

## Medical therapy for advanced gastro-entero-pancreatic and bronchopulmonary neuroendocrine tumors

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### ABSTRACT

Neuroendocrine tumors (NETs) represent a spectrum of rare neoplasms arising in different organism sites. Depending on the site of onset, they also can be distinguished using lab exams (secreting vs. nonsecreting), clinical symptoms (functioning vs. nonfunctioning), behavioral, morphological characteristics (tumor cells' architectural growth patterns, mitotic and Ki-67 index, presence of necrosis), and grade of cellular differentiation. The aim of this review is to focus on the main signaling pathways targeted by medical treatments of advanced sporadic gastro-entero-pancreatic (GEP) and bronchopulmonary (BP) neuroendocrine neoplasms. The scientific literature regarding treatment of advanced GEP and BP-NETs has been extensively reviewed using MEDLINE and PubMed databases, selecting principal and more recent research articles, clinical trials, and updated guidelines. Somatostatin analogues represent a valid approach to control symptoms in functioning tumors and to inhibit tumor progression in certain categories on the basis of the typical somatostatin receptor expression observed in NETs. The pathogenesis of NETs has been the subject of increased interest in recent years. Many driver mutations pathway genes have been identified as important factors in the carcinogenesis process and, therefore, as potential targets for new anticancer therapies. Activating mutations have been shown in epidermal growth factor receptor, stem cell factor receptor, platelet-derived growth factor receptor, vascular endothelial growth factor, basic-fibroblastic growth factor, transforming growth factor, insulin-like growth factor-1, and their receptors. Effective M-Tor inhibition pathway modulation has led to the approval of drugs in this field such as everolimus. New drugs and several combination regimens with targeted and newer biological agents are being developed and tested in recently conducted and ongoing trials.

**Key words:** Gastrointestinal and bronchopulmonary neuroendocrine tumors; advanced disease; medical treatment; targeted agents

### INTRODUCTION

Neuroendocrine neoplasms typically occur in gastrointestinal and bronchopulmonary tracts. Gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NENs) originate from neuroendocrine cells of the gastrointestinal tract and pancreatic islets.<sup>[1]</sup>

Three-tiered grading systems have been proposed for GEP-NENs classification, according to their morphological features and ki-67 index:<sup>[2]</sup> neuroendocrine tumors (NETs), involving G1 (ki67 < 3%) and G2 (ki67 ≥ 3 and ≤ 20%) neoplasms, and neuroendocrine carcinomas, G3 with ki67 > 20%. Neuroendocrine carcinomas show worse prognosis, and platinum-based chemotherapy is currently considered the standard of care.<sup>[3,4]</sup>

Identification of many driver mutations in pathway genes involved in the pathogenesis of well- and moderately-differentiated NENs has promoted the development of specific targeted therapies.<sup>[5-7]</sup>

Conversely, bronchopulmonary NETs are approximately 20-25% of all lung malignancies.<sup>[8-12]</sup> On the basis of 2004, World Health Organization classification, pulmonary NETs can be divided into three groups:<sup>[13]</sup> carcinoid tumors (typical carcinoids/atypical carcinoids) (1-2%), large-cell neuroendocrine (LCNEC) (3%), and small-cell carcinomas (SCLC) (15-20%). According to immunohistochemical markers, these neuroendocrine

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entities are further summarized into 2 groups based on their grade of biological aggressiveness: well-differentiated neoplasms including typical and atypical carcinoids, and poorly differentiated ones involving LCNEC and SCLC.

Despite comprehensive and notable medical progress, therapeutic options are still inadequate for gastrointestinal and bronchopulmonary (BP) neuroendocrine tumors, due to the lack of in-depth knowledge of molecular mechanisms and predictive factors. This review aims to summarize the current knowledge about pathways involved in advanced, sporadic well- and moderately differentiated GEP-NETs and in BP carcinoids, highlighting available evidences on biological and targeted therapies.

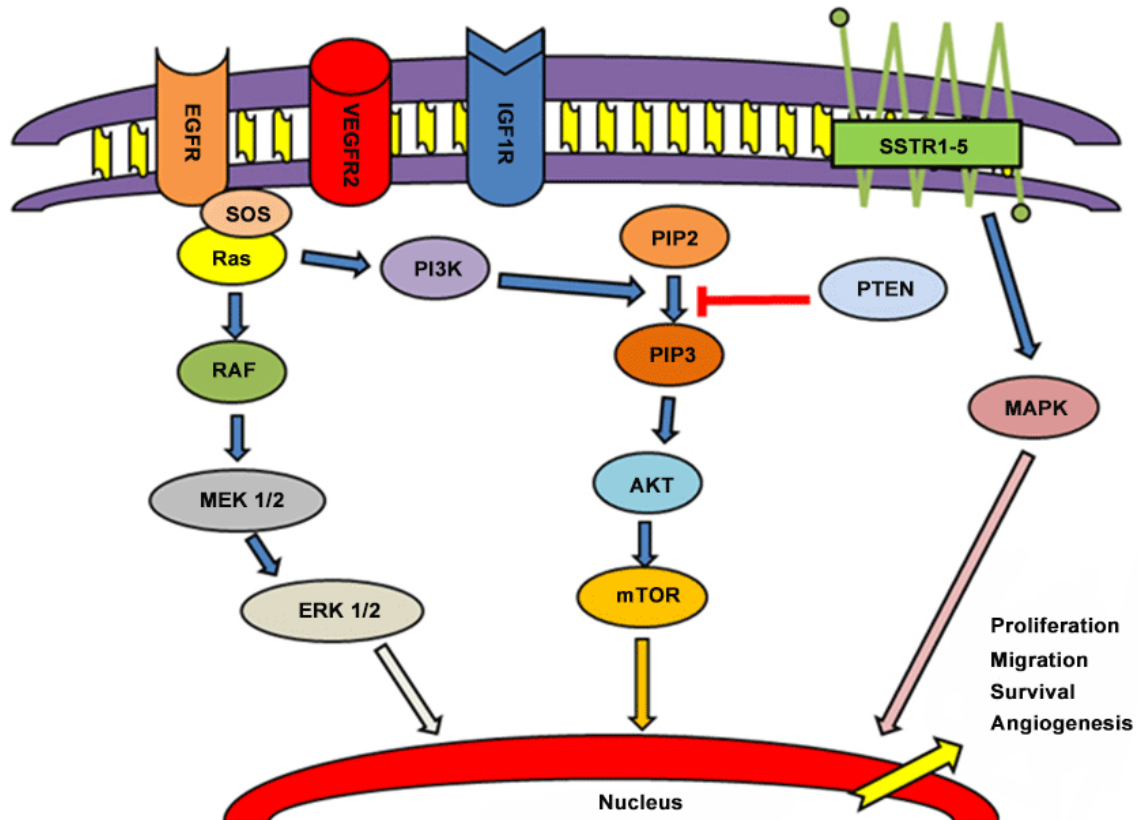
### SHORT SYNTHETIC ANALOGUES OF SOMATOSTATIN

The primary treatment objective for patients with NETs is cure. Symptom control and limitation of disease progression represent the secondary goals. The traditional first and only possible radical approach is surgery. However, NETs are frequently diagnosed in advanced stages when curative surgery is generally not possible. Medical management with the principal objective of

relieving symptoms and, in recent years, of suppressing tumor growth and spread is a necessary option for advanced NETs that are unsuitable for surgery.<sup>[14]</sup>

Among medical therapies, Short synthetic analogues of somatostatin (SSAs) represent one of the possible options in the presence of carcinoid syndrome. SSAs include octreotide, lanreotide, vapreotide, seglitide, and pasireotide. SSAs' affinity for the distinct receptor subtypes is different than that of native somatostatin.<sup>[15-17]</sup> Five different somatostatin receptor (SSTR) subtypes have been characterized in humans (SSTR1-SSTR5) [Figure 1].<sup>[18-22]</sup> SSTR2 represents the principal target for octreotide, lanreotide, vapreotide, seglitide, and pasireotide. Furthermore, pasireotide shows a higher binding capacity towards SSTR1, activating also SSTR 3 and 5.<sup>[23-25]</sup> For this reason, different SSAs show a distinct affinity with their own ligands, eliciting various biological and clinical activities<sup>[16]</sup> in the same cell type through the activation of subsets of disparate intracellular mediators.<sup>[23,24,26]</sup> Nevertheless, the natural ligands of SSTR1-5 can bind all somatostatin receptors with high affinity.

