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REVIEW

Vascular endothelial growth factor (VEGF) pathway and neuroendocrine neoplasms (NENs): prognostic and therapeutic considerations

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Abstract Neuroendocrine neoplasms (NENs) consist of a large heterogeneous group of epithelial tumors with neuroendocrine differentiation, as proved by immune reactivity for neuroendocrine markers. From the very first studies of vascular endothelial growth factor (VEGF) pathway, VEGF has been considered an important prognostic marker in NENs. Consequently, a number of preclinical experiences and clinical trials have examined the efficacy of VEGF-targeted therapeutics in NENs. Bevacizumab and sorafenib were clinically tested in NENs and they showed modest activity, while on the other hand, they present significant toxicity problems. More interesting in gastroenteropancreatic (GEP)-NENs seems to be the demonstrated efficacy of sunitinib. Preclinical as well as clinical sunitinib data in this regard provide a new hope in that direction. The use of other novel VEGF-targeted agents like aflibercept as well as VEGFR-TKI is being investigated in a number of phase II studies; the results of which are greatly awaited. Additionally, the use of potential biomarkers to select patients for VEGF-targeted therapy may be considered for further clinical evaluation. Thus, this article reviews the basic science as well as clinical data of VEGF signaling in advanced NENs with special emphasis on the different VEGF-targeting agents tested previously in this disease and the future prospective in that field.

Keywords Neuroendocrine neoplasms · VEGF · Bevacizumab · Sunitinib

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Introduction

Neuroendocrine neoplasms (NENs) consist of a large heterogeneous group of epithelial tumors with neuroendocrine differentiation, as proved by immune reactivity for neuroendocrine markers [1]. They include a group of pathologically interrelated albeit heterogeneous neoplasms that can arise in almost all organs of the body [2]. Although this group of tumors shares common pathological and clinical characteristics, significant differences are present among different tumor subtypes in terms of biology as well as clinical characteristics [1, 3–5].

Multiple classification schemes have been suggested for this group of diseases; first, these neoplasms were classified on a clinical basis as either functioning or nonfunctioning based on the presence or absence of clinical endocrine manifestations related to hormone production [6]. However, this classification does not provide sufficient prognostic information nor does it guide therapeutic choices particularly in the era of molecular-targeted therapeutics for NENs [7].

Thus; in 2000, the World Health Organization (WHO) has provided another approach to classify NENs through its classification [8], where a combination of pathological/clinical criteria was used to categorize NENs. Most authorities around the globe have adopted this classification scheme, significantly impacting the guidelines of various scientific societies [9–13]. This has been further refined in the 2010 WHO classification for NENs, and the designation of NET G1, NET G2, and NEC G3 has been confirmed [14–17].

Another classification of NENs has been proposed based on the anatomical location dividing them into gastroenteropancreatic (GEP)-NENs, thoracic NENs (including thymic and bronchial NENs), medullary thyroid carcinoma (MTC), and other rare anatomical presentation of NENs. Different incidences, clinical presentations, and biological characteristics have been proposed for different anatomical

subtypes of NENs. For example, the incidence of GEP-NENs has been estimated to be 5.25/100,000/year [9], while the incidence of thoracic NENs has been estimated to be 1.57/100,000/year.

The spectrum of available treatments for advanced NENs is diverse, including chemotherapeutic agents, somatostatin analog (SSA), interferon (IFN), and peptide radio-receptor therapy (PRRT) in addition to molecular-targeted agents. In the context of an intense search for prognostic and predictive factors for response and efficacy of different therapies, a number of molecular targets have been identified, opening new avenues for potential therapeutic opportunities [6]. Such molecular targets include mTOR pathway alterations, MAP kinase pathway alterations, as well as VEGF pathway-related alterations.

In this review, we will provide an overview of the various considerations relating to vascular endothelial growth factor (VEGF) pathway as a potentially novel therapeutic approach for NENs.

