Sequential Everolimus and Sunitinib Treatment in Pancreatic Metastatic Well-Differentiated Neuroendocrine Tumours Resistant to Prior Treatments

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Keywords

Everolimus · Sunitinib · Pancreas · Molecular agents · Survival · Neuroendocrine tumour

Abstract

Objective: Alternating treatment with sunitinib and everolimus has been shown to be efficacious in renal cell carcinoma. However, no data currently exist for the role of alternate sequence administration of these agents in well-differentiated pancreatic neuroendocrine tumours (pNETs). **Methods:** Thirty-one patients were administered one compound and upon progression were switched to the other. All patients had grade 1 or 2 tumours and stage IV disease with similar metastatic load. The primary end point included estimation of the median overall progression-free survival (mPFS) along with each drug's mPFS as a first-line (mPFS1) and a second-line treatment (mPFS2); tolerability and serious adverse events were also evaluated. Secondary end points included overall survival (OS), 2-year mortality rate, and incidence of disease progression. **Results:** Overall, mPFS did not differ between the everolimus to sunitinib group (36.5 months) and the sunitinib to everolimus group (31.6 months) with a hazard ratio of 0.94 ([95% CI, 0.45–1.97], $p = 0.7$). Although

mPFS1 after first-line everolimus was longer (16.3 months) compared to sunitinib (9 months), this was not statistically significant ($p = 0.15$). Sequential second-line treatment showed no difference in the mPFS2 ($p = 0.3$). No difference in OS between the 2 groups was observed. Tolerability was better for everolimus compared to sunitinib. **Conclusions:** Treatment with sequential molecular target agents was well tolerated and associated with similar overall mPFS in both schemes of administration. Larger prospective studies are required to investigate the long-term efficacy and sequence of administration of alternate therapy with molecular targeting agents in metastatic pNETs and their effect on OS.

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Introduction

Digestive neuroendocrine tumours (NETs) arise from neuroendocrine cells of the pancreas (pNETs) and the gastrointestinal tract [1]. Although pNETs represent ap-

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proximately 1.3% of all cases of pancreatic cancer, their incidence and prevalence are increasing [1]. The majority of pNETs is non-functional, and they are frequently diagnosed at a late stage in the presence of metastatic disease [1–3]. One of the largest series of patients with pNETs showed that those presenting with unresectable or metastatic disease exhibit a worse prognosis with a median overall survival (OS) of 17 months (95% confidence interval [CI], 14–19) compared to those with localized disease that exert a median OS of 100 months (95% CI, 68–148) [2]. However, during the last years the median survival of patients with metastatic pNETs has improved substantially, reaching 41.7 months following the introduction of newer chemotherapeutic agents [4]. Although recent findings suggest that the prognosis of patients with pNETs has probably improved, more robust data on OS estimates are required.

Recently, the findings of a number of phase II and III studies have also documented the efficacy of molecular targeted therapies (MTT) in pNETs [2, 3, 5]. Everolimus inhibits the mammalian target of rapamycin (mTOR), a serine-threonine kinase that stimulates cell growth, proliferation, and angiogenesis [3, 5]. Treatment with everolimus has obtained a 65% reduction in the estimated risk for disease progression or death compared to placebo and a significant 6.4 months increase in median progression-free survival (mPFS) in advanced pNETs that had progressed despite previous treatment [2].

Vascular endothelial growth factor (VEGF) receptor inhibitors are also an alternative therapeutic option for patients with metastatic pNETs [6]. Sunitinib, a potent multi-targeted tyrosine kinase inhibitor with direct anti-tumour and anti-vascular effect, has been shown to selectively inhibit multiple receptor tyrosine kinases that are implicated in tumour growth, neoangiogenesis and metastatic progression of cancer [7, 8]. A phase III trial that evaluated sunitinib versus placebo in patients with low- and intermediate-grade progressive pNETs found a statistically significant improvement in the PFS in the sunitinib arm (11.4 vs. 5.5 months; $p < 0.001$) [6].

Recent position statements and guidelines propose that everolimus and sunitinib are both approved in progressive grade 1 and 2 pNETs, irrespective of Ki-67 value and tumour burden, and are considered as different treatment options in pNETs [9]. Thus, both agents may be used as first- or second-line options with respect to chemotherapy or subsequent to therapy with long-acting somatostatin analogues (SSAs) [9–11].