SSTRs were expressed in over 80% of well-differentiated GEP-NENs. SSTR in particular has been observed to predominate in both gastrointestinal-NENs (90%) and primitive-NETs (P-NETs), especially in gastrinomas,



**Figure 1:** Principal pathways involved in carcinogenesis and progression of NENs. EGFR: epidermal growth factor receptor; VEGFR2: vascular endothelial growth factor receptor 2; IGF1R: insulin-like growth factor 1 receptor; SSTR: somatostatin receptors; SOS: save our souls; PI3K: phosphoinositide 3-kinase; PIP2: phosphatidylinositol biphosphate 2; PIP3: phosphatidylinositol biphosphate 3; PTEN: phosphatase and tensin homolog; MEK: methyl ethyl ketone; ERK: extracellular signal-regulated kinase; AKT: protein kinase B; mTOR: mammalian target of rapamycin; MAPK: mitogen-activated protein kinase

**Table 1a: SSAs approved for NETs treatment**

Author/trials	Regimen	Patients enrolled	Results	Adverse reactions (grade > 3)
Rinke <i>et al.</i> <sup>[49]</sup> Arnold <i>et al.</i> <sup>[28]</sup> PROMID (Phase III)	Octreotide vs. placebo	Advanced GEP or NETs of unknown origin	mTTP: 14.3 vs. 6 months; SD: 64% vs. 37.2%	Diarrhea
Caplin <i>et al.</i> <sup>[50]</sup> Clarinet (Phase III)	Lanreotide vs. placebo	Advanced GEP or NETs of unknown origin	mPFS: NR vs. 18 months	Diarrhea
Filosso <i>et al.</i> <sup>[57]</sup>	Octreotide	Metastatic atypical bronchial carcinoid with carcinoid syndrome (diarrhea)	RR = 60%	None

GEP: gastro-entero-pancreatic; NETs: neuroendocrine tumors; mTTP: median time to progression; mPFS: median progression free survival; SD: stable disease; NR: not reached; RR: response rate

**Table 1b: SSAs not yet approved for NETs treatment**

Author/trials	Regimen	Patients enrolled	Results	Adverse reactions (grade > 3)
Wolin <i>et al.</i> <sup>[53]</sup> (Phase III)	Pasireotide vs. octreotide	Advanced GEP- NETs	mPFS: 11.8 vs. 6.8 months; SD: 60.8% vs. 42.3%	Hyperglycemia, diarrhea

SSAs: short synthetic analogues of somatostatin; NETs: neuroendocrine tumors; GEP-NETs: gastro-entero-pancreatic neuroendocrine tumors; mPFS: median progression free survival; SD: stable disease

**Table 1c: Drug not yet approved for the treatment of refractory carcinoid syndrome**

Author/trials	Regimen	Patients enrolled	Results	Adverse reactions (grade >3)
Kulke <i>et al.</i> <sup>[54]</sup>	Telotristat	Metastatic GEP-NETs with carcinoid syndrome	Reduction of BMs: 30%	Gastrointestinal symptoms: nausea, vomiting, or abdominal discomfort
Pavel <i>et al.</i> <sup>[55]</sup>	Telotristat	Metastatic well-differentiated NETs with carcinoid syndrome (diarrhea)	Reduction of BMs: 43.5%	Gastrointestinal symptoms: nausea, vomiting, or abdominal discomfort

GEP-NETs: gastro-entero-pancreatic neuroendocrine tumors; mTTP: median time to progression; mPFS: median progression free survival; BMs: bowel movements; RR: response rate

glucagonomas, and VIPomas (80-100%).<sup>[27,28]</sup> However, insulinomas express SSTR in 50-70% of cases, showing a prevalence of SSTR5 mRNA expression that is positively correlated with aggressive pathological characteristics.<sup>[29]</sup>

SSTR2 is usually expressed in NENs, and its loss could be highly correlated with the dysregulation of tumor proliferation, consequently promoting tumor growth.<sup>[30,31]</sup>

SSTR1 and SSTR5 are less expressed in NENs and correlate with a major risk of angioinvasion and distant metastasis. SSTR3 is even less present, and SSTR4 is almost absent.<sup>[32-34]</sup>

Reductions of receptor density, changes in their subtype pattern, and probably also their downregulation seem to be a consequence of tumor dedifferentiation. Thus, the presence of SSTRs might also be useful as a specific predictor of prognosis.<sup>[16]</sup> However, any significant association between the expressed receptors subtypes and the primary tumor site at onset is observed in relation to high and heterogeneous expression of SSTRs, or to a specific hormone secretion.<sup>[35-37]</sup>

SSTR functioning appears different and dependent on the presence in several types of cancer cell, various distributions on cellular surface, and intrinsic features (ability of desensitization, internalization, and cross talk).<sup>[26,38]</sup> However, their activity causes a blockage of cellular survival, proliferation, differentiation, and hormone secretion, except for SSTR4, promoting cell mitosis through overregulation of Mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 (MAPK/ERK1/2) pathway.

In fact SSTR1 acts on starting MAPK pathway; SSTR2 augments Src homology region 2 domain-containing phosphatase-1 and epidermal growth factor receptor (EGFR) activity, over-regulates p21 and Rb, reducing MAPK activity and blocking cellular proliferation. p53 and Bax, involved in apoptosis, are induced by SSTR3. It also blocks vascular endothelial growth factor receptor (VEGFR). Finally, protein tyrosine phosphatases are targeted by SSTR5.<sup>[18,39]</sup>

The role of SSAs, as mentioned, is to reduce active symptoms and to have an antiproliferative effect in secreting and nonsecreting neuroendocrine tumors.

**Table 2: Principal studies with inhibitors of mTOR**

Author/trials	Regimen	Patients enrolled	Results	Adverse reactions (grade > 3)
Yao <i>et al.</i> <sup>[64]</sup> RADIANT-1 (Phase II)	Stratum 1: everolimus Stratum 2: everolimus plus octreotide LAR	Metastatic P-NETs after progression	Stratum 1: mPFS: 9.7 months Stratum 2: mPFS: 16.7 months	Stomatitis, diarrhea
Pavel <i>et al.</i> <sup>[66]</sup> RADIANT-2 (Phase III)	Everolimus plus octreotide LAR vs. placebo plus octreotide LAR	Advanced NETs with carcinoid syndrome after progression	mPFS: 16.4 vs. 11.3 months	Stomatitis, diarrhea, fatigue
Fazio <i>et al.</i> <sup>[67]</sup> RADIANT-2 (Phase III)-exploratory analysis	Everolimus plus octreotide LAR vs. placebo plus octreotide LAR	Low- to intermediate-grade advanced lung NETs	mPFS: 13.6 vs. 5.6 months	Stomatitis, rash, diarrhea, fatigue
Yao <i>et al.</i> <sup>[65]</sup> RADIANT-3 (Phase III)	Everolimus vs. placebo	Advanced P-NETs after progression	mPFS: 11.0 vs. 4.6 months	Stomatitis, diarrhea, fatigue, nausea, rash

mTOR : mammalian target of rapamycin; P-NETs: primitive neuroendocrine tumors; mPFS: median progression free survival; LAR: long-acting release

**Table 3: Anti-IGF-R1 drugs in NETs**

Author/trials	Regimen	Patients enrolled	Results	Adverse reactions (grade > 3)
Naing <i>et al.</i> <sup>[99]</sup> (Phase I)	Cixutumumab plus temsirolimus	Advanced solid tumors pre-treated (neuroendocrine tumors)	SD = 47%	Hyperglycemia, hypertriglyceridemia, hypercholesterolemia, thrombocytopenia, mucositis
Rothenberg <i>et al.</i> <sup>[100]</sup> (Phase I)	Ganitumumab	Advanced solid tumors pre-treated (neuroendocrine tumors)	PR = 20% SD = 80%	Diarrhea
Strosberg <i>et al.</i> <sup>[101]</sup> (Phase II)	Ganitumumab	Metastatic progressive carcinoid or P-NETs	No objective responders by RECIST. mPFS = 6.3 months: 10.5 months for carcinoid patients, and 4.2 months for P-NET patients. OS rate at 12 months = 66%; mOS = NR	Hyperglycemia, neutropenia, thrombocytopenia, infusion reaction

IGF: insulinlike growth factor; NETs: neuroendocrine tumors; SD: stable disease; PR: partial response; P-NETs: primitive neuroendocrine tumors; RECIST: response evaluation criteria in solid tumors; mPFS: median progression free survival; OS: overall survival; mOS: median overall survival; NR: not reached

If presented on tumor cells' surface, the blockage of SSTRs operates directly on cell proliferation, stimulating antimitotic and apoptotic activities. SSAs also induce cell growth inhibition with indirect activities (not requiring SSTR neoplasm expression),<sup>[40-44]</sup> such as angiogenesis inhibition and immunomodulation mechanism, mediated by stimulation of the production of natural-killer cells and blockage of growth factors.<sup>[45-48]</sup>

The results of two international studies (PROMID, using octreotide, and CLARINET trial, using lanreotide) represent the principal reason for using SSAs as first-line medical and systemic therapy in GEP tumors or neuroendocrine tumors of unknown origin, especially for data about progression-free survival (PFS).<sup>[49,50]</sup> As

shown by Rinke *et al.*<sup>[49]</sup> in the PROMID study, advanced midgut NENs gained an advantage in time to progression, response rate, and risk reduction of tumor progression from use of octreotide long-acting release (LAR) compared to placebo. Furthermore, octreotide LAR also extends overall survival (OS), but only in the subgroup of patients with metastatic midgut NETs and a low hepatic load ( $\leq 10\%$  at study entry)<sup>[51]</sup>. [Table 1a]