VEGF pathway and carcinogenesis

VEGF is one of the most commonly studied biomarkers in different diseases; it was initially identified as an endothelial cell-specific mitogen that has the capacity to induce physiological and pathological angiogenesis [18, 19]. Downstream signaling of VEGF in tumor cells is mediated by a family of receptor tyrosine kinases (RTKs). These include VEGFR1, VEGFR2, and VEGFR3 [20]. Most of these receptors are expressed by endothelial cells as well as many tumor types, and the expression pattern of these receptors in some tumors has been linked with some clinical parameters [21, 22].

A number of pathophysiological mechanisms that contribute to increasing the level of VEGF have been described [23]. The first mechanism is hypoxia-mediated angiogenesis, via the hypoxia-induced factor 1 (HIF-1) pathway and in hypoxic environment; HIF-1a induces the expression of a number of growth factors, of which VEGF is the most important [24, 25]. The second mechanism is through deregulated production of some growth factors (platelet-derived growth factor (PDGF)), insulin-like growth factor 1 (IGF-1), transforming growth factor-alpha (TGF-alpha), and transforming growth factor-beta (TGF-beta), which may lead also to an increased VEGF production. The third mechanism is through mutations leading to continuous proliferation signals and consequently increased VEGF production [26].

Once the production of VEGF overshoots the local antiangiogenic factors, angiogenesis occurs. Consequently, this will lead to the enhancement of the invasive and metastatic potential of cancer cells as well as enhancing the immune evasion of the tumor cells [27, 28].

Prognostic value of VEGF pathway alterations in NENs

Accordingly, VEGF overexpression (both in serum and tissue) has been proposed as an adverse prognostic as well as predictive factor in a number of solid tumors including NENs [29]; to support this hypothesis, a number of preclinical studies have been conducted with inconsistent results. The majority of published data relate to GEP-NENs; however, VEGF alterations prognostic values have also been evaluated in other subcategories of NENs.

I. Prognostic value of VEGF pathway alterations in GEP-NENs:

- Zhang and coworkers evaluated using immunohistochemistry, VEGF, and Sp1 expression patterns in 50 cases of human gastrointestinal neuroendocrine tumor having various clinicopathologic characteristics. They found that overexpression of VEGF promotes the growth of human neuroendocrine tumors in part through up-regulation of angiogenesis [30]. Similarly, Pavel and coworkers evaluated prognostic value of circulating levels of VEGF and IL-8 in 38 patients with advanced neuroendocrine carcinomas. They found that VEGF and IL-8 are associated with tumor progression and might qualify as markers of prognosis and therapy control in patients with neuroendocrine carcinomas [31].
- Moreover, Pinato et al. have evaluated the prognostic value of an expression signature of the angiogenic response in gastrointestinal neuroendocrine tumors; they found that tumors with preserved SSTR-2 and low Hif-1α expression have an indolent phenotype and may be offered less aggressive management and less stringent follow-up [32].
- On the other hand, a study by Kuiper and coworkers evaluated the potential prognostic value of angiogenic markers endoglin and vascular endothelial growth factor in gastroenteropancreatic neuroendocrine tumors. They found that increased endoglin tissue expression in tumors was significantly related to tumor size ($P<0.01$), presence of metastases ($P=0.04$), and a more advanced tumor stage ($P=0.02$), whereas expression of VEGF was not [33].
- Additionally, Poncet and coworkers have used an experimental orthotopic xenograft model to analyze the relations between angiogenic activity and tumor progression in digestive neuroendocrine tumors. They compared two endocrine cell lines: STC-1, a low vascular endothelial growth factor (VEGF)-producing cell line, and INS-r3, a high VEGF-producing cell line They found that in well-differentiated digestive neuroendocrine tumors, angiogenesis is disconnected

from tumor progression: the development of a highly vascular tumor microenvironment is correlated with VEGF secretion but is not associated with invasive and metastatic properties [34].

