Management of Neuroendocrine Tumors of the Pancreas and Digestive Tract

From Surgery to Targeted Therapies: A Multidisciplinary Approach

Eric Raymond · Sandrine Faivre
Philippe Ruszniewski *Editors*

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Preface

Complexity of Patient Care in Neuroendocrine Tumors of the Digestive Tract

Neuroendocrine tumors (NETs) have emerged as paradigm tumors for which multidisciplinary care is required. NETs are known as rare tumors. However, the increasing incidence of NET renders it likely that physicians caring for cancers may have either already faced or may be certainly exposed during their career to the challenging issues of discussing the case of a patient with NET. During the last 5 years, several novel therapeutic options have emerged for NET, profoundly challenging practices that had been previously set for decades. This moving field has generated some confusion, leading to novel treatment algorithms to guide medical decisions. To either better understand or handle the multidisciplinary approaches that are required for optimizing the care of NET patients, physicians are now looking for references from experts and comprehensive reviews summarizing the current knowledge on treatments of patients with NET.

NETs are fascinating multifaceted diseases that can primarily localize in many organs with various presentations. Few patients may present with symptomatic tumors at diagnosis due to endocrine secretions and/or bulky tumor masses. In some instances, emergency care may even be required to speedup diagnosis and therapy. More frequently, NETs are diagnosed at late stages due to the lack of symptoms and the relative indolence of the disease, even in the presence of multiple metastases. Therefore, the vast majority of patients with NET may present at diagnosis with advanced primary and already developed metastasis, the liver being the primary site of digestive NET dissemination. Although only a small number of patients may undergo surgical resection, surgery remains the only curative approach and shall therefore be discussed along with other options even in the presence of metastases. Since most patients will develop multiple non-operable liver metastases early on during the natural history of their disease, curative surgery is often impossible and instead debulking liver-resection and liver-directed therapy, such as chemoembolization of radiofrequency ablation, may have palliative benefits for patients with liver-dominant metastases. Interestingly, NET cells often express somatostatin receptors that can control hormonal secretions and stimulate tumor proliferation. Somatostatin analogs, inhibiting somatostatin

receptor functions, are often prescribed to relieve symptoms resulting from hormonal hypersecretion in functioning tumors such as diarrheas and flushing episodes. Recently, data also demonstrated that somatostatin analogs could also delay tumor progression in selected patients with carcinoid tumors, although this demonstration has not yet been fully demonstrated for patients with pancreatic NETs (PNETs). Taking advantage of the presence of somatostatin receptors at the surface of cancer cells, somatostatin analogs loaded with radionucleotides have been used to selectively target cancer cells and deliver metabolic radiotherapy to disseminated NET metastases. Based on large retrospective clinical experiences, Peptide Receptor Radionucleotide Therapy (PRRT) is now frequently proposed to patients with advanced NET. Although evidences suggest activity of PRRT in NET, the overall benefit and long-term safety of this therapeutic approach remains to be validated prospectively. For patients with advanced NET, chemotherapy has been an important part in the history of treatment for NET. Chemotherapy was the first treatment option demonstrating significant benefits, delaying tumor progression, controlling symptoms, and in some circumstances improving overall survival. While midgut carcinoid tumors showed poor sensitivity to chemotherapy, PNETs have been acknowledged to be more sensitive to chemotherapy. Chemotherapy, such as streptozocin, either combined with doxorubicin or fluorouracil, has been the only systemic treatment approved for many years in advanced PNETs, though the magnitude of benefit has been often challenged in recent publications. Temozolomide, an oral methylating chemotherapy with mechanisms of action similar to DTIC, has been evaluated in retrospective series. Temozolomide demonstrated evidence of activity, possibly related to the lack of methyl guanine transferase expression, the enzyme that repairs DNA insults caused by temozolomide. More recently, large prospective trials using sunitinib and everolimus demonstrated that progression of PNET could be delayed using small molecules targeting cell signaling. Inhibition of mTOR using everolimus may cause inhibition of cancer cell proliferation and can alter metabolic function of NET cancer cell, delaying tumor progression in advanced well-differentiated tumors. In addition, sunitinib, inhibiting NET angiogenesis at the level of endothelial cells and pericytes was also shown to delay tumor progression in well-differentiated PNET. These two drugs have been recently approved in advanced PNET and now offer more opportunities in the NET armamentarium to delay progression. While treatment options have progressed, imaging techniques and endoscopy have also gained in precision allowing earlier diagnosis, better sensitivity in the detection of metastases, and more efficient criteria for evaluating drug efficacy. Considering the multiple treatment options in PNET, strategies are now required to optimize the sequential use of somatostatin analogs, PRRT, chemotherapy, and targeted therapies in patients with advanced PNETs that are not amenable to curative surgery. Another important issue in the care of patients with NET shall also consider how quality of life could be impacted by treatment decisions.

The multiple options for treatment of patients with NET require multidisciplinary approaches and discussions from experts from various specialties to select

the best treatment choice for each individual case. Multidisciplinary boards developed in expert centers are aiming to encompass the various needs for care of patients with NET and should be promoted, eventually using networking though teleconferences in centers that cannot develop expertise in all the domains. In this book, we have aimed to keep the spirit of multidisciplinary board meetings, asking experts to deliver chapters where readers may find data to make their own opinions. Authors have been selected from centers of expertise for NET in Europe and in the United States. Authors have been requested to provide updated information about current knowledge for various aspects of treatment of patients with NET. We expect that readers will find inspiring ideas and information that may help them to better understand options and optimize the care of patients with NET.

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Contents

Chapter 1
Scintigraphy in Endocrine Tumors of the Gut

Rachida Lebtahi

Abstract This review provides an overview of the currently used nuclear medicine imaging modalities and ongoing developments in the imaging of neuroendocrine tumors (NETs). Most NETs overexpress the somatostatin receptor mainly sst2. Somatostatin receptor scintigraphy with ^{111}In-DTPA-0octreotide has proven its role in the diagnosis and staging of gastroenteropancreatic NETs. The use of ^{68}Ga-labeled analogs of octreotide for PET imaging, with of different radiolabelled somatostatin analogues with higher affinity and different affinity profiles to the somatostatin receptor subtypes such as DOTATOC, DOTANOC, and DOTATATE, are in clinical application in nuclear medicine. The development PET tracers for NET imaging include Fluorodihydroxyphenylalanine (^{18}FDOPA) and fluorodeoxyglucose (^{18}FDG). ^{18}FDOPA-PET appears to be a major tool for the management of carcinoid tumors with excellent diagnostic performances. The role of ^{18}FFDG PET-CT in the prognosis of neuroendocrine tumors should be evaluated.

Keywords Neuroendocrine tumors · Somatostatin receptor scintigraphy · ^{68}Ga-DOTATOC · ^{68}Ga-DOTANOC · ^{68}Ga-DOTATATE · ^{18}FDOPA-PET

Introduction

Nuclear imaging procedures of neuroendocrine tumors (NETs) consist in images performed with a hybrid camera combining single-photon emission computed tomography with computed tomography (SPECT-CT) and/or images with a positron emission tomography camera (PET).

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The first imaging procedure used radiolabeled somatostatin analogs for the detection of NETs [1, 2]. A high density of somatostatin receptors with high affinity for octreotide (somatostatin analog) has been demonstrated in almost all NETs [3]. Five subtypes of somatostatin receptor were identified (from sst1 to sst5 subtypes) [4]. In the same tumor, different subtypes of receptors may be expressed, and most NETs express more than one of five somatostatin receptor subtypes. For the detection of NETs, Krenning et al. [1] and Lamberts et al. [2] reported the first results of somatostatin receptor scintigraphy using radiolabeled somatostatin analogs. The technique most often used today is somatostatin receptor scintigraphy with SPECT-CT using ^{111}In-DTPA-octreotide (Octreoscan®) [5–7]. The uptake of ^{111}In-DTPA-octreotide is based on a specific receptor mechanism. Octreoscan® can therefore visualize tumors which express these receptors, such as NETs. With Octreoscan®, the uptake within the tumor depends on the presence of somatostatin receptors (mainly sst-2), and the intensity of this uptake is related to the density of sst-2 receptors [3, 8, 9]. The localization of the tumor and determination of the extent are essential for the management of patients with NETs [6, 7].

Somatostatin Receptor SPECT-CT

Octreoscan® scintigraphy has been proven useful in functional or nonfunctional neuroendocrine tumors. The sensitivity for the detection reported by the literature is estimated at 70–100 % [6–8]. Scintigraphy permits staging workup and/or the follow-up after treatment [5–7, 10, 11]. The sensitivity of Octreoscan® scintigraphy for detecting neuroendocrine tumors of the gut has been well studied [5–7, 12, 13]. The major diagnostic value of this method is to be complementary to other conventional imaging techniques. Almost all studies demonstrated that scintigraphy has greater sensitivity for detecting both hepatic and extrahepatic metastases. The Octreoscan® scintigraphy confirms known lesions and reveals lesions not visualized by other imaging techniques [11]. It suggests the character of an endocrine tumor already revealed by conventional imaging. The positivity of somatostatin receptor scintigraphy has been reported to be a strong predictive factor of response to treatment with radiolabeled analogs. More recently, it has been used to select patients likely to receive peptide-receptor radionuclide therapy (PRRT) [12]. Its positivity suggests that it is a good prognosis marker of the neuroendocrine nature of a tumor [9, 13]. The recommended protocol is intravenous injection of about 200 MBq of ^{111}In-pentetreotide (with 10 μg of the somatostatin analogs) [5–7]. Images should be performed at 4 and 24 h post injection, using planar images and systematically abdominal SPECT-CT at 24 h post injection. Normal imaging results show a physiologic low-level uptake in the pituitary, thyroid, and breasts. The accumulation is also shown in the liver (with always homogeneous repartition), the kidneys, and the spleen. In addition, the gallbladder is often visualized. The visualization of pituitary, thyroid, and spleen is due to specific receptor binding. There is a predominant kidney clearance, and the

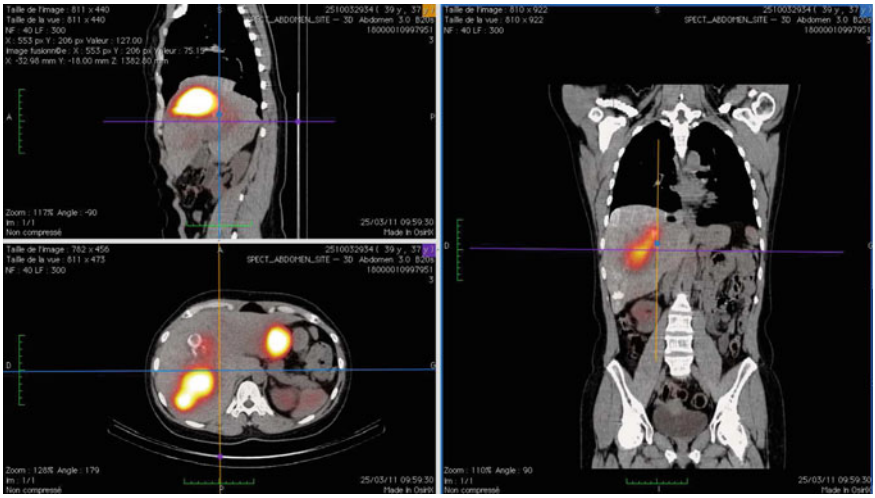

Fig. 1.1 Patient with well-differentiated neuroendocrine tumors: Octreoscan® SPECT-CT showed liver metastases

renal uptake is related to reabsorption of the radiolabeled peptide in the renal tubular cells. Hepatobiliary clearance into the bowel also occurs, leading to the acquisition of delayed abdominal images or the use of laxatives in order to differentiate tumoral from physiologic uptake (Fig. 1.1).

Despite greater sensitivity, limitations of Octreoscan® scintigraphy should be noted. The methodology clearly influenced the sensitivity of the examination. Routine use of planar images and SPECT-CT images of the abdomen (24 h after injection) rather than whole body images are recommended. Octreoscan®x cannot provide information on the size of the tumor. The density and type of the somatostatin receptors vary with the histologic type of the tumors: Insulinomas have a low affinity for octreotide, related to a low expression of sst subtype-2 [5]. Garin et al. [13] reported that negative Octreoscan® scintigraphy in well-differentiated endocrine tumors is negative prognostic factor.

Specificity of Octreoscan® should be noted. Some other tumoral and nontumoral diseases can show positivity of Octreoscan® [7].

Somatostatin Receptor PET-CT

Positron emission tomography (PET) scan is becoming more widely used and may be a useful localizing modality for neuroendocrine tumors as different radiolabeled substances can be used as metabolic substrate. After the development of a PET tracer for somatostatin analogs, ^{68}Ga-DOTA-NOC (tetra-azacyclododecane

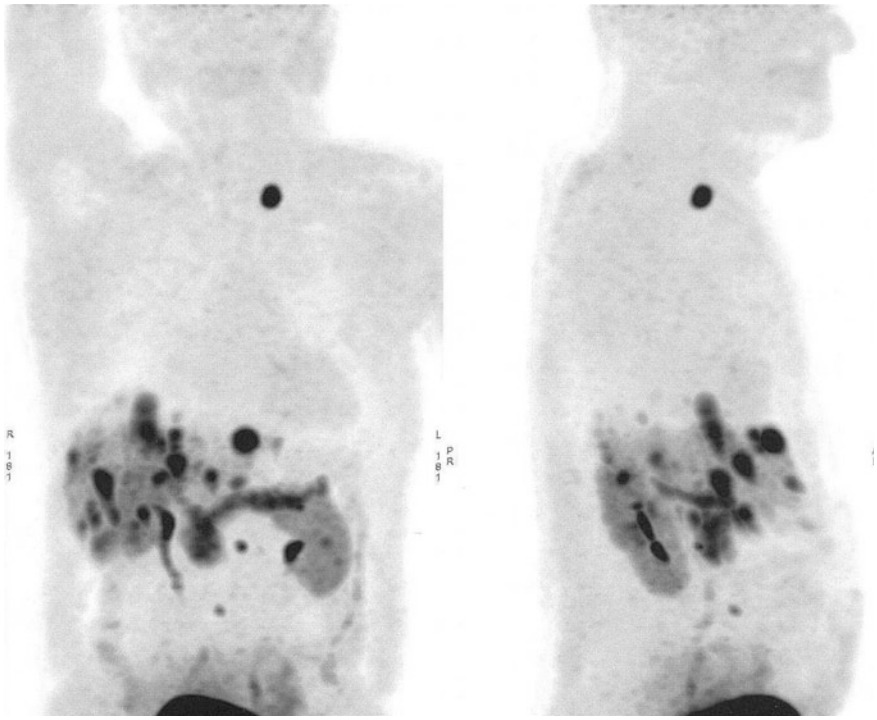

Fig. 1.2 Patient with well-differentiated neuroendocrine tumors: ^{18}FDOPA PET-CT showed multiple liver metastases and sus-clavicular left lymph node

tetra-acetic acid-[1-Nal3]-octreotide) has been introduced. This compound for PET imaging has a high affinity for sst2 and sst5 and has been used for the detection of NETs in preliminary studies. The uptake of ^{68}Ga-DOTA-NOC is based on a receptor mechanism and although this has not yet been adequately assessed, it seems to have higher sensitivity for NETs than Octreoscan®, thereby increasing diagnostic accuracy. Additionally, it has several advantages over Octreoscan®: increased spatial resolution and the possibility of images with a short uptake time (60 min), and relatively easy synthesis [14].

The two other compounds most often used in functional imaging with PET are ^{68}Ga-DOTATOC and ^{68}Ga-DOTATATE. ^{68}Ga-DOTATOC and ^{68}Ga-DOTA-TATE possess similar diagnostic accuracy for detection of NET lesions. The increasing availability of ^{68}Ga somatostatin analogs PET-CT now offers superior accuracy for localization and functional characterization of NETs. However, studies are needed to enable imaging of NET with optimal targeting of tumor receptors.

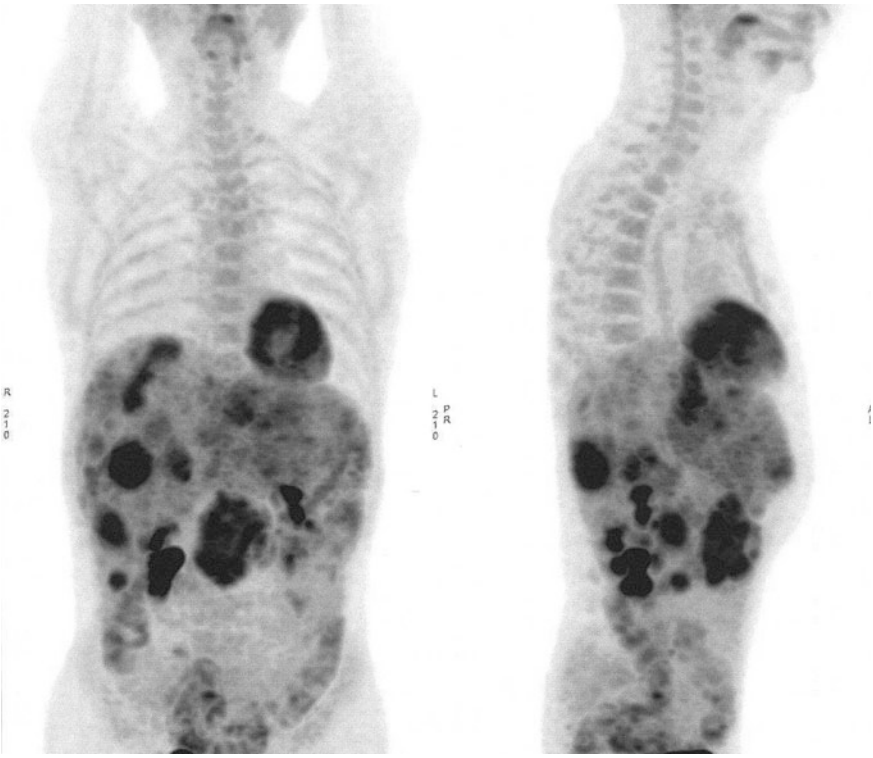

Fig. 1.3 Patient with well-differentiated neuroendocrine tumors grade 2 (KI 67: 15 %).^{18}FDG-PET-CT showed liver metastases and mesenteric lymph node

^{18}F-DOPA PET-CT

Fluorodihydroxyphenylalanine-(^{18}F-FDOPA) PET is a recent imaging modality used to localize neuroendocrine tumors [15]. These tumors have the ability to produce biogenic amines and polypeptide hormones, and they take up and decarboxylate their amine precursors, L-dihydroxyphenylalanine. ^{18}FDOPA-PET appears to be a major tool for the management of carcinoid tumors with excellent diagnostic performances (65–96 %) related to these capacities to concentrate amino acids inside the vesicules of cytoplasmatic space through metabolic mechanism. ^{18}FDOPA-PET is less sensitive and less useful for the management of noncarcinoid tumors (Fig. 1.2).

^{18}FDG PET-CT

Although ^{18}F-2-Deoxy-D-glucose (^{18}F-FDG) PET is the most widely used and accepted type of PET in clinical oncology, it has limited use in well-differentiated tumors such as NETs due to their low expression of glucose transporters and low proliferative activity. However, several studies have evaluated ^{18}F-FDG PET-CT in well-differentiated NET [13, 16, 17]. Garin et al. reported that ^{18}FDG uptake is a poor prognostic factor in NETS, in relation to tumor aggressiveness and is related to a lower overall survival (Fig. 1.3).

Conclusions

All of these performances highlight the significant contribution of the scintigraphic procedures from a diagnostic point of view and the management of therapy of patients with NETs. PET imaging could be of major interest for the diagnosis, evaluation of progression and treatment response in NETs. ^{18}FDG-PET even though still not validated, carries major prognostic information and may influence determination of the optimal therapeutic strategy. The role of ^{18}F-DOPA is clearly recommended before surgery for the detection of carcinoid tumors. The different new somatostatin analogs with ^{68}Ga radiolabeling must be evaluated.

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Chapter 2
Profiling mTOR Pathway in Neuroendocrine Tumors

S. Cingarlini, M. Bonomi, C. Trentin, V. Corbo, A. Scarpa and G. Tortora

Abstract The serine/threonine kinase mammalian target of rapamycin (mTOR) plays a central role in regulating critical cellular processes such as growth, proliferation, and protein synthesis. The study of cancer predisposing syndromes within which neuroendocrine tumors (NETs) may arise has furnished clues on the involvement of mTOR pathway in sporadic diseases so far. Recent comprehensive analyses have definitely shown activation of mTOR pathway in both experimental and human sporadic NETs. Upstream regulators of mTOR (PTEN and TSC2) have been found mutated in sporadic PNETs. Activation of mTOR pathways in NETs is already demonstrated by expression profiles analysis that revealed downregulation of TSC2 gene and alterations of TSC2 and PTEN protein expression in the vast majority of tumors well-differentiated tumors. Moreover, a global microRNA expression analysis revealed the overexpression, in highly aggressive tumors, of a microRNA (miR-21) that targets PTEN reducing its expression and therefore leading to mTOR activation as well. Overall, these clues have furnished the rationale for the use of mTOR inhibitors the treatment for PNETs. With the recent approval of everolimus (mTOR-targeted drug) for the treatment of advanced PNETs, this paradigm has been effectively translated into the clinical setting. In this review, we discuss mTOR pathway involvement in NETs, the clinical evidence supporting the use of mTOR inhibitors in cancer treatment, and the current clinical issues that remain to be elucidated to improve patients' management.

The pathway of the mammalian target of rapamycin (mTOR) plays a central role both in cell proliferation and in the survival rate. Physiologically, it finely tunes anabolic and catabolic processes according to the available energy sources to

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warrant cell proliferation and homeostasis [1]. mTOR is also involved in many pathological conditions other than cancer such as diabetes, neurodegeneration, and obesity. Aberrant signaling caused by molecular alterations within the cascade may contribute to cancer development and progression [2–4].

The great amount of extracellular and intracellular inputs converging on it (or on its singular components) makes mTOR a crucial crossroad whose outputs influence essential cellular functions (such as protein/lipid synthesis, autophagy, or cytoskeletal organization). Growth factors stimuli (acting on mTORC1 and triggering the downstream anabolic signaling), energy depletion and low oxygen levels (activating mainly AMPK and thereby inhibiting mTOR complex either directly or through TSC2), DNA damage (which leads to a PTEN- and TSC2-mediated inactivation of mTOR), and amino acids levels (whose presence is essential for mTOR signaling but whose exact mechanism of action is still unraveled) are some of the most significant examples of the plethora of inputs and outputs coming to and from mTOR [1].

In neuroendocrine tumors (NETs), nearly all the members of PI3K/Akt/mTOR pathway, from the upstream RTK inducers to its final effectors, can be molecularly altered and one or more than one of the above-mentioned alterations can be detected in the same cancer cell. The involvement of mTOR pathway in neuroendocrine tumorigenesis is suggested by a series of evidences:

- Familial syndromes such as multiple endocrine neoplasia type 1 (MEN1), von Hippel–Lindau (VHL) syndrome, type 1 neurofibromatosis (NF1), and tuberous sclerosis complex (TSC). Single pathogenic molecular alterations may trigger the development of NETs with a higher incidence if compared to the generic population. Inactivation of VHL is associated with an increased steady-state level of HIF-1, whose expression is dependent on mTOR-mediated translational regulation [5, 6]. Loss of NF1 is associated with constitutive mTOR activation (depending upon Ras and PI3K) [7]. Loss of function mutations of either TSC1 or TSC2, whose encoded proteins form the TSC complex, can negatively regulate mTOR.
- Sporadic disease: The majority of primary pancreatic neuroendocrine tumors (PNETs) show reduced protein levels of either one or both of the two main inhibitors of the mTOR pathway, TSC2 and PTEN [2]. Allelic loss of PTEN at the level of the chromosome arm 10q is frequent, and somatic inactivating mutations affecting PTEN and TSC2 genes have been reported in nearly 10 % of PNETS [8–10]. Reduced PTEN expression may also be ascribed to the miR-21 overexpression, a noncoding microRNA regulating protein expression on a post-transcriptional level [11]. Oncogene mutations affecting mTOR pathway are rarely, if ever, observed [12, 13].
- A phase III clinical trial showing that the mTOR inhibitor everolimus gave a clinically meaningful benefit in treated patients.

Alterations of mTOR Pathway and Therapeutic Opportunities

The engagement of upstream RTKs by growth factors switches on PI3K signaling axis. **PI3K** is then recruited to plasma membrane-anchored receptors and activated; its activation status leads to phosphorylation of PIP2 to PIP3. **Akt**, through its pleckstrin homology (PH) domain, binds PIP3 activating **mTOR**, as part of the mTORC1 complex, by suppressing the suppressor **TSC 1/2 complex**. The two best-established substrates of mTORC1, S6K1 and 4EBP1, control various aspects of translation. **p-S6K1** leads to activation of eIF3 translation complex; substrates of p-S6K1 includes other translation-related proteins such as S6, eIFB4, eEF2K, PDCD4, CBP80, and SKAR. By contrast, phosphorylation of **4EBP1** by activated mTORC1 leads to a "loss of function" of its translation repressor physiological activity; 4EBP1 phosphorylation-mediated dissociation from eIF4E allows eIF4G and eIF4A to assemble with eIF4E, a complex known as eIF4F, and to initiate translation. PI3K/Akt/mTOR pathway is regulated by main proteins. PTEN seems to be one of the main negative regulators of this pathway with its phosphatase activity on both protein and lipid substrates. In particular, it antagonizes PI3K, taking a phosphate away from PIP3, thereby partially switching-off Akt activity [1].

PI3K

Jiao et al. [12] by sequencing the exome of nearly 18,000 protein-coding genes in a set of ten PNETs and with the validation in 58 additional ones found mutations along mTOR pathway in nearly 15 % of the tumors. **Mutations** in PI3KCA (p110α) was identified in 1.4 % of PNETs (1/68). This percentage faces with higher ones described in other histotypes (breast 27 %, endometrial 24 %, colon 15 %, etc.) [14, 15]. No p85α mutations are to date described in NETs contrary to other histotypes (8 % glioblastoma, 8 % colon cancer, 17 % pancreatic cancer, 2 % breast cancer). **PI3K amplification** was detected in 53 % of lung squamous cell carcinomas, 69 % of cervical tumors, and 32 % of head and neck squamous cell carcinomas. To date, no data relative to PI3K amplification are available in NETs.

Preclinical studies in NETs with first-generation **PI3K inhibitors** outlined the evidence that PI3K signaling plays a role in in vitro neuroendocrine cell growth. *LY294002* alone, a morpholine derivative of quercetin and a potent PI3K inhibitor, reduced tumor cell proliferation both in lung (NCI-H727) and in GI (BON) neuroendocrine tumor cell lines, together with a consensual decrease in pAkt levels [16]. *LY294002* treatment of murine endocrine cell lines synergize with rapamycin in inhibiting cell growth [17]. In other neuroendocrine tumor cell lines (BON, GOT-1, and NCI-H727), *BEZ235*, a dual PI3K and mTOR inhibitor, is similarly able to limit the triggering of MAPK cascade [18]. These data are in agreement

with the evidence that MAPK pathway activation occurs during mTOR inhibition through a PI3K-mediated feedback loop [19].

Neither clinical experience has so far been reported with pure **PI3K** inhibitors nor with dual PI3K/mTOR inhibitors in NETs.

Akt

Analysis of gene copy number shows the relation between **amplification** of Akt family members and cancer. Akt2 amplifications in particular were reported in 14, 20, and 30 % of ovarian, pancreas, and head–neck cancers, respectively [20, 21]. Akt1 gene amplification was detected in a single gastric carcinoma out of a series of more than 200 human malignancies [22]. No literature data are to date available concerning Akt amplification in NETs. A comprehensive screening of human malignancies for genetic **mutations** in the catalytic domain of nearly 240 Ser/Thr kinases did not reveal any mutations in Akt1, Akt2, and Akt3 exome sequences. A further analysis, instead, showed a unique mutation in the PHD of Akt1 (E17K) in 8, 6, and 2 % of breast, colorectal, and ovarian cancers, respectively [23, 24]. Genome-wide analysis of a set of ten PNETs did not reveal alteration in Akt-coding genes.

Activation of Akt is described in many human tumors; the phosphorylation rate of Akt ranges from 61–76 % in two different series including GEP-NETs [25]. Activated status was not in relation to grading, dimension, or stage of the disease.

Different kinds of **Akt inhibitors** have been described, and an increasing number of new molecules are under way. Among them: (a) *Phosphoinositides analogues* able to replace PIP3 at the Akt PH site, thereby preventing plasma membrane localization and phosphorylation of Akt; the perifosine belongs to this class of inhibitors, for which encouraging phase II data have been obtained in renal cell carcinoma, colorectal cancer, and multiple myeloma. Recently, the pan-Akt inhibitor, perifosine, shows very effective inhibitory activity on Akt phosphorylation and on NET tumor cells viability [26]. (b) *Substrate analogues* work as Akt inhibitors, but no clinical data are to date available with such inhibitors. (c) *ATP-competitive ligands* represent another class of new molecules. GDC-0068 is an highly selective pan-Akt inhibitor that paradoxically increases phosphorylation of Akt in cells while locking it in a nonfunctional state [27]. The preferential targeting of activated ATP-bound Akt by such an inhibitor can lead to an increase in the therapeutic index (i.e., drug more active against tumor cells with highly activated Akt rather than normal cells showing low Akt activity). An open-label phase Ib, dose-escalation study assessing safety, tolerability, and pharmacokinetics of GDC-0068 in combination with docetaxel or fluoropyrimidines in patients with advanced solid tumors is ongoing. (d) A small *pan-Akt inhibitor*, named triciribine, is able to inhibit the cell growth and increase apoptosis in human cancer cells that harbor constitutive activation of Akt due to overexpression of Akt or other genetic aberrations such as PTEN inactivation. In vitro experiences with triciribine on

NET cell lines (BON, CM, STC-1) showed that inhibition of Akt conferred a growth inhibitory effect together with a consensual reduction of pAkt levels in sensitive cell lines (STC-1 and CM). BON cells are resistant to *in vivo* effective doses of drug; lower basal level of pAkt and higher level of PTEN compared to sensitive cells are probably related with insensitivity to Akt inhibition [28]. (e) *Allosteric inhibitors* represent the last generation, isoenzyme-specific Akt inhibitors; the inhibitory properties result from a change in the shape of Akt active site after their binding to an allosteric Akt site. In NET cell lines, knockdown models blocking Akt isoforms 1 and 3 seemed to have the highest efficacy in lowering Akt phosphorylation and inhibiting cell tumor growth. According to these preclinical data, selective targeting of Akt-1 and/or Akt-3 in NETs seems to be a promising approach. In two carcinoid cell lines (i.e., pancreatic carcinoid BON and bronchopulmonary H727), the treatment with MK-2206, an allosteric inhibitor of Akt, was able to suppress AKT phosphorylation and significantly reduced cell proliferation in a dose-dependent manner. MK-2206 leads to an increase in the levels of cleaved PARP and cleaved caspase-3, with a concomitant reduction in the levels of Mcl-1 and XIAP, indicating that its antiproliferative effect probably occurs through the induction of apoptosis [29].

A first in human **clinical trial** with an allosteric Akt inhibitor (MK-2206), including, among other histotypes, three NETs, has been recently published. Two of these NETs bearing patients achieved tumor shrinkage of −13 and −17 % and both remained on trial for 32 weeks. Ras mutations and PTEN loss were described among partial responding patients with other histotypes. Recently, a new trial has just started with MK-2206 in PNET [30].

mTOR

In NETs, there is evidence that **mutations** and other genetic alterations can affect PI3K/Akt/mTOR pathway (i.e., PTEN and TSC2 loss/mutations, PI3KCA mutations) [12, 31].

Despite the importance of mTOR activation in human cancer, activating **mutations** in its coding gene were only recently reported. By mining cancer genome database, Sato et al. [32] identified ten mutations in the mTOR gene from 750 cancer samples. Among them, two different mutations (S2215Y and R2505P in colon and kidney cancers, respectively) are able to confer growth factors-independent mTORC1 activation. These mutations have not yet been reported to have a transforming activity, besides the "promoting" one, remains unclear [31, 32]. No data are now available in NETs with regard to mTOR genetic defects.

Phosphorylation status of "nodal" proteins, having many putative specific phosphorylation sites, cannot be investigated with an antibody specific to only one of them. mTOR in particular possesses four known phosphorylation sites (i.e., Ser^{2448}, Ser^{2481}, Thr^{2446}, and Ser^{1261}), each one having a cognate "phosphorylator" and a different biological significance. Phospho-mTOR (pmTOR) for example was

analyzed by Righi et al. [33] in a series of 218 surgically resected lung NETs using an antibody specific for Ser^{2448}, originally believed to be an "Akt-restricted" phosphorylation site but recently identified as "S6K1-cognate" one. In this series, mTOR **activation** was significantly higher in low-to-intermediate grade tumors as compared to high-grade ones, although no correlation with survival was showed. mTOR and pmTOR expressions were also detected, respectively, in 70 and 61 % of PNETs in a series of 34 patients described by Zhou et al. [34]. In a series reported by Kasajima et al. [35], mTOR positivity was also detected in 67 % of gastric and pancreatic NETs compared to 16 % of duodenal NETs.

In a preclinical setting, the reduction in tumor cell viability after the treatment with **mTOR inhibitors** supports the hypothesis of an important biological role for mTOR in tumor cell biology. There are to date two different classes of mTOR inhibitors:

(a) *Rapamycin analogues*, allosteric inhibitors of mTORC1 which, by forming a complex with the intracellular receptor FKBP12, bind to mTOR and inhibit mTORC1 downstream signaling. They are partial mTORC1 inhibitors and cell-type-specific mTORC2 inhibitors. Sirolimus, temsirolimus, everolimus, and deforolimus are members of this family. Everolimus treatment leads to NET **cell growth inhibition** in different experimental settings; RAD001 inhibited BON (a human PNET cell line) and INS1 (a rat insulinoma cell line) proliferation in nanomolar ranges [36, 37]. In 24 primary cultures from bronchial carcinoids, a different sensitivity to RAD001 treatment was observed; more aggressive histopathological features (i.e., higher proliferation index and nodal metastatic status) and higher expression of the molecular targets (i.e., mTOR-specific mRNA amount and basal phosphorylated and total mTOR levels) predict response to mTOR inhibition. In another study, PI3KCA and/or PTEN genetic defects, higher basal pAkt, greater inhibition of pS6K, and greater increase in pAkt during the treatment were hallmarks of mTOR inhibition [38].

(b) *Small molecules* mTOR kinase inhibitors. They can act only on mTOR, since they are ATP-competitive inhibitors (i.e., AZD8055 and WYE-354) or mTOR kinase inhibitors (i.e., PP30, PP242, and torin1), or they can be dual PI3K and mTOR inhibitors (i.e., primarily BEZ235 and XL765). As described below and in contrast to FHIT- or VHL-deficient kidney cancers or PTEN-deficient glioblastomas, everolimus has to date a limited clinical activity once tested in clinical trials in the absence of molecular and genetic stratification. This could be related to the inability to prevent mTORC2-mediated activation of Akt. The dual mTORC1/mTORC2 inhibitor CC-223 has recently showed ability to address mTORC2-mediated escape mechanisms; a phase I evaluation in advanced solid and hematologic cancers is ongoing. Also, the dual mTOR/PI3K inhibitor NVP-BEZ235 has proved to be more effective than single inhibitors in limiting NET cell lines growth [39].

In the clinical setting, **mTOR** inhibition led to encouraging results in an otherwise daunting scenario. In the first study of the "RADIANT saga" (RADIANT-1), everolimus was given alone or in combination with octreotide LAR if such a treatment was ongoing at baseline. Primary endpoint was response rate in the largest

stratum of everolimus monotherapy ($n = 115$ patients). A RR of 9.6 % was observed in the everolimus "stratum" as against 4.4 % in the everolimus + octreotide one. PFS in the stratum of SSA and everolimus is longer than the one of everolimus alone (PFS 16.7 vs. 9.7 months) [40]. In RADIANT-2 phase III study, the role of everolimus in association with octreotide LAR in patients with low-to-intermediate grade NETs was explored versus placebo. Median progression-free survival by central review was 16.4 months in the everolimus plus octreotide LAR group and 11.3 months in the placebo group [41]. RADIANT-3 study further explored the role of everolimus in the management of advanced PNETs randomizing patients versus placebo; pretreatment with chemotherapy was a stratification criteria and SSA treatment was allowed. The trial design allowed also the crossover at PD. A total of 5 % of patients had PR according to RECIST criteria in the everolimus arm, but a total of 64 % of patients receiving the drug experienced some degree of tumor shrinkage as compared to 21 % in the placebo arm. In addition to this, everolimus reduced tumor proliferation as shown by lowered Ki67 values on paired re-biopsies. But the most striking benefit following the treatment with everolimus is the lengthening of time to disease progression; central review PFS was 11.4 and 5.4 months for the everolimus and placebo arm, respectively, resulting in a reduction of the risk of progression for the experimental arm of nearly 65 %. No subgroup was disadvantaged; neither chemo-pretreated patients nor tumors with a moderately grade of differentiation [42].

TSC2 and PTEN

PTEN and the TSC complex are the major upstream-negative regulators of PI3K-dependent mTORC1 activation. A recent expression profiling of PNETs leads to evidences for a frequent activation of mTOR pathway in primitive disease and the alteration of TSC2 and PTEN protein expression in the vast majority of cases [2]. These observations were confirmed by the finding of mutations in TSC2 or PTEN in about 16 % of cases [12]. Interestingly, altered expression of either TSC2 or PTEN was found in tumors showing an aggressive clinical behavior. The authors commented that the deficiency of one of those genes could help in overcoming the impairment of mTOR activity due to the hypoxic condition in which these aggressive tumors growth. The presence of multiple alterations along the pathway may help to bypass this negative feedback, as suggested by the fact that tumors bearing reduced expressions of both PTEN and TSC2 are those that developed metastases and showed progression of disease. Furthermore, the results of a global microRNA expression analysis revealed overexpression of miR-21, which has PTEN among its targets, in NETs showing the highest proliferation indexes [11, 43].

The development of a molecularly target agent should be sustained by the identification of biomarkers predictive of efficacy to adequately select those patients more likely to benefit from the treatment and thereby optimizing the therapeutic index.

In this setting, the activation status and the molecular alterations of PI3K members (as well as those of downstream effectors or of molecules belonging to parallel and interacting pathways) have been evaluated both on cell lines and *in vivo* with sometimes discrepant results.

- **pAKT** predicts sensitivity to molecular inhibitors both in JFCR39 (a panel of 39 well-characterized cell lines) analyzed in silico and in other in vitro and *in vivo* models [44]. Moreover, pAKT levels positively correlated with sensitivity to everolimus in treated patients, both baseline and during drug administration. In the latter case, there was an evidence of compensatory activation of Akt as a consequence of mTOR inhibition [38].
- Predictive role of **PI3KCA** mutation and **PTEN** loss on breast [45] and neuroendocrine cell lines [38] was not confirmed in other settings [44].
- **KRAS** and **BRAF** mutations showed a negative predictive role for PI3K pathway inhibitors [44]. A single nucleotide polymorphism on the **FGFR** was found to have a negative prognostic and predictive role both in PNETs in preclinical models and patients [46].
- **c-MYC** and **4EIF** amplification were detected in human cells becoming resistant to BEZ235, a dual PI3KCA and mTOR inhibitor [47]. The role of c-MYC (and NOTCH) in PI3K inhibitors resistance was also confirmed in an analysis of breast cancer cell lines [48].

These fragmented evidences, derived from heterogenous preclinical models, are still too immature and limited to draw significant conclusions and to provide for a rationale to design clinical trials on molecularly selected patients.

mTOR-Interacting Pathways and Therapeutic Opportunities

mTOR pathway is part of a complex network. Thousands of molecular interplays occur: synergistic, additive, or (partially) redundant effects of the above-mentioned alterations, associated with positive or negative feedback loops, outline cancer real landscape. Nevertheless, most studies have focused on singular PI3K members and analyzed this signaling pathway as a vertical, one way, straightforward axis. NETs do not represent an exception. This approach does not mirror cancer cell biology and may have been responsible of the so far limited (and sometimes discouraging) results of target therapies in "PI3K-addicted" tumors, either in preclinical or, unavoidably, in clinical setting. In fact, each molecule and each pathway (PI3K included) are part of the complex and dynamic cancer signaling network. The understanding of the interactions between the different signaling intracellular processes is crucial to develop more effective therapeutic strategies.

Examples of such complex interactions in NETs are the following:

- **Cell proliferation-related pathways**

mTOR is a crucial crossroad on which both extracellular and intracellular stimuli induced by hypoxia, growth factors, oxidative stress, amino acid depletion converge to trigger adaptive reactions. One of the outputs deriving from these complex interactions is the regulation of cell proliferation.

Growth factor receptors are involved in mTOR pathway regulation both as activating factors and as pivotal players of complex feedback loops. Activated molecules along PI3K/Akt/mTOR pathway often act as negative regulators of upstream molecules. This is the case of receptor tyrosine kinases whose transducing activity and even levels of expression are lowered once downstream signaling is elevated. That is not true once oncogenic hits, such as PTEN loss or PI3KCA mutation/amplification, are probably refractory to these negative regulations.

Activation of Akt by any of several mechanisms (loss of PTEN, activation of PI3K or Akt) also inhibits the expression of PDGF and IGFR receptors [49]. In a similar way, once mTOR is activated, it phosphorylates S6K1, which may be able to negatively modulate RTKs transcription, thus preventing further IGF1-/other growth factors-mediated signal transduction through this pathway [50, 51].

A cross talk between PI3K and **Raf/MAPK pathways** has been demonstrated. In cancers bearing mutant, RTKs or oncogenes able to activate both the above-mentioned pathways, blocking mTORC1 leads to a feedback increase in activity of RTK/IRS1/PI3K pathway and a "shunt-effect" toward Ras/MAPK one, which in turn becomes able to drive tumor growth by itself [19, 52]. In NET cell lines, the treatment with rapalogs leads, through suppression of the pS6K-IRS-mediated negative feedback loop, to a global upregulation of upstream RTK/PI3K/Akt pathway and therefore to cross-activation of Ras/Raf/Erk signaling; an upregulation of VEGF secretion, through both a raise of NFkB-mediated VEGF transcriptional levels and HIF-α induction, has also been observed [18, 19, 53]. Therefore, the increase in pAkt levels, besides being an "early" marker of mTOR inhibition sensitivity as stated above, is also a pathogenetic step in mTOR-resistance development as observed in other tumor models. Synergistic antitumor effects were observed combining RAD001 and MEK inhibitors [26, 54]. Pharmacologic inhibition of PI3K, together with mTOR inhibition, prevents pERK increase.

A backflow is also outlined from piecemeal evidences: Ras can directly bind to and activate PI3K [55]; active ERK/RSK can phosphorylate and dissociate TSC1/TSC2 complex, thereby activating mTORC1 [56]; Raf inhibition leads to an increase in pAkt levels [26].

The combination between MEK inhibitors and PI3K-mTOR pathway inhibitors depicts one of the most interesting areas of the contemporary **clinical scenario**. The combination of GDC-0973 (MEK inhibitor) and GDC-0941 (PI3K inhibitor) was evaluated in 78 patients with advanced solid malignancies. Partial responses were observed in three patients who have BRAF- or KRAS-mutant tumors. The combination of trametinib (MEK inhibitor) and BKM120 was evaluated in 49 patients with advanced RAS- or BRAF-mutant tumors; partial responses were observed in three patients. To date, no clinical experience is available in NET tumors.

- **Angiogenesis-related pathways**

The real role of angiogenesis in "well-differentiated" NETs is not yet fully elucidated in a context in which a rich vascularization in NET mirrors the physiology of healthy tissue/organ counterpart. Similarly, VEGF expression in NETs may correspond to the persistence of normal functional parameters of neuroendocrine cells, which are physiologically committed to produce a finely regulated amount of VEGF. Low-grade NETs have the capacity to synthesize, store, and secrete VEGF, which is inconstant and heterogeneous in high-grade NETs. The so-called **neuroendocrine paradox** is also reflected in the fact that, in NETs, the density of the vascular network is a marker of differentiation rather than of aggressiveness: The most vascularized tumors are less aggressive, the more differentiated are the less angiogenic. Therefore, the rich and mainly mature vascularization represents one of the hallmarks of NETs. Moreover, in human NETs, the solid tumor with mature co-opted vascularization represents the majority of the disease burden, while synchronous angiogenic islets represent only a small compartment in the "druggable" sprouting angiogenesis. In conclusion, the boundary between innate resistance to antiangiogenic treatments and early development of acquired resistance, simply due to the removal of a small drug-sensitive subpopulation, is subtle although relevant in treatment planning.

mTOR is able to integrate signals regulating cellular energy and nutrient status, thus establishing a close relationship also with angiogenesis. In fact, under hypoxia mTORC1 activity is downmodulated through different mechanisms including activation of AMP-activated protein kinase (AMPK) and of mTOR-suppressing TSC1–TSC2 complex through some HIF-target genes. Another process of mTOR inhibition under hypoxia is mTOR accumulation in the nucleus, via promyelocytic leukemia gene (PML), which prevents its activating interaction with the small cytoplasmic GTPase Rheb.

The resulting mTOR inhibition leads to the expression of proteins able to face hypoxic situations (i.e., HIF-1a and VEGF-A) [57]. **HIF-1** and other proteins involved in cellular response to hypoxia in fact require the selective translation of specific mRNA despite global inhibition of translation.

Hypoxia lacks its efficacy in mediating mTOR suppression once other negative regulators are lost and overall during malignant transformation. This is the case of PML and TSC. In TSC *null* cells, HIF-1 accumulates at higher levels compared with wild-type cells under conditions of hypoxia, and this can be prevented by the treatment with rapamycin. Similarly, PML, a tumor suppressor gene known to be involved in cellular senescence and apoptosis, has also a critical role in neoangiogenesis inhibition. In hypoxic conditions, PML *null* cells synthesize higher HIF-1a compared to wild-type counterpart and this effect is abolished by rapamycin [58].

These evidences represent an apparent paradox remembering that mTOR activity inhibits translation of genes such as HIF-1 and VEGF. Anyway inhibition of mTOR activity is able to inhibit HIF translation and tumor growth in many preclinical models [5, 6]. Similar observations were reported also for HIF-1a-regulated genes such as the one coding for **VEGF** [56].

But if mTOR downregulation during hypoxia leads to an increase in pro-angiogenic HIF-1 levels, why do we observe an antiangiogenic effect using mTOR inhibitors during malignant transformation? The expression of hypoxia-modulated genes could vary between normal and tumor cells and could be further modulated by different microenvironmental conditions. But a better understanding of the pathways involved and how they are interconnected is required in order to optimize type and schedule of the treatment; acceleration of metastasis observed in a preclinical model of short-term mTOR suppression deserves further investigations. Sustained suppression of mTOR pathway may in fact lead to a rebound in tumor growth similarly to what observed during VEGFR/PDGFR inhibition [59]. A recent survey on the patterns of failure of PNETs patients treated with everolimus did not show significant differences in comparison with the ones in the placebo arm. The fraction of progression events due to new metastases only, growth of preexisting lesions and new metastases together with growth of preexisting ones were in fact similar [60]. Knowledge of the exact balance between different mTOR regulating and modulated processes is mandatory in order to optimize therapeutic interventions in humans. Because of potential synergy between VEGF pathway and mTOR inhibitors a **clinical phase** I trial recently evaluated the combination between sorafenib and everolimus in NETs. Despite toxicity concerns that will probably preclude widespread clinical use of this combination, tumor shrinkage was observed in nearly 60 % of patients [61].

- "**Death-related" pathways**

Cell death can occur because of several mechanisms and the phenotypic changes accompanying cell death can vary depending on the stimulus and cell setting.

Apoptosis is the first, although not the only one, genetically programmed death process identified.

The PI3K/Akt/mTOR pathway integrates survival signals provided by extracellular and intracellular stimuli mediating pro-survival signals. Among its various functions, Akt inhibits apoptosis either directly by phosphorylating apoptosis-signaling molecules or indirectly by modulating the activity of transcription factors. Recent evidences showed that also PI3K is implicated in the apoptotic process. Pharmacological inhibition of PI3K restored TRAIL sensitivity in numerous cancers [62]. NET cell lines of heterogeneous origin exhibit a range of TRAIL sensitivities and that TRAIL sensitivity correlates with the expression of FLIPS, caspase-8, and Bcl-2. In the NET cell lines tested, neither single mTOR inhibition by everolimus nor dual mTOR/PI3K inhibition by NVP-BEZ235 was able to enhance TRAIL susceptibility in any of the tested cell lines [63].

More recently **autophagy**, a process in which de novo-formed membrane-enclosed vesicles engulf and deplete cellular components, has been shown to engage in a complex interplay with apoptosis. In some cellular settings, it can serve as a cell survival pathway, while in others it can lead to death either in collaboration with apoptosis or as a backup mechanism when the former is defective. This cross talk is not straightforward and sometimes contradictory.

Autophagy in fact does not always lead to cell death, but in some cellular contexts it is able to attenuate apoptosis by creating a cellular milieu in which survival is favored.

mTOR negatively regulates autophagy by phosphorylating and inactivating Ulk1, a serine/threonine kinase that acts at the initiation step of autophagy. There is increasing evidence that PI3K/Akt/mTOR inhibitors initiate autophagy as a survival program that may interfere with their antitumor activity. Consequently, inhibition of autophagy was used as a strategy to enhance the efficacy of PI3K/Akt/mTOR inhibitors in different cancers [64]. In this context, BEZ235 stimulates the enlargement of the lysosomal compartment and generation of reactive oxygen species (ROS), both related to a stimulation of autophagy, while chloroquine promotes lysosomal membrane permeabilization (LMP). So in combination, BEZ235 and chloroquine cooperate to trigger LMP, Bax activation, loss of mitochondrial membrane potential (MMP) and caspase-dependent apoptosis. Lysosome-mediated apoptosis occurs in a ROS-dependent manner, as ROS scavengers significantly reduce BEZ235-/CQ-induced loss of MMP, LMP, and apoptosis [65].

For the above-listed explanations, mTOR pathway, in particular in NETs, represents a cornerstone in the complex cellular regulation mechanisms; due to such a key role, it embodies a highly important therapeutic target.

Box 1. Components of PI3K/Akt/mTOR Pathway [1]

- PI3K

PI3Ks are a family of **lipid kinases** that share the ability to phosphorylate the 3-hydroxyl group of phosphoinositides. To date, **three classes of PI3Ks** are known with different structure and substrate. *Class I PI3Ks* are heterodimeric proteins with a catalytic and a regulatory isoform. Catalytic subunits are expressed by separate genes coding for the cognate proteins (PI3KCα, PI3KCβ, and PI3KCD). PI3KCA is the only catalytic subunit gene found to be mutated in cancers; mutations often cause gain in kinase activity. *Class II PI3Ks* consists of a single catalytic subunit presenting three different isoforms (PI3KC2α, PI3KC2β, and PI3KC2γ). Accumulating evidence suggests that the class II isoform PI3KC2β may play a role in cancer development. *Class III PI3Ks* similarly consists of a single catalytic subunit. They probably have a role in regulating cell growth.

- Akt

Akt is a serine/threonine protein kinase that tunes a plethora of cellular functions, including glucose metabolism, cell proliferation, and migration. Three **family members** are known so far: *Akt1* involved in cellular survival

pathways ranging from regulating apoptotic processes to protein synthesis; *Akt2* an important signaling molecule in the insulin signaling pathway. *Akt3* has to date no clear role. Both PDK1 and mTORC2 cooperatively act in plasma membrane recruitment and activation of Akt. Upon membrane translocation and subsequent phosphorylation, Akt changes its conformation and becomes a catalytically competent kinase. More than 100 substrates are to date identified; one of them, TSC2, is phosphorylated and thus inhibited, allowing downstream RHEB to activate mTORC1. Negative regulation of Akt activity is primarily mediated by PTEN which acts de-phosphorylating Akt.

- mTOR

mTOR forms the catalytic core of at least two functional complexes TOR complex 1 (mTORC1) and TOR complex 2 (mTORC2). **mTORC1** senses and integrates different intra- and extracellular inputs to promote cellular anabolic processes. It is primarily composed of mTOR catalytic subunit, raptor (regulatory-associated protein of mTOR), and PRAS40. Raptor functions as a scaffolding protein able to bind directly to TOR signaling motifs (TOS) on downstream targets (i.e., S6K1 and 4EBP1); PRAS40, once phosphorylated by mTOR or by Akt, has a likely negative regulatory function on mTOR itself. The best-characterized downstream targets of mTORC1 are S6K1 and 4EBP1, which are members of AGC family kinases and both of which control unique aspects of translation. S6K1 and 4EBP1 act as translation enhancer and repressor, respectively. **mTORC2** is the second mTOR complex, which consists of mTOR, rictor (rapamycin-insensitive companion of mTOR), Sin1, mLST8, and protor (protein associated with rictor). The activity of mTORC2 is mainly regulated by PI3K and, as opposite to mTORC1, is insensitive to nutrients or energy conditions. TSC complex also may promote mTORC2 signaling in contrast to its inhibitory effect on mTORC1. Similarly to mTORC1, also mTORC2 has, as main substrates, a different subgroup of AGC family kinases, including Akt, SGK1, and PKC. PKC, once phosphorylated, becomes able to activate PDK1, thereby producing a positive downstream signal on Akt pathway. Furthermore, mTORC2 directly phosphorylates Akt. Another substrate of mTORC2 is serum glucocorticoid-induced protein kinase 1 (SGK1), which exhibits overlapping substrate specificity with other AGC kinases, but it seems to carry out elective regulation of channels, carriers, and Na(+)/K(+)-ATPase, enzymes as well as several transcription factors.

Regulation of mTORC1 activity is especially complex counting both growth factors- and an energy/nutrient/stress-sensing arm. Growth factors mediate signals through both PI3K- and MAPK-dependent pathways. TSC1/2 complex represents a regulatory node because both MAPK and AKT are able to phosphorylate it, through PI3K-independent and PI3K-dependent

pathway, respectively, suppressing its function in response to the different growth factor-related milieu. By contrast, elevation of intracellular AMP/ATP ratio together with positive feedback loop mediated by LKB1 activate AMPK, which acts as master regulator in cellular energy metabolism; AMPK then phosphorylates TSC2 on a different site and activates it, thereby suppressing mTORC1 signaling. Feedback loops and cross talk between pathways further complicate the understanding of mTORC regulation. When mTOR is activated, it phosphorylates S6K1 which in turn induces a negative ***feedback loop*** uncoupling insulin receptor substrate-1 (IRS-1) from PI3K, thus preventing further signal transduction through this pathway. S6K1 is also able to phosphorylate rictor of the mTOR complex 2 (mTORC2), so preventing mTORC2-mediated activating phosphorylation of Akt and thereby lowering PI3K-driven signaling.

Box 2. RIP-Tag2 Mouse Model in NET Translational Preclinical Studies

In the RIP-Tag2 mouse model in which pancreatic neuroendocrine tumorigenesis is driven by Rb and p53 SV40-mediated "silencing," different phases of the neuroendocrine disease follow one another, from development of hyperplastic islets (mice of 3–4 week of age), through angiogenic islets to solid tumors, which moreover represent only a very small quote of the initial hyper plastic islets. In this context, once external (i.e., pharmacological) perturbations occur many adaptive features appear.

VEGF/VEGFR inhibition:

Mechanisms underlying adaptive behavior of NETs in response to pharmacologic drug perturbation are still lacking in preclinical models "other-than-RIP-Tag2" and even more in clinical setting. In VEGF-A gene-specific knockout RIP1-Tag2 mice, both angiogenic switching and pancreatic neuroendocrine tumor growth were severely disrupted [66]. Although the role of VEGF-B is not fully understood and although high expression level of VEGF-B is detected in many types of tumors, unexpectedly in RIP1-Tag2 mice the transgenic expression of VEGF-B leads to a reduced growth of the naturally occurring PNET. 12-week-old RIP-Tag2 mice treated for 4 weeks with anti-VEGFR2 antibody (DC101) showed, after an initial phase of tumor burden and vessel density reduction, a re-growth phase leading to aberrant vessel density re-establishment and expression of pro-angiogenic factors. In this model, the authors observed a clear trend toward an increased invasiveness and metastasization of experimental tumors during antiangiogenic

monotherapies. All these data apparently challenge the predictivity of this model with regard to the recent registrative phase III study of sunitinib in PNETs [67]. Many intersections between VEGFR axis and signaling pathways from other RTKs are to date described and probably cooperate in conferring resistant phenotype to single-agent treatment approach.

EGFR inhibition:

EGFR mRNA increases significantly during RT2 PNET malignant progression together with concomitant activation of PI3K pathway. EGFR-specific TKIs decrease in tumor burden both in intervention and in regression trials (treating mice from 11–14 and 12–16 weeks of age, respectively). mTOR and EGFR dual inhibition in RIP-Tag2 mice is more effective than single-agent treatment in reducing tumor growth, and most notably the reactivation of mTOR pathway observed in adaptive resistance to rapalog treatment was obviated by combination treatment.

