

# From Targets to Treatments: A Review of Molecular Targets in Pancreatic Neuroendocrine Tumors

Bertram Wiedenmann<sup>a</sup> Marianne Pavel<sup>a</sup> Beata Kos-Kudla<sup>b</sup>

<sup>a</sup>Department of Hepatology, Gastroenterology and Endocrinology, Charité Medical School, Berlin, Germany;

<sup>b</sup>Department of Pathophysiology and Endocrinology, Silesian Medical University, Katowice, Poland

## Key Words

Molecular targets · Pancreatic neuroendocrine tumors · Targeted therapies

## Abstract

Pancreatic neuroendocrine tumors (pancreatic NET) are relatively rare, slowly growing tumors, although their incidence is increasing, and patients may survive for several years with metastatic disease. Apart from symptomatic relief, there have been few treatment options for these tumors in the past. More recently, investigators have explored the potential of molecularly targeted agents in treating pancreatic NET, with some success. In this review, we consider the data supporting exploitation of different targets in pancreatic NET, including peptide receptors, receptor tyrosine kinases (involved in tumor angiogenesis and more directly supporting tumor growth), and intracellular targets, such as the mammalian target of rapamycin (mTOR), which has a central role in regulating cell growth, metabolism, and apoptosis. Probably due to the paucity of pancreatic NET, many clinical trials to date have included heterogeneous NET populations, and there are few randomized studies of this specific patient population. Very recently, promising results have been achieved in placebo-controlled, phase III trials with the multitargeted tyrosine kinase inhibitor, sunitinib, and the mTOR inhibitor, everolimus. These agents have been approved or

are currently being reviewed by authorities for use in patients with pancreatic NET. Here we review potential molecular targets in pancreatic NET and summarize the available data for targeted agents from phase II and III trials open to patients with this tumor.

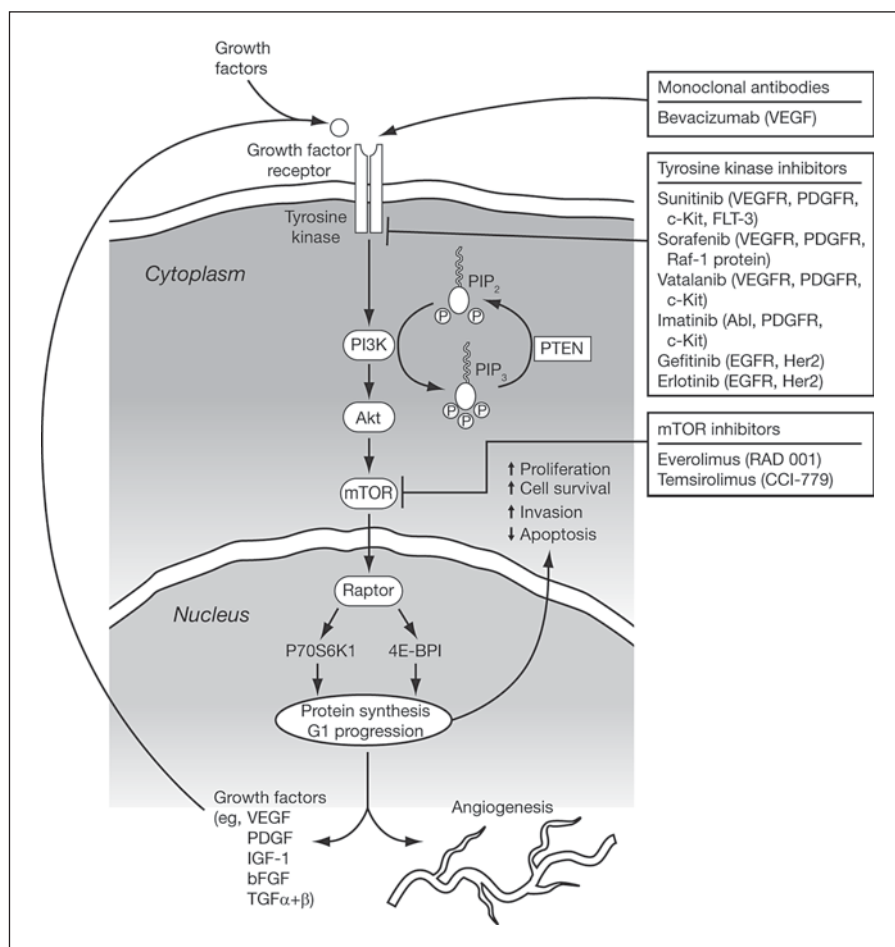
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## Introduction

Pancreatic neuroendocrine tumors (pancreatic NET) are increasing in incidence [1, 2] and, with improved diagnostics and better recognition within the medical community, their prevalence is higher than previously thought [3]. The natural course of the disease varies according to the type of primary tumor, tumor size, and histological grade. Symptoms arising from hypersecretion of hormones or amines occur in less than half of all cases. The lack of specific symptoms in nonfunctional tumors leads to an often higher tumor mass at first presentation compared with their functional counterparts. Approximately two thirds of patients with pancreatic NET have distant metastases at diagnosis [1].