- Another Japanese group has evaluated the prognostic value of expression of angiogenic molecules in 37 patients with pancreatic endocrine tumors. They found that the expression of vascular endothelial growth factor-A did not separate aggressive pancreatic neuroendocrine tumors (pNETs); however, they found the high expression of another marker (CXCL-12) in tumor cells to be significantly associated with aggressive variables like tumor growth and hematogenous tumor spread [35].
- More interestingly, Silva and coworkers evaluated another marker in the VEGF pathway, that is, VEGFR-2 and they found that although VEGFR-2 is expressed in BON carcinoid cells, reduction in VEGFR-2 expression actually enhanced proliferation, invasion, and migration of the BON cell line. Also, expression of VEGFR-2 was inversely related to PI3K signaling. Carcinoid liver metastases in mice demonstrated decreased VEGFR-2 expression [36]. This observation actually lends a number of questions related to whether VEGFR-2 activation rather than inhibition may be a reasonable therapeutic strategy in certain subsets of NENs.

II. Prognostic value of VEGF pathway alterations in other NEN subcategories:

Another study by Marton and coworkers has evaluated the prognostic significance of HIF-1α and VEGF-C in neuroendocrine breast cancer. They found that HIF-1α overexpression indicated unfavorable prognosis and could serve as an additional prognostic factor in neuroendocrine breast carcinomas (NEBC). Moreover, patients with NEBC exhibiting moderate or strong VEGF-C expression could be candidates for a specific VEGF-C antibody therapy [37].

The above data collectively indicate that serum and tissue VEGF level may not be an optimal prognostic biomarker for GEP-NENs while the data is still insufficient for other NEN subcategories; however, other related angiogenic biomarkers may be good candidates for further evaluation in prognostic and predictive settings.

Current VEGF-targeted therapeutics in clinical use

Currently, the available VEGF-targeted therapeutics include monoclonal antibodies, tyrosine kinase inhibitors (TKIs), or metronomic chemotherapeutics. The most commonly evaluated monoclonal antibody has been bevacizumab, which is a monoclonal antibody directed against VEGF, while the spectrum of VEGFR-targeted TKIs has been very broad encompassing numerous agents like sorafenib, sunitinib, pazopanib, axitinib, cediranib, regorafenib, vandetanib, cabozantinib, brivanib, as well as many other agents in different phases of clinical development [22, 38].

A number of characteristic toxicities have been described in association with VEGFR-TKI including mucocutaneous toxicities, hypertension, as well as thyroid dysfunction [39–41].

Preclinical experience with VEGF pathway-targeted therapeutics in NENs

i. Bevacizumab:

A number of preclinical studies have evaluated the potential antitumor activity of bevacizumab in NENs (mainly GEP-NENs) (Table 1).

- A Japanese group from Tokyo University has conducted two studies in that regard; the first of which evaluated the tumor inhibitory effect of bevacizumab single agent on QGP-1 pancreatic NEN cell lines. They found that single agent bevacizumab exhibits a marked tumor growth-inhibitory effect [42].
- Another study by the same group evaluated the combination therapy of gemcitabine or oral S-1 with the anti-VEGF monoclonal antibody bevacizumab for pancreatic neuroendocrine carcinoma QGP-1 xenografted into mice. They found that the tumor volume became smaller (from the maximum volume) in the group treated with bevacizumab, gemcitabine, and S-1 (BGS) and the group treated with bevacizumab and gemcitabine (BG) [43].

ii. VEGFR-targeted tyrosine kinase inhibitors:

- Allen et al. evaluated brivanib (a dual FGF/VEGF inhibitor), for mouse pancreatic neuroendocrine tumors developing adaptive/evasive resistance to VEGF inhibition; they found that brivanib produced enduring tumor stasis and angiogenic blockade, both first and second line following the failure of sorafenib [44].

Clinical experience with VEGF pathway-targeted therapeutics in NENs

Clinical data for GEP-NENs

i. Bevacizumab:

A number of phase I and II studies have been conducted to evaluate bevacizumab-based combination in advanced NENs (Table 1).