Multi-target inhibition:

- In 12-week-old RIP-Tag2 mice, 4 weeks treatment with anti-VEGFR2 antibody led to an hypoxia-driven change in the repertoire of pro-angiogenic molecules, such as FGF, and adding an FGF-trap treatment to anti-VEGFR2 approach or upfront use of brivanib (dual FGF/VEGF inhibitor) allowed a significant delay in tumor re-growth [68, 69].
- In 12-week-old RIP-Tag2 mice, 4 weeks treatment with anti-VEGFR2 antibody led to an increase in co-opted α-SMA+ pericytes inside re-growing tumors, which co-stained with PDFGR-α; concomitant targeting of PDGFR and VEGFR could probably be useful in preventing anti-VEGFR2 resistance [70].
- In 12-week-old RIP-Tag2 mice, 4 weeks treatment with anti-VEGFR2 antibody led to increased tumor hypoxia, hypoxia-inducible factor-1α, and c-Met activation. Upfront treatment with XL880 or XL184 reduced by an 80 % tumor vasculature, delayed tumor regrow after withdrawal of drugs, reduced pericytes and basement membrane sleeves that probably provide a scaffold for re-growing blood vessels.

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Chapter 3
Relevance of Angiogenesis in Neuroendocrine Tumors

Alexandre Teulé, Laura Martín and Oriol Casanovas

Abstract While traditional cytotoxic drugs have shown limited efficacy in neuroendocrine tumors (NETs), their biological features have been characterized and can be exploited therapeutically. Their most prominent trait is an extraordinary vascularization in low-grade NETs and a hypoxia-dependent angiogenesis in high-grade NETs, which is associated with a significant expression of many pro-angiogenic molecules. Therefore, several antiangiogenic compounds have been tested in these malignancies, and among these, sunitinib has demonstrated activity in pancreatic NET patients by dually targeting the vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) pathways. In spite of these efficacious clinical results, apparent resistance to antiangiogenic therapies has been described in NET animal models and in clinical trials. Therefore, overcoming antiangiogenic resistance is a crucial step in the subsequent development of antiangiogenic therapies. Several strategies have been postulated to fight resistance, but preclinical studies and clinical trials will investigate and address these therapeutic approaches in the coming years in order to overcome resistance of antiangiogenic therapies in NETs.

Keywords Neuroendocrine tumor · Angiogenesis · Sunitinib · Molecular target

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Introduction

Neuroendocrine tumors (NETs) are rare malignancies, but their incidence and prevalence has increased in the last decades [1]. This type of tumors comprises a heterogeneous family with a wide and complex spectrum of clinical behavior. The limited effectiveness of traditional DNA-damaging agents has led to the exploration of new targeted drugs based on the molecular features of these tumors, in order to improve their systemic treatment.

NETs have a number of biological features that can be exploited therapeutically, such as an extraordinary tumor vascularization with high expression of several pro-angiogenic molecules, being vascular endothelial growth factor (VEGF) the major mediator of tumor angiogenesis due to its properties of potent endothelial cell mitogen and vascular permeability-inducing agent. The presence of VEGF may be required to maintain the differentiated state of capillary vessels in the hypervascular tumors [2, 3]. Indeed, most NETs are hypervascular, as it is characteristic of normal endocrine glands, which have a dense vascular network that facilitates hormone secretion and dumping to the bloodstream. Specifically, NETs show a microvascular density ranging from 10- to 20-fold higher than in typical carcinomas. However, many studies have shown that in pancreatic NETs, microvascular density is higher in benign, low-grade tumors than in malignant, high-grade tumors [4]. Furthermore, these studies have demonstrated that intratumoral vessel density is associated with a good prognosis and prolonged survival [5], which is completely the opposite of other digestive epithelial tumors and most carcinomas in general. Thus, an intriguing characteristic of NETs is their physiologically derived high vessel density in low-grade tumors that is diminished over tumor progression and aggressiveness. Nevertheless, high-grade NETs typically show hypoxic areas and upregulation of hypoxia-response transcription factors (HIFs) and genes of cellular response to hypoxia (carbonic anhydrase IX, glucose transporters, etc.) [6]. The upregulation of HIFs involves the induction of various pro-angiogenic factors as hypoxia is a mechanism that induces angiogenic responses. Thus, while vessel density is lower in high-grade NETs, they show a very robust pro-angiogenic response that is clearly observed by increased endothelial proliferation and vascular overgrowth.

The high vascularization of NETs has its molecular base on the specific repertoire of secreted molecules from neuroendocrine cells. Indeed, neuroendocrine cells physiologically express a high level of pro-angiogenic molecules, particularly in the pancreas, but also in peptidergic endocrine cells, which constitutively synthesize several members of the VEGF family [7]. Consistently, NETs also typically express a variety of pro-angiogenic cytokines and growth factors, including vascular endothelial growth factors (VEGF-A, VEGF-C), fibroblast growth factors (FGFs), ephrins, or angiopoietins, among others. For example, neuroendocrine tumors (NETs) and their derived cell lines demonstrate a high capacity to synthesize and secrete high levels of several VEGF family members [2].

Overall, NETs not only show a dense vascular structure, but also have an angiogenic capacity that is characteristic of vessel-dependent tumors and thus evidencing a strong rationale for the use of antiangiogenic therapies in this type of malignancies. Therefore, several antiangiogenic compounds are currently undergoing clinical evaluation in NETs, either as monotherapy or in combination with chemotherapy or other targeted drugs. We will mention the biology of each of these mechanisms of angiogenesis and discuss the clinical data that are available to date.

Early Days, "Early" Antiangiogenic Drugs

Experimental evidence of the sensitivity of NETs to antiangiogenic drugs is based on preclinical studies in animal models, where promising results were described in the mid- and late 1990s with strikingly efficacious effects ranging from tumor stabilization to tumor regression depending on the model used. In particular, several antiangiogenic drugs have been evaluated in a transgenic mouse model of insulinoma, the RIP-Tag2, developed by Douglas Hanahan [8]. In this transgenic mouse model, an angiogenic switch occurs in premalignant lesions followed by a persistent angiogenesis during progression to expansive solid tumors and invasive carcinomas. For this reason, different angiogenesis inhibitors, such as the naturally occurring antiangiogenic molecules angiostatin and endostatin, were tested at distinct stages of disease progression. The different antiangiogenic treatments have proved to prevent the antiangiogenic switch in premalignant lesions, intervene in the rapid expansion of small tumors, or induce the regression of large end-stage cancers. Thus, antiangiogenic drugs may prove most efficacious when they are targeted to specific stages of cancer [9]. Early studies with the aminopeptidase inhibitor TNP-470, minocycline, and interferon-α/β demonstrated an antiangiogenic effect together with an effective tumor growth impairment [10].

These preclinical results are associated with the clinical use of thalidomide in NETs. Thalidomide is an orally bioavailable immunomodulatory drug with antiangiogenic properties due to its capacity to inhibit tumor necrosis factor-alpha (TNF-α) production and also VEGF and basic fibroblast growth factor (bFGF) pathways. The first small clinical study ($n = 18$) in NETs with thalidomide in monotherapy did not show objective responses [11]. Nevertheless, the combination of thalidomide and temozolomide was evaluated in another phase II study with a radiological response rate of 45 % in pancreatic NETs and 7 % in carcinoid tumors, respectively. However, a high incidence of grade 3–4 of lymphopenia was reported and 10 % of the patients had opportunistic infections [12].

The use of endostatin in the clinic has also demonstrated some benefit. *Endostatin* is a 20-kDa proteolytic fragment of collagen XVIII with antiangiogenic and antitumor activity in preclinical studies (Fig. 3.1). The antiangiogenic function of endostatin has been well documented during the past decade. However, the exact mechanism that endostatin executes its antiangiogenic functions remains elusive.

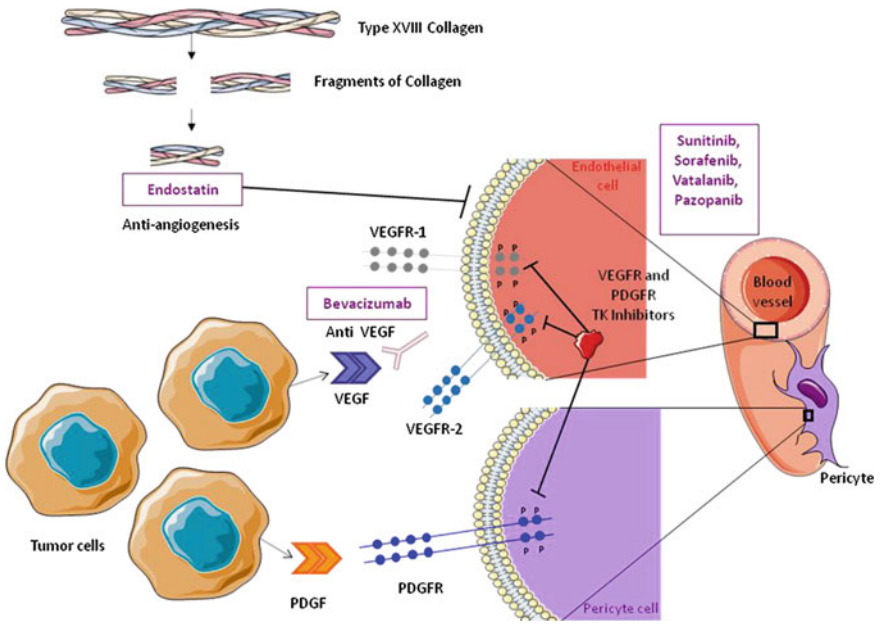

Fig. 3.1 Target pathways for antiangiogenic therapy in NETs. Image depicts the cellular and molecular components that drive angiogenesis in NETs (tumor cells, endothelial cells, pericytes, and extracellular matrix). Furthermore, in order to block the main pro-angiogenic pathways (VEGF/VEGFR and PDGF/PDGFR), different drugs such as endogenous inhibitors (endostatin), antibodies (bevacizumab) or small molecule inhibitors (sunitinib, sorafenib, vatalanib, and pazopanib) that can target vascular or perivascular cells have been developed

Both preclinical and human phase I studies of recombinant human endostatin (rhEndostatin) indicated activity in NETs. However, the phase II study performed in 40 patients with advanced NETs showed a high rate of stable disease (80 %) but did not result in significant tumor regression. The toxicity was minimal [13].

The VEGF/VEGFR Axis

The key mediator of angiogenesis is the VEGF, and VEGF signaling inhibition has been shown to result in significant tumor growth delay in a wide range of animal models [14]. The inhibition of VEGF signaling not only arrests endothelial cells (ECs) proliferation and prevents vessel growth, but also induces regression of existing vessels by increasing EC death. VEGF inhibitors also suppress the mobilization of endothelial progenitor cells (EPCs) from the bone marrow and improve cytotoxic drug delivery by normalizing the chaotic and abnormal architecture of tumor vessels and reducing vascular permeability. Consistently, several antiangiogenic therapies targeting the VEGF/VEGFR2/KDR signaling axis have

shown to be effective in mouse models of NETs. In particular, a monoclonal antibody that blocks VEGF-A ligand (AF-493-NA) and a blocking antibody of the VEGFR2 (DC101) has been tested in the RIP-Tag2 mouse model of insulinoma with consistent antiangiogenic effects in microvessel density, endothelial cell proliferation, and antitumor activity with increased apoptosis [3, 15]. Bevacizumab, a humanized monoclonal antibody that recognizes and blocks VEGF (Fig. 3.1), failed to inhibit growth NETs cells in vitro, but reduced their angiogenic potential by blocking the cells' ability to stimulate endothelial cell tube formation and proliferation and impaired tumor growth in animals [16].

Clinically, the activity of bevacizumab in NETs was tested in a randomized phase II study [17]. Forty-four patients on stable doses of octreotide were randomly assigned to 18 weeks of treatment with bevacizumab or PEG interferon alfa-2b. At disease progression (DP) or at the end of 18 weeks (whichever occurred earlier), patients received bevacizumab plus PEG interferon until progression. In the bevacizumab arm, four patients (18 %) achieved confirmed partial response (PR), 17 patients (77 %) had stable disease (SD), and one patient (5 %) had PD. No objective responses were observed in PEG interferon arm. Progression-free survival (PFS) rates after 18 weeks of monotherapy were 95 % in bevacizumab arm versus 68 % on the PEG interferon arm. Bevacizumab therapy also resulted in a significant reduction of tumor blood flow measured by functional CT scans.

A larger randomized phase III in patients with unresectable metastatic or locally advanced carcinoid tumors comparing depot octreotide acetate and interferon alfa-2b versus depot octreotide acetate and bevacizumab is being conducted since 2007 (SWOG S0518, clinicaltrials.gov NCT00569127). The results of this study are awaited in the near future.

Bevacizumab has also been tested in combination with cytotoxic drugs. Kulke et al. explored the efficacy and safety of the combination of bevacizumab plus temozolomide in a small phase II trial [18]. The combination showed an objective response rate of 24 % in pancreatic NETs but 0 % in carcinoid tumors. A phase II study of capecitabine, oxaliplatin, and bevacizumab for metastatic or unresectable NETs was reported in 2010 ASCO Annual Meeting. PR was observed in 7 pts (23 %), SD in 22 pts (71 %), and PD in 2 pts (6 %). Of the patients who achieved a PR, 6 had pancreatic NETs [19]. The combination with FOLFOX (oxaliplatin, leucovorin, and 5-fluorouracil) has also been tested with similar results [20]. Recently, a new phase II trial has been reported using the combination of bevacizumab with capecitabine in 49 patients with intestinal NETs. Nine (18.4 %) PR and 34 (69.4 %) SD were observed [21]. Another phase II trial testing bevacizumab plus traditional chemotherapy 5-FU/streptozotocin achieved an encouraging 55 % of PR with an acceptable toxicity profile [22]. Further phase III trials are warranted to establish the efficacy of adding bevacizumab to chemotherapy in NETs.

Other Vascular Players: PDGFR Axis and the Pericytes

Not only vascular cells are important for angiogenesis, but also the periendothelial support cells of the microvasculature or pericytes have shown to be relevant targets for effective antiangiogenesis. These cells mediate the stabilization of the vessels based on the synthesis of new basement membrane and tight association with endothelial cells; thus, endothelial cells can induce pericyte recruitment to protect themselves from death consequent to the lack of the crucial tumor-derived survival signals conveyed by VEGF [23]. Molecularly, a specific cross talk between endothelial cells and pericytes that implicates VEGF and PDGF is key for the vascular formation and maintenance and creates a crucial therapeutic opportunity that has been exploited [24]. For its supportive cooperative function aiding the endothelial cell stabilization and function, PDGFR inhibition has been developed in the context of dual inhibition of VEGFR and PDGFR [9]. Indeed, experimental studies with the RIP-Tag2 transgenic mouse model demonstrate a significant synergy when both endothelial cells and pericytes are dually blocked with VEGFR and PDGFR small molecule inhibitors such as sunitinib, which elicits detachment of pericytes and disruption of tumor vascularity in multiple stages in tumorigenesis, most notably in the often-intractable late-stage solid tumor [25, 26]. Although these positive results of the dual targeting of VEGFR and PDGFR, undesirable effects could emerge because a severe reduction or lack of pericyte coverage may disrupt the integrity of the vasculature, enabling tumor cells to transit into the circulatory system, thereby facilitating metastasis [23, 27].

On the clinical side, PDGFRs have been characterized in human pancreatic NET samples. PDGFR-α and PDGFR-β are commonly expressed both on tumor cells and tumor stroma [28]. The clinical approach to dually inhibit both VEGFR and PDGFR in NETs has been developed using several small molecule compounds such as sunitinib, sorafenib, vatalanib, and pazopanib (Fig. 3.1).

Sunitinib is the only antiangiogenic drug tested in a randomized phase III placebo-controlled trial [29] in patients with progressive well-differentiated pancreatic NETs, which is statistically positive in progression-free survival (11.4 months in sunitinib arm vs. 5.5 months in placebo arm). Sunitinib 37.5 mg/day was administered orally in a continuous schedule. The objective response rate was 9.3 % in the sunitinib group versus 0 % in the placebo group. This study was the first positive phase III trial with antiangiogenic drugs in the field and has changed the daily clinical practice in NETs. In a previous phase II study, 107 patients (41 carcinoid tumors and 66 pancreatic NETs) with documented disease progression were treated with repeated six-week cycles of sunitinib 50 mg/day, four weeks on and two weeks off. The overall objective response rate was 16.7 % in pancreatic NETs and 2.4 % in carcinoid tumors [30].

Sorafenib is an orally active, multikinase inhibitor with selectivity for the VEGFR-2, VEGFR-3, PDGFR-β, FLT3, c-kit, RET and RAF kinases. Sorafenib monotherapy has been evaluated in a phase II trial in 93 patients with NETs. The overall response rate was 10 % in both pancreatic and carcinoid NETs [31].

Vatalanib inhibits all known VEGFRs, with particular selectivity for VEGFR-2. At higher concentrations, vatalanib also inhibits PDGFR- β and c-kit. Two phase II studies were reported in 2008 in NETs, but both showed no significant radiological responses [32, 33]. Finally, pazopanib, another potent inhibitor of VEGFR, PDFGR-α/β, and c-kit, has been tested in 33 patients, most of them previously treated with mTOR inhibitors or other antiangiogénica drugs, with a 6 % of PR 79 % SD. This trial may introduce the concept of treatment sequencing with novel targeted agents in NETs [34]. Pazopanib has also been tested in combination with octreotide LAR in pancreatic NETs with 17 % of PR and a PFS of 11.7 months [35]. Axitinib, another potent inhibitor of VEGFR 1-3, PDFGR-β, and c-kit, is also been tested in this field.

Antiangiogenic Resistance

Clinical results using antiangiogenic drugs demonstrate only moderate gains in time to progression, and scarce benefits in overall survival, despite the long-term treatment. Why are there such modest and short-lasted benefits of antiangiogenic therapies in the clinic? The initial hypothesis was that antiangiogenesis therapy would not induce resistance (it would be "resistant to resistance") because it targeted endothelial cells instead of the tumor cell itself [36]. Nevertheless, clinical and experimental evidence indicates that a vascular regrowth in tumors is present after reversal of VEGF inhibition [37]. In some cases, there is a period of benefit followed by progression and mortality that reflects an adaptive response by tumors. Tumors can manifest an "evasive response" by upregulating alternative pro-angiogenic signals (such as ephrins or angiopoietins), recruiting pro-angiogenic inflammatory cells or pericytes, accentuating invasiveness of tumor cells into local tissue to co-opt normal vasculature, and increasing metastatic seeding and tumor cell growth in lymph nodes and distant organs. By contrast, patients for whom there is no tangible benefit at the beginning of the therapy indicate that an intrinsic resistance to angiogenesis inhibitors exists [38].

VEGF inhibition produces vascular trimming and hypoxia, which leads to upregulation of multiple pro-angiogenic molecules, including VEGFs, FGFs, and angiopoietins, which can contribute to eventual resistance [3, 38]. Tumor hypoxia could select for tumor populations able to grow in low oxygen environments [39, 40] and/or provide alternate compensatory pro-angiogenic pathways to allow persistent neovascularization [41] Furthermore, studies in the RIP-Tag2 model have described progression of NETs in course of antiangiogenic therapies targeting the VEGF/VEGFR signaling axis. Thus, genetic or pharmacological potent angiogenesis inhibition can alter the natural history of tumors by triggering resistance to therapy and increasing invasion and lymphatic or distant metastasis [42, 43]. Similar results have been observed in other models [44].

Acquired resistance can also be developed due to rapid vascular remodeling of tumor-associated vessels as a consequence of antiangiogenic therapy. The mature

Table 3.1 Multi-target inhibitory profile of antiangiogenic drugs to address resistance

Antiangiogenic drug	PDGFR	VEGF	VEGFR	FGFR	FLT-3	HIF-alfa
Sunitinib	■		■		■	
Bevacizumab		■				
Sorafenib	■		■		■	
Pazopanib	■		■			
Dovitinib	■		■	■	■	
Vatalanib	■		■			
Axitinib	■		■			
Brivanib			■	■		

remodeled vessels are resistant to antiangiogenic drugs, which usually target relatively immature vessels [45, 46].

Strategies to overcome this resistance mechanism are warranted. Based on preclinical data, several authors have proposed some strategies to overcome the antiangiogenic resistance that are based in combinatorial targeting of the VEGF pathway with other "escape" pathways that could be used for resistance (Table 3.1). In particular, some of these strategies, such as dual-targeted therapies, have been tested in xenografts [47]. The combination of bevacizumab and HIF-1 or Sp1 inhibitors may increase the therapeutic efficacy of antiangiogenic treatment [48, 49]. In another study, Allen et al. [50] suggest that co-targeting of VEGF and FGF signaling pathways can improve efficacy and overcome adaptive resistance to VEGF inhibition in the RIP-Tag2 model of pancreatic NETs. They tested the dual-FGFR/VEGFR tyrosine kinase inhibitor brivanib in both first and second line following the failure of anti-VEGFR2 antibody (DC101) or sorafenib showing promising results in overcoming resistance to VEGF-selective therapy.

On the clinical side, some phase II studies have tested the combination of antiangiogenic drugs. 2-Methoxyestradiol (2ME2) administered in combination with bevacizumab has been evaluated in a prospective study in thirty-one patients with metastatic carcinoid tumors [51]. No confirmed radiological responses by RECIST were observed. However, 68 % of the radiologically evaluable patients experienced at least some degree of tumor reduction, and the median PFS time was 11.3 months. The results of a study [52] with the combination of sorafenib and bevacizumab were reported in 2011 ASCO Annual Meeting. The overall response ratio was 9.8 %, and the disease control rate at 6 months was 95.1 %. Median progression-free survival was 12.4 months. The most common grade 3–4 toxicities were hand–foot syndrome and asthenia, which occurred in 20.5 % and 15.9 % of patients, respectively. Another trial has tested the combination of bevacizumab and everolimus in NETs. Addition of everolimus to bevacizumab was associated with further decrease in tumor blood flow (15 %; $p = 0.02$) than bevacizumab alone. By intention-to-treat (ITT) analyses, there were 26 % of PR and 27 % of SD. The median PFS was 14.4 months [53]. Recently, preliminary results of another phase II trial with the combination of bevacizumab and temsirolimus, another mTOR

inhibitor, have been reported. Confirmed PR was documented in 11 of the first 25 (44 %) evaluable patients [54].

On the other hand, the identification of biomarkers for response or resistance to a particular antiangiogenic regimen is imperative in order to monitor the efficacy of antiangiogenic therapy. A study in the RIP-Tag2 model of pancreatic NETs described that tumors refractory to therapy following long-term treatment with a vascular endothelial growth factor receptor-2 blocking antibody contained blood vessels with a prolific investment of pericytes expressing α-smooth muscle actin. This is a response of resistant tumors to the therapy, which is impairing neovascularization and/or eliciting vascular regression, and in order to maintain a core of preexisting blood vessels alive and functional, they increase the amount of pericytes [23]. Further studies are warranted to validate the occurrence of pericytes expressing α-smooth muscle actin as a biomarker for tumors refractory to therapy [55].

A Perspective

Morphological, histological, and molecular features of NETs strongly support the notion that angiogenesis is a promising target in these malignancies. Indeed, several antiangiogenic drugs have been clinically validated, and two of those have been recently approved and are being incorporated in the daily clinical practice of pancreatic NETs. Nevertheless, not all patients respond to these therapies, demonstrating upfront refractoriness to therapy or *intrinsic resistance*. This patient population has to be carefully studied and detected in the future to find the most appropriate patient selection marker or characteristic in order to effectively treat these refractory patients. On the other hand, antiangiogenic drugs demonstrate clinical efficacy in many NETs patients, but these clinical benefits are overshadowed by apparent *acquired resistance* to antiangiogenic therapies emerging in NETs. Therefore, overcoming antiangiogenic resistance is a crucial step in the future development of antiangiogenic therapies. Several strategies have been postulated to fight resistance, including multi-pathway inhibitors or multi-combination of antiangiogenic drugs that target different pathways that can revert resistance caused by the upregulation of alternative pro-angiogenic signaling molecules, the recruitment of vascular progenitor cells or pericytes to the forming blood vessels, and also in order to fight against the increased capabilities for invasion without angiogenesis observed in some animal models. In this sense, clinical studies that investigate and address these approaches in the coming years are warranted.

Nevertheless, preclinical data in the RIP-Tag2 model indicate that many of these mechanisms of resistance show reversibility after antiangiogenic therapy has been stopped (Pàez-Ribes and Casanovas, unpublished observations). This confirms that these forms of resistance may reflect adaptations to therapy rather than irreversibly acquired capabilities and thus suggest that switching to a non-angiogenic drug in these resistant patients could revert their angiogenesis dependence and resensitize these patients to antiangiogenic drugs. Following this hypothesis, sequential

treatment with an antiangiogenic drug followed by a non-antiangiogenic drug (i.e., another targeted therapy or chemotherapy) could resensitize patients to another antiangiogenic drug as a third line of treatment. Obviously, many studies are warranted to unravel the preclinical basis and clinical potential of this hypothetical sequential treatment and to finally determine its clinical benefit for NETs patients.

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Chapter 4
Advances with Somatostatin Analogs in Neuroendocrine Tumors; The Promise of Radionuclides in Neuroendocrine Tumors

Cindy Neuzillet, Olivia Hentic, Eric Raymond and Philippe Ruszniewski

Abstract Somatostatin (SST) analogs form the cornerstone of medical therapy of well-differentiated neuroendocrine tumors (NETs). These tumors are commonly characterized by high SST receptor expression levels, mainly of subtype 2 (SSTR2), which is the basis for SST use in imaging and therapeutic strategies in digestive NETs. Since the 1980s, SST analogs (octreotide and lanreotide) have been widely prescribed to relieve symptoms resulting from hormonal hypersecretion in functioning tumors, such as carcinoid syndrome. In the last few years, two phase III studies have demonstrated that in addition to their antisecretory effects, SST analogs also exert antiproliferative effects in selected patients. Moreover, underlying molecular mechanisms of action have been elucidated, paving the way for strategies to overcome acquired resistance, such as dose optimization, combination with other targeted agents, and dual-targeting chimeric molecules. Conjugation of SST analogs with radionuclides has also been used for peptide-receptor-targeted radiotherapy (PRRT) in advanced NETs, with promising results. This chapter summarizes key preclinical and clinical data on the antisecretory and antitumor effects of SST analogs and provides an overview of recent advances with these agents and SST-based PRRT.

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Introduction

Neuroendocrine tumors (NETs) are considered rare malignancies, although their incidence and prevalence have significantly increased over the last two decades [1]. NETs arise from neuroendocrine cells, which are scattered throughout the digestive tract. Digestive NETs are classified on the basis of their site of primary origin, differentiation, and histologic grade, as well as their functional status which is based on the presence of a clinical hormonal syndrome caused by excessive hormone secretion by cancer cells (30–40 % of digestive NETs). In specialized centers, 80–90 % of patients with midgut NETs and 60–70 % of those with pancreatic NETs present with distant metastases at initial diagnosis [2]. Therapeutic approaches for metastatic disease include surgical, medical, radiologic, and nuclear medicine strategies [2].

Somatostatin (SST) analogs form the cornerstone of medical therapy of well-differentiated NETs. Since the discovery of SST more than 40 years ago, analogs have been widely prescribed to relieve symptoms resulting from hormonal hypersecretion in functioning tumors, such as carcinoid syndrome [3]. SST analogs have been further used for diagnosis (see Chap. 2) and more recently for therapy with peptide-receptor-targeted radiotherapy (PRRT). In the last few years, two phase III studies have demonstrated that in addition to their antisecretory effect, SST analogs can also delay tumor progression in some NET patients. Moreover, underlying molecular mechanisms of action and resistance have been elucidated, giving a rationale for dose optimization and combination therapy with other targeted agents. This chapter provides an overview of these recent advances with SST analogs and SST-based PRRT.

Somatostatin and Expression of its Receptors in NETs

SST is a polypeptide hormone first isolated from the hypothalamus in the late 1960s [4, 5]. It is produced by paracrine cells located in the gastrointestinal tract, pancreas, lung, and central nervous system. SST is a regulatory molecule and can act in an autocrine, paracrine, and endocrine manner, with a broad range of inhibitory actions over many physiologic functions. These include: (1) reducing gastrointestinal endocrine and exocrine fluid secretions, pancreatic enzyme and bile flow; (2) slowing gastric and intestinal motility and gallbladder contraction; (3) decreasing liver and splanchnic blood flow; and (4) regulating the functioning of activated immune cells and inhibiting angiogenesis and cell proliferation [6].

SST is a small cyclic molecule and has two natural biologically active forms, SST-14 and SST-28. Active forms are generated by proteolytic cleavage from prosomatostatin (prohormone), derived from the precursor preprosomatostatin (preprohormone) made up of 116 amino acids. Circulating SST plasma half-life is remarkably short ($\approx$ 2 min) due to enzymatic degradation.

SST effects are mediated through a family of five high-affinity G protein-coupled receptors (SSTR1–SSTR5), which were cloned and characterized in the early 1990s [7]. Upon ligand binding, adaptor proteins and enzymes are recruited to the cell membrane and activate secondary messengers and cytoplasmic targets, triggering the modulation of several intracellular signaling pathways, in a G inhibitory protein (Gi)-dependent or Gi-independent manner [7]. Each SSTR subtype activates or inhibits a specific spectrum of transducing pathways, involving common but also unique signaling cascades. SSTR signaling is further regulated by receptor endocytosis and trafficking [8].

Well-differentiated NETs are commonly characterized by high SSTR expression levels. SSTR2 predominates and is expressed in more than 80 % of midgut and pancreatic NETs, except for the case of insulinomas where less than 50 % express this receptor subtype [9]. Other SSTR subtypes are also found in digestive NETs but to a lesser extent [10]. High SSTR2 expression is the basis for SST use in diagnostic and therapeutic strategies in digestive NETs.

Somatostatin Analogs

The very short half-life of native SST made it unsuitable for routine clinical use (i.e., requiring continuous intravenous administration) and prompted the development of more potent and stable analogs. Modifications to the chemical structure of SST were designed to make the molecule more resistant to enzymatic degradation [11]. Initially, short-acting analogs with half-lives of 6–8 h (e.g., octreotide, lanreotide) which could be administered by intravenous or subcutaneous injection were developed, followed by long-acting prolonged-release formulations of octreotide (intramuscular injection) and lanreotide (deep subcutaneous injection), allowing 4 week intervals between injections [11]. Licensed doses for prolonged-release formulations octreotide LAR® and lanreotide Autogel® are 10, 20, and 30 mg and 60, 90 and 120 mg, every 4 weeks, respectively.

Unlike natural SST, both octreotide and lanreotide display high-affinity binding for SSTR2 and SSTR5, medium affinity for SSTR3, and low affinity for SSTR1 and SSTR4 [3, 12]. More recently, pasireotide, a novel stable cyclohexapeptide with a pan-SSTR inhibitory profile mimicking the action of natural SST, has been developed. Pasireotide binds SSTR1, SSTR2, SSTR3, and SSTR5 with high affinity and has a 30- to 40-fold higher affinity for SSTR1 and SSTR5 compared with octreotide or lanreotide [13]. A long-lasting form of pasireotide administered by intramuscular injection every 4 weeks has been developed and is currently under evaluation in clinical trials [14, 15].

SST analogs are remarkably well tolerated by patients with NETs, and safety is rarely the cause for treatment discontinuation. Most side effects are mild and transitory, and involve the injection site (local reaction), the gastrointestinal tract (nausea, abdominal pain, diarrhea partially caused by malabsorption), glucose metabolism (hyper or hypoglycemia due to insulin/glucagon secretion modulation), and biliary effects (sludge or gallstones due to decreased gallbladder motility). Some patients also experience nonspecific symptoms such as generalized pain, arthropathy, rash, fatigue, headache, or dizziness. The absence of rebound hormone hypersecretion with SST analogs is particularly valuable.

Antisecretory Effects

Historically, SST analogs were developed to control NET symptoms related to hormonal hypersecretion.

Molecular Basis

SST inhibits the synthesis and secretion of many hormones, including gastrin, glucagon, insulin, cholecystokinin, secretin, and vasoactive intestinal peptide (VIP), mainly via inhibition of exocytosis. This antisecretory effect is mediated through SSTR2, SSTR5, and SSTR1 [16]. Several intracellular effector pathways have been described (Fig. 4.1): (1) a Gi-dependent inhibition of adenylate cyclase with a subsequent decrease in intracellular cyclic adenosine monophosphate (cAMP) production, resulting in down-regulation of cAMP-dependent protein kinase A (PKA); (2) modulation of intracellular Ca^{2+} concentration, via Gi-dependent stimulation of various K^+ channels inducing membrane hyperpolarization and inhibition of voltage-dependent Ca^{2+} channels, and Go-dependent inhibition of these Ca^{2+} channels, both mechanisms decreasing transmembrane Ca^{2+} influx and intracellular Ca^{2+} concentration; (3) activation of downstream protein phosphatases such as the Ca^{2+}-dependent phosphatase calcineurin, which inhibits exocytosis [3, 7, 16].

Indications

Octreotide is approved for the control of hormone-related symptoms in NETs in the U.S. and in Europe, and lanreotide is approved for the same indication in Europe only. Since their introduction in routine clinical practice in the 1980–1990s, SST analogs have clearly improved therapeutic management of patients with functional NETs [17]. The first clinical trial with octreotide, published in 1986, involved 25

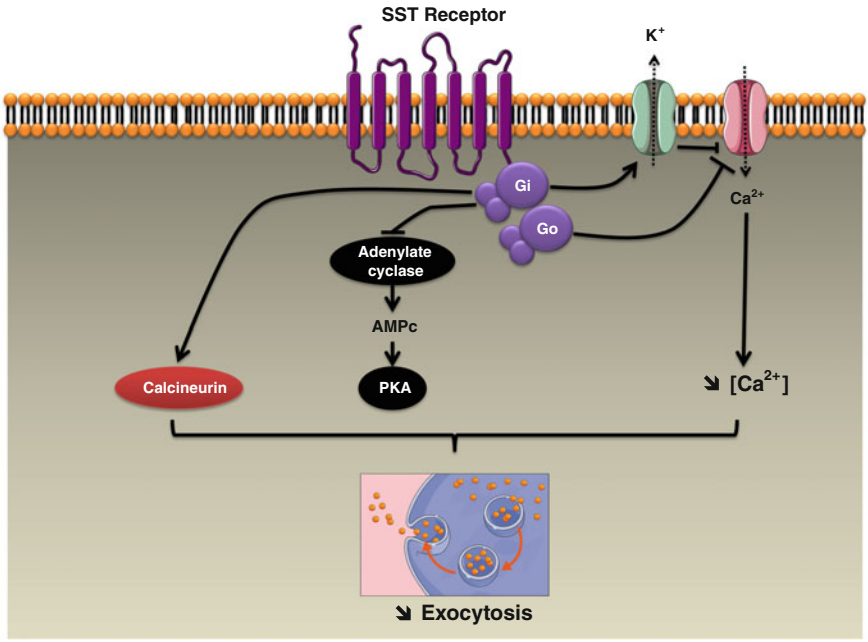

Fig. 4.1 Schematic representation of the main molecular mechanisms involved in the antisecretory effects of somatostatin analogs. *cAMP* cyclic adenosine monophosphate; *Gi* G inhibitory protein; *SST* somatostatin; *PKA* protein kinase A

patients with carcinoid syndrome [18]. Flushing and diarrhea were promptly relieved in 22 patients (88 %), and 18 (72 %) had a decrease of ≥50 % in their urinary 5-HIAA levels compared with pretreatment values, leading to approval of octreotide by the Food and Drug Administration (FDA). Subsequently, about 30 studies were conducted with SST analogs, involving more than 500 patients, most of whom had carcinoid syndrome (review in [3]). The mean symptom control rate was 73.2 %, and no difference was found between the various agents and formulations. SST analogs appeared more effective against flushing than diarrhea. In a study published in 2004 by Ruszniewski et al. [19], 71 patients with carcinoid syndrome were enrolled to receive lanreotide Autogel® at a dose of 90 mg every 4 weeks for two injections, after which the dose was adapted over 6 months according to the patient's response. Flushing episodes and diarrhea were significantly decreased at 6 months compared with baseline (p <0.001 and $p = 0.006$, respectively), and biochemical response (decrease of ≥ 50 % in serum chromogranin A and/or urinary 5-HIAA levels) was observed in 30 % of patients. In a crossover study comparing octreotide with lanreotide in 33 patients with carcinoid syndrome, O'Toole et al. [20] demonstrated equivalent efficacy for the two compounds in terms of symptom control and reduction in tumor markers. Pooled data analysis from 15 studies confirmed that octreotide LAR® and lanreotide Autogel® yield similar symptom control rates of

74.2 % (95 % confidence interval [CI], 61.9–92.8 %) and 67.5 % (95 % CI, 40.0–100 %), respectively, as well as for biochemical response rates (51.4 % [95 % CI, 31.5–100 %] and 39.0 % [95 % CI, 17.9–58 %], respectively) (Fig. 4.2, part 1) [3]. These findings were also reported in acute situations, such as during carcinoid crisis, which may occur spontaneously or during tumor manipulation in surgical or radiologic procedures [21]. A phase III study of pasireotide LAR® versus octreotide LAR® in metastatic NET patients with functional symptoms inadequately controlled by SST analogs was presented at the ASCO 2013 meeting (abstract #4031, NCT00690430). A total of 110 patients have been randomized and were stratified by predominant symptom at baseline (diarrhea, flushing, or both). Symptom response rates at 6 months were not significantly different between pasireotide LAR® and octreotide LAR® (21 vs. 27 %; odds ratio [OR] = 0.73, $p = 0.53$).

SST analogs are also effective against other NET hormone-related symptoms (review in [22, 23]). While insulinomas and gastrinomas are the most frequent functional pancreatic NETs, use of SST analogs in these indications is limited [9, 17]. In gastrinomas, high doses of proton-pump inhibitors are essential to control Zollinger–Ellison syndrome caused by gastrin hypersecretion, and the use of SST analogs is limited to rare refractory cases. In insulinomas, the effect of SST analogs on glycemia is unpredictable and hypoglycemia may be either improved or worsened by SST analogs due to glucagon suppression, requiring careful management of this treatment with initiation during hospitalization. Moreover, other treatments are available and effective in controlling insulin hypersecretion: diazoxide, which inhibits insulin release by direct action on β cells, or more recently, everolimus [24]. In contrast, the beneficial role of SST analogs has been well documented in less common types of functional pancreatic NETs [9, 17]. In glucagonomas, SST analogs are effective in controlling necrolytic migratory erythema in 80–90 % of patients, although their effect on the associated diabetes and weight loss is less pronounced [9, 17]. In VIPomas, diarrhea and electrolyte imbalance, also known as Werner–Morrisson syndrome or WDHA (watery diarrhea, hypokalemia, achlorhydria) syndrome, are significantly improved by SST analogs in 80–90 % of patients, although their effect may be limited by tachyphylaxis [9, 17].

Antitumor Effects

Recently, SST analogs were demonstrated to exert antitumor effects in selected NET patients and underlying molecular mechanisms have been elucidated.

Molecular Basis

The molecular mechanisms responsible for the antitumor effects of SST analogs are classified as direct and indirect mechanisms [7]. Direct mechanisms are

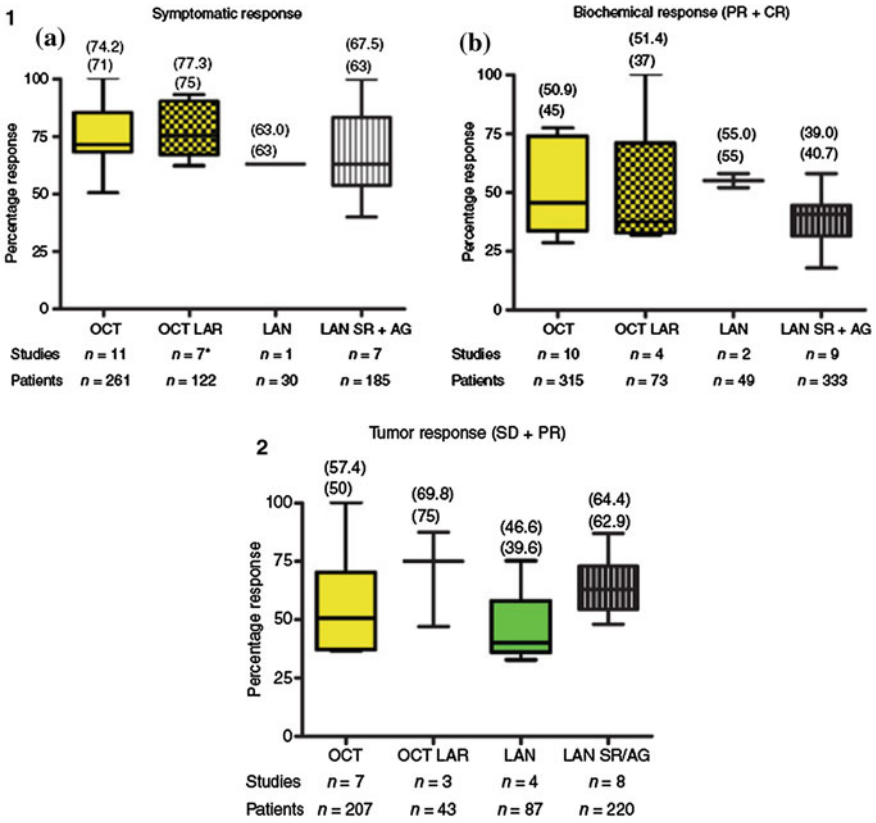

Fig. 4.2 Efficacy of different somatostatin analogs and formulations in terms of antisecretory effects and antitumor effects (data compilation from 15 studies, *in* [3]). *OCT* octreotide; *OCT LAR* octreotide LAR®; *LAN* lanreotide; *LAN SR/AG* lanreotide Autogel®; *PR* partial response; *CR* complete response; *SD* stable disease Mean (*top*) and median in brackets. (1) Antisecretory effects, (2) Antitumor effects

associated with cell cycle arrest and/or apoptosis downstream of SSTR activation. Mechanisms inhibiting cancer cell proliferation are complex and involve several intracellular signaling pathways which depend on the SSTR subtype (Fig. 4.3). All SSTRs induce expression of cell cycle inhibitors, including p27 and p21, leading to cell cycle arrest at the G1/2 (SSTR1, 2, 4, and 5) or G2/M (SSTR3) phase [7, 16]. SSTR1, 2, 3, and 4 trigger the recruitment and activation of phosphotyrosine phosphatases (PTPs) in a Gi/Go-dependent manner [7]. These PTPs (SHP-1, SHP-2, PTPη) subsequently dephosphorylate growth-factor-bound tyrosine kinase receptors (TKRs) and phosphorylated tyrosine residues of TKR targets (e.g., c-Src), thereby inhibiting growth factor signaling and modulating downstream effectors such as mitogen-activated protein (MAP) kinases (SSTR1, 2, 3, 4), PI3 K/AKT (SSTR2), and nitric oxide (NO) pathways (SSTR1, 2, 3) [7, 16]. SSTR5, on the other hand, does not require PTP to exert its antiproliferative effect.

It is mediated by Gi/Gq-dependent inhibition of: (1) phospholipase C (PLC) and IP3 which regulate Ca^{2+} influx, (2) cGMP and downstream MAP kinase signaling, and (3) the src-like tyrosine kinase p60Src that inactivates NO synthase [7]. Moreover, SSTR2 and SSTR3 were also shown to induce apoptosis through p53-dependent (SSTR3) or p53-independent (SSTR2) mechanisms [7, 16]. Both apoptotic pathways are affected by SST, the extrinsic pathway, through sensitization to death receptors of TNF-α, TRAIL, and Fas-Ligand (SSTR2 and SSTR3), and the intrinsic (mitochondrial) pathway, through inhibition of anti-apoptotic proteins such as Bcl-2 (SSTR2) and induction of pro-apoptotic proteins such as Bax (SSTR3) [7, 16]. The apoptotic effect of SSTR2 also involves the inhibition of survival signals mediated by MAP kinases and PI3 K/AKT pathways [7, 16].

Overall, growth inhibition is mediated mainly by SSTR2 and SSTR5, while apoptosis is triggered by SSTR3, which may explain why octreotide and lanreotide, targeting mainly SSTR2 and SSTR5, often display cytostatic rather than cytotoxic effects. In addition, SST also displays anti-invasive properties in certain tumor types. This effect may be due to SSTR1, 2, 3, and 4-mediated inhibition of the small GTPases Rac or Rho, both of which regulate cytoskeleton organization and cell migration [7, 16].

Indirect mechanisms mainly involve SST-induced inhibition of growth factor secretion and tumor angiogenesis, and modulation of immune cells [7, 16]. SST impacts the growth hormone (GH)/insulin growth factor (IGF)-1 axis both centrally, through inhibition of GH synthesis (SSTR2 and SSTR5), and peripherally, through down-regulation of STAT5b-mediated IGF-1 gene transcription in the liver (SSTR2 and SSTR3) [7, 16]. In addition, endothelial cells express SSTR1, 2, 3 and 5, and SST decreases their proliferation and migration [7, 16]. Inhibition of endothelial NO synthase and expression of proangiogenic factors such as VEGF, PDGF, or bFGF, also contributes to the antiangiogenic effect of SST [7, 16].

Defining the Indications: The PROMID and CLARINET Studies

Tumor responses were reported early in the clinical development of SST analogs, and a large number of clinical trials were initiated to investigate the antitumor effect of these agents, although the underlying molecular mechanisms were not yet clearly understood (Fig. 4.2, part 2) [17]. In a systematic review of 28 prospective studies (17 octreotide trials and 11 lanreotide trials) published from 1989–2011, partial response (PR) rates ranged from 0–31 % and stable disease (SD) from 15–89 % [25].

The PROMID study was the first phase III trial to demonstrate that long-term administration of SST analogs can control tumor growth in NET patients [26]. In this study, 85 treatment-naïve midgut NET patients were randomly assigned to receive either monthly octreotide LAR® 30 mg (n = 42) or placebo (n = 43) until

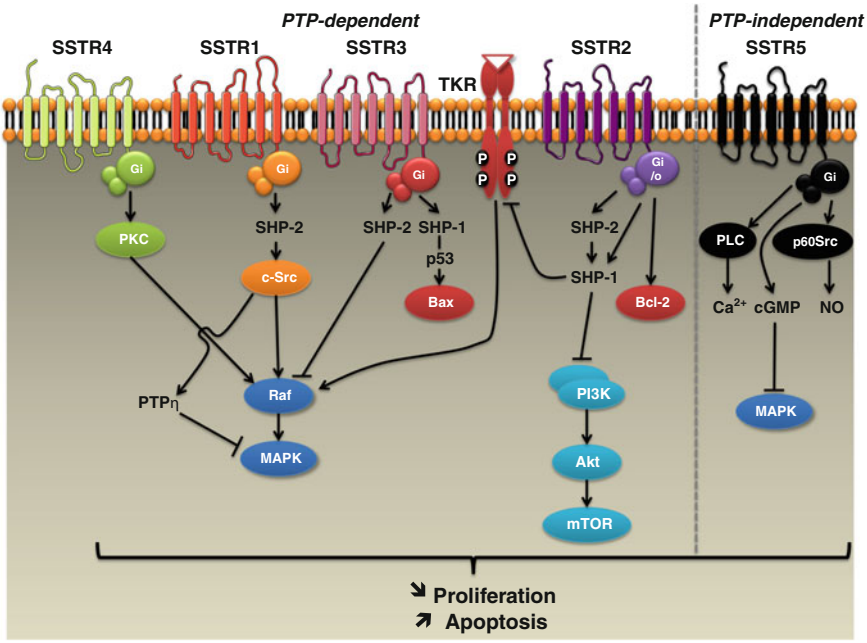

Fig. 4.3 Schematic representation of main molecular mechanisms involved in antitumor effects of somatostatin analogs. *MAPK* mitogen-activated protein kinase; *PKC* protein kinase C; *PLC* phospholipase C; *PTP* phosphotyrosine phosphatase; *SSTR* somatostatin receptor; *TKR*: tyrosine kinase receptor

tumor progression or death. The majority (61 % of patients) had nonfunctioning tumors. Most patients had tumors with a Ki67 proliferation index less than 2 % (95 % of patients) and were octreoscan-positive (74 % of patients). Median time to progression (TTP) in the octreotide LAR® arm was 14.3 months compared with 6.0 months in the placebo arm (hazard ratio [HR] = 0.34, $p = 0.000072$). After 6 months of treatment, SD was observed in 66.7 % of patients in the octreotide LAR® arm versus 37.2 % in the placebo arm ($p = 0.0079$). The greatest benefit was observed in patients with low hepatic tumor burden ($\leq$10 %) and resected primary tumor. The effect was independent of tumor functionality. The study was not designed to demonstrate superiority in terms of overall survival since more than 90 % of patients with tumor progression in the placebo arm received octreotide LAR. Based on the results of this study, the National Comprehensive Cancer Network (NCCN) guidelines were revised to include the use of SST analogs as antineoplastic agent for metastatic midgut NETs.

However, many criticisms emerged subsequent to the publication of this study. The major reproach was the lack of progression status prior to inclusion. Since evidence of progressive disease (PD) was not a requirement for study entry, the indication for starting active treatment with an SST analog in otherwise asymptomatic patients is unclear [27]. Moreover, there was a significant imbalance in the

time since diagnosis between the study arms in favor of the octreotide LAR® arm, with a median of 7.5 months compared with 3.3 months for the placebo arm ($p = 0.0096$). Longer time between diagnosis and treatment suggests a more indolent disease [27]. Finally, these results left partially unanswered the question of which patients are most likely to benefit from SST analog treatment, since the benefit was more important in, but probably not limited to, patients with metastatic midgut NETs with Ki67 proliferation index ≤2 %, hepatic tumor burden ≤10 % and resected primary tumor. In a retrospective study conducted in 68 patients with metastatic digestive NETs, pretreatment stability (HR = 0.241, $p = 0.008$), Ki67 proliferation index ≤5 % (HR = 0.262, $p = 0.009$), and hepatic tumor burden ≤25 % (HR = 0.237, $p = 0.004$) were significantly associated with SD under lanreotide therapy in multivariate analysis, suggesting that PROMID-derived criteria could be expanded [28].

More recently, the results of the CLARINET study were presented at the ESMO 2013 meeting (abstract #E17-7103, NCT00353496). CLARINET is a phase III trial evaluating the antiproliferative effects of lanreotide Autogel® in patients with advanced gastroenteropancreatic NETs. A total of 204 patients with well or moderately differentiated (Ki67 proliferation index <10 %), octreoscan-positive, nonfunctioning NETs who had not received SST analogs or other treatment in the prior 6 months were enrolled. They were randomized to receive lanreotide Autogel® 120 mg (n = 101) or placebo (n = 103) every 4 weeks for 96 weeks, or until tumor progression or death. Primary tumor locations were pancreatic (45 %), midgut (36 %), hindgut (7 %), and unknown (13 %). Most patients were treatment-naïve (81 %) and had SD (96 %) prior to inclusion. Twenty-two percent of patients had WHO grade 2 tumors (Ki67 = 3–10 %), and 33 % had a hepatic tumor burden of more than 25 %.

After two years of treatment, median progression-free survival (PFS) was 18.0 months in the placebo arm and had not been reached in the lanreotide Autogel® arm (HR = 0.47, $p = 0.0002$), while 22 % of placebo subjects were alive compared with 62 % in the lanreotide group (HR = 0.47, $p = 0.0002$). In subgroup analysis, the increase in PFS with lanreotide Autogel® was significant compared with placebo for patients with midgut tumors (HR = 0.35, $p = 0.0091$), but not for patients with pancreatic tumors (HR = 0.58, $p = 0.0637$). Benefit was similar for WHO grade 1 and grade 2 tumors and was greater for patients with a lower hepatic tumor burden (≤25 %, HR = 0.34, $p = 0.0002$) but remained significant in patients with a greater hepatic tumor burden (>25 %, HR = 0.45, $p = 0.0170$). The results of this study contribute to expand and better define the indications of SST analogs in digestive NETs.

Data for pasireotide are scarcer. In the phase III study evaluating symptomatic effect of pasireotide LAR® versus octreotide LAR® presented at the ASCO 2013 meeting (abstract #4031, NCT00690430), patients on pasireotide LAR® had a 5 month longer PFS than patients on octreotide LAR® (investigator assessment), despite no differences in symptom response rates. These results warrant a large phase III trial to clarify the antitumor role of pasireotide.

Current Limits and Perspectives with SST Analogs

Given their mechanisms of action, SST analogs can be considered targeted agents directed against SSTR. As for all targeted therapies, their use in the clinic is limited by the emergence of acquired resistance, also known as tachyphylaxis. Furthermore, in some NET patients, SST analogs lose effectiveness within months of treatment initiation, whereas in other patients, tumor symptoms and growth can be controlled for several years. The reasons for tachyphylaxis are unclear but may be due to reduced SSTR expression on NET cells, activation of alternative pathways, and/or changes in SST analog pharmacokinetics when chronically administered. Three major strategies have been developed to enhance SST analogs efficacy and/or overcome acquired resistance: dose optimization, combination with other agents, and new SSTR-binding molecules.

Dose Optimization

In some patients, increasing the dose may restore the original response [17]. In clinical practice, this may be achieved either by a higher dose or by shortening the interval between injections (review in [29]). In a retrospective 8-year study in 108 patients with metastatic midgut NETs with carcinoid syndrome, 24 % had a sustained symptomatic response [30]. In the remaining patients, loss of symptomatic response with the initial dose was noted within 3–60 months. In 17 % of them, symptoms were controlled by an increase in octreotide LAR® dose, while the other patients required additional treatment.

The highest approved dose of octreotide LAR® is 30 mg administered every 4 weeks. A retrospective study of digestive NET patients requiring higher (40–90 mg) doses to control symptoms refractory to conventional doses of octreotide showed that dose escalation was safe [31]. In the dose-titration study of lanreotide Autogel®, 45 (63 %) of the 71 patients were treated with 120 mg/4 weeks, 11 (16 %) with 90 mg/4 weeks (initial dose), and 15 (21 %) with 60 mg/4 weeks. Twenty-seven (38 %) responded to doses of 120 mg or less, 15 (21 %) to 90 mg or less, and 11 (15 %) to 60 mg [19]. Dose optimization was well tolerated and caused a reduction in episodes of flushing and diarrhea by a mean of 1.3 and 1.1 episodes/day, respectively ($p < 0.001$). Welin et al. [32] evaluated the effect of a high-dose octreotide regimen (octreotide 160 mg/2 weeks) in 12 patients with progressive advanced midgut carcinoid tumors. Ten patients had symptomatic improvement of flush and diarrhea, and tumor size and biochemical markers were stabilized for a median of 12 months in 75 % of the patients. The results of the HIDONET trial (NCT00990535) evaluating a more frequent dosing schedule of octreotide LAR® (30 mg/3 weeks) in patients with progressive NETs are pending.

In addition, a study reported a decrease of 50–70 % of SST analogs plasma levels over a 2 year period in patients chronically treated with SST analogs [33].

This may be explained by either: (1) diminished bioavailability of the agent due to granulomatous reaction at the injection site, (2) development of antibodies against the agent, or (3) altered pharmacokinetics and metabolism of the agent due to changes in disease status or the patient (such as body mass index) [17]. However, the usefulness of plasma SST analog concentrations for treatment optimization is not clearly established, and plasma concentration monitoring during chronic SST analog therapy is not recommended [17].

Combination Therapy

The cytostatic rather than cytotoxic activity of SST analogs, thus inhibiting proliferation rather than inducing apoptosis, opens the opportunity for combination therapy with other agents, either chemotherapy or targeted agents. In vitro and *in vivo* data using SSTR2-positive colon cancer cell lines showed that SST analogs may have an additive/synergistic effect when combined with chemotherapy, such as 5-FU or mitomycin C [17]. Adding SST analogs potentiated the antiproliferative effect (S-phase block) and increased apoptosis of cancer cells. SST analogs can also be combined with targeted agents. Recently, phase III trials have demonstrated that targeted therapies directed against receptors of VEGF (sunitinib) or mTOR (everolimus) produced clinically significant improvement in patients with digestive NETs [34, 35].