These 2 agents are standard therapies for other types of cancer such as metastatic renal carcinoma [12, 13].

Current treatment guidelines for patients with metastatic renal cell cancer recommend a first-line challenge with a VEGF inhibitor, including sunitinib, followed by everolimus at progression [12, 13]. Recent trials have shown the non-inferiority of everolimus compared to sunitinib as first-line treatment and support the standard treatment paradigm of first-line sunitinib followed by everolimus at progression [14].

In the present study, we aim to investigate whether MTT with everolimus and sunitinib administrated sequentially in patients with advanced well-differentiated pNETs, that developed disease progression following the administration of one of these agents, could be tolerated and affect disease progression and survival.

Patients and Methods

Patients

We studied 31 patients with advanced well-differentiated pNETs and stage IV/IIIB disease at initial diagnosis and evaluated their response to sequential treatment with MTT according to Response Evaluation Criteria in Solid Tumours (RECIST) criteria. Before entering the study, all patients had disease progression documented by RECIST besides different prior therapeutic modalities (surgery, peptide receptor radiotherapy treatment [PRRT], chemotherapy and SSA). In particular, 18 patients had already received PRRT (median 6 [range 3–9] cycles), 12 patients had already received chemotherapy (1 patient had received 4 cycles of combination therapy with capecitabine/temozolomide, 6 patients streptozotocin and 5-fluorouracil, 2 patients cisplatin-etoposide, and 3 patients temozolomide alone), and 22 patients were treated with SSAs concomitantly with other treatments.

All patients had stage IV disease at the time of initiation of MTT, similar tumour load, in respect of the different organs involved (liver, chest, bone, lymph nodes), the number and the size of metastases, and similar grading as shown by the Ki-67% labelling index value. All patients of each group entered the second-line treatment with MTT when the disease had progressed according to findings of imaging modalities including computed tomography and magnetic resonance imaging or when there was no tolerability of MTT because of serious adverse events (AEs). Radiological imaging to document disease progression was performed every 3–6 months.

Patients had no contraindications for the initiation of MTT or other serious comorbidities except well-controlled hypertension in 6 patients and diabetes mellitus type 2 in 8 patients (3 out of 31 were receiving concomitantly at least 1 anti-hypertensive drug and/or 1 anti-diabetic agent). Before treatment initiation with an MTT, all patients had pre-defined haematology and biochemistry analysis (baseline values) which included a complete blood count control, urea and electrolytes, renal and hepatic function, bone metabolism, and thyroid function.

Study Design and Treatment

In this retrospective study, data were obtained and analyzed from 2 institutional collected databases from 2 centres. The study

included consecutive patients with metastatic pNETs (stage IV) resistant to prior therapeutic modalities who received an MTT, either everolimus ($n = 20$) as first-line treatment followed by sunitinib as second challenge (everolimus to sunitinib group) or sunitinib ($n = 11$) as a first-line treatment followed by everolimus (sunitinib to everolimus group) as second challenge. The allocation of the sequence of treatment with either MTT was decided by the treating physicians based on local availability and personal preferences.

Patients received each drug until documented disease progression according to RECIST criteria or development of unacceptable toxicity. The everolimus full dosage was 10 mg daily, and the sunitinib 37.5 mg daily. However, dose modifications were permitted in the presence of AEs, so that everolimus could be decreased to 5 mg and sunitinib to 25 mg daily, respectively.

The study protocol was approved by the ethics committee of both participating centres, according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research. All patients gave informed consent according to the Declaration of Helsinki and Good Clinical Practice guidelines.

Primary and Secondary End Points

The primary end point of the study was to assess overall mPFS after the introduction of both agents in each treatment group, as well as the mPFS after first- and second-line treatment with either MTT agent used in a sequential manner. In particular, mPFS after the first-line treatment (mPFS1) was defined as the time from the first initiation of an MTT (with either everolimus or sunitinib) to treatment discontinuation either due to disease progression or development of serious AEs. mPFS for the second-line treatment (mPFS2) was defined as the time after the switch from one MTT agent to the other, until treatment discontinuation either due to disease progression or development of serious AEs or death.

Tolerability and AEs were also included as primary end points. AEs were classified as grade 1 (mild and transient effect), grade 2 (moderate), grade 3 (severe necessitating medical intervention), and grade 4 (potentially life threatening necessitating hospitalization). Dose modifications were introduced for grade 1, 2, and 3 AEs. Discontinuation of the treatment was decided when the patients had progressed according to RECIST or when patients developed unacceptable toxicity (grade 3 or 4).