Recent years have seen a dramatic increase in the number of molecularly targeted agents to treat cancer as a result of our increased understanding of the processes and pathways involved in tumorigenesis. This paper fo-

**Fig. 1.** Schematic diagram of a theoretical pancreatic endocrine tumor cell, smooth muscle cell (pericyte) or endothelial cell demonstrating the sites and mechanism of action of novel agents for the management of metastatic pancreatic neuroendocrine tumors. Adapted from Metz and Jensen [4], copyright permission obtained.



cuses on molecules identified as potential treatment targets in pancreatic NET and reviews the latest data on experimental antitumor agents acting at these targets.

### Molecular Targets in Pancreatic NET

A number of potential therapeutic targets have been identified and are currently under investigation for treating pancreatic NET (fig. 1) [4].

#### Peptide Receptors

The first 'targeted' treatments for pancreatic and other types of NET were somatostatin analogs, used since the 1980s to alleviate symptoms of hormonal hypersecretion. Somatostatin analogs are still a key component of treatment today, and further exploration of the somatostatin receptors (SSTRs) as relevant targets has yielded new approaches to diagnosis and treatment.

#### Somatostatin and Its Receptors

Somatostatin, an endogenous cyclic peptide, regulates the secretion of growth hormone, insulin, glucagon and gastrin [5]. It acts through a family of seven G protein-coupled transmembrane receptors with five distinct subtypes (SSTR1–5) [6, 7]. Activation of SSTRs has a variety of direct and indirect effects [8]. Direct antiproliferative effects include inhibition of the cell cycle, inhibition of growth factor effects, and induction of apoptosis. The literature suggests that these effects may be mediated by the PI3K/mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK), and Ras/extracellular signal-regulated kinase (ERK) signaling pathways [9, 10]. Indirect effects include inhibition of the release of growth factors and trophic hormones, inhibition of angiogenesis, and modulation of the immune system [8]. Studies of receptor distribution in different types of pancreatic NET show that receptor expression is widespread (50–100% of tumor samples across different stud-

ies), with more than one subtype commonly expressed in a given tumor [11–14]. However, expression appears to vary with histological type, as well as between patients with the same tumor type. Nevertheless, expression of SSTR2 and SSTR5 is found in approximately 90 and 80% of pancreatic NET tumor cells, respectively, and pancreatic NET is potentially sensitive to hormone treatment [15]. Somatostatin itself has a very short half-life (~3–4 min) [7]. Attempts have therefore been made to synthesize somatostatin analogues such as octreotide and lanreotide that have high affinity to SSTR2 and SSTR5 but lack the disadvantages associated with somatostatin [7, 16].

#### Other Peptide Receptors

Successful exploitation of the SSTR for both diagnostic and therapeutic purposes in managing pancreatic NET has led to increased interest in the distribution of other peptide receptors. The limited evidence available suggests that receptors for bombesin, cholecystokinin, and vasoactive intestinal peptide are all expressed to some degree on at least some types of pancreatic NET [17–21].

#### Receptor Tyrosine Kinases and Angiogenic Mediators

Angiogenesis is a central and complex process in tumor growth and metastasis, and involves a number of receptor tyrosine kinases (RTKs) and their ligands. The importance of angiogenesis in the development of pancreatic NET is supported by studies in the RIP1-Tag2 mouse model [22, 23], discussed in more depth below.

#### Vascular Endothelial Growth Factor Receptor

Both vascular endothelial growth factor (VEGF)-A and its receptors, VEGFR-1 and VEGFR-2, are constitutively expressed in normal islets as well as in pancreatic RIP1-Tag2 tumors [24]. Using the same model, VEGF-A was shown to be critical for the angiogenic switch, as well as for tumor growth [25]. Inhibitors of VEGFR significantly reduce pancreatic tumor growth or cause regression of established tumors in treated mice, compared with controls, and also disrupt tumor vasculature [26–30].