SST analogs and everolimus both act on the PI3 K/AKT/mTOR signaling cascade, controlling protein synthesis and cell survival. Synergistic effects are exerted by enhancing signal inhibition on the downstream target 4E-BP1 [16]. SST analogs (through their action on endothelial cells and inhibition of VEGF production) and everolimus (through inhibition of the mTOR-HIFα axis) may also synergistically decrease angiogenesis. Finally, by reducing IGF-1 levels, SST analogs may overcome resistance from everolimus-induced regulation of IGF-1 pathway. The RADIANT-1 open-label phase II study assessed the clinical activity of everolimus (10 mg/day), with or without octreotide LAR® (30 mg/4 weeks) based on prior octreotide therapy, in patients with pancreatic NETs, after failure of chemotherapy [36]. Median PFS was 16.7 and 9.7 months with combination therapy and monotherapy, respectively. Subsequently, the RADIANT-2 phase III study compared everolimus (10 mg/day) plus octreotide LAR® (30 mg/4 weeks) to placebo plus octreotide LAR® in 429 patients with progressive low- or intermediate-grade advanced NETs [37]. Median PFS was significantly improved in the everolimus plus octreotide LAR® group (16.4 months vs. 11.3 months in the placebo plus octreotide LAR® group, HR = 0.77, $p = 0.026$). Drug-related adverse events were mostly grade 1 or 2, including stomatitis, rash, fatigue, and diarrhea. Phase I (NCT00804336, NCT01263353, NCT01590199) and II (NCT01374451) studies of pasireotide LAR® in combination with everolimus in digestive NETs are currently ongoing.

There is also a rationale for combining SST analogs with antiangiogenics. Well-differentiated NETs are remarkably highly vascularized and display sensitivity to intra-arterial (chemo) embolization and antiangiogenic agents (see Chap. 12). Combination of SST analogs with anti-VEGF antibodies (e.g., bevacizumab) or tyrosine kinase inhibitors (e.g., sunitinib) may yield enhanced antiangiogenic and antitumoral effects. Combination of octreotide LAR® (30 mg/4 weeks) with temozolomide (100 mg/day) and bevacizumab (7.5 mg/m^2/3 weeks) in 15 patients with advanced NETs, mainly WHO grade 2, showed interesting activity, with tumor control in 13 (86 %) patients (1 complete response, 8 PR, 3 SD) and a median TTP of 36 weeks [38]. A phase II study compared combinations of octreotide LAR® (continued at pre-study dose) with either bevacizumab (15 mg/m^2/3 weeks) or pegylated interferon α-2b or in patients with advanced extrapancreatic NETs [39]. Bevacizumab plus octreotide LAR® resulted in a high tumor control rate (95 %, including 18 % PR and 77 % SD), significant decrease in tumor blood flow ($p < 0.01$) and longer PFS (18 week PFS rate of 95 vs. 68 % in the interferon plus octreotide arm). The results of the subsequent phase III study (NCT00569127) are pending, along with the phase II study of combined bevacizumab, pertuzumab (HER1/HER2 inhibitor) and octreotide LAR® for advanced NETs (NCT01121939), and the phase II study of everolimus and octreotide LAR® with or without bevacizumab for advanced pancreatic NETs (NCT01229943). However, these studies were not designed to assess the benefit of combination therapy versus SST analog monotherapy. The SUNLAND phase II study, which evaluates sunitinib versus placebo in combination with lanreotide Autogel® in patients with progressive advanced midgut NETs (NCT01731925), is ongoing.

To summarize, there is emerging evidence for additive/synergistic activity of SST analogs combined with targeted agents. Such combinations may be of particular interest in patients with NETs progressing under SST analogs. However, the underlying mechanisms are not clearly defined and appropriate clinical studies are required to specifically assess the effect of combination therapy versus SST analog monotherapy.

New SSTR-binding Molecules

Advances in the understanding of SSTR signaling, trafficking, and interactions have led to the development of new SSTR-binding molecules. Besides pan-SSTR analogs such as pasireotide, compounds cotargeting SSTRs and other receptors have emerged as a promising strategy. The observation that the majority of well-differentiated NETs coexpress SSTRs and the dopamine type 2 receptor (D2R) and that SSTR and D2R can form heterodimers with enhanced functional activity, provided a rationale for the development of new chimeric compounds that can bind both receptor types [40–42]. BIM-23A760 was the first molecule of this family. It was designed to have high affinity for SSTR2 and D2R, while its affinity for SSTR5 was intentionally low to reduce the risk of hyperglycemia. Phase II

clinical studies have been performed in patients with carcinoid syndrome (NCT01018953) and acromegaly (NCT00994214) [9]. However, its clinical development has been stopped and new chimeric molecules are currently being developed. Among them, BIM-23A758 (which is an SSTR2/D2R chimeric compound as well) induced significant antitumor effects in human GOT1 midgut carcinoid cells and may be a promising new molecule for NET therapy [43].

Peptide-receptor-targeted Radiotherapy

Peptide-receptor scintigraphy such as octreoscan is useful in NET imaging for diagnosis and staging (see Chap. 2). Moreover, it can be used to select patients for the therapeutic strategy using radiolabeled SST analogs, PRRT [44]. Different radiolabeled SST analogs have been used, all of which share a common structure, comprising three parts: the radionuclide itself (Indium-111 [^{111}In], Yttrium 90 [^{90}Y], or Lutetium 177 [^{177}Lu]), a chelator (diethylene triamine penta-acetic acid [DOTA] or 1,4,7,10-tetra-azacyclododecane-1,4,7,20-tetra-acetic acid [DTPA]), and an SST analog (octreotide or lanreotide). Modifications in the radionuclide and chelator can considerably affect compound characteristics, including pharmakocinetics and SSTR-binding affinity [45].

Early studies were performed with high doses of ^{111}In-DTPA^{0}-octreotide, the same radiolabeled peptide as is used for octreoscan imaging. ^{111}In is a γ-emitter creating Auger electrons with short range and low tissue penetration (review in [46]), which may only be effective for the treatment of micrometastatic or low-burden disease, and is unsuitable for treating bulky tumors. This may explain the disappointing results of ^{111}In-coupled peptides in clinical studies in metastatic NET patients, with low rates of objective tumor response (0–17 %) compared with ^{90}Y- or ^{177}Lu-coupled peptides. ^{90}Y- or ^{177}Lu are β-emitter with higher energy and tissue penetration (reviewed in [47] and [48], respectively). ^{90}Y-DOTA-octreotide (DOTATOC) and ^{177}Lu-DOTA-octreotate (DOTATATE) are the two most widely used radiopeptides for PRRT in metastatic NETs, both yielding similar response rates (15–35 %) [45, 49]. More than 500 patients have been treated with ^{177}Lu-DOTATATE and more than 300 with ^{90}Y-DOTATOC in clinical studies, respectively. Results are summarized in Table 4.1. Differing antitumor effects between studies may be due to different administered doses and dosing schemes, total tumor burden including the extent of liver involvement, and patient characteristics.

Side effects with this treatment are infrequent, however, may be serious. Main toxicities are renal, hematologic, and hepatic. The kidneys are the dose-limiting organs for PRRT, particularly with ^{90}Y-DOTATOC. Concomitant infusion of amino acid solutions can be performed to reduce kidney uptake of radiopeptides and limit renal toxicity. In a study of long-term follow-up of renal function after PRRT, median decline in creatinine clearance of about 7 %/year with ^{90}Y-DOTATOC and 4 %/year with ^{177}Lu-DOTATATE was reported [50]. All

Table 4.1 Summary of main clinical studies of peptide-receptor radionuclide therapy (PRRT) with ^{90}Y- or ^{177}Lu-labeled somatostatin analogs in patients with digestive neuroendocrine tumors (adapted from [45, 49])

Reference	No. of patients	Progression at inclusion (% patients)	Reported response					Criteria
			CR	PR	MR	SD	PD	
^{90}Y-DOTA-octreotide								
Otte et al. [51]	16	NA	0	1 (6 %)	NA	14 (88 %)	1 (6 %)	NA
Waldherr et al. [52]	37	84	1 (3 %)	9 (24 %)	NA	23 (62 %)	4 (11 %)	WHO
Waldherr et al. [53]	37	100	1 (3 %)	7 (19 %)	NA	6 (70 %)	3 (8 %)	WHO
Bodei et al. [54]	21	NA	0	6 (29 %)	NA	11 (52 %)	4 (19 %)	WHO
Valkema et al. [55]	58	81	0	5 (9 %)	7 (12 %)	29 (50 %)	14 (24 %)	SWOG
Bushnell et al. [56]	90	100	0	4 (4 %)	NA	63 (70 %)	15 (17 %)	SWOG
Pfeifer et al. [57]	53	77	2 (4 %)	10 (19 %)	NA	34 (64 %)	7 (13 %)	RECIST
^{90}Y-DOTA-lanreotide								
Virgolini et al. [58]	39	100	0	0	8 (20 %)	17 (44 %)	14 (36 %)	WHO
^{90}Y-DOTA-octreotate								
Baum et al. [59]	75	89	0	28 (37 %)	NA	39 (52 %)	8 (11 %)	NA
Cwikla et al. [60]	60	100	0	13 (23 %)	NA	44 (77 %)	3 (5 %)	RECIST
^{177}Lu-DOTA-octreotate								
Kwekkeboom et al. [61]	310	38	5 (2 %)	86 (28 %)	51 (16 %)	107 (35 %)	61 (20 %)	SWOG
Swärd et al. [62]	16	NA	0	6 (38 %)	NA	8 (50 %)	2 (13 %)	RECIST
Garkavij et al. [63]	12	NA	0	2 (17 %)	3 (25 %)	5 (42 %)	2 (17 %)	RECIST
Bodei et al. [64]	39	NA	1 (3 %)	12 (31 %)	7 (18 %)	10 (26 %)	9 (23 %)	RECIST

CR complete response, *PR* partial response, *MR* minor response, *SD* stable disease, *PD* progressive disease, *NA* not applicable or nonavailable

Response Evaluation Criteria in Solid Tumors (RECIST): CR; PR, ≥30 % reduction in tumor size; SD, <30 % reduction or <20 % increase in tumor size; PD, ≥20 % increase in tumor size or new lesion(s). Measurements: bidimensional

World Health Organization (WHO): CR; PR, ≥50 % reduction in tumor size; SD, <50 % reduction or <25 % increase in tumor size; PD, ≥25 % increase in tumor size or new lesion(s). Measurements: bidimensional

Southwest Oncology Group (SWOG): CR; PR, ≥50 % reduction in tumor size; MR, between 25 and 50 % reduction in tumor size; SD, not qualifying for CR/PR/PD; PD, >50 % increase in tumor size. Measurements: unidimensional

patients were infused with renoprotective amino acids during administration of the radioactive peptides. PRRT is contraindicated in case of renal impairment with creatinine clearance <40–50 mL/min or severe cardiac impairment [44]. Exclusion of patients with risk factors for renal function alteration after PRRT (i.e., age >70 years, hypertension, diabetes, renal morphological abnormalities) may also be recommended.

Side effects of ^{177}Lu-DOTATATE have been analyzed in 504 patients with digestive NETs [61]. Hematologic toxicity grade 3 or 4 occurred after 3.6 % of administrations, or in 9.5 % of patients. Factors associated with a higher risk of grade 3–4 hematologic toxicity were age >70 years, previous chemotherapy, creatinine clearance ≤60 mL/min, and bone metastasis. Three patients with extensive liver metastases had serious liver toxicity, two of which were probably caused by the therapeutic radiation dose to the liver and were reversible. Accordingly, PRRT is contraindicated in patients with impaired hematologic function (hemoglobin <8 g/dL, platelets $<75 \times 10^9$/L, WBC $<2 \times 10^9$/L) or severe liver function impairment (total bilirubin >3 times the upper limit of normal, albumin <30 g/L, prothrombin time increased). Lastly, four patients developed myelodysplastic syndrome (0.8 %), which could be attributed to prior chemotherapy in one patient, but was more likely related to PRRT in the other three patients. Overall, serious delayed toxicity probably attributable to PRRT was present in about 1 % of patients.

Based on these efficacy data and an acceptable toxicity profile, PRRT appears to be a promising therapeutic strategy in NETs. Patients with inoperable octreoscan-avid NETs and no contraindication (pregnancy and lactation, and appropriate renal, cardiac, hematologic, or liver function impairment) are eligible for PRRT. The question of the best timing for PRRT in the NET management strategy is as yet unanswered. Currently, PRRT is mainly proposed to metastatic NET patients, no exclusively metastatic to the liver, after failure of other medical strategies. However, PRRT may also be beneficial in other settings, including neoadjuvant use in selected cases [45]. Various options have been proposed to improve PRRT, including combination of compounds (^{90}Y- and ^{177}Lu-radiolabeled peptides), locoregional administration of radiopeptides via selective hepatic intra-arterial injection, combination with radiosensitizing drugs (5FU or capecitabine), individualized tailored dosimetry, and α-emitters (e.g., ^{213}Bismuth) with short path length for treatment of small tumors [45, 49, 65].

Conclusion

In conclusion, SSTR targeting with SST analogs and derived strategies such as PRRT have revolutionized the management of patients with NETs. The PROMID and the CLARINET studies have demonstrated that in addition to their antisecretory effect, SST analogs also exert antiproliferative effects in selected patients. Underlying molecular mechanisms of action have been elucidated, paving the way

for strategies to overcome acquired resistance, such as dose optimization, combination with other targeted agents, and dual-targeting chimeric molecules. Results of ongoing studies will contribute to the optimization of SST-based therapy.

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Chapter 5
Streptozocin-Based Chemotherapy: Still a Standard of Care for Neuroendocrine Tumours?

Saira Khalique and Tim Meyer

Abstract Streptozocin (STZ)-based chemotherapy has been used for over 40 years in the treatment for neuroendocrine tumours (NETs); however, there have been few randomized trials, and STZ remains unlicensed in many countries. With the recent approval of sunitinib and everolimus for pancreatic NETs (PNETs), and the emergence of a more stratified approach to cancer therapy, it is timely to re-evaluate the role of STZ for NETs. Here, we review the evidence base for STZ-based chemotherapy, the toxicity associated with treatment and the position of STZ in the current therapeutic algorithm. Although there are no trials comparing chemotherapy with best supportive care, there is evidence that multi-agent STZ-containing regimens are associated with improved survival compared with control therapy. Additionally, in PNETs, chemotherapy appears to be associated with higher response rates compared with targeted therapies and this may be important in those who are symptomatic from tumour burden and those with locally advanced disease who may be down-staged for resection. The role of Ki67 and other predictive markers requires further assessment in prospective studies as does the relative efficacy of alternative agents such as temozolomide (TMZ).

Keywords Neuroendocrine · Chemotherapy · Streptozocin · Ki67 · Carcinoid

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Introduction

Neuroendocrine tumours (NETs) are a heterogeneous group of tumours that arise from different sites in the body and have a wide range of clinical behaviour. Those patients with low grade, indolent disease may live for up to 20 years, whereas those with aggressive high-grade tumours have a median survival of around 6 months [32]. At presentation, the majority of patients have unresectable disease due to local extension or metastases and non-curative, palliative interventions may be offered. These include somatostatin analogues that can suppress the symptoms of hormonal production in functional tumours, reductive surgery, hepatic artery embolisation or ablation, peptide receptor radionuclide therapy (PRRT) and systemic therapy [21]. However, lack of prospective studies and randomised trials has made the development of evidence-based therapeutic algorithms difficult. Our purpose here is to review the evidence for streptozocin (STZ)-based chemotherapy and define its current role.

Evidence for Chemotherapy

A limited number of cytotoxic drugs have been widely used for the treatment for NETs. Broadly, these can be classified as: alkylation agents including STZ, temozolomide (TMZ) and dacarbazine (DTIC); anti-metabolites such as fluorouracil (5FU); topoisomerase inhibitors such as doxorubicin (DOX) and etoposide; intercalating agents such as cisplatin and carboplatin; and mitotic spindle poisons such as the taxanes. There have been no randomised controlled trials in which chemotherapy has been compared with best supportive care and very few randomised trials comparing different chemotherapy regimens.

STZ is widely regarded as the standard of care for combination chemotherapy based on over 40 years of experience. STZ is an antibiotic derived from Streptomyces achromogenes, and in vitro evidence shows that STZ is preferentially taken up by the β islet cells in the pancreas causing specific islet cell damage and diabetes in animal models. The first report of therapeutic benefit was a case report of one patient with an islet cell carcinoma [18] and a subsequent review of 52 patients with islet cell tumours treated with STZ also suggested encouraging activity [1]. Subsequently, STZ has been evaluated in numerous case series, and a limited number of prospective phase II and small randomised trials. Response rates reported for PNETs tend to be higher than those of non-pancreatic NETs (NP-NETs), and the two groups are therefore considered separately.

Table 5.1 Outcomes for streptozocin-containing regimens in pancreatic NETs

Publication	Chemotherapy	Number of patients	RR (%)	PFS (months)	OS (months)	P value For RCT for OS
Broder and Carter [1]	STZ	52	50	–	–	
Moertel et al. [16]	STZ	42	36[a]	17	16.5	NSD
	STZ + 5-FU	42	63	17	26	
Eriksson et al. [9]	STZ + 5-FU	19	58[a]	36	–	
	STZ + DOX	25	36	22	–	
Bukowski et al. [3]	CTZ + 5-FU	44	32[a]	11	25	
Moertel et al. [17]	CTZ	33	30[a]	17	18	0.03[b]
	STZ + 5-FU	34	45	14	16.8	0.004[b]
	STZ + DOX	38	69	18	26.4	
Cheng and Saltz [5]	STZ + DOX	16	6	–	–	
Rivera and Ajani [25]	STZ + 5-FU + DOX	11	55	15	21	
Kouvaraki et al. [12]	STZ + 5-FU + DOX	84	39	18	37	
McCollum et al. [14]	STZ + DOX	16	6	3.9	20	
Delaunoit et al. [7]	STZ + DOX	45	36	16	24	
Fjallskog et al. [10]	STZ + DOX (L)	30	40	13	52	
Turner et al. [28]	5FU/CIS/STZ	49	38	–	–	

[a] RR assessed using tumour measurements and biochemical response
[b] Compared with STZ + DOX arm
STZ streptozocin; *5-FU* 5-fluorouracil; *CTZ* chlorozotocin; *DOX* doxorubicin; (*L*) liposomal; *CIS* cisplatin; *RR* response rate; *PFS* median progression-free survival; *OS* median overall survival

Chemotherapy for Pancreatic NETs

Moertel and colleagues conducted the seminal trials in patients with PNETs in 1980 and 1992 (Table 5.1). In the first, 103 patients with advanced PNETs were randomised to STZ (500 mg/m^2 D1-5) alone or in combination with 5-FU (400 mg/m^2 D1-5) in 6 weekly cycles [16]. Response was assessed by a combination of criteria including radiological, reduction in hepatomegaly or biochemical makers of 'enodocrine hyperfunction', and according to these criteria, the response rates were 36 % for the single agent and 63 % for the combination. The median overall survival (OS) was 26 months in the 5-FU/STZ group and 16.5 months in the STZ-alone group ($P > 0.05$). The second trial randomised 125 patients of which 105 were subsequently deemed to be eligible for analysis [17]. The three treatment regimens were as follows: 5-FU/STZ according to the schedule used in the initial trial and DOX/STZ (DOX 50 mg/m^2, 3 weekly and STZ 500 mg D1-5, 6 weekly) or chlorozotocin 150 mg/m^2 every 6 weeks. Using the same response criteria described in the first trial, the DOX/STZ group had significantly higher response rates (69 %) than either the 5-FU/STZ group (45 %) or the CTZ (30 %). The median duration of regression was 14 months for 5-FU/STZ, 17 months for chlorozotocin and 18 months for DOX/STZ. Median OS was 1.5 and 1.4 years in the chlorozotocin and 5FU/STZ groups, respectively, and 2.2 years in the DOX/STZ

group. The DOX/STZ arm was significantly better to the two other arms with respect to OS. Predictors of survival were performance status and age between 40 and 60 years [17]. On the basis of these trials, STZ-based combination chemotherapy became a standard of care for PNETs.

Subsequently, there have been a number of retrospective case series that have applied conventional radiological response criteria, including WHO and Response Evaluation Criteria in Solid Tumours (RECIST) criteria [27], with variable outcomes. Two small studies initially questioned the high response rates reported in the Moertel studies: Cheng et al. reviewed the outcome of 16 patients who had received the DOX/STZ over a 6 year period at Memorial Sloan-Kettering Cancer Centre. Duration of treatment ranged from 1.5–18 months. Applying WHO response criteria, only 6 % achieved a partial response, 56 % had SD and 38 % had PD [5]. Similarly, McCollum et al. carried out a multicentre retrospective review of 16 patients with PNETs who had received the DOX-STZ regimen. According to the RECIST criteria, 6 % had a PR, 38 % had SD as their best response and 56 % had PD. The median progression-free survival (PFS) was 3.9 months (95 % CI 2.8–8.8 months), and median OS was 20.2 months, which was thought to be more a reflection of the indolent nature of PNETs rather than as a result of the chemotherapeutic regimen [14]. More recently, three larger retrospective series have reported more encouraging activity for STZ-based combinations. Delaunoit et al. carried out a review of 45 consecutive patients with PNETs treated with the DOX–STZ regimen. According to WHO criteria, 36 % achieved a PR, 16 % had a MR, 9 % had SD and 40 % had PD [7]. Patients treated with DOX/STZ first-line had a median survival of 22.4 months compared with 5.5 months for patients previously treated with chemotherapy ($P = 0.013$). Fjallskog et al. also reported a response rate of 40 % in 30 patients treated with STZ and a liposomal formulation of DOX [10].

STZ-based combinations using three drug regimens have also produced encouraging results. Kouvaraki et al. reported the outcome for 84 patients with PNETs treated with the 4 weekly FAS regimen (5FU 400 mg/m^2 D1-5, STZ 400 mg/m^2 D1-5 and DOX 400 mg/m^2 [12]. The response rate was 39 % while 50 % had SD and 11 % had PD. Four responding patients were able to have curative resection. The median duration of response was 9.3 months, and the median time to response (TTR) was 3.9 months. None of the 11 patients with metastatic gastrinomas responded to chemotherapy, compared with 45 % (33/73) of patients with all other tumour types. Overall, median PFS and OS were 18 and 37 months, respectively. The extent of liver disease (>75 %) was significantly associated with a shorter PFS and OS. Most recently, Turner et al reviewed outcome for patients treated with the FCiSt combination (5-FU 500 mg/m^2, STZ 1 g/m^2 and cisplatin 70 mg/m^2) given on D1 of a 21 day cycle. Where glomerular filtration rate (GFR) was less than 60 ml/min cisplatin was substituted with carboplatin AUC 5 mg/ml/min. Of 47 evaluable patients with PNETs, 38 % had a PR, 51 % had SD and 11 % had PD. The median TTR was 20 weeks [28].

Table 5.2 Outcomes for streptozocin-containing regimens in non-pancreatic NETs

Publication	Chemotherapy	Number of patients	RR (%)	PFS (months)	OS (months)	P value For RCT for OS
Moertel and Hanley [15]	STZ + 5-FU	42	33[a]	–	–	
	STZ + C	47	26	–	–	
Engstrom et al. [8]	STZ + 5-FU	86	22[a]	–	14.7	NSD
	DOX	86	21	–	11.1	
Oberg et al. [20]	STZ + 5-FU	24	8[a]	–	18	
Bukowski et al. [2]	FAC-S	56	31[a]	–	12.9	
	FC-S	9	22	–	7.6	
Sun et al. [26]	DOX + 5-FU	85	16	4.7	15.7	0.0267
	STZ + 5-FU	78	16	4.8	24.3	
Dahan et al. [6]	STZ + 5-FU	32	3	5.5	30.4	0.83
	IFN − α	32	9	14.1	44.3	
Turner et al. [28]	5FU/CIS/STZ	33	25	–	–	

[a] RR assessed using tumour measurements and biochemical response

STZ streptozocin; *5-FU* 5-fluorouracil; *DOX* doxorubicin; *C* cyclophosphamide, *CIS* cisplatin; *RR* response rate; *PFS* median progression-free survival; *OS* median overall survival; *FAC-S* 5-fluorouracil, adriamycin, cyclophosphamide, streptozocin; *FC-S* 5-fluorouracil, cyclophosphamide, streptozocin; *IFN-α* interferon-alpha; *CIS* cisplatin

In summary, for PNETs, there is evidence from randomised trials that combination of STZ-based chemotherapy is associated improved survival and response rates from recent retrospective series applying WHO or RECIST criteria are in the region of 30–40 %.

Chemotherapy for Non-Pancreatic NETs

The first randomised trial exploring STZ in metastatic NP-NETs was reported by Moertel and Hanely in 1979 [15] (Table 5.2). In this study, the combination of STZ with 5FU or cyclophosphamide was compared and response rates of 44 and 37 %, respectively, were reported among the mid-gut NETs using combined radiological or biochemical criteria. Two further randomised trials have been undertaken in this group. The first study reported the outcome of EST 5275 Eastern Cooperative Oncology Group (ECOG) trial that recruited 210 patients between 1976 and 1981. In an attempt to reduce the toxicity, a less intensive 10 week cycle was used for the combination of 5FU/STZ (5FU 400 mg/m^2 D1-5 and D36-40, STZ 500 mg/m^2 D1-5) and DOX was administered at 60 mg/m^2 D1, 22, 43 and 4 weekly thereafter. Of 161 patients treated first-line, the response rate for the 5FU/STZ combination was 22 % and for DOX was 21 % using radiological, clinical or biochemical criteria. Two patients on the 5-FU/STZ arm died following myelosuppression, and 2 patients treated with DOX died of heart failure.

One patient also died of renal failure having received a total of 43.2 g STZ. Median OS was 48 weeks for DOX and 64 weeks for 5-FU/STZ but the difference was not significant. Since the median duration of response was short (26 and 31 weeks for DOX and 5-FU/STZ, respectively) and there were only 3 cases of CR, the authors felt that neither regimen should be considered as standard therapy for 'carcinoid' tumours.

A follow-up ECOG trial (study E1281) randomised 176 patients with metastatic carcinoid tumour to receive either 5-FU/STZ according to the same schedule as EST 5275 or 5-FU/DOX (5FU 400 mg/m 500 mg/m^2 D1-5 and DOX 40 mg/m^2 D1 both 5 weekly) [26]. Among the 163 evaluable patients, 2.4 % had CR and 13.5 % had PR in the 5-FU/DOX arm while 16 % had PR in the 5-FU/STZ arm according to WHO criteria. Overall, median PFS was 4.7 months. Further analysis showed that PFS was superior in those patients with a better PS ($P = 0.0013$). Median OS was 18.4 months, with 5-FU/STZ demonstrating a significantly improved OS in comparison with 5-FU/DOX (24.5 vs. 15.7 months; $P = 0.0267$). Significant toxicity was observed in some patients with four treatment-related fatalities. In the 5FU/DOX group, there were 2 deaths from infection and one from liver failure while in the 5FU/STZ arm one patient died from haematologic toxicity. Also in the 5FU/STZ arm, renal toxicity was reported in 34.8 % patients and two had life-threatening renal failure. More recently, a small randomised controlled trial compared 5FU/STZ to interferon in which the majority of patients were mid-gut tumours NETs and only one of the 32 patients (3 %) had a partial response by WHO criteria [6]. Among a retrospective series of patients treated with FCiSt regimen described above, 25 % of the 32 NP-NETs responded by RECIST criteria [28].

In summary, the response rate for NP-NETs appears lower than for PNETs when either combined or conventional response criteria are applied. Furthermore, only one study has demonstrated improved survival comparing two treatment arms suggesting that 5FU/STZ is the current standard.

Delayed Response to Chemotherapy

Unlike most tumours, the response of NETs to cytotoxic therapy can be slow, and delayed response (DR) has been demonstrated in at least two studies. Kouvaraki et al. [12] reported a median TTR for 3.9 months (range, 1–14) in patients with PNETs treated with FAS regimen and argued that the DR warranted persisting with therapy to achieve response. However, Turner et al. also observed DR in 19 % of responding patients treated with FCiSt who had SD at the end of the treatment but had ongoing tumour shrinkage during post-treatment surveillance [28]. The median TTR was 4.4 months (range, 2–11) that was similar to that reported by Kouvaraki et al. These observations suggest that response may be underestimated unless post-treatment surveillance is undertaken. The mechanism of the DR requires further work to understand the underlying biology.

Predictors of Response to Chemotherapy

For some molecularly targeted agents, efficacy requires the expression of a known actionable target. However, for cytotoxic chemotherapy, predictors of response are less well defined. Tumour grade, as defined by the WHO criteria, has clearly been shown to be prognostic in NETs [23, 24] and has also been proposed as a means of selecting patients for chemotherapy [29]. Some evidence for this has been provided by Turner et al. who found that both mitotic index (MI) and Ki67 were associated with response to chemotherapy [28]. For MI, the RR increased from 15 % for tumour with MI 0-1 to 55 % for MI $\geq$ 5 while for Ki67, RR increased from 18 % for a ki67 $<$ 10 to 52 % for Ki67 $>$ 24 %. O'Toole et al. investigated a number of therapeutic biomarkers that could predict response to chemotherapy in 46 patients with gastrointestinal NETs who were receiving systemic chemotherapy. Overall, human mutL homologue 1 (hLMH1) and phosphatase and tensin homologue (PTEN) expression correlated with treatment response, whereas Ki67 and p53 expression were associated with lack or response or progression on therapy. High mean hLMH1 and PTEN expression were significantly associated with a response to therapy for patients on STZ and less significantly with DOX. Ki67 and p53 expressions were associated with progressive disease on STZ. Weak expression of Akt and CA9 was associated with a response to DOX. No markers were associated with a response to 5-FU, although lack of response was associated with Ki67, multidrug resistance protein-1 (MDR-1) and p53 [19]. There is also interest in the expression of DNA repair enzyme O6-methylguanine DNA methyltransferase (MGMT) as a predictive marker of response to alkalating agents. MGMT removes the cytotoxic O(6)-alkylguanine adducts from DNA contributing to resistance to alkylators. Promotor methylation leading to epigenetic silencing of *MGMT* results in reduced protein expression and increased benefit from TMZ in patients with glioblastoma [11]. However, the results in NETs are conflicting. In a small series of 16 carcinoid tumours and 11 PNETs, methylation of MGMT was more common in carcinoid tumours than PNETs (25 % vd 0 % $p = 0.03$) [4], and in a recent study in poorly differentiated NETs, only one of 25 had MGMT methylation [31]. In contrast, Kulke et al. found MGMT was deficient in 57 % (19/37) PNETs and 0 % (0/60) of carcinoid tumours and that MGMT expression was associated with response to TMZ. The discrepancy may arise from the techniques applied since some studies examine promoter methylation while others use immunohistochemical analysis of protein expression. Whether MGMT levels impact on sensitivity to STZ is an important question that has not been addressed in NETs.

Toxicity Associated with STZ

STZ is selectively transported into cells by the glucose transport protein GLUT2 and is used experimentally to induce diabetes through destruction of the pancreatic β-cells that express high levels of GLUT2 [30]. Diabetes is therefore a potential

toxicity for patients undergoing treatment, and some investigators have used lower doses in diabetics but to our knowledge diabetes has not been reported clinically relevant toxicity when using STZ for the treatment for NETs. In some studies, nephrotoxicity has proved to be a significant problem associated with STZ. In Moertel's 1980 trial, one third developed mild renal impairment [16] but of the 105 patients treated in a study by Moertel (1992), 9 developed renal failure and 7 required dialysis [17]. It is not clear what proportion of those with severe renal impairment were treated with chlorozotocin; however, 11 of 82 patients treated with STZ-containing regimen were reported to have chronic renal insufficiency. In the E1281 trial, 40 patients (34.8 %) of those patients allocated to STZ/5FU had renal toxicity, and although this was mild to moderate in most cases, two patients had life-threatening renal failure [26]. However, there was no grade 3 or 4 nephrotoxicity in a number of recent studies, suggesting that a proactive approach to monitoring and dose reductions may avoid major toxicity [7, 12, 28]. STZ is also highly emetogenic, and in early studies, nausea and vomiting were common [16, 17], but improvements in supportive therapy and the introduction of 5HT3 antagonists have significantly reduced the severity of this side effect. Recent studies report grade 3 or 4 nausea and vomiting in 1–17 % patients [7, 12, 28]. As with all cytotoxics, myelosuppression is a side effect of STZ particularly when used in combination but is only relevant when associated with sepsis.

The Place of Chemotherapy in the Therapeutic Algorithm

Recently, the tyrosine-kinase inhibitor sunitinib and the mTOR inhibitor everolimus have been approved for the treatment for well-differentiated PNETs on the basis of two multicentre placebo-controlled randomised trials [22, 33]. In both trials, the primary endpoint was PFS and both drugs were associated with an improved PFS of around 6 months as compared with placebo. The predominant benefit of both drugs appeared to be disease stabilisation, and objective response rates were at 9.3 % for sunitinib and 5 % for everolimus. Everolimus has also been evaluated in NP-NETs with carcinoid syndrome in combination with octreotide long-acting repeatable (LAR). Compared with LAR alone, the PFS improved from 11.3–16.4 but this did not meet the pre-specified statistical boundary for significance. The on-going Radiant-4 trial is evaluating everolimus in non-syndromic patients NP-NETs. There are no phase III data for sunitinib in NP-NETs but in a phase II trial, the response rates were only 2.4 % in carcinoid tumours compared with 16.7 % in PNETs [13].

For PNETs, there are now three treatments with level 1 evidence for efficacy, and the clinical challenge is to define the appropriate algorithm for their use. Drug resistance remains a significant challenge and all patients ultimately progress on any given therapy. Since patients often live with their disease for many years, it is now likely that patients will progress through several lines of different treatment and the selection of first-line treatment is a key question. The evidence to date

suggests that chemotherapy is associated with a superior response rate particularly in those with a higher Ki67 % proliferation index. Therefore, STZ-based chemotherapy appears to be the rational first-line therapy for PNETs with a proliferation rate above 10 %. In addition, it should be first-line in those patients who are symptomatic from tumour burden or those with locally advanced disease who may become surgically resectable with down-staging therapy. For NP-NETs, the response rates to chemotherapy appear less but there are no other therapies associated with a superior response rate to date and chemotherapy remains an option in selected patients.

Conclusion

STZ-based chemotherapy is an effective and important treatment option for patients with NETs. In PNETs, there is a proven survival advantage and the response rates are superior to other interventions. Further work is required to define the best cytotoxic regimen and evaluate the role of drugs such as TMZ in comparison with STZ. Advances in molecular pathology may also help to further define predictive markers so that patients may be appropriately stratified to maximise benefit and minimise toxicity. Trials are also required to establish the optimum sequence of therapy during the course of, what is commonly, a chronic illness. The relative rarity of this disease and heterogeneity require international collaboration and the successful completion of the recent trials of sunitinib, and everolimus demonstrated that this is achievable.

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Chapter 6
Place of Surgical Resection in the Treatment Strategy for Gastrointestinal Neuroendocrine Tumors

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Abstract Neuroendocrine tumors (NETs) of the digestive tract are usually slow-growing neoplasms carrying an overall favorable prognosis. Even if most of patients have metastatic or locally advanced tumors, surgery, from resection to transplantation, remains the only potential curative option for these patients and should always be considered. Nevertheless, because of the very few randomized controlled trials available, the optimal place of surgery within a global treatment strategy remains controversial.

Keywords Neuroendocrine tumors · Surgical resection · Primary tumor · Liver metastases · Debulking surgery · Transplantation · Surgical strategy

Introduction

Neuroendocrine tumors (NETs) of the digestive tract NET are usually slow-growing neoplasms carrying an overall favorable prognosis compared with their adenocarcinoma counterpart. These fascinating tumors are best treated with a multimodal management including oncologists, radiologists, pathologists, and surgeons. Despite an extensive scientific literature on these tumors, high-level evidence are sparse and the optimal treatment for patients with NET remains

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controversial [1] and should be tailored according to tumor's characteristics, especially their site, grade, and relation to genetic syndromes. Surgical resection definitely has a cornerstone role within a global treatment strategy. Nevertheless, if there are some recent large randomized controlled trials for medical treatment for metastatic NET, there is, to date, no randomized trial concerning surgical management or comparing different treatment strategies. Overall, surgery, from resection to transplantation, remains the only potential curative option for these patients and should always be considered, even in the presence of synchronous metastases or locally advanced tumors [2–18].

We herein discuss the role of surgical resection in the treatment for NET of the digestive tract.

Preoperative Workup: The Surgeon's Perspective

From the surgeon's perspective, preoperative workup aims:

- To exclude genetic syndromes such as multiple endocrine neoplasia type 1 (MEN 1), von Hippel–Lindau's disease, or neurofibromatosis type 1. Indeed, these inherited diseases call for a specific preoperative workup, management, as well as postoperative follow-up.
- To characterize the primary tumor, i.e., evaluate its local extension and relationship to adjacent organs, stage the regional (node) and distant (liver, bone, lung) disease extension and assess its secreting status.
- To assess, when possible, the natural history of NET, which is highly variable, depending of tumor location, secretion, size, Tumor, Node, Metastasis (TNM) status, differentiation, and grade. It is important to remember that most of them are slowly growing neoplasms.
- And, finally, to estimate the benefit–risk balance of surgery for a specific patient in order to tailor the management.

This preoperative workup is best multimodal including clinical examination, biological tests, and tumor markers [such as chromogranin A, urine 5-hydroxyindoleacetic acid (5-HIAA) or specific secreted hormones (gastrin, glucagon, somatostatine, etc.)], and various imaging modalities including computed tomography (CT), magnetic resonance imaging (MRI), and nuclear imaging. High-quality CT scan, including three phases spiral multidetector CT acquisitions with contiguously reconstructed sections, as well as dedicated liver MRI sequence are required, especially for liver metastasis detection [19]. In this setting, high sensitivity of diffusion-weighted MRI appears to be a useful tool [20]. Enterography (by CT or MRI) can be extremely helpful in detecting small intestinal lesions, especially when liver metastases are discovered but no primary tumor is found through other tests [21, 22]. In this specific condition, colonoscopy can be useful in searching for colorectal and terminal ileus neoplasms as well. Technique of nuclear imaging

Table 6.1 WHO 2010 histological classification of neuroendocrine tumor [29]

Grade	Mitotic per 10 high-power microscopic fields (HPF)	Proliferative index/Ki-67 index (%)
Low grade (G1)	<2	≤2
Intermediate grade (G2)	2–20	3–20
High grade (G3)	>20	>20

should be chosen according to tumor size, location, and characteristics: for all NET, ^{111}In-somatostatin receptor scintigraphy; for pancreatic, NET ^{68}GA-DOTATOC PET; for aggressive NET, ^{18}F-FDG-PETs, and for small bowel NET, ^{18}F-DOPA-PET, even though there is, at the present time, no clear consensus on the choice of tracer [23–26]. Endoscopic ultrasound is a very accurate tool to detect small pancreatic NET, especially sporadic insulinoma [27], and to stage gastroduodenal and rectal NET.

Histoprognostic Classification

Several classifications, recently updated, have been used for NET [28]. The World Health Organization (WHO) recently published an update on its classification for neuroendocrine tumor (Table 6.1) [29]. This histological classification divides neuroendocrine tumor into grades, according to mitotic rate and proliferative index (Ki-67 assessed by immunohistological staining). This classification is the one that now should be used in addition to the seventh TNM UICC stage [30]. If the value of the new 2010 WHO classification has been recently confirmed [31], it raises question especially regarding G3 tumors. Indeed, tumors with a mitotic count and/or Ki-67 above 20 % can have well- or poorly differentiated features, and consequently very different biological behavior, requiring different therapeutic strategy.

Aim of Surgery

Surgery aims either to provide/improve local control of disease burden, or to stop the natural course of the disease and, above all, definitively cure the patient. Treatment strategy and surgical indications are highly variable according to tumor site, genetic origin, local and regional extension, and biological behavior of the tumor.

Despite lack of high-level evidence such as randomized controlled trials, surgical strategies for NET are now better defined. Most of these recommendations are detailed in the European Neuroendocrine Tumor Society (ENETS) guidelines

Table 6.2 Gastric neuroendocrine tumor: risk of malignancy, surgical indication, and disease-specific mortality

Tumor type	Risk of malignancy	Surgical indication	Disease-specific mortality (%)
Type 1	Very low (2–5 %)	Surgery if >1 cm, not endoscopically resectable, deep parietal invasion (beyond submucosa), nodal or distant spread	±0
Type 2	Low (10 %)	Local excision surgery if large or positive margins	<10
Type 3	High (>50 %)	Formal gastrectomy in all cases	25–30

recently published and available online (www.enets.org). If some surgical indications are consensual, surgery should also sometimes be avoided.

Overall, surgical resection is rarely an "oncological emergency," and a temporary "*wait and see*" policy is often acceptable to better assess the tumor natural history. If some surgical indications are consensual, surgery should also sometimes be avoided. Considering the rarity of such disease, surgery needs to be discussed on a case-by-case basis in a multidisciplinary neuroendocrine tumor board.

When Surgery is Required

First, it is noteworthy that surgery is the single most effective therapy for NET. Whatever abdominal procedure is planned, cholecystectomy should always be performed during primary resection of the tumor [32]. This is justified by the risk of gallstone-related complications or acute cholecystitis if patients are later treated with somatostatin analogs or liver arterial embolization [33, 34].

Some surgical indications are consensual, because of the clear benefit on long-term outcome.

Gastric Neuroendocrine Tumor

Gastric NETs represent less than 10 % of digestive NET. They can be due to chronically elevated gastrin (ECLomas) because of achlorydia from atrophic fundic gastritis (type 1, the most frequent) or to gastrin tumoral secretion (type 2, associated with Zollinger–Ellison Syndrome—ZES) (Table 6.2). Patients with type 1 or type 2 gastric NET above 1 cm with deep gastric parietal wall invasion and/or positive margins after endoscopic resection should undergo surgical resection [35–37]. It is important to recognize the low malignancy risk of type 1 lesions. These lesions can be treated either by local resection or antrectomy (by open or laparoscopic approach) [38], and this latter anatomical resection can, in theory, suppress the source of gastrin and decrease recurrence rate [35]. Overall,

total gastrectomy should be avoided when possible, and its indications are limited to widely diffuse and large or malignant lesions. Whatever procedure performed, endoscopic surveillance is recommended thereafter [39].

Regarding rare sporadic primary gastric NET (type 3), they carry an overall dismal prognosis and should undergo curative-intent (R0) resection, by formal gastrectomy according to tumor location with regional lymphadenectomy, similar to gastric adenocarcinoma.

Duodenopancreatic Neuroendocrine Tumor

Insulinomas are the most common functioning endocrine neoplasms of the pancreas, frequently presenting with nonspecific symptoms due to hypoglycemia, such as weakness, confusion, headaches, sweating, tremors, palpitation, and visual disturbances. They are most of the time sporadic and benign, with less than 5 % of them being associated with MEN 1 and less than 10 % being malignant (i.e., with distant or nodal metastasis) [40, 41]. Accurate preoperative localization and characterization (including endoscopic ultrasonography (EUS) [42, 43], triphasic CT scan, or MRI [44]) are mandatory in order to tailor surgical treatment and to avoid blind distal pancreatectomy as it used to be recommended. Usually, allow adequate tumor localization and can avoid invasive exams such as intra-arterial calcium stimulation with hepatic venous sampling [44, 45]. Additionally, EUS can accurately assess relationship between the tumor and the main pancreatic duct. Surgery is the treatment of choice with an overall cure rate close to 100 % for benign lesions, if complete resection is achieved [46]. Procedures should be performed by an experienced team in pancreatic surgery and can be either performed laparoscopically or through open approach [41, 47]. After additional intraoperative localization of the tumor by palpation and ultrasonography, the lesion can be either enucleated or resected by standard pancreatectomy such as pancreaticoduodenectomy or distal pancreatectomy. Whenever possible, parenchyma-sparing resection [48] including central pancreatectomy or enucleation should be preferred because of a better long-term exocrine and endocrine function. Enucleation is best indicated for small benign lesions located in the head of the pancreas and far enough, i.e., 2–3 mm, from the main pancreatic duct [49]. Interestingly, preoperative EUS can accurately help the surgeon to choose the adequate surgical procedure assessing relationship between the tumor and the main pancreatic duct.

In patients with MEN 1, insulinoma can be multiple in about 10 % of patients and one should keep in mind of it in order to locate all lesions, pre and/or intraoperatively, thus avoiding blind pancreatectomy.

Treatment for other rare duodenopancreatic secreting lesions is in first line surgical resection [50], as for sporadic gastrinoma, glucagonoma, or vipoma. Most of these lesions are malignant, and standard pancreatectomy with formal lymphadenectomy is required. Regarding duodenal gastrinoma that even when malignant, often grows slowly, routine duodenotomy during surgical exploration usually allows accurate identification, and consequently, local resection can be

performed. It is important to note that local lymphadenectomy should be systematically performed in order to decrease recurrence rate. Nodal extension of disease can occur in about 45 % of both duodenal and pancreatic gastrinomas [51].

Nonsecreting tumors began to be more incidentally diagnosed because of the widespread use of cross-sectional imaging, representing up to 75 % in recent surgical series [52, 53]. Their natural history is heterogeneous and difficult to assess during preoperative workup, and whether an incidental finding is associated with improved prognosis is still a matter of debate [52, 53]. Nevertheless, size being an important prognostic factor, surgical resection with regional lymphadenectomy is required for lesions above 2 cm [52]. Indeed, in this setting, the risk to develop metastatic disease during the follow-up is above 10 % [54].

Small Bowel Neuroendocrine Tumor

They represent from 25–40 % [55–57] of gastrointestinal NET. They mostly occur after 60 years of age, can be multiple in up to 40 % of cases, and they present with carcinoid syndrome in about a quarter of patients [55, 57]. Their prognosis is not as good as for other gastrointestinal tumors justifying an aggressive surgical management. However, the overall survival is around 60 % and can be up to 85 % in cases with curative resection [17, 58]. If possible, primary should be localized before surgery, using double balloon enteroscopy, capsule endoscopy, CT/MRI enterography as well as cross-sectional, and nuclear imaging such as 18F-DOPA-PET.

Even small and asymptomatic lesions need to undergo surgery because lesion size does not correlate with biological behavior, and they can be associated with node or liver metastases [15]. Small bowel lesions need to go through segmental resection with a formal wide lymphadenectomy including all gross metastatic nodes even when they are located around the superior mesenteric artery origin. Nevertheless, a special attention must be paid to avoid large resections leading to small bowel syndrome. Surgery for the primary tumor should always be considered even in the presence of metastatic disease, since the primary lesion can be responsible for local complications such as intussusception, small bowel obstruction, or ischemia. Additionally, also some lesions can present with important peritumoral fibrosis involving the mesentery root and the retroperitoneum, leading to occlusion, hydronephrosis, and chronic pain. Studies showed better results among patients who had at least their primary tumor resected along with nodal resection, with a better disease-free and overall survival [15, 17]. Since abdominal complications remain one of the major causes of death, we believe that in the setting of unresectable lesion, 90 % cytoreductive surgery can be considered, if a long enough small bowel can be conserved [56]. Surgery is at best performed after medical control of carcinoid syndrome, if present, by somatostatin analogs. Preoperatively, a special attention must be paid to carcinoid heart disease assessment in case of patient with carcinoid syndrome. Up to now, laparoscopic approach in this setting has been poorly studied, but we believe that open approach

should be preferred in order to explore the full length of small bowel and to achieve a large lymphadenectomy.

Despite complete surgical resection of small bowel carcinoids, recurrence can occur in about 30–40 % of cases [16, 59]. Liver recurrence is best treated with resection in the case it is possible, which occurs usually in less than 20 %. Otherwise, other modalities of local treatment such as chemoembolization and pure embolization can be used as well as systemic therapy, from somatostatin analogs to chemotherapy.

Appendiceal Neuroendocrine Tumor

Appendiceal NET is currently diagnosed incidentally after appendectomy. They represent about a third of all gastrointestinal endocrine tumors and are the most benign of carcinoids. Although the overall 5-year survival is around 85 %, size is the most important prognostic factor. Tumors below 1 cm are cured by appendectomy alone, without need for any additional treatment. For tumors above 2 cm, a right colectomy is needed [60], due to the risk of lymph node extension. Between 1 and 2 cm, the risk-to-benefit ratio needs to be determined on a case-by-case basis.

Colonic and Rectal Neuroendocrine Tumor

These rare tumors are often incidentally diagnosed during colonoscopy and are nonfunctioning in most of the cases.

For well-differentiated colonic tumors, colonic resection with standard oncologic criteria should be performed in a similar way to adenocarcinoma, because of the poor 5-year prognosis of these tumors, between 40 and 70 %.

For well-differentiated rectal tumors below 1 cm, developed within the submucosal layer (T1) and without nodal involvement on preoperative EUS and MRI, local resection, either endoscopical or surgical is appropriate. For tumors above 2 cm, standard oncologic anterior resection is required. Between 1 and 2 cm, the risk-to-benefit ratio needs to be determined on a case-by-case basis.

Concerning endoscopic resection, endoscopic submucosal resection has replaced standard polypectomy as the preferred technique for tumors below 1 cm, and newer techniques such as submucosal resection with band ligation or endoscopic submucosal dissection are likely to be associated with less residual disease [61]. Submucosal resection with band ligation has the advantages of being easier to perform, demanding a shorter procedure time and having better negative margin rates; thus, it may be considered the treatment of choice for small rectal carcinoid tumors [62, 63].

Table 6.3 Frequency and type of pancreatic neuroendocrine tumors in patients with genetic syndrome

Genetic syndrome	Frequency of pancreatic neuroendocrine tumors (%)	Types of pancreatic neuroendocrine tumor
MEN 1	80–100	Nonfunctioning tumors, gastrinomas, insulinomas
VHL	12–20	Usually nonfunctioning tumors
NF-1 (von Recklinghausen)	<10	Mainly somatostatinoma; gastrinomas, insulinomas, and nonfunctioning tumors
Tuberous sclerosis	<5	Nonfunctioning tumors, gastrinoma, insulinoma

Table 6.4 Types of tumor and prevalence in MEN type 1 patients

Type of tumor	Prevalence (%)
Nonfunctioning	80–100 % (microadenomas); 55–80 % (>1 cm)
Gastrinomas	25–50
Insulinomas	20
Glucagonomas	3
Vipomas	1
Somatostatinomas	1
GRHomas	1

Neuroendocrine Tumor Within a Genetic Background

NET can be associated with multiple endocrine neoplasia type 1, or less frequently with von Hippel–Lindau's disease, von Recklinghausen's disease, or tuberous sclerosis (Table 6.3) [64].

Regarding MEN 1, tumors such as nonfunctioning tumors above 2 cm [65], glucagonoma, vipoma, somatostatinoma, or GRFoma should undergo standard resection, since malignant NET is one of the main determinants of long-term survival in this disease. Insulinoma [66] represents a formal indication of resection because of its potentially harmful secretion (Table 6.4) [67].

In VHL disease, pancreatic NETs are usually nonfunctioning and have a better prognosis than sporadic nonfunctioning pancreatic NETS (metastatic disease in 10–20 vs. 60–90 %), and patients are usually younger about 30 years [68–70]. Tumors can also be multiple in about 20 % and locate throughout the pancreas. Usually, nonsurgical management is recommended for those patients, but criteria predicting poor prognosis have been described, being: tumor size $\geq$3 cm, tumor doubling time $\leq$500 days, and mutation in exon 3. In the case that more than two of these criteria are present, surgery is to be considered. Otherwise, surveillance with CT/MRI can be advocated [68, 69]. Patients with VHL should be screened for pancreatic lesions starting at 12 years of age and resecting PNETs should be considered if the patient is having an exploratory laparotomy for another manifestation of VHL [70].

NETs related to NF-1 are usually somatostatinomas frequently located around the papilla. These tumors metastasize in one-third to half of cases irrespective of primary tumor size. Although some experts advocate local excision for tumors smaller than 2 cm and surgical excision for tumors larger than 2 cm, as many as 70 % of surgical cases are associated with regional or liver metastasis, an aggressive surgical approach is best indicated in the form of pancreaticoduodenectomy [70–73].

Tuberous sclerosis-related pancreatic NETs are very rare (about 10 cases reported in the literature), and pancreatic NET in these patients can be both nonfunctional and functional. Because of the possible malignant potential of these tumors, surgical resection should be considered [51, 70, 74].

When Surgery Should be Avoided

Surgery should only be avoided after a complete clinical, biological, and imaging workup and discussion in a multidisciplinary neuroendocrine tumor board. Workup should include complete clinical examination, static and dynamic biological tests adapted to tumor origin and secretion. Imaging workup should at least include thoraco-abdominal CT scan, to assess tumor burden. When liver is involved, because of the frequent numerous small metastases [75], a liver MRI should be added routinely. If in doubt, nuclear imaging should be performed to better assess tumor biology (^{18}F-FDG-PET CT) or tumor burden (^{111}In-somatostatin receptor scintigraphy, ^{68}GA-DOTATOC PET, or ^{18}F-DOPA-PET).

Gastric Neuroendocrine Tumor

In patients with gastric NET associated with chronic atrophic gastritis (type 1) or with Zollinger–Ellison syndrome with multiple endocrine neoplasia type 1 (type 2), tumors under 1 cm can undergo endoscopic resection and follow-up with yearly endoscopic surveillance (every 6–12 months) with gastric biopsies is necessary [35–37], because of their very low risk of lymph node extent.

High-grade and Poorly Differentiated Tumors

These tumors are characterized by a mitotic count over 20 per high-power field and/or a Ki-67 index over 20 % and a poorly differentiated pathologic pattern. These tumors have a specific and very aggressive biological behavior totally different from G1 and G2 tumors. Platin-based chemotherapy is the first line treatment for these tumors. Surgical indications are very limited and can be considered for very localized tumors and/or tumors well controlled under chemotherapy.

When Surgery Should be Discussed

Metastastic Tumor to the Liver

The presence of metastases, most commonly located in the liver, is a major adverse prognostic factor [76–78] and affects up to 75 % of patients. Metastases are often bilobar, numerous, and synchronous. Despite a lack of high-level evidence, complete (R0) resection is the only hope for cure in these patients, and an aggressive surgical approach—including resection of metastatic liver disease—is widely accepted [2–18]. Nevertheless, intra- and extrahepatic recurrences are frequent, and a careful patient selection is needed. Patient selection is based on operative risk and general status, but mainly on tumor biological behavior. Surgery should be only discussed for patients with well-differentiated tumor (G1 and G2), at low risk of postoperative mortality and with no extrahepatic disease, when R0 resection is technically doable. On a technical point of view, two-step hepatectomy or intraoperative ablation enables complete resection with low mortality in patients with bilobar liver metastases, with a 5-year overall survival above 90 % and a 5-year disease-free survival around 50 % [79]. It is important to note that metastases are sometimes far more numerous than what is previously suggested by imaging [75].

Unresectability is defined as the technical impossibility to achieve R0 resection: metastases involving the right or left hepatic pedicle and abutting the contralateral pedicle, or involving or abutting the vena cava, or involving two hepatic veins and abutting the third one, or lesions that would leave <25 % of functional liver after resection. It is important to note that unresectability should be only defined par an experienced team including a hepatobiliary surgeon. Whether, in this setting, debulking surgery is justified remains highly controversial and should be limited to highly selected cases if at least 90 % of the tumor could be resected.

A recent meta-analysis demonstrated that surgery was superior to chemoembolization or other treatment modalities (non surgical) in the treatment for liver metastases of NET, with a significant longer survival [80].

Orthotopic liver transplantation has been proposed as an alternative option. Indeed, liver metastases are one of the main causes of dead, are usually confined to the liver for a long time, and can be responsible of debilitating symptoms.

Main criteria for transplantation are the absence of extrahepatic disease, a low Ki-67, and symptomatic disease refractory to previous therapies. In a French multicentric study, overall 5-year survival was 50 % and poor prognostic factors included upper-abdominal exenteration, primary tumor in duodenum or pancreas and hepatomegaly [81]. A review including 150 transplanted patients extracted from the UNOS database [82] demonstrated a long-term survival similar to that of patients with hepatocellular carcinoma, especially in patients with stabilized disease. Additionally, a recent European multicentric study of 213 patients showed an overall survival of up to 60 % after liver transplantation and suggested hepatomegaly, concurrent resections and age over 45 years as poor outcome predictors in the more recent cases [83]. Nguyen et al. also showed 5-year survival rate of near

Table 6.5 Survival after liver transplantation for neuroendocrine tumors

Authors/ years	Number	Prognostic factors	5-year disease-free survival (%)	5-year overall survival (%)
Le Treut/2008	85	Upper-abdominal exenteration Primary tumor in duodenum or pancreas, hepatomegaly	20	47
Gedaly/2011	150	Progressive disease	30	49
Nguyen/2011	184	Higher donor creatinine level; need for early retransplantation	–	About 50
Máthé/2011	89	Age over 55 years; Simultaneous pancreatic resection	–	44
Le Treut/2013	213	Hepatomegaly Concomitant resections Age over 45 years	30	52

60 % after 2002 and introduction of the Model for End-Stage Liver Disease (MELD) criteria [84]. Máthé et al. [85] found that age over 55 years and simultaneous pancreatic resection were poor prognostic factors in a study with 89 patients (Table 6.5).

In conclusion, in highly selected patients with unresectable liver metastasis, liver transplantation is a valid option that should be considered and discussed on a case-by-case basis. But still, optimal time for the transplant, if during stable or progressive disease, remains unclear [83].

Small Nonsecreting Pancreatic Neuroendocrine Tumor and Incidentaloma

Whether NET discovered as incidentalomas carry a better prognosis remains controversial [52, 53, 86]. If operative management is mandatory for symptomatic lesions, nonoperative management can be discussed on a case-by-case basis according to the individual risk-to-benefit evaluation. Since tumor size correlates with malignancy [52], for lesions below 2 cm and especially for those below 1 cm requiring aggressive surgery such as pancreaticoduodenectomy because of their anatomical localization, surveillance should be considered as an option. In this setting, preoperative assessment of grade or malignancy remains difficult. Fine needle aspiration cytology could estimate the Ki-67 value preoperatively, but its accuracy has been assessed in small series only [87, 88]. New imaging modalities, such as perfusion CT [89], diffusion-weighted MRI [90], or nuclear medicine assessment, including 18-FDG-PET [91], are currently in development and could help to identify tumors with a more aggressive behavior, which will need to be resected.

Appendiceal Neuroendocrine Tumor

Surgical indications should be discussed on a case-by-case basis for tumors between 1 and 2 cm, and patients are usually warranted a right hemicolectomy according to the presence of deep mesoappendiceal invasion (>3 mm), positive lymph node, positive margin, microscopic lymphatic, or venous invasion.

Neuroendocrine Tumor Within a Genetic Background

In patients with MEN 1, the situation is controversial regarding small, i.e., below 2 cm, often multiple nonfunctioning tumors [65]. A better understanding of their natural history shows most of the time-indolent growth, and regular follow-up can be advocated. Resection can be discussed when tumor is growing, especially over 2 cm.

If insulinoma should always be resected, the situation regarding gastrinoma is more complex. Treatment for gastrinomas in patients with MEN 1 has evolved with the development of highly efficient drugs such as proton pumps inhibitor to treat gastric acid hypersecretion. Gastrinomas are present in about half of patients with MEN 1, and their management has long been controversial [51]. If diagnosis is most of the time easily done, tumor localization is more challenging. Gastrinomas are often small, multiple, associated with metastatic lymph node and located in the duodenum in about 80 % of cases, but hard to accurately localized even with duodenotomy and intraoperative endoscopy. Local resection or duodenectomy is unlikely to cure patients, whom, however, have a long life expectancy. Pancreaticoduodenectomy has been advocated by some [92] with encouraging results, but is not recommended by most of the teams because of the postoperative mortality of this procedure and its long-term side effects for a slow-growing neoplasm. Overall, resection could be recommended for lesions above 2 cm because of a higher risk of aggressive tumor growth and liver metastatic disease [65]. It is important to take into account that pancreatic surgery should only be done after surgical treatment for hyperparathyroidism and should include local lymphadenectomy.

Conclusion and Perspectives

NET represents a wide range of neoplasms with various biological behaviors. Treatment strategy pursued for each patient needs to be individualized and discussed in a multidisciplinary tumor board specialized in neuroendocrine tumor. Surgery represents the only chance for cure and should always be discussed, even in the setting of advanced or metastatic disease.

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Chapter 7
Liver-Directed Therapies in Neuroendocrine Tumors

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Abstract The presence of liver metastases (LM) in neuroendocrine tumors (NET) is a major factor altering both quality of life and prognosis. Surgery is recognized as the sole curative treatment. When it is not possible, radiological directed therapies are crucial. This chapter addresses the various roles, technical issues, clinical efficacy and safety of thermal ablation (radiofrequency, microwave, and cryotherapy), and transarterial embolization (bland embolization, chemoembolization, and radioembolization) as liver-directed therapies. The choice of management depends on liver burden and metastases pattern, but also on origin of the primary tumor, tumor differentiation, and tumor proliferative activity. The treatment for neuroendocrine LM still needs to be standardized. Management in centers of expertise should be strongly encouraged in order to enable a multidisciplinary approach to limit morbidity and mortality.

Introduction

The presence of liver metastases (LM) secondary to neuroendocrine tumors (NET) of jejunum/ileum and pancreatic origin has been shown as a major factor altering both quality of life and prognosis regardless the primary site [1]. Moreover, liver is the predominant site for NET metastases besides regional lymph nodes [1].

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At diagnosis, NET in the jejunum/ileum and in the pancreas is associated with regional or distant metastases in 71–89 and 54–88 % of cases, respectively [2].

Studies based on histological cohorts of untreated patients with NET have shown a dismal prognosis in patients with LM compared to patients without (0–40 vs. 75–99 %) [3–5].

Surgery of LM is the standard of care and the sole curative treatment. Surgery is recommended when complete resection or debulking more than 90 % seems feasible [2]. This option justifies aggressive surgical approach, which could require either 2-step surgery in synchronous bilobar LM or patient preparation to surgery as portal vein ligation or embolization [6].

Complete resection of LM is definitely the goal to achieve with a 5-year survival of 80 % [7]. Yet, overall survival is still satisfactory in R1 resection with a 5-year survival of 70 % [7].

In a large retrospective series of patients with LM from NET of the jejunum/ileum and pancreatic origin who were treated with best supportive care or hepatic arterial embolization or liver resection, the only significant factor on multivariate analysis was curative intent to treat [8]. Furthermore, the 5-year survival rates for patients treated with medical therapy, hepatic arterial embolization, and operation was <25, 50, and 76 %, respectively [8].

However, surgery cannot be proposed to all patients with LM. In Chamberlain's paper, only 34 patients out of 85 had surgical resection [8]. Hence, nonsurgical treatments and especially liver-directed treatments are discussed in tumor boards for many patients with LM.

Imaging

The role of imaging in the management of patients with LM is crucial. The aims of imaging are threefold:

1. To assess the presence of LM
2. To analyze the tumor burden within the liver (number, distribution, location to major liver vessels and percentage of liver tumor involvement)
3. To determine the different tumor characteristics.

Somatostatin receptor scintigraphy (SRS), CT scan, and MR imaging have been evaluated and compared for the detection and staging of LM. The best results were obtained with MR imaging with hepatic arterial phase and T2-weighted fast spin echo being the most sensitive sequences [9]. In 40 patients with LM, SRS, CT, and MR imaging detected a total of 204, 325 and 394 lesions, respectively [9].

Diffusion-weighted MR imaging has been shown to improve detection of liver metastases, especially that from colorectal origin. A recent retrospective study has compared the sensitivity and specificity of DW-MR imaging for assessing LM from NET to T2-weighted fast spin-echo and 3D dynamic gadolinium-enhanced sequences, using surgical and histopathological findings as the standard of

reference [10]. In a series of 162 neuroendocrine LM, it was shown that DW-MR was significantly more sensitive (71–71.6 %) than T2-weighted fast spin-echo (47.5–55.6 %), and dynamic gadolinium-enhanced sequences (48.1–55.6 %). The increased sensitivity was also observed in neuroendocrine LM smaller than 1 cm. Interestingly, the specificity of these three sequences was similar and ranged from 88.9–100 %.

LM from NET are known to be numerous and scattered throughout the liver, and a surgical paper with histopathological examination of 3–4-mm-thick serial slices has clearly demonstrated that preoperative imaging including MR imaging underestimates the number of LM [11]. In this series, only 50 % of LM were detected preoperatively [11].

Last, imaging enables assessment of tumor characteristics. Appearance of LM from NET varies from hypervascular tumors to hypovascular or cystic tumors [12]. This various presentation has an impact on patient management. For instance, tumor enhancement on arterial-phase imaging is predictor of tumor response to chemoembolization and time to progression in those patients [12].

Thermal Ablative Techniques

Thermal ablative ablation is based on the cytotoxic effects of nonphysiologic temperature that are locally administrated by probes placed within the liver. Radiofrequency ablation (RFA) and microwave ablation (MWA) are by far the most popular methods.

Radiofrequency Ablation

With RFA, high-frequency current is transmitted to the liver through one or several electrode needles (uni- or multipolar technique). The ionic vibrations generated by the high-frequency current induce heat that denatures intracellular proteins and leads to apoptosis and cell death. Pathologically, the destroyed tumor is replaced by coagulation necrosis. Thermal ablation zone should include the tumor and sufficient margins to prevent from local recurrence.

RFA can be performed percutaneously under CT or US guidance or intra-operatively mostly in combination with liver resection using either laparoscopic or open approach. Follow-up by imaging (CT and or MR imaging) is essential to assess complete tumor necrosis (Fig. 7.1).

Classical indications of RFA are LM fewer than five lesions and tumor size less than 5 cm [2]. Yet, two other issues should be discussed in LM from NET: the role of RFA in tumor debulking and in controlling functional syndromes due to specific hormones excess. This explains that most series of patients with LM from NET had more than 5 ablated tumors with intra-operative RFA during one session [13, 14].

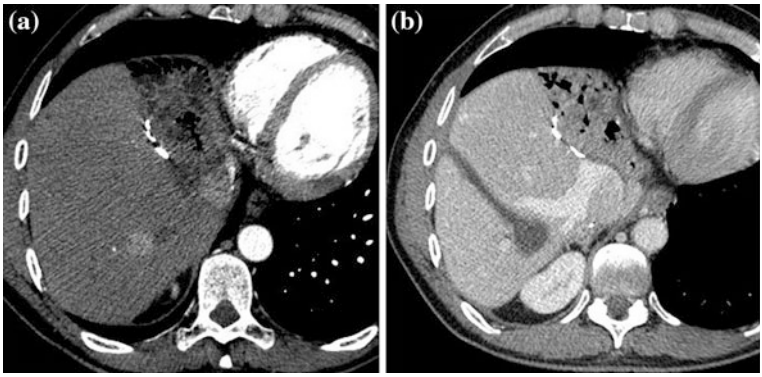

Fig. 7.1 45-year-old man with small bowel neuroendocrine tumor, with left hepatectomy for liver metastasis. **a** Axial CT scan (arterial phase) shows new metastatic liver lesion in the remnant right liver. **b** Axial CT scan (portal venous phase) obtained after RFA of the lesion shows hypoattenuation with no residual lesion

In Elias's series, 16 patients had combined liver surgery and RFA [13]. A mean of 15 and 12 LM per patient were surgically removed and RF ablated, respectively. Morbidity was observed in 69 % of the cases. The 3-year overall survival and disease-free survival were similar to their previous experience of liver resection alone of LM from NET.

In Akyildiz's series, 119 laparoscopic RFAs without liver resection were performed in 89 patients with LM from NET. The mean tumor size was 3.6 cm and the mean number of tumors was 6 (range 1–16) [14]. Perioperative morbidity was 6 % and 30-day morbidity was 1 %. Fourty four patients had hormonal symptoms prior to the procedure. One week after RFA, 97 % of these patients reported at least partial symptoms relief, and 73 % had significant or complete relief. The symptomatic response lasted for a median of 14 ± 5 months [14]. Median disease-free survival was 1.3 year and overall survival was 6 years after RFA.

One of the major problems is the recurrence of metastases within the liver as new tumors are reported up to 63 % in the largest series of patients treated with RFA [14]. Conversely, local liver recurrence was observed from 3.3–7.9 % per lesion [14, 15]. Interestingly, in a meta-analysis including 5.224 ablated tumors of various origins, the rate of local recurrence was lower in neuroendocrine LM than in others [15]. This might be due to tumor characteristics such as well-circumscribed margins or to natural history of these tumors [15].

As in other liver malignancies, factors predictive of tumor recurrence are tumor size, ablation margin, and blood vessel proximity [16]. In a multivariate analysis, statistically significant determinants of survival were only gender (with males having the worse prognosis) and size of the dominant liver metastasis (a tumor size exceeding 3 cm was associated with a greater mortality) [17].

Complications observed after RFA are not related to tumor type. They include pain, bile leakage, liver abscess, intra-abdominal hemorrhage, bowel perforation and pulmonary complications [2, 14, 16, 17].

In some patients, RFA is considered in patients with LM who had previous Whipple procedure and bilioenteric anastomosis. We have to keep in mind that there is an increased risk of liver abscess formation in those patients (40 vs. 0.4 %) [16].

In summary, RFA of LM from NETs differs from other LM due to the large number of lesions per patient. Then, RFA is mostly palliative aiming at debulking and controlling hormonal symptoms. This explains why intra-operative approach with or without combined liver resection is preferred rather than percutaneous approach.

Microwave Ablation

MWA uses electromagnetic devices with frequencies ≥900 MHz. The principle of this technique is similar to RFA but has several theoretical advantages. First, the intra-tumoral temperatures are consistently higher than can be achieved with RFA. Second, MWA is overcoming the "heat sink" effect observed in RFA due to the cooling effect of blood flow in large vessels close to the tumor, both resulting in a better tumor control.

MWA has not been extensively evaluated in LM from NET. Only one series reported 11 patients with LM from NET out of 100 patients [18]. As with RFA, most procedures were performed intra-operatively either with concomitant hepatic resection (7/11) or with concomitant extrahepatic tumor resection (6/11). The median number of ablated LM was 4 ranging from 1–13 tumors. Complications were observed in 3 patients. No local liver recurrence was noticed [18].

Cryotherapy

Cryotherapy is based on the decreased cell viability at low temperatures. The obtained tissue temperature should be −50 °C to achieve necrosis in neoplastic tissue.

To our knowledge, only three series have evaluated cryotherapy in LM from NETs (the largest with 19 patients) [19–21]. As with other thermal ablative techniques, hormonal symptoms relief was observed in the vast majority of patients. Notably, post-procedural coagulopathy has been found in all patients of the two main series [20, 21] requiring transfusion of either platelets or fresh frozen plasma. In one of these series, 2 patients required intra-abdominal packing and transfusion of clotting factors [21]. The authors have not observed similar complications in any other liver malignancies and speculated that the necrosing

carcinoid tumors were releasing substances that may disrupt the coagulation cascade [21].

Despite the efficacy on hormonal symptoms, cryotherapy has been gradually replaced by RFA, mainly for safety reasons.

Radioembolization

Radioembolization is defined as the injection of micron-sized embolic particles loaded with radioisotope by use of percutaneous transarterial techniques. Radioembolization with Yttrium-90 microspheres involves infusion of embolic microparticles of glass or resin impregnated with the isotope Yttrium-90 through a catheter directly into the hepatic arteries. Yttrium-90 is a pure β emitter and decays to stable Zr-90 with a physical half-life of 64.1 h. The average energy of the β particles is 0.9367 MeV, has a mean tissue penetration of 2.5 mm, and has a maximum penetration of 10 mm.

The efficacy of this radioembolization technique is based on the fact that intrahepatic malignancies derive their blood supply almost entirely from the hepatic artery, as opposed to the normal liver, which mainly depends on the portal vein. The microspheres are injected selectively into the proper hepatic artery and subsequently become lodged in the microvasculature surrounding the tumor. Very high irradiation doses are delivered to the tumors, whereas the surrounding liver parenchyma is largely spared.

The use of Yttrium-90 for the treatment for primary and secondary liver malignancies is no longer investigational or experimental and both devices have got FDA and European approval.

The technique comprises two steps:

The first step is patient eligibility and conditioning. Selective mesenteric and hepatic angiography and scintigraphy are performed for several reasons:

- to document the visceral anatomy and identify hepatic arterial anatomic variants,
- to isolate the hepatic circulation by occluding extrahepatic vessels with prophylactic embolization of extrahepatic arteries (e.g., right gastric, gastroduodenal artery),
- to evaluate the hepatic arterial supply of the tumors, and
- to calculate the lung shunting.

The second step is the radioembolization therapy itself. Several days after patient eligibility and conditioning, treatment is performed with microsphere infusion proceeding at flow rates similar to that of the native hepatic artery. Treatment for the contralateral lobe, if needed, is usually performed 30–60 days.

The largest series of selective interval radiation therapy (SIRT) of LM from NET is a retrospective review of 148 patients from 10 institutions. Complete and

partial tumor responses were seen in 2.7 and 60.5 % of the cases according to RECIST criteria, respectively [22]. Stable disease was observed in 22.7 % of the cases and progressive disease occurred in only 4.9 % of the cases [22]. Similar results were reported in the other series including a prospective one [23–28] Paprottka et al. have observed that 97.5 % of LM become necrotic or hypovascular explaining the high rate of overall response when using imaging criteria which aim to depict tumor changes such as EASL or mRECIST criteria.

Symptomatic responses were observed in 55–100 % [23, 29, 30].

Low toxicity is another advantage of radioembolization. Side effects are mainly represented by fatigue, nausea or vomiting, and abdominal pain; No Grade 4 toxicities but one were seen in articles which detail complications [22, 28, 30] after the procedure. Moreover, no radiation-induced liver failure was described in those patients [22, 28, 30].

Transarterial Chemoembolization and Bland Embolization

Rationale and Results

The rationale for transarterial hepatic embolization (TAE) is based on the fact that most LM from NET are hypervascular and derive their blood supply from hepatic artery. The goal of TAE is to induce ischemia of tumor cells thereby reducing hormone output and causing necrosis. Various particles have been used including gelfoam, polyvinyl alcohol particles, and more recently microspheres.

In the 1990s, transarterial chemoembolization (TACE) has been developed based on the principle that ischemia of the tumor cells increases sensitivity to chemotherapeutic substances [31]. Another advantage of TACE over TAE is the higher drug concentration obtained by regional delivery of chemotherapy. Various drugs have been used: doxorubicin and streptozotocin being the most common injected and, alone or in combination, mitomycin C, cisplatin, and gemcitabine. Even some teams have injected a mixture of doxorubicin, mitomycin, and cisplatin. In TACE, embolization is performed immediately after intra-arterial injection of cytotoxic agents. As injection of streptozotocin has been reported to be painful, the procedure is then performed under general anesthesia [32].

More recently, three trials have evaluated drug-eluting beads with doxorubicin in LM from NETs. Drug-eluting beads are particles which are preloaded with any chemotherapeutic agent. The principle is to deliver high dose and more sustained release of drug into the tumor compared to systemic chemotherapy [33, 34].

Despite the large number of TACE or TAE studies performed in patients with LM from NET, there are no randomized trials. Table 7.1 summarizes the main results of these treatments. Most of these studies have evaluated clinical, biological, and morphological responses. Partial or complete symptoms' relief was observed in 42–100 % (Table 7.1) which lasts between 9 and 24 months [35, 36].

Table 7.1 Results of TACE or TAE studies performed in patients with LM from NET

Author	Years	Patients/ session	Tumor	Treatment	Methods	PR	SD	PD	TTP	OS	Symptom relief (%)	Bioch resp (%)
Carrasco [37]	86	25/79	16 SB 9 other	TAE	Sponge	94			11	16	87	100
Ajani [65]	88	22/97	GP	TAE	PVA	60				34	60	60
Ruszniewski [54]	93	23/71	18 SB 5 GP	TACE	Doxo+sponge	61 20	22 40	17 40	14 12		73	57
Therasse [36]	93	28	SB	TACE	Doxo+sponge	35	24		29	24	100	91
Diamandidou [66]	98	20/60	17 SB 3 GP	TACE	Microencapsulate cisplatine	78	22				67	73
Eriksson [40]	98	41/55	29 SB 12 GP	TAE	Sponge	38 17	43 8	19 16	12 10	80 20	52 50	38 42
Brown [52]	99	35/63	21 SB 14 GP	TAE	PVA				15	21	89	
Kim [39]	99	30	16 SB 14 GP	TACE	Cispl+doxo 5FU+STZ	25 50			24	15		75 90
Dominguez [32]	00	15/45	8 SB 7 GP	TACE	STZ	53			11		60	50
Gupta [67]	03	81	SB	TAE 50 TACE 31	PVA or sponge No precision	75	16	9	19	31	63	
Kress [38]	03	26/62	12 SB 10 GP 4 UK	TACE	No precision	8	53	19				
Loewe [68]	03	33/75	SB	TAE	Cyanoacrylate	73	23	4			56	62
Roche [51]	03	14	SB	TACE	Doxo+sponge	72	14	14		47	90	75

(continued)

Table 7.1 (continued)

Author	Years	Patients/ session	Tumor	Treatment	Methods	PR	SD	PD	TTP	OS	Symptom relief (%)	Bioch resp (%)
Roche [50]	04	64/186		TACE	Doxo+sponge	74			15		93	52
Gupta [44]	05	123	69 SB 54 GP	42TAE+27TACE 32TAE+22TACE	PVA or sponge No precision	67 35			23 16	34 23		
Osborne [69]	06	59	42 SB 17 GP	TAE	PVA or embosphères				22	24	81	
Strosberg [46]	06	84	59 SB 20 GP 5 other	TAE	PVA or embosphères	48	52	0		36	80	91
Bloomston [70]	07	122	SB	TACE	Doxo+mito+cispl Sp, PVA or emb	82	12		19	33	92	80
Granberg [35]	07	15/23	7 SB 8 other	TAE	Embosphères	35	56	9	6		42	13
Ho [45]	07	46/93	31 SB 15 GP	TAE 7 TACE 86	Sponge ou PVA Doxo+mito+cispl	45 45	32 45	23 9	42	42	78	
Marrache [12]	07	67/163	48 SB 19 GP	TACE	STZ(44) ou doxo(23)	37	36	27	15		91	65
Ruutiainen [43]	07	67/219		TAE 23 TACE 44	PVA Doxo+mito+cispl				15 7			
De Baere [33]	08	20/34		TACE	Deb Doxo	80	15	5	15			
Kamat [71]	08	38	7 SB 10 GP 21 other	TAE ou TACE	PVA or sponge TACE various	44			9	19	65	
Pitt [41]	08	100	56 SB 44 GP	TAE 49 TACE 51	Sp, PVA, emb Cispl,adria,mito				56	26	TAE 76 TACE 69	
Sward [72]	09	107/213	SB	TAE	Sponge or PVA						71	60

(continued)

Table 7.1 (continued)

Author	Years	Patients/ session	Tumor	Treatment	Methods	PR	SD	PD	TTP	OS	Symptom relief (%)	Bioch resp (%)
Gaur [34]	11	18/32	SB	TACE	Deb Doxo	58	42	0	14			
Maire [42]	12	26	SB	TAE 12 TACE 14	Sponge Doxo	65	30	5	24 19			75
Dong [73]	11	123	15 GP 21 SB 87 other	TACE	Doxo ou STZ Sponge ou emb	62	24	14		65		
Baghat [49]	12	13/27		TACE	Deb Doxo	10	80	10			100	

Abbreviations

PR Partial response (%), *SD* Stable disease (%), *PD* Progressive disease (%), *TTP* Time to progression (in month), *OS* overall survival (in month), *SB* Small bowel tumor, *GP* Gastropancreatic tumors, *UK* Unknown primitive tumor, *PVA* Polyvinyl alcohol particles, *Sp* Sponge, *Emb* Embospheres, *STZ* Streptozotocin, *doxo* Doxorubicin, *cispl* Cisplatin, *mito* Mitomycin

Significant decrease in tumor markers occurred in 13–100 % [35, 37]. Morphological response (either complete or partial) was seen in 8–94 % [37, 38]. Yet, imaging criteria for assessing tumor response have not been detailed in all published articles. When evaluated, overall survival since TAE or TACE initiation ranges from 15–80 months [39, 40].

Technical Issues

Careful analysis of the literature highlights many disagreements on technical issues.

Embolization Versus Chemoembolization

Several studies have retrospectively compared TAE and TACE in patients with LM from NETs. In all studies but one, treated patients had NET from the jejunum/ileum and NET from pancreatic origin and no subgroup analysis has been performed. In two studies, no differences have been shown in terms of patient survival and tumor response [41, 42]. In one study, chemoembolization demonstrated trends toward improvement, in time to progression, symptom control, and survival (although not significant) [43]. Furthermore these authors, as others have shown that chemoembolization was not associated with a higher degree of toxicity than bland embolization [43].

Gupta et al. [44] have separately analyzed their results in small intestinal tumors and pancreatic tumors. They have shown that the addition of intra-arterial chemotherapy to embolization did not improve the overall survival, nor progression-free survival in patients with small intestinal tumors. Moreover, it had a deleterious effect on the morphologic response rate. In contrast, a tendency toward prolonged survival and improved response rate was noted in patients with pancreatic tumors treated with TACE compared with TAE [44].

A prospective comparison between TAE and TACE in neuroendocrine LM from the midgut has been published recently [42]. Primary endpoint was progression-free survival. The expected number of enrolled patients was not achieved explaining that this study may suffer from a lack of power. Yet, no difference was seen in the two groups [42]. The first-year progressive-free survival rates were 91.6 and 90 % in the TAE and TACE arms, respectively. The median PFS was 24 months and 19 months in the TAE and TACE arms, respectively. There results confirm that the addition of intra-arterial chemotherapy to embolization does not prolong PFS.

In summary, TACE has not been proved superior to TAE in LM from the jejunum/ileum. The question is still open in LM from NET of the pancreas.

Which Cytotoxic Drug?

Most cytotoxic drugs that have been injected during TACE procedure are drugs that are currently used with systemic chemotherapy. Most teams recommend doxorubicin in small intestinal tumors and streptozotocin in pancreatic tumors [12, 39]. As drug assignment was not controlled nor randomized, it is not possible to determine which drug is more efficient. However, authors see potential advantage in using streptozotocin, especially in LM from the pancreas, which may save doxorubicin for subsequent use and chemotherapy [12] (Fig. 7.2).

Which Embolization Particles?

No comparison between absorbable and nonabsorbable particles has been made in LM from NETs. Moreover, most studies have included patients treated with absorbable and nonabsorbable particles [41, 44–46].

Only one study has focused on TAE with triaxyl gelatin microspheres (embosphere®). Hepatic embolization was performed using either particles sized 300–500, 500–700, and/or 700–900 μm. Absence of disease progression was seen in 91 % of the cases, and 35 % of the patients had partial response on imaging using RECIST criteria despite the fact that some patients had extensive tumor necrosis [35]. No major complications occurred in this series. Notably, all patients with bilobar involvement were treated sequentially [35] (Fig. 7.3).

Should We Use Drug-Eluting Beads?

Studies have compared the conventional TACE technique and the drug-eluting beads technique and have shown a more prolonged retention of drug within hepatocellular carcinoma in the latter [47]. Preloaded in LM from NET is doxorubicin (DC Bead, Terumo, Japan) [33, 34]. Stabilization or partial response on imaging was observed in 95 and 100 % of cases. The mean PFS was 14 and 15 months, respectively [33, 34]. Again, no comparison has been made with conventional TACE in those patients. Yet, the PFS rates were in the range of the others (Table 7.1). Interestingly biliary and liver injuries such as dilated bile ducts, portal vein narrowing, portal venous thrombosis, and biloma/liver infarcts have been reported in patients with LM from NET and are more often observed than in patients with hepatocellular developed on cirrhosis [48]. This first observation was largely confirmed by a study which showed that 7/13 (54 %) patients with LM from NET developed bilomas which forced interruption of the trial. Notably, all of these patients had multiple small LM [49]. It is hypothesized that hypertrophied peribiliary plexus observed in cirrhosis could protect against the ischemic/chemical insult of bile ducts suggesting caution when using drug-eluting beads in noncirrhotic liver [48].

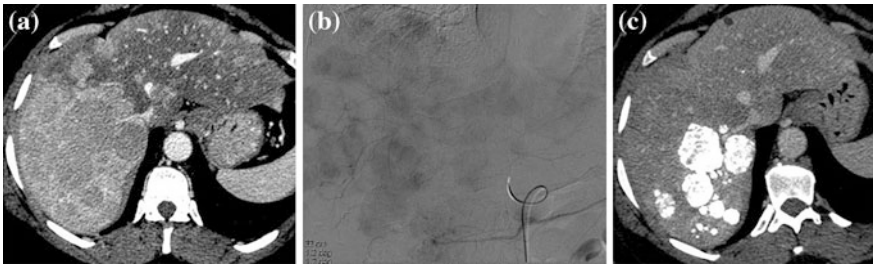

Fig. 7.2 51-year-old woman with pancreatic neuroendocrine tumor and multiple liver metastatases predominantly on the right liver. **a** Axial CT scan shows hypervascular lesions at the arterial phase (in a steatotic liver). **b** Angiogram before chemoembolization demonstrates multiple blushes predominantly in the right liver, and **c** axial CT scan after chemoembolization of the right liver using streptozotocin shows a major lipiodol uptake of lesions suggesting complete response

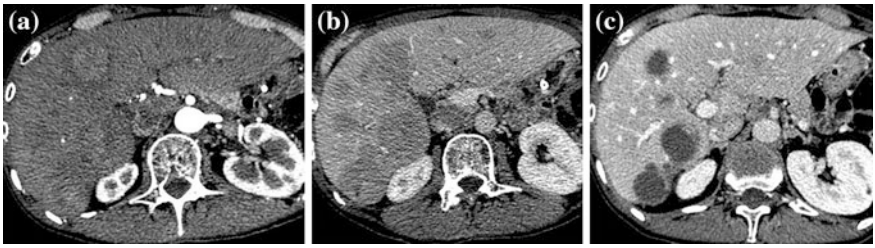

Fig. 7.3 35-year-old man with small bowel neuroendocrine tumor. **a** Axial CT scan shows hypervascular LM (arterial phase). **b** Axial CT scan at the portal venous phase shows same lesions which become hypoattenuating, and **c** axial CT scan at the portal phase after bland embolization of the right liver with microspheres shows complete response with necrosis and decrease in size of all lesions

Which Liver Volume Should be Treated in the Same Session?

Tumoral liver involvement is an important issue for both efficacy and toxicity. Best morphological responses are obtained in patients with limited liver involvement (<30 or <50 %) [38, 50]. On the other hand, toxicity is increased in major liver involvement (>70 or 75 %) [38, 44, 51, 52]. This threshold has been first used as an exclusion criterion by many teams. However, Gupta et al. [44] have been able to treat many patients >75 % liver involvement successfully and safely by treating only a small portion of the liver in each embolization session.

Which Timing?

TAE and TACE can be repeated safely in patients with LM from neuroendocrine tumors, and especially in patients with disease progression [53]. The complication rate after repeat TACE is lower than after first TACE [53]. As in other indications, TACE used to be performed at fixed delays whatever the tumor response. The trend is now to adapt the number of sessions and the interval between sessions to the tumor response.

Predictive factors of tumor response after TAE or TACE have been identified. Some of them depend on LM characteristics such as tumor liver involvement <30 % and tumor enhancement on arterial-phase CT images [12, 50]. Primary tumor of the jejunum/ileum is associated with a better tumor response of the LM than pancreatic tumor [12, 40, 44, 50, 54].

Embolization and Chemoembolization Versus Radioembolization

To date, there has been no randomized trial but a review paper and a multicenter, prospective treatment registry with radioembolization which have evaluated the efficacy of radioembolization and TAE/TACE in neuroendocrine LM [55, 56]. Treatment efficacy seems similar. TAE/TACE seems more appropriate in patients with bulky and large tumors which require a segmental targeted approach whereas radioembolization could be more advantageous in patients with small LM that have a miliary bilobar distribution.

Bland Embolization Combined with Targeted Therapy

It is known that embolization stimulates release of VEGF into the circulation. Authors have speculated that sunitinib, an oral VEGFR inhibitor, could be administered following embolization [57]. They observed high rates of PFS (15.2 mo) and OS (95 and 59 % at 1 and 4 years, respectively) associated with this sequence of therapies.

Complications

In a retrospective series of 72 patients with neuroendocrine LM, the median length of stay was 4 days [58]. The most common and classical complication is the post-embolization syndrome which is seen in up to 80–90 % of the patients [44, 59]. It includes fever, leukocytosis, abdominal pain, nausea, and a transient increase in liver enzymes. Some of the severe complications are also observed in other liver malignancies such as liver failure, cholecystitis, gastric ulcers, and bleeding, whereas some others such as carcinoid crisis are specific of LM from

neuroendocrine origin [2]. In a retrospective series of 489 TACE performed in various tumors, the 3 patients who developed abscess formation had a neuroendocrine tumor and a bilioenteric anastomosis [60].

Portal vein thrombosis and hepatic insufficiency are considered exclusion criteria for both TAE and TACE [1]. As the odd ratio of developing abscess in patients with bilioenteric anastomosis is very high (894), TACE should be avoided in those patients [61]. If it must be performed, very broad spectrum prophylactic antibiotics and bowel preparation before the procedure should be considered [62].

Indications of Liver-Directed Treatments

The presence of LM largely influences prognosis in all types of NET [63]. Prognosis has improved with significant overall survival increasing in both patients with LM from the jejunum/ileum and the pancreas undergoing multidisciplinary treatment [63]. This includes hepatobiliary surgery, locoregional, and/or medical therapies.

Patient management depends on LM characteristics (tumor pattern and tumor burden), tumor differentiation and proliferative activity, and natural history of LM.

LM may be defined according to three different macroscopic patterns: (1) a *simple* pattern corresponds to LM confined to one liver lobe or limited to two adjacent segments, (2) a *complex* pattern is assessed when LM primarily affect one lobe but with smaller satellites contra laterally, (3) the *diffuse* pattern corresponds to diffuse, multifocal LM [63].

Tumor differentiation and proliferative activity are also important factors for patient management. In this article, we will only consider liver-targeted therapy in Grade 1 and Grade 2 neuroendocrine LM as systemic chemotherapy is the recommended treatment in Grade 3 tumors.

Last, natural history course is also a key factor. The "watch and wait" attitude is recommended in nonprogressive and nonsymptomatic LM in patients with limited tumor burden (30–50 %) [64].

In single pattern LM, the standard of care is surgical resection if possible. Local ablative therapies (mainly RF ablation) are performed when surgery is contraindicated.

In complex pattern of LM, local ablative treatments (mainly intra-operatively) may be used in combination to surgical resection.

In diffuse LM, surgery and local ablative therapies are no longer indicated. The role of intra-arterial treatment (TAE, TACE, radioembolization) is crucial and particularly in LM from the jejunum/ileum because efficacy of systemic chemotherapy has not been proved in these tumors. In LM secondary to NET of the pancreas, intra-arterial treatments are competing with systemic therapy including targeted therapy.

In conclusion, liver-directed therapies are widely performed in LM from neuroendocrine tumors. As these tumors largely differ from the other LMs (number,

imaging findings, prognosis, treatment, etc.), tumor boards dedicated to NET are advisable. Interventional radiologists should also be aware of the indications and specific contra-indications of liver-directed therapies in these tumors.

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Chapter 8
Inhibition of mTOR in Neuroendocrine Neoplasms of the Digestive Tract

Eric Raymond and Marianne Pavel

Abstract The clinical behavior of neuroendocrine neoplasms (NEN) is highly variable. NEN may present as indolent tumors, usually well differentiated, that have limited impact on performance status but are detected at late stages. NEN may also display more aggressive behaviors when cancer cells are poorly differentiated, leading to very rapid tumor growth severely impairing patient general conditions. The term carcinoid was formerly used to identify well-differentiated slowly growing NEN and is actually replaced by the term neuroendocrine tumors G1/G2. Somatostatin analogs have improved the clinical management of patients with NEN by controlling carcinoid symptoms (flushing, diarrhea) and delaying tumor progression. The mammalian target of rapamycin (mTOR), a main protein kinase downstream to the phosphoinositide 3-kinase/Akt signaling pathway, appears as an important intracellular mediator involved in multiple cellular functions including proliferation, differentiation, apoptosis, tumorigenesis, and angiogenesis. Alterations in the normal activity of mTOR and of mTOR-related kinases in this pathway have been found in a diversity of human tumors, including NEN; therefore, mTOR pathway represents an attractive target for new anticancer therapies. While mTOR inhibitors, such as everolimus, are established therapy in pancreatic NET, results from recent clinical trials indicate that mTOR inhibitors may be also of value in the management of non-pancreatic NET. However, ongoing clinical trials will have to confirm efficacy and elucidate, in which subtypes and in which setting these drugs might be most usefully applied.

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Introduction

Neuroendocrine neoplasms (NEN) are uncommon malignancies arising from the neuroendocrine cells and could virtually develop in all organs, including the digestive tract and the lung [1]. Survey data have shown that the incidence of NEN is increasing worldwide [2]. The more frequent diagnosis and the prolonged survival of patients have an impact on the high prevalence of this disease and make it ranking as the second cause of tumors of the digestive tract after colon cancer [2]. Symptoms may attract attention to patients with functioning tumors who are then often diagnosed earlier than non-functioning tumors. The clinical outcome of individuals with NET remains difficult to predict. Indolence may be observed for months or years until eventually the tumor may be more rapidly growing.

Surgery is the backbone of treatment for a limited number of patients [3]. However, since most patients are presenting liver metastasis, liver resection and liver-directed therapy such as bland embolization, chemoembolization, or radiofrequency ablation are often proposed as palliative approaches [4]. Somatostatin analogs have been developed to relieve symptoms [5] and were recently shown to delay tumor progression in selected patients with midgut NET [6] and pancreatic neuroendocrine tumors with rather low proliferative activity. Streptozocin, either combined with doxorubicin or combined with fluorouracil [7, 8], remains the only chemotherapy approved in few countries in advanced pancreatic endocrine tumors (pNET), although the benefit of chemotherapy has been questioned in few reports [9, 10]. As a result of the complexity of care in patients with endocrine tumors of the digestive tract, the current treatment algorithm is based on a relatively complex multidisciplinary approach. Clinico-pathological parameters such as proliferative activity, functionality, somatostatin receptor status, tumor extent, and tumor slope represent important components for therapeutic decision making.

Activation of the mammalian target of rapamycin (mTOR)-signaling pathway mediated either through upstream insulin or insulin-like growth factor receptor-1 or through direct activation through nutrients (amino acids have been identified as activators of mTOR signaling and are required for maximal activation of mTOR signaling by growth factors such as insulin) under specific conditions such as hypoxia has also been frequently implicated in the proliferation of neuroendocrine tumor cells [11]. Consistent with this observation is the finding that inhibition of mTOR has significant antiproliferative effects in endocrine tumor cell lines [12]. Based on laboratory results and clinical data from phase II/III clinical trials, evidence has accumulated, suggesting that mTOR inhibitors may have substantial activity in patients with advanced pNET and some activity in other NENs. In this review, we describe the role of mTOR signaling in the biology of NEN and update recent results of clinical trials in pNETs and non-pancreatic NET ("carcinoids").

Molecular Characteristics of NEN

Recent pathological studies have deciphered the biological features characterizing subgroups of sporadic NEN. In this literature, somatic mutations of *VHL* were considered to represent a rare event in sporadic pNET [13, 14] as compared to hereditary form of pNET (Illustrations). However, Schmitt et al. [15] have recently reported that up to 25 % of sporadic pNETs display genomic alterations in the *VHL* gene. Indeed, in this study including mainly well-differentiated pNET, 14/78 cases (18 %) had deletion of the *VHL* gene detected by FISH, while 2/35 (6 %) informative cases showed methylation of the *VHL* promoter region. Interestingly, these genomic abnormalities were associated with under expression of VHL RNA in 25 % (8/32 cases) of these tumors. Consistently, approximately one-third of tumor samples showed positive staining of hypoxia target proteins including HIF-1α, CA-9, and GLUT-1 in 29, 44, and 34 % of pNET, respectively. By correlating *VHL* alterations and hypoxia with survival parameters, the authors observed that both *VHL* mutations and positive CA9 expressions were associated with higher risks of recurrence and poor survival [16]. Further evidence has suggested that the expression of other proteins acting as oxygen sensors in cells such as prolyl hydroxylase domain proteins (PHD)-1, PHD-2, and PHD-3 also correlated with the presence of tumor metastases, tumor recurrence, and poor prognosis [17]. These forms of sporadic pNETs with adverse outcome are interesting to consider with regard to recent therapeutic approaches targeting hypoxia-associated angiogenesis (HIF-1α-dependent VEGFR activation) and/or mTOR signaling pathway. This was recently confirmed by genetic testing of patient tissues from pNET, identifying that genes such as DAXX/ATRX and MEN1, as well as other genetic alterations in the mTOR pathway, are frequently observed in pNETs [18]. In this study, mutations in the PI3K pathway were observed in about 15 % of cases.

mTOR Signaling in NEN

Several studies have shown that mTOR plays a central role in several signaling pathways activated by growth factors and nutritional status, receiving stimulatory signals from Ras and phosphoinositide 3-kinase (PI3K). PI3K localizes Akt to the cell membrane where it can be phosphorylated and activated by PDK1. Activated Akt phosphorylates tuberin (TSC2), resulting in TSC1/2 complex instability and inhibition of the tumor suppressor function of the TSC2. Rheb, a small tyrosine phosphatase, is inhibited by the TSC2–TSC1 complex and positively modulates mTOR function. Phosphorylation of mTOR at the Ser2448 site promotes phosphorylation of p70S6K, resulting in the activation of p70S6K [19, 20]. Noteworthy, several components of the Ras/MAPK/ERK and PI3K signaling pathways are mutated in subsets of most human cancers, inducing abnormal regulation of mTOR signaling and, therefore, possibly increasing its susceptibility to mTOR inhibitors [21, 22]. Data recently showed that genetic and metabolic changes

accompanying malignant transformation might cause hypersensitivity to mTOR inhibition [16]. Rapamycin, a specific mTOR inhibitor, is produced by the bacterium *Streptomyces hygroscopicus*. Rapamycin derivatives, temsirolimus (CCI-779), everolimus (RAD001), and deforolimus (AP23573), known as rapalogs, specifically inhibit mTOR functions, inactivate the ribosomal p70S6 kinase, and inhibit translation, resulting in an arrest in G1 phase of the cell cycle phase and apoptosis [23]. Preclinical studies indicate that rapamycin and its derivatives are potent inhibitors of the proliferation of numerous tumor cell lines in culture and of tumor models or human xenografts, including endocrine cancers [24]. Everolimus, an orally bioavailable derivative of rapamycin, used in lower dose to prevent kidney and heart transplant rejection, has been explored in phases I, II, and III clinical trials as an anticancer agent [25, 26], showing promising activity with an overall good safety profile. Emerging results suggest that inhibition of mTOR signaling can be exploited as a potential tumor-selective therapeutic strategy [27].

Development of Everolimus in Patients with pNET

Background Rationale for Everolimus in pNET

Tuberous sclerosis, neurofibromatosis, and von Hippel–Lindau disease display deregulation of the mTOR signaling pathway and have been linked to the development of pNET [24]. Sporadic neuroendocrine tumors have been frequently associated with downregulation of TSC2 and PTEN proteins. Furthermore, mTOR signaling pathway may be directly activated by the insulin-like growth factor receptor 1, energy and nutrient deprivation, and oxygen depletion. Therefore, rapamycin derivatives have early emerged as potential anticancer agents for the treatment for neuroendocrine tumors. Everolimus (Afinitor®; Novartis, Basel, Switzerland) belongs to the macrolide antibiotic family of rapamycin and as such binds the cytosolic immunophilin protein FKBP12 (FK-binding protein 12). Everolimus is not per se a kinase inhibitor but stands as an allosteric inhibitor of mTORC1, and because it requires FKBP12 to inhibit mTOR function, everolimus shall be considered as a highly specific mTOR inhibitor with no direct effects on mTORC2 and without any other significant inhibitory effects on other kinases associated with the mTOR pathway [23]. Preclinical evidence has shown that inhibition of mTOR using rapamycin or other rapamycin derivatives including everolimus is associated with significant antiproliferative effects in endocrine tumor cell lines [17]. The mechanism of actions of everolimus is not fully elucidated although several antitumor properties have been demonstrated in cancer cells. The antiproliferative effects of everolimus may be related to the inhibition of the PI3K survival pathways and/or to the direct inhibitory effects of the drug on protein translation and cell cycle progression. In tumors addicted to the activation of mTOR signaling, the use of rapamycin or everolimus may also lead to cell death either by apoptosis, senescence, or autophagy. Additional data were provided that the inhibition of the

mTOR signaling pathway on endothelial cell may be responsible for antiangiogenic effects of rapamycin derivatives [28]. These data support a strong preclinical rationale for the use of mTOR inhibitors in patients with neuroendocrine tumors.

Evidence of Antitumor Activity in Phase I–II Trials

Sporadic tumor responses have been reported in patients with neuroendocrine tumors during the phase I trial program with everolimus [29]. Evidence of antitumor activity was reported in a phase II non-randomized program that evaluated two groups of patients with advanced neuroendocrine tumors: one group treated with everolimus alone at the daily dose of 10 mg/day and the other using everolimus in combination with octreotide long-acting release [30]. This phase II program reported 9.6 % partial responses, 67.8 % stable diseases, and a median progression-free survival (PFS) of 9.7 months in patients treated with everolimus alone. The response rate in the group of patients treated with everolimus plus somatostatin analogs was 4.4 %, 80 % of patients had stable disease, and the median PFS was 16.7 months. As this study was not intended to compare the two groups, the patient populations treated with single-agent everolimus were quite different from the one receiving everolimus along with somatostatin analogs and a direct comparison of results between the two groups is not appropriate. The apparent PFS advantage observed in patients treated with the combination needs to be interpreted with caution. For instance, patients treated with everolimus and somatostatin analogs had assumedly more indolent diseases and a strikingly longer survival (median OS not reached vs. 24.9 months in the stratum with everolimus monotherapy). Nevertheless, this trial showed that everolimus alone and in combination with somatostatin analogs had activity and can be safely administered to patients with pNET and set up the basis for the launch of a larger program with two large randomized placebo-controlled trials looking at the activity of everolimus in patients with pNETs or advanced NET associated with the carcinoid syndrome (carcinoid tumors).

Everolimus Phase III Study Design in pNET

The effects of everolimus were reported in a large multicenter international double-blinded randomized phase III trial comparing everolimus given at the daily dose of 10 mg to placebo in patients with advanced pNETs [31]. Patients entering this trial were required to have a well- or moderately differentiated advanced pNET not amenable to curative surgery. Twenty-four percent of patients had functioning tumors, 40 % received concomitant somatostatin analogs, and about 50 % of patients were previously treated with chemotherapy and somatostatin analogs. Patients were required to demonstrate tumor progression within a year prior to enrollment in the study. The trial was designed to detect a 50 %

improvement in progression-free survival as adjudicated by local investigators and was competed without interim analysis enrolling a total of 410 patients in 82 centers and 18 countries. At the cutoff date of February 2010, 141 and 177 patients had discontinued treatment with everolimus and placebo, respectively. The primary reasons for study termination were disease progression in 92 patients treated with everolimus and 163 patients treated with placebo or adverse event in 36 patients treated with everolimus and 7 patients treated with placebo. The number of deaths was almost identical in the two arms (51 events in the everolimus arm and 50 events in the placebo arm) [32].

Everolimus Phase III Study Results in pNETs

Results of this trial showed that the median PFS of patients treated with everolimus was 11.0 months as compared with 4.6 months in patients treated with placebo (hazard ratio 0.35, p *value* < 0.001) [32]. The findings were confirmed by an independent central review of CT scans with a median PFS according to the central assessment of 11.4 months with everolimus compared with 5.4 months with placebo (hazard ratio 0.34, $p < 0.001$). Subgroup analysis showed that the PFS benefit of everolimus was consistent, regardless of the tumor histology, prior use of somatostatin analogs, or prior treatment with chemotherapy. Objective response rates were low (5 and 2 %, respectively) in the everolimus and the placebo arms. The overall benefits of everolimus treatment appeared to be reflected better by looking at the disease control rate with 64 % of patients had some degrees of tumor shrinkage when treated with everolimus. A total of 148 patients (73 %) treated with placebo crossed over to everolimus at the time of tumor progression. As expected, no difference in overall survival was observed between the everolimus- and the placebo-treated patients. The safety profile of everolimus in this study appears to be consistent with that previously reported in other tumor types of cancers such as renal cell carcinoma, stomatitis, rash, diarrhea, fatigue, and infection being the most commonly reported adverse events. The frequency and severity of pneumonitis/pulmonary infections were also consistent with previously published papers.

Based on these data, the FDA granted approval of everolimus in patients with advanced non-resectable pNETs.

Development of Everolimus in Patients with Advanced Non-pancreatic NET/Carcinoid Tumors

Background Rationale for Everolimus in Carcinoids

Formerly, so-called “carcinoids” are suspected to share several common biological characteristics with pNETs. However, there is only a few if any preclinical works that could support the use of everolimus as an anticancer drug in “carcinoid

tumors." In clinical trials, objective responses (17 %) and sustained tumor stabilization have been described in patients with carcinoids and served as supportive data for investigating everolimus in a large randomized trial [32].

Everolimus Phase III Study Design in Advanced NET with Carcinoid Syndrome (Carcinoids)

A randomized, double-blinded, placebo-controlled, multicenter phase III study was conducted in patients with low- or intermediate-grade advanced carcinoid tumor receiving long-acting release (LAR) octreotide 30 mg q/28 days and placebo or octreotide LAR 30 mg q/28 days and 10 mg/day everolimus [33]. Data are extensively described in the ODAC review made public on April 2011. Patients were required to experience disease progression in the 12 months that preceded the study entry. No stratification was planned. The primary end point was median PFS as determined by an independent review committee. Secondary end points were response rate, overall survival, safety, and pharmacokinetic and biomarker evaluations. At the time of progression, patients randomized in the placebo arm were allowed to crossover to everolimus arm. A total of 429 patients were entered in this trial, including 216 patients in the everolimus arm and 213 in the placebo arm. The study had preplanned interim analysis for PFS. Adjusted for two interim analyses, the prespecified boundary at final analysis for median PFS based on central adjudicated reading was $p \leq 0.0246$. The primary end point was narrowly missed; there was a discrepancy between local reading and central reading that had led to the loss of events for the assessment of the primary end point due to earlier disease progression as judged by the local readers. Finally, although the study included a large number of patients, it did not reach the number of events predefined to reach a significant outcome (223 events were reached instead of a calculated number of 287 events which would yield 92.2 % power with the use of an unstratified log rank test at a one-sided significance level of 2.5 %). Furthermore, there were randomization imbalances in the study, largely because prognostic factors had not been well defined at the start of the study, but were needed to stratify patients. These included performance status, primary tumor site, and elevated biomarkers (chromogranin A and 5-hydroxyindoleacetic acid) [33, 34].

Everolimus Phase III Study Results in Carcinoids

In this trial [33], despite randomization, the study population was unexpectedly heterogeneous and unbalanced with a higher proportion of patients with poor prognosis entered in the everolimus group as compared to that in the placebo group. For instance, more patients with ECOG 1–2 performance status, with intermediate-grade tumors, with lung primary, and with prior systemic chemotherapy, were

entered in the everolimus arm. It is likely to induce bias in the interpretations of data. Median PFS assessed by central review was 16.4 months (95 % CI 13.7–21.2) in the everolimus plus octreotide LAR arm and 11.3 months (95 % CI 8.4–14.6) in the placebo plus octreotide LAR arm ($p = 0.026$). Everolimus plus octreotide LAR was associated with a 23 % reduction in the estimated risk for progression (HR 0.77; 95 % CI 0.59–1.00). Based on the local investigator assessment, median PFS was 12.0 months (95 % CI 10.6–16.1) in the everolimus plus octreotide LAR arm and 8.6 months (95 % CI 8.1–11.1) in the placebo plus octreotide LAR arm ($p = 0.018$). Results were consistent with the central review (HR 0.78; 95 % CI 0.62–0.98).

For the apparent loss of PFS events (see Everolimus Phase III Study Design) in the central review, and to adjust for suggested informative censoring that may have impacted the results, inverse probability of censoring weights (IPCW) methodology was applied to assess for biases introduced by informative censoring. This analysis confirmed that informative censoring impacted the results of the central review analysis (median PFS everolimus + octreotide LAR, 13.8 months; placebo + octreotide LAR, 8.3 months; HR = 0.60; 95 % CI = 0.44–0.84; $P = 0.0014$). The P value was obtained from a one-sided log rank test. The hazard ratio was obtained from an unadjusted Cox model. Median overall survival was not reached at the time of analysis and was not different between groups ($p = 0.908$). An updated analysis showing a median overall survival of 29.2 months in the everolimus group as compared to 35.2 months in the placebo group was not significantly different between both groups (unadjusted hazard ratio 1.16 (95 % CI, 0.91–1.49); adjusted for baseline covariates [age, gender, race, WHO PS, and prior SSA usage] 1.06 (95 % CI, 0.82–1.36)) [35]. Reasons for treatment discontinuation were different for the two groups, patients treated with placebo-discontinued treatment mainly for progression (69 % in the placebo group compared to 44 % in the everolimus group), while the proportion of patients discontinuing for adverse events was higher in the everolimus group as compared to the placebo group (19 vs. 3 %). Six patients discontinued due to death in the everolimus arm versus 2 patients in the placebo arm. Serious adverse event and grade 3–4 events (infection, diarrhea, fatigue, hyperglycemia, and renal insufficiency) were more frequently reported in the everolimus group as compared to the placebo group. Based on the results of this trial, it appears that everolimus cannot in general be recommended for the treatment of patients with advanced neuroendocrine tumors associated with carcinoid syndrome and that further trials will be required to properly evaluate the effects of everolimus in patients with different primary tumor sites. In consequence, the RADIANT-4 trial was constructed to evaluate everolimus as monotherapy in advanced non-resectable intestinal and lung NET (NCT01524783). Accrual of this trial with 279 patients has been completed. In an international setting, the LUNA trial is actually recruiting patients with lung and thymic NET where there is no standard therapy available to further evaluate efficacy of everolimus versus pasireotide (a novel somatostatin analog with universal binding to SSTR) versus combination of both drugs (NCT01563354).

Conclusion

mTOR plays a central role in the regulation of cell growth, proliferation, metabolism, and angiogenesis in different types of cancers including NEN. Activation of the mammalian target of rapamycin (mTOR)-signaling pathway (mediated either through upstream insulin-like growth factor receptor 1 or through direct activation through nutrients (glucose, amino acids)) and its modulation by hypoxia have been implicated in the proliferation of pancreatic neuroendocrine tumors and carcinoids driving on everolimus research in clinical studies. Everolimus has been recently approved based on improvement in progression-free survival in patients with advanced pancreatic neuroendocrine tumors in a large international multicentre placebo-controlled double-blinded randomized trial. Although no statistical significant PFS benefit was observed in a heterogeneous patient population with advanced NET with carcinoid syndrome treated with everolimus as compared to placebo, subgroups of patients seem to have a benefit and data were sufficient to launch novel trials (RADIANT-4, LUNA) in this indication. Everolimus trials have defined progression-free survival as a valid end point for future clinical trials and have set new standards of care for patients with progressive advanced/metastatic pancreatic neuroendocrine tumors. Everolimus is now part of treatment options for patients with NET in ENETS guidelines [36] (Fig. 8.1).

Illustrations

Genetic diseases associated with the development of digestive neuroendocrine tumors. Neuroendocrine tumors of the digestive tract are usually sporadic but can arise in multiple endocrine neoplasia type 1 (MEN1) and more rarely in other syndromes, including von Hippel–Lindau (VHL) syndrome and tuberous sclerosis. MEN1 is a tumor suppressor gene that, when mutated in the germline, predisposes to MEN1 syndrome. Biallelic inactivation of the *MEN*1 (multiple endocrine neoplasia type 1) gene, usually through a mutation in one allele coupled with the loss of the remaining wild-type allele, occurs with pancreatic NET. Mutations affecting *VHL*, *NF*1, and *TSC*1 genes are associated with an increased risk of developing malignant pancreatic neuroendocrine tumors in patients with von Hippel–Lindau disease, type 1 neurofibromatosis, and tuberous sclerosis syndromes, respectively.

Hereditary forms of neuroendocrine tumors have highlighted the crucial role of genes regulating hypoxia signaling. In von Hippel–Lindau disease, loss of pVHL protein function, which usually tags the hypoxia-inducible factor 1

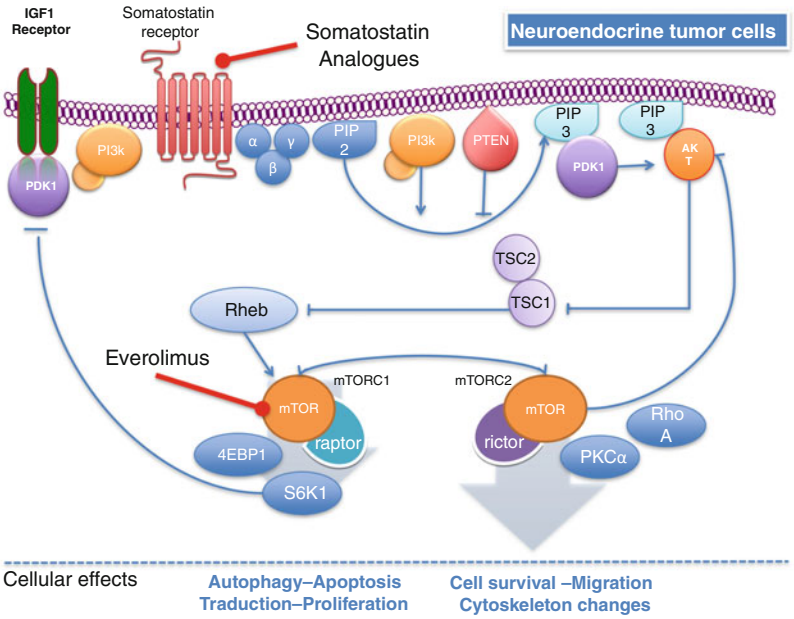

Fig. 8.1 Upstream activation of the mTOR signaling pathway and consequences of mTORC1 inhibition using everolimus in pancreatic neuroendocrine tumors

(HIF1) for proteasomal degradation, results in nuclear accumulation of HIF1α, yielding increased transcription of a number of hypoxia-inducible genes, such as CA-9 and GLUT-1 (glucose transporter 1). In type 1 neurofibromatosis and tuberous sclerosis syndromes, HIF-1α is indirectly activated through mTOR (mammalian target of rapamycin), due to loss of function of *NF*1 and *TSC*1 genes. Of note, such cases of familial pNET are acknowledged to be associated with adverse outcome as compared to MEN1 tumors.

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Chapter 9
Angiogenesis Inhibition Using Sunitinib in Pancreatic Neuroendocrine Tumors

Cindy Neuzillet, Sandrine Faivre, Pascal Hammel, Chantal Dreyer and Eric Raymond

Abstract Well-differentiated pancreatic neuroendocrine tumors are highly angiogenic malignancies that rely on vascular endothelial growth factor (VEGF) receptor (VEGFR) activation for endothelial proliferation and on platelet-derived growth factor receptor (PDGFR) function for pericyte coverage. Expression of VEGF by cancer cells is thought to be induced by hypoxia-inducing factor 1 alpha that can be caused either by hypoxia or by genetic abnormalities such as mutations of *VHL*. Sunitinib is a potent multitarget tyrosine kinase inhibitor blocking activation of both VEGFR and PDGFR in cellular and animal models. Sunitinib was shown to disrupt the tumor vascular network in the RIP-TAG pancreatic neuroendocrine tumor model. Data from phase I/II clinical trials have shown sticking activities in the treatment of patients with advanced neuroendocrine tumors, yielding to a phase III randomized registration trial. In this later study, sunitinib demonstrated significant benefits in terms of progression-free survival and response rate in patients with advanced well-differentiated pancreatic neuroendocrine tumors. The safety profile in this patient population was consistent with previously reported data in other tumor types. Interestingly, safety issues for sunitinib in patients with neuroendocrine tumors did not alter the patient quality of life. In this chapter, we review the rational for using sunitinib in pancreatic neuroendocrine tumors, recent clinical results, and provide some insight into how and when to use this novel drug in the inventory of treatment options for pancreatic neuroendocrine tumors.

Keywords Angiogenesis · Pancreatic neuroendocrine tumor · PNET · Carcinoids · VEGFR · PDGFR · mTOR inhibitor · Endocrine tumors · Somatostatin analogs · Combinations

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Introduction

Neuroendocrine tumors (NETs) are rare malignancies arising from endocrine cells in the digestive tract [1]. Databases in the United States showed that the incidence of well-differentiated NETs is increasing and the prevalence of this disease makes it one of the most frequent tumors of the digestive tract [2]. For example, the prevalence of pancreatic neuroendocrine tumors (PNETs) makes it more frequent than pancreatic adenocarcinoma that despite displaying higher incidence also shows poorer survival, resulting in a lower prevalence compared to PNET. PNETs may either be functioning or be non-functioning depending on the ability of cancer cells to secrete hormones. Symptoms being more often associated with functioning PNETs, patients with functioning PNETs are often diagnosed earlier than non-functioning tumors. At diagnosis, almost 50 % of patients are diagnosed with localized tumors and about 25 % of patients have either regional or distant metastases. Surgery remains the only treatment that can cure patients but indeed remains limited to patients with operable diseases [3]. Since most tumors will develop unresectable liver metastases and/or extrahepatic metastases early on, curative surgery (liver resection and/or radiofrequency ablation) is often impossible and instead of surgery, liver-directed therapy, such as chemoembolization may have palliative benefits for patients with liver-dominant metastases [4]. Somatostatin analogs are often prescribed to relieve symptoms resulting from hormonal hypersecretion in functioning tumors such as diarrhea and flushing episodes [5]. Recently, data also demonstrated that somatostatin analogs could also delay tumor progression in selected patients with carcinoid tumors [6], although this demonstration has not yet been made for patients with PNETs. PNETs are acknowledged to be sensitive to chemotherapy contrary to carcinoid tumors. Chemotherapy, such as streptozotocin, combined with either doxorubicin or fluorouracil [7, 8] was the only systemic treatment approved for many years in advanced PNETs, though the magnitude of benefit has been challenged in recent reports [9, 10]. However, no significant effect has yet been demonstrated in endocrine tumors of the digestive tract outside PNETs.

Well-differentiated PNETs of the digestive tract are often slow-growing tumors with a low mitotic index and low Ki-67 staining. However, PNETs are known to be highly vascularized, as measured by CD31 immunoassay and express high levels of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1α (HIF-1α). Conversely, undifferentiated PNETs demonstrate active proliferation with a high mitotic index and Ki-67 expression, remaining poorly vascularized, and display low levels of VEGF and HIF-1α [11]. HIF-1α-dependent VEGF expression is a key driver of angiogenesis [12] in well-differentiated PNETs. Malignant PNET tissue also shows widespread expression of platelet-derived growth factor receptors (PDGFR)-α and PDGFR-β, stem cell factor receptor (c-KIT), and vascular endothelial growth factor receptors (VEGFR)-2 and VEGFR-3 (Fig. 9.1) [13]. Several preclinical studies have supported the rational of using antiangiogenic agents such as drugs inhibiting VEGF, VEGFR, and PDGFR [14]. Downstream activation of

the mammalian target of rapamycin (mTOR), mediated either through upstream insulin-like growth factor receptor-1 (IGFR-1) and somatostatin receptors or through direct activation through the lack of nutriments and hypoxia, has also been shown to stimulate proliferation in PNETs [15]. Consistently, inhibition of mTOR yielded significant antiproliferative effects in endocrine tumor cells [16].

Laboratory results and clinical data from phase I/II clinical trials showed evidence, suggesting that antiangiogenic agents may have potent activity in patients with advanced PNETs. In this chapter, we will review the biology of PNETs along with mechanisms yielding to the development of tumor angiogenesis. We will focus on the results obtained with sunitinib in patients with well-differentiated PNET.

Characteristics of Angiogenesis in PNETs

Well-differentiated PNETs are often characterized by the abundance of tumor vessels. Contrasting with angiogenesis in other tumor types such as glioblastomas and colorectal and breast carcinomas, in which angiogenesis is associated with a poor prognosis, angiogenesis in well-differentiated PNETs is eventually associated with a good prognosis [17]. Tumor differentiation and angiogenesis (as measured by CD31 staining) in pathological specimens are often correlated with radiological features using CT scan. Paradoxically, well-differentiated tumors are far more angiogenic than poorly differentiated carcinomas. Consistently, vascular density is correlated with tumor enhancement at the pancreatic phase of the CT scan. In univariate analysis, high microvessel density (immunostaining) and vascular enhancement in the tumors in CT scan have been associated with a favorable survival [18]. In addition, Couvelard et al. [19] have reported that high microvascular density was prevalent in well-differentiated PNETs compared to poorly differentiated carcinomas that displayed fewer vessels. Moreover, well-differentiated tumors usually have high cytoplasmic expression of VEGF and HIF-1α. In contrast, poorly differentiated carcinomas are associated with nuclear HIF-1α and high membrane expression of carbonic anhydrase-9 (CA-9). Low microvascular density and high CA-9 membrane expression have been associated with a significantly poorer survival.

Data from pathological series have elucidated biological features characterizing subgroups of sporadic PNETs. In the literature, somatic mutations of *VHL* were considered to represent a rare event in sporadic PNETs [20, 21] as compared to hereditary form of PNET. However, Schmitt et al. [22] recently reported that up to 25 % of sporadic PNETs display alterations in the *VHL* expression. Importantly, in this study performed mainly in well-differentiated PNETs, 14/78 cases (18 %) had deletion of the *VHL* gene (FISH), while 2/35 (6 %) cases showed methylation of the *VHL* promoter region. Interestingly, these genomic abnormalities were associated with low expression of *VHL* RNA in 25 % (8/32 cases). Consistently, approximately one-third of tumor samples showed positive staining of hypoxia

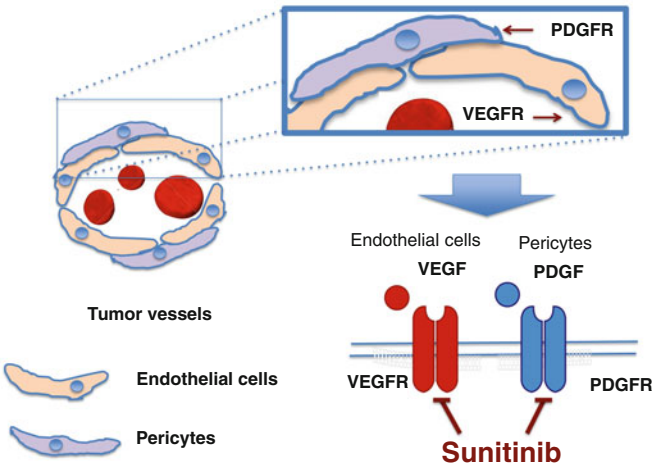

Fig. 9.1 *PNET angiogenesis*. Angiogenesis in PNET is primarily made from endothelial cells covered by pericytes. Endothelial cells are dependent on VEGF and VEGFR for maintenance and survival. Pericytes are dependent on PDGF and PDGFR. Sunitinib is a tyrosine kinase inhibitor that inhibits VEGFR and PDGFR, inhibiting endothelial cells and pericytes survival

target proteins including HIF-1α, CA-9, and GLUT-1 in 29, 44, and 34 % of PNET, respectively. Correlating *VHL* alterations and hypoxia with survival parameters, the authors observed that both *VHL* mutation and positive CA-9 expression were associated with poor outcome [22]. Furthermore, evidence has suggested that the expression of other proteins acting as oxygen sensors in cells such as prolyl hydroxylase domain proteins (PHD)-1, PHD-2, and PHD-3 was also correlated with a poor outcome such as tumor metastases, tumor recurrence, and lower survival [23]. Changes in metabolism and chromatin remodeling were recently observed in genetic investigations, identifying that DAXX/ATRX and MEN1, along with genetic disorders in the mTOR pathway, are frequently observed in PNETs [24]. Sporadic PNETs with adverse outcome remain good clinical models for therapeutic approaches targeting hypoxia-associated angiogenesis (HIF-1α-dependent VEGFR activation) and/or mTOR signaling pathway.

Studies of Angiogenesis in RIP-TAG Transgenic Mice

For three decades, Hanahan et al. have contributed to the field of endocrine malignant tumors by developing in 1985 a mouse model named RIP1-Tag2 (RT2). In this model, the insulin promoter was coupled with the large-T antigen of SV40,

yielding transgenic mice that develop islet cell dysplasia, in situ carcinoma that could subsequently evolve according to a comprehensive multistage carcinogenesis mimicking human PNETs [25]. Unlike xenograft models transplanted in immune-deficient mice in which the murine stroma participate in tumor angiogenesis, the RT2 transgenic model offers the opportunity to explore tumor cells within their own physiological environment and angiogenesis. The RT2 model appears particularly important to explore interactions between cancer cells and "non-tumor" stromal cells, such as endothelial cells and pericytes that are responsible for the development of tumor angiogenesis. This model is also recognized as a prototype model for stepwise processes of tumor development and progression via multiple stages. For instance, Langerhans islet cells display stages of multifocal carcinogenesis such as in situ carcinoma, eventually undergoing the angiogenic switch that results in invasive, bulky, and potentially lethal endocrine carcinoma.

Multiple signaling pathways related to IGF/IGF-1R, MMP-9 and MMP-2, VEGF-A/VEGFR2, mTOR, EGFR, and PDGF-B/PDGFRβ [12, 26–28] are frequently activated in several cell types such as endothelial and tumor cells, pericytes, and stromal cells in PNETs [27–29]. The RT2 model appears highly reproducible and has been frequently used as a tool to explore pharmacological strategies targeting different stages of PNET carcinogenesis [29–31].

However, the RT2 model also suffers limitations. For instance, the incidence of tumor necrosis and hypoxia-related signaling activation remains limited in RT2 as compared to human PNETs. Another limiting factor is the low incidence of metastases occurring at late stages of RT2, as most of the mice may die at advanced stages from tumor-induced hypoglycemia before developing distant metastases. This limitation was addressed by developing a variant model of RT2 that consisted of a double transgenic RIP1-Tag2 and RIP7-Igf-1R mouse model, overexpressing the type I insulin-like growth factor receptor (IGF-1R) in pancreatic islets [26]. This model yields to a more invasive and metastatic phenotype. Although the life span of mice in this later model is reduced compared to RT2 counterparts, this model has been considered to be more relevant to study PNET metastases as it reproduces features closer to advanced human PNETs.

Evaluation of Antiangiogenic Agents in the RT2 Model

Targeted agents including antiangiogenic agents have been explored using the RT2 model (Table 9.1). Initially, preclinical evaluations have focused on matrix metalloproteinase (MMP) inhibitors, angiostatin, and endostatin. Preclinical evaluations using [29, 30] these compounds failed to translate into clinical benefits due to poor pharmacokinetic parameters (angiostatin, endostatin) or unexpected toxicities (MMP inhibitors). In recent years, new compounds, including tyrosine kinase inhibitors against VEGFR and PDGFR and mTOR inhibitors, have been extensively investigated. VEGFR2 inhibitors and mTOR inhibitors were able to interfere efficiently with angiogenesis and tumor development in RT2 models [31].

Table 9.1 All-grade emergent adverse events (%) occurring in ≥20 % of patients in either sunitinib or placebo arm in the sunitinib phase III trial [32]

	Sunitinib ($n = 83$)	Placebo ($n = 82$)
Diarrhea	49 (59)	32 (39)
Nausea	37 (45)	24 (29)
Asthenia	28 (34)	22 (27)
Vomiting	28 (34)	25 (30)
Fatigue	27 (32)	22 (27)
Hair color changes	24 (29)	1 (1)
Neutropenia	24 (29)	3 (4)
Abdominal pain	23 (28)	26 (32)
Hypertension	22 (26)	4 (5)
Hand-foot syndrome	19 (23)	2 (2)
Anorexia	18 (22)	17 (21)
Stomatitis	18 (22)	2 (2)
Taste disturbances	17 (20)	4 (5)
Epistaxis	17 (20)	4 (5)

VEGFR2 inhibition almost completely prevented dysplastic lesions to undergo the angiogenic switch in prevention experiments and also significantly reduced the size of tumors in intervention and regression experiments. The antitumor effects of antiangiogenic agents and mTOR inhibitors were associated with a significant reduction in vessel density and permeability. Immunohistochemistry studies revealed that rapamycin also increased significantly the frequency of apoptotic cancer cells [31]. The promising results reported in these experiments are consistent with the recent clinical trials using sunitinib and everolimus that were recently conducted in patients with PNET [32, 33].

Understanding Resistance to VEGFR Inhibitors in RT2 Mice

Emerging resistance to VEGFR inhibitors appears as an important issue in clinical trials that remains poorly understood. Several studies using the RT2 model have noticed that continuous exposure to antiangiogenic agents such as VEGFR inhibitors may eventually be associated with the emergence of acquired resistance. Casanovas et al. [34] have reported that short-term treatment with VEGFR2-blocking antibodies resulted in smaller tumors. However, more prolonged exposures that were associated with initial tumor shrinkage were subsequently followed by a tumor regrowth mimicking tumor progression occurring in patients with acquired resistance to antiangiogenic agents. When regrowing, tumors were shown harboring an invasive phenotype. Islet tumor cells that developed under VEGFR2 therapies were more prompt to invade surrounding exocrine pancreatic tissues and induce capsule breakage. Despite a sustained inhibition of VEGFR2, most tumors analyzed at the time of progression still displayed a high microvascular density

with leaky vessels and microvascular hemorrhages comparable to untreated tumors. This suggested that tumor angiogenesis might have developed using VEGFR2-independent mechanisms at the time of tumor resistance. In this paper, the authors pointed out the important role of hypoxia and HIF-1α in resistant tumors. Further analysis revealed that several proangiogenic factors, including fibroblast growth factors (FGFs), ephrins, and angiopoietins, were upregulated in resistant tumors. Interestingly, these proangiogenic factors were mainly upregulated in tumor cells compared to endothelial cells. These data suggest that hypoxia triggered by VEGFR2 inhibitors may induce multiple alternative factors of angiogenesis and facilitate a transition toward a more invasive phenotype. Consistently, our team also reported such an induced epithelial-to-mesenchymal transition in patients receiving long duration of treatment with sunitinib in patients with hepatocellular carcinomas [35]. More recently, data have also suggested that resistance to VEGFR inhibitors may be associated with c-MET activation in part explaining the reasons why tumor progression in PNETs may be associated with the development of an invasive and metastatic phenotype.

Sunitinib as a Prototype Drug-Inhibiting Angiogenesis in PNET

Sunitinib malate (SUTENT®; Pfizer Inc, NY, USA) is an antiangiogenic agent initially approved for the treatment for advanced renal cell carcinoma and imatinib-resistant/imatinib-intolerant gastrointestinal stromal tumors (GIST). Sunitinib inhibits VEGFR-1, VEGFR-2, and VEGFR-3, PDGFR-α and PDGFR-β, and c-KIT, in addition to FMS-like tyrosine kinase 3 (FLT3), colony-stimulating factor-1 receptor (CSF-1R), and glial cell line-derived neurotrophic factor receptor (rearranged during transfection (RET)) [36]. The above-mentioned kinase receptors have been described in NETs including PNETs, providing a rationale for the clinical evaluation of sunitinib in PNET [12, 28]. Furthermore, as a result of inhibition of VEGFR and PDGFR, data on sunitinib in the RT2 mouse model demonstrated a significant reduction in endothelial cells and pericyte coverage of tumor vessels [37]. Experiments performed in this model showed that the inhibition of either PDGFR or VEGFR might inhibit the growth of tumors by preventing the malignant transformation and by acting on established tumors. The magnitude of the effects was greater when both receptors were inhibited together than either VEGFR or PDGFR alone, suggesting a potential for greater therapeutic benefit when both of the receptor families were inhibited concurrently. As a result, therapeutic experiments using sunitinib in mice with established RT2 tumors demonstrated significant reduction in tumor size and extended survival in mice treated with sunitinib as compared to a saline placebo, establishing the relevance of this model for clinical applications [38].

Activity of Sunitinib in Phase I–II Clinical Trials

In the first-in-man phase I trial with sunitinib, potent antitumor activity (unusually high number of objective radiological responses) was observed in several tumor types [39]. Patients who were enrolled in the phase I trial presented tumors that expressed VEGFR, PDGFR, and c-KIT, that were highly angiogenic, and that were resistant to cytotoxic agents. Objective responses were observed in renal cell carcinoma and imatinib-resistant GIST, leading to phase II/III trials that subsequently demonstrated the efficacy of sunitinib in those two indications. Three patients with NETs entered in the phase I trial. These patients were primarily referred for tumor progression after several lines of chemotherapy. One patient experienced a prolonged partial response, and two patients achieved sustained minor responses [39]. Subsequently, a multicentre phase II trial was launched with sunitinib (50 mg/day 4 weeks on/2 weeks off) in patients with carcinoids and PNETs [40]. Among 66 patients with advanced PNETs (28.8 % of patients with functioning tumors), the objective response rate was 16.7 % with 56.1 % of patients experiencing tumor stabilization for more than 6 months, leading to a median time-to-tumor progression of 7.7 months. Patient-reported outcome data in this phase II trial showed no detrimental effects of sunitinib on quality of life across repeated cycles.

Dataset to Evaluate the Efficacy of Sunitinib in PNET

The efficacy of sunitinib in PNET was demonstrated in a large placebo-controlled multicenter phase III trial. In this study [32], sunitinib was given continuously at the daily dose of 37.5 mg (with no dose interruption). The continuous daily dosing yields same dose intensity than the 50 mg/day dosing given for four weeks every six weeks. This dosing was selected to avoid dose interruption, potential flair-up in tumor angiogenesis following treatment discontinuation, and was though to induce less acute, i.e., more manageable side effects in a patient population expected to receive sunitinib over a very long period of time. The study was designed to detect a 50 % improvement in the progression-free survival (PFS) in patients receiving sunitinib compared to placebo, expecting that placebo-treated patients would achieve a median PFS of less than 6 months. Based on theses assumptions, 340 patients would have been required to complete this trial. Since PFS was the primary end point in this trial, patients were allowed to crossover to sunitinib at the time of tumor progression in open-label sunitinib continuation studies. This study was terminated earlier than anticipated based on advice provided by an independent data monitoring committee who accessed unblinded data. This committee detected a higher occurrence of deaths and serious adverse events in patients receiving placebo. The committee also identify that at the time of their evaluation, the PFS in patients receiving sunitinib was double of that in the placebo group. Despite a relatively low number of events, this observation of 100 % improvement

in PFS made unlikely that the trial would have been missing its primary end point of 50 % improvement in PFS at the time of the formal primary analysis. Furthermore, it was considered unethical to continue randomizing patients in the placebo group, which was resulting in a greater risk of early death and adverse events. Therefore, the recommendation was made to discontinue randomizing patients and give a chance to patients previously randomized to placebo to access open-label sunitinib. This recommendation was subject to criticism. It was primarily argued that this early termination, occurring before the expected number of events, might have reduced the power of the study and overestimated the magnitude of PFS benefit of sunitinib in this patient population. However, the PFS benefit (as reflected by the hazard ratio) in this study was very high and has been correlating with a high tumor control rate and an improved overall survival. Those benefits observed on secondary end points provided additional supportive evidence of the drug efficacy and easily set aside most of reservations based predominantly on pure statistical considerations.

Efficacy of Sunitinib in Patients with PNET

Results from this trial showed that the median PFS of 11.4 months in patients treated with sunitinib was significantly higher to the 5.5-month PFS in patients treated with placebo (hazard ratio 0.397, $p < 0.001$) [32]. These results were further confirmed by an independent blinded review of CT scans in this patient population. The magnitude of benefit was independent of most relevant clinical factors, including the percentage of liver involvement by metastases, prior or concurrent use of somatostatin analogs, prior use of systemic chemotherapy, and Ki-67 index (evaluated on primary tumor biopsy). Importantly, a slight but meaningful survival improvement was detected in patients treated with sunitinib despite crossover that usually tends to reduce survival benefits in randomized trials. Interestingly, a trend toward overall survival differences seems to be maintained over time in updated analyses. The radiological objective response rate was 9.3 % in patients treated with sunitinib with an additional number of patients who experienced unconfirmed partial responses (primarily due to the early study termination that prevented subsequent radiological confirmation of responses in the database) and, thus, may have underestimated the objective response rate of sunitinib in this trial. Furthermore, several patients receiving sunitinib with liver metastasis also develop large area of tumor hypodensity that usually reflects tumor necrosis without significant changes in the tumor size. This peculiar feature was previously reported with antiangiogenic agents in other tumor types and is usually considered as evidence of antitumor activity. The safety of sunitinib was also evaluated in patients with PNET in this trial (Table 9.1). Adverse events were similar to those observed in other sunitinib studies and mainly consisted of neutropenia, hypertension, diarrhea, and hand-foot syndrome and were manageable and reversible. Quality of life of patients was also evaluated under treatment with

sunitinib. Interestingly, quality of life was maintained over multiple cycles of sunitinib. Interestingly, sunitinib can be combined safely with somatostatin analogs. In the sunitinib phase III trial, 35 % of the patients received somatostatin analogs. No difference in the safety profile of patients treated or not treated with somatostatin analogs was detectable in this trial. Furthermore, the PFS benefit of sunitinib was similar in patients receiving or not receiving somatostatin analogs. Based on data from this phase III trial, the FDA and the EMA have approved sunitinib in patients with advanced well-differentiated PNET.

Sunitinib in the PNET Treatment Algorithm

See Fig. 9.2.

Most of the treatment options for the last 20 years have been based on small, mostly uncontrolled phase II trials or retrospective clinical data. The last randomized clinical trials leading to drug approval 30 years ago were studies published by Moertel [7, 8]. These trials established streptozotocin-based chemotherapy as a standard of care in PNETs for more than 30 years. However, the methodology that was used in those trials used response criteria based on clinical examination that is not anymore considered reliable for trials. The extent of benefit of streptozotocin-based chemotherapy has been revisited in recent years using radiological CT-scan-based criteria, such as RECIST-defined response criteria. Those recent trials confirmed the activity of streptozotocin but stressed that the magnitude of benefit may have been overestimated in original publications. Furthermore, chemotherapy, including streptozotocin, is not considered as a standard of care in many countries. Therefore, in the sunitinib phase III trials, only a proportion of 57.5 % of patients had prior treatments with chemotherapy. This allowed subgroup analyses looking at PFS in patients previously treated or not treated with chemotherapy. In the sunitinib phase III trial, the hazard ratio for PFS benefit was similar in patients previously treated or not treated with chemotherapy. Therefore, it is tempting to consider that targeted agents such as sunitinib may be considered for first-line treatment prior to chemotherapy or alternatively in patients who progress under chemotherapy. However, we are missing clinical studies directly comparing sunitinib to streptozotocin or other more recent chemotherapy regimens (such as the orally administered temozolomide–capecitabine combination) as first-line treatment for well-differentiated PNETs. Of course, the selection of the first treatment option for patient with advanced non-operable well-differentiated PNET will also be made according to drug availability and reimbursement issues in many communities and countries. Importantly, we are still missing scientific data on how to rationally select the appropriate first-line therapy in PNETs. Ki-67 (MIB-1) index has been proposed to identify patients with high level of cancer cell proliferation who may benefit most from an antiproliferative treatment such as chemotherapy [41]. However, Ki-67 index is often obtained from tumor biopsy made in the tumor primarily that may have happened years prior to

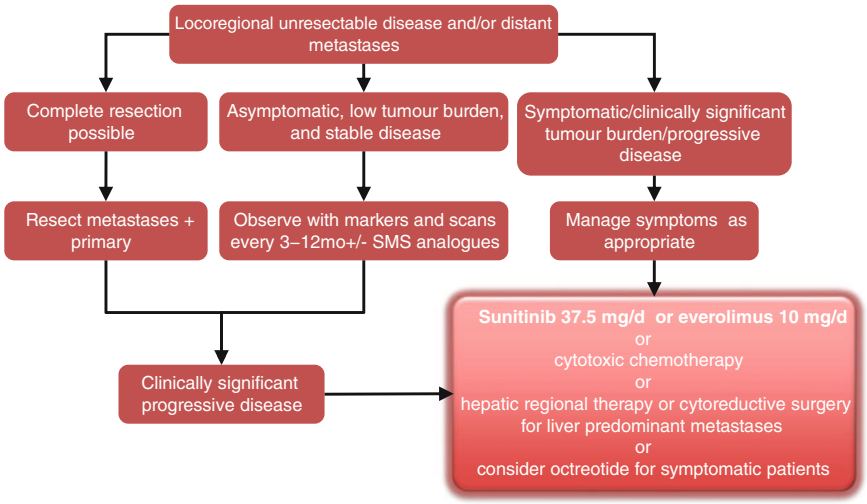

Fig. 9.2 Treatment algorithm for patients with PNET

consider systemic treatment with chemotherapy. Only a few patients are rebiopsied on the metastasis at the time of systemic tumor progression. Data available from tumors that were rebiopsied at the time of tumor progression tend to suggest that the Ki-67 index would increase, most patients having a Ki-67 index >5 %.

Convenience and Compliance Using Sunitinib in PNET

Convenience has been also regarded as a potential advantage of targeted therapies, limiting discomfort associated with intravenous infusions, central venous line implantation, and in-patient hospitalization. This may be particularly important for patients who wish to continue working or continue social activities. However, patient compliance to self-administered targeted agent has emerged as a major issue for sunitinib as well as mTOR inhibitors. Compliance shall be continuously evaluated to ensure the optimal management of patients treated with sunitinib. This requires a careful evaluation of treatment-related side effects that shall be either prevented or treated early to avoid treatment interruption.

Conclusion

Conventional streptozotocin-based chemotherapy displays limited efficacy in PNETs and almost no activity in carcinoid tumors, and therefore, the search for novel therapies was highly warranted. Data from two large placebo-controlled

phase III trials have demonstrated that sunitinib and everolimus produced clinically significant improvements in PFS in patients with unresectable, locally advanced, or metastatic well-differentiated PNETs. This led to the recent approval of these two drugs in patients with advanced PNETs. More clinical data will urgently be needed to answer critical clinical questions such as the optimal sequence for the use of chemotherapy, sunitinib, and everolimus. Recent publications have shown that performing large trials in PNETs was feasible. Furthermore, those trials established that PFS could be regarded as a valid end point for the clinical development of novel anticancer agents. Future trials are underway or will be initiated to evaluate the activity of combinations and to optimize the use of sunitinib at earlier stages of PNETs, at the time of surgery, or in combination with liver-targeted therapies. The search for novel drugs in carcinoid tumors is another challenge for future clinical trials.

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Chapter 10
Clinical Management of Targeted Therapies in Neuroendocrine Tumours

L. Carter, R. A. Hubner and J. W. Valle

Abstract Targeted treatments such as sunitinib and everolimus are providing exciting new options for the management of pancreatic neuroendocrine tumours (pNETs). Clinical management of patients receiving targeted therapies aims to maximise the benefits patients achieve whilst minimising the impact of side effects to maintain quality of life. Adverse event management requires physicians to control co-morbidities, carefully review medication histories and to educate and support patients prior to receiving new treatments. Individual strategies to control specific common side effects such as fatigue, diarrhoea and stomatitis should be employed to allow optimal treatment duration and maintenance of dose intensity; all of which are key to ensure maximum benefit is derived from any treatment option. Recognising and acknowledging the difficulties patients may experience with adherence to chronic medications, and providing strategies to overcome them is a further important component of patient care. At the core of clinical management is effective communication between patients and physicians, which ensures patients are fully involved in decisions concerning their care, and will allow advances in the use of targeted therapies to be translated into benefits for individual patients.

Keywords Neuroendocrine tumour · Targeted therapy · Side effects · Adverse events · Anti-angiogenesis · mTOR inhibition

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Introduction

Neuroendocrine Tumours (NETs) are a group of malignancies arising from neuroendocrine cells in a variety of organs, most commonly the respiratory and gastrointestinal tracts. Although often considered uncommon, recent data indicate an increasing incidence of NETs over the past three decades [1] which, combined with their indolent behaviour, contributes to a prevalence that approaches or exceeds that of other cancers for many primary sites. Clinical presentation is generally either due to hormone secretion giving rise to characteristic syndromes, or symptoms and signs of advanced malignancy in non-functional tumours. Potentially, curative surgery forms the mainstay of treatment in early-stage disease; however, many patients present with advanced disease or relapse after resection, and for these patients, therapeutic options are all of palliative intent although some patients will live for a number of years. Tumour grade and differentiation have a powerful influence on prognosis. The median survival in patients with localised grade (G) 1–2 tumours is 18.6 years, whilst for patients with regional disease, it is 9.25 years [1]. In patients with advanced disease, those with well differentiated tumours (including G1 or G2) have a median survival of 33 months, compared to 5 months for those with poorly differentiated, G3, tumours [1]. The site of the primary tumour is also relevant in estimating prognosis, with advanced tumours of the small bowel and duodenum having the most favourable outcomes, whilst those of the liver and colon have the poorest. Thus, there are a number of factors which influence the disease course for patients with NETs, and these can also guide medical practitioners in the selection and timing of the most appropriate therapeutic strategies.

Recent progress has been made in the treatment of patients with advanced, progressive pancreatic neuroendocrine tumours (pNETs) with targeted therapies. Sunitinib, an inhibitor of multiple receptor tyrosine kinases, significantly improved the median progression-free survival (PFS) to 11.4 months compared to 5.5 months ($p < 0.001$) for placebo in a phase III trial [2]. In a similar study, the mammalian target of rapamycin (mTOR) inhibitor everolimus also significantly improved median PFS to 11.0 months compared to 4.6 months ($p < 0.001$) with placebo [3]. Furthermore, patients with low- or intermediate-grade small bowel NETs treated with octreotide long-acting repeatable (LAR) and everolimus experienced a median progression-free survival of 16.4 months, compared to 11.3 months with octreotide LAR and placebo although this difference just failed to reach the level of statistical significance ($p = 0.026$ with a preset boundary set at $p \leq 0.0246$) [4]. These landmark trials not only led to regulatory approval of both sunitinib and everolimus in pNETs but also facilitated the investigation of these and other targeted agents, such as bevacizumab and pazopanib, in the treatment of both pancreatic and non-pancreatic NETS.

Many patients with NETs will not have significant symptoms until their disease is advanced so careful consideration as to how to utilise treatments to maximise efficacy whilst maintaining patients' quality of life is required. Optimising the

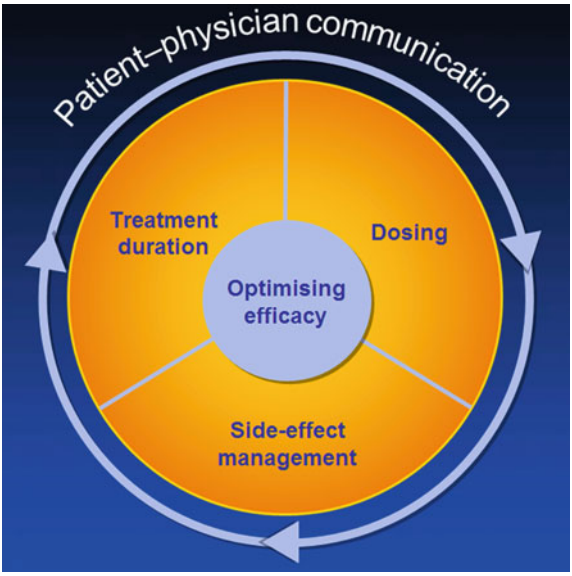

Fig. 10.1 Key factors for successful therapy management with targeted agents [5]

efficacy of treatment requires appropriate maintenance of dose intensity, maximising treatment duration and improving adherence to therapy. To achieve these physicians must not only appropriately manage side effects but also ensure they effectively communicate with patients. These are not discrete areas of management but have significant overlap when treating patients with NETs as highlighted in Fig. 10.1.

Adverse Event Management

Targeted therapies such as sunitinib and everolimus have distinct typical adverse event profiles which differ to cytotoxic chemotherapies. The continual and sometimes prolonged nature of treatment with targeted therapies also leads to challenges in managing side effects. Raymond et al. [2] reported the most commonly experienced adverse events in patients with advanced pNETs receiving sunitinib; these were diarrhoea, nausea, asthenia, vomiting and fatigue (see Table 10.1). The majority of adverse events were grade 1 or 2 whilst the most common grade 3 and 4 adverse events were neutropenia (noted in 12 % of patients) and hypertension (noted in 10 % of patients). The adverse events which most commonly resulted in discontinuation of sunitinib were fatigue (4 % of patients), diarrhoea (2 %) and cardiac failure (2 %).

Treatment of patients with pNETs with everolimus also resulted in primarily grade 1 and 2 toxicities [3]. The commonest adverse events with everolimus were stomatitis, rash, diarrhoea and fatigue. The most common grade 3/4 toxicities were

Table 10.1 Commonest side effects experienced in patients receiving sunitinib and everolimus in the phase III trials

Side effect	Sunitinib		Everolimus	
	All grades (%)	Grades 3/4 (%)	All grades (%)	Grades 3/4 (%)
Diarrhoea	59	5	34	3
Nausea	45	1	20	2
Asthenia	34	5	13	1
Vomiting	34	0	15	0
Fatigue	32	5	31	2
Stomatitis	22	4	64	7
Rash	18	0	49	<1
Infections	Not known	Not known	23	2

anaemia, hyperglycaemia, stomatitis, thrombocytopenia, diarrhoea, hypophosphatemia and neutropenia, all occurring with a frequency of 7 % or less. Adverse events led to discontinuation of treatment in 13 % of cases; the most common adverse events to result in discontinuation of treatment were pneumonitis, fatigue and interstitial lung disease (breakdown by event not provided). Everolimus in combination with octreotide LAR produced a similar pattern of adverse events with the most common adverse events being stomatitis, rash, fatigue and diarrhoea [4].

The trials examining the use of sunitinib and everolimus in NETs confirm that they are not without toxicity. The chronic nature of the treatment with targeted therapies also raises concerns about cumulative toxicity and potential late side effects. Assessment of patients with metastatic renal cell carcinoma (RCC) in an expanded access programme for sunitinib confirmed that with longer lengths of treatment (over 6 months) patients experienced a comparative increase in the incidence of adverse events compared to less than 6 months treatment (it must be noted that the schedule of administration differs between the two indications; 37.5 mg/day continuous daily dosing in pNET and 50 mg/day for 4 weeks followed by 2 weeks' rest in RCC). There were, however, no new or unexpected toxicities seen and no increase in the rate of serious cardiac toxicity (grade 3 or greater) [6, 7]. This highlights the continual need to address emergent adverse event management with patients.

1. **Prior to therapy:** Adverse event management for pNET patients treated with targeted agents should begin prior to starting therapy. Careful history taking and examination is required to assess for co-morbidities, particularly those which overlap with the toxicity of the proposed treatment such as hypertension [8, 9]. Optimising a patient's overall fitness is also important through for example addressing nutrition. It is also imperative to assess a patient's psychological well-being and assess for conditions such as undiagnosed depression. An assessment of patients' regular medication including complementary medicines should also be performed to rule out medications which could interact with the targeted medication, for example as sunitinib is predominantly metabolised by the cytochrome p450 (CYP), enzyme CY3A4 medications

which affect CYP3A4 could influence sunitinib levels [10]; for example ketoconazole, an inhibitor of CYP3A4 doubled the area under the concentration–time curve (AUC) of sunitinib whilst rifampicin, an inducer of CYP3A4 reduced the AUC by half [11]. It is also important to screen for medications that may exacerbate potential side effects of the targeted agents such as QT prolongation. Pre-existing infections should be treated before a patient starts everolimus [12]. Everolimus has the potential to re-activate viral hepatitis infection; it is therefore necessary assess for risk factors of Hepatitis B and C and to undertake formal testing if at increased risk. Involving a multidisciplinary team in the patient's management such as a podiatrist if there is pre-existing foot pathology prior to starting sunitinib may be helpful [8].

Routine blood tests should be performed along with specific bloods such as thyroid function tests for patients to be treated with sunitinib and glucose and lipid levels for patients to be treated with everolimus to rule out any undiagnosed conditions that may impact on a patient's ability to tolerate treatment. It is then prudent to continue to intermittently monitor full blood counts, renal profile liver function tests and drug-specific test such as thyroid function [12, 13]. There is no standard routine for how often to check blood tests, but they are usually performed once in each 28-day treatment cycle provided the patient is stable with the exception of thyroid function tests which are often performed every 2 cycles [14].

2. **During therapy:** It is also important that patients are educated appropriately about the side effects of the targeted agents they are to receive and how to tackle side effects [9, 15, 16]. It has been shown that patients may tolerate treatment better if they are prepared and can then tackle side effects prior to them affecting dose intensity [17]. It is also helpful if the patients have written information to back up what was discussed in the clinic. Illustrative aids may also be useful to aid patients recognising side effects such as PPE and everolimus induced rashes [15, 18]. Reinforcing the need for patients (or carers) to contact the specialist team promptly if they develop respiratory symptoms whilst receiving everolimus is also important. It may also be helpful to the patient if their family and friends are involved in the discussions about adverse event management to help them to manage adverse events outside the oncology clinic [15]. Frequent monitoring will be necessary initially, particularly during the first two cycles as patients are likely to require additional support and sources of advice such as contact with a specialist nurse to help them adjust to the new treatment and manage emerging adverse events [18]. In the initial cycle, it would be common to review the patient after 2 weeks of treatment and then review them at the start of each subsequent cycle.

Actively tackling adverse events as they occur is important to minimise their impact. It is important to distinguish between clinically relevant and less relevant side effects. Hypothyroidism, hair and skin depigmentation, neutropenia, PPE and diarrhoea seldom lead to treatment interruption or permanent dose reduction if

appropriately managed. Fatigue and hypertension occasionally lead to treatment interruption or dose reduction. Pneumonitis, interstitial lung disease and cardiac failure are potentially life-threatening and may require treatment interruptions or discontinuation [2, 3]. It is important to consider techniques to manage adverse events whilst preserving dose intensity if possible. Strategies to tackle common adverse events experienced with everolimus and sunitinib will now be considered.

Adverse Event Management Strategies

Fatigue

Fatigue is a common adverse event associated with many targeted therapies and can be very disabling for patients significantly impacting on their quality of life. Fatigue is often multi-factorial with the patient's cancer and co-morbidities potentially contributing to its severity in addition to the therapy itself. Identification and correction of reversible factors such as hypothyroidism and anaemia may improve fatigue. Fatigue can also be caused by underlying psychosocial problems or depression which should also be addressed if present. Dehydration can exacerbate fatigue so patients should be asked to maintain a good intake of fluids [18]. Counselling regarding adaptation of daily activities and routines according to energy levels may enable patients to cope with fatigue more effectively. Physical exercise may be beneficial for patients experiencing fatigue [15, 18] though some authors advocate daytime rest [9].

Diarrhoea

Diarrhoea is a common adverse event associated with both sunitinib and everolimus. Patient education again remains central to the management of diarrhoea [19]. Patients should be encouraged to keep diaries listing the severity and frequency of episodes of diarrhoea along with associated symptoms to aid physicians in managing it [20]. Recommendations about adapting diet include increasing foods that are high in fibre whilst avoiding foods noted to aggravate the diarrhoea such as spicy foods. The involvement of dieticians to enable patients to adapt their diets whilst maintaining good nutrition may be beneficial [18]. Dehydration associated with diarrhoea should be aggressively tackled through maintaining a high fluid intake and if necessary utilising rehydration solutions [8, 19]. Medication such as loperamide, diphenoxylate or opiates may be required to control diarrhoea. Patients should be given clear instructions about the most effective way in which to take these medications to control their symptoms and their adherence to this should be subsequently assessed.

Dermatological Adverse Events

Targeted agents can cause a range of dermatological adverse events including rashes, PPE, hair depigmentation, skin discoloration, pruritus and acute folliculitis. The causative mechanism for the frequent dermatological side effects is not fully known, but proposed mechanisms include microtrauma to capillaries leading to impaired repair, accumulation of breakdown products in keratinocytes and excretion of substances in sweat leading to high concentrations on certain areas of skin [21]. PPE, in which painful blisters and calluses occur in areas of friction and pressure, is a frequent adverse event associated with sunitinib. Patients should be advised to wear comfortable shoes with extra support from cotton socks and gel insole liners for cushioning. Patients may benefit from the involvement of podiatrists [15]. Application of topical alcohol-free emollients to the palms of the hands and soles of the feet after baths and overnight should be encouraged [18].

Rashes can be managed by encouraging regular moisturisation and the application of urea containing lotions to the skin. Long-term topical steroid use should be discouraged due to the risk of infection. Anti-histamines may help alleviate associated pruritus. Distinguishing a rash that is not clinically serious from a rash associated with hypersensitivity such as Stevens–Johnson syndrome involves assessment for bullous lesions, mucosal involvement and associated clinical symptoms such as elevated temperature [19].

Stomatitis

Stomatitis (usually oral) was noted as one of the commonest adverse events associated with everolimus therapy in trials with neuroendocrine patients. Patients require education about stomatitis and how to tackle it. Dietary modifications such as avoiding spicy, salty or acidic foods and eating foods which required less chewing may help [19]. Patients experiencing stomatitis may benefit from switching to paediatric toothpastes and using soft toothbrushes [8, 15]. They should be encouraged to use regular alcohol-free mouthwashes such as bicarbonate mouthwashes. They may experience symptomatic benefit from using mouthwashes containing paracetamol and using codeine and morphine if required to control pain. Topical anaesthetics and ice chips may also help alleviate the discomfort [15, 19].

Hypertension and Cardiovascular Adverse Events

Pre-existing hypertension should be corrected prior to commencing patients on sunitinib. Blood pressure should be regularly monitored either daily or three times a week [15] whilst a patient continues to receive sunitinib. If hypertension is subsequently diagnosed patients should be treated with appropriate anti-hypertensive according to their individual circumstances [19] and local/national guidelines. The optimal anti-hypertensive to use in patients receiving targeted agents is still an area

of debate. Due to their renoprotective effects, the use of ACE inhibitors and AT-II receptor antagonists is recommended by some authors [22]. A number of anti-hypertensive agents such as verapamil and diltiazem use should carefully be considered in patients treated with sunitinib as they may affect metabolism by CYP3A4 [9]. Caution in the use of diuretics is also advised to the risk of developing diarrhoea and thus dehydration with targeted agents [22]. Lifestyle measures to help with hypertension such as regular exercise and a low-salt diet should also be recommended [8].

Cardiovascular adverse events such as congestive heart failure, left ventricular dysfunction, cardiomyopathy and prolongation of QT interval are associated with sunitinib with 2.4 % of patients with pNET developing cardiac failure in the phase III trial [2]. A thorough cardiovascular history and examination should be undertaken prior to commencing sunitinib [9]. Patients with myocardial infarctions, severe/unstable angina, symptomatic congestive heart failure, cerebrovascular accidents and transient ischaemic attacks within 12 months were all excluded from the phase III trial of sunitinib in patients with pNETs [2] and therefore would not be ideal candidates for treatment. Baseline electrocardiograms and if clinically indicated echocardiograms should be performed with regular electrocardiograms thereafter [18]. Patients should be educated about cardiovascular risks and the symptoms to monitor for. Oncologists also need to ensure they actively seek out symptoms or signs of cardiovascular compromise so that prompt investigation and treatment is undertaken if necessary.

Hypothyroidism

The rates of sunitinib induced thyroid dysfunction vary between studies up to 85 % in one retrospective review of patients with RCC [23]. In patients with pNET, 6 of the 86 patients randomised to treatment with sunitinib in the phase III study developed hypothyroidism [2]. The exact cause is unknown, but it is hypothesised to be due to prevention of appropriate binding of VEGF to thyroid cells and by impairing thyroid blood flow [23]. Prior to commencing treatment patients, thyroid function should be checked, and if any abnormalities are noted, these should be investigated and treated as appropriate. When patients are receiving sunitinib regular monitoring of thyroid function should be undertaken for example every other cycle [14]. Once detected, hypothyroidism should be managed as per standard medical practice.

Quality of Life

Targeted therapies are used in neuroendocrine patients with advanced incurable disease in whom the aims must be to balance the toxicity of therapies with their response to preserve a patient's quality of life. However, patients with NETs have

a worse health-related quality of life than the general population [24]. Patients with carcinoid syndrome or increased rates of bowel movements or flushing report worsened health-related quality of life than patients with NETs without those characteristics. The quality of life of the population being treated is therefore already affected by their cancer so they are not experiencing adverse events as their only symptoms. Quality of life data were not collected in the phase III trial of everolimus in patients with pNET [3] whilst assessment of quality of life in neuroendocrine patients receiving sunitinib compared to patients receiving placebo showed no clinically or statistically significant difference in symptoms apart from diarrhoea or global health-related quality of life. Delay of deterioration of global health-related quality of life and five functional scales covering emotional and physical aspects of a patient's life was seen with sunitinib treatment [25]. This delay was dependent on the effect of sunitinib on PFS. Therefore to preserve patient's quality of life, effective treatment with targeted agents is required.

Dose Maintenance and Treatment Duration

Dose intensity has been shown to be important in achieving maximal benefit from a number of oncological therapies in differing disease sites. A retrospective analysis of breast cancer patients who received 85 % or less of their planned adjuvant treatment showed they had both a shorter time to relapse and overall survival compared to those who received more complete treatment. Patients who received less than 65 % of their planned cytotoxic chemotherapy had a relapse-free survival of just 48 % compared to 77 % for those who had received the full treatment [26]. A meta-analysis of patients receiving the targeted agent sunitinib for the treatment of metastatic renal cell cancer (mRCC), gastrointestinal stromal tumours and solid tumours also demonstrated increased sunitinib exposure was associated with improved clinical outcomes including time to progression (TTP), overall survival and response rates [27]. In mRCC patients ($n = 146$), there was a statistically significant relationship between exposure and the probability of receiving a complete or partial response ($P = 0.00001$). However, there was also a trend towards increased adverse events including fatigue, neutropenia and diastolic blood pressure with increased exposure to sunitinib. Although a positive relationship between exposure to sunitinib and the incidence of fatigue was observed, there was no relationship with the severity of fatigue indicating the relationship between side effects and dose is not straight forward. When managing adverse events, dose reductions should be carefully considered therefore to ensure they are only carried out when the side effects are definitely related to the targeted therapy rather than the disease, are of a severity to warrant intervention and cannot be controlled in other ways to allow maintenance of dose intensity.

Maximising treatment duration is also an important consideration to ensure optimal results for patients [28]. This can be achieved by managing side effects appropriately and addressing patients' expectations which will be discussed in

further detail later in this chapter. As there are limited treatment options for patients with NETs, each treatment needs to be utilised to its full potential for as long as the patient is responding and not experiencing excessive clinically relevant adverse events. The optimal duration of therapy with targeted treatments is not yet clear but currently patient are treated until they develop progressive disease or are unable to tolerate the treatment. In RCC, trials are underway to assess the optimal schedules with which to treat patients with targeted agents, but this issue has not yet been addressed in pNETs. Unlike many traditional cytotoxic chemotherapies, targeted agents may cause a disease stabilisation through a cytostatic response rather than a disease reduction through a cytotoxic response which can make it harder to assess response with traditional criteria such as RECIST. A study looking at tumour growth rates in addition to RECIST criteria in patients treated with targeted agents indicated that the treatments may be being stopped prematurely in patients who could be benefiting from them [29]. Careful consideration of a patient's overall benefit from targeted therapies as well as the scan response needs to be made before stopping or switching therapies.

Adherence

Adherence can be defined as the extent to which a patient's overall behaviour coincides with medical advice [30]. Compliance is sometimes used interchangeably with adherence but can also be defined as taking medication as directed, e.g. with or without food [17] whilst persistence can be defined as continuing to take medications for the recommended length of time (though they could be taken either correctly or incorrectly) [31]. The definitions and methodologies utilised to assess adherence vary significantly in published data [30]. Methods such as patient-reported adherence, pill counts, drug metabolite levels, microelectronic monitoring system (utilising intelligent tablet bottles which record the time and date when the cap was removed), and analysing pharmacy and insurance records have all been used. Each of these methods has potential limitations and may produce variable results when considering the data produced. Adherence remains, however, a key area of assessment when dealing with oncology patients.

Adherence rates for many therapies can be strikingly low, but it has often been thought that cancer patients, given the gravity of their conditions, would be motivated and therefore adherent to medication. It has also perhaps not been a key concern amongst oncologists as until recent years intravenous chemotherapies have been predominately used where adherence in the community was not a consideration. Adherence rates in oncology studies, however, range between 20 and 100 % [30] and are felt to be lower outside clinical trials [32]. Levine et al. [33] assessed adherence in 108 newly diagnosed haematological patients over a 6-month period noting just 16.8 % of patients were fully adherent to allopurinol and 26.8 % of patients adherent to prednisolone, prescribed as part of their treatment regimen.

Adherence to treatment depends on a number of factors including socio-demographical characteristics of the patient, features of the treatment such as complexity and side effects and features of the illness such as disability and duration [34, 35]. Although there is variability in the literature as to factors influencing adherence, oncologists should be particularly aware of the possibility of poor adherence in patients with a history of mental illness such as depression [35], patients with poor knowledge or insight into their disease, patients with poor social support systems, patients with cognitive impairment and patients at the extremes of age—adolescents and the elderly. Adherence rates depend on part on a patient's perceptions of the risks, benefits and costs of an intervention [36]. It is therefore important to explore a patient's beliefs about their illness and the treatment proposed with them [37].

Improving Adherence

Poor adherence can result in patients' conditions worsening, unnecessary diagnostic tests or changes in regimens for patients. If a patient is not taking a drug, they will not benefit from it so utilising strategies to ensure good adherence is an important part of all consultations. Many patients worry about admitting poor adherence to health professionals as they perceive it may be construed as "bad behaviour" [30]. Within consultations, therefore simply asking patients about adherence in a non-judgemental manner, acknowledging that it can be difficult, may help. It will allow the patient to discuss the barriers to good adherence enabling the patient and physician to develop strategies to tackle these together [34, 38].

Patient education has been demonstrated to improve adherence to treatments [33, 39, 40]. The aims of patient education should include developing a patient's knowledge of their diagnosis and prognosis and the treatment options available including their associated risks and benefits thus empowering them to manage their own illness [17]. When discussing a therapeutic regimen, it is important to provide clear and simple instructions with written information to provide reinforcement for the patient [41]. Dosing schedules can be improved through methods such as the use of pill boxes [42], simplifying regimes if possible, and utilising cues to daily events to remind the patients to take medications [34, 38]. It is also important to enlist family members, friends and ancillary staff such as nurses and pharmacists to provide support and assistance to the patient in maintaining adherence [17, 34, 41].

Patient–Physician Communication

As previously alluded to, effective patient–physician communication is pivotal for successful therapy management as in all oncological management. Clear communication is necessary to inform patients about their condition and prognosis and

thus manage their expectations. Good communication, as well as strengthening the patient–physician relationship, has been shown to improve patient outcomes. It increases patient satisfaction with their care and may help them cope better with their illness [37, 43] and improve outcomes [44].

Research has shown that patients want to receive detailed information about their disease, prognosis and treatment options but want to feel able to negotiate how they receive this [45], for example the amount of information they want to receive in a visit. Good interpersonal communication should include friendliness, respect, interest, empathy, be genuine and include unconditional acceptance [45, 46]. Utilising similar language to the patient [17] is important as is summarising and checking patients' understanding regularly [45]. Above all listening to patients and their wishes and concerns is essential for good communication. As sustained improvement in communication skills has been demonstrated through training [47] it is worth considering the role improving oncologists' communication skills could have on therapy management given the central importance of good communication skills.

Conclusions

Effective clinical management of targeted therapies is a multifaceted process with the patient at its centre.

Ensuring good adherence to treatments is critical for the success of targeted therapies and can be improved by openly discussing this issue with patients. Patient education can improve adherence and patients' ability to manage their disease including the adverse events associated with treatment. Active management of adverse events including preventative measures will enable dose intensity to be maintained. The foundation of all oncological management is effective communication between patients and physicians ensuring joint input into management plans. Optimising the clinical management of targeted therapies in neuroendocrine patients will ensure they receive the maximum benefits from therapies whilst maintaining their quality of life.

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Chapter 11
Imaging of Neuroendocrine Tumors and Challenges in Response Evaluation for Targeted Therapies

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Abstract Targeted therapies such as sunitinib and everolimus have emerged as novel treatment options for patients with pancreatic neuroendocrine tumors (PNET). Clinical trials are also pending for non-pancreatic neuroendocrine tumors. Large randomized trials using sunitinib and everolimus in PNET demonstrated that response rate by Response Evaluation Criteria in Solid Tumors (RECIST) was insufficient to predict the overall patients' outcome and did not correlate with progression-free survival. Using RECIST, most patients receiving targeted agents have experienced tumor stabilization. Disappointedly, tumor stabilization by RECIST made impossible to recognize during the course of therapy the subset of patients who were truly benefiting from treatments. Therefore, investigators have started to seek for other imaging methods and criteria that could help identifying true responders from patients receiving targeted agents. In this chapter, we aimed to review the various imaging techniques used to characterize neuroendocrine tumors in routine clinic and clinical trials. We also challenge the potential

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advantages of those imaging techniques for the evaluation of response in patients with neuroendocrine tumors. Finally, we discuss novel criteria that are not only based on measurement of tumor dimension but also rely on tumor density such as Choi criteria.

Keywords Angiogenesis · Hypodensity · Necrosis · RECIST · Choi

Introduction

Digestive neuroendocrine carcinomas are rare malignancies of increasing incidence. Among them, well-differentiated tumors are characterized by a rich vasculature and display specific characteristics using contrast-enhanced imaging that are critical to consider given therapeutic implications. Indeed, only few medical options were available for decades, based on classical cytotoxics and/or somatostatin analogs. Recently, oral-targeted therapies designed at blocking tumor vasculature were approved in well-differentiated malignant pancreatic neuroendocrine tumors, opening a new era in the therapeutic armamentarium. Clinical observations using both sunitinib and everolimus in pNET showed less than 10 % objective response by classical dimensional RECIST, contrasting with marked survival improvement [1, 2]. Such results have renewed the debate on response evaluation, suggesting the insufficiency of RECIST to translate the benefit of targeted therapies, as previously discussed by Choi and colleagues since 2007 while using imatinib in GIST [3, 4] and our team by using sunitinib in advanced hepatocellular carcinoma [5]. In this chapter, we review specific imaging features of neuroendocrine tumors and discuss challenges for response evaluation for targeted therapies in well-differentiated advanced neuroendocrine tumors.

Imaging Characteristics of Neuroendocrine Tumors

Imaging plays a central role in the management of patients with neuroendocrine tumors. A variety of imaging modalities are available for assessing these solid lesions, including ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), endoscopic US, and hybrid nuclear imaging techniques such as single-photon emission computed tomography-CT (SPECT-CT), and positron emission tomography-CT (PET-CT); each of which has its own strengths and limitations. Accurate diagnosis can be challenging. The use of a multimodality imaging approach is often helpful in equivocal or complex cases and depends both on the localization of the lesions and on the oncological setting: detection and characterization of the primary tumor, tumoral staging or grading, evaluation of the tumoral response, detection of recurrences, etc.

Percutaneous US remains the first-line examination for most patients, mainly because it is non-invasive, cheap, and widely available. It has been shown to be useful in the detection of primary tumors, mainly located in the pancreas, but also for the liver metastases, which is a central question in this type of tumors. Pancreatic tumors typically appear as hypoechoic and well-delineated masses. In young patients, the lesion may appear as iso- or hyperechoic in comparison with the surrounding pancreatic parenchyma, which is classically less echoic than in elder patients. The cephalic region of the pancreas is easier to explore, and the examination is often impaired by the presence of digestive gas. This is why US is not used for the detection of tumors of the midgut or the hindgut [6].

Endoscopic ultrasound (EUS), or echoendoscopy, enables a better detection of the primary tumors and is considered to be a key examination in the initial diagnosis. Its sensitivity and specificity are 89 and 90 %, respectively, with a low morbidity rate [7, 8]. Aside from the description of the lesion, the operator can assess the vascular involvement, detect associated nodes, and perform a fine-needle biopsy, when necessary. The main limitations are the small duodenal gastrinoma (sensitivity of 50 %), the peripancreatic nodes, and the caudal lesions with a sensitivity of 55 % [9]. The stomach can also be explored in the context of a MEN-1, in order to detect ulcerations, and gastric neuroendocrine tumors.

The classical imaging evaluation relies on both contrast-enhanced CT and MR, even if their detection sensitivity is slightly lower than that of EUS. For the diagnosis of pancreatic neuroendocrine tumors, they show similar sensitivities ranging from 73–85 % [10–14]. The spectrum of imaging findings associated with NET is wide and depends of the size and the differentiation of the lesions. Small tumors are generally solid and homogeneous, whereas larger tumors are heterogeneous and may show cystic-necrotic degeneration and calcification.

Interestingly, in contrast to other tumor types in which angiogenesis correlates with tumor aggressiveness, high angiogenesis reported in well-differentiated pancreatic neuroendocrine tumors is associated with a good prognosis. Tumor vascularization measured by CD31 staining on pathological specimens is often correlated with radiological features using contrast-enhanced imaging, well-differentiated tumors being more angiogenic than poorly differentiated carcinomas. Consistently, in the study of Rodallec et al., vascular density assessed by light microscopy was significantly correlated with tumor enhancement at the pancreatic phase of the CT. By univariate analysis, both high microvessel density as measured by immunostaining and vascular enhancement in the tumors in CT were associated with a favorable survival [15]. Conversely, undifferentiated tumors are poorly vascularized and prompt for proliferation, thus display standard imaging characteristics of solid tumors with rapid kinetics: larger and heterogeneous lesions showing hypodensity on precontrast CT, hypointensity on T1-weighted images, variable intensity on T2-weighted sequences, and mild enhancement after contrast agent injection in comparison with the surrounding parenchyma. The presence of a venous tumoral invasion must be identified and is very specific of this type of tumors. In contrast, well-differentiated neuroendocrine tumors are often characterized by a low mitotic index and are associated with a rich

vascularization. These lesions appear as well-delineated and spontaneously hypodense or isodense lesions on precontrast CT, hypointense on T1-weighted images, and hyperintense on T2-weighted acquisitions. After contrast medium injection, lesions strongly enhance on the arterial phase acquisition. On the portal and delayed phases, the lesions appear less dense or intense than the surrounding parenchyma (a phenomenon referred to as "washout"). This "typical" enhancement pattern is in fact described in 45–55 % of the cases, mostly in insulinomas. Another pattern, referred to as "fibrotic," is encountered in 30–40 % of the NETs, especially gastrinomas. The lesions share the same precontrast features but present with a progressive enhancement after contrast medium injection without washout [6, 15, 16]. On diffusion-weighted imaging (DWI) with low b values (0 or 50 s/mm^2), some small benign endocrine neoplasms may have high signal intensity, and high ADC values may be measured on the ADC map images. In comparison, malignant endocrine neoplasms may have high signal intensity on DWI with high b values and low ADC values [17].

The somatostatin receptor scintigraphy (SRS), also called octreotide scan or octreoscan, is a type of scintigraphy that uses Octreotide®, a drug similar to somatostatin, radiolabeled with indium-111. The radioactive octreotide enables the visualization of the tumor cells that express receptors for somatostatin; this is why this technique is mostly useful for well-differentiated lesions. Its sensitivity depends on the size of the lesion, its localization, the tumoral differentiation, and the secretion type, but is not influenced by somatostatin analog treatments. For gastrinomas, SRS has a sensitivity of 30–90 % for lesions <1 cm to >2 cm, respectively [18]. For insulinomas, its sensitivity is close to 50 % and around 73 % for all the other tumoral subtypes [19, 20]. False positive corresponds to infections, postoperative aspects, lymphoma, and some solid pseudopapillary tumors.

To date, the indications of ^{18}FDG-PET are the initial evaluation of the tumoral extension and the follow-up of undifferentiated lesions with negative SRS. Indeed, and as opposed to SRS, there is a correlation between the degree of dedifferentiation and the fixation of ^{18}F-FDG [21]. Finally, the recent introduction of somatostatin receptor PET-CT imaging, using 68 germanium or 68 gallium (^{68}Ga-DOTA-TOC and ^{68}Ga-DOTA-NOC), is promising and might lead to the replacement of the more classical SRS by these techniques [22–25].

The liver is the most frequent site for metastatic dissemination of digestive neuroendocrine tumors. Liver metastases are usually highly vascularized, with their blood supply arising primarily from the hepatic artery. Therefore, in most cases, hepatic arterial phase images should provide the best results in the detection of metastases regardless of the imaging technique used (CT or MR imaging). Dromain et al. [20] have reported that liver metastases have a typical hypervascular enhancement in 73 % of patients, an atypical hypovascular or delayed enhancement in 16 % of patients, and a peripheral enhancement with progressive fill-in mimicking hemangioma in 11 % of patients (Fig. 11.1). Tumor lesions, especially bulky ones, are frequently associated with area of necrosis translating

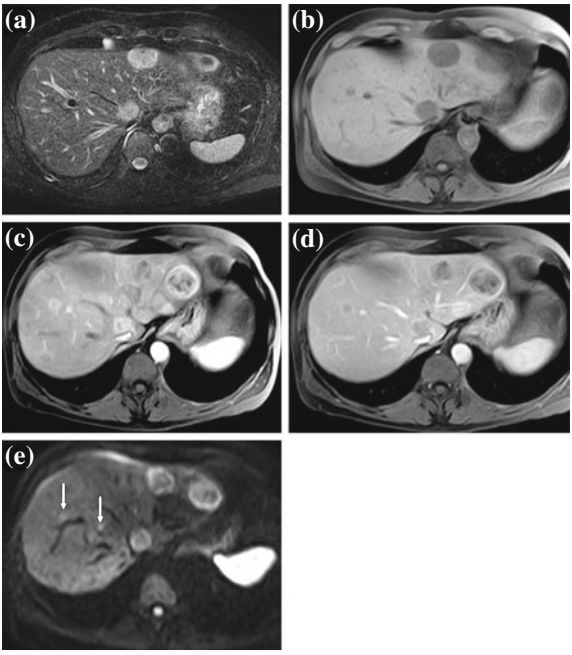

Fig. 11.1 Representative MR imaging aspects of liver metastases of well-differentiated pancreatic neuroendocrine tumors. Lesions present with a hyperintensity on T2-weighted images (**a**) and hypointensity on fat-saturated T1-weighted images (**b**). After contrast medium injection, the lesions are characterized by a marked and heterogeneous enhancement on the arterial phase acquisition (**c**) and a signal decrease in comparison with the surrounding liver parenchyma on the portal phase acquisition (washout, **d**). On the diffusion-weighted images with high b value, the metastases appear as hyperintense lesions (**e**). This sequence enables the detection of very small lesions (*white arrows* in **e**) that were not clearly depicted on the other sequences

into hypodensity at CT-scan. Therefore, large liver metastases of well-differentiated endocrine tumors appear spontaneously heterogeneous on CT-scan with a well-vascularized contrast-enhanced peripheral rim contrasting with central tumor hypodensity. MR has been shown to be superior to CT for the detection of liver metastases. In a series of 64 patients comparing the performances of SRS, CT and MR, Elias et al. showed that MR detected significantly more lesions than the two other techniques (49 vs. 24 % and 38 %, for SRS and CT, respectively) [26]. Moreover, the characterization of the lesions was better. Interestingly, this study did not include DWI sequences that have been shown to increase the detection rate of focal liver lesions in various benign and malignant conditions, especially colorectal liver metastases. Very recently, d'Assignies et al. confirmed these results in TNE liver metastases, showing the superiority of DWI over conventional MR, with a sensitivity of 72 %, and a specificity of 92 % [27]. The association of DWI and contrast medium injection increased the detection rate (sensitivity and specificity of 78 and 97 %, respectively).

Limitations of Recist Criteria to Evaluate Response to Targeted Therapies in Neuroendocrine Tumors

In hypervascularized neuroendocrine tumors, morphological changes are clearly observed after exposure to targeted antiangiogenic agents. As described in early trials using VEGFR inhibitors, the central part of the tumor may show a total disappearance of vascularization suggesting tumor necrosis. Within the first weeks of treatment, patients with bulky tumor masses may display an increase in lactate dehydrogenase in plasma, consistent with the occurrence of tumor necrosis [28, 29]. Occurrence of necrosis has been further confirmed in few patients who benefited from posttreatment surgical resection of residual disease.

Macroscopically, morphological changes that will be detectable on imaging will reflect the early decrease in number of tumor vessels along with the reduced blood flow in the central area of the tumor, rather than tumor shrinkage. Several imaging techniques including high-frequency Doppler ultrasound, blood flow computerized tomography scans, and dynamic contrast magnetic resonance imaging have been proposed to capture the effects of targeted agents on tumor angiogenesis in several clinical trials [5, 30–32]. However, those functional techniques are not routinely performed when treating patients with antiangiogenics outside prospective trials.

Response Evaluation Criteria in Solid Tumors (RECIST), which are simple and reproducible criteria, have been established for many years to assess tumor response when using classical cytotoxics in clinical trials [33, 34]. While RECIST criteria are acknowledged to reflect adequately tumor progression, RECIST have been recently increasingly criticized for their limitations to assess the activity of targeted and antiangiogenic therapies [32]. Since 2007, Choi and colleagues expressed their concern about the insufficiency of traditional RECIST dimension criteria to evaluate the effect of imatinib in GIST and were the first to propose to evaluate changes in tumor density using contrast-enhanced computed tomography [3] as an alternative method for tumor evaluation. Choi criteria included both tumor size and tumor density and were showed to identify more adequately than RECIST patients benefiting from imatinib in GIST [4]. Beyond GIST, the approval of sorafenib in advanced hepatocellular carcinoma [35] and of sunitinib and everolimus in advanced digestive neuroendocrine tumors [36] has focused the attention on response criteria since both agents were registered in pivotal trials while reporting less than 10 % objective response rate. Indeed, in the pivotal trial with sunitinib, RECIST were the selected criteria of response in pancreatic neuroendocrine tumors. The results of pivotal phase III trials with sunitinib and everolimus in pancreatic neuroendocrine tumors showed objective response rate lower than that usually expected for a drug with activity in cancer. In the sunitinib trial, the response rate was 9.1 % [1]. In most patients, only minor responses that meet criteria of tumor stabilization by RECIST were reported. During the course of the phase III trial with sunitinib, several patients with liver metastasis developed large area of hypodensity that usually reflects tumor necrosis with or without

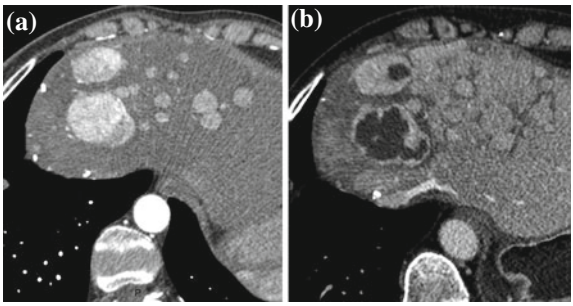

Fig. 11.2 Sunitinib induces decrease in tumor density in patients with advanced pancreatic neuroendocrine tumors. **a** Prior to treatment, liver metastases of pancreatic neuroendocrine tumors with high vascularization. **b** Treatment with sunitinib induces large area of tumor hypodensity

significant changes in tumor size (Fig. 11.2). This pattern was previously reported with other antiangiogenic agents in other tumor types and is usually considered as evidence of antitumor activity [37, 38]. Taken together, it was suggested that this peculiar feature that is not captures by RECIST would explain why PFS and overall survival benefit could be observed with a drug displaying only few responses by RECIST. Similar findings were reported in the everolimus trial with 5 % objective responses according to RECIST, even though a total of 64 % of the patients receiving everolimus had some degree of tumor shrinkage [2]. Therefore, tumor density on CT-scan, rather than measurement of tumor size, has been discussed as a surrogate endpoint of activity for patients treated with targeted therapies for advanced neuroendocrine tumors, as this has been previously proposed for imatinib in GIST by Choi and collaborators.

Perspectives of New Response Criteria for Neuroendocrine Tumors Using CT-Scans

In contrast to functional imaging, which may be costly or uneasy to access, CT-scan is a readily available technique that can be considered as a reliable method to assess both tumor size and tissue density. The first intent to include both tumor size and tumor density was achieved by Choi and colleagues for the assessment of response to imatinib in GIST-treated patients. They defined a partial response as a $\geq$10 % decrease in one-dimensional size or a $\geq$15 % decrease in tumor density on a contrast-enhanced CT-scan (Table 11.1). Choi criteria yielded a response rate of 80 % as compared to 43 % by RECIST and were significantly more predictive of time to tumor progression (TTP) and OS in GIST patients treated with imatinib [4]. In the field of hepatocellular carcinoma, our team has recently investigated the applicability of Choi criteria across two cohorts of patients with advanced HCC treated with VEGFR inhibitors. In the phase II study exploring sunitinib in advanced HCC, investigators have noticed that sustained tumor stabilization was usually associated with decreased tumor density on CT-scans [37]. This led us to perform a post-hoc ancillary study in the 26 patients

Table 11.1 Definition of RECIST and Choi criteria

	RECIST	Choi
CR	Disappearance of all lesions	Disappearance of all lesions
	No new lesions	No new lesions
PR	A decrease in size of ≥30 % (sum of diameters)	A decrease in size of ≥10 % or a decrease in tumor density (HU) ≥15 % on CT
		No new lesions
		No obvious progression of non-measurable disease
SD	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD	Does not meet the criteria for CR, PR, or PD
		No symptomatic deterioration attributed to tumor progression
PD	An increase in size of ≥20 % (sum of diameters)	An increase in tumor size of ≥10 % and does not meet criteria of PR by tumor density (HU) on CT
		New lesions
		New intratumoral nodules or increase in the size of the existing intratumoral nodules

CR complete response, *PR* partial response, *HU* Hounsfield unit, *CT* computed tomography, *SD* stable disease, *PD* progression of disease, *RECIST* Response Evaluation Criteria in Solid Tumors. The sum of longest diameters of target lesions as defined in RECIST [33]

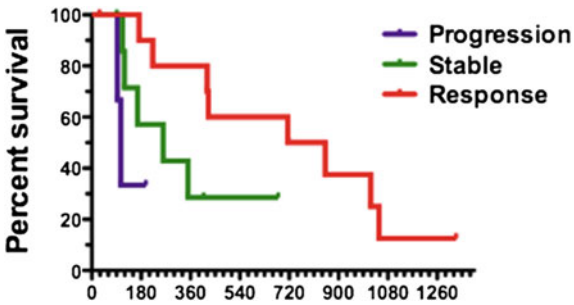

Fig. 11.3 Median progression-free survival (PFS) according to the Choi criteria evaluation. Patients classified as objective responders at 4 weeks according to Choi had a better PFS (median: 783 days) than those classified as stable disease (median: 260 days) or progressive disease (median: 106 days), $p = 0.0385$

evaluable for changes in tumor density on CT-scans. While only 3.8 % of patients had objective response according to RECIST, 65.4 % of patients were reclassified as responders according to Choi criteria [5]. Interestingly, patients reclassified as responders according to Choi criteria experienced a significantly longer median TTP (7.5 months), than non-responders (4.8 months; HR = 0.33; 95 % CI, 0.04–0.75, $p = 0.0182$). We also applied Choi criteria to a second cohort of advanced HCC patients treated in our center with sorafenib on a routine basis, using a blinded independent central response assessment was conducted with RECIST and Choi criteria. Among 64 patients available for both RECIST and Choi criteria, 3 % had response according to RECIST and 51 % according to Choi

criteria. Interestingly, better OS (≥22 months) was observed in patients with complete/partial response than in patients with stable disease and progression, regardless the type of radiological criteria. However, in this second independent cohort, Choi criteria identified more adequately than RECIST subgroups of patients with complete/partial response that also experienced longer survival [39].

The experience of Choi criteria is very limited in neuroendocrine tumors but represents an exciting challenge. In our institution, we have reviewed 22 cases of patients with well-differentiated pancreatic neuroendocrine tumors treated either with sunitinib or everolimus according to both RECIST and Choi criteria [40]. At the time of the first evaluation, two patients (9 %) were responders according to RECIST whereas 11 patients (50 %) presented an objective response according to Choi criteria. Despite the low number of patients, Fig. 11.3 shows that patients responding at the first evaluation according to Choi that had better PFS (median: 783 days) than those classified as stable (median: 260 days) or progressive (median: 106 days), with a p value of 0.0385. These results will require further validation on a larger number of patients, and discussion in terms of technical endpoints (choice of CT phase acquisition, selection of target lesions for hypodensity evaluation), but these preliminary data suggest that Choi criteria could be useful to investigate in neuroendocrine tumors treated with targeted therapies.

Conclusion

Well-differentiated neuroendocrine tumors, especially from pancreatic origin, are highly addicted to angiogenesis and display specific features on contrast-enhanced CT-scans. In a majority of patients treated with targeted therapy, a significant decrease in tumor density can be detected despite only limited reduction in tumor size. Therefore, classical RECIST might be insufficient to capture the full effect of such antiangiogenics agents, warranting additional assessments according to Choi criteria, which might be useful to identify patients benefiting from targeted therapies with better overall survival.

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Chapter 12
Overcoming Resistance to Targeted Therapies: The Next Challenge in Pancreatic Neuroendocrine Tumors (PNETs) Treatment

Annemilaï Tijeras-Raballand, Cindy Neuzillet, Anne Couvelard, Maria Serova, Armand de Gramont, Pascal Hammel, Eric Raymond and Sandrine Faivre

Abstract Chemotherapy in pancreatic neuroendocrine tumors (PNET) has remained for decades the only validated therapeutic option, albeit with debated efficacy. Recently, data from two large placebo controlled phase III trials have changed the therapeutic landscape. They demonstrated that targeted therapies directed against receptor of vascular endothelial growth factor (VEGFR) (sunitinib) and mammalian target of rapamycin (mTOR) (everolimus) produced clinically significant improvement in patients with advanced PNETs, resulting in a doubling of progression-free survival and leading to FDA approval. However, with an increasing number of patients being treated with these drugs following their approval, resistance has emerged as a critical clinical issue. In this review, we aim to summarize the current knowledge about primary (i.e., early progression) and acquired (i.e., tumor regrowth after initial response) resistance to antiangiogenic agents and mTOR inhibitors, using data available from preclinical and clinical studies in various malignancies. Herein, we also describe how these general mechanisms of resistance

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may emerge in patients with PNET treated with sunitinib and everolimus. Overcoming such resistances is likely to be the next challenge for clinicians in advanced PNETs management, warranting seeking for new anticancer strategies.

Keywords Resistance · Pancreatic neuroendocrine tumors · Targeted therapies · Antiangiogenic agents · Sunitinib · mTOR · Everolimus

Introduction

Management of advanced pancreatic neuroendocrine tumors (PNET) has remained a clinical challenge for decades. Due to debated efficacy of chemotherapy in PNETs, there has been a growing interest for targeted therapies. Recently, the antiangiogenic sunitinib and the mammalian target of rapamycin (mTOR) inhibitor everolimus obtained approval from the FDA following two phase III clinical trials showing improvement of progression-free survival (PFS) (11.4 vs. 5.5 months and 11.0 vs. 4.6 months, respectively) [1, 2]. These agents target two key pathways driving PNET biology, as exposed in previous chapters.

Sunitinib and everolimus are now used for several years in multiple malignancies, allowing the understanding of general mechanisms associated with resistance to those targeted agents [3]. First, patients may exhibit an absence of objective benefit in case of primary resistance mechanisms, such as preexisting mutations in the targeted pathways. Second, initial response followed by progression, thereby affording appreciable but limited survival advantage, reflects acquired resistance. Stage of the disease, treatment history, genomic identity, and host microenvironment may interact to convey indifference to targeted therapies [3].

The aim of this chapter is to comprehensively provide the readers with the current knowledge on general mechanisms of resistance to antiangiogenic agents and mTOR inhibitors. Targeted agents have been only recently incorporated in the armamentarium for treatment of PNETs, thus specific preclinical and clinical data remain limited. Describing molecular features in PNETs and data from the RIP-TAG model may serve as basis to understand how resistance may emerge in PNETs under targeted therapies. Finally, we will report a case to illustrate resistance in the clinical setting in a figure and outline potential strategies to overcome it.

General Mechanisms of Resistance to Antiangiogenic Agents and mTOR Inhibitors

Antiangiogenic Agents

Sustained angiogenesis is a hallmark of cancer and is crucial for tumor development (Fig. 12.1) [4]. It is a complex and multistep process which involves many

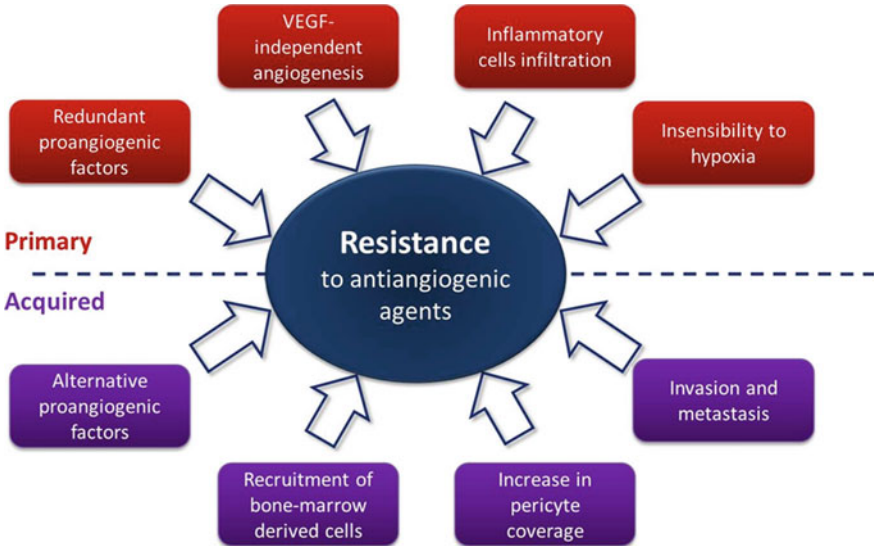

Fig. 12.1 Tijeras-Raballand—overview of the mechanisms of primary and acquired resistance to antiangiogenic agents

growth factors [5]. VEGF has been identified as the key driver of angiogenesis and a target of choice for antiangiogenic treatments. The pioneers of the clinical proof-of-concept for angiogenesis inhibition are (1) bevacizumab, an anti-VEGF antibody, (2) sorafenib, a tyrosine kinase inhibitor (TKI) targeting Raf-1, VEGF receptor (VEGFR), PDGFR-β, and c-Kit, and (3) sunitinib, a TKI targeting VEGFR-2 and PDGFR-β. Few tumors are intrinsically refractory to these inhibitors, but most eventually develop resistance. Given their interesting effects on disease control and broad potential, many efforts have been made to understand the underlying mechanisms of relative inefficacy of antiangiogenics on improving overall survival [3].

Primary resistance may be due to the fact that some tumors produce redundant pro-angiogenic factors besides VEGF and are thus relatively insensitive to VEGF-dependent pathway inhibition. Moreover, tumors may exhibit modes of vessel growth (such as vessel co-option, vascular mimicry, and intussusception) that are less dependent on VEGF pathway and then less sensitive to its blockade [6]. Effects of anti-VEGF agents may also be impaired by preexisting infiltration of inflammatory cells. More precisely, M2-type macrophages express various factors, such as MMP-9, CXC chemokines, and pro-inflammatory cytokines (IL-6, TNF-α, IL-1), that promote angiogenesis and may protect tumor vessels from VEGF pathway inhibition. Finally, some tumor cells can be indifferent to hypoxia. For example, pancreatic ductal adenocarcinomas primarily display a poorly vascularized stroma. Such vascular hypoxic microenvironments promote survival of anaerobic tumor cells that are intrinsically refractory to antiangiogenic treatment.

Other tumors may acquire resistance, thus become unresponsive to the anti-angiogenic treatment. Most experimental evidence suggests that at least four distinct adaptive mechanisms are involved in acquired resistance to antiangiogenic agents [3]. The first mechanism is hypoxia-induced activation and/or upregulation of alternative pro-angiogenic pathways such as angiopoietin, ephrins, or FGF. Second, hypoxia also triggers recruitment of bone marrow-derived cells that can contribute to tumor vascularization in a VEGF-independent way. Third, increase in pericyte coverage of the tumor vasculature can protect endothelial cells from death consequent to the lack of VEGF-mediated survival signaling [7]. Finally, hypoxia may enhance tumor cells invasiveness into local tissue and metastatic seeding to lymph nodes and distant organs, providing access to non-tumoral tissue with mature vasculature, less sensitive to anti-VEGF therapy.

mTOR Inhibitors

The PI3K/AKT/mTOR pathway mediates signal transduction downstream many growth factor receptors. mTOR functions as a sensor of nutritional/metabolic stress during cell growth and promotes survival and protein synthesis during nutrient or energy rich periods (Fig. 12.2) [8]. This pathway is often deregulated in cancer and thus represents an interesting therapeutic target [9]. Rapalogs, which include rapamycin and associated analogs (temsirolimus, everolimus, and deferolimus), inhibit mTOR activity through association with its partner FK506 binding protein 12 (FKBP-12) [9, 10]. However, as observed with antiangiogenic agents, clinical benefit of rapalogs remains sporadic. Therefore, attempts have been made to identify predictive markers in response to rapalogs and to understand resistance mechanisms.

Primary resistance may be due to activation of alternative pathways, mainly Erk pathway, such as in tumors harboring KRas or BRaf mutations. Second, one of the primary downstream substrates of mTOR, 4EBP1, suppresses eIF4E activity and then represses mRNA translation; low levels of 4EBP1 or high levels of eIF4E may confer resistance to rapalogs [8]. Third, rapamycin treatment prevents p27 downregulation, thereby inhibiting proliferation; accordingly, cells with low levels of p27 may be less responsive to rapalog-mediated growth inhibition [8]. Moreover, tumor cells in which apoptotic pathways are nonfunctional are resistant to rapalog-induced cell death. In contrast, to cells with constitutive activation of PI3K/AKT/mTOR pathway, cyclin D1 overexpression and/or functional apoptotic pathways have been shown to be more sensitive to mTOR inhibition [11].

Many mechanisms of acquired resistance to rapalog therapy have been identified that bypass, within the mTOR pathway, the blockade of the target. They include: (1) activation of feedback loops and alternative pathways (e.g., PIM kinases that can phosphorylate and activate 4EBP1 in an mTOR-independent way), (2) mutations in rapalog targets FKBP-12 or mTOR, (3) loss of function of PP2A, a phosphatase involved in dephosphorylation and inactivation of AKT, and (4) stimulation of autophagy, which is competitive to apoptosis [8].

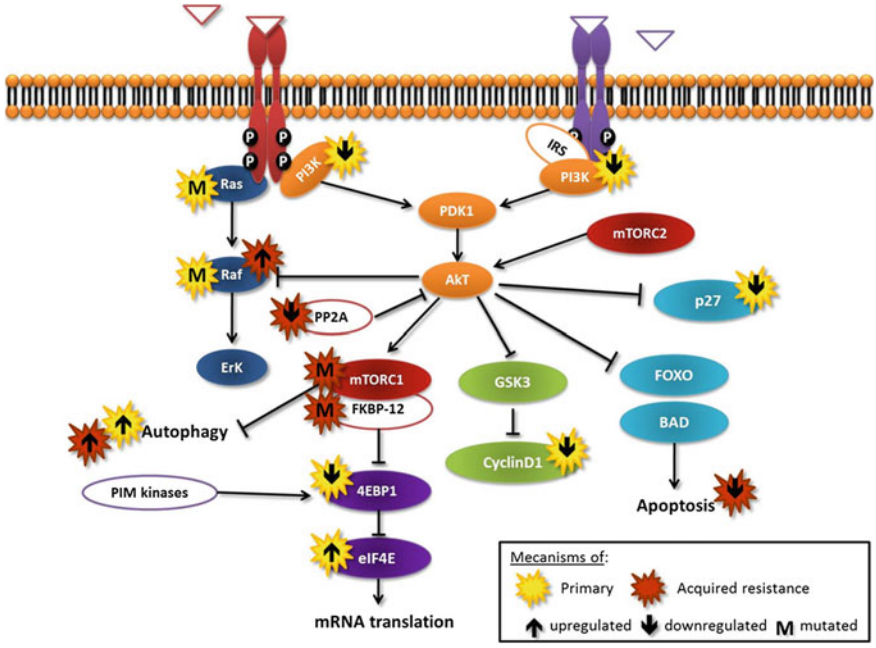

Fig. 12.2 Tijeras-Raballand—schematic representation of the Pi3K-Akt-mTOR cascade deregulations and relevant cross talks involved in resistance to mTOR inhibitors

Pathological and Molecular Characteristics of PNETs Involved in Resistance to Antiangiogenic Agents and mTOR Inhibitors

Resistance to Antiangiogenic Agents

Well-differentiated PNETs are highly vascularized tumors as measured by CD31 immunoassay and express high levels of VEGF, VEGFR-2 and VEGFR-3 [12]. They thus appear as good candidates for antiangiogenic therapy.

The transcription factor hypoxia-inducible factor-1alpha (HIF-1α) is one of the key drivers of angiogenesis in PNETs. The classical HIF targets (also known as hypoxia-related genes) include (1) genes which increase oxygen supply and delivery, such as VEGF and erythropoietin (EPO), and (2) genes that induce glycolysis, such as GLUT-1, hexokinase, and PDK-1. HIF-1α is regulated by the von Hippel–Lindau protein (pVHL), which forms the recognition component of an E3 ubiquitin ligase complex (Fig. 12.3). Under normoxic conditions, HIF-1α is hydroxylated on critical proline residues by prolyl-hydroxylase domain (PHD) proteins, which act as oxygen sensors. Following prolyl-hydroxylation, pVHL binds to HIF-1α, leading to its ubiquitination and subsequent proteasome

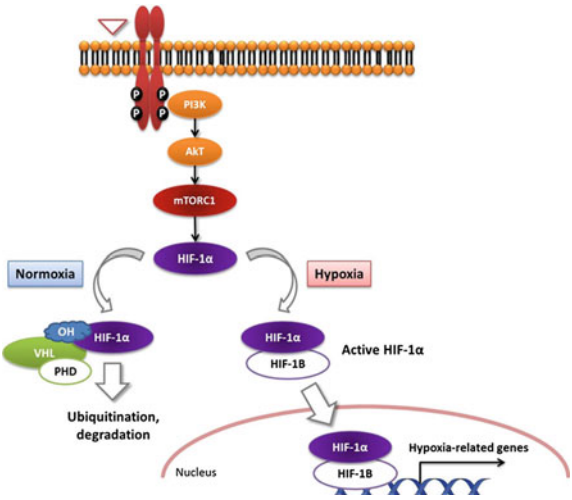

Fig. 12.3 Tijeras-Raballand—schematic representation of HIF-1 transcription factor regulation under hypoxic and normoxic conditions and the role of VHL

degradation. Hypoxia-related genes are thus silenced. Conversely, under hypoxia, HIF-1α is no longer hydroxylated by PHDs and cannot be recognized by pVHL. This results in expression of hypoxia-related genes [13].

Two distinct mechanisms can lead to HIF-1α activation in PNETs (Fig. 12.4). First, *VHL* gene can be inactivated by genetic or epigenetic mechanisms, resulting in the accumulation of HIF-1α and transcription of hypoxia-related genes regardless of the oxygenation status [3, 14]. Hereditary PNETs are observed in VHL disease, a rare autosomal dominant neoplastic syndrome (about one in 36,000 live births) caused by germline mutations in the *VHL* gene on the short arm of chromosome 3 [15]. It is characterized by the development of various benign and malignant tumors and cysts in multiple organs: hemangioblastomas of the central nervous system, retinal angiomas, pancreatic cysts and neuroendocrine tumors, renal cell carcinoma and cysts, epididymal cystadenomas, phaeochromocytomas, and endolymphatic sac tumors [15]. PNETs occur in 10–15 % of patients with VHL disease and are frequently multiple (>30 %). VHL-associated tumors (including PNETs) are usually highly vascularized, due to upregulation of hypoxia-related genes. Alternatively, somatic mutations of *VHL* gene were considered to be a rare event in sporadic PNETs [8, 16]. However, Schmitt and colleagues [17] have recently reported that up to 25 % of sporadic PNETs display genomic alterations of the *VHL* gene that resulted in gene silencing, such as deletion and promoter methylation. These genomic abnormalities were associated with underexpression of *VHL*-RNA in 25 % of these tumors. Consistently, approximately one-third of tumor samples showed positive staining for HIF-1α and its targets CA9 and GLUT-1 [17].

Following HIF-1α activation, canonical pathways linked to VEGF/VEGFR are activated and drive tumor angiogenesis. Thus, this uncontrolled activation of HIF-1α may convey sensitivity to anti-VEGF inhibitors. These data gave rationale

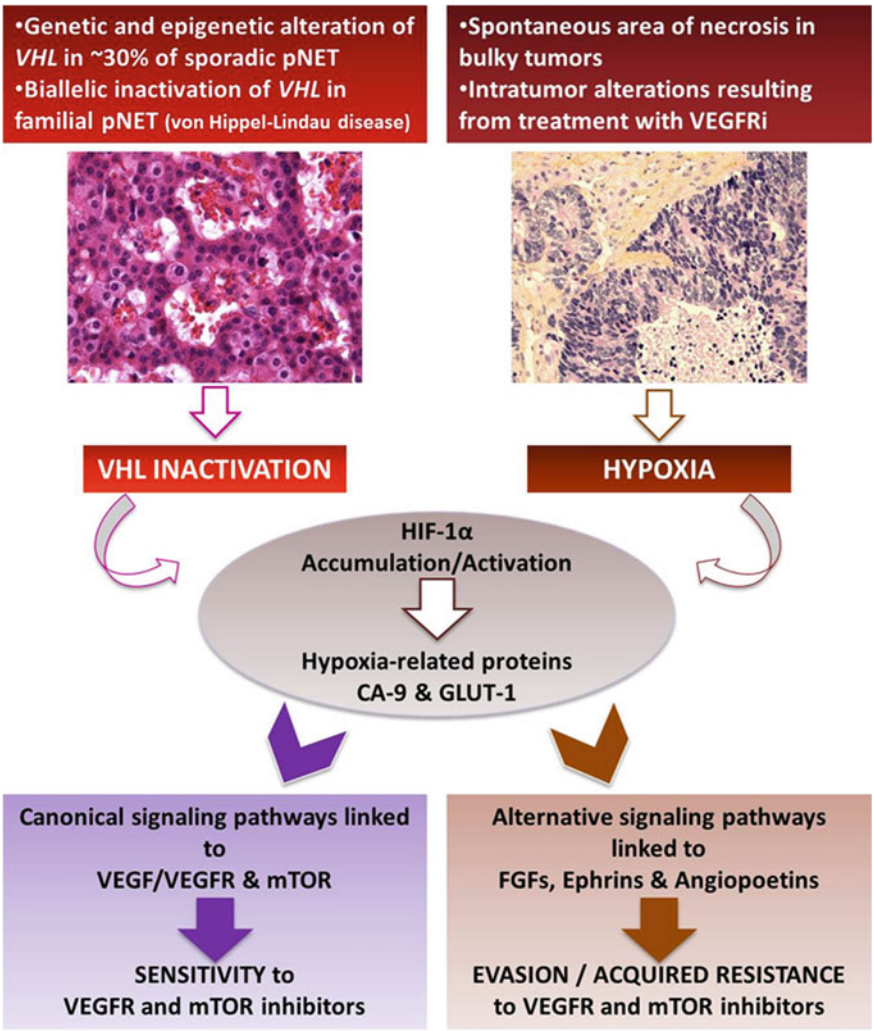

Fig. 12.4 Tijeras-Raballand—VHL inactivation and hypoxia as important events associated with pancreatic neuroendocrine tumor aggressiveness and resistance to targeted therapies

for anti-VEGF-targeted therapies in PNETs and was confirmed in preclinical and clinical studies [1, 16, 18].

Alternatively, HIF-1α activation can result from tumor hypoxia. This is observed at late stage of bulky tumors' natural history in which central hypoxia followed by necrosis spontaneously occurs, due to high tumor volume. Interestingly, high expression of PHD proteins, reflecting tumor hypoxia, has been shown to be associated with higher risks of recurrence and poor survival [13]. Hypoxia-induced activation of HIF-1α triggers canonical signaling pathways

(hypoxia-related genes) but also alternative pathways, which are involved in angiogenesis, epithelial-to-mesenchymal transition (EMT), and cell survival, thus increasing tumor aggressiveness. Noticeably, hypoxia can also be the consequence of antiangiogenic treatments and thereby contribute to acquired resistance to VEGFR inhibitors. Indeed, it has been shown that several pro-angiogenic factors, including predominantly fibroblast growth factors (FGFs), ephrins, and angiopoietins (alternative-pathway genes), were upregulated in resistant tumors to VEGFR inhibitors [16]. All together, these results suggest that VEGFR inhibitor-induced hypoxia may upregulate multiple pro-angiogenic alternative factors and trigger a transition toward a resistant and more invasive phenotype.

Consequently, HIF-1α activation can be both a factor of sensitivity (when hypoxia-unrelated) and resistance (when resulting from tumor hypoxia) to anti-VEGFR therapy in PNETs.

Resistance to mTOR Inhibitors

The same ambivalence regarding consequences of HIF-1α activation exists in response to mTOR inhibitors. Experimentally, mTOR inhibition decreases HIF-1α levels. Thus, tumors that express high level of HIF-1α, such as tumors harboring *VHL* inactivation, may be hypersensitive to rapalog therapy. Alternatively, one of the major mechanisms proposed to underlie the anticancer activity of rapalogs is an antiangiogenic effect by inhibiting signal transduction downstream VEGFR and PDGFR. This latter leads to hypoxia, induction of HIF-1α, and its "collateral damages" as described above and may drive tumor resistance toward these agents.

Development of a Preclinical Model for PNETs Study

Preclinical models available for PNETs study are quite limited as compared with other tumor types. Indeed, PNET cell lines are difficult to obtain. No human cell line has been established to date, and only a few murine, hamster, or rat cell lines have been developed. Most murine cell lines required an SV40-based transformation. Regarding *in vivo* studies, almost all of them have been performed using the specific RIP1-Tag2 (RT2) mouse model developed by Hanahan et al. [19]. They used a fusion transgene composed of the SV40 large-T oncogene and the insulin-promoter, yielding to transgenic mice. Following oncoprotein expression, these mice developed multifocal islet cell dysplasia and in situ carcinomas, ultimately resulting in invasive, yet potentially lethal, PNETs. These tumors evolved according to a multistage carcinogenesis mimicking human PNETs [19]. Noticeably, as observed in human PNETs, this model reproduced the crucial step of angiogenic switch.

This transgenic model offers the opportunity to explore murine tumor cells within their physiological tissue environment. Moreover, this model appears particularly interesting to study the interactions between cancer cells and "non-tumor" cells, including those responsible for tumor angiogenesis. The RT2 model also appears highly reproducible and, therefore, represents a dedicated tool to explore pharmacological strategies targeting different stages of endocrine pancreatic carcinogenesis [16, 20–22].

A possible limitation of the RT2 model is that unlike human bulky PNETs, tumor necrosis is infrequent, leading to limited hypoxia-related signaling activation. Another limitation is the relatively low incidence of distant metastases at late stage, as mice mainly die from tumor-induced hypoglycemia. Therefore, an interesting variant of the RT2 model was developed and consisted of a double transgenic RIP1-Tag2 and RIP7-*Igf*-1R mouse model, overexpressing the type I insulin-like growth factor receptor (IGF-1R) in pancreatic islets [23]. This particular phenotype yields to more invasive and metastatic tumor patterns. Although the life span of these latter mice is slightly reduced in comparison with their RT2 counterparts, this model may be interesting to consider since it reproduces features close to the latest stages of human PNETs.

RT2 model has been widely used to study multiple signaling pathways—mainly related to IGF-II/IGF-1R, MMP-9 and MMP-2, VEGF-A/VEGFR2, mTOR, EGFR, and possibly PDGF-B/PDGFRβ [23–26]—and several cell types—tumor and stromal cells, endothelial cells, and pericytes [21, 24, 26]—involved in PNET development. These findings reinforce the rationale for development of targeted therapies directed against VEGF and mTOR pathways.

Another *in vivo* model has been marginally used. Rat pancreas was treated with azaserine, leading to a chimio-induced carcinoma. CA20948 rat PNET cell line was, thereby, established by isolating cells of acinar origin [27]. This cell line was then injected subcutaneously in the lower flank of rats. This heterotopic model does not allow the study of tumor/stroma interactions, which represents its major limitation for drug evaluation.

VEGF Pathway Inhibitors

In PNETs, antiangiogenic agents have been exclusively studied in the RT2 model. VEGF pathway inhibition was shown to decrease angiogenesis and tumor development.

In prevention experiments, VEGFR2 inhibition by anti-VEGFR2 antibody almost completely abrogated the angiogenic switch and reduced by more than 50 % the tumor burden in both intervention and regression experiments. These effects were associated with a significant reduction in vessel density and permeability [16].

Moreover, VEGFR and PDGFR inhibition in the RT2 model demonstrated, respectively, a 75 % reduction in endothelial cells and a 63 % reduction in

pericyte coverage of tumor vessels [28]. PDGFR or VEGFR inhibition may slow down tumor growth by preventing malignant transformation and by acting directly on already established tumors. Synergistic effects were observed by dual VEGFR/PDGFR inhibition compared to single inhibition. These results suggested a greater potential therapeutic effect when both receptor families are inhibited concurrently, giving a strong rationale for the use of sunitinib.

However, continuous exposure to antiangiogenic agents such as VEGFR inhibitors may eventually be associated with the emergence of acquired resistance in the RT2 model. Casanovas et al. [16] reported that prolonged treatment (4 weeks) with VEGFR2-blocking antibodies resulted in initial response with tumor shrinkage, followed by tumor progression. At the stage of regrowth, tumors harbored a more invasive phenotype. PNETs progressing under VEGFR-targeted therapies were more prompt to invade surrounding exocrine pancreatic tissues and induce capsule breakage [16]. Despite sustained inhibition of VEGFR2, most tumors analyzed at the time of progression still displayed a high microvascular density with leaky vessels and microvascular hemorrhages comparable to untreated tumors. The authors suggested that tumor angiogenesis may have developed through VEGFR2-independent mechanisms (i.e., FGF, ephrins, and angiopoietins) [16]. These observations warrant further comprehensive investigations to elaborate future therapeutic strategies to anticipate and counteract acquired resistances to targeted therapies.

mTOR Pathway Inhibitors

In the RT2 model, rapamycin was shown to interfere efficiently with angiogenesis and tumor development [22]. Immunostaining studies on tumor tissues revealed that rapamycin increased significantly the frequency of apoptotic cells [22]. Rapamycin also increased significantly the survival of treated mice in comparison with the control group. These data provided a strong rationale for the clinical use of mTOR inhibitors in patients with PNETs.

As VEGFR inhibitors, rapamycin treatment in the RT2 model showed transient efficacy followed by tumor progression [22]. Data regarding PNET resistance to mTOR inhibitors are scarce. A unique study performed in the heterotopic rat model showed no efficacy of everolimus on primary tumor progression, suggesting primary tumor resistance. Moreover, everolimus was shown to promote liver, lung, and lymph nodes metastasis [29]. This may be due to suboptimal drug exposure with intermittent administration schedule, raising concern for potential emerging of resistance in noncompliant patients. Contrary to other cancer types, no predictive marker for response or resistance to mTOR inhibitors has been identified in PNETs [30–32].

Strategies to Overcome Resistance to Targeted Therapies in PNETs

Dual Inhibition in Angiogenic Pathways

FGF signaling cooperates with VEGF pathway in tumor angiogenesis. The key role of FGF signaling in acquired resistance to VEGFR inhibitors has been demonstrated in RT2 model (Fig. 12.5) [16]. Anti-FGF treatment in the regrowth phase under VEGFR-targeted treatment produced a significant decrease in tumor progression. These results prompted investigation of brivanib, a dual FGF/VEGF inhibitor. In the RT2 model, brivanib monotherapy showed efficacy both in first- and second-line therapy following the failure of anti-VEGFR2 monoclonal antibody [33]. Noticeably, tumor stability and vascular inhibition were extended by first-line brivanib monotherapy, as compared single VEGFR inhibitor. In the majority of samples analyzed, brivanib produced no signs of revascularization (up to 11-week treatment), in contrast to the demonstrable and earlier onset of adaptive resistance via revascularization with single VEGFR inhibitor. These data suggest that brivanib may delay induction of adaptative resistance.

Thus, two strategies using brivanib may be considered: in first-line therapy, to increase response duration, or after failure of VEGFR inhibitor monotherapy.

Dual Inhibition in mTOR Pathway

mTOR mediates signal transduction downstream EGFR. Adaptive resistance to mTOR inhibitors might involve EGFR-driven upregulation of Akt by suppressing negative feedback loops. Then, the resistance may be overcome by EGFR inhibitors. This gave rationale to mTOR/EGFR dual inhibition by a rapamycin/erlotinib combination treatment [22]. Addition of erlotinib to rapamycin therapy suppressed the development of adaptive resistance and provided survival benefit in the RT2 model.

Sequential Treatment

Another strategy may be to consider mTOR inhibitors after failure of VEGFR inhibitors. Emerging preclinical evidence suggest that resistance to VEGFR-targeted therapies is mediated via tumor and environmental changes [34, 35]. These changes enable a tumor growth that is less dependent on VEGFR, through the activation of other growth factors signaling including FGF/FGFR, HGF/MET, G-coupled protein receptors, and TGF-beta receptor [34, 36]. Given that mTOR transduces signal downstream of many of these receptors, it appears as a relevant target in the setting of tumors resistant to VEGFR inhibitors (Fig. 12.5).

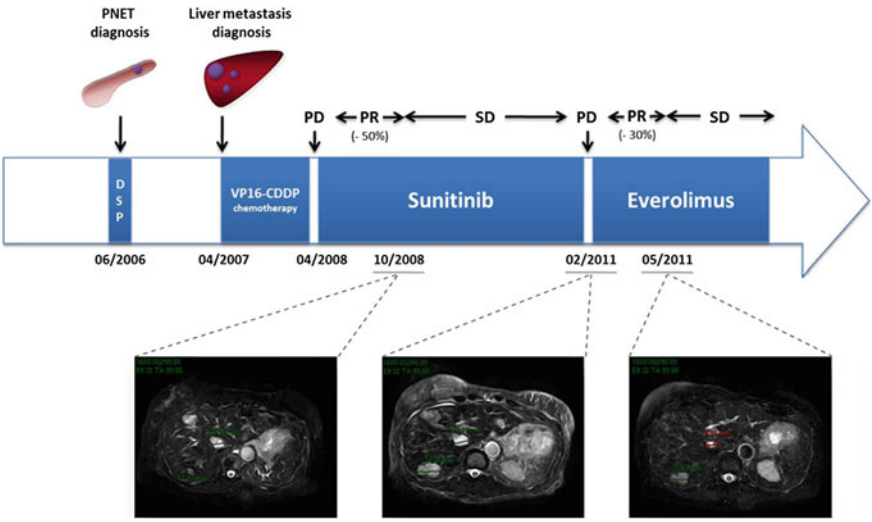

Fig. 12.5 Tijeras-Raballand—example of a 63-year-old female patient with liver metastasis from PNET illustrating acquired resistance: The patient had a history of PNET of the pancreatic tail which had been surgically resected (distal splenopancreatectomy). Ten months after primary tumor resection, liver metastasis was diagnosed. She was first treated by VP16-CDDP chemotherapy. One year after starting this regimen, disease progression (*PD* progressive disease) was observed and the patient was enrolled in a clinical trial evaluating sunitinib. Sunitinib treatment resulted in partial response (PR: partial response) according to RECIST with a 50 % decrease in the size of liver target lesions for nearly 3 years. Sunitinib was then stopped due to tumor progression and a switch to everolimus was decided by multidisciplinary tumor board. Early PR (−30 %) was observed after 3 months of everolimus, still ongoing after 1 year of treatment. Abdominal magnetic resonance imaging pictures show best tumor response under sunitinib treatment, disease progression under sunitinib (acquired resistance), and partial tumor response after 3 months of treatment with everolimus

Conclusion

Data from two large placebo controlled phase III trials have demonstrated that targeted therapies directed against VEGFR (sunitinib) and mTOR (everolimus) produced clinically significant improvements in PFS in patients with unresectable, locally advanced or metastatic PNETs. However, as in other tumors, resistance to these targeted therapies will emerge in PNETs. Overcoming such resistances will be the next challenge for clinicians. Preclinical studies (mainly based on the RT2 mouse model), along with clinical experience will help to answer critical questions such as the optimal sequences for the use of targeted therapies in PNETs and will lead to the development of strategies to limit or counteract acquired resistance.

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Chapter 13
New Anticancer Agents in Neuroendocrine Tumors

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Abstract Neuroendocrine tumors (NETs) are a heterogeneous family of neoplasms of increasing incidence and challenging clinical management. Although generally more indolent than carcinomas, they have a widely variable clinical behavior and are on occasions associated with a very aggressive clinical course. For many decades, available medical options for the systemic treatment of advanced disease have been scant and of limited value in the control of disease progression. In this context, sunitinib and everolimus have triggered great enthusiasm in the field as they have proved for the first time in well-designed controlled clinical trials that there are agents able to improve the clinical outcome of this complex disease. In this chapter, we will review emergent data on new drugs for the treatment of G1–2 NETs, including recently approved angiogenesis and mTOR inhibitors, as well as other novel-targeted agents in perspective.

Keywords Targeted agents · Neuroendocrine · Gastroenteropancreatic · Therapy · Everolimus · Sunitinib · PI3K · HER · IGFR · HDAC

Introduction

Neuroendocrine tumors (NETs) are a family of neoplasms with a complex spectrum of clinical behavior. Their wide anatomical location and heterogenous biology, together with their unique ability to secrete different peptides and neuramines that may cause distinct clinical syndromes, have made these tumors particularly challenging for clinical management. Although traditionally considered

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"rare" tumors, their incidence has substantially increased over the last decades (2.5–5 cases per 100,000 in Caucasian populations). This increasing incidence has been at least partially attributed to improved diagnostic techniques and clinical awareness. The recent development of new anticancer agents in this context has certainly significantly contributed to it [1–4].

Surgery is the only potentially curative therapeutic strategy in localized disease and may also play a role in advanced stages. Patients with advanced disease have, however, limited therapeutic options. In patients with predominant liver metastasis, a number of ablative techniques or chemoembolization may be considered. They are generally employed with palliative purposes in patients with slow growing functional tumors refractory to medical therapy to improve symptom control, but may also be useful to reduce tumor burden and control disease progression in non-functioning tumors. Peptide receptor radionuclide therapy is an alternative option when available for those with octreoscan positive NETs. Nevertheless, the benefit-risk balance of these approaches has never been properly assessed in adequately powered controlled clinical trials.

Regarding systemic drug therapy, long-acting somatostatin analogs remain the best means to achieve symptomatic relief in functioning tumors and some limited data suggest they may also retard disease progression in well-differentiated midgut NETs. Interferon has also demonstrated some efficacy in terms of symptomatic control of the hormonal syndrome, although its use is associated with substantial adverse effects and its impact in terms of tumor growth control has not been consistently demonstrated in small randomized trials. For tumors progressive or refractory to these therapeutic strategies, however, treatment options are scarce. Indeed, conventional cytotoxic therapy, such as combinations of streptozotocin with doxorubicin or 5-fluorouracil, has been reported to induce response rates of 8–20 % in recent series. These chemotherapy schedules showed improved response rates and/or survival compared with single-agent therapy in some old classical randomized clinical trials, but the antitumor efficacy of these agents against placebo or best supportive care has never been formally evaluated. In any case, well-differentiated NETs, particularly those of enteric or non-pancreatic origin, generally exhibit low susceptibility to conventional chemotherapy. In this context, the recently demonstrated antitumor activity against NETs of two new targeted agents, sunitinib and everolimus, is of particular relevance, as they have demonstrated for the first time a clinically relevant antiproliferative effect in well-differentiated NETs of pancreatic origin. Everolimus has also shown some limited activity in non-pancreatic NETs. This has triggered great enthusiasm in the field, and a number of other targeted agents are currently being evaluated in clinical trials. These recent advances will be reviewed in the current chapter, as well as some of the more promising new agents in development that may eventually add to the treatment armamentarium in the near future [5].

Role of Angiogenesis in NETs

NETs are highly vascularized tumors, and vascular endothelial growth factor (VEGF) and its receptors (VEGF-R) are overexpressed in 60–84 % of carcinoids and pancreatic islet cells NETs. Other pro-angiogenic factors such as the platelet-derived growth factor (PDGF) and the fibroblast growth factor (FGF) have also been involved in NET development and progression. VEGF is one of the most important growth factors that regulate angiogenesis under both physiological and pathological conditions. It stimulates both proliferation and migration of endothelial cells, enhances microvascular permeability, and is essential for revascularization during tumor formation [6, 7]. Some authors have correlated VEGF expression with increased angiogenesis, metastases, and decreased progression-free survival among patients with GEP-NETs. Moreover, activation of the HIF pathway has been correlated with a shortened disease-free survival in pancreatic endocrine tumors [8].

The highly vascular-dependent nature of these tumors has led to the conduction of an increasing number of trials testing the activity of different agents with antiangiogenic properties in this setting, including drugs targeting VEGF (bevacizumab), small molecules that inhibit the receptor tyrosine kinase domains of VEGFR and other related receptors (sunitinib, sorafenib, pazopanib, axitinib), and other antiangiogenic compounds with different mechanisms of action (endostatin, thalidomide). Some of them are starting to show these agents are to play a relevant role in the management of this disease.

Sunitinib

Sunitinib malate is an oral tyrosine kinase inhibitor with multiple targets, including VEGFR-1,2,3, PDGFR-α,β, c-KIT (stem-cell factor receptor), RET, FMS-like tyrosine kinase 3 (FLT-3), and colony-stimulating factor-1 receptor (CSF-1R). In preclinical models (RIP1-Tag2 transgenic mouse model), sunitinib has shown to reduce tumor burden and increase animal survival by inhibiting the proliferation of VEGFR-dependent endothelial cell and by reducing the PDGFR-dependent pericyte coverage [9]. First evidence of sunitinib activity in GEP-NETs was already documented in the first-in-man phase I clinical trial conducted in patients with advanced solid tumors [10]. In the phase II trial that followed, sunitinib efficacy was evaluated in 107 patients with advanced NETs [11]. Patients were treated with repeated 6-week cycles of oral sunitinib (50 mg/d for 4 weeks, followed by 2 weeks off treatment). Among patients with pancreatic endocrine tumors (PNETs) ($n = 66$), objective responses (OR) were documented in 16.7 % and stable disease (SD) in 68 %. Among those with carcinoid tumors ($n = 41$), however, OR were only achieved in 2.4 % of patients, while 83 % had SD. Despite a higher rate of tumor response, median time to tumor progression was

shorter for patients with pancreatic versus carcinoid tumors (7.7 and 10.2 months, respectively), with very similar rates of survival at one year (81.1 and 83.4 %). The toxicity profile of sunitinib was similar to that observed in trials of sunitinib in other disease types, being the most common treatment-related toxicities, as expected, hypertension (16 %), constitutional symptoms (fatigue and anorexia), and gastrointestinal adverse events (diarrhea and nausea). Despite this, no significant differences from baseline in patient-reported quality of life or fatigue were observed during treatment. These results led to the design of a double-blind, placebo-controlled randomized phase III study comparing 37.5 mg sunitinib continuous daily dosing with placebo in patients with progressive well-differentiated pancreatic NETs not suitable for curative surgery [12]. Crossover of placebo patients to sunitinib at disease progression was not initially permitted. About two-third of patients had received prior chemotherapy, and one third received concomitant somatostatin analogs in both study arms. This study aimed to include 340 patients but was prematurely closed with 171 patients due to the excess of deaths observed in the placebo arm (Table 13.1). After unblinding at study closure, patients were offered open-label sunitinib therapy. Overall, toxicity was manageable and allowed maintenance of quality of life across multiple cycles. The most frequent adverse events observed with sunitinib were diarrhea, nausea, vomiting, asthenia, and fatigue. Although objective responses were only observed in 9 % of patients in active treatment, progression-free survival (PFS) documented in sunitinib-treated patients was more than double of that observed in patients receiving placebo (11.4 months vs. 5.5 months; HR 0.42; $P < 0.001$). A Cox proportional hazards analysis of PFS according to baseline characteristics favored sunitinib in all subgroups studied. The magnitude of benefit seemed to be also independent of previous treatments, prior or concurrent use of somatostatin analogs, Ki-67 proliferative index, or bulk of liver involvement by the tumor. At the initial data cutoff point, 9 deaths were reported in the sunitinib group (10 %) versus 21 deaths in the placebo group (25 %) (HR for death, 0.41; 95 % CI 0.19–0.89; $p = 0.02$). With further follow-up, and after 69 % of patients had crossed over to sunitinib therapy, overall survival still favored the sunitinib arm (30.5 vs. 24.4 months), although this difference lost statistical significance (HR = 0.737, $P = 0.19$). Based on these data, the European Medicines Agency (EMA) granted sunitinib approval in November 2010 for the treatment of advanced well-differentiated PNETs.

Bevacizumab

Bevacizumab is a humanized anti-VEGF monoclonal antibody that has demonstrated efficacy in a wide spectrum of solid tumors, including colorectal, breast, renal, and non-small cell lung cancer. A small randomized phase II trial conducted by Yao et al. also suggested this drug could have some activity in GEP-NETs [13]. In this study, 44 patients with metastasic or unresectable carcinoid tumors on

Table 13.1 Randomized phase III trials of targeted agents in GEP-NETs

Study	Tumor	*N*	Treatment	Crossover (%)	Prior CT (%)	Prior SSA (%)	Concurrent SSA (%)	ORR (%)	*P*	PFS (m)	HR	*P*	OS	HR	*P*
NCT00428597	Pancreatic NETs	171	Sunitinib	69	57	35	27	9.3	<0.007	11.4	0.42	<0.001	30.5 m	0.74	NS
			Placebo		61	38	30	0		5.5			25.4 m		
RADIANT-3	Pancreatic NETs	410	Everolimus	73	50	50	39	5	NS	11.4	0.34	<0.001	73 % (18 m)	1.05	NS
			Placebo		50	50	40	2		5.4			74 % (18 m)		
RADIANT-2	Functional NETs	429	Everolimus + octreotide LAR	58	35	80	–	3	NS	16.4	0.77	= 0.026 (one-ided)	71 % (18 m)	1.22	NS
			Placebo + octreotide LAR		26	78	–	2		11.3			74 % (18 m)		

NETs neuroendocrine tumors, *CT* chemotherapy, *SSA* somatostatin analogs, *LAR* long-acting repeatable, *ORR* objective response rate, *PFS* progression-free survival, *OS* overall survival, *HR* hazard ratio, *m* months; *NS* non-significant

stable doses of octreotide were randomly assigned to 18 weeks of treatment with bevacizumab (15 mg/kg intravenously once every 3 weeks) or PEG interferon alfa-2b (0.5 mcg/kg subcutaneously once per week). After the completion of the 18 week therapy or at disease progression (whichever occurred earlier), patients were allowed to receive the combination of the two drugs. The bevacizumab arm showed higher response (18 vs. 0 %) and PFS rates after 18 weeks of treatment (95 vs. 68 %). In addition, a significant decrease in tumor blood flow (BF) as measured by paired functional CT scans was observed among patients treated with bevacizumab but not among those treated with interferon. Regarding toxicity, neutropenia was more frequently observed in the PEG interferon alfa-2b arm (14 vs. 0 %, $p = 0.02$) and hypertension in the bevacizumab arm (18 vs. 0 %, $p = 0.01$). Other than that no significant differences were observed among study arms in terms of fatigue, nausea, vomiting, headache, or myalgia. A large phase III study is currently ongoing (SWOG S0518) to try to confirm these promising results (trial estimated completion date: January 2012; expected enrollment: 400 patients). Another randomized run-in study of bevacizumab versus everolimus for 21 days, followed by the combination of both drugs, is currently assessing the role of functional CT scans as surrogate markers for selection of patients likely to benefit from antiangiogenic therapies [14]. Preliminary results of this study, that included 39 patients with low- to intermediate-grade NETs, showed that treatment with bevacizumab significantly decreased tumor BF, and this was further reduced with the addition of everolimus. Moreover, a significant association was observed between objective responses (21 %) and functional CT scan parameters such as higher baseline permeability surface, higher post-treatment mean transit time (MTT), higher percentage decrease in BF, and higher percentage increase in MTT. Confirmatory studies of these provocative findings are warranted. Also suggesting enhanced antitumor effects with combined mTOR and VEGF-targeted therapy are the preliminary results recently reported of a multicenter phase II trial testing the combination of temsirolimus and bevacizumab in patients with progressive well to moderately differentiated PNETs, with an objective response rate (44 %) that well exceeds that expected from single agent therapy. Finally, results from the BETTER trial have been recently reported, which tested the combination of bevacizumab and capecitabine in 49 patients with non-pancreatic NETs of the GI tract (40 from the small intestine, 3 from the cecum, 4 from the rectum, and 2 from the stomach), all with Ki-67 proliferative index <15 % (35 % of 0–2 %) [15]. Tumor control rate was observed in 88 % of patients, including partial responses in 9 (18 %), and the PFS rate at 18 months was 55 %. Grade 3/4 adverse events occurred in 41 patients (84 %), mainly gastrointestinal toxicities (29 %) and hypertension (31 %). Several additional phase II trials are evaluating safety and efficacy of a number of bevacizumab combinations with other cytotoxic drugs [(FOLFOX, XELOX, Temozolomide) or targeted agents (temsirolimus, pertuzumab, sorafenib) (see below)].

Other Angiogenesis Inhibitors

Sorafenib is a small molecule currently approved for the treatment of hepatocellular and renal cell carcinoma, which inhibits tumor-cell proliferation and angiogenesis by inhibiting, among others, the serine–threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3, and platelet-derived growth factor receptor β (PDGFR-β). A phase II trial reported the outcome of 93 patients with advanced PNETs and carcinoid tumors that were treated with sorafenib 400 mg bid [16]. Among evaluable patients, 4 of 41 patients (10 %) in each group achieved a partial response, and 3 and 9 minor responses were observed in patients with PNETs and carcinoid tumors, respectively. Preliminary results (presented at ASCO 2007, but not yet published), showed a 6 month PFS rate of 40 % for carcinoid tumors and 61 % for PNETs. With regard to safety, grade 3–4 adverse events occurred in 43 % of patients, being fatigue (9 %), skin (20 %) and gastrointestinal toxicities (7 %) the most commonly encountered. Although these results were considered to indicate a modest activity of sorafenib in this patient population, and are not substantially different from that observed for some of the recently approved drugs for this indication, further development in prospective randomized trials has not followed. However, different combinations of sorafenib with other biological or cytotoxic agents, such as everolimus or cyclophosphamide, are being explored in non-controlled trials. In addition, combinations with other antiangiogenic agents are also being assessed. Indeed, the Spanish Neuroendocrine Tumor Group (Grupo Español de Tumores Neuroendocrinos or GETNE) has recently reported the early results of a phase II study evaluating the combination of sorafenib (200 mg bid, days 1–5 of each week) and bevacizumab (5 mg/kg once every 2 weeks), based on the hypothesis of a potential synergistic effect derived from the complementary VEGF "vertical" signaling inhibition [17]. Forty-four patients with advanced G1–2 NETs were enrolled in this trial ($n = 31$ carcinoid tumors and 13 with PNETs). Most common grade 3–4 toxicities were hand-foot syndrome (23 %), asthenia (16 %), hypertension (9 %), and mucositis (7 %). Seven patients prematurely ended the study treatment due to adverse events. The overall response rate by RECIST criteria was 9.8 %. Disease control rate (95 %) and median PFS (12.4 months) are encouraging, although further follow-up is required for mature data. Other ongoing trials include combinations of sorafenib with conventional cytotoxic agents (cyclophosphamide-NCT00605566) or other targeted agents such as everolimus.

Pazopanib is a second-generation orally available multitargeted tyrosine kinase inhibitor against vascular endothelial growth factor receptor-1, 2, and 3, platelet-derived growth factor receptor-α, platelet-derived growth factor receptor-β, and c-kit. Preclinical evaluation has demonstrated significant antiangiogenic properties and antitumor activity in a variety of tumor types, and phase I clinical trials have revealed manageable toxicities as well as activity in renal cancer and several other malignancies. A prospective phase II study evaluated the combination of

pazopanib and depot octreotide in 2 cohorts of patients with PNETs ($n = 30$) and carcinoid tumors ($n = 22$) following a two-stage design, with early stopping rules if no RECIST-defined response was observed among the first 20 evaluable patients enrolled per cohort [18]. Prior VEGF-targeted therapy was not allowed. No objective responses were documented during the first stage in patients with carcinoid tumors and trial accrual was consequently stopped. Objective response rate, however, is not an appropriate endpoint for this type of drugs in general, and for this tumor type in particular. Certainly, a cytostatic effect cannot be ruled out with this study design. Median PFS for this cohort was 12.7 months. Among PNET patients, objective responses were observed in 17 % with a median PFS of 11.7 months. A subsequent study by the Spanish Neuroendocrine Tumor Group (GETNE) is currently assessing safety and efficacy of pazopanib in patients with progressive advanced NETs [19]. The major difference with the prior one is that this trial did allow prior targeted therapy with antiangiogenic agents or mTOR inhibitor, and of note, 83 % of included patients ($N = 44$) had been pretreated with at least one of these agents. The primary endpoint was clinical benefit rate (CBR = CR + PR + SD) at 6 months according to RECIST v1.0 criteria. A number of potential predictive biomarkers are also being evaluated, including VEGF and soluble VEGFR-2 plasma levels, as well as circulating tumor and endothelial cells. Preliminary analysis were recently presented at ESMO 2012 and reported a CBR of 100 % in patients with no previous targeted therapy (7 pts), 89 % in patients previously treated with mTOR inhibitors (9 pts), 83 % in those pretreated with antiangiogenics (12 pts), and of 60 % in patients that had received both antiangiogenics and mTOR inhibitors prior to study entry (5 pts). Grade 3–4 toxicities included asthenia (18 %), hypertension (9 %), diarrhea (9 %), and ALT elevation (11 %). These encouraging results suggest a role for sequential targeted agent therapy in this disease.

Other less successfully tested antiangiogenic agents include *Vatalanib*, *Thalidomide*, *Atiprimod,* and *Endostatin*. *Vatalanib* is an orally administered small molecule targeting VEGFR-1,2,3, PDGFR-β, and cKIT. Two small phase II trials with this drug showed limited efficacy with an unfavorable toxicity profile (liver toxicity, dizziness, emesis, hypertension, and proteinuria); and therefore, its development in NETs has been halted [20, 21]. *Thalidomide* is an agent with antiangiogenic properties of unknown mechanism of action that has proven to be active against multiple myeloma. In NETs, it has been tested in combination with temozolomide [22]. Although results of this phase II trial were encouraging—a radiological response rate of 25 %, a biochemical response rate of 40 % and an overall survival at 2 years of 61 %—it is difficult to estimate the individual contribution of each of these agents to the overall outcome. The combination was tolerable, being the most common toxicity lymphopenia with opportunistic infections documented in 10 % of treated patients. *Atiprimod* is a JAK2/JAK3 small molecule inhibitor with significant antiproliferative, antiangiogenic, and proapoptotic effects in preclinical models. Atiprimod inhibits the phosphorylation of signal transducer and activator of transcription 3 (STAT3), blocking the signaling pathways of interleukin-6 and VEGF and down regulating the antiapoptotic

proteins Bcl-2, Bcl-XL, and Mcl-1, thereby inhibiting cell proliferation, and inducing cell cycle arrest and apoptosis. Preliminary results of a phase II proof-of-concept study of atiprimod in patients with advanced low—to intermediate-grade neuroendocrine carcinomas reported 91 % stabilizations in the 23 patients that completed 2 cycles of therapy with tolerable toxicity profile [23]. Finally, *Endostatin* is a 20 kd fragment derived from the C-terminal region of mouse collagen XVIII, an extracellular matrix heparin sulfate proteoglycan that is an abundant constituent of blood vessels and most basal laminae in organs distributed throughout the body. Treatment with recombinant murine endostatin induced the regression of experimental tumors growing in mice to dormant, microscopic lesions. The antiproliferative activity of endostatin seems to result specifically from effects directed against endothelial cells. Preclinical studies showed promising antimetastatic and growth inhibitory activity against several tumor models with no discernible host toxicity, and a minor durable response was observed in a patient with a non-functioning pancreatic NET treated with endostatin in the first-in-man phase I clinical trial [24]. Based on this, a phase II trial was conducted in 42 patients with advanced pancreatic NETs and carcinoid tumors [25]. No objective radiological responses were however documented, and the authors concluded that this agent did not have significant antitumor activity in these patients.

More recently, *Axitinib,* a potent inhibitor of VEGF receptors 1, 2, 3, PDGFR and cKIT, with promising preliminary activity against several vascular-dependent solid tumors, is also undergoing clinical evaluation in NETs. Indeed, the Spanish GETNE group is currently conducting a randomized double-blind placebo-controlled phase II trial (EUDRACT: 2011-001550-29) that aims to accrue 80 patients with advanced and progressive well-differentiated NETs of non-pancreatic origin. Patients are randomly assigned to receive Octreotide LAR in combination with either Axitinib or Placebo. Prior therapy with angiogenesis inhibitors is not permitted. The primary endpoint of the study is PFS. Plasma samples and paraffin-embedded tumor tissue will be collected from all patients to explore the potential prognostic and predictive value of different intracellular pathways involved in VEGFR, PDGFR, and other related RTKs signaling. As of February 2013, 43 patients have been enrolled in the trial, complete accrual is expected within 1 year, and the first interim analysis is preplanned to be performed 6 months following enrollment of 50 % of the study population (that is by August 2013).

Role of the Mammalian Target of Rapamycin Pathway in NETs

Mammalian target of rapamycin (mTOR) is a serine–threonine kinase that plays a key role in regulation of cellular metabolism, growth, and proliferation. mTOR integrates multiple upstream signals including growth factors and mitogens, and

active signaling results in an increase in translation of proteins that are important in regulating cell cycle progression and metabolism. mTOR is also involved in angiogenesis control by regulating the translation of hypoxia-inducible factor 1α (HIF1α). In the setting of reduced nutrients or other cellular signals to limit growth, mTOR is inhibited and this leads to increased levels of CDK2 and cell cycle inhibition. The PI3k/AKT/mTOR may be stimulated by upstream activation of VEGFR, PDGFR, and Insulin growth factor receptor (IGFR) and is under negative control of two tumor suppressor genes, tuberous sclerosis 2 (TSC2), and phosphatase and tensin homolog (PTEN). Up to 50 % of human cancers present aberrant activation of this pathway through several mechanisms, including over-expression or amplification of growth factor receptors, activating mutations of the pathway kinases (PI3K, AKT) or loss of function of inhibitory proteins (PTEN, TSC2). In particular, several genetic disorders that have constitutive activation of this pathway, such as multiple endocrine neoplasia type I (MEN1), Von Hippel–Lindau (VHL) or tuberous sclerosis complex (TSC), are associated with an increased incidence of NETs. Loss of TSC2 or PTEN expression reduce the inhibition of mTOR activity caused by hypoxia, and increases the survival and growth of hypoxic tumor cells thus contributing to tumor progression [26]. Autocrine activation of the mTOR signaling pathway mediated through IGF-1 has been implicated in the proliferation of PNET tumor cells. On the other hand, expression profiling assays have shown that TSC2 and PTEN are commonly downregulated in PNETs, and this is inversely correlated with prognosis. Further confirming the relevance of this pathway in the pathogenesis of NETs is the work by Jiao and colleges, which determined the exomic sequence of ∼18,000 protein-coding genes in a Discovery set of ten well-characterized sporadic PNETs, and then screened the most commonly mutated genes in 58 additional PNETs [27]. Among others, they found mutations in genes in the mTOR pathway in 14 % of the tumors, including PTEN, PIK3CA, and TSC2. Consistent with these observations, inhibition of mTOR has a significant antiproliferative effect on NET cell lines. All these findings suggest that molecular tumor profiling could potentially play a role for the selection of patients most likely to benefit from mTOR-targeted therapy [28].

Early efforts to modulate aberrant mTOR activity in malignancy initially employed rapamycin (sirolimus). Rapamycin is a macrolide antibiotic that binds to the cytosolic protein, FK binding protein 12 (FKBP-12), thus interacting with the mTOR complex 1 and preventing downstream signaling. Subsequently, several rapamycin derivatives developed ("rapalogues"), with improved pharmacological properties for clinical development, have demonstrated antiproliferative effects in vitro and *in vivo* in PTEN deficient cancer cells and in different preclinical models of carcinoid tumor cells. Inhibitors of the mTOR pathway have now been successfully tested in a number of malignancies that are associated with aberrant activation of the mTOR signaling pathway, including lymphomas, breast, or renal cancer, and also NETs. In the section that follows we will summarize the most relevant information regarding performance of this class of drugs in NETs [29, 30].

Temsirolimus

Temsirolimus is a rapamycin analog with greater solubility and improved therapeutic index compared with its parent compound sirolimus. This mTOR inhibitor was evaluated in a phase II study conducted in 37 patients with advanced neuroendocrine carcinomas (21 carcinoid, 15 pancreatic) [31]. Although pharmacodynamic analysis revealed effective mTOR pathway downregulation, and 54 % of patients achieved some degree of tumor shrinkage (1–29 %), activity was deemed to be modest as the objective response rate was only 5.6 %, the median TTP was 6 months and the 1 year OS rate was 72 %. Higher baseline levels of pmTOR ($P = 0.01$) predicted for a better response and increases in pAKT ($P = 0.041$) and decreases in pmTOR ($P = 0.048$) after treatment were associated with an increased TTP. Results were considered, at the time the study was reported, not clinically relevant enough to pursue further development of this agent in NETs, although experts agree today that objective radiological responses are probably not the best way to assess efficacy of this class of agents.

More recently, renewed interest on this agent has emerged following the positive results documented with everolimus in NETs. Indeed, a multicenter phase II trial is evaluating a combination of temsirolimus with bevacizumab in progressive well or moderately differentiated pancreatic NETs [32]. Substantial preliminary activity has been reported for this regimen, with confirmed PR documented in 13 of the first 25 (52 %) evaluable patients and 84 % of patients progression free at 6 months. The combination had an acceptable toxicity profile, and most common grade 3–4 adverse events were hypertension (14 %), leukopenia (11 %), lymphopenia (11 %), hyperglycemia (11 %), mucositis (8 %), hypokalemia (8 %), and fatigue (8 %). Accrual is ongoing, and mature results are awaited with interest.

Everolimus

Everolimus (RAD001) is an oral mTOR inhibitor that has been extensively studied in NETs. The first evidence of activity of this agent in NETs was reported by Yao et al. in 2008. These investigators conducted a phase II study that included 30 patients with carcinoid tumors and 30 patients with PNETs in two consecutive cohorts [33]. The first cohort ($n = 30$) received the combination of depot octreotide (30 mg intramuscularly every 28 days) and everolimus at a dose of 5 mg daily, and the second cohort ($n = 30$) received the same dose of octreotide and 10 mg daily of everolimus. Encouraging antitumor activity was observed, with a RR of 27 % for PNETs and 17 % for carcinoid tumors, and a median PFS of 63 and 50 weeks, respectively. By dose level, median PFS of patients treated with 5 and 10 mg of everolimus was 50 weeks, respectively. When tumor type, dose level, prior octreotide use, and disease status at time of study entry were analyzed in a Cox proportional hazard model, everolimus dose of 10 mg was associated with superior PFS (HR = 0.5; 95 % CI, 0.3–0.98), and progression at study entry

was associated with shorter PFS (HR = 3.3; 95 % CI, 1.5–7.2). Treatment was generally well tolerated and the most frequent grade 3–4 adverse events were diarrhea, fatigue, hyperglycemia, hypophosphatemia, and mucositis. In addition, small case series or case reports have suggested a relevant role of everolimus for the control of the hormone secretion syndrome, such as hypoglycemia in insulinomas or the carcinoid syndrome in intestinal NETs.

Based on these results, a program named RADIANT (RAD001 in Advanced NETs) was launched to further explore the efficacy of everolimus in different sets of NETs. The first trial, RADIANT-1, was a large open-label phase II study conducted in patients with metastatic pancreatic NETs who had experienced progression on or after chemotherapy [34]. Patients were stratified by prior octreotide therapy (stratum 1: everolimus 10 mg/d, $n = 115$; stratum 2: everolimus 10 mg/d plus octreotide long-acting release [LAR], $n = 45$). In stratum 1, 11 patients achieved a partial response (9.6 %) and 78 patients had SD (67.8 %), while in stratum 2, there were two partial responses (4.4 %) and 36 disease stabilizations (80 %). Median PFS by central radiology review was 9.7 months in stratum 1 and 16.7 months in stratum 2. Patients with an early chromogranin A (CgA) response had a significantly longer PFS compared with patients without an early response (13.3 vs. 7.5 months, HR = 0.25, $P = 0.00004$). Most adverse events were mild to moderate and were consistent with those previously seen with everolimus in other trials.

RADIANT-2 was a study that included 429 patients with low- or intermediate-grade advanced NETs with a history of carcinoid syndrome (the great majority of non-pancreatic origin) [35]. These patients were randomly allocated to receive octreotide LAR with placebo or with everolimus, with crossover to everolimus allowed at disease progression for placebo-allocated patients (Table 13.1). Drug-related adverse events (everolimus plus octreotide LAR vs. placebo plus octreotide LAR) were mostly grade 1 or 2, and adverse events of all grades included stomatitis (62 vs. 14 %), rash (37 vs. 12 %), fatigue (31 vs. 23 %), and diarrhea (27 vs. 16 %). Objective responses were rare (about 2 % in both study arms), although the proportion of patients experiencing some degree of tumor shrinkage was higher among everolimus-treated patients (75 %) than among those receiving placebo (45 %). Patients treated with everolimus and octreotide also achieved higher proportions of CgA and 5-hydroxyindoleacetic acid responses (46 and 61 %) compared with those treated with placebo and octreotide (36 and 54 %). PFS evaluated by local investigators was 12 months versus. 8.6 months for everolimus versus placebo-treated patients, respectively, (HR 0.78, 95 % CI [0.62–0.98] $P_{\text{unilateral}} = 0.018$), although this benefit was of borderline statistical significance by blinded central review (HR 0.77; 95 % CI [0.59–1.00] $P_{\text{unilateral}} = 0.026$). Of note, some imbalances occurred in patient characteristics among study arms, including a higher proportion of patients allocated to the everolimus plus octreotide LAR arm with a WHO performance status >0 (45 vs. 34 %), lung as the primary tumor site (15 vs. 5 %), or prior use of chemotherapy (35 vs. 26 %), generally associated with a poorer prognosis, may have potentially biased results against the investigational arm (everolimus plus octreotide). Furthermore, a high proportion of patients on the

placebo arm crossed over to everolimus at disease progression (58 %) and no differences were observed in OS among study arms.

A third large study, the RADIANT-3, was conducted in 410 patients with progressive advanced low- or intermediate-grade pancreatic NETs who were randomly assigned to everolimus or placebo with a double-blind crossover study design (Table 13.1) [36]. Fifty percent of patients had received prior chemotherapy. The safety profile of everolimus was acceptable, with mucositis, infections, and pulmonary events as the major severe adverse events. Again, objective responses were low (5 vs. 2 %), but disease control rate (78 vs. 53 %) and PFS were significantly greater in patients receiving everolimus than in those treated with placebo (11.4 vs. 5.4 months, HR 0.34, $p < 0.0001$). In addition, everolimus produced sustained decreases in CgA and NSE levels that were significantly greater than changes observed with placebo. The benefit in PFS was consistent across all subgroups of patients regardless of age, gender, race, performance status, time since diagnosis, prior or concomitant somatostatin analog treatment, previous chemotherapy, tumor burden, or tumor grade. Baseline CgA and neurospecific enolase (NSE) were prognostic for PFS, although everolimus benefit was observed in both patients with and without elevated baseline CgA and NSE levels. No impact was observed on survival, but this may be explained by the fact that 73 % of patients on placebo crossed over to everolimus at disease progression, similar to what was observed in the prior study.

Based on these results, regulatory agencies have granted marketing authorization to everolimus for the treatment of progressive advanced PNETs (FDA—May 2011; EMA—September 2011) but not for NETs of non-pancreatic origin. However, a forth study, RADIANT-4, is at present being initiated comparing everolimus plus best supportive care versus placebo in patients with advanced NETs of gastrointestinal or lung origin. This study aims to recruit 279 patients over a period of time of 19 months and shall clarify if everolimus is to play a role in the treatment of non-pancreatic NETs. The CALGB has also initiated a randomized study (CALGB80701) to assess the combination of everolimus and octreotide, with or without bevacizumab, in patients with pancreatic NETs. Finally, a number of other studies are currently addressing the efficacy of everolimus in combination with other drugs including somatostatin analogs (SOM230 (COOPERATE-2 study)) and other targeted agents (sorafenib, erlotinib).

Potential Role of Other Pathways in NETs

Insulin Growth Factor Receptor Pathway Inhibitors

Several lines of evidence support the role of IGFs in cancer development and progression. First, epidemiological case–control and cohort studies have shown that insulin resistance status characterized by hyperinsulinemia, is associated with an

increased risk for a number of malignancies, including carcinomas of the breast, lung, prostate, colon, and kidney. On the other hand, many preclinical studies have demonstrated that both insulin and IGFs are mitogenic to a variety of cell types, including NET cell lines, and play a role in cancer initiation, progression, and metastasis. In addition, IGF-1 activates the PI3K/AKT and ERK/MAPK pathways, which also contribute to the initiation of hypoxia-inducible factor 1 (HIF1) and VEGF secretion. Finally, multiple oncogenes require the presence of IGF-1R to achieve cellular transformation, and IGF-1 signaling confers resistance to many antineoplastic therapies. All these observations have provoked considerable interest over recent years on the IGF pathway as a novel therapeutic target in cancer. With more than 30 compounds under investigation targeting the IGF-1R, this has become an exciting area of intense research including the field of NETs [37].

A phase II trial with *dalotuzumab* (MK-0646, a monoclonal IgG1 antibody targeting IGFR1) in NETs reported, however, disappointing results. No responses were observed in 25 patients (10 carcinoids and 15 pancreatic), and only 20 % achieved non-durable SD [38]. The drug was tolerable, being the most common side effects mild to moderate hyperglycemia (25 %), drug infusion reactions (4 %), and asthenia (8 %). Another monoclonal antibody against IGFR1, *ganitumab* (AMG-479), is also under clinical investigation in advanced progressive NETs. This trial has enrolled 60 patients (30 carcinoids and 30 pancreatic), and although no objective responses were observed by RECIST criteria, some degree of tumor shrinkage (1–29 %) was documented in 37 % of carcinoid tumors and 31 % of pancreatic NETs [39]. Median progression-free survival for the whole cohort was 6.3 months (carcinoids: 10.5 months; pancreatic: 4.2 months), and 70 % of patients were alive at 1 year. Hyperglycemia (4 %), neutropenia (4 %), thrombocytopenia (4 %), and infusion reaction (1 %) were the most common grade 3–4 drug-related events. Other IGFR inhibitor currently undergoing phase I–II clinical testing in NETs is cixutumumab (IMC-A12, a monoclonal anti-IGFR1 antibody), in combination with octreotide, or with octreotide and everolimus [40].

Epidermal Growth Factor Receptor Pathway Inhibitors

Over-expression of epidermal growth factor receptor (EGFR) has been documented in NETs and has been associated with poor prognosis and resistance to anticancer agents in a number of tumor types. A phase II trial has been conducted with *gefitinib*, a tyrosine kinase inhibitor targeting EGFR, in 96 patients with NETs (57 carcinoids, 39 pancreatic) [41]. Progressive disease was required for study entry. Objective response rates were low (1 partial response in a patient with a carcinoid tumor and 2 responses in patients with pancreatic NETs), and the 6-month progression-free survival rate was 61 % for carcinoids and 31 % for pancreatic NETs. Several phase II trials are currently ongoing exploring the combination of HER-targeted agents with antiangiogenic agents or other drugs targeting the PI3K/AKT/mTOR pathway. Study NCT01121939 recently reported preliminary results for the

combination of bevacizumab and *pertuzumab* [42]. Pertuzumab is a humanized monoclonal antibody directed at the dimerization domain of the receptor tyrosine-protein kinase erbB-2 (HER2) receptor. This combination was tested in 43 patients with advanced NETs (32 carcinoids, 11 pancreatic). Response rate was 16 % and median progression-free survival was 8.2 months. Median overall survival had not been reached, and toxicity was manageable (grade 3 hypertension 28 %, reduced heart ejection fraction 9 % and diarrhea 7 %; no grade 4 toxicities). Other ongoing NET trials with this class of agents include trial NCT00843531, exploring the combination of everolimus and erlotinib (accrual halted in carcinoids for lack of efficacy, but recruitment ongoing in pancreatic NETs), and trial NCT00947167, exploring the combination of pertuzumab and erlotinib.

Histone Deacetylase Inhibitors

A novel class of drugs that inhibit the histone deacetylase (HDAC) enzymes are capable of targeting epigenetic silencing mechanisms, resulting in reversal of crucial steps in carcinogenesis, and thus hold significant potential as anticancer therapy. Indeed, HDAC inhibitors have been shown to suppress tumor growth and induce apoptosis in a variety of solid tumors in preclinical models, including NETs. Early clinical development of *depsipeptide* (FK-228), an HDAC inhibitor, documented a long-lasting minor response in a patient with an islet cell tumor. Based on this and its unique mechanism of action, a phase II clinical trial was undertaken in patients with metastatic carcinoid/islet cell tumors [43]. However, the study was terminated prematurely with only 15 patients accrued due to an unexpected high number of serious cardiac adverse events so the objective response rate could not be determined. Also disappointing were results recently communicated with another HDAC inhibitor tested in NETs, *panobinostat* (LBH589) [44]. The study was stopped early at the planned interim analysis based on lack of meaningful clinical efficacy outlined in the Simon two-stage design, as no objective responses were documented in the first 15 patients enrolled (67 % carcinoid, 33 % pancreatic NET). Median progression-free survival was 11.8 months, and thrombocytopenia and fatigue were the most common treatment-related severe toxicities, most of them of grade 3. Therefore, this class of agents do not seem to hold great promise in the field of NETs.

Conclusions

NETs represent a heterogenous family of tumors with growing incidence and challenging clinical management. Although generally more indolent than carcinomas, they often have unpredictable biological behavior and are on occasions associated with a very aggressive clinical course. In addition, their susceptibility to

conventional cytotoxic therapy is rather limited. In this context, results of the recently published randomised trials with sunitinib and everolimus are particularly relevant, as they have demonstrated for the first time that there are agents able to positively impact the outcome of this disease. These targeted agents are indeed new effective options in patients with low- or intermediate-grade pancreatic NETs in whom disease progression has been documented. Whether these agents shall be employed before or after chemotherapy failure, or in patients with NETs of non-pancreatic origin is still a matter of debate. The efficacy of both agents seems similar, although no formal head-to-head comparisons exist nor are expected to be performed in the near future. Nevertheless, there is much room for improvement, and more efforts in basic, translational and clinical research will be necessary in the following years for progress to be made. Meanwhile, as the molecular pathways governing NET development and progression are unraveled, development of predictive biomarkers to help select subgroups of patients that are more likely to benefit from specific therapies are certainly warranted. In addition, a number of novel agents in the horizon shall eventually contribute to further improve the prognosis of these patients.

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Chapter 14
Measuring the Relationship of Quality of Life and Health Status: Including Tumor Burden, Symptoms, and Biochemical Measures in Patients with Neuroendocrine Tumors

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Abstract The measurement of health-related quality of life (HRQoL) has become essential for evaluating the impact of neuroendocrine tumors (NETs) on symptoms, as well as the social, emotional, psychological, and physical functioning of these patients. In this chapter, we describe two tools that have been developed to assess the wide spectrum of NET symptoms, determine the impact of this disease on patient's overall well-being, and discriminate between patients with tumors from those who are free of disease. We discuss the importance of adequate sensitivity, specificity, and reproducibility. Psychometric factor analysis was utilized to explore the various ways that these tumors manifest themselves and to help determine a patient's tumor burden, biochemical and hormonal status. First, we present data on the use of generic tools to evaluate responses to NET interventions. We then focus on two specific tools that have been developed and validated specifically to quantify health-related quality of life (QoL) in patients with NETs. There are distinct similarities between these tools, the EORTC QLQ-GI.NET21 and the Norfolk QoL-NET, but the unique differences favor the use of the Norfolk QoL-NET for clinical trials.

Introduction: A Historical Perspective

Concern for quality of life (QoL) and respect for the sanctity of life were both concepts expressed by the earliest medical and philosophical writings of ancient Greece. In the Christian world, the *sanctity of life* was extolled as paramount. For

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the ancient Greeks and Romans, and in many post-Renaissance philosophies, *QoL* assumed greater importance. These two opposing themes are woven into western history and opponents for each philosophy exist today [7].

"The term, QoL, was first mentioned in modern times by Pigou [8], in his book, 'The Economics of Welfare' wherein he proposed, 'the surroundings of work react on the QoL of workers'" [8].

After the Second World War, the World Health Organization (WHO) revived the social concept of QoL in 1948 and broadened the definition to include health, defining it as, "A state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." This new definition of health-related quality of life (HRQoL) led to conjecture on whether or not HRQoL could be measured.

In theory, the concept of HRQoL was generally accepted. However, its actual usefulness and validity remained questionable, as reflected in this statement from Fallowfield: "Hundreds of generic and specific tests purporting to measure different aspects of QoL have been developed. Acknowledgment that QoL is a valid outcome measure in clinical trials has been hampered by a variety of factors, including the conceptual vagueness of QoL, the use of assessment tools of dubious validity and reliability, the inappropriateness of methods, and the weakness of statistical analysis of the resulting data…. Consequently we have a responsibility to ensure that the tests employed to measure QoL are psychometrically sound, and that they are administered thoughtfully and analyzed correctly" [9].

In "Assessing QoL in clinical research: from where have we come and where are we going?" Wood Dauphine describes the history of health-related QoL assessment, discusses its current status, and suggests challenges for the future [10]. She wrote, "The development of generic measures began in the early 1970s and continues today. Disease-specific measures have also proliferated. The 1980s and 1990s saw an increase in methodological rigor, and additional emphasis on analytic approaches, interpretation of scale scores, cultural and language issues, as well as on the development of shorter measures. Future challenges include conceptualization and testing of theoretical models, further refinement of individualized measures for use in routine clinical practice, the use of computer adapted testing in QoL assessment, and the inclusion of QoL information in health databases" [10].

Neuroendocrine Tumors

Neuroendocrine tumors (NETs) have always been regarded as a "Cinderella" condition. Their distribution and frequency are shown in Fig. 14.1. Yet it is estimated that there are now more than 100,000 patients with gastroenteropancreatic NETs in the USA [11]. NETs are more prevalent than stomach and gastric cancer combined [12]. According to the SEER registry, NETs have risen from 1/100,000 peoples in 1973 to 5/100,000 in 2004 and this number appears to be continuing to

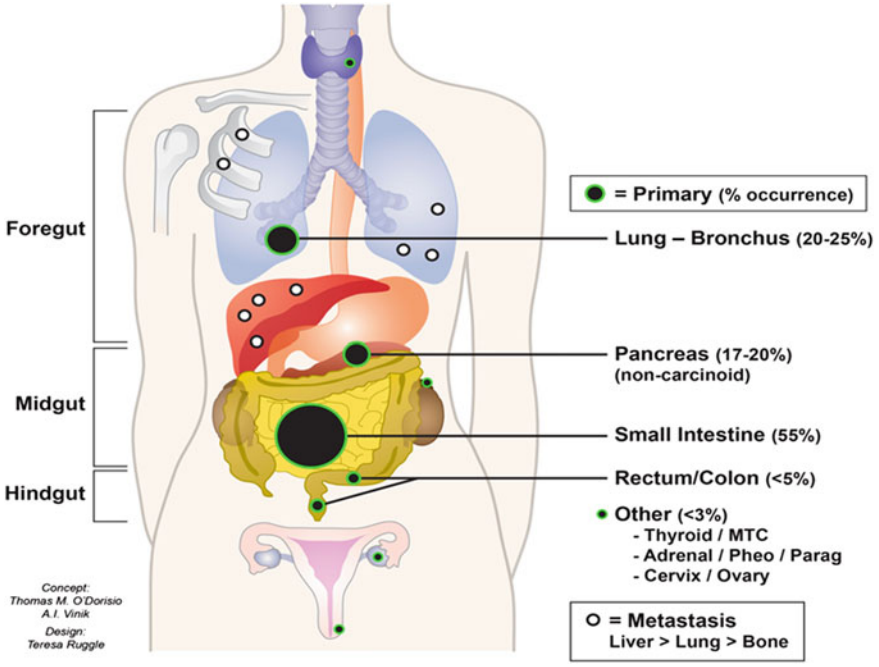

Fig. 14.1 Distribution and frequency of NETs

increase [13]. Patients with well-differentiated NETs have a 65–90 % 5-year survival for all sites of NETs; for localized NETs with regional metastasis, survival is 46–78 %, and in patients with poorly differentiated tumors, this falls to 25–54 % [14, 15]. The survival of patients with well-differentiated NETs can be markedly improved if diagnoses occur before the advent of metastases, increasing median survival from 30–120 months if the disease remains localized. This demonstrates the importance of early recognition [14, 15]. However, the majority of cases are diagnosed in the advanced stages. Studies have demonstrated that the delay from first appearance of symptoms to diagnosis is usually 9.2 years [16, 17].

It is clear that both patients and practitioners need a heightened awareness of the symptoms of the condition that can often masquerade as other disease states (Fig. 14.2). Early recognition, together with the advent of new approaches to therapy—the use of somatostatin analogs alone and in combinations with other chemotherapeutic, surgical, and advanced technological procedures—has had a very significant impact on the course of the disease, which we now may regard as chronic rather than a rapidly progressive and fatal condition. In this milieu, there was a need for developing a questionnaire to capture patients' responses, that would be able to help define those patients who have the condition while excluding those without it, and have the ability to distinguish between the impact of the

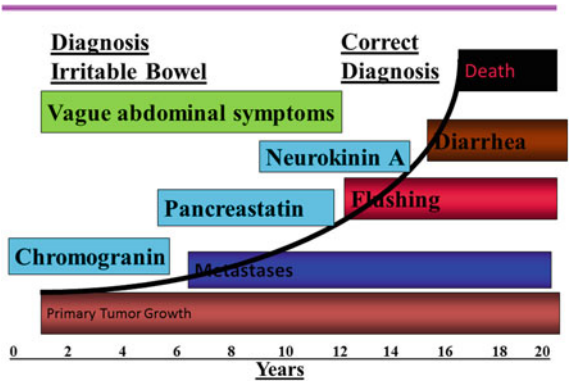

Fig. 14.2 Natural history of NETs and the appropriate diagnostic, staging, and prognostic biomarkers. Modified from Vinik and Moattari [4]

disease itself on QoL as opposed to the effects of various intervention and drugs used as therapy.

With regard to therapy, a fundamental objective of any health care intervention is the enhancement of the patient's QoL and overall well-being. A patient's health-related QoL encompasses their experience as a result of the underlying condition, their response to medical treatment, and consequently how their illness impacts their overall well-being [18]. Consideration of a patient's QoL has become increasingly important in evaluating the adverse health effects resulting from chronic illnesses such as NETs. Knox et al. [19] found that advanced therapy like surgical resection for NETs is associated with a significantly improved and sustained functional QoL. On the contrary, QoL may be severely impaired by the effects of many chemotherapeutic agents causing nausea, vomiting, and fatigue. The effects of radiation, too, can compromise QoL (Fig. 14.3).

In evaluating QoL, one needs to balance the impact of an intervention with of the impact of the underlying disease. The patient should actively participate in a decision to embark on therapy giving due consideration to the potential negative side effects as a result of the intervention versus any known benefit of the intervention itself. Remaining life, in terms of not only time but also quality, should be evaluated. QoL is a powerful tool to empower patients and their healthcare providers to share in this vital aspect of improving health outcomes.

Comparison of QoL in Patients with NETs and the General Population

Beaumont et al. [2] evaluated the HRQoL in patients with NETs and determined the association with demographic and clinical features of these tumors. Patients with NETs were invited to complete 2 standardized generic measures of QoL including Patient-Reported Outcomes Measurement Information System (PROMIS)-29 and the SF-36 with a set of standard demographic and disease-related questions. General

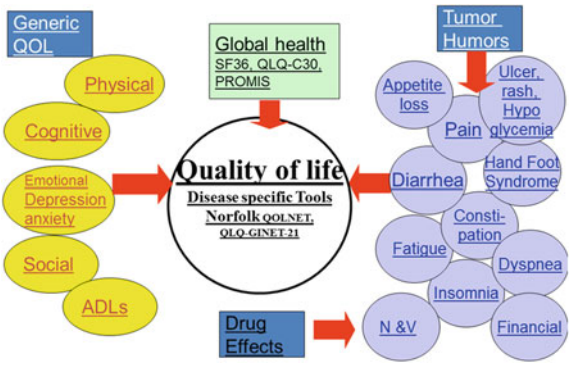

Fig. 14.3 Differences between disease-specific QoL tools and the impact of the disease and the medications used to treat the disease on quality of life [5, 6]

linear models were used to evaluate the associations between HRQoL and demographic and clinical characteristics. They entered a total of 663 patients who demonstrated worse HRQoL when compared to the general population and to a sample of mixed cancer patents and survivors. Patients with a current NET, either not surgically removed or recurrent after surgery, and patients with carcinoid symptoms (flushing and diarrhea) experienced a worse total QoL as well as impaired physical function, social activity, limitation of their physical role, depression, fatigue, pain interference with life, general health, and vitality using the combination of the PROMIS-29 and SF-36 tools (Fig. 14.4).

This study clearly illustrates that patients with NETs have a worse QoL than the general US population including patients with other small bowel neoplasms. Furthermore, patients with carcinoid syndrome fared worse than patient's with non-functioning tumors. The greatest limitation of the study was that it was cross-sectional, observational of a non-probability-based study sample. Objective information on tumor burden and biochemical markers was not available, and therefore, no clinical correlations could be derived. Furthermore, the prognostic and discriminatory capacity of the tools could not be assessed with a control population and of the group of subjects followed longitudinally. While this study clearly showed the impact carcinoid syndrome has on general QoL, it fell short and could not define the health relatedness of the various features, for example depression, physical function, and fatigue. This will be explored further below.

Patient-Reported Outcomes in Clinical Trials Using Generic Tools

In the current patient-centered environment, there has been an increasing interest in incorporating patients' assessments of their health status, giving rise to questionnaires designed to collect and analyze patient-reported outcomes (PROs).

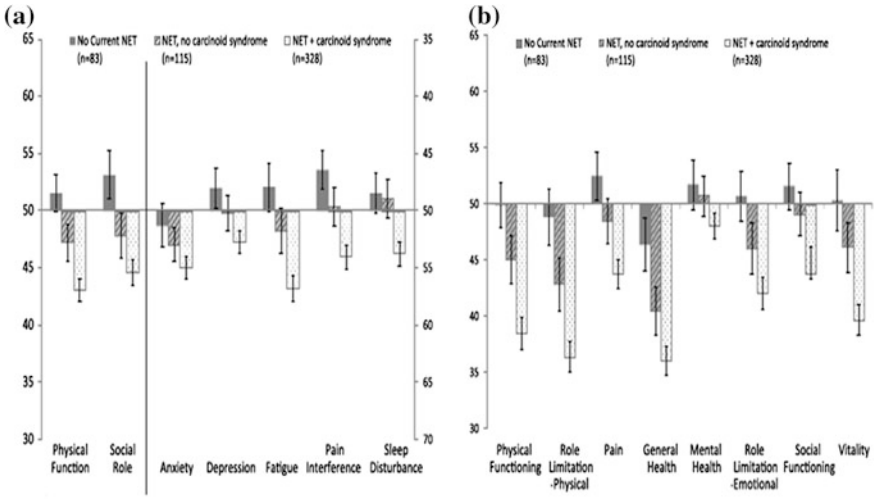

Fig. 14.4 Comparison of HRQoL in patients with neuroendocrine tumors compared with the general US population [2]

The abbreviation "QoL" used in this chapter will denote HRQoL. Thus, subjective, self-reported patient' assessments of their health status as it affects their QoL reflect health outcomes related to QoL. Although QoL as a marker for health outcomes is of newly recognized value, the importance of QoL measures for evaluating results of clinical research is indisputable. Clinical trials for a new therapy will not pass through the FDA without the use of a suitable validated HRQoL instrument to assess patient-reported outcomes. QoL measures are also used to discriminate the presence or absence of a condition, discriminate the different levels of severity within a condition, correlate subjective and objective measures, and, most importantly, monitor patient progress. QoL questionnaires administered to patients may help to bridge the gap between patient and physician and may also serve to help touch on issues too sensitive for the patient to address personally. As reported by Clauser et al. [20], patient-reported outcomes are used in a variety of cancer clinical trials to better understand the burden of cancer and the adverse effects of cancer therapy such as pain, fatigue, and nausea. Also mentioned in this article is the fact that the evolution of patient-reported outcomes in cancer trials has been documented by the National Cancer Institutes (NCI)-supported Cancer Outcomes Measurement Working Group. In their endeavor to measure QoL across a wide range of cancers and other diseases, the NCI created the PROMIS [21].

In Europe, the European Organization for Research and Treatment of Cancer (EORTC) developed the first generation of a core questionnaire in 1987 for the measurement of patient-reported outcomes in cancer clinical trials, EORTC QLQ-C36 [22]. Subsequently, EORTC QLQ-C36 was modified to EORTC QLQ-C30, and then again to EORTC QLQ-C30 (version 3.0) in December 1997, and is now

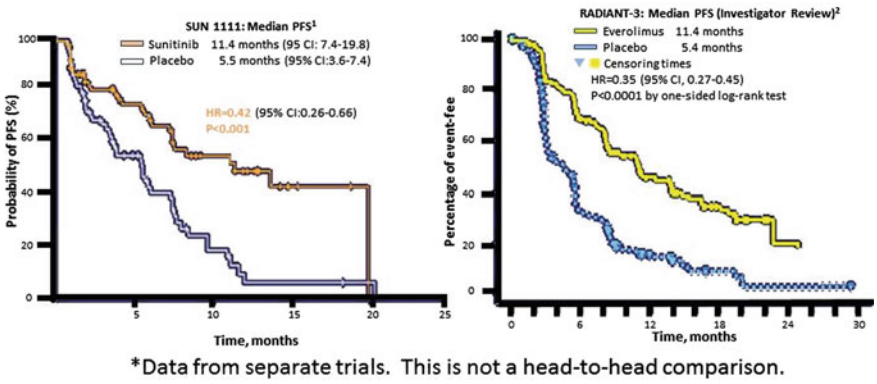

Fig. 14.5 Comparison of sunitinib versus everolimus on primary endpoints [1, 3]

the recommended version for new studies. While there was a concerted effort in Europe and later in the USA toward the development of cancer-related questionnaires, no specific tool was available for assessing subjective QoL outcomes in patients with NETs. In fact, it was recognized that although the EORTC QLQ-C30 was an important tool to measure generic aspects of cancer, it had limitations for capturing specific aspects of cancer-related diseases. This lack of disease-specific self-reported QoL measures motivated the development of disease-specific modules.

The first randomized, controlled pancreatic neuroendocrine tumor (pNET) trial to include QoL assessment was the phase III study of sunitinib [1, 5] (Fig. 14.5). The study used the EORTC QLQ-C30, a well-validated general oncological HRQoL instrument suitable for a clinical trial setting, but not specific to pNETs.

The QLQ-C30 is composed of both multi-item scales (we also refer to these as domains) and single-item measures. These include five functional scales, three symptom scales, a global health status/QoL scale, and six single items (five of which are also symptoms). Each of the multi-item scales includes a different set of items, and no item occurs in more than one scale. Each domain/scale/item is independent. For all domains/scales/items (with the exception of global HRQoL), patients are asked a series of questions regarding their status over the past week and they respond to one of four choices (a: Not at All; b: A Little; c: Quite a Bit; d: Very Much). There is no "total" score across any of the domains/scales/items.

In interpreting the scores, high scores for a functional domain/scale and the global health status represent a high or healthy level of functioning, while a high score for a symptom scale/item represents a high level of symptomatology or problems. Subjects were included in this study if they completed their baseline EORTC QLQ-C30 assessment and at least one additional post-baseline assessment while on treatment.

Assessments of QoL measures were made every 4 weeks, and the rate of compliance was >80 % (73 of 86 patients in the sunitinib group and 71 of 85

patients in the placebo group). No differences were found between sunitinib and placebo on the cognitive, emotional, physical, role, social functioning, and symptom scales, with the exception of diarrhea, which was significantly worse for sunitinib patients.

Having seen the efficacy data, as well as the results from the repeated measures mixed-effects model (which was comparable across the sunitinib and placebo arms), a post hoc analysis was performed to understand how sunitinib fared in delaying deterioration in global HRQoL and functioning scales. Given that deterioration can be comprised of several factors, for this study deterioration was described as a composite endpoint—death, or first progression, or two consecutive cycles of clinically significant change in a specific HRQoL scale.

Note Given that the 15 scales of the EORTC QLQ-C30 (whether multi- or single items) are independent and cannot be summed up for a total score, each HRQoL scale was evaluated separately as part of the composite endpoint (e.g., death, PFS or global HRQoL; death, PFS or physical functioning).

In order to understand the effect attributable to PFS and death, a sensitivity analysis was carried out by controlling for these two variables by evaluating time to deterioration for the HRQoL scale alone (Tables 14.1, 14.2, 14.3, 14.4).

Patient Disposition

PRO Completion Rate Within the Phase III Study Exceeded 80 % of Patients Eligible for Completing the QLQ-C30 at Each Cycle Within the First 10 Cycles

In a priori analyses, sunitinib was associated with a clinically and statistically significant worsening of diarrhea (diff. = 21.38; $P < 0.001$) and a statistically significant trend toward worsening of insomnia (diff. = 7.753, $P = 0.0372$) compared with placebo. However, sunitinib did not differ from placebo in any of the functioning scales (cognitive, emotional, physical, role, social), other symptoms, or in global HRQoL.

In a post hoc analysis, sunitinib significantly delayed deterioration in two of the five functioning scales (emotional and physical) and global HRQoL based on the composite endpoints of PFS, death, or MID. Figure 14.6 presents the results from the log-rank test comparing TTD between the two treatment arms in global HRQoL and functioning scales.

The vertical axis represents the median time to deterioration in months. There was a statistically significant difference in TTD for global HRQoL, emotional and physical functioning scales favoring sunitinib. This means that sunitinib delayed deterioration in these scales, where deterioration was evaluated as the composite endpoint of PFS, death, or MID. TTD was also evaluated in the symptom scales, and there was not a statistically significant delay in deterioration, with the exception of constipation (in favor of sunitinib) [5].

Table 14.1 Patient-reported outcomes assessment

- Patient-reported outcomes (PROs) were measured using the validated, self-administered 15-domain European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) version 3.0 [23, 24] which consists of three independent domains
  - Global HRQoL
  - Functional scales
    Cognitive, emotional, physical, role and social functioning
  - Symptom items/scales[a]
    Appetite loss, constipation, diarrhea, dyspnea, fatigue, insomnia, nausea and vomiting, pain
- Patients completed the questionnaire at baseline (Cycle 1, Day 1), and Day 1 of every cycle thereafter (cycle = 4 weeks), and at the end of treatment or withdrawal

[a] Financial difficulties were considered to be a separate item from symptoms

Table 14.2 A priori statistical analysis: EORTC QLQ-C30

- The EORTC QLQ-C30 questionnaire was scored using the EORTC QLQ-C30 scoring manual [23, 24] and interpreted using a minimal important difference (MID) approach [25]
  - Clinical significance (MID): ≥10 points in mean change from baseline
  - Statistical significance: 0.05 level based on a two-sided test
- Repeated measures mixed-effects models were used as the primary model for between-treatment comparison
- Descriptive statistics were used for the observed mean and mean change from baseline, and for the proportion of patients who improved or worsened (clinically significant), or who remained stable

In a separate study, QoL was evaluated with EORTC QoL 30 in 265 patients with gastroenteropancreatic or bronchial NETs treated with [177 Lu-DOTA, Tyr3] octreotate [26]. Regardless of the treatment outcome, insomnia, appetite loss, and diarrhea improved significantly. Patients with bone metastases or a decrease of 50 % or more in the biomarker chromogranin A (CgA) had improvement in their EORTC scores by at least 10 points. In a subgroup of patients (36 %) with decreased GHS/QoL or symptoms at the start of therapy GHS/QoL improved: Fatigue in 49 %, nausea in 70 %, vomiting in 53 %, pain in 44 %, dyspnea in 59 %, insomnia in 63 %, appetite loss in 60 %, constipation in diarrhea 67 % [26].

Development of Two Disease-Specific Questionnaires

Health-Related QoL in Patients with NETs

As novel treatments are prolonging survival, NETs have become a chronic condition for many patients, making QoL an increasingly important consideration. Patients with NETs have a significantly worse QoL than the general population

Table 14.3 Baseline EORTC QLQ-C30 scores were comparable with no clinically significant differences[a]

	Sunitinib ($N = 73$)	Placebo ($N = 71$)
Global HRQoL	67.0 (62.0, 72.0)	64.0 (58.4, 69.6)
Functional scales		
Cognitive functioning	87.1 (83.0, 91.1)	87.1 (82.6, 91.6)
Emotional functioning	75.2 (69.6, 80.9)	73.7 (67.3, 80.2)
Physical functioning	83.1 (78.1, 88.1)	83.1 (78.0, 88.1)
Role Functioning	84.3 (78.6, 90.0)	77.5 (70.7, 84.3)
Social functioning	79.4 (72.7, 86.0)	77.0 (69.3, 84.8)
Symptom items/scales		
Appetite loss	16.9 (10.7, 23.1)	18.7 (11.9, 25.4)
Constipation	14.9 (8.9, 21.0)	14.9 (9.0, 20.7)
Diarrhea	20.4 (13.2, 27.6)	19.2 (12.3, 26.1)
Dyspnea	15.9 (10.7, 21.1)	19.7 (12.5, 26.9)
Fatigue	29.4 (23.6, 35.1)	34.5 (28.0, 41.0)
Insomnia	25.4 (18.1, 32.6)	26.3 (19.4, 33.1)
Nausea and vomiting	6.7 (3.7, 9.7)	12.6 (7.2, 18.1)
Pain	22.9 (16.9, 28.8)	22.5 (15.4, 29.6)
Financial difficulties	19.9 (12, 27.8)	15.7 (8.9, 24.4)

[a] Clinically significant difference is defined as MID $\geq$ 10 points
Figures in parentheses are 95 % confidence intervals

Table 14.4 Results of a priori analysis: overall post-baseline EORTC QLQ-C30 scores (mixed-effects model) [5]

	Sunitinib	Placebo	Difference	P-value
Global HRQoL	62.44	61.28	1.15	0.6799
Functional scales				
Cognitive functioning	79.94	81.38	−1.44	0.6058
Emotional functioning	72.59	76.15	−3.56	0.3008
Physical functioning	78.92	76.13	2.79	0.3230
Role functioning	70.88	69.37	1.51	0.7113
Social functioning	74.44	76.11	−1.67	0.6487
Symptom items/scales				
Appetite loss	24.95	23.07	1.88	0.6545
Constipation	10.70	14.70	−4.00	0.1936
Diarrhea	37.19	15.81	21.38	<0.0001
Dyspnea	22.31	17.08	5.23	0.1339
Fatigue	40.52	38.74	1.78	0.6138
Insomnia	32.61	24.86	7.75	0.0372
Nausea and vomiting	14.29	13.15	1.15	0.6939
Pain	25.48	28.99	−3.51	0.3711
Financial difficulties	17.28	17.00	0.28	0.9367

Clinically significant (≥10 points)
Statistically significant (P<0.05)

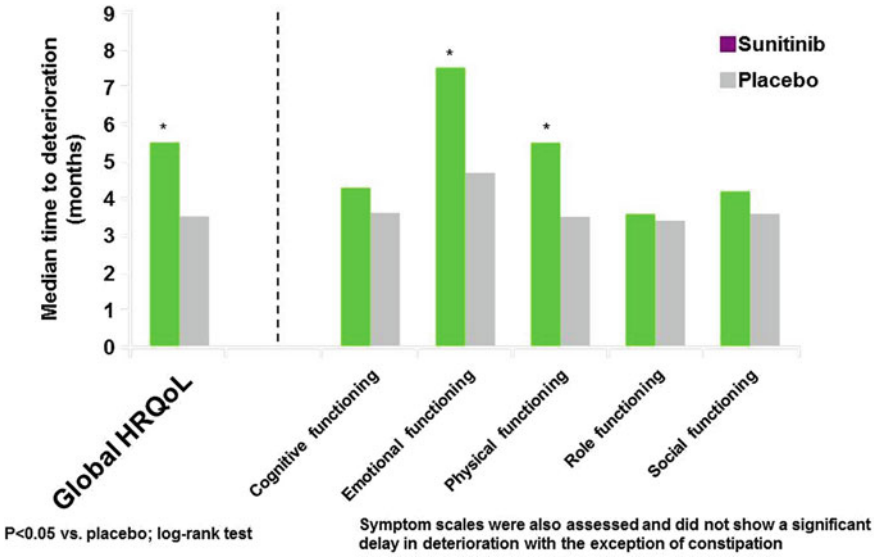

Fig. 14.6 Post hoc analysis: TTD in global HRQoL and functional scales by treatment arm [5]

[27–30]. The symptoms of functioning pNETs, such as diarrhea, rash, and sweating, the toxicity associated with chemotherapy and radiation, and the emotional, social, and cognitive impact of the disease can have a profoundly negative effect on a patient's QoL. Measurement of QoL using questionnaires to assess PROs in the setting of clinical trials is an important means of evaluating the benefit of treatments. PROs help to bridge the gap between the patient and physician, especially regarding sensitive personal issues related to treatment. Measurement of PROs in cancer trials has become a priority in the USA and Europe. Instruments include the National Cancer Institute's PROMIS and the European Organization for Research and Treatment of Cancer's EORTC QLQ-36 [24]. Questionnaires developed specifically to assess health-related QoL in NET patients include the Norfolk QoL-NET and the EORTC QLQ-C30 GI.NET21 [6, 20]. Until recently, only a limited body of research on carcinoid and NETs existed, and as previously mentioned, there was no disease-specific tool to measure health-related QoL in patients with this disease. To fill this need, two questionnaires were simultaneously, but independently developed—on two different continents—to measure the subjective, self-reported effects of NETs on QoL. While there are some distinct similarities, there are also notable differences in each; however, both have the common goal of measuring QoL. The Norfolk QoL-NET, a 72-item all-inclusive single questionnaire was developed in 2004, at the Neuroendocrine Unit, a department within Eastern Virginia Medical School (EVMS), located in Norfolk, Virginia. The development process, which extended over 3 years in different patient populations in the United States, has been described and published [6]. In the interim, the European group working in the NET field also developed a 21-item

disease-specific QoL questionnaire to supplement the EORTC QLQ-C30 (their generic cancer measure updated in 1995), mentioned above [31]. They named the new, disease-specific tool, EORTC QLQ-GI.NET21 [22].

Description of Two Disease-Specific Questionnaires

The Norfolk QoL-NET is a fully validated 72-item questionnaire, with excellent internal consistency [6]. It covers seven domains: [1] depression, [2] flushing, [3] respiratory, [4] gastrointestinal (GI), [5] cardiovascular, [6] physical functioning, and [7] positive attitude. Measurements are related to a 4-weeks time frame and capture 11 symptoms, and it measures both **frequency** and **severity** of symptoms: flushing, joint/bone pain, other pain, peripheral edema, wheezing, diarrhea/constipation, rash, cyanosis, telangiectasia, fatigue, and coughing. The questionnaire assesses the impact of these symptoms on daily activities, including work, family life, and psychosocial activities. We have shown that the burden of disease plays a major role on physical functioning and consequently on QoL [32]. Twenty questions in the Norfolk QoL-NET were designed to capture activities of daily living and physical functioning.

The EORTC QLQ-C30 GI.NET21 is a 21-item questionnaire to supplement the QLQ-36 and focuses specifically on NETs of the gut (Fig. 14.6). Measurements span a 1-week time frame, with the exception of sexual activity, which uses a 4-weeks time frame [6]. It does not measure the frequency or severity of symptoms and does not address physical functioning. Additionally, it lacks items related to cardiovascular symptoms. It uses three defined multi-item symptom scales, endocrine symptoms, GI symptoms, and treatment side effects, and has two single-item symptoms: bone/muscle pain and worry about weight loss. It also uses two psychosocial scales (social functioning and disease-related worries) and includes two other single items (sexuality and communication). The questionnaire also includes items related to generic cancer concerns.

Certain symptoms in the QLQ-GI.NET21, such as headaches, night sweats, and abdominal bloating, are not included in the Norfolk QoL-NET. However, these are generic cancer questions and not specific to NETs. Also featured in the QLQ-GI.NET21 are items that deal with worry about general cancer issues, but an important NET-specific question related to coughing is not included, nor are items related to diarrhea and constipation. Norfolk QoL-NET does include diarrhea and constipation, specific to NETs and addresses these symptoms in depth. “Have you had diarrhea even if you did not eat?” “Have you had *continuous* diarrhea even if you did not eat?” “Have you had a cough, not related to a cold or allergies?”

During the psychometric validation process of the Norfolk QoL-NET, factor analysis confirmed the subscales/factors and items. Seven different factors emerged from the analyses, Cronbach α values ranged from 0.86–0.97 showing excellent internal consistency of the items in each factor. This analysis has not been shown to date in the European version.

In the course of the development process of the Norfolk QoL-NET [6], we related measures of QoL to tumor burden, biochemical values, and symptoms, using the results of both questionnaires to demonstrate criterion validity. In this chapter, we evaluate the structure of both questionnaires in more detail and discuss the ability of each to capture the salient features of the disease, as well as each questionnaire's capacity to correlate patient-related QoL scores with objective health measures.

Study Methods for Completing Both Questionnaires

During clinic visits to the Neuroendocrine Unit at the EVMS, patients with a diagnosed NET (from August through November 2008) were informed about the study and asked to participate. Those in agreement, signed the consent form and completed the Norfolk QoL-NET and the EORTC QLQ-C30 GI.NET21 questionnaires. At the end of the study period, 29 patients were enrolled; information about the status of the disease in terms of the tumor burden, biochemistry, and carcinoid symptoms was extracted from their files matching the date they completed the questionnaires.

For the evaluation of the tumor burden, a scale was developed, from 1–6, "1" representing status post tumor resection, "2" no evidence of tumor, "3" single tumor without metastasis, "4" tumor with metastasis to liver or elsewhere but not to bone, "5" tumor with metastasis to bone, and "6" tumor with metastasis to bone *and* liver or elsewhere. Most of the blood samples for biochemical values were assayed and analyzed at the Norfolk Sentara Laboratory System. The markers for this study were Chromogranin A (CgA), urinary 5-hydroxyindoleacetic acid (5-HIAA), and serotonin, with normal values as follows: Chromogranin A 0–5 nmol/L, 5-HIAA 2–8 mg per 24 h, and serotonin 12–44 pg/ml.

The Norfolk Carcinoid Symptom Score is another tool developed in the Endocrine Unit at EVMS to address the usual symptoms present in individuals with NETs (see Appendix). It has a total of eighteen questions: Four questions relate to flushing, four to respiratory symptoms, three to gastrointestinal symptoms, two to cardiovascular, and three to physical functioning. The remaining two questions are about family and personal history. Each question is scored from 0–1, "0" denoting absence of the symptom and "1" denoting that the symptom is present. The total possible score (worst scenario) is eighteen points.

Statistical analysis: For all 29 patients, the means and SE were calculated for the total scores of both questionnaires, biochemical values, and symptom scores. The median was calculated for tumor burden. Since these data were not a Gaussian distribution, nonparametric correlations were used to explore the relationship between total QoL scores from both questionnaires, each individual domain of the Norfolk QoL-NET, tumor burden, biochemical markers, and symptom scores. P values <0.05 were accepted as statistically significant.

Table 14.5 Validation of the EORTC QLQ-GI.NET21 questionnaire for assessing quality of life of patients with gastrointestinal neuroendocrine tumors

• 253 Patients, 10 centers – 124 Non-functioning CgA – 111 5-HIAA (carcinoid) – 5 Insulinomas – 4 Gastrinomas – 3 Glucagonomas – 1 VIPomas – 5 Unknown • Completed EORTC core questionnaire (the QLQ-C30), version 3.0; and QLQ-GI.NET21	• 21 Items • Four single items – To muscle and/or bone pain (MBP), body image (BI), information (INF), and sexual functioning (SX) • 17 items • Five scales: – Endocrine (ED; 3 items) – GI symptoms (GI; 5 items) – Treatment related (TR; 3 items) – Social functioning (SF; 3 items) – Disease-related worries (3 items)

Yadegarfar et al. [40]

Table 14.6 Comparison of the Norfolk QoL-NET with the EORTC QLQ-GI.NET21 tools

NORFOLK QoL-NET (made up of 7 domains, totaling 72 items)		EORTC QLQ-NET21 (made up of 21 items)	
Domains	Items	Domains	Items
• Depression	10	• 4 Single items	
		– Muscle and/or bone pain	(MBP)
• Flushing	8	– Body image	(BI)
		– Information	(INF)
		– Sexual functioning	(SX)
		• 5 Scales with 17 items in total	
		– Endocrine (ED)	5
		– Gastrointestinal symptoms (GI)	5
• Respiratory	8	– Treatment related (TR)	5
– Cardiovascular	6	– Social functioning (SF)	5
• Gastrointestinal	7	– Disease-related worries	5
• Physical functioning	30		
– Positive attitude	3		

The results for the Norfolk QoL-NET and the EORTC QLQ-C30 QLQ-GI.NET21 were compared using Spearman's nonparametric correlations. The results showed a strong correlation between the total scores of the two questionnaires ($r = 0.93, p < 0.0001$); all the domains of the Norfolk QoL-NET correlated positively with the EORTC QLQ-C30 QLQ-GI.NET21 total score—except for the cardiovascular domain which did not correlate at all (Table 14.5).

Regression analysis was used to determine the predictability of the scores of each domain for the Norfolk QoL-NET correlated with the total scores of the Norfolk QoL-NET and the EORTC QLQ-C30 GI.NET21 questionnaires (Table 14.6). Both questionnaires correlated strongly with the Norfolk Carcinoid

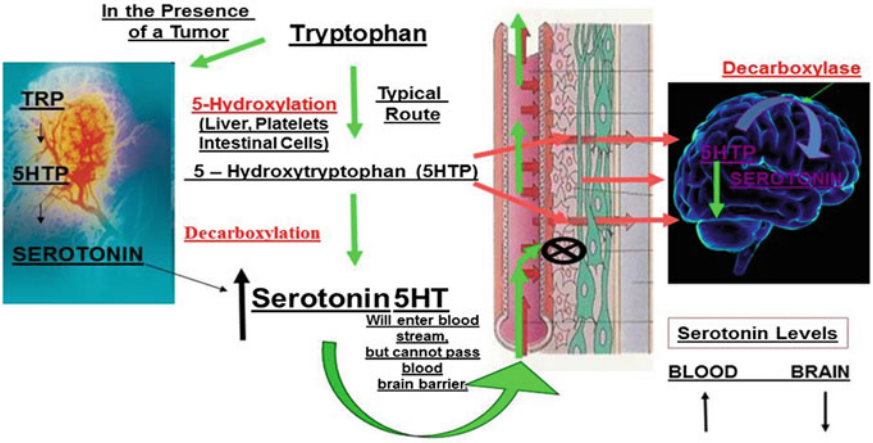

Fig. 14.7 Biosynthesis of serotonin in the presence of a carcinoid tumor and illustration of the deviation of transport of the precursor 5-hydroxytryptophan to the brain resulting in a net reduction in synthesis of brain serotonin as a mechanism contributing to the depression. This figure also explains the apparent paradox of an elevated serotonin in the blood with depression related to low brain serotonin since serotonin cannot cross the blood brain barrier. Copyright © 2009 Aaron and Etta Vinik

Symptom Score and also with tumor burden. No correlation was found between the QoL scores and CgA values. The analysis of the data from the serotonin values that matched the date the patients completed the questionnaires, showed a positive strong correlation between serotonin and QoL and assessed either by the Norfolk QoL-NET or by the EORTC QLQ-C30 GI.NET21 questionnaires; and this marker also correlated positively with three of the Norfolk QoL-NET domains (Fig. 14.7). Table 14.7 shows the r and p values of these correlations.

Missing data were handled in the following way: For CgA values, the mean of the results for each variable was used, provided that more than two-thirds of the values were available. For the analysis of the other biochemical markers, 5-HIAA and serotonin, only patients who had these measures were included (Table 14.8).

When comparing the Norfolk QoL-NET with the QLQ-C30 GI.NET21, certain differences emerged. While there was a good correlation between the total scores with the two tools a clear distinction emerged when the correlations between the different domains of the Norfolk QoL-NET and the total scores of the two tools were examined.

All the domains of Norfolk QoL-NET—physical functioning, depression, gastrointestinal, flushing, respiratory, positive attitude, and cardiovascular correlated strongly with the total QoL Norfolk NET score with p values <0.05; in contrast, only three domains of the Norfolk QoL-NET predicted the total QoL score of the EORTC QLQ-C30 GI.NET21. These were physical functioning, gastrointestinal, and respiratory which reached significance. The remaining four

Table 14.7 Comparison of the Norfolk QoL-NET total and domain scores, with tumor burden, biochemical markers, and the Norfolk carcinoid symptom scores with the EORTC QLQ-C30 GI.NET21 scores

	Total Norfolk QoL		Total European QoL		Domain 1 depression		Domain 2 flushing		Domain 3 respiratory		Domain 4 gastrointestinal		Domain 5 cardiovascular		Domain 6 physical functioning		Domain 7 positive attitude	
	R	p	r	p	r	P	r	P	r	p	r	p	r	p	r	p	r	p
Total Norfolk QoL			0.94	<0.001	0.73	<0.0001	0.62	0.0003	0.65	0.0002	0.78	<0.0001	0.46	0.012	0.96	<0	0.52	0.004
Tumor burden	0.52	0.004	0.5	0.005	0.42	0.023	0.24	0.216	0.02	0.935	0.58	0.001	0.18	0.343	0.56	0.002	0.18	0.346
Serotonin	0.62	0.013	0.71	0.003	0.56	0.03	0.08	0.78	0.32	0.25	0.62	0.013	0.29	0.3	0.62	0.013	0.12	0.67
CgA	0.06	0.764	0.06	0.765	−0.15	0.433	0.26	0.176	0.08	0.663	0.03	0.891	0.34	0.07	0.07	0.735	0.12	0.55
Carcinoid Symptom Score	0.67	<0.0001	0.67	<0.0001	0.37	0.051	0.58	0.001	0.53	0.003	0.6	0.0006	0.55	0.0018	0.7	<0.0001	0.59	0.0009

Table 14.8 Correlations between serotonin, total QoL scores, and domains of the Norfolk QoL-NET in patients with NETs

Total QoL and domain scores	Serotonin	
	r	P
Total scores Norfolk QoL-NET	0.62	0.013
Total scores EORTC C30 GI.NET21	0.71	0.003
Domain 1: depression	0.56	0.03
Domain 2: flushing	0.08	0.78
Domain 3: respiratory	0.32	0.25
Domain 4: gastrointestinal	0.62	0.013
Domain 5: cardiovascular	0.29	0.3
Domain 6: physical functioning	0.62	0.013
Domain 7: positive attitude	0.12	0.67

domains, flushing, depression, cardiovascular, and positive attitude, failed to reach significance.

However, correlation of the domains of the Norfolk QoL-NET, physical functioning, gastrointestinal, depression, flushing, respiratory, and positive attitude, was stronger with the total Norfolk QoL-NET score than it was with the total scores of the EORTC QLQ-C30 QLQ-GI.NET21; similarly, there was a stronger correlation of QoL-NET domains with tumor burden and the Norfolk Carcinoid Symptom Score.

Overall, a strong correlation has been demonstrated between Norfolk QoL-NET and the EORTC QLQ-C30 GI.NET21. Either tool can be considered for use in the clinical trial setting and QoL as measured by both instruments has been correlated with Norfolk Carcinoid Symptom Score, tumor burden, and serotonin level [6].

Conclusions

Patient-reported outcomes have become an essential component of evaluating patients with NETs. QoL is affected by the tumor itself but also by the treatment modality used—both of which must be assessed. There are clear relationships between changes in QoL and time to tumor progression, tumor bulk and amines/peptides secreted. There are generic tools for evaluating of QoL, but these do not address the specific impact of NETs (e.g., flushing, diarrhea, wheezing). However, historically, disease-specific tools were not available to measure these key factors. In both the USA and Europe, the need arose to evaluate the QoL in patients with NETs as more patients were diagnosed (or misdiagnosed) with this disease. Because of the lack of specific questionnaires to assess the spectrum of symptoms present in this disease, two disease-specific QoL questionnaires, the Norfolk QoL-NET and the combination EORTC QLQ-C30 GI.NET21, were developed.

The Norfolk QoL-NET questionnaire is an all-inclusive single tool of 72 questions for measuring subjective, self-reported effects of NETs on QoL. It measures both frequency and severity of symptoms; the measurements relate to a 4-weeks time frame as opposed to the single-week time frame in the EORTC QLQ-C30 GI.NET21. The Norfolk QoL-NET captures clinical symptoms related to NETs that have been classified by factor analysis into domains. There are ten questions in the depression domain and eight in the flushing domain that include actual flushing, rash, and telangiectasia, while the European tool has only two questions related to flushing. In the respiratory domain, there are eight questions related to shortness of breath, wheezing, and coughing compared to only one question related to shortness of breath present in the European questionnaire. In contrast to the lack of cardiovascular questions in the European questionnaire, the Norfolk QoL-NET has six questions assessing the presence of edema and cyanosis. The physical functioning domain in the Norfolk QoL-NET is comprised of twenty-six questions. Additionally, there are three questions related to positive attitude and four related to the impact of treatment with somatostatin on the QoL of these patients. The Norfolk QoL-NET assesses the impact of treatment with somatostatin on the QoL of patients with NETs. Since new treatment options are being developed that may affect the QoL of patients in different ways, this issue should be addressed and the questionnaire should be modified. The European tool has no questions addressing this concern.

The psychometric analysis performed on the Norfolk QoL-NET tool resulted in seven domains, providing a structure for entering, analyzing, and interpreting patient data for proposed interventions, directed to specific problems. As yet, there is no publication to show that psychometric analysis has been performed on EORTC QLQ-C30 GI.NET21.

There is a strong positive correlation between the Norfolk QoL-NET and the European questionnaires. All the domains of the Norfolk, QoL-NET, except for the cardiovascular domain, correlated with the EORTC QLQ-C30 GI.NET21 total score. This might be (as stated above), because the European questionnaire has no questions related to cardiovascular symptoms, while there are six questions addressing this issue in the Norfolk tool.

The strongest correlation found between the total scores of both questionnaires and the Norfolk QoL-NET domains was with the physical functioning domain. This domain was also found to be the biggest predictor of total score for both questionnaires after logistic regression analysis.

Both questionnaires and each domain correlate positively with the Carcinoid Symptom Score, except for depression; this might be because there are no questions about depression in the Carcinoid Symptom Score tool.

Tumor burden correlates with both questionnaires and with the depression, gastrointestinal and physical functioning domains. We recommend the use of the tumor burden scale, proposed in this study, for future studies since it gives an easy and reproducible way to assess, classify, and compare this variable. Of the biochemical markers investigated in this study, only serotonin was found to have a significant positive correlation with the total QoL scores assessed with either

questionnaire; it also correlated with three domains—depression, gastrointestinal, and physical functioning. Serotonin regulates numerous biological processes including cardiovascular function, bowel motility, ejaculatory latency, and bladder control, as well as other processes including platelet aggregation [33]. Serotonin may be incriminated in a number of the symptoms of carcinoid particularly diarrhea and cardiomyopathy. While the relationship between serotonin and total QoL (in particular depression) appears counterintuitive, there may be a plausible explanation. Serotonin does not cross the blood brain barrier, but 5-HTP (the precursor to serotonin) is able to and is required for brain synthesis of 5-HT (serotonin). Since the tumor deviates 5-HTP into production of 5-HT, which is released into the circulation, high levels of blood 5-HT reflect a deficiency of 5-HT in the brain. We are continuing to study this fascinating phenomenon to ascertain whether our proposed hypothesis on the inverse relationship between high blood serotonin levels and depression can be confirmed in a larger population of patients with NETs [34].

Finally, we demonstrated a strong correlation between Norfolk QoL-NET and the EORTC QLQ-C30 GI.NET21, indicating that either tool can be considered for use in a clinical trial. However, the Norfolk QoL-NET captures additional features—a larger number of questions covering the flushing and respiratory domains, and, in particular, the cardiovascular impact, which has been found in 37 % of patients with carcinoid tumors [35]. Nevertheless, it should be mentioned that the prevalence of cardiovascular complications in NETs may be falling for reasons that are not obvious [36]. We believe that the Norfolk QoL-NET is an important tool for measuring a patients' perception of the burden of their disease and impact of treatment modalities on their QoL and may be a useful guide in deciding changes in therapy to alter apparent health status. Norfolk QoL-NET should be particularly sensitive to symptom change, physical functioning, respiratory and cardiovascular disease progression or remission, and in this respect, it may have advantages over the EORTC QLQ-C30 GI.NET21. This remains to be seen when longitudinal studies are completed.

It has been reported that there has been very little change in the natural history of carcinoid tumors [37]. However, with the recent advent of new drugs for the treatment of NETs [13, 38], there are new inroads into the "Rapid Pace of Non Progress" [11, 37] or the "Odyssey in the Land of Slow Growing Tumors" [39]. To this end, tools that can identify the selective impact of these agents on the different domains determining health status and QoL should be welcome, particularly if they pave the way for new therapeutic options which derive their logic from health-related QoL measures.

Targeted therapies have revolutionized treatment for advanced pNETs, improving efficacy and helping to maintain patient QoL. Clinical trials examining the use of targeted therapies in combination with other systemic treatments, as well as in adjuvant and neoadjuvant settings, may further extend the usefulness of these agents. Better understanding of biomarkers to measure treatment response and prognosis in pNETs may also allow more effective use of targeted and other therapies in the future.

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Chapter 15
Clinical Approaches of Emergencies in Neuroendocrine Tumors

Geertrui Mertens, Saskia Carton, Chris Verslype and Eric Van Cutsem

Abstract Neuroendocrine tumors (NET) are a rare and heterogeneous group of tumors that represent a wide variety of clinical problems leading to many challenges for the clinician, including several emergencies. In this chapter, we will detail on selection of relevant emergencies that occur as complications of NETs. We will focus on the following topics: carcinoid syndrome, carcinoid crisis, and preoperative, intraoperative, and postoperative management to prevent a carcinoid crisis, carcinoid heart disease, approach of MEN-1, insulinoma, and VIPoma, and at least the challenge of a pheochromocytoma.

Carcinoid Syndrome and Carcinoid Crisis

The carcinoid syndrome was first described in 1950s. It occurs in 20 % of all well-differentiated NETs of the jejunum and ileum (midgut). The clinical features are episodic flushing, diarrhea, abdominal pain, heart failure, telangiectasia, bronchospasm, and pellagra (Table 15.1). Carcinoid syndrome is rare in tumors of other origin, even more uncommon in rectal NETs [1–7].

The development of carcinoid syndrome is due to liver metastases, which secrete vasoactive mediators directly into the systemic circulation, bypassing the liver. However, it may also occur in the absence of liver metastases, if there is direct retroperitoneal involvement, primary ovarian, or bronchial neuroendocrine tumor. At the time of diagnosis, 20–30 % presents with an acute carcinoid

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Table 15.1 Clinical characteristic of carcinoid tumors

Clinical features	Frequency %	Mediators
Flushing	85–90	Kallikreine, histamine, 5-HT, prostaglandins, substance P
• Pink/red/purple		
• Face, neck, upper chest		
• Limited duration		
Diarrhea	70	Gastrin, 5-HT, prostaglandins, histamine, vasoactive intestinal peptide
• Secretory (watery)		
Abdominal pain	35	Obstruction, mesenteric fibrosis, hepatomegaly
• Variable		
• Colic or ischemic type		
Heart failure		5-HT, substance P
• Right	30	
• Left	10	
Telangiectasia	25	Unknown
• Purplish vascular lesions		
• Nose, upper lip, malar areas		
Bronchospasm	10–20	Histamine, 5-HT
• Wheezing		
• Dyspnea		
Pellagra	5	Niacin deficiency
• Dermatitis		
• Glossitis		
• Stomatitis		
• Mental confusion		

Modified from: [47]. 5-HT: 5-hydroxytryptophan

syndrome [3, 5, 6, 8]. Carcinoid syndrome associated with bronchial NETs is often atypical and presents as episodic flushing, diaphoresis, tremor, periorbital edema, lacrimation, salivation, and edema [5, 6]. Carcinoid crisis is a life-threatening expression of the carcinoid syndrome that arises when overwhelming amounts of humoral factors are released into the systemic circulation. It can occur spontaneously or iatrogenic. A list of provoking factors is given in Table 15.2 [5, 6, 9, 10].

It is characterized by profound flushing, hemodynamic instability (tachycardia and hypotension), arrhythmias, bronchoconstriction, reversible right ventricular dysfunction, and altered mental state [5, 9, 12].

Pathophysiology

As many as 40 secretory products have been identified in various carcinoid tumors (Table 15.1). They can be divided into three main groups: the amines, the polypeptides, and the prostaglandins.

Table 15.2 Provoking factors for carcinoid syndrome and crisis: strong and mild evidence [6, 9, 11,12]

Strong evidence	Mild evidence (reported in case reports and patients' guidelines)
Palpation	*Alimentary triggers*
• Bedside	• Alcohol
• Intraoperative	• Amine-containing food (e.g., caffeine drinks, chocolate, wine, etc.)
• Abdominal ultrasound	• Serotonin containing food (e.g., bananas, pineapples, tomatoes, etc.)
Procedures	*Stress*
• Chemotherapy	• Emotional
• Hepatic artery ligation	• Physical
• Embolization	
• Radio nucleotide therapy	
• Fine needle biopsy	
• Induction of anesthesia	
Drugs	*Drugs*
• Beta-adrenergic agonists	• SSRIs
• (Nor)epinephrine	

The three most important substances are serotonin, histamine, and the kinin peptides (tachykinins and bradykinins), which can induce the carcinoid syndrome. Other less important mediators are prostaglandines, vasoactive intestinal peptide, adrenocorticotrophic hormone, and motilin [5].

These vasoactive substances are secreted into the portal circulation and metabolized within the liver. As a result of this first-pass effect, most NETs do not cause the features of a carcinoid syndrome. The syndrome occurs when the venous drainage empties directly into the systemic circulation, which is typically seen in patients where hepatic metastases are present, when there is retroperitoneal involvement or where the primary tumor is situated outside the gastrointestinal tract (e.g., bronchial, ovarian, or testicular neuroendocrine tumors) and releases hormones directly in the systemic circulation [6].

Most of the tumors of the jejunum, ileum, proximal colon, and appendix (>70 %) and some NETs of the stomach and respiratory tract (10–35 %) secrete serotonin [5-hydroxytryptamine (5-HT)], a metabolic derivate from tryptophan (Fig. 15.1) [8]. Serotonin is primarily found in the gastrointestinal tract, platelets, and in the central nervous system. However, most of the serotonin (>90 %) is located in the enterochromaffin cells in the gastrointestinal tract. There it plays an important role in controlling the gastrointestinal motility, sensitivity, and secretion. Furthermore, when it is stored in platelets, it plays an important role in the hemostasis. In the brain, it has various functions, controlling cognitive functions, mood, appetite, and sleep. Adrenergic stimulation is responsible for the release of serotonin into the circulation [13, 14].

In patients without the carcinoid syndrome, approximately 1 % of dietary tryptophan is converted to serotonin. This value may increase to 70 % in patients

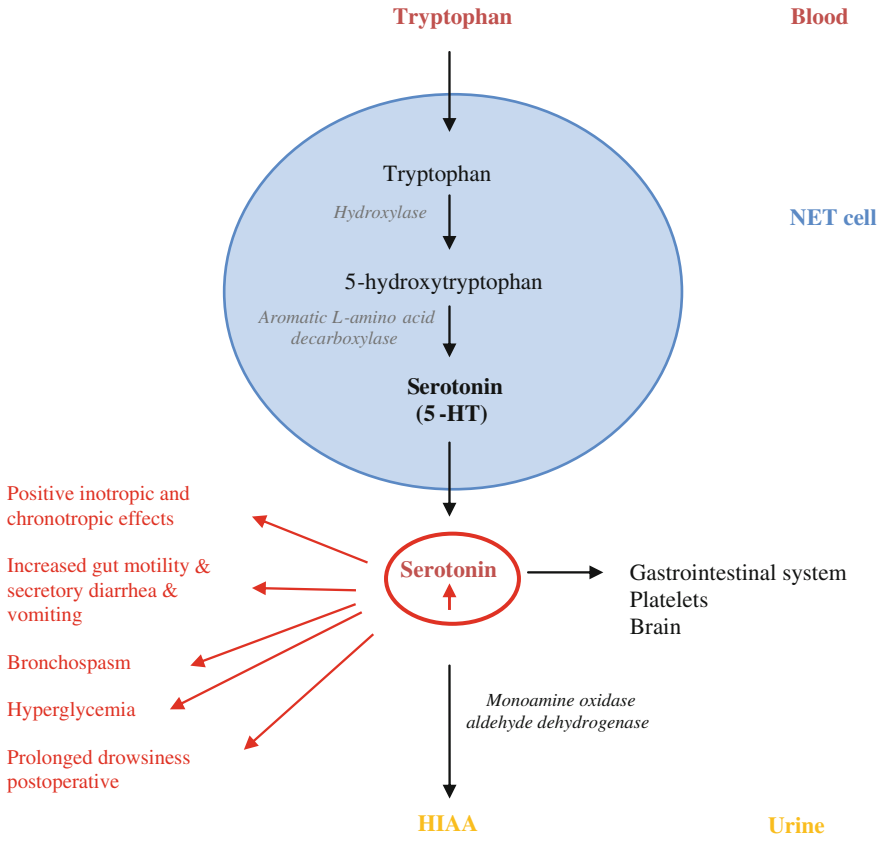

Fig. 15.1 Pathways of serotonin metabolism (5-HT: 5-hydroxytryptamin; 5-HIAA: 5-hydroxyindoleacetic acid)

with carcinoid syndrome [5, 6]. Tryptophan is used for the synthesis of serotonin, proteins, and nicotinic acid. As a result of tryptophan deficiency, decreased protein synthesis, hypoalbuminemia, nicotinic acid deficiency, and pellagra can develop. Once serotonin is secreted, it is oxidized and dehydrogenated by aldehyde dehydrogenase and monoamine oxidase to 5-hydroxyindoleacetic acid (5-HIAA), which is ultimately excreted by the kidney. Elevated levels of 5-HIAA are monitored in a 24-h urine sample as a marker of excess serotonin production and represents the presence of a NET. The elevated levels of serotonin can cause positive inotropic and chronotropic responses, increased gut motility, secretory diarrhea, vomiting, bronchospasm, hyperglycemia, and prolonged drowsiness following anesthesia (Fig. 15.1). Histamine release is mostly seen in patients with foregut NET and is probably responsible for the bronchoconstriction and flushing. This theory is however controversial. At least, kinins (e.g., bradykinins and tachykins) can also be released. Peripheral effects of bradykinin, a kinin produced

by kallikrein, include hypotension, vasodilatation with flushing, increased capillary permeability, and bronchoconstriction. Tachykinins (e.g., substance P, …) are involved in the development of carcinoid heart syndrome or episodic flushing.

Diagnosis

Diagnosis is often delayed due to the atypical presentation of NETs [1, 6]. Histologic diagnosis requires expertise and includes the use of immunohistochemistry for chromogranine and synaptophysin and also in some tumors for other peptides and the determination of the proliferation index (Ki67) and of the mitotic index [15]. Moreover, imaging (radiology, endoscopy, and nuclear imaging) plays a crucial role in the diagnosis and staging of NETs. Serum markers also contribute to diagnosis: Serum chromogranin A (CgA) is usually found in high concentrations regardless of hormone-related symptoms. However, CgA can be elevated in patients with atrophic gastritis and anacidity, in patients taking proton pomp inhibitors, and in patients with renal insufficiency [8, 15]. In case of a serotonin-producing NET, the work-up includes a 24 h urine sample for 5-HIAA [1]. Urinary 5-HIAA appears to be a good biological marker for the assessment of carcinoid symptoms by a NET and its association with perioperative morbidity. Overall, high preoperative urinary 5-HIAA measurement is a risk factor for perioperative complications, including death. Serial measurements of 5-HIAA are used to monitor disease progression in these patients, although today the serial measurement of serum chromogranin A is more often done [10].

Normal levels of 24 h urinary 5-HIAA are less than 10 mg. Levels greater than 25 mg per 24 h have been considered to be diagnostic for carcinoid tumors. During the collection of the 24 h urine sample, patients should avoid serotonin-rich food (e.g., bananas, pineapples, tomatoes, kiwis, eggplant, plums, plantain, and walnuts) and drugs which will affect urinary excretion of 5-HIAA (e.g., naproxen, paracetamol, etc.) [1, 6, 15]. However, up to 20 % of patients with carcinoid symptoms have normal 5-HIAA levels. Additional screening for insulin, C-peptide, gastrin, VIP, pancreatic polypeptide, glucagon, and calcitonin should depend on the clinical symptoms, histological features, and functional status of the tumor.

Management of Carcinoid Syndrome

The primary treatment goal, and currently the only possible cure, for patients with gastrointestinal NETs, is curative surgery. Curative surgery is, however, often not possible, since most patients present with metastases at the moment of diagnosis. As curative treatment is not possible, many patients require chronic medical management to relieve symptoms and to suppress tumor growth. Symptom control can be done by somatostatine analogs (SSA). At this moment, three SSAs are

available: octreotide, lanreotide, and pasireotide. SSAs act by binding to somatostatin receptors, which are expressed on the majority of NETs. There are five receptors (SST1–SST5), linked with several intracellular systems: reduction in calcium inflow, inhibition of adenylyl cyclase, and in some tissues stimulation of tyrosine phosphatase activity. Activation of SST1 and SST2 is associated with antimitotic activity of SSA. Activation of SST5 is responsible for the reduction in calcium inflow, which is correlated with inhibition of cell proliferation. The SST3 receptor mediates apoptosis. Another working mechanism of SSA is inhibition of growth factor and angiogenesis, as well as immunomodulatory effects. The SST2 receptor is the most frequent somatostatin receptor on NETs (90 % of serotonin-secreting NET and 80 % on pancreatic NET). Native somatostatine binds to all receptors, but octreotide has high affinity for SST2 and lower affinity for SST3 and SST5 [7, 16–18].

Octreotide was the first approved SSA. It can be administered as a short-acting octeotride that is usually administered three times daily subcutaneously (SC) (Sandostatine® 0.1–0.5 mg) and a slow-releasing form of octreotide injected intramuscular monthly (Sandostatine LAR® 20–30 mg), both controlling symptoms equally. If a patient is changed to the long-acting formulation, the time required to reach steady-state levels makes continuation of subcutaneous octreotide administration necessary for at least 2 more weeks [19]. The antitumor efficacy of SSA appears weak, even if it is used in high dosages. However, disease stabilization occurs in 50–60 %. Recently, the first randomized, double-blind, placebo-controlled, multicenter, phase III study of octreotide LAR in patients with metastastic well-differentiated neuroendocrine midgut tumors was published. This study (PROMID) shows that octreotide LAR inhibits tumor growth in patients with metastatic midgut NETs, without a difference between functioning and non-functioning tumors. The antiproliferative effect was presumably more in patients with low (<10 %) hepatic tumor load or after a resected primary tumor. Although further studies are needed, the number of patients with high tumor load was low [5, 6, 20, 21]. Another SSA is lanreotide (BIM-23014, Somatuline Depot®), a long-acting formulation, which is available in monthly injections at doses ranging from 60–120 mg SC every 4 weeks. Octreotide and lanreotide have similar clinical efficacy and tolerability for the treatment of carcinoid syndrome [15]. Patients receiving octreotide or lanreotide may experience an ‘escape from response’ 6–18 months after initiation due to progression of the disease or tachyphylaxis. Pasireotide (SOM-230), a newer SSA, with high binding affinity for SST1, SST2, SST3, and SST5, is under development in patients with symptomatic NETs. The most common side effects of SSAs are abdominal bloating and discomfort; mostly, they are mild and disappear spontaneously within the first week. Diminished gallbladder contractility can develop, leading to gallstones or sludge, but only a small proportion of patients develop symptoms. According the ENET guidelines, preventive cholecystectomy is not required [15].

In NET patients with carcinoid symptoms, with progressive disease under and/or symptoms not controlled by SSA, other antitumoral therapies have to be considered. Interferon-α therapy (IFN, Intron®) is generally recommended as a

second-line approach in patients with functional and non-functioning NETs and low proliferation index, to control refractory symptoms. It is used at doses titrated by side effects and leukocyte count. The effect of IFN on symptom control is similar to that of somatostatin analogs; however, they do not act as rapidly, and it has a less favorable safety profile. NET patients, with symptoms not under control by SSA and with a slowly progressive tumor with a low proliferation index and a negative somatostatin receptor scintigraphy, are potential candidates for IFN. Liver-targeted therapies to debulk tumors and improve symptoms, such as surgical resection, transarterial embolization (TAE) with or without selective artery infusion of chemotherapy (TACE), and radiofrequency ablation (RFA), play an important role in the management of patients with advanced NET with liver metastases. It can control tumor growth and the carcinoid and/or tumor-related symptoms when medical therapy failed. However, if palliative surgery is performed, it is often recommended to resect at least 90 % of the tumor. It is still unclear which percentage of the tumor bulk has to be resected to achieve improvement in symptom control and outcome. More prospective clinical trials are clearly needed [15, 21, 23]. Everolimus and sunitinib are recommended in patients with good or moderately differentiated progressive pancreatic NETs. Chemotherapy is recommended in progressive pancreatic NETs and also in the poorly differentiated NETs of any site. Peptide receptor radionucleotide therapy (PRRT) with ^{90}Y- and/or Lu-DOTATOC or –DOTATATE may be used to treat metastatic somatostatin receptor-expressing NET [15]. Patients with end-stage liver disease and uncontrollable symptoms unresponsive to any other therapy have been considered for orthotopic liver transplantation. Although the shortage of organs in combination with low survival data suggests that liver transplantation should only be considered in exceptional circumstances and further studies are needed [8].

Antidiarrheal agents such as loperamide, diphenoxylate, or atropine can be used for refractory symptoms. For more severe diarrhea, opiates (tincture of opium) may be prescribed. Several studies propose serotonin receptor antagonists; however, the benefit is small. Patients with bile acid malabsorption, occurring after resection of the small bowel, can be treated with cholestyramine or colestipol [24, 25]. Fluid and electrolyte abnormalities should be corrected.

Preoperative, Intraoperative, and Postoperative Management to Prevent a Carcinoid Crisis

Preoperative management should include a detailed medical history and clinical examination to uncover the presence and the severity of the carcinoid syndrome. Furthermore, presenting triggering factors should be determined. Routine investigation should include a complete laboratory screening with blood count, glucose, liver and renal function and electrolyte screening, as well as a urinary 5-HIAA

determination, electrocardiography, and echocardiography. The tumor staging and classification should be complete.

Preoperative management is focused on relieving symptoms and prevention of a potential carcinoid crisis. This is achieved by antagonizing the mediators of carcinoid disease and blocking their release. The effect of histamine is antagonized by H1-blocker (e.g., cetirizine) and H2-blocker (e.g., ranitidine). Bradykinin can be blocked by aprotinin, a kallikrein inhibitor, which is useful for the treatment of flushing and perioperative hypotension, refractory to octreotide. There are, however, conflicting case reports for the treatment of perioperative hypotension [6]. Several other agents have been used in perioperative management, including steroids for bronchospasm, methylsergide, and cyproheptadine for gastrointestinal manifestations and ketanserin, which blocks the effect of serotonin mediated at the 5-HT2 receptor, namely vasoconstriction, bronchoconstriction, and platelet aggregation, and can be used to treat hypertension [5, 6]. Anxiolytic premedication (e.g., alprazolam) that does not have histamine-releasing properties is recommended to reduce the release of catecholamine as a result of preoperative stress [5, 6]. The prevention of a carcinoid crisis should be performed prior to surgery and locoregional interventions, using SC or IV SSA. Various regimes for dosing SSA preoperatively have been reported; however, the optimal dose had not been studied systematically. Subcutaneously, it is given at a dose of 0.5 mg three times daily, 2 weeks prior to surgery, and given for 1 week after surgery. If it is to be discontinued postoperatively, it should be reduced slowly over the first postoperative week. Intravenously, it is used at a dose of 50–100 μg per hour, initiated 24 h before, and given for 24–48 h after the surgical intervention. Furthermore, the patient's maintenance medications should be continued [8, 15]. Octreotide has been successfully used during orthotopic liver transplant for NET at a dose of 0.05 mg IV injection prior to incision, followed by a continuous infusion of 0.05 mg per hour, and additional 0.05 mg boluses to treat episodes of hemodynamic instability. Following this management, there were still episodes of hypotension, related to surgical manipulation of the liver, but never life-threatening [9].

Intraoperative invasive monitoring starting before induction, with an arterial and central venous pressure (CVP) catheter to detect hypovolemia and hypotension, airway pressure monitoring to detect the onset of bronchospasm, temperature monitoring, to detect hypothermia and monitoring of the urinary output, is recommended. In patients with cardiac dysfunction, monitoring of left ventricular function by a Swan-Ganz catheter can be useful. The ideal induction agent is propofol; however, hypotension should be avoided [26]. Etomidate can cause histamine release and may not suppress laryngeal reflexes, but is cardiovascularly more safe [27, 28]. Furthermore, only non-depolarizing neuromuscular blocking agents that do not cause histamine release should be used. The best choice is vecuronium or rocuronium [6, 28]. Opioids that do not cause histamine release should be used for analgesia. Intravenous fentanyl has been used with good effect and no adverse events. We suggest to give 0.5 mg octreotide IV before induction to prevent mediator release.

PREOPERATIVE MANAGEMENT	INTRAOPERATIVE MANAGEMENT	POSTOPERATIVE MANAGEMENT
UPDATE: - Medical history - Clinical examination - Laboratory screening - Urinary 5-HIAA control - Electrocardiogram - Echocardiography - Complete tumor staging/classification PREOPERATIVE MEDICATION: 1. Two weeks prior to operation: - H1-blockers (cetirizine 10 mg, once daily) - H2-blockers (ranitidine 150 mg, two times daily) - Octreotide 0.5 mg, 3 times daily SC 2. Day of operation: - H1-and H2-blockers - Benzodiazepine (alprazolam 0.5mg) - Octreotide 0.5mg, 3 times daily SC or 50-100 µg IVper hour, initiated 24hours before, and given for 24-48 hour after the surgical intervention If diarrhea: loperamide. If bronchospasm: ipratropium	MONITORING PATIENT: - Arterial and CVP monitoring - Airway pressure - Temperature - Glycaemia - Urine output (- Swan-ganz catheter) INDUCTION: - Before induction: Octreotide 0,5 mg SC - Propofol NEUROMUSCULAR BLOCKING: - Vecuronium - Rocuronium ANALGESIA: - Fentanyl TREAT COMPLICATIONS: - Bronchospasm:H1-blockers & ipratropium - Hyperglycemia:insulin - Hypertension:beta-blockade, ketanserin, octreotide, increasing depth of anesthesia CARCINOID CRISIS (+ hypotension): - Fluid filling - Octreotide bolus up to 1 mg IV - Interrupt tumor manipulation	MONITORING PATIENT: - Intensive care (if possible) - Arterial and CVP monitoring - Airway pressure - Temperature - Glycaemia - Urine output (- Swan-ganzcatheter) POSTOPERATIVE MEDICATION: - Reducing octreotide slowly over 1 week - H1-blockers (cetirizine 10 mg, once daily) - H2-blockers (ranitidine 150 mg, two times daily) - Analgesia (if necessary): fentanyl

Fig. 15.2 Preoperative, intraoperative, and postoperative management to prevent a crisis

Early management of perioperative complications is required to prevent progression to carcinoid crisis. When a carcinoid crisis occurs, treatment differs from that of other causes of acute intraoperative hypotension. Symptoms are usually refractory to fluid resuscitation alone. The blood pressure should be supported by infusion of octreotide up to 1 mg. Catecholamines may provoke release of mediators from the tumor and worsen the crisis and therefore should be avoided. Nebulized ipratropium has been used with good results for bronchoconstriction. Octreotide is useful for bronchospasm resistant to other treatments. Beta-adrenergic agonists for the treatment of bronchospasm should be used with caution, since they may exacerbate the symptoms, due to further histamine release (Fig. 15.2).

Hyperglycemia, caused by elevated serotonin levels, should be monitored intraoperative and treated if necessary with an insulin infusion.

In summary, special considerations in the anesthetic management of patients with carcinoid syndrome include preoperative optimization of carcinoid symptom control, intensified intraoperative blockade of serotonin receptors, avoidance of drugs that might stimulate release of vasoactive substances from tumor cells, and maintenance of vigilance well into the postoperative period.

Carcinoid Heart Disease

The association between cardiac disease and NETs was first described in the 1952. In more than 50 % of patients with carcinoid syndrome, carcinoid heart disease develops. In 20 % of patients, carcinoid heart disease is the primary presentation

of metastatic carcinoid disease. Cardiac involvement predicts a decline in mean survival from 4.6–1.6 years [29, 30]. Carcinoid heart disease can present as valvular dysfunction or, less frequently, as a cardiac metastasis. Mostly, it is characterized by plaque-like deposits of fibrous tissue, typically found on the leaflets, subvalvular apparatus, and chordae tendinae as on the tips of papillary muscles. The tricuspid valve is most commonly affected. In a series of 74 patients, it was observed in 97 %. The pulmonary valve was involved in 88 % of patients. Mitral and aortic valve involvement is less frequent. Left-sided cardiac involvement is rare in bronchopulmonal NET and in patients with a patent foramen ovale. Cardiac metastases are uncommon, and they can be present without carcinoid cardiomyopathy [29, 31–34]. The pathophysiology of carcinoid heart disease is not well understood, but is probably an effect of serotonin. High levels of urinary 5-HIAA are not only a marker for the severity of the valvular disease, they are also a marker for progression of underlying valvulopathy [35]. The diagnosis may be delayed by the fact that cardiac symptoms are often absent or subtle. Diagnosis is based on the combination of suggestive pathologic features seen on echocardiography and an elevated urinary 5-HIAA [33].

Right heart failure should be treated with salt and fluid restriction in addition to diuretics. Carcinoid syndrome associated with symptomatic right heart failure (NYHA classification III–IV) has an unfavorable prognosis, if only treated medically, because most of the times, there is an evolution to progressive heart failure and death, rather than progression of the tumor [29]. Valve replacement should be considered in a selected population by expert surgeons. Early recognition and early surgical intervention before advanced clinical heart failure has occurred result in a better outcome [30, 33].

Primary Hyperparathyroidism in Patients with Multiple Endocrine Neoplasia Type 1 (Men1)

MEN-1 is characterized by the combined occurrence of parathyroid hyperplasia, pancreatic endocrine tumor, and pituitary adenoma. The contemporary definition of MEN-1 is the coincidence of at least two of the above-mentioned tumors. A familial MEN-1 requires, besides that, a first-degree relative with at least one of the three tumors [36, 37]. Primary hyperparathyroidism is the most important clinical manifestation. In most of the cases, it is a mild hypercalcemia with a normal range serum parathyroid hormone (PTH), detected during the second decade of life. Demineralization and/or recurrent kidney stones are the main clinical manifestation. In very rare cases, there is a severe hypercalcemia (>14 mg/dL), with weakness, confusion, stupor, and coma, at diagnosis. The standard therapy is hydration with saline infusion (4–6 l/day) and correction of possible volume depletion, in association with a loop diuretic, to further increase urinary calcium excretion. Glucocorticoids can also be useful. Saline therapy

requires careful monitoring. In the carriers of MEN-1 mutation, biochemical measurement of calcium, PTH, gastrin, pancreatic polypeptide, glucose, prolactin, insulin, and glucagon is recommended every 1–3 years. The only sufficient screening test for hyperparathyroidism is total serum calcium concentration corrected for albumin level or ionized calcium fraction.

The therapy of choice for primary hyperparathyroidism in MEN-1 is a total parathyroidectomy and thymectomy with autotransplantation of parathyroid tissue. Calcimimetics are a new effective and until now unlicensed therapeutic option for MEN-1-associated hyperparathyroidism. Even SSAs have been reported to improve hypercalcemia [8].

Functional Pancreatic Endocrine Tumor Syndromes

Functional pancreatic islet cell tumors have been traditionally named according to the hormones which are secreted. There are gastrinomas, glucagonomas, VIPomas, insulinomas, and somatostatinomas. In the setting of emergencies of neuroendocrine tumors, we will focus on the insulinomas and also on the very rare VIPomas [37–39].

Insulinomas

Insulinomas are the most frequent functioning NET of the pancreas. The incidence is being estimated at only 1–3 new cases per one million persons per year. Most of the insulinomas are sporadic (90 %), solitary (83–92 %), and benign (90 %); 5 % is hereditary and non-sporadic and seen in MEN-1; less than 10 % is malignant [39]. Clinically, it presents as hypoglycemic symptoms, including diplopia, blurred vision, confusion, behavioral changes, amnesia, seizures, and coma. Additionally, there are adrenergic symptoms when the glucose level drops below 55 mg/dL, representing as sweating, weakness, hunger, tremor, nausea, feelings of warmth, anxiety, and palpitations [40]. A recent gain in body weight is present in the majority of patients. The main duration of symptoms at diagnosis is 3 years [41]. The Whipple's triad defines the clinical criteria. It includes symptoms of hypoglycemia during periods of exercise or fasting, blood sugar less than 40 mg/dL while symptomatic and resolution of symptoms after administration of glucose [38]. The golden standard in the diagnosis is the 72 h fasting test. During this test, patients are monitored for symptoms of hypoglycemia. Once the patient becomes symptomatic, glucose, insulin, proinsulin, C-peptide, beta-hydroxybutyrate levels should be documented. In case of an underlying insulinoma, a low serum glucose, elevated insulin, proinsulin, C-peptide and beta-hydroxybutyrate levels are usually seen. The most sensitive criteria to diagnose insulinoma was the combination of elevated proinsuline levels with a fasting glucose <45 mg/dL [41].

Table 15.3 Treatment options for insulinomas

Surgery
Lifestyle modification
• Multiple small meals
• A meal before sleep/soon after waking up
• Avoid strenuous exercise
• Glucose snacks/drinks on hand
Drug therapy
• Diazoxide [50–300 mg/day (up to 600 mg/day)]
• Verapamil/dephenylhydantoin
• Glucocorticosteroids
• Everolimus
• IFN-α
• SSA
Radionucleotide therapy
• PRRT
Hepatic interventions
• Hepatic artery embolization
• Chemoembolization

The curative treatment of choice is surgery. Symptomatic disease or non-resectable tumors can benefit from lifestyle modifications. Medical treatment is indicated when symptoms are not controlled by diet alone. First-line therapy is diazoxide, a non-diuretic benzothiadiazine, which inhibits insulin release by direct acting on the β cells. However, the efficacy of diazoxide is transient and associated with side effects in elderly patients (edema, weight gain, renal impairment, and hirsutism). Verapamil and diphenylhydantoin are also successful in controlling hypoglycemia. Glucocorticoids can be useful in refractory cases. SSAs are effective in 50 % of patients. Everolimus and PRRT are recommended in metastatic insulinoma resistant to the standard medical therapy. Hepatic interventions should be targeted at patients with later stage disease of symptomatic liver metastases. In case of asymptomatic hypoglycemia, a IV bolus of glucose (10–25 g) is advised, followed by application of a glucose 10 % infusion [15, 37] (Table 15.3).

The preoperative, intraoperative, and postoperative management of insulinomas in curative surgery are summarized in Table 15.4 [41].

VIPoma

Also known as Verner–Morrison syndrome and pancreatic cholera syndrome, VIPomas are rare NETs that secrete vasoactive intestinal polypeptide (VIP). The incidence of VIPoma is 0.05–0.2 new cases per million persons per year. Forty to seventy percentage is malignant, and 6 % is associated with MEN-1. Clinically, there is severe watery diarrhea, hypokalemia, and acidosis, all of them resulting in

Table 15.4 Preoperative, intraoperative, and postoperative management of insulinomas in curative surgery

Preoperative management	Intraoperative management	Postoperative management
Update	*Monitoring*	*Monitoring*
• Medical history	• Blood glucose	• Blood glucose
• Clinical examination		
• Laboratory screening		
• Tumor staging/classification		
Preoperative medication	*Intraoperative medication*	*Postoperative medication*
• Glucose 10 % (start 24 h prior to surgery until induction)	• If glucose <40–50 mg/dL: glucose IV	• Insulin (if necessary during the first days postoperative)

Table 15.5 Management of VIPomas

Surgery
Drug therapy
• Replacement of fluid loss
• Correction of electrolytes
• SSAs
• IFN-α
• Everolimus
• Chemotherapy
Liver-targeted therapy
• Resection
• Embolization
• RFA
• Cryoablation
• (Transplantation)

severe dehydration [41]. The diagnosis of VIPoma is established by the presence of unexplained severe, watery diarrhea (often in volumes of >5 liter a day) and an elevated serum VIP concentration (>75 pg/ml). Although VIP secretion may be episodic and normal between diarrheal episodes, additional repeated VIP levels should be measured if there is a clinical suspicion. The imaging procedure of choice, to secure the diagnosis, is a CT scan or MRI [41].

The treatment of choice is replacement of fluid losses and correction of electrolyte abnormalities. To treat the severe diarrhea, octreotide is recommended. In case of refractory diarrhea to octreotide, IFN-α is an option or everolimus in case of pancreatic vipoma. Liver-targeted therapy is suggested in patients with metastatic disease (Table 15.5) [37].

Table 15.6 Adapted from [45]. Frequency: highest (++++) to lowest (+)

Signs		Symptoms	
Hypertension	++++	Headaches	++++
Postural hypotension	+	Palpitation	++++
Tachycardia/reflex bradycardia	+++	Anxiety/nervousness	+++
Sweating	++++	Tremulousness	++
		Weakness, fatigue	++
Pallor	++	Nausea/vomiting	+
Flushing	+	Abdominal or chest pain	+
Weight loss	+	Dizziness or faintness	+
Fasting hyperglycemia	++	Paresthesias	+
Decreased gastrointestinal motility	+	Constipation (rarely diarrhea)	+
Tachypnea	+	Visual disturbances	+

Pheochromocytoma

A pheochromocytoma is a rare catecholamine-producing tumor. The estimated annual incidence is 0.8 per 100,000 persons per year. They are mostly arising from catecholamine-producing chromaffin cells in the adrenal medulla. In 10 %, the tumor cells are located in the deparasympathic ganglia [43, 44]. The clinical features of a pheochromocytoma are summarized in Table 15.6 [45].

The clinician should be aware about the diagnosis in a patient with paroxysmal hypertension in association with the classic triad of headache, palpitation, and sweating. The diagnose can be confirmed by measurement of metanephrines, epinephrine, and norepinephrine in a 24 h urine collection. The test is positive when it is more than two times the upper limit of normal. The values can be false positive after emotional or physical stress, sickness, alcohol consumption, and when taking some specific medications (some psychiatric medication and levodopa). When the values are elevated, confirmation of the diagnosis is done by CT scan (without intravenous contrast) and MRI. The treatment of choice is surgery. All patients with pheochromocytoma need to undergo preoperative α-adrenergic blockade, and if necessary β-blockade, to control the tension and prevent a hypertensive crisis, as well as volume expansion. β-adrenergic blockade should never be started first. Postoperative hypotension can be avoided by adequate fluid replacement and glucose infusion to prevent hypoglycemia. After tumor removal, catecholamine secretion should fall to normal values in approximately 1 week. During a hypertensive crisis, the standard treatment is labetolol (Trandate®) at a dose of 20 mg IV (infusion time 1–5 min), repeating until effect, following by a continuous infusion of 10–100 mg labetolol per hour. An alternative regime is phentolamine (Regitine®), a short-acting, non-selective alpha-adrenergic blocker, given at a dose of 1–10 mg IV, following by a continuous infusion of 0.25–1 mg/min. After adequate alpha-adrenergic blockade has been achieved, β-adrenergic blockade can be started (Seloken®, Tenormin®) at a dose of 5–10 mg intravenously [46].

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