Secondary end points included estimation of OS in months defined as the time from the initiation of the MTT to death as well as the 2-year survival rate. Incidence of the disease progression was also analyzed. Hazard ratios (HR) were calculated for both the PFS and OS.

Statistical Analysis

Statistical analysis was performed using Stata 10 (StataCorp, College Station, TX, USA). The survival analysis was performed using the Kaplan-Meier method, and the results were compared using the log-rank test. Cox proportional hazard models (HR) with 95% CI were applied adapted for confounding factors such as age and gender. Results were considered significant at $p < 0.05$. The distribution of continuous variables is reported as median values or mean $\pm$ standard deviation (SD). Recurrent events analysis was performed using the Andersen-Gill Cox regression model [15].

Results

Patient Population

Thirty-one patients (8 females, median age 52.5 years) were recruited from 2 European Centres (Department of Pathophysiology, Sector of Endocrinology, Laiko Hospital, University of Athens, Greece, and Department of Internal Medicine, Sector of Endocrinology, Erasmus MC, Rotterdam, Netherlands). The characteristics of the studied population are presented in Table 1. Patients had no differences in respect of their baseline characteristics such as age, sex, grade, and disease stage. Ten patients (32%) had a functional pNET, 18% in the sunitinib to everolimus group, and 40% in the everolimus to sunitinib group albeit with no statistical significant differences ($p = 0.2$). Twenty-three patients had stage IV and 4 patients had stage IIIB disease at initial diagnosis; however, at the initiation of MTT, all patients had stage IV disease. Nineteen (61%) patients had grade 2 and 8 (26%) grade 1 tumours (in the remaining 4, the grade was unknown). Twenty patients received first-line everolimus followed by sunitinib, and 11 were assigned to first-line sunitinib followed by everolimus. The most frequent previous therapeutic modalities included SSAs (in 71% of the cases), PRRT (in 58% of the cases), surgical ablation (in 58% of the cases), and chemotherapy (in 39% of the cases). PRRT was a more frequent treatment modality in the everolimus to sunitinib group versus sunitinib to everolimus group (70 vs. 36%, $p = 0.07$), whereas liver-directed therapy in the form of radiofrequency ablation was more frequent in the sunitinib to everolimus group (27 vs. 5%, $p = 0.08$), albeit without both reaching statistical significance (Table 1).

Tumour load and distribution were similar in all patients. The median Ki-67 value was 5% in both groups (mean values = 6.0 ± 3.5 for the everolimus to sunitinib group vs. 5.0 ± 3.9 for the sunitinib to everolimus group, $p = 0.33$). Seven patients had a Ki-67 $>10\%$ and 20 had a Ki-67 $<10\%$, whereas in 4 patients the Ki-67 was not available. All 20 patients from the group that received first-line everolimus had liver metastases; 13 of these patients (65%) had multiple (>5) metastases, 4 also had bone metastases, and 2 had pulmonary metastases. All 11 patients from the first-line sunitinib group had liver metastases; in 7 of them (63%), the metastases were multiple (>5), 1 also had bone metastases, 1 peritoneal metastases, and 1 had pulmonary metastases.

The time interval between the first-line treatment and the second challenge during which the vast majority of

Table 1. Baseline patient characteristics and comparison of the patients in the 2 studied groups (sunitinib to everolimus vs. everolimus to sunitinib)

