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-I, 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.1

Three-tiered grading systems have been proposed for GEP-NENs classification, according to their morphological features and ki-67 index:2 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.3,4 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.5-7 Conversely, bronchopulmonary NETs are approximately 20-25% of all lung malignancies.8-12 On the basis of 2004, World Health Organization classification, pulmonary NETs can be divided into three groups:13 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...
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.\[14\]

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.\[15-17\]

Five different somatostatin receptor (SSTR) subtypes have been characterized in humans (SSTR1-SSTR5) [Figure 1].\[18-22\] 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.\[23-25\] For this reason, different SSAs show a distinct affinity with their own ligands, eliciting various biological and clinical activities\[16\] in the same cell type through the activation of subsets of disparate intracellular mediators.\[22,24,26\] 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-NE-Ns. SSTR in particular has been observed to predominate in both gastrointestinal-NE-Ns (90%) and primitive-NE-Ns (P-NE-Ns), especially in gastrinomas,

![Figure 1: Principal pathways involved in carcinogenesis and progression of NE-Ns. 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](image-url)
glucagonomas, and VIPomas (80-100%). However, insulinomas express SSTR in 50-70% of cases, showing a prevalence of SSTR5 mRNA expression that is positively correlated with aggressive pathological characteristics. SSTR2 is usually expressed in NENs, and its loss could be highly correlated with the dysregulation of tumor proliferation, consequently promoting tumor growth.

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.

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. 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.

SSR 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). 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.

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

Table 1a: SSAs approved for NETs treatment

<table>
<thead>
<tr>
<th>Author/trials</th>
<th>Regimen</th>
<th>Patients enrolled</th>
<th>Results</th>
<th>Adverse reactions (grade &gt; 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinke et al. [9] Arnold et al. [24]</td>
<td>Octreotide vs. placebo</td>
<td>Advanced GEP or NETs of unknown origin</td>
<td>mTTP: 14.3 vs. 6 months; SD: 64% vs. 37.2%</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Caplin et al. [25] Clarinet (Phase III)</td>
<td>Lanreotide vs. placebo</td>
<td>Advanced GEP or NETs of unknown origin</td>
<td>mPFS: NR vs. 18 months</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Filosso et al. [26]</td>
<td>Octreotide</td>
<td>Metastatic atypical bronchial carcinoid with carcinoid syndrome (diarrhea)</td>
<td>RR = 60%</td>
<td>None</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Author/trials</th>
<th>Regimen</th>
<th>Patients enrolled</th>
<th>Results</th>
<th>Adverse reactions (grade &gt; 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolin et al. [27] (Phase III)</td>
<td>Pasireotide vs. octreotide</td>
<td>Advanced GEP-NETs</td>
<td>mPFS: 11.8 vs. 6.8 months; SD: 60.8% vs. 42.3%</td>
<td>Hyperglycemia, diarrhea</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Author/trials</th>
<th>Regimen</th>
<th>Patients enrolled</th>
<th>Results</th>
<th>Adverse reactions (grade &gt;3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kulke et al. [28]</td>
<td>Telotristat</td>
<td>Metastatic GEP-NETs with carcinoid syndrome</td>
<td>Reduction of BMs: 30%</td>
<td>Gastrointestinal symptoms: nausea, vomiting, or abdominal discomfort</td>
</tr>
<tr>
<td>Pavel et al. [29]</td>
<td>Telotristat</td>
<td>Metastatic well-differentiated NETs with carcinoid syndrome (diarrhea)</td>
<td>Reduction of BMs: 43.5%</td>
<td>Gastrointestinal symptoms: nausea, vomiting, or abdominal discomfort</td>
</tr>
</tbody>
</table>

GEP-NETs: gastro-entero-pancreatic neuroendocrine tumors; mTTP: median time to progression; mPFS: median progression free survival; BMs: bowel movements; RR: response rate
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), such as angiogenesis inhibition and immunomodulation mechanism, mediated by stimulation of the production of natural-killer cells and blockage of growth factors.

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) as shown by Rinke et al. 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 (≤ 10% at study entry).