Several studies have investigated the expression of VEGF, VEGFR, and other markers of angiogenesis in tumor biopsy samples from patients with pancreatic NET (table 1). Taken together, the studies suggest that VEGF may be only weakly expressed in many pancreatic NET, but is strongly expressed in at least some cases. There are conflicting data on whether VEGF expression

is highest in benign tumors [31, 32] or (specifically VEGF-C expression) in well differentiated neuroendocrine carcinomas [33, 34], and on whether or not microvascular density (MVD) is correlated with VEGF expression [31–34].

In a single study, expression of VEGFR-2 and VEGFR-3 in neoplastic cells was variable, but (in a preliminary comparison) was possibly correlated with ligand expression in both primary and metastatic tissue, suggesting that VEGFR may be involved in regulating growth or survival of some pancreatic NET [33].

#### Angiopoietin-2

A recent study reported high uniform expression of Angiopoietin-2 (Ang-2) messenger RNA (mRNA) in endothelial cells of both nontransformed pancreatic tissue and pancreatic NET tissue [35]. Interestingly, epithelial mRNA Ang-2 expression occurred exclusively in pancreatic NET cells. Increased microvessel density and enhanced lymphatic metastasis were evident in Ang-2-expressing tumors, indicating a functional role of Ang-2 in experimental NET. Consistent with this notion, circulating Ang-2 was significantly elevated in patients with NET, compared with healthy controls. Furthermore, elevated Ang-2 levels were correlated with presence of metastatic disease, with the highest concentrations found in patients with liver metastasis. Ang-2 concentrations above the 75th percentile predicted shorter survival ( $p = 0.0003$ ). These findings are supported by other studies of patients with NET [36, 37]. Significantly elevated levels of serum Ang-2 levels ( $p = 0.01$ ) were found in patients with NET ( $n = 47$ ; 17 with pancreatic NET), compared with healthy controls ( $n = 44$ ), and the time to disease progression was shorter in those patients with serum Ang-2 levels above 4,756 pg/ml ( $p = 0.04$ ) [37]. The induction of Ang-2 in pancreatic NET may, therefore, represent a clinically relevant mechanism of disease progression and could constitute an adverse prognostic marker [35].

#### Platelet-Derived Growth Factor Receptor

In a single series of human pancreatic NET samples, both platelet-derived growth factor receptor (PDGFR)- $\alpha$  and PDGFR- $\beta$  were commonly expressed in primary and metastatic tumor cells, as well as tumor stroma [38]. PDGFR- $\beta$  expression was upregulated in primary pancreatic NET and metastases compared with normal endocrine pancreatic tissue, and in tumor stroma compared with normal pancreatic stroma [39]. In a gene profiling study, PDGFR- $\beta$  was upregulated in pancreatic neuroen-

**Table 1.** Summary of published studies investigating the expression of VEGF, VEGFR, and microvascular density in tumor biopsy samples from patients with pancreatic NET