Recently, the CLARINET trial enrolled nonfunctioning GEP-NENs randomized to receive depot lanreotide or placebo and demonstrated an improvement in PFS for patients in the treatment arm [Table 1a]. Due these significant data, octreotide LAR and depot lanreotide have been approved as treatment for patients with newly

**Table 4: Anti-angiogenic drugs in NETs**

Author/trials	Regimen	Patients enrolled	Results	Adverse reactions (grade > 3)
Faivre <i>et al.</i> <sup>[115]</sup> (Phase I)	Sunitinib	Metastatic solid tumors pre-treated: (neuroendocrine tumors)	ORR = 20%	Fatigue, hypertension
Kulke <i>et al.</i> <sup>[116]</sup> (Phase II)	Sunitinib	Carcinoid or pancreatic neuroendocrine tumor not candidates for curative surgery	SD = 82.9% in carcinoid patients. SD = 68.2% in P-NETs mTTP in carcinoid tumors = 10.2 months mTTP in P-NETs = 7.7 months OS rate at 12 months in carcinoid patients: 83.4% OS rate at 12 months 81.1% in P-NETs	Fatigue, hypertension, GI hemorrhage, pulmonary embolism, increased lipase, cardiac congestive failure, cerebrovascular accident, hyponatremia
Raymond <i>et al.</i> <sup>[117]</sup> (Phase III)	Sunitinib vs. placebo	Low- and intermediate-grade advanced P-NETs	mPFS = 11.4 vs. 5.5 months ORR = 9.3% vs. 0 OS rate = 25% vs. 10%	Diarrhea, nausea, vomiting, fatigue
Yao <i>et al.</i> <sup>[118]</sup> (Phase II)	Octreotide plus bevacizumab vs. octreotide plus pegylated IFN $\alpha$ 2b	Metastatic or unresectable carcinoid tumors	SD = 77% vs. 68% PFS rate: 95% vs. 68%	Granulocytopenia, headache, hypertension
Chan <i>et al.</i> <sup>[120]</sup> (Phase II)	Bevacizumab plus temozolomide	Locally advanced or metastatic NETs	ORR = 15% (33% in P-NETs and 0% in carcinoid tumors) mPFS = 11.0 months (14.3 for P-NETs vs. 7.3 months for carcinoid tumors). mOS = 33.3 months (41.7 for P-NETs vs. 18.8 months for carcinoid tumors)	Lymphopenia, thrombocytopenia
YAO <i>et al.</i> <sup>[121]</sup> (Phase II)	Everolimus alone with the combination of everolimus and bevacizumab	Advanced P-NETs	ORR = 26%	-
Ahn <i>et al.</i> <sup>[123]</sup> (Phase II)	Pazopanib	Advanced GEP NENs, not amenable to loco-regional therapies	ORR = 18.9% SD = 56.8% DCR = 75.7%	Proteinuria, neutropaenia, hypertension, diarrhea, anorexia, abdominal pain, AST/ALT elevation
	Pazopanib plus octreotide	Metastatic or locally advanced grade 1-2 carcinoid tumours or P-NETs	ORR = 21.9% of P-NETs ORR = 0% in GI-NETs PFS: 14.2 months in P-NETs, PFS = 12 months in GI-NETs	Hypertriglyceridemia, thrombosis.

ORR: overall response rate; SD: stable disease; P-NETs: primitive neuroendocrine tumors; GI: gastrointestinal; OS: overall survival; mPFS: median progression free survival; mOS: median overall survival; GEP NENs: Gastro-entero-pancreatic neuroendocrine neoplasms; DCR: disease control rate; IFN: Interferon; AST/ALT: aspartate transaminase/alanine transaminase; GI-NETs: gastrointestinal neuroendocrine tumors; PFS: progression free survival

diagnosed, recurrent, and advanced neuroendocrine primary tumor, hormone-secreting status, and presence tumors in progressive disease, irrespective of site of of symptoms.



Pasireotide, a new somatostatin analogue, may represent an effective therapeutic option in tumors that are refractory to octreotide or lanreotide.<sup>[52]</sup> In a phase III randomized, blinded study, pasireotide showed symptom control comparable to octreotide but with an improved PFS ( $P = 0.045$ ).<sup>[53]</sup> [Table 1b]

Another drug, telotristat etiprate, inhibitor of serotonin synthesis, was studied in patients with carcinoid syndrome characterized by diarrhea. Kulke *et al.*<sup>[54]</sup> and Pavel *et al.*<sup>[55]</sup> conducted a prospective single-arm study in patients with functional tumor and diarrhea ( $\geq 4$  bowel movements/day) not well controlled by octreotide. Telotristat etiprate was shown to reduce both the frequency of bowel movements and biochemical markers of carcinoid syndrome [Table 1c].

In contrast, there are no validated prospective clinical trials that guide the treatment of advanced bronchopulmonary carcinoids. Small retrospective mono-institutional data and subgroup analysis of some multicentric trials involving gastro-entero-pancreatic NETs represent the only available results. In particular SSAs seem to produce tumor stabilization in about 30-70% of patients with low-grade BP-NETs.<sup>[56]</sup>

Filosso *et al.*<sup>[57]</sup> demonstrated that octreotide is effective in reducing symptoms of carcinoid syndrome and urinary 5-hydroxyindoleacetic acid values in patients with liver metastases of radically resected atypical bronchial carcinoid. The efficacy of the drug seemed to be related to the expression of SST2 somatostatin receptors in the pathologic tissue, as demonstrated by polymerase chain reaction method [Table 1a]. In the setting of thoracic NETs, the first multicentric randomized prospective trial investigating either pasireotide in combination with Mammalian target of rapamycin (mTOR) inhibitor or pasireotide alone is still ongoing.

## mTOR INHIBITORS

Everolimus, mTOR inhibitor, represents another important option for NETs treatment. In fact, mTOR has been identified as a kinase activated in the Ras/Raf, MAPK, Phosphoinositide 3-Kinase (PI3K)-Protein Kinase B (AKT) pathway of GEP and BP-NETs.<sup>[58]</sup> [Figure 1]

Recently, overexpression of mTOR and/or its pathway targets has been shown to be very common in GEP-NETs, resulting in higher proliferative activity and adverse clinical outcomes.<sup>[59,60]</sup> Furthermore, somatic mutations of PI3K are individuated in a minority of P-NETs and are described also in bronchopulmonary carcinoids. PI3K/AKT/mTOR pathway, then, is especially switched on among P-NETs promoting the principal cellular functions.<sup>[61-63]</sup> Currently, a phase Ib trial with everolimus in association with PI3K inhibitor is ongoing (ClinicalTrials. Gov Identifier: NCT02077933).

Tumorigenesis and metastatic power in NENs seem to be conditioned by a great number of intracellular pathways, as transduction mechanisms involving receptor tyrosine kinases and G-protein coupled receptors. mTOR and Jun N-terminal kinase seem to modulate their action by contributing to increased cell growth and number.

Everolimus plus octreotide demonstrated a benefit in PFS for GEP-NETs patients with progressive disease. These data emerged from the phase II RAD001 in advanced neuroendocrine tumors trial (RADIANT-1).<sup>[64]</sup> [Table 2]

Everolimus is currently approved for the treatment of P-NETs in progressive disease, with or without concomitant SSAs therapies, on the basis of the results achieved from RADIANT-3 trial.<sup>[65]</sup> [Table 2]

A large prospective phase III multicentric study (RADIANT-4) investigating the efficacy of everolimus vs. placebo in progressive GI and BP-NETs has recently been completed. Everolimus has received approval for this indication in early 2016.

The mTOR inhibitors have rapidly become of clinical interest in thoracic NETs. Everolimus (alone or in combination with SSAs) was effective, according to exploratory analysis of low- to intermediate-grade advanced lung NETs in the large multicentric phase 3, randomized, placebo-controlled RADIANT II study. These clinically significant data reinforce the necessity of further research of everolimus treatment regimens in this patient setting.<sup>[66,67]</sup> [Table 2]

For this reason, the LUNA trial, exclusively enrolling patients with thoracic NETs after disease progression, has been performed and awaits definite data consolidation. It has examined the efficacy of everolimus in monotherapy, everolimus in association with pasireotide, or pasireotide alone. (ClinicalTrials. Gov Identifier: NCT01563354)

Another mTOR inhibitor, temsirolimus, was investigated in NETs without any report of success.<sup>[68]</sup> However, a resistance to mTOR inhibition and a greater propensity toward further metastasis was observed and seems to be related to the loss of another fundamental target, phosphatase and tensin homologue (PTEN).<sup>[69-73]</sup> PTEN is localized in the cytosol and in the nucleus, blocking PI3K activity in the cytosol and securing the genome in the nucleus. Its starting through internalization correlates with to a reduction of AKT.<sup>[74-76]</sup> PTEN is frequently mutated in P-NETs and its low expression correlates with high grading.<sup>[77]</sup> In particular, low expression in cytosol of lung NETs indicates a category of patient with poor prognosis.<sup>[78]</sup>