Table 1 A number of preclinical experiences with VEGF-targeted agents in NENs

Authors	Type of study	Cellular population	Drugs	Methods (cell viability)	Results in vitro/in vivo
Kasuya et al. [42]	Preclinical (cell lines)	QGP-1	Bevacizumab	The ability of the cell lines to proliferate and secrete VEGF in vitro, the antitumor effect of bevacizumab administration in vivo	The number of intratumoral blood vessels decreased and the percentage of proliferating cells was approximately the same.
Kasuya et al. [43]	Preclinical (xenograft)	QGP-1	Gemcitabine or oral S-1 with bevacizumab	The antitumor effect and side effects were evaluated by measuring the tumor volume and weight and by changes in body weight, respectively.	The tumor volume became smaller (from the maximum volume) in the group treated with bevacizumab, gemcitabine, and S-1 (BGS) and the group treated with bevacizumab and gemcitabine (BG).
Allen et al. [44]	Preclinical (xenograft)	RIP-Tag2 mouse model of PNET	Brivanib	Tumor growth, vascularity, hypoxia, invasion, and metastasis	Brivanib produced enduring tumor stasis and angiogenic blockade, both first and second line following the failure of sorafenib.
Scholz et al. [45]	Preclinical (xenograft)	BON and QGP-1 human NET cells	ZK 304709 (novel VEGFR-TKI)	Primary tumor growth and metastatic spread, apoptosis, microvessel density, and lymphatic vessel density were determined.	In this model, ZK 304709 achieved efficacious tumor growth control via induction of apoptosis and inhibition of tumor-induced angiogenesis.

- Berruti and coworkers have evaluated bevacizumab plus octreotide and metronomic capecitabine in a phase II trial that included 45 patients with advanced GEP-NENs; this study has showed a median progression-free survival (PFS) of 14.9 months while the OS was not reached (Table 2). The principal grade 3–4 toxicities include hand and foot syndrome (11.1 %), proteinuria (4.4 %), and renal toxicity (2.2 %) [46].
- Additionally, the Spanish neuroendocrine tumor group conducted a phase II study of sorafenib and bevacizumab combination in patients with advanced NENs (31 carcinoids and 13 pancreatic). The majority of included patients (42 patients) had a well-differentiated NEN. Target lesions were present mainly in the liver (86 %) and lymphatic nodules (32 %). This study showed a median PFS of 12.4 months, median time to progression (TTP) of 14.5 months, overall response rate (ORR) of 9.4 %, and disease control rate (DCR) of 95.1 %. However, toxicity was particularly problematic with 11.4 % G3-4 asthenia and 16 % G3-4 hand-foot syndrome [50].
- On the other hand, Chan and coworkers evaluated the combination of bevacizumab plus temozolomide in patients with advanced GEP-NENs. Thirty-four patients (56 % with carcinoid, 44 % with pancreatic NETs) were included in this study, and notably, the outcome measures differed between pancreatic and nonpancreatic NENs. Response rates were 33 % for pancreatic NENs and 0 % for carcinoid tumors. The median progression-free survival was 14.3 months for pancreatic NETs vs. 7.3 months for carcinoid tumors. The median overall survival was 41.7 months for pancreatic NETs vs. 18.8 months for carcinoid tumors [47].
- In another study, Yao and colleagues evaluated Depot Octreotide with bevacizumab and Pegylated Interferon Alfa-2b combination in a phase II study for patients with advanced GEP-NENs; this study included 44 patients and the median PFS for the whole group was 66 weeks while the principal grade 3–4 toxicity was hypertension [49].
- Additionally, Koumarianou and coworkers evaluated double antiangiogenic strategies through using combination treatment with metronomic temozolomide, bevacizumab, and long-acting octreotide for