Reference	Number*	Type(s) of pancreatic NET <sup>a</sup>	Parameters investigated <sup>b</sup>	Key findings
Terris et al. [107], 1998	20	<ul style="list-style-type: none"> <li>- Gastrinoma (n = 2)</li> <li>- Insulinoma (n = 3)</li> <li>- Thyrocalcitoninoma (n = 2)</li> <li>- Glucagonoma (n = 2)</li> <li>- Non-functioning (n = 11)</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF expression</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF staining detected in 16 of 20 samples, but most commonly weak (&lt;25% positive cells)</li> <li>- VEGF reactivity detected by Western blot in all cases positive by immunohistochemistry</li> </ul>
Zhang et al. [94], 2007	15	Not specified	<ul style="list-style-type: none"> <li>- VEGF expression</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF staining detected in 12 of 15 samples (weak in 7, strong in 5)</li> </ul>
Couvelard et al. [32], 2005	45	<ul style="list-style-type: none"> <li>- Benign tumor (WHO stage 1; n = 8)</li> <li>- Tumor of uncertain behavior (WHO stage 2; n = 11)</li> <li>- Well-differentiated endocrine carcinoma (WHO stage 3; n = 18)</li> <li>- Poorly differentiated endocrine carcinoma (WHO stage 4; n = 8)</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF expression</li> <li>- MVD</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF expression in 73% of patients (weak, n = 18; moderate, n = 10; strong, n = 4)</li> <li>- VEGF expression negatively correlated with WHO stage (i.e. highest in benign tumors)</li> <li>- VEGF score significantly higher in tumors with low proliferation index, no necrosis, and no fibrotic focus, but was not related to survival</li> <li>- MVD decreased significantly with disease progression according to WHO classification</li> <li>- Low MVD significantly associated with poorer survival</li> </ul>
Marion-Audibert et al. [31], 2003	77 (82 tumors total, as 3 patients had 2 tumors and 1 patients had 3 tumors)	<ul style="list-style-type: none"> <li>- Benign tumor (n = 23)</li> <li>- Tumor of uncertain behavior (n = 23)</li> <li>- Well-differentiated endocrine carcinoma (n = 35)</li> <li>- Poorly differentiated endocrine carcinoma (n = 1)</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF expression</li> <li>- MVD</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF expression barely detectable or only weak in 66% of cases overall</li> <li>- Strong VEGF expression (overall 34% of cases) more common in benign tumors (61%) than in tumors of uncertain behavior (48%) or well-differentiated carcinomas (8%)</li> <li>- MVD and VEGF expression not closely correlated</li> <li>- MVD significantly higher in benign tumors than in tumors of uncertain behavior and in carcinomas</li> <li>- MVD significantly higher in tumors &lt;2 cm diameter, proliferation index &lt;2%, and no metastasis</li> </ul>
Hansel et al. [33], 2003	19 (19 primary tumors, plus 7 liver metastases from these patients)	<ul style="list-style-type: none"> <li>- Benign tumor (n = 3)</li> <li>- Tumor of uncertain behavior (n = 6)</li> <li>- Low-grade malignant (n = 10)</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF-A expression</li> <li>- VEGF-C expression</li> <li>- VEGFR-2 expression</li> <li>- VEGFR-3 expression</li> <li>- MVD</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF-A expression limited and not correlated with MVD</li> <li>- VEGF-C expression low to moderate in primary tumors; higher in tumors of uncertain behavior/low-grade malignant tumors than in benign tumors</li> <li>- VEGF-C expression significantly higher in liver metastases than in primary tumors</li> <li>- VEGF-C expression not correlated with MVD in either primary tumor or metastatic tissue</li> <li>- High levels of VEGFR-2 expression in endothelial cells of all primary and metastatic tissue examined</li> <li>- VEGFR-3 expression limited on endothelial cells</li> <li>- Variable VEGFR-2 and -3 expression on neoplastic cells</li> </ul>
Rubbia-Brandt et al. [34], 2004	39	<ul style="list-style-type: none"> <li>- Benign tumor (n = 19)</li> <li>- Tumor of uncertain behavior (n = 9)</li> <li>- Well-differentiated, low-grade malignant (n = 11)</li> </ul>	<ul style="list-style-type: none"> <li>- VEGF-C</li> <li>- MVD<sup>c</sup></li> </ul>	<ul style="list-style-type: none"> <li>- VEGF-C expression in 19 of 39 tumors</li> <li>- VEGF-C expression significantly higher in well-differentiated carcinomas than in benign or borderline tumors</li> <li>- MVD significantly higher in well-differentiated carcinomas than in benign or borderline tumors by one immunostaining method but not another</li> <li>- VEGF-C expression and MVD not correlated</li> </ul>

\* Number of patients with pancreatic NET in series. <sup>a</sup> Classification cited as in publication. <sup>b</sup> Not necessarily a comprehensive list for each reference: relevant parameters only (i.e. VEGF, VEGFR or MVD) included in this table. <sup>c</sup> Lymphatic vessel density.



doocrine carcinomas with metastases compared with benign pancreatic NET [40].

Inhibitors of PDGFR significantly reduce tumor growth or cause regression of established pancreatic tumors in treated mice compared with controls, and also disrupt tumor vasculature [26, 27, 29, 30].

#### Stem Cell Factor Receptor (c-Kit)

Several studies have demonstrated expression of c-kit in human pancreatic NET tissue samples [38, 41–43]. The proportion of positive samples ranged widely from approximately 14–90% between reports, and within one study varied substantially with the type of antibody used to detect c-kit [41]. Inconsistencies between studies may therefore be related to technique rather than necessarily reflecting a real variation in c-kit levels. Recently, c-kit expression has been identified as an independent prognostic marker for pancreatic NET [44].

#### Epidermal Growth Factor Receptor

Several immunohistochemical studies have reported epidermal growth factor receptor (EGFR) expression in human pancreatic NET samples [38, 45–49]. Estimates of the proportion of samples expressing EGFR range from 18 to 65%, with variation in the intensity of staining also reported. The variation between studies may reflect differences in the patient populations or antibodies used [48]. Although there is some debate as to whether EGFR expression can be used as a marker of malignancy in NET [46, 50], a recent study found that the expression of EGFR correlated significantly with the grade of malignancy in pancreatic NET, increasing from low levels of expression in benign tumors and those of uncertain behavior to high levels of expression in well- and poorly differentiated tumors [49]. Furthermore, patients with pancreatic NET expressing activated EGFR have been found to have significantly worse prognosis than those with tumors not expressing activated EGFR [48]. Targeted therapy against the EGFR tyrosine kinase domain may therefore prove to be a useful therapeutic approach for pancreatic NET.