Characteristics	Whole group	Sunitinib to everolimus group	Everolimus to sunitinib group	<i>p</i> value
Patients, <i>n</i>	31	11	20	
Median age, years	52.5	51.46	57.9	0.3
Males, %	23	9 (81%)	14 (80%)	0.5
Stage	all stage IV	all stage IV	all stage IV	
Grade				
1	8 (26%)	4 (36%)	4 (25%)	0.8
2	19 (61%)	6 (55%)	13 (65%)	0.8
Unknown	4 (13%)	1 (1%)	3 (15%)	
Familial syndrome	4 (13%)	1 (MEN-1)	3 (all MEN-1)	
Functional syndrome	10 (32%)	2 (18%)	8 (40%)	0.2
Other comorbidities				
HTA	6 (19%)	–	–	
DM2	8 (26%)	–	–	
Previous treatments				
Surgery	18 (58%)	7 (63%)	11 (55%)	0.6
Chemotherapy	12 (39%)	4 (36%)	8 (40%)	0.8
PPRT	18 (58%)	4 (36%)	14 (70%)	0.07
SSA	22 (71%)	8 (72%)	13 (65%)	0.65
TACE	3 (10%)	1 (9%)	2 (10%)	0.4
Radiofrequency	4 (13%)	3 (27%)	1 (5%)	0.08
Ki-67, %	6±0.5 (5)	5.0±3.9 (5)	6.0±3.5 (5)	0.33
Follow-up, months		11.7±11.8 (7.4)	14.3±12.9 (10.4)	0.6
PFS for pNETs ^a	12.5 (7.7–27.7)	9 (6.0–9.4)	16.3 (7.8–27.7)	0.15
PFS for pNETs ^b	12.4 (3.4–22.6)	15.5 (4.3–25)	10.4 (2.4–13.3)	0.3
Disease progression				
After first-line treatment	25 (81%)	7 (63%)	18 (90%)	0.3
After second-line treatment	16 (52%)	7 (55%)	9 (60%)	0.4
Mortality	11 (35%)	3 (27%)	8 (40%)	0.5

HTA, hypertension; DM2, diabetes mellitus type 2; PPRT, peptide receptor radiotherapy; SSA, somatostatin analogues; TACE, transarterial chemo-embolization; PFS, progression-free survival; pNET, pancreatic neuroendocrine tumour. Values for Ki-67 and follow-up are expressed as mean ± SD (median). ^a After first-line treatment with everolimus or sunitinib (before the switch); median (25–75%). ^b After second-line treatment with everolimus or sunitinib (after the switch); median (25–75%).

patients received no other treatment except SSAs was similar in the 2 groups: 3.92 ± 5.0 months for the everolimus to sunitinib group versus 4.08 ± 5.9 months for the sunitinib to everolimus group ($p = 0.78$).

Primary End Points

Progression-Free Survival

No significant differences were found for overall mPFS in both treatment groups. In particular, the overall mPFS for the everolimus to sunitinib group was 31.6 months and for the sunitinib to everolimus 36.5 months with an HR of 0.94 (95% CI, 0.45–1.97), suggesting that the order

of the sequencing of the treatment did not exert a significant effect on mPFS. The mPFS1 after first-line everolimus was longer (16.3 months, $n = 20$) compared to sunitinib (9 months, $n = 11$), although not statistically significant ($p = 0.15$) with an HR of 1.98 (95% CI, 0.77–5.14) for the first-line sunitinib vs. the everolimus group (Fig. 1). The mPFS2 after the sequential second-line treatment also showed no difference ($p = 0.3$) between the 2 agents (15.5 months for everolimus, $n = 11$, vs. 10.3 months for sunitinib, $n = 20$) with an HR of 1.66 (95% CI, 0.63–4.36) for the second-line sunitinib vs. the everolimus group (Fig. 2).

Fig. 1. Kaplan-Meier plots for progression-free survival (PFS) after first-line treatment with everolimus (E) and first-line treatment with sunitinib (S). The median PFS1 for the first-line treatment with everolimus was 16.3 months, and with sunitinib 9 months ($p = 0.15$, HR [95% CI] = 1.98 [0.77–5.14]).

Fig. 2. Kaplan-Meier plots for progression-free survival (PFS) after second-line treatment with everolimus (E) and sunitinib (S). The median PFS2 after the sequential second-line treatment showed no difference ($p = 0.3$) between the 2 agents (15.5 months for everolimus vs. 10.3 months for sunitinib, HR [95% CI] = 1.66 [0.63–4.36]).

Table 2. Number of patients who developed AEs following MTT with everolimus and sunitinib during the first-line and the second-line treatment