## IGF1 INHIBITORS

Insulin growth factor 1 (IGF1), a factor involved in tumor progression, is secreted by neuroendocrine

neoplasms.<sup>[79-80]</sup> IGF-1 receptors (IGF-1R), binding IGF-1, activate signals inside normal neuroendocrine cell, through components of the PI3K/Akt/mTOR and the Ras/Raf/MEK/ERK pathways,<sup>[82-86]</sup> inducing cellular proliferation and over-regulating antiapoptotic activity.<sup>[81]</sup> [Figure 1] IGF-1 receptors, then, are usually overexpressed in NETs,<sup>[87-90]</sup> especially in symptomatic and functioning ones. This represents a possible role in tumorigenesis of GEP and bronchial NETs and a potential target for therapy.<sup>[91-93]</sup> The rationale for the use of IGF1R inhibitors depends on their theoretical capability to reduce AKT phosphorylation induced by mTOR inhibitors.<sup>[94-96]</sup>

In this regard, cixutumumab, a fully human immunoglobulin G1 monoclonal antibody competitively binding IGF-1R, is in the early phases of clinical progress.<sup>[97]</sup> Cixutumumab is still studied in association with octreotide LAR in an ongoing phase II study enrolling patients with progressing metastatic P-NETs and midgut carcinoid tumors.<sup>[98]</sup> Also, the combination of cixutumumab, everolimus, and octreotide is being evaluated in a phase I trial conducted in patients with advanced low- or intermediate-grade neuroendocrine tumors for which standard curative measures do not exist (Clinical Trial: NCT01204476). Another similar phase I trial was performed in advanced cancer patients, with candidates receiving temsirolimus with cixutumumab. The preliminary results showed good tolerance.<sup>[99]</sup> [Table 3]

Similarly, ganitumumab, another fully human monoclonal antibody against IGF-1R, is undergoing evaluation in clinical trials. Rothenberg *et al.*<sup>[100]</sup> demonstrated encouraging activity and good tolerance in a phase I trial including previously treated metastatic NET patients [Table 3]. Strosberg *et al.*<sup>[101]</sup> performed a phase II study of ganitumumab in patients with metastatic progressive low- and intermediate-grade carcinoids or P-NETs. This trial showed a good tolerance of ganitumumab, but no objective responders [Table 3]. Further studies are necessary to deepen the role of cixutumumab and ganitumumab and to identify other IGF-1R targets.

## VEGF AND ITS RECEPTOR INHIBITORS

Neuroendocrine neoplasms, especially for midgut and P-NETs and bronchial carcinoids, are highly vascularized and overexpress vascular endothelial growth factor (VEGF) and its receptors.<sup>[102,103]</sup> Four VEGF forms are individuated and examined: VEGF-A, VEGF-B, VEGF-C, and VEGF-D,<sup>[104-108]</sup> with a different affinity to their three own receptors.<sup>[109-113]</sup> [Figure 1] For these reasons, the interest of angiogenesis inhibition was encouraged.

The small molecule tyrosine kinase inhibitor (TKI) sunitinib has been studied as a targeted therapy option in NENs. Based on these results in term of response rate that were observed in phase I trial with sunitinib,<sup>[114,115]</sup>

Kulke *et al.*<sup>[116]</sup> conducted a phase II trial evaluating the efficacy of sunitinib in GEP-NETs. They showed a significant antitumor activity in P-NETs vs. carcinoid tumors and good tolerance. In addition, in a phase III trial involving low- and intermediate-grade advanced P-NETs, Raymond *et al.*<sup>[117]</sup> demonstrated a better PFS in the arm of sunitinib compared to placebo. The improved PFS did not depend on previous treatments or concomitant SSAs. Therefore, sunitinib is approved for the treatment of P-NETs after disease progression.

Considering the importance of VEGF in the pathogenesis of NENs, bevacizumab, an anti-VEGF antibody, has been used either alone or in combination with other drugs with favorable results. A phase II trial, in particular, enrolled patients with advanced carcinoid tumors with stable doses of octreotide to receive either bevacizumab or pegylated Interferon  $\alpha$ 2b. Bevacizumab showed superiority in objective responses, reduction of tumor blood flow, and PFS.<sup>[118,119]</sup> Bevacizumab in association with temozolomide in patients with metastatic NETs also showed a major response rate, PFS, and OS in P-NETs.<sup>[120]</sup>

In another recently completed phase II study, everolimus and bevacizumab were shown to be associated with an overall tumor response rate of 26% and good tolerance in advanced P-NETs.<sup>[121]</sup> Therefore, a further phase II trial will compare everolimus alone with the combination of everolimus and bevacizumab in patients with P-NETs, in order to find supplementary function of antiangiogenic agents in this setting of patients (ClinicalTrials. Gov Identifier: NCT01229943). Randomized studies of anti-VEGF TKI should also be evaluated in patients with advanced carcinoid tumors.

Pazopanib is an oral bioavailable, multitargeted tyrosine kinase inhibitor (VEGF receptors 1, 2, and 3), involved in reducing neoplastic growth and dissemination.<sup>[122]</sup> Ahn *et al.*<sup>[123]</sup> demonstrated, in a non-randomized, open-labeled, single-center phase II trial, that pazopanib in monotherapy was as effective as the other available targeted therapies, not only in P-NETs, but also in GI NETs [Table 4]. Phan *et al.*<sup>[124,125]</sup> found that pazopanib in combination with octreotide LAR depot was more effective in advanced G1-G2 P-NETs than in advanced carcinoid tumors [Table 4].

Other trials with pazopanib, and with other multitarget agents such as famitinib (c-kit, platelet-derived growth factor receptor (PDGFR), VEGFR2, VEGFR3, Flt1 and Flt3 inhibitor), regorafenib (c-Raf; BRAF, VEGFR-1,2,3; PDGFR $\alpha$ , Fibroblast Growth Factor Receptor (FGFR)-1; c-kit; RET; Flt-3 inhibitor), and nintedanib (VEGFR, FGFR, PDGFR inhibitor) are ongoing. Some of them are also enrolling patients with bronchopulmonary NETs (Clinical Trial: NCT01280201; NCT01994213; NCT02259725; NCT02399215).<sup>[126-128]</sup>

## EGF AND ITS RECEPTOR AND TGF $\alpha$

EGFR/AKT/mTOR pathway activation could be shown in all entities of NETs and was observed especially in tumors with high grading and poor prognosis. Typical and atypical bronchopulmonary carcinoids<sup>[129]</sup> and gastrointestinal-neuroendocrine tumours (GI-NETs) and P-NETs present and over-regulate EGFRs.<sup>[130]</sup> [Figure 1] Papouchado *et al.*<sup>[131]</sup> in particular, described a higher presence of EGFR (> 91%) in GI-NETs, especially rectal NETs, than in P-NETs (< 25%).

An elevated presence of EGFR and transforming growth factor alpha (TGF $\alpha$ ) in P-NETs was observed by Srivastava *et al.*<sup>[132]</sup> An elevated amount of secreted TGF $\alpha$  was detected in cultures of carcinoid tumors and pheochromocytomas, and the administration octreotide and anti-EGFR monoclonal antibodies seemed to reduce the secretion and the proliferative effect of TGF $\alpha$ .<sup>[133]</sup> Krishnamurthy *et al.*<sup>[134]</sup> showed a high expression of TGF $\alpha$  in GI NETs (72%) without any correlation with tumor size, grading, and other pathologic features, but only depending on the technique used (immunohistochemistry or northern blot analysis).<sup>[133]</sup> In rectal NENs TGF- $\alpha$  expression seemed to be increased in lesions larger than 5 mm and tumors with higher Ki67 index.<sup>[135]</sup> Despite the heterogeneity of these results, EGFR and its signal transduction pathways (RAS-RAF-MAPK) might represent an interesting target for the treatment of NETs.

In fact, a synergistic effect in determining apoptosis in atypical carcinoid cell lines was demonstrated by the association of epidermal growth factor (EGF) receptor inhibitors (erlotinib) with everolimus in *in-vitro* studies.<sup>[129]</sup>

A phase II trial evaluated gefitinib in 96 pretreated patients affected by GEP-NETs achieved prolonged disease control with rare objective responses; the study drug was well-tolerated.<sup>[136]</sup>

## OTHER TYROSINE KINASE INHIBITORS AND IMMUNOTHERAPY

Beta fibroblast growth factor (bFGF) and c-kit/Platelet Derived Growth Factor (PDGF) inhibitors are being developed, based upon the variable expression of bFGF, c-kit and PDGF in NETs.<sup>[137-139]</sup>

Despite little systematic and rigorous in-depth analysis of immunotherapy in NETs (interferon and dendritic cell vaccines), the recent progress in targeting of Cytotoxic T lymphocyte antigen-4 and PD-1 provide opportunities for future advances.<sup>[140]</sup> Further studies are necessary to examine the variable expression of PD-1, PD-L1/L2 in NENs.

## CONCLUSION

The predictive and prognostic characteristics of NETs are still under investigation to individuate a pattern of peculiar molecular genetic alterations in each kind of neoplasm. The aim is to find a correlation of specific abnormalities implicated in carcinogenesis and dissemination that may provide potential targets for tailored biotherapy.

In GEP and lung NETs, carcinogenesis and dissemination often involves SSTRs, mTOR/Akt/PI3K and PTEN, IGF-1, VEGF, EGF, TGF, FGF and c-kit/PDGF and its corresponding receptors, markers whose established value may more thoroughly define an appropriate course of treatment.

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No patient involved.

## Ethics approval

This article does not contain any studies with human participants or animals.

## REFERENCES

1. Cives M, Strosberg J. An update on gastroenteropancreatic neuroendocrine tumours. *Oncology (Williston Park)* 2014;28:749-56, 758.
2. Rindi G, Arnold R, Bosman FT, Capella C, Klimstra DS, Kloppel G, Komminoth P, Solcia E. Nomenclature and classification of neuroendocrine neoplasms of the digestive system. In WHO Classification of Tumours of the Digestive System, Eds. FT Bosman, F Carneiro, RH Hruban & N Theise. Lyon: IARC Press. 2010. p. 13-14.
3. Sorbye H, Strosberg J, Baudin E, Klimstra DS, Yao JC. Gastroenteropancreatic high-grade neuroendocrine carcinoma. *Cancer* 2014;120:2814-23.
4. La Rosa S, Sessa F. High-grade poorly differentiated neuroendocrine carcinomas of the gastroenteropancreatic system: from morphology to proliferation and back. *Endocr Pathol* 2014;25:193-8.
5. Capdevila J, Salazar R, Halperin I, Abad A, Yao JC. Innovations therapy: mammalian target of rapamycin (mTOR) inhibitors for the treatment of neuroendocrine tumours. *Cancer Metastasis Rev* 2011;30:27-34.
6. Capdevila J, Taberero J. A shining light in the darkness for the treatment of pancreatic neuroendocrine tumours. *Cancer Discov* 2011;1:213-21.
7. Hilfenhaus G, Gohrig A, Pape UF, Neumann T, Jann H, Zdunek D, Hess G, Stassen JM, Wiedenmann B, Detjen K, Pavel M, Fischer C. Placental growth factor supports neuroendocrine tumour growth and predicts disease prognosis in patients. *Endocr Relat Cancer* 2013;20:305-19.
8. Vallières E, Shepherd FA, Crowley J, Van Houtte P, Postmus PE, Carney D, Chansky K, Shaikh Z, Goldstraw P; International Association for the Study of Lung Cancer International Staging Committee and Participating Institutions. The IASLC Lung Cancer



- Staging Project: proposals regarding the relevance of TNM in the pathologic staging of small cell lung cancer in the forthcoming (seventh) edition of the TNM classification for lung cancer. *J Thorac Oncol* 2009;4:1049-59.
9. Faggiano A, Sabourin JC, Ducreux M, Lumbroso J, Duvillard P, Leboulleux S, Dromain C, Colao A, Schlumberger M, Baudin E. Pulmonary and extrapulmonary poorly differentiated large cell neuroendocrine carcinomas: diagnostic and prognostic features. *Cancer* 2007;110:265-74.
  10. Travis WD. Advances in neuroendocrine lung tumors. *Ann Oncol* 2010; 21 Suppl 7:vii65-71.
  11. Rekhmann N. Neuroendocrine tumors of the lung: an update. *Arch Pathol Lab Med* 2010;134:1628-38.
  12. Bertino EM, Confer PD, Colonna JE, Ross P, Otterson GA. Pulmonary neuroendocrine/carcinoid tumors. *Cancer* 2009;115:4434-41.
  13. Valente M, Catena L, Milione M, Pusceddu S, Formisano B, Bajetta E. Common diagnostic challenges in the histopathologic diagnosis of neuroendocrine lung tumors: a case report. *Case Rep Oncol* 2010;3:202-7.
  14. Oberg KE. The management of neuroendocrine tumours: current and future medical therapy options. *Clin Oncol (R Coll Radiol)* 2012;24:282-93.
  15. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev* 2003;24:389-427.
  16. Modlin IM, Pavel M, Kidd M, Gustafsson BI. Review article: somatostatin analogues in the treatment of gastroenteropancreatic neuroendocrine (carcinoid) tumours. *Aliment Pharmacol Ther* 2010;31:169-88.
  17. Bruns C, Lewis I, Briner U, Meno-Tetang G, Weckbecker G. SOM230: a novel somatostatin peptidomimetic with broad somatotropin release inhibiting factor (SRIF) receptor binding and a unique antisecretory profile. *Eur J Endocrinol* 2002;146:707-16.
  18. Patel YC. Somatostatin and its receptor family. *Front Neuroendocrinol* 1999;20:157-98.
  19. Møller LN, Stidsen CE, Hartmann B, Holst JJ. Somatostatin receptors. *Biochim Biophys Acta* 2003;1616:1-84.
  20. Ruzsniewski P, Ducreux M, Charyvialle JA, Blumberg J, Cloarec D, Michel H, Raymond JM, Dupas JL, Gouerou H, Jian R, Genestin E, Bernades P, Rougier P. Treatment of the carcinoid syndrome with the long acting somatostatin analogue lanreotide: a prospective study in 39 patients. *Gut* 1996;39:279-83.
  21. Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. *Proc Natl Acad Sci U S A* 1992;89:251-5.
  22. Yamada Y, Reisine T, Law SF, Ihara Y, Kubota A, Kagimoto S, Seino M, Seino Y, Bell GI, Seino S. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylate cyclase. *Mol Endocrinol* 1992;6:2136-42.
  23. Ben-Shlomo A, Zhou C, Pichurin O, Chesnokova V, Liu NA, Culler MD, Melmed S. Constitutive somatostatin receptor activity determines tonic pituitary cell response. *Mol Endocrinol* 2009;23:337-48.
  24. Cescato R, Loesch KA, Waser B, Mäcke HR, Rivier JE, Reubi JC, Schonbrunn A. Agonist-biased signalling at the sst2A receptor: the multi-somatostatin analogs KE108 and SOM230 activate and antagonize distinct signalling pathways. *Mol Endocrinol* 2010;24:240-9.
  25. Reubi JC, Eisenwiener KP, Rink H, Waser B, Mäcke HR. A new peptidic somatostatin agonist with high affinity to all five somatostatin receptors. *Eur J Pharmacol* 2002;456:45-9.
  26. Schonbrunn A. Selective agonism in somatostatin receptor signalling and regulation. *Mol Cell Endocrinol* 2008;286:35-9.
  27. Ferone D, Arvigo M, Semino C, Jaquet P, Saveanu A, Taylor JE, Moreau JP, Culler MD, Albertelli M, Minuto F, Barreca A. Somatostatin and dopamine receptor expression in lung carcinoma cells and effects of chimeric somatostatin-dopamine molecules on cell proliferation. *Am J Physiol Endocrinol Metab* 2005;289:E1044-50.
  28. Arnold R, Trautmann ME, Creutzfeldt W, Benning R, Benning M, Neuhaus C, Jürgensen R, Stein K, Schäfer H, Bruns C, Dennler HJ. Somatostatin analogue octreotide and inhibition of tumour growth in metastatic endocrine gastroenteropancreatic tumours. *Gut* 1996;38:430-8.
  29. De Sà SV, Corrêa-Giannella ML, Machado MC, de Souza JJ, Pereira MA, Patzina RA, Siqueira SA, Machado MC, Giannella-Neto D. Somatostatin receptors subtype 5 (SSTR5) mRNA expression is related to histopathological features of cell proliferation in insulinomas. *Endocr Relat Cancer* 2006;13:69-78.
  30. Schmid HA, Lambertini C, van Vugt HH, Barzaghi-Rinaudo P, Schafer J, Hillenbrand R, Sailer AW, Kaufmann M, Nuciforo P. Monoclonal antibodies against the human somatostatin receptor subtypes 1-5: development and immunohistochemical application in neuroendocrine tumours. *Neuroendocrinology* 2012;95:232-47.
  31. Reubi JC, Laissue J, Krenning E, Lamberts SW. Somatostatin receptors in human cancer: incidence, characteristics, functional correlates and clinical implications. *J Steroid Biochem Mol Biol* 1992;4:27-35.
  32. Reubi JC. Peptide receptors as molecular targets for cancer diagnosis and therapy. *Endocr Rev* 2003;24:389-427.
  33. Reubi JC, Waser B. Concomitant expression of several peptide receptors in neuroendocrine tumours: molecular basis for *in vivo* multireceptor tumour targeting. *Eur J Nucl Med Mol Imaging* 2003;30:781-93.
  34. Reubi JC. Somatostatin and other Peptide receptors as tools for tumour diagnosis and treatment. *Neuroendocrinology* 2004;80 Suppl 1:51-6.
  35. Reubi JC, Kappeler A, Wase B, Laissue J, Hipkin RW, Schonbrunn A. Immunohistochemical localisation of somatostatin receptors SSTR2A in human tumours. *Am J Pathol* 1998;153:233-45.
  36. Papotti M, Bongiovanni M, Volante M, Allia E, Landolfi S, Helboe L, Schindler M, Cole SL, Bussolati G. Expression of somatostatin receptors type 1-5 in 81 cases of gastrointestinal and pancreatic endocrine tumours. A correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis. *Virchows Arch* 2002;440:461-75.
  37. Volante M, Rosas R, Allia E, Granata R, Baragli A, Muccioli G, Papotti M. Somatostatin, cortistatin and their receptors in tumours. *Mol Cell Endocrinol* 2008;286:219-29.
  38. Lahlou H, Guillermet J, Hortala M, Vernejoul F, Pyronnet S, Bousquet C, Susini C. Molecular signalling of somatostatin receptors. *Ann N Y Acad Sci* 2004;1014:121-31.
  39. Reisine T, Bell GI. Molecular biology of somatostatin receptors. *Endocr Rev* 1995;16:427-42.
  40. Lamberts SW, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumours. *Endocr Rev* 1991;12:450-82.
  41. Bousquet C, Puente E, Buscail L, Vaysse N, Susini C. Antiproliferative effect of somatostatin and analogs. *Chemotherapy* 2001;47 Suppl 2:30-9.
  42. Butturini G, Bettini R, Missiaglia E, Mantovani W, Dalai I, Capelli P, Ferdeghini M, Pederzoli P, Scarpa A, Falconi M. Predictive factors of efficacy of the somatostatin analogue octreotide as first line therapy for advanced pancreatic endocrine carcinoma. *Endocr Relat Cancer* 2006;13:1213-21.
  43. Schally AV. Oncological applications of somatostatin analogues. *Cancer Res* 1988;48:6977-85.
  44. Weckbecker G, Raulf F, Stolz B, Bruns C. Somatostatin analogs for diagnosis and treatment of cancer. *Pharmacol Ther* 1993;60:245-64.
  45. Pollak MN, Schally AV. Mechanisms of antineoplastic action of somatostatin analogs. *Proc Soc Exp Biol Med* 1998;217:143-52.
  46. Froidevaux S, Eberle AN. Somatostatin analogs and radiolabeled peptides in cancer therapy. *Biopolymers* 2002;66:161-83.
  47. Schally AV, Nagy A. Chemotherapy targeted to cancers through tumoural hormone receptors. *Trends Endocrinol Metab* 2004;15:300-10.

48. Pyronnet S, Bousquet C, Najib S, Azar R, Laklai H, Susini C. Antitumour effects of somatostatin. *Mol Cell Endocrinol* 2008;286:230-7.
49. Rinke A, Müller HH, Schade-Brittinger C, Klose KJ, Barth P, Wied M, Mayer C, Aminossadati B, Pape UF, Bläker M, Harder J, Arnold C, Gress T, Arnold R; PROMID Study Group. Placebo-controlled, double-blind, prospective, randomized study on the effect of octreotide LAR in the control of tumour growth in patients with metastatic neuroendocrine midgut tumours: a report from the PROMID Study Group. *J Clin Oncol* 2009;27:4656-63.
50. Caplin ME, Pavel M, Čwikla JB, Phan AT, Raderer M, Sedláčková E, Cadiot G, Wolin EM, Capdevila J, Wall L, Rindi G, Langley A, Martinez S, Blumberg J, Ruzniewski P; CLARINET Investigators. Lanreotide in metastatic enteropancreatic neuroendocrine tumours. *N Engl J Med* 2014;371:224-33.
51. Arnold R, Wittenberg M, Rinke A, Schade-Brittinger C, Aminossadati B, Ronicke Gress TM, Mueller HH; PROMID Study Group; Philipps University Marburg, Department of Internal Medicine, Division of Gastroenterology and Endocrinology, Marburg, Germany; KKS Marburg, Marburg, Germany; Philipps University Marburg; Department of Gastroenterology, Marburg, Germany; Koordinierungszentrum für Klinische Studien der Philipps-Universität Marburg, Marburg, Germany; Institute of Medical Informatics, Biometry and Epidemiology, LMU Munich, München, Germany. PROMID Study Group. Placebo controlled, double blind, prospective, randomized study on the effect of octreotide LAR in the control of tumour growth in patients with metastatic neuroendocrine midgut tumours (PROMID): results on long-term survival. *ASCO* 2013;31:Abstr 4030.
52. Schmid HA. Pasireotide (SOM230): development, mechanism of action and potential applications. *Mol Cell Endocrinol* 2008;286:69-74.
53. Wolin EM, Jarzab B, Eriksson B, Walter T, Toumpanakis C, Morse MA, Tomassetti P, Weber MM, Fogelman DR, Ramage J, Poon D, Gadbow B, Li J, Pasięka JL, Mahamat A, Swahn F, Newell-Price J, Mansoor W, Öberg K. A multicenter, randomized, blinded, phase III study of pasireotide LAR versus octreotide LAR in patients with metastatic neuroendocrine tumours (NET) with disease-related symptoms inadequately controlled by somatostatin analogs. *J Clin Oncol* 2013;31:abstr s4031.
54. Kulke MH, O'Dorisio T, Phan A, Bergsland E, Law L, Banks P, Freiman J, Frazier K, Jackson J, Yao JC, Kvols L, Lapuerta P, Zambrowicz B, Fleming D, Sands A. Telotristat etiprate, a novel serotonin synthesis inhibitor, in patients with carcinoid syndrome and diarrhea not adequately controlled by octreotide. *Endocr Relat Cancer* 2014;21:705-14.
55. Pavel M, Hörsch D, Caplin M, Ramage J, Seufferlein T, Valle J, Banks P, Lapuerta P, Sands A, Zambrowicz B, Fleming D, Wiedenmann B. Telotristat etiprate for carcinoid syndrome: a single-arm, multicenter trial. *J Clin Endocrinol Metab* 2015;100:1511-9.
56. Aparicio T, Ducreux M, Baudin E, Sabourin JC, De Baere T, Mitry E, Schlumberger M, Rougier P. Antitumour activity of somatostatin analogues in progressive metastatic neuroendocrine tumours. *Eur J Cancer* 2001;37:1014-9.
57. Filosso PL, Ruffini E, Oliaro A, Papalia E, Donati G, Rena O. Long-term survival of atypical bronchial carcinoids with liver metastases, treated with octreotide. *Eur J Cardiothorac Surg* 2002;21:913-7.
58. Hay N. The Akt-mTOR tango and its relevance to cancer. *Cancer Cell* 2005;8:179-83.
59. Alonso-Gordoa T, Capdevila J, Grande E. GEP-NETs Update: Biotherapy for neuroendocrine tumours. *Eur J Endocrinol* 2015;172:R31-46.
60. Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, Schulick RD, Tang LH, Wolfgang CL, Choti MA, Velculescu VE, Diaz LA Jr, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumours. *Science* 2011;331:1199-203.
61. Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, Piemonti L, Capurso G, Di Florio A, delle Fave G, Pederzoli P, Croce CM, Scarpa A. Pancreatic endocrine tumours: expression profiling evidences a role for AKT-mTOR pathway. *J Clin Oncol* 2010;28:245-55.
62. Humar R, Kiefer FN, Berns H, Resink TJ, Bettegay EJ. Hypoxia enhances vascular cell proliferation and angiogenesis *in vitro* via rapamycin (mTOR)-dependent signalling. *FASEB J* 2002;16:771-80.
63. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144:646-74.
64. Yao JC, Lombard-Bohas C, Baudin E, Kvols LK, Rougier P, Ruzniewski P, Hoosen S, St Peter J, Haas T, Lebwohl D, Van Cutsem E, Kulke MH, Hobday TJ, O'Dorisio TM, Shah MH, Cadiot G, Luppi G, Posey JA, Wiedenmann B. Daily oral everolimus activity in patients with metastatic pancreatic neuroendocrine tumours after failure of cytotoxic chemotherapy: a phase II trial. *J Clin Oncol* 2010;28:69-76.
65. 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, Öberg K; RAD001 in Advanced Neuroendocrine Tumors, Third Trial (RADIANT-3) Study Group. Everolimus for advanced pancreatic neuroendocrine tumours. *N Engl J Med* 2011;364:514-23.
66. Pavel ME, Hainsworth JD, Baudin E, Peeters M, Hörsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin EM, Oberg K, Van Cutsem E, Yao JC; RADIANT-2 Study Group. 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* 2011;378:2005-12.
67. Fazio N, Granberg D, Grossman A, Saletan S, Klimovsky J, Panneerselvam A, Wolin EM. Everolimus plus octreotide long-acting repeatable in patients with advanced lung neuroendocrine tumors: analysis of the phase 3, randomized, placebo-controlled RADIANT-2 study. *Chest* 2013;143:955-62.
68. Duran I, Kortmansky J, Singh D, Hirte H, Kocha W, Goss G, Le L, Oza A, Nicklee T, Ho J, Birl D, Pond GR, Arboine D, Dancy J, Aviel-Ronen S, Tsao MS, Hedley D, Siu LL. A phase II clinical and pharmacodynamic study of temsirolimus in advanced neuroendocrine carcinomas. *Br J Cancer* 2006;95:1148-54.
69. Hafsi S, Pezzino FM, Candido S, Ligresti G, Spandidos DA, Souza Z, McCubrey JA, Travalis S, Libra M. Gene alterations in the PI3K/PTEN/AKT pathway as a mechanism of drug-resistance (review). *Int J Oncol* 2012;40:639-44.
70. Zhang S, Yu D. PI(3)King apart PTEN's role in cancer. *Clin Cancer Res* 2010;16:4325-30.
71. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, Roberts TM, Sellers WR. Regulation of G1 progression by the PTEN tumour suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. *Proc Natl Acad Sci U S A* 1999;96:2110-5.
72. Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK. The lipid phosphatase activity of PTEN is critical for its tumour suppressor function. *Proc Natl Acad Sci U S A* 1998;95:13513-8.
73. Furnari FB, Huang HJ, Cavenee WK. The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells. *Cancer Res* 1998;58:5002-8.
74. Putz U, Howitt J, Doan A, Goh CP, Low LH, Silke J, Tan SS. The tumour suppressor PTEN is exported in exosomes and has phosphatase activity in recipient cells. *Sci Signal* 2012;5:ra70.
75. Tamura M, Gu J, Takino T, Yamada KM. Tumour suppressor PTEN inhibition of cell invasion, migration, and growth: differential involvement of focal adhesion kinase and p130Cas. *Cancer Res* 1999;59:442-9.
76. Kim JS, Xu X, Li H, Solomon D, Lane WS, Jin T, Waldman T. Mechanistic analysis of a DNA damage-induced, PTEN-dependent size checkpoint in human cells. *Mol Cell Biol* 2011;31:2756-71.
77. Wang L, Ignat A, Axiotis CA. Differential expression of the PTEN tumour suppressor protein in fetal and adult neuroendocrine tissues and tumours: progressive loss of PTEN expression in poorly differentiated neuroendocrine neoplasms. *Appl Immunohistochem*

*Mol Morphol* 2002;10:139-46.

78. Collaud S, Tischler V, Atanassoff A, Wiedl T, Komminoth P, Oehlschlegel C, Weder W, Soltermann A. Lung neuroendocrine tumors: correlation of ubiquitinylation 1 and sumoylation with nucleo-cytosolic partitioning of PTEN. *BMC Cancer* 2015;15:74.
79. Samani AA, Yakar S, LeRoith D, Brodt P. The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocr Rev* 2007;28:20-47.
80. Furukawa M, Raffeld M, Mateo C, Sakamoto A, Moody TW, Ito T, Venzon DJ, Serrano J, Jensen RT. Increased expression of insulin-like growth factor I and/or its receptor in gastrinomas is associated with low curability, increased growth, and development of metastases. *Clin Cancer Res* 2005;11:3233-42.
81. Chaves J, Saif MW. IGF system in cancer: from bench to clinic. *Anticancer Drugs* 2011;22:206-12.
82. von Wichert G, Jehle PM, Hoefflich A, Koschnick S, Dralle H, Wolf E, Wiedenmann B, Boehm BO, Adler G, Seufferlein T. Insulin-like growth factor-I is an autocrine regulator of chromogranin A secretion and growth in human neuroendocrine tumor cells. *Cancer Res* 2000;60:4573-81.
83. Nilsson O, Wangberg B, Theodorsson E, Skottner A, Ahlman H. Presence of IGF-I in human midgut carcinoid tumours--an autocrine regulator of carcinoid tumour growth? *Int J Cancer* 1992;51:195-203.
84. Nilsson O, Wangberg B, McRae A, Dahlstrom A, Ahlman H. Growth factors and carcinoid tumours. *Acta Oncol* 1993;32:115-24.
85. Wulbrand U, Wied M, Zöfel P, Göke B, Arnold R, Fehmann H. Growth factor receptor expression in human gastroenteropancreatic neuroendocrine tumours. *Eur J Clin Invest* 1998;28:1038-49.
86. Höpfner M, Baradari V, Huether A, Schöfl C, Scherübl H. The insulin-like growth factor receptor 1 is a promising target for novel treatment approaches in neuroendocrine gastrointestinal tumours. *Endocr Relat Cancer* 2006;13:135-49.
87. Vitale L, Lenzi L, Huntsman SA, Canaider S, Frabetti F, Casadei R, Facchin F, Carinci P, Zannotti M, Coppola D, Strippoli P. Differential expression of alternatively spliced mRNA forms of the insulin-like growth factor 1 receptor in human neuroendocrine tumours. *Oncol Rep* 2006;15:1249-56.
88. Richardson PG, Mitsiades CS, Laubach JP, Lonial S, Chanan-Khan AA, Anderson KC. Inhibition of heat shock protein 90 (HSP90) as a therapeutic strategy for the treatment of myeloma and other cancers. *Br J Haematol* 2011;152:367-79.
89. Gloesenkamp C, Nitzsche B, Lim AR, Normant E, Vosburgh E, Schrader M, Ocker M, Scherübl H, Höpfner M. Heat shock protein 90 is a promising target for effective growth inhibition of gastrointestinal neuroendocrine tumours. *Int J Oncol* 2012;40:1659-67.
90. Gilbert JA, Adhikari LJ, Lloyd RV, Halfdanarson TR, Muders MH, Ames MM. Molecular markers for novel therapeutic strategies in pancreatic endocrine tumors. *Pancreas* 2013;42:411-21.
91. Froesch ER, Schmid C, Schwander J, Zapf J. Actions of insulin-like growth factors. *Ann Rev Physiol* 1985;47:443-67.
92. Schoenle E, Zapf J, Humbel RE, Froesch ER. Insulin-like growth factor I stimulates growth of hypophysectomised rats. *Nature* 1982;296:252-3.
93. Adams SO, Nissley SP, Handwerger S, Rechler MM. Developmental patterns of insulin-like growth factor-I and -II synthesis and regulation in rat fibroblasts. *Nature* 1983;302:150-3.
94. Shi Y, Yan H, Frost P, Gera J, Lichtenstein A. Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade. *Mol Cancer Ther* 2005;4:1533-40.
95. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene* 2007;26:1932-40.
96. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006;66:1500-8.
97. Rowinsky EK, Youssefian H, Tonra JR, Solomon P, Burtrum D, Ludwig DL. IMC-A12, a human IgG1 monoclonal antibody to the insulin-like growth factor I receptor. *Clin Cancer Res* 2007;13:5549-55s.
98. Anthony LB, Loehrer PJ, Leong S, Shah MH, Safran H, Senzer NN, Zojwalla NJ, Youssefian H. Phase II study of cixutumumab (IMC-A12) plus depot octreotide for patients with metastatic carcinoid or islet cell carcinoma. *J Clin Oncol* 2010;28:15s.
99. Naing A, Kurzrock R, Burger A, Gupta S, Lei X, Busaidy N, Hong D, Chen HX, Doyle LA, Heilbrun LK, Rohren E, Ng C, Chandhasin C, LoRusso P. Phase I Trial of Cixutumumab Combined with Temsirolimus in Patients with Advanced Cancer. *Clin Cancer Res* 2011;17:6052-60.
100. Rothenberg ML, Tolcher AW, Sarantopoulos J, Rodon J, Friberg G, Deng H, McCaffery I, Hwang Y, Puzanov I. AMG 479 monotherapy to treat patients with advanced GI carcinoid tumors: a subset analysis from the first-in-human study. Presented at the ASCO Gastrointestinal Cancers Symposium, San Francisco, January 2009.
101. Strosberg JR, Chan JA, Ryan DP, Meyerhardt JA, Fuchs CS, Abrams T, Regan E, Brady R, Weber J, Campos T, Kvols LK, Kulke MH. A multi-institutional, phase II open-label study of ganitumab (AMG 479) in advanced carcinoid and pancreatic neuroendocrine tumors. *Endocr Relat Cancer* 2013;20:383-90.
102. Sartelet H, Decaussin M, Devouassoux G, Nawrocki-Raby B, Brichon PY, Brambilla C, Brambilla E. Expression of vascular endothelial growth factor (VEGF) and its receptors (VEGF-R1 [Flt-1] and VEGF-R2 [KDR/Flk-1]) in tumor lets and in neuroendocrine cell hyperplasia of the lung. *Hum Pathol* 2004;35:1210-7.
103. Bertino EM, Confer PD, Colonna JE, Ross P, Otterson GA. Pulmonary neuroendocrine/carcinoid tumors. *Cancer* 2009;115:4434-41.
104. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995;1:27-31.
105. Berger DP, Herbstreit L, Dengler WA, Marmé D, Mertelmann R, Fiebig HH. Vascular endothelial growth factor (VEGF) mRNA expression in human tumour models of different histologies. *Ann Oncol* 1995;6:817-25.
106. Lawnicka H, Stepień H, Wyczółkowska J, Kolago B, Kunert-Radek J, Komorowski J. Effect of somatostatin and octreotide on proliferation and vascular endothelial growth factor secretion from murine endothelial cell line (HECa10) culture. *Biochem Biophys Res Commun* 2000;268:567-71.
107. Kuo CJ, Farnebo F, Yu EY, Christofferson R, Swearingen RA, Carter R, von Recum HA, Yuan J, Kamihara J, Flynn E, D'Amato R, Folkman J, Mulligan RC. Comparative evaluation of the antitumour activity of antiangiogenic proteins delivered by gene transfer. *Proc Natl Acad Sci U S A* 2001;98:4605-10.
108. Matsumoto T, Claesson-Welsh L. VEGF receptor signal transduction. *Sci STKE* 2001;2001:re21.
109. Klagsbrun M, Takashima S, Mamluk R. The role of neuropilin in vascular and tumour biology. *Adv Exp Med Biol* 2002;515:33-48.
110. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;9:669-76.
111. Treiber G, Wex T, Röcken C, Fostitsch P, Malfertheiner P. Impact of biomarkers on disease survival and progression in patients treated with octreotide for advanced hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2006;132:699-708.
112. Dimitroulopoulos D, Xinopoulos D, Tsamakidis K, Zisimopoulos A, Andriotis E, Panagiotakos D, Fotopoulou A, Chrysohoou C, Bazinis A, Daskalopoulou D, Paraskevas E. Long acting octreotide in the treatment of advanced hepatocellular cancer and overexpression of somatostatin receptors: randomized placebo-controlled trial. *World J Gastroenterol* 2007;13:3164-70.
113. Ho QT, Kuo CJ. Vascular endothelial growth factor: Biology and therapeutic applications. *Int J Biochem Cell Biol* 2007;39:1349-57.
114. O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW, Wong LM, Hong W, Lee LB, Town A, Smolich BD, Manning WC,