#### Sarcoma Kinase

Sarcoma (Src) family kinase activity has been shown to regulate adhesion, spreading, and migration of pancreatic NET cells in vitro [51]. The Src-like kinase, LCK (lymphocyte-specific protein tyrosine kinase), was found to be significantly upregulated at the RNA level in both primary and metastatic pancreatic NET compared with normal islets, with a 4.35-fold difference ( $p = 0.02$ ) [52].

Immunohistochemical findings also supported the up-regulation of LCK. However, there was no significant correlation between LCK staining and stage, differentiation, proliferation, functional status, or clinical outcome.

#### Combining Angiogenic Targets

The pathways involved in angiogenesis are heavily interlinked. For example, dual inhibition of VEGFR and PDGFR results in a greater antiangiogenic effect in the RIP1-Tag2 model than selective inhibition of VEGFR or PDGFR alone, with substantial decreases in both endothelial cells and pericytes [53]. A therapeutic approach combining several targeted agents or using a single, multitargeted agent, may therefore be of greater value than using an agent directed at a single target alone.

#### Intracellular and Downstream Targets

Targeting intracellular molecules, such as those mediating signal transduction downstream of RTKs, offers yet another potential therapeutic approach. One established target is mTOR, a serine/threonine kinase with a central role in regulating cell growth, metabolism, and apoptosis.

#### mTOR

Mutations in specific tumor suppressor genes known to regulate mTOR appear to be associated with an increased risk of developing pancreatic NET. These regulatory genes include, most notably, phosphatase and tensin homolog (PTEN) [54, 55], the tuberous sclerosis complex 2 gene (TSC2) [56–58] and, possibly, neurofibromatosis type 1 [59, 60]. In a recent gene expression profiling study using a large panel of pancreatic NET, PTEN and TSC2 were downregulated in most primary tumors examined [61]. Downregulation was significantly associated with shorter disease-free and overall survival (OS), supporting a role for the PI3K/Akt/mTOR pathway in the development of pancreatic NET.

#### Other Potential Targets

Several additional potential therapeutic targets have been identified by research into pancreatic NET. Expression of these targets is summarized and referenced in table 2. One target worth more detailed discussion is the dopamine receptor; targeting this receptor is showing potential in treating other tumors and may be of benefit in NET.

#### Dopamine Receptors

Dopaminergic drugs have been shown to inhibit human small-cell lung cancer growth both in vivo and in vi-

**Table 2.** Additional potential therapeutic targets in pancreatic neuroendocrine tumors

Target	Target expression and/or antitumor efficacy of drug directed at target shown in:		
	cell lines in vitro	animal model	human tumor samples
IGF system (including IGF-1; IGF-2; IGF-1R; IGF-2R; and IGF-binding proteins)	Antiproliferative effects of IGF-1R inhibitor [108]	IGF-1R upregulated [109]	mRNA expression upregulated [110, 111]; increased IGF-1 mRNA correlated with tumor growth/aggressiveness [111]
B-Raf	Inhibition of downstream signaling and anti-proliferative effects by B-raf inhibitor (sorafenib) [112]	Not evaluated	Protein expression upregulated [112]
Cyclooxygenase 2 (COX-2)	Significant, dose-dependent reduction of cell viability associated with increased apoptosis in BON cells and mouse insulinoma $\beta$ TC-3 [49]	Not evaluated	Protein expression upregulated [113, 114]; may be linked to tumor progression [113]
Metastasis-associated gene 1 (MTA-1)	Not evaluated	Not evaluated	Expression associated with malignant behavior [115]
Histone deacetylase (HDAC)	Antiproliferative effects of HDAC inhibitor [116, 117]	Not evaluated	Not evaluated
Cyclin-dependent kinase 4 (CDK4)	Not evaluated	Not evaluated	Protein and mRNA expression upregulated [118]
Claudin 3 and 7	Not evaluated	Not evaluated	Protein and mRNA expression upregulated [119]
MAGE1 (target antigen for autologous cytotoxic T lymphocytes)	Not evaluated	Not evaluated	Protein expression upregulated [120]
p53 pathway and its negative regulators MDM2, MDM4, and WIP1	MDM2 gene amplification, increased mRNA and protein levels [121]	Not evaluated	MDM2, MDM4 and/or WIP1 gene amplification [121]
PI3K/Akt	Antiproliferative effects of PI3K and Akt inhibitors in BON cells, with decreased levels of phosphorylated Akt [122]	Not evaluated	Activated Akt detected [123]
D114/Notch and Ephrin-B2/EphB4 pathways	Not evaluated	In the RIP1-Tag2 model, inhibition of D114/EphB4 disrupted tumor angiogenesis and reduced tumor size [124]	Not evaluated