AEs	1st line everolimus (n = 20)		1st line sunitinib (n = 11)		2nd line everolimus (n = 11)		2nd line sunitinib (n = 20)	
	grade 1/2	grade 3	grade 1/2	grade 3	grade 1/2	grade 3	grade 1/2	grade 3
Haematological ^a	4 (20)	1 (5)	2 (18)	2 (18)	2 (18)	1 (9)	4 (20)	2 (10)
Gastrointestinal intolerance ^b	3 (15)	–	4 (36)	2 (18)	3 (36)	1 (9)	–	2 (10)
Fatigue	2 (10)	–	3 (36)	2 (18)	2 (18)	–	2 (10)	2 (10)
Liver dysfunction	2 (10)	–	–	–	1 (9)	–	–	1 (5)
Abdominal pain	–	–	2 (18)	1 (9)	–	–	1 (5)	3 (15)
Dermatitis	5 (25)	–	1 (9)	–	2 (18)	–	1 (5)	–
Hyperglycaemia	4 (20)	2 (10)	–	–	2 (18)	–	–	–
Pneumonitis	4 (20)	1 (5)	–	–	1 (9)	1 (9)	–	–
Kidney failure	2 (10)	–	1 (9)	–	1 (9)	–	1 (5)	–
Hypothyroidism	1 (5)	–	–	–	–	–	4 (20)	–
Stomatitis	2 (10)	–	3 (36)	–	1 (9)	–	1 (5)	1 (5)

Data are presented as n (%). AEs, adverse effects; MTT, molecular targeted therapy. ^a Includes anaemia, leucopenia, thrombopenia, ^b Includes nausea, vomiting, diarrhoea, change of taste.

Adverse Events and Tolerance

Fifteen out of 20 (75%) patients developed AEs (all grades) during the first-line treatment with everolimus compared to 7 patients out of 11 (64%) during the first-line treatment with sunitinib. Five out of 11 (45%) developed AEs (all grades) during the second-line treatment with everolimus compared to 11 out of 20 (55%) during the second-line treatment with sunitinib. Four out of 20

(20%) patients who received first-line treatment with everolimus had grade 3 AEs, and 2 of them (10%) had to discontinue treatment with everolimus and switch to the second challenge treatment with sunitinib because of intolerant AEs (Table 2). Five out of 11 (45%) patients who received first-line treatment with sunitinib had grade 3 AEs, and 4 of them (36%) had to switch to the second challenge treatment with everolimus because of intoler-

Fig. 3. Kaplan-Meier plots for overall survival (OS) for the everolimus to sunitinib group (E→S) compared to sunitinib to everolimus group (S→E). No statistical significant differences were found in the median OS time between both groups ($p = 0.875$, HR [95% CI] = 0.90 [0.23–3.45]).

ant AEs. During the second-line treatment, 3 out of 11 (27.7%) patients who received everolimus as second-line treatment had grade 3 AEs, and 1 of them (9%) discontinued treatment. Seven out of the 20 (35%) patients who received sunitinib as second-line treatment had grade 3 AEs, and 3 (15%) of them discontinued treatment (Table 2).

None of the patients developed grade 4 AEs. In all the other cases, AEs were well tolerated especially after dose adaptation. Serious AEs after first-line treatment with either single agent were less compared to those after the sequential treatment with the 2 agents (serious AEs presented in 10% of patients who received everolimus as first-line treatment vs. 25% of patients who received the sequential treatment of everolimus followed by sunitinib, $p = 0.4$, and in 36% of patients who received sunitinib as first-line treatment vs. 45% of patients who received the sequential treatment of sunitinib followed by everolimus, $p = 1.0$).

Anaemia, hyperglycaemia and pneumonitis as well as cutaneous manifestations were the most frequent AEs in the everolimus to sunitinib group, whereas fatigue and abdominal pain in the sunitinib to everolimus group.

Secondary End Points

No statistically significant differences were found in the median OS time in both groups ($p = 0.875$) (Fig. 3). The HR for the sunitinib to everolimus group versus the everolimus to sunitinib group was 0.90 (95% CI, 0.23–3.45). The 2-year survival rate in the everolimus to sunitinib group was 0.83 (95% CI, 0.57–0.94) compared to 0.69 (95% CI, 0.31–0.89) in the sunitinib to everolimus group, but without statistical significance.

Disease progression after the first-line treatment was observed in 18 out of 20 patients with pNETs (90%) after a median 13.6 months' exposure to everolimus and in 7 out of 11 patients (63%) after a median 7.4 months' exposure to sunitinib. Disease progression after the second-line treatment was observed in 9 out of 20 patients (45%) after a median 7.2 months' exposure to sunitinib and in 7 out of 11 patients (63%) after a median 7 months' exposure to everolimus. Second-line treatment was discontinued because of death in 8 patients (40%) from the everolimus to sunitinib group and in 3 patients (27%) from the sunitinib to everolimus group. The overall mortality rate for both groups was 35% (11 patients out of 31). All deaths were considered disease-related and were observed only during the second-line treatment period with either agent.