<table>
<thead>
<tr>
<th>Table 2: Principal studies with inhibitors of mTOR</th>
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<tbody>
<tr>
<td><strong>Author/trials</strong></td>
</tr>
<tr>
<td>Yao et al. (Phase II) RADIANT-1</td>
</tr>
<tr>
<td>Pavel et al. (Phase III) RADIANT-2</td>
</tr>
<tr>
<td>Fazio et al. (RADIANT-2 Phase III) exploratory analysis</td>
</tr>
<tr>
<td>Yao et al. (Phase III) RADIANT-3</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Table 3: Anti-IGF-R1 drugs in NETs</th>
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</thead>
<tbody>
<tr>
<td><strong>Author/trials</strong></td>
</tr>
<tr>
<td>Naing et al. (Phase I)</td>
</tr>
<tr>
<td>Rothenberg et al. (Phase I)</td>
</tr>
<tr>
<td>Strosberg et al. (Phase II)</td>
</tr>
</tbody>
</table>

IGF: insulin-like 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

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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. Due these significant data, octreotide LAR and depot lanreotide have been approved as treatment for patients with newly developed diseases.
Table 4: Anti-angiogenic drugs in NETs

<table>
<thead>
<tr>
<th>Author/trials</th>
<th>Regimen</th>
<th>Patients enrolled</th>
<th>Results</th>
<th>Adverse reactions (grade &gt; 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faivre et al.[115] (Phase I)</td>
<td>Sunitinib</td>
<td>Metastatic solid tumors pre-treated: (neuroendocrine tumors)</td>
<td>ORR = 20%</td>
<td>Fatigue, hypertension</td>
</tr>
<tr>
<td>Kulke et al.[116] (Phase II)</td>
<td>Sunitinib</td>
<td>Carcinoid or pancreatic neuroendocrine tumor not candidates for curative surgery</td>
<td>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</td>
<td>Fatigue, hypertension, GI hemorrhage, pulmonary embolism, increased lipase, cardiac congestive failure, cerebrovascular accident, hyponatremia</td>
</tr>
<tr>
<td>Kulke et al.[116] (Phase II)</td>
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<td>Fatigue, hypertension, GI hemorrhage, pulmonary embolism, increased lipase, cardiac congestive failure, cerebrovascular accident, hyponatremia</td>
</tr>
<tr>
<td>Raymond et al.[117] (Phase II)</td>
<td>Sunitinib vs. placebo</td>
<td>Low- and intermediate-grade advanced P-NETs</td>
<td>mPFS = 11.4 vs. 5.5 months ORR = 9.3% vs. 0% OS rate = 25% vs. 10%</td>
<td>Diarrhea, nausea, vomiting, fatigue</td>
</tr>
<tr>
<td>Yao et al.[118] (Phase II)</td>
<td>Octreotide plus bevacizumab vs. octreotide plus pegylated IFN α2b</td>
<td>Metastatic or unresectable carcinoid tumors</td>
<td>SD = 77% vs. 68% PFS rate: 95% vs. 68%</td>
<td>Granulocytopenia, headache, hypertension</td>
</tr>
<tr>
<td>Chan et al.[119] (Phase II)</td>
<td>Bevacizumab plus temozolomide</td>
<td>Locally advanced or metastatic NETs</td>
<td>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)</td>
<td>Lymphopenia, thrombocytopenia</td>
</tr>
<tr>
<td>YAO et al.[120] (Phase II)</td>
<td>Everolimus alone with the combination of everolimus and bevacizumab</td>
<td>Advanced P-NETs</td>
<td>ORR = 26%</td>
<td>-</td>
</tr>
<tr>
<td>Ahn et al.[121] (Phase II)</td>
<td>Pazopanib</td>
<td>Advanced GEP NENs, not amenable to loco-regional therapies</td>
<td>ORR = 18.9% SD = 56.8% DCR = 75.7%</td>
<td>Proteinuria, neutropaenia, hypertension, diarrhea, anorexia, abdominal pain, AST/ALT elevation</td>
</tr>
<tr>
<td>Ahn et al.[121] (Phase II)</td>
<td>Pazopanib plus octreotide</td>
<td>Metastatic or locally advanced grade 1-2 carcinoid tumours or P-NETs</td>
<td>ORR = 21.9% of P-NETs ORR = 0% in GI-NETs PFS: 14.2 months in P-NETs, PFS = 12 months in GI-NETs</td>
<td>Hypertriglyceridemia, thrombosis.</td>
</tr>
</tbody>
</table>

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

Another drug, telotristat etiprate, inhibitor of serotonin synthesis, was studied in patients with carcinoid syndrome characterized by diarrhea. Kulke \textit{et al.}\textsuperscript{[54]} and Pavel \textit{et al.}\textsuperscript{[55]} 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.\textsuperscript{[56]}

Filosso \textit{et al.}\textsuperscript{[57]} 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.\textsuperscript{[58]} [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.\textsuperscript{[59,60]} 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.\textsuperscript{[61-63]} 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).\textsuperscript{[64]} [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.\textsuperscript{[65]} [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.\textsuperscript{[66,67]} [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.\textsuperscript{[68]} 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).\textsuperscript{[69-71]} 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 a reduction of AKT.\textsuperscript{[74-76]} PTEN is frequently mutated in P-NETs and its low expression correlates with high grading.\textsuperscript{[77]} In particular, low expression in cytosol of lung NETs indicates a category of patient with poor prognosis.\textsuperscript{[78]}

**IGF1 INHIBITORS**

Insulin growth factor 1 (IGF1), a factor involved in tumor progression, is secreted by neuroendocrine
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, inducing cellular proliferation and over-regulating antiapoptotic activity. [101] [Figure 1] IGF-1 receptors, then, are usually overexpressed in NETs, especially in symptomatic and functioning ones. This represents a possible role in tumorigenesis of GEP and bronchial NETs and a potential target for therapy. [91-93] The rationale for the use of IGF1R inhibitors depends on their theoretical capability to reduce AKT phosphorylation induced by mTOR inhibitors. [94-96]

In this regard, cixutumumab, a fully human immunoglobulin G1 monoclonal antibody competitively binding IGF-1R, is in the early phases of clinical progress. [97] 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. [98] 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. [99] [Table 3]

Similarly, ganitumumab, another fully human monoclonal antibody against IGF-1R, is undergoing evaluation in clinical trials. Rothenberg et al. [100] demonstrated encouraging activity and good tolerance in a phase I trial including previously treated metastatic NET patients [Table 3]. Strosberg et al. [101] 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. [102,103] Four VEGF forms are individuated and examined: VEGF-A, VEGF-B, VEGF-C, and VEGF-D. [104-108] with a different affinity to their three own receptors. [109-113] [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, [114,115] Kulke et al. [116] 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. [117] 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 α2b. Bevacizumab showed superiority in objective responses, reduction of tumor blood flow, and PFS. [118,119] Bevacizumab in association with temozolomide in patients with metastatic NETs also showed a major response rate, PFS, and OS in P-NETs. [120]

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. [121] 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 antiangiogenetic 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. [122] Ahn et al. [123] 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. [124,125] 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; PDGFRAu, 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). [126-128]
EGF AND ITS RECEPTOR AND TGFα

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 carcinoid, and gastrointestinal-neuroendocrine tumours (GI-NETs) and P-NETs present and over-regulate EGFRs and P-NETs present and over-regulate EGFRs and Gastrointestinal-neuroendocrine tumours (GI-NETs) and P-NETs present and over-regulate EGFRs. In particular, described a higher presence of EGFR (> 91%) and in GI-NETs, especially rectal NETs, than in P-NETs (< 25%).

An elevated presence of EGFR and transforming growth factor alpha (TGFα) in P-NETs was observed by Srivastava et al. An elevated amount of secreted TGFα 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 TGFα. Krishnamurthy et al. showed a high expression of TGFα 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). In rectal NENs TGF-α expression seemed to be increased in lesions larger than 5 mm and tumors with higher Ki67 index. 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.

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.

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.

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. 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/P13K and PTEN, IGF-1, VEGF, EGF, TGF, FGFR and c-kit/PDGF and its corresponding receptors, markers whose established value may more thoroughly define an appropriate course of treatment.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

Patient consent
No patient involved.

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

REFERENCES


51. Arnold R, Wittenberg M, Rinke A, Schade-Bittering C, Aminossadati B, Ronicke Gress TM, Mueller HH; PROMID Study Group; Philips University Marburg, Department of Internal Medicine, Division of Gastroenterology and Endocrinology, Marburg, Germany; KKS Marburg, Marburg, Germany; Philips University Marburg; Department of Gastroenterology, Marburg, Germany; Koordinierungszentrum für Klinische Studien der Philips-Universität Marburg, Marburg, Germany; Institute of Medical Informatics, Biometry and Epidemiology, LMU Munich, Munich, 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.


dependent mechanism. induces feedback activation of Akt signaling through an IGF-1R-

receptor substrate-1/phosphatidylinositol 3-kinase cascade. Ito T, Venzon DJ, Serrano J, Jensen RT. Increased expression of insulin-like growth factor I and/or its receptor in gastrinomas is linked to increased, increased growth, and development of metastases. Clin Cancer Res 2005;11:3233-42.