tro [62], and it has been proposed that they may also have an antiproliferative effect in secreting pancreatic NET [63]. Several studies have investigated dopamine 2 expression in NETs [64–67]. Grossrubatscher et al. [65] showed high expression of these receptors in 85% of NET tumors studied; most tumors were located in the pancreas (n = 15) and the lung (n = 14). Dopamine 2 receptor immunoreactivity was present in 93% of the islet cell tumors studied. Generally, high positivity was reported in more than 70% of tumor cells, particularly in bronchial and pancreatic tumors. The authors conclude that there may be a role for dopaminergic drugs in inhibiting secretion and/or cell proliferation in NETs. Interestingly, co-expression of the dopamine 2 receptors with SSTR2 and SSTR5 has also been found, with higher expression of the dopamine receptors in low-grade

rather than high-grade NET [67]. Kidd et al. [68] report variable expression of dopamine 2 and somatostatin receptors depending on cell type and tissue of origin, with differential cytotoxicity induced by chimeric compounds. A somatostatin/dopamine chimeric compound may therefore be a viable therapeutic option.

#### *Progress in Pancreatic NET Using Molecularly Targeted Therapies*

This section focuses on agents in phase II or later development (table 3). The clinical data are notable for their heterogeneity with respect to the enrolled patient population and for the current lack of randomized trials, stemming in part from the rarity of the tumors.

**Table 3.** Summary of targeted agents with phase II or III clinical data that include patients with pancreatic NET

Agent <sup>a</sup>	Target(s)	Clinical development phase <sup>b</sup>	Population <sup>b</sup> (n patients total)	Outcomes	References
<sup>177</sup> Lu-DOTATATE	Somatostatin receptors	II	Mixed gastroenteropancreatic NET, retrospective analysis (n = 310; 72 with nonfunctioning pancreatic NET; 12 with gastrinoma; 5 with insulinoma; 2 with VIPoma)	Study population 21% CR; 28% PR; median TTP = 40 months in a mixed patient population, prior tumor progression not required; median OS from start of treatment = 46 months Nonfunctioning pancreatic NET (n = 72): 6% CR; 36% PR; 18% MR; 26% SD; 14% PD Gastrinoma (n = 12): 5 PR; 4 MR; 2 SD; 1 PD Insulinoma (n = 5): 3 PR; 1 SD; 1 PD VIPoma (n = 2): 1 PR; 1 PD	Kwekkeboom et al. [84], 2008
Sunitinib	VEGFR-1-3, PDGFR- $\alpha$ and - $\beta$ , c-kit, RET, CSF-1R, FLT3	III (vs. placebo)	Well-differentiated pancreatic NET (n = 171; 86 received sunitinib)	Pancreatic NET: median PFS = 11.4 vs. 5.5 months with placebo; ORR = 9.3 vs. 0% with placebo	Raymond et al. [86], 2011
		II	Advanced carcinoid (n = 41) and pancreatic NET (n = 66)	Pancreatic NET: median TTP = 7.7 months; 1-year survival = 81.1%; ORR = 16.7%; SD = 68%	Kulke et al. [85], 2008
Sorafenib	VEGFR-2-3, PDGFR- $\beta$ , FLT3, c-kit, RET	II	Two cohorts: pancreatic NET and gastrointestinal NET (n = 93; 43 with pancreatic NET)	Pancreatic NET: ORR = 10%; 61% PF at 6 months	Hobday et al. [87], 2007
Vatalanib	VEGFR-1-3, PDGFR- $\beta$ , c-kit	II	Mixed NET (n = 20; 4 with pancreatic NET)	Study population: 50% SD at 6 months; median TTP = 7 months	Pavel et al. [89], 2008
Imatinib	PDGFR- $\alpha$ and - $\beta$ , BCR-ABL, c-kit	II	Mixed NET (n = 15; 1 with pancreatic NET)	Pancreatic NET: PD in 1 patient	Gross et al. [90], 2006
Gefitinib	EGFR	II	Two cohorts: pancreatic and gastrointestinal NET (n = 96; 39 with pancreatic NET)	Pancreatic NET: 31% PF at 6 months; 7% PR; 14% SD	Hobday et al. [93], 2006
Bevacizumab	VEGF (all biologically active forms)	II (+ temozolomide)	Pancreatic or gastrointestinal NET (n = 34; 18 with pancreatic NET)	Pancreatic NET: 24% PR; 70% SD	Kulke et al. [95], 2006
		II (+ FOLFOX)	Mixed NET (n = 13; 6 with pancreatic NET)	Pancreatic NET: 33% PR; 67% SD	Venook et al. [97], 2008
		II (+ capecitabine + oxaliplatin)	Mixed NET (n = 13; number of patients with pancreatic NET not reported)	Study population: 31% PR; 46% SD No specific pancreatic data	Kunz et al. [98], 2010
Everolimus	mTOR	II (+ octreotide)	Mixed NET (n = 60; 30 with pancreatic NET)	Pancreatic NET: 27% PR; 60% SD; 13% PD; median PFS = 50 weeks; OS NR	Yao et al. [102], 2008b
		II (Stratum 1: monotherapy Stratum 2: + LAR octreotide (in those who had received octreotide previously))	Pancreatic NET (n = 160; stratum 1 = 115; stratum 2 = 45)	Pancreatic NET: Stratum 1: 9.6% PR; 67.8% SD; 13.9% = PD; median PFS = 9.7 months Stratum 2: 4.4% PR; 80% SD; 0% = PD; median PFS = 16.7 months	Yao et al. [103], 2010
		III	Pancreatic NET (410; 207 received everolimus)	Pancreatic NET: median PFS 11.0 vs. 4.6 months with placebo Estimate for proportion PF at 18 months = 34%	Yao et al. [104], 2011
Temsirolimus	mTOR	II	Pancreatic or gastrointestinal NET (37; one did not receive treatment; 13 with pancreatic NET)	Study population: 5.6% PR; median TTP = 6 months; 1-year OS rate = 71.5% Pancreatic NET: 6.7% PR; 60% SD; 26.7% PD; median TTP 6 months = 51.6%; 1-year OS rate 71.5%	Duran et al. [105], 2006