Table 2 Clinical experience with VEGF-targeted agents in NENs

Study	Phase	Therapy	Number of patients	ORR	PFS	OS	Grade ¾ toxicities
1. Bevacizumab studies							
Berruti et al. [46]	Phase II	Bevacizumab plus octreotide and metronomic capecitabine	45 patients	17.8 %	14.9 months	Not attained	Hand and foot syndrome (11.1 %), proteinuria (4.4 %), and renal toxicity (2.2 %)
Chan et al. [47]	Phase II	Bevacizumab plus temozolomide	34 patients (56 % with carcinoid, 44 % with pancreatic NETs)	15 %	11.0 months (14.3 months for pancreatic NETs vs. 7.3 months for carcinoid tumors)	33.3 months (41.7 months for pancreatic NETs vs. 18.8 months for carcinoid tumors)	Lymphopenia (53 %) and thrombocytopenia (18 %)
Kulke et al. [48]	Phase II	Methoxyestradiol in combination with bevacizumab	31 patients	0 %	11.3 months	N/R	Hypertension, diarrhea, GI hemorrhage
Yao et al. [49]	Phase II	Depot Octreotide with bevacizumab and Pegylated Interferon Alfa-2b	44 patients	Bevacizumab arm, 18 % INF Alfa-2b arm, 5 %	For all patients, 66 weeks	N/R	Hypertension
Castellano D. et al. [50]	Phase II	Sorafenib 200 mg bid+bevacizumab	44 patients (31 carcinoids and 13 pNETs)	9.4 %	12.4 months	Not reached	Not reported
Firdaus et al. [51]	Phase II	Bevacizumab, pertuzumab, and sandostatin	43 patients (32 (74 %) carcinoid, 11 (26 %) PNET)	16 %	For the entire group, 8.2 months For carcinoid patients, 8.5 months PFS for pNET patients, 6.4 months	Has not been reached	Hypertension (28 %), LVEF dysfunction (9 %) (reversible with trial drug hold), and diarrhea (7 %)
Koumarianou et al. [52]	Phase II	Metronomic temozolomide, bevacizumab, and long-acting octreotide	Advanced NETs, mainly grade II tumors with Ki-67 labeling index (LI) 3–19 %	75 %	36 weeks	N/R	N/R
2. Sunirinib studies							
Strosberg et al. [53]	Phase II	Sunitinib following hepatic transarterial embolization	39 patients with metastatic NENs to the liver	72 %	15.2 months	1 year survival=95 %	N/R
Kulke et al. [54]	Phase II	Sunitinib monotherapy	107 (carcinoid, n=41; pancreatic endocrine tumor, n=66)	Pancreatic NETs, 16.7 % Carcinoid, 2.4 %	Pancreatic NETs, 7.7 months Carcinoid, 10.2 months	1 year survival=81 % for pNETs and 83 % for carcinoid	Hypertension, vomiting, anorexia
Raymond et al. [55]	Phase III	Sunitinib monotherapy vs. placebo	171 patients randomly assigned (in a 1:1 ratio)	9.3 vs. 0 %	11.4 vs. 5.5 months	Nonestimable	Diarrhea, neutropenia, and hand-foot syndrome
3. Sorafenib studies							
Chan et al. [56]	I	Sorafenib+everolimus (MTD)$^{\delta}$	18 carcinoids 3 PNETs $^{\alpha}$	9 %	N/A$^{¥}$	N/A	Skin rash

Table 2 (continued)

Study	Phase	Therapy	Number of patients	ORR	PFS	OS	Grade ¾ toxicities
Hobday et al. [57]	II	Sorafenib	50 carcinoids 43 PNETs	22 %	9.6 months	N/A	N/R
Lam et al. [58]	II	Sorafenib	All MTC[β]	6 %	17.9 months	Not reached	Hypertension, hand-foot syndrome, and diarrhea
Quintela-Fandino 2013 [59]	II	Sorafenib and low-dose cyclophosphamide	18 carcinoids 4 PNETs	5 %	3 months	11.7 months	N/R
4. Pazopanib							
Ahn et al. [60]	II	Pazopanib 800 mg daily continuously	37 patients	18.9 %	N/R	N/R	N/R
5. Cabozantinib							
Kurzrock et al. [61]	I	Cabozantinib 175 mg daily	37 patients	29 %	N/R	N/R	Palmar-plantar erythrodysesthesia (PPE), mucositis, and AST, ALT, and lipase elevations
Elisei et al. [62]	III	Cabozantinib 140 mg daily	330 patients	28 % (in cabozantinib arm)	11.2 vs. 4 months	N/R	Diarrhea, palmar-plantar erythrodysesthesia, decreased weight and appetite, nausea, and fatigue
6. Vandetanib							
Wells et al. [63]	II	Vandetanib 300 mg daily	30 patients	20 %	27.9 months	N/R	Diarrhea, rash, fatigue, and nausea
Wells et al. [64]	III	Vandetanib 300 mg daily	331 patients	N/R	Not reached (vandetanib group) vs. 19.3 months (placebo group)	N/R	Diarrhea, rash, nausea, hypertension
7. Thalidomide-based regimens							
Kulke et al. [65]	II	Temozolomide and thalidomide	29 patients	25 % (45 % among pancreatic endocrine tumors, 33 % among pheochromocytomas, and 7 % among carcinoid tumors)	N/R	N/R	Lymphopenia, opportunistic infections
Varker et al. [66]	II	Thalidomide single agent	18	0 %	N/R	N/R	Orthostatic hypotension, sensory neuropathy, and deep venous thrombosis

N/R not reported,
OS overall survival,
ORR overall response rate, *PFS* progression-free survival

malignant neuroendocrine tumors. From January 2007 until January 2009, 15 patients with advanced GEP-NETs, mainly grade II tumors with Ki-67 labeling index (LI) 3–19 %, were treated with the abovementioned combination. The median reported PFS was 36 weeks [52].

- In another interesting article, Ng and coworkers assessed perfusion CT findings for patients with advanced GEP-NENs receiving bevacizumab and interferon therapy. They found that perfusion CT detects significant changes in perfusion parameters in metastatic carcinoid tumors treated with bevacizumab. Such changes are apparent just 2 days into therapy, are sustained, and are significantly different from those associated with IFN treatment. Tumor blood flow decreased with bevacizumab treatment by a relatively fixed percentage relative to baseline measurements. These data can have important implications as a potential prognostic and predictive tool in carcinoid tumor patients treated with bevacizumab-based regimens [38, 67].

Other bevacizumab phase II studies are summarized in Table 2.

ii. Sunitinib

Sunitinib is the most commonly tested VEGFR-TKI in advanced NENs with numerous phase II and III studies published in this indication.

- Following an encouraging phase II study by Kulke et al. [54], an international phase III study has been conducted for sunitinib in advanced pancreatic neuroendocrine tumors (pNETs) [55]. This study enrolled 171 patients with random assignment (in a 1:1 ratio) to either sunitinib or placebo. The study showed that continuous daily administration of sunitinib at a dose of 37.5 mg improved progression-free survival, overall survival, and the objective response rate as compared with placebo among patients with advanced pancreatic neuroendocrine tumors and following the results of this study, sunitinib has been adopted as one of the standard treatment options for advanced pNETs.
- Moreover, sunitinib has been investigated in a phase II study in a post chemoembolization setting for patient with advance pNETs metastatic to the liver and the results were encouraging (PFS=15.2 months) and 1 year survival=95 % [53].

iii. Sorafenib

Sorafenib is one of the most extensively studied VEGFR-TKI in solid tumors [29, 39, 68]; in advanced NENs, it has been investigated in a number of settings, both as a single agent and in combination and both in phase I and phase II studies.

- A phase I study to examine the potential for combined use of sorafenib plus everolimus has been conducted in patients with advanced GEP-NENs. Patients included in this study had locally unresectable or metastatic carcinoid or pancreatic neuroendocrine tumors of low- or intermediate-grade of malignancy. Sorafenib 200 mg twice daily with everolimus 10 mg daily represented the maximum tolerated dose (MTD). However, toxicity concerns from such a combination may preclude more widespread use [56].
- Additionally, Hobday and coworkers treated 93 patients with metastatic GEP-NENs (50 carcinoid tumors and 43 islet cell pancreatic tumors) with sorafenib 400 mg twice daily. A 10 % partial response rate was observed both in carcinoids and in pancreatic tumors, with a 40 % of 6-month progression-free survival (PFS) in carcinoid and 60 % in pancreatic tumors. Grade (G) 3–4 toxicity occurred in 43 % of patients, mainly skin toxicity [57].

iv. Pazopanib

- Ahn and colleagues have evaluated pazopanib monotherapy for metastatic GEP-NENs in a phase II study that included 37 patients with encouraging objective response rate of 18.9 % [60]. This result encourages further evaluation of pazopanib in this setting in a randomized phase III study.

v. Thalidomide-based regimens as a VEGF-targeted strategy

Another interesting VEGF-targeted strategy in GEP-NENs has been the use of thalidomide-based regimens which is an immunomodulating agent with known antiangiogenic mechanism of action [69].

- Thalidomide has been tested both as a single agent and in combination with temozolomide in two phase II studies and despite being found fairly well tolerated in patients with advanced enteropancreatic NENs, it failed to reveal significant objective responses [65, 66].

Clinical data for MTC

vi. Cabozantinib:

- A phase I study by Kurzrock and coworkers has evaluated the activity of cabozantinib in 37 patients diagnosed with MTC. Partial response was documented in 29 % and stable disease and 41 % has documented stable disease [61].
- These encouraging results prompted the evaluation of cabozantinib in another phase III study which was

reported in 2013; this study has included 330 patients with progressive MTC who were randomized to cabozantinib vs. placebo. The estimated median PFS was 11.2 months for cabozantinib versus 4.0 months for placebo ($P<.001$). Accordingly, cabozantinib has been approved for progressive MTC as it represents an important new treatment option for patients with this rare disease [62].

vii. Vandetanib:

- In a phase II study by Wells and coworkers, the activity of vandetanib in patients with locally advanced or metastatic hereditary MTC was evaluated. Thirty patients were enrolled in this study, partial response was documented in 20 % of patients, and median PFS was 27.9 months [63]. These results have prompted further evaluation of the drug in a larger phase III study where 331 locally advanced or metastatic MTC patients were enrolled. The study met its primary objective of PFS prolongation with vandetanib versus placebo (hazard ratio [HR], 0.46; 95 % CI, 0.31 to 0.69; $P<.001$), and thus, vandetanib has been approved for this indication [64].

vii. Sorafenib:

A phase II study, from the USA, has been conducted in MTC. In this study, patients with histologically confirmed metastatic or locally advanced MTC received sorafenib monotherapy at a dose of 400 mg twice daily. Sorafenib has been shown to be well tolerated, with suggestion of clinical benefit for patients with sporadic MTC [58].

Novel approaches for optimizing VEGF-targeted therapy

A number of approaches have been suggested to optimize VEGF-targeted therapy either by using novel agents or by combination with other targeted therapies.

- For example, Sennino et al. have evaluated the value of concurrent inhibition of c-Met and VEGF signaling in pancreatic neuroendocrine tumors in a xenograft model. They found that treatment of pancreatic neuroendocrine tumors in RIP-Tag2 mice with a neutralizing anti-VEGF antibody reduced tumor burden but increased tumor hypoxia, hypoxia-inducible factor-1α, and c-Met activation and thus increased invasion and metastasis. However, invasion and metastasis were reduced by concurrent inhibition of c-Met by PF-04217903 or PF-02341066 (crizotinib). A similar benefit was found in orthotopic Panc-1 pancreatic carcinomas treated with sunitinib plus PF-04217903 and in RIP-Tag2 tumors treated with XL184 (cabozantinib), which simultaneously blocks VEGF and c-Met signaling. These findings document that invasion

Table 3 Ongoing trials for VEGF-targeted agents in neuroendocrine tumors

Title of the study	Clinicaltrials.gov identifier	Study locations	Phase	Therapy	Status	Estimated completion date
Everolimus and octreotide with or without bevacizumab in treating patients with locally advanced or metastatic pancreatic neuroendocrine tumors that cannot be removed by surgery	NCT01229943	401 North American centers (USA and Canada)	Phase II	Everolimus and octreotide with or without bevacizumab	Ongoing, but not recruiting participants	December 2014
Capecitabine, temozolomide, and bevacizumab for metastatic or unresectable pancreatic neuroendocrine tumors	NCT01525082	Three USA centers	Phase II	Capecitabine, temozolomide, and bevacizumab	Recruiting	December 2014
Phase II study of axitinib (AG-013736) with evaluation of the VEGF pathway in metastatic, recurrent, or primary unresectable pheochromocytoma/paraganglioma	NCT01967576	NCI, USA	Phase II	Axitinib	Recruiting	October 2016
Ziv-aflibercept for advanced progressive carcinoid tumors	NCT01782443	Three USA centers	Phase II	Ziv-aflibercept	Recruiting	March 2015
Octreotide acetate and recombinant interferon alfa-2b or bevacizumab in treating patients with metastatic or locally advanced, high-risk neuroendocrine tumor	NCT00569127	USA	Phase III	Octreotide acetate and recombinant interferon alfa-2b or bevacizumab	Closed for recruitment	N/A

N/A not applicable

and metastases are promoted by selective inhibition of VEGF signaling and can be reduced by the concurrent inhibition of c-Met [70]. And thus, this provides a rationale for a possible combination strategy of concurrent inhibition of c-MET and VEGF in pancreatic NENs.

Ongoing studies

Across the globe, a number of cooperative groups are conducting clinical studies on multiple VEGF-targeting agents in multiple phases of development (Table 3); results of these studies are expected within the next 2 years. These studies span the whole spectrum of VEGF-targeting agents (newer monoclonal antibodies, e.g., ziv-aflibercept as well as newer VEGFR TKIs, e.g., axitinib).

Conclusions and future perspectives

VEGF pathway has been extensively studied in preclinical and clinical settings of NENs. From the very first studies of VEGF pathway, VEGF has been considered an important prognostic marker in NENs. Consequently, a number of preclinical experiences have examined the efficacy of VEGF-targeted therapeutics in NEN xenograft and cell line models. These preclinical experiences provide a good rationale for proceeding forward with a number of clinical studies of bevacizumab-based combination in this indication.

Bevacizumab and sorafenib were clinically tested in NENs and they showed modest activity, while on the other hand, they present toxicity problems. Another interesting treatment option in NENs is sunitinib as supported by its demonstrated efficacy. Preclinical as well as clinical sunitinib data in this regard provide a new hope in that direction. In addition to bevacizumab, sunitinib, and sorafenib, a number of VEGF-targeted molecular agents have been studied in advanced NENs, including pazopanib, which has been studied in a phase II study for GEP-NENs with initially encouraging results, cabozantinib and vandetanib (for advanced MTC). Additionally, thalidomide—which is an immunomodulating agent with known antiangiogenic mechanism of action—has been tested both as a single agent and in combination with temozolomide in two phase II studies and despite being found fairly well tolerated in patients with advanced enteropancreatic NENs, it failed to reveal significant objective responses. Moreover, across the globe, a number of cooperative groups are conducting clinical studies on multiple newer VEGF-targeting agents in multiple phases of development; the results of these studies are expected within the next 2 years. These studies span the whole spectrum of VEGF-targeting agents (newer monoclonal antibodies, e.g., ziv-aflibercept as well as newer VEGFR tyrosine kinase inhibitors, e.g., axitinib).

Given the biological and clinical heterogeneity of advanced NENs, the biggest challenge to the success of VEGF-targeted treatments in advanced NENs seems to be the lack of biologically driven randomized controlled trials that can stratify patients into different molecularly driven subsets with determination of sensitive subsets that drive the best benefit from one treatment over the other. Thus, the use of potential biomarkers to select patients for VEGF-targeted therapy should be considered as a priority in all future clinical trials and the efforts exerted by different groups around the world to further explore the biological heterogeneity of NENs should be further supported by respective institutional bodies. A particularly interesting subject for discussion in this regard is the use of gene expression profiling and liquid biopsies to evaluate the biological subtype as well as newer imaging technologies (including diffusion-weighted MRI) to assess vascularity and perfusion as an indicator of possible responsiveness to VEGF-targeted therapeutics.

Additionally, the use of novel combinations between VEGF-targeted therapeutics and other targeted or nontargeted systemic agents (including cytotoxic chemotherapy and hormonal therapy) is very appealing, given the different mechanisms of action of these agents that can enhance further the ability of these regimens to combat the disease. Moreover, traditional methods of targeting the VEGF pathway (including metronomic chemotherapy and somatostatin analogues) should be further reevaluated innovatively in prospective randomized studies.

Conflicts of interest None

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