- Murray LJ, Heinrich MC, Cherrington JM. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. *Blood* 2003;101:3597-605.
115. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, Raymond E. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006;24:25-35.
  116. Kulke MH, Lenz HJ, Meropol NJ, Posey J, Ryan DP, Picus J, Bergsland E, Stuart K, Tye L, Huang X, Li JZ, Baum CM, Fuchs CS. Activity of sunitinib in patients with advanced neuroendocrine tumours. *J Clin Oncol* 2008;26:3403-10.
  117. Raymond E, Dahan L, Raoul JL, Bang YJ, Borbath I, Lombard-Bohas C, Valle J, Metrakos P, Smith D, Vinik A, Chen JS, Hörsch D, Hammel P, Wiedenmann B, Van Cutsem E, Patyna S, Lu DR, Blanckmeister C, Chao R, Ruzsniowski P. Sunitinib malate for the treatment of pancreatic neuroendocrine tumours. *N Engl J Med* 2011;364:501-13.
  118. Yao JC, Phan AT, Hoff PM, Chen HX, Charnsangavej C, Yeung SC, Hess K, Ng C, Abbruzzese JL, Ajani JA. Targeting vascular endothelial growth factor in advanced carcinoid tumour: a random assignment phase II study of depot octreotide with bevacizumab and pegylated interferon alpha-2b. *J Clin Oncol* 2008;26:1316-23.
  119. Faivre S, Sablin MP, Dreyer C, Raymond E. Novel anticancer agents in clinical trials for well-differentiated neuroendocrine tumours. *Endocrinol Metab Clin North Am* 2010;39:811-26.
  120. Chan JA, Stuart K, Earle CC, Clark JW, Bhargava P, Miksad R, Blaszkowsky L, Enzinger PC, Meyerhardt JA, Zheng H, Fuchs CS, Kulke MH. Prospective Study of bevacizumab plus temozolomide in patients with advanced neuroendocrine tumors. *J Clin Oncol* 2012;30:2963-8.
  121. Yao JC, Phan AT, Fogelman D, Ng CS, Jacobs CB, Dagohoy CD, Leary C, Hess KR. Randomized run-in study of bevacizumab (B) and everolimus (E) in low- to intermediate-grade neuroendocrine tumors (LGNETs) using perfusion CT as functional biomarker. *J Clin Oncol* 2010;28 suppl 15:abstr 4002.
  122. Sonpavde G, Hutson TE. Pazopanib: a novel multitargeted tyrosine kinase inhibitor. *Curr Oncol Rep* 2007;9:115-9.
  123. Ahn HK, Choi JY, Kim KM, Kim H, Choi SH, Park SH, Park JO, Lim HY, Kang WK, Lee J, Park YS. Phase II study of pazopanib monotherapy in metastatic gastroenteropancreatic neuroendocrine tumours. *Br J Cancer* 2013;109:1414-9.
  124. Phan AT, Yao JC, Fogelman DR, Hess KR, Ng CS, Bullock SA, Malinowski P, Regan E, Kulke M. A prospective, multi-institutional phase II study of GW786034 (pazopanib) and depot octreotide (sandostatin LAR) in advanced low-grade neuroendocrine carcinoma (LGNEC). *Proc Am Soc Clin Oncol* 2010;28:abstr 4001.
  125. Phan AT, Halperin DM, Chan JA, Hess KR, Malinowski P, Regan E, Ng CS, Yao JC, Kulke MH. Pazopanib and depot octreotide in advanced, well-differentiated neuroendocrine tumours: a multicentre, single-group, phase 2 study. *Lancet Oncol* 2015;16:695-703.
  126. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA. BAY 43-9006 exhibits broad spectrum oral antitumour activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumour progression and angiogenesis. *Cancer Res* 2004;64:7099-109.
  127. Wilhelm SM, Dumas J, Adnane L, Lynch M, Carter CA, Schütz G, Thierauch KH, Zopf D. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumour activity. *Int J Cancer* 2011;129:245-55.
  128. Bill R, Fagiani E, Zumsteg A, Antoniadis H, Johansson D, Haefliger S, Albrecht I, Hilberg F, Christofori G. Nintedanib Is a Highly Effective Therapeutic for Neuroendocrine Carcinoma of the Pancreas (PNET) in the Rip1Tag2 Transgenic Mouse Model. *Clin Cancer Res* 2015;21:4856-67.
  129. Bago-Horvath Z, Sieghart W, Grusch M, Lackner A, Hayden H, Pirker C, Komina O, Węsierska-Gądek J, Haitel A, Filipits M, Berger W, Schmid K. Synergistic effects of erlotinib and everolimus on bronchial carcinoids and large-cell neuroendocrine carcinomas with activated EGFR/AKT/ mTOR pathway. *Neuroendocrinology* 2012;96:228-37.
  130. Shah T, Hochhauser D, Frow R, Quaglia A, Dhillon AP, Caplin ME. Epidermal growth factor receptor expression and activation in neuroendocrine tumours. *J Neuroendocrinol* 2006;18:355-60.
  131. Papouchado B, Erickson LA, Rohlinger AL, Hobday TJ, Erlichman C, Ames MM, Lloyd RV. Epidermal growth factor receptor and activated epidermal growth factor receptor expression in gastrointestinal carcinoids and pancreatic endocrine carcinomas. *Mod Pathol* 2005;18:1329-35.
  132. Srivastava A, Alexander J, Lomakin I, Dayal Y. Immunohistochemical expression of transforming growth factor alpha and epidermal growth factor receptor in pancreatic endocrine tumours. *Hum Pathol* 2001;32:1184-9.
  133. Nilsson O, Wangberg B, Kolby L, Schultz GS, Ahlman H. Expression of transforming growth factor alpha and its receptor in human neuroendocrine tumours. *Int J Cancer* 1995;60:645-51.
  134. Krishnamurthy S, Dayal Y. Immunohistochemical expression of transforming growth factor alpha and epidermal growth factor receptor in gastrointestinal carcinoids. *Am J Surg Pathol* 1997;21:327-33.
  135. Shimizu T, Tanaka S, Haruma K, Kitadai Y, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F. Growth characteristics of rectal carcinoid tumours. *Oncology* 2000;59:229-37.
  136. Hobday TJ, Holen K, Donehower RC, Camoriano J, Kim G, Picus J, Philip P, Lloyd R, Mahoney M, Erlichman C. A phase II trial of gefitinib in patients (pts) with progressive metastatic neuroendocrine tumours (NET): a phase II consortium (P2C) study. *J Clin Oncol* 2006;24:189S. Available from: URL: [http://meeting.ascpubs.org/cgi/content/abstract/24/18\\_suppl/4043](http://meeting.ascpubs.org/cgi/content/abstract/24/18_suppl/4043).
  137. Modlin IM, Shapiro MD, Kidd M. Carcinoid tumor and fibrosis: an association with no explanation. *Am J Gastroenterol* 2004;99:2466-78.
  138. Yao JC, Zhang JX, Rashid A, Yeung SC, Szklaruk J, Hess K, Xie K, Ellis L, Abbruzzese JL, Ajani JA. Clinical and *in vitro* studies of imatinib in advanced carcinoid tumours. *Clin Cancer Res* 2007;13:234-40.
  139. Lankat-Buttgereit B, Hörsch D, Barth P, Arnold R, Blöcker S, Göke R. Effects of the tyrosine kinase inhibitor imatinib on neuroendocrine tumour cell growth. *Digestion* 2005;71:131-40.
  140. Schott M, Feldkamp J, Lettmann M, Simon D, Scherbaum WA, Seissler J. Dendritic cell immunotherapy in a neuroendocrine pancreas carcinoma. *Clin Endocrinol (Oxf)* 2001;55:271-7.