<sup>a</sup> As specified in each publication. <sup>b</sup> Most advanced phase (all prospective studies). CSF-1R = Colony-stimulating factor 1 receptor; RET = glial cell line-derived neurotrophic factor receptor (rearranged during transfection); FLT3 = FMS-like tyrosine kinase-3; PD = progressive disease; PF = progression-free; NR = not reached; CR = complete response; MR = minimal response.

## Somatostatin Analogs and Peptide Receptor Radiotherapy

### Somatostatin Analogs

Currently available somatostatin analogs comprise octreotide and lanreotide, available as depot formulations; octreotide is also available as an immediate-release formulation. The question of whether somatostatin analogs – predominantly used for symptom relief – have any antitumor activity has been hotly debated. Prospective and retrospective clinical studies have revealed that the use of somatostatin analogs is associated with tumor growth stabilization in 50–60% of gastroenteropancreatic NET patients, whereas partial tumor remissions rarely occur [69, 70]. However, there are limited data in pancreatic NET exclusively. In a prospective study of 21 patients with pancreatic NET treated with octreotide long-acting release (LAR), 38% had stable disease (SD) after a median follow-up of 49.5 months. While one prospective placebo-controlled study confirmed an antitumor effect in midgut NET, the applicability of the data to pancreatic NET remains unclear [71–73]. Recent data have shed light on the biological effect of octreotide, with the demonstration that SSTR2 (located in the cell membrane in untreated patients) becomes internalized following high-dose octreotide therapy in patients with NET [74].

Potential advances in this area include the development of somatostatin/dopamine chimeric compounds [68]; the pan-somatostatin analog pasireotide, which has affinity for SSTR1, SSTR2, SSTR3, and SSTR5 [75, 76]; and also non-peptidic somatostatin analogs with high affinity and selectivity for SSTR1 [77–79] and SSTR3 [80]. These compounds have yet to be tested for efficacy.

