS, TW
Approval of the manuscript: all authors

Compliance with ethical standards

Conflict of interest T Walter, JY Scoazec have acted as advisory board member for Ipsen, Pfizer and Novartis.
All other authors: no conflict of interest.

Funding This study received financial support from Novartis and la Ligue Contre le Cancer du Rhône, France.

C Vercherat is recipient of a post-doctoral grant from LYric grant INCa-DGOS 4664.

References

1. Yao JC, Hassan M, Phan A, et al. (2008) One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35, 825 cases in the United States. *J Clin Oncol Off J Am Soc Clin Oncol* 26:3063–3072
2. Pavel ME, Hainsworth JD, Baudin E, et al. (2011) Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet* 378:2005–2012
3. Yao JC, Fazio N, Singh S et al. (2016) Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. *Lancet* 387(10022):968–77. doi: 10.1016/S0140-6736(15)00817-X.
4. Yao JC, Shah MH, Ito T, et al. (2011) Everolimus for advanced pancreatic neuroendocrine tumors. *N Engl J Med* 364:514–523
5. Galanis E, Buckner JC, Maurer MJ, et al. (2005) Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a north central cancer treatment group study. *J Clin Oncol Off J Am Soc Clin Oncol* 23:5294–5304
6. Lee CK, Marschner IC, Simes RJ, et al. (2012) Increase in cholesterol predicts survival advantage in renal cell carcinoma patients treated with temsirolimus. *Clin Cancer Res: An Official Journal of the American Association for Cancer Research* 18:3188–3196
7. Ravaud A, Urva SR, Grosch K, et al. (2014) Relationship between everolimus exposure and safety and efficacy: meta-analysis of clinical trials in oncology. *Eur J Cancer* 50:486–495
8. Neshat MS, Mellinghoff IK, Tran C, et al. (2001) Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci U S A* 98:10314–10319
9. Janku F, Wheler JJ, Westin SN, et al. (2012) PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring PIK3CA mutations. *J Clin Oncol Off J Am Soc Clin Oncol* 30:777–782
10. Iyer G, Hanrahan AJ, Milowsky MI, et al. (2012) Genome sequencing identifies a basis for everolimus sensitivity. *Science* 338:221
11. Bollard J, Couderc C, Blanc M, et al. (2013) Antitumor effect of everolimus in preclinical models of high-grade gastroenteropancreatic neuroendocrine carcinomas. *Neuroendocrinology* 97:331–340
12. Cho D, Signoretti S, Dabora S, et al. (2007) Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. *Clin Genitourin Cancer* 5:379–385
13. Duran I, Kortmansky J, Singh D, et al. (2006) A phase II clinical and pharmacodynamic study of temsirolimus in advanced neuroendocrine carcinomas. *Br J Cancer* 95:1148–1154
14. Yoon DH, Ryu MH, Park YS, et al. (2012) Phase II study of everolimus with biomarker exploration in patients with advanced gastric cancer refractory to chemotherapy including fluoropyrimidine and platinum. *Br J Cancer* 106:1039–1044
15. Lombard-Bohas C, Cariou B, Verges B, et al. (2014) Management of metabolic disorders induced by everolimus in patients with differentiated neuroendocrine tumors: expert proposals. *Bull Cancer* 101:175–183
16. Verges B, Walter T, Cariou B (2014) ENDOCRINE SIDE EFFECTS OF ANTI-CANCER DRUGS effects of anti-cancer targeted therapies on lipid and glucose metabolism. *Eur J Endocrinol* 170:R43–R55
17. Bilimoria KY, Talamonti MS, Tomlinson JS, et al. (2008) Prognostic score predicting survival after resection of pancreatic neuroendocrine tumors: analysis of 3851 patients. *Ann Surg* 247:490–500
18. Khan MS, Kirkwood A, Tsigani T, et al. (2013) Circulating tumor cells as prognostic markers in neuroendocrine tumors. *J Clin Oncol Off J Am Soc Clin Oncol* 31:365–372
19. Capurso G, Archibugi L, Delle Fave G (2015) Molecular pathogenesis and targeted therapy of sporadic pancreatic neuroendocrine tumors. *J Hepatobiliary Pancreat Sci* 22(8):594–601. doi:10.1002/jhbp.210.
20. Missiaglia E, Dalai I, Barbi S, et al. (2009) Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. *J Clin Oncol Off J Am Soc Clin Oncol* 28:245–255
21. Qian ZR, Ter-Minassian M, Chan JA, et al. (2013) Prognostic significance of mTOR pathway component expression in neuroendocrine tumors. *J Clin Oncol Off J Am Soc Clin Oncol* 31:3418–3425
22. Sharpe LJ, Brown AJ (2008) Rapamycin down-regulates LDL-receptor expression independently of SREBP-2. *Biochem Biophys Res Commun* 373:670–674

23. Custodio AJ-FP, Alonso-Orduña V, López López C, Alonso T, Guillermo C, Carmona-Bayonas A, Álvarez-Escolá CSM, Capdevila J, Grande E, Barriuso J, Feliu J, Aller J (2015) Everolimus (EVE)-induced hyperglycemia (HG) in patients (pts) with advanced G1-G2 neuroendocrine tumors (NETs): clinical relevance and predictive value. ENETS - The European Neuroendocrine Tumor Society. Barcelona, In
24. Rugo HS, Hortobagyi GN, Yao J, Pavel M, Ravaud A, Franz D, Ringeisen F, Gallo J, Rouyre N, Anak O, Motzer R (2016) Meta-analysis of stomatitis in clinical studies of everolimus: incidence and relationship with efficacy. *Ann Oncol* 27(3):519–525. doi:[10.1093/annonc/mdv595](https://doi.org/10.1093/annonc/mdv595).
25. Templeton AJ, Dutoit V, Cathomas R, et al. (2013) Phase 2 trial of single-agent everolimus in chemotherapy-naive patients with castration-resistant prostate cancer (SAKK 08/08). *Eur Urol* 64:150–158