Discussion

This is the first study in metastatic pNETs and progressive disease despite previous treatments investigating the tolerability and the role of sequential treatment using the currently approved MTT agents, everolimus and sunitinib, in respect of PFS and OS. The comparison of first-line treatment with either everolimus or sunitinib did not show any statistical difference in PFS in favour of any of the 2 agents used. In addition, sequential treatment was not associated with any difference in OS between the 2 therapeutic schemes. Serious AEs necessitating treatment discontinuation were observed in 25% of patients in the everolimus to sunitinib group and in 45% of patients in the sunitinib to everolimus group. Patients seemed to tolerate better everolimus compared to sunitinib albeit without statistical significance.

The PFS obtained when either agent was used as second-line treatment compares favourably to that obtained in the placebo group of patients included in the RADIANT-2 and the study evaluating the efficacy of sunitinib in progressive pNETs [6, 11]. Although indirect, these findings suggest that sequential administration of MTT agents results in clinically meaningful responses with prolongation of PFS following disease progression to the initial challenge with MTT. Although there was no statistical significance in overall mPFS between the 2 schemes, everolimus as first-line treatment was associated with a better mPFS1 compared to sunitinib (16.3 vs. 9.0 months,

respectively), whereas sunitinib as second-line treatment was associated with a better mPFS2 compared to everolimus (15.5 vs. 10.4 months, respectively). A possible continuous anti-proliferative effect of previously applied PRRT that was mostly utilized in the everolimus to sunitinib group could have accounted for this effect; however, patients in the sunitinib to everolimus group had more liver-directed cytoreductive interventions. Although robust conclusions regarding the ideal sequence of treatment cannot be derived from this study, it is probable that previous treatments could affect first-line treatment and the efficacy of the sequential treatment. It is expected that the on-going characterization of the molecular profiling of pNETs will make possible the selection of the sequence of the most appropriate agent [16].

pNETs are in their great majority slowly growing tumours and generally more indolent than adenocarcinomas; however, once these tumours progress beyond surgical resectability, they are essentially incurable [17]. Systemic treatment options have substantially been expanded in recent years with long-acting SSAs regarded as the first therapeutic option for well-differentiated pNETs with a Ki-67 value <10% [18]. However, the majority of patients will eventually develop progressive disease necessitating further systemic therapy. Following disease progression, different therapeutic options are available including cytotoxic chemotherapy, PRRT and the administration of MTT agents [19, 20]. Currently, there are no comparative studies evaluating the ideal sequence of these therapies upon progression, although several algorithms have been proposed by relevant scientific societies taking into consideration parameters such as the Ki-67 value, tumour growth rate, and disease burden [10, 19].

Although MTT agents have been used following disease progression to SSAs, the issue of sequential treatment with currently available MTT agents in pNETs has not yet been formally addressed as in other malignancies [10]. Alternate sequence of sunitinib and everolimus has been well studied in advanced renal cell carcinoma where sunitinib and everolimus are both approved as first-line and second-line therapies, respectively [12, 13]. A prospective clinical trial in patients with advanced renal cancer (RECORD-3) comparing sunitinib until progression followed by everolimus versus the reverse sequence demonstrated the non-inferiority of everolimus compared to sunitinib in terms of PFS and OS, with important crossover of patients (43–45%) from one group to the other [14]. Sunitinib exhibited a slightly longer PFS and OS without reaching statistical significance, though

[14]. In the RECORD-1 trial, that compared everolimus with placebo after sunitinib or sorafenib failure in advanced renal cell carcinoma, the mPFS was 4.9 vs. 1.9 months compared to the placebo group [21]. These data suggest that a programmed sequential strategy administering sunitinib and everolimus without waiting for disease progression may be considered as a potential option in patients with advanced renal cell carcinoma. In the present study, sequential therapy with both MTT schemes showed increased PFS compared to monotherapy with each agent alone, without the sequence of administration being important.

Although there are no clinical data evaluating the effects of a second-line challenge treatment with an MTT agent in pNETs, *in vitro* studies offer a better understanding of the mechanism underlying the role of the sequence of treatment of MTT agents in cancerous tissues. The sequential combinations of everolimus and sunitinib in a renal cancer mouse model induced anti-angiogenic effects, leading to tumour necrosis [22]. In particular, the addition of everolimus after 5 weeks' exposure of renal cancer cells to sunitinib slowed tumour growth further [22]. Alternate sequence of sunitinib and everolimus mitigated the development of mesenchymal phenotype compared with sunitinib as single agent [23]. In particular, mice treated with sunitinib monotherapy presented controlled tumours characterized by lower vessel size compared to progressive tumours, while everolimus was more efficient on mature vessels than sunitinib that mainly affected unstable neoangiogenesis [23].

Besides sequential treatment, multiple combination therapies have been explored in phase I/II trials either as a *de novo* combination of 2 targeted drugs, or of targeted drugs with SSAs, but also with chemotherapy, PRRT, or even locoregional therapies of the liver. In one of these trials, everolimus was explored in combination with the novel SSA pasireotide, an upstream activator of the mTOR pathway without any benefit in the PFS [24]. Another trial investigated the combination of everolimus and the angiogenesis inhibitor bevacizumab that binds circulating VEGF compared to everolimus alone [25]. Although PFS was statistically significantly different between the 2 arms, the clear benefit was only modest (2.7 months) in the combination treatment of everolimus with bevacizumab, at the expense of more side effects. Due to more side effects, treatment was discontinued in 38% of the patients receiving the combination treatment compared to 16% with everolimus alone [25]. Another promising combination therapy is that of metformin in association with everolimus. Metformin activates ade-

nosine monophosphate-activated protein kinase, and might enhance the anti-proliferative efficacy of everolimus. Both drugs are currently investigated in pNETs along with the administration of octreotide [26].

While the choice of targeted therapies in other malignancies is frequently driven by the findings of the precise molecular alterations present in the tumour, no such study has been done in NETs. This is particularly important given that the survival of patients with advanced NETs appears to be different based on the driver mutation(s) present in the tumour. An ongoing study includes the analysis of the involved cell signalling pathways for the treatment of patients with advanced metastatic gastrointestinal NETs and pNETs with currently approved MTT agents according to the mutations present (sunitinib for *MEN1/PDGFR/KIT/FLT3* mutations or everolimus for *NF1/PTEN/PI3K/AKT/mTOR/VHL/TP53* mutations) [16].

In this study, both combinations with the 2 MTT agents were relatively well tolerated by the majority of the patients. When comparing the tolerability of each agent when administered alone as first-line treatment with other studies, dermatitis of any grade was the most frequent AE (25%) in our patients treated with first-line everolimus, and hyperglycaemia was the most serious one (10%) (grade 3). However, in the RADIANT-2 [11] and RADIANT-4 [27] trials, stomatitis (7 and 9%, respectively) was the most frequent and the most serious AE followed by diarrhoea (6 and 7%). In addition, diarrhoea, fatigue, and stomatitis were the most frequent AEs (36%) in our patients treated with first-line sunitinib, and neutropenia, diarrhoea, and fatigue (18% for each symptom) the most serious ones (grade 3/4). Similarly, in the SUN III [6] study, diarrhoea was also the most frequent AE of any grade (59%) and neutropenia (12%) the most frequent serious AE (grade 3/4).

The major inherent limitation of the present study lies, due to its design, in the retrospective nature of the analysis. This limitation, a common feature in most observational studies, may explain the allocation of a different number of patients in each group and the different duration of treatment. Unfortunately, the small sample size did not provide sufficient statistical power for any of our findings. Additionally, the lack of a separate group of patients receiving monotherapy with an MTT agent did not permit a direct comparison between the single agent treatment and the combination of the 2 agents regarding PFS, OS, and mortality. However, this is the first clinical study comparing sequential challenge treatment in this rare category of neoplasms and showing that this treat-

ment is well tolerated and can be used even in previously heavily treated patients. The rationale of performing such studies has also been highlighted in a recent comprehensive review of the effect of available MTT on PFS and OS in patients with pNETs [28].

In conclusion, overall mPFS and OS did not seem to differ significantly among patients who received sequential treatment with both everolimus and sunitinib irrespective of the order of administration. The sequential treatment was well tolerated in both schemes of administration, but the small sample size and the retrospective nature of our study did not allow a robust conclusion to be made. However, these data provide evidence that programmed alternating sequential strategies studied in a prospective manner could demonstrate the therapeutic efficacy of this approach in patients with advanced pNETs.

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