### Peptide Receptor Radiotherapy

The presence of SSTRs on gastrointestinal and pancreatic NETs has been exploited to achieve targeted delivery of radiotherapy, using radiolabeled somatostatin analogs (indium-111, yttrium-90, or lutetium-177). Results from phase II trials of patients with various types of NET have shown that radionuclide treatment using  $^{90}\text{Y}$ -DOTATOC ( $^{90}\text{Y}$ -DOTA<sup>0</sup>-D-Phe<sup>1</sup>-Tyr<sup>3</sup>-octreotide) produces partial responses (PR) in 4–35% of patients [81, 82]. Recently, Cwikla et al. [83] found  $^{90}\text{Y}$ -DOTATATE ( $^{90}\text{Y}$ -DOTA<sup>0</sup>, D-Phe<sup>1</sup>-Tyr<sup>3</sup>-octreotate) to be effective in patients (n = 60) with gastroenteropancreatic NET; 23% had PR and the remaining 77% of patients had SD. Response rates with  $^{177}\text{Lu}$ -DOTATATE ( $^{177}\text{Lu}$ -DOTA<sup>0</sup>, Tyr<sup>3</sup>-octreotate) are also encouraging in patients with several types of pancreatic and other gastrointestinal NET, with an objective response rate (ORR) of approximately 43% (com-

plete response 4.4% and PR 38.5%) in the pancreatic NET group [84]. To date, prospective studies comparing ‘hot’ with ‘cold’ somatostatin analogs with respect to their symptom-controlling as well as their antiproliferative efficacy are lacking.

### RTK Inhibitors and Antiangiogenic Agents

#### Sunitinib

Sunitinib is an oral, multitargeted RTK inhibitor of VEGFR-1–3, PDGFR, c-kit, RET, CSF-1R, and FLT3, with direct antitumor and antiangiogenic effects. In the RIP1-Tag2 transgenic mouse model of pancreatic NET, sunitinib reduced tumor burden and increased survival, reduced the endothelial cell population, and reduced pericyte coverage [29, 53]. In a phase II clinical trial in patients with advanced carcinoid (n = 41) or pancreatic NET (n = 66), the overall ORR was 16.7% in patients with pancreatic NET, and a further 68% had SD [85]. Median time to tumor progression (TTP) was 7.7 months and 1-year survival was 81.1% in patients with pancreatic NET. A phase III, randomized, double-blind trial of sunitinib versus placebo in patients with progressive, well-differentiated, malignant pancreatic NET was closed early because of the greater risk of progression and death in patients assigned to placebo [86]. Median progression-free survival (PFS) with sunitinib was 11.4 months and with placebo was 5.5 months (hazard ratio 0.42; 95% CI 0.26–0.66; p < 0.001). Based on these positive results, the European Commission approved sunitinib (SUTENT<sup>®</sup>, Pfizer Inc.) for the treatment of unresectable or metastatic, well-differentiated pancreatic NET with disease progression in December 2010.

#### Sorafenib

Sorafenib is an orally active, multikinase inhibitor with selectivity for the RTKs VEGFR-2, VEGFR-3, PDGFR- $\beta$ , FLT3, c-kit, and RET, as well as for the serine/threonine RAF kinases, B-Raf and Raf-1/C-Raf, which are associated with activation of these RTKs. To date, results have been reported from a single phase II study of sorafenib, which included two cohorts of patients with, respectively, gastrointestinal tumors (n = 50) and pancreatic NET (n = 43) [87]. In patients with pancreatic NET, the ORR was 10%, and 14 of 23 evaluable patients (61%) were progression-free at 6 months.

#### Vatalanib

Vatalanib inhibits all known VEGFRs, with particular selectivity for VEGFR-2; at higher concentrations vatalanib also inhibits PDGFR- $\beta$  and c-kit. One phase II trial is ongoing in patients with progressive, advanced



NET (NCT00590343); however, no specific data on pancreatic NET have been published [88]. In another study, 4 of 20 patients enrolled had pancreatic NET; overall, the best response was SD (8/16 patients at 6 months) in heavily pretreated patients, with a median TTP of 7 months [89].

#### Imatinib

Imatinib is an orally available phenylaminopyrimidine analog which specifically inhibits tyrosine kinase activity associated with *c-kit*, PDGFR- $\alpha$ , PDGFR- $\beta$ , and BCR-ABL. Imatinib inhibited cell proliferation and induced apoptosis in both *c-kit*-positive and *c-kit*-negative neuroendocrine cells in vitro [42]. Although imatinib has been investigated in some types of NET [90, 91], few patients with pancreatic NET have been enrolled and there is currently no evidence of clinical activity in this tumor type.

#### Gefitinib

Gefitinib is an EGFR-specific tyrosine kinase inhibitor. Blockade of EGFR using gefitinib inhibited growth of pancreatic NET cell lines in vitro, inducing apoptosis and cell cycle arrest [92].

The activity of gefitinib has been assessed in a phase II study of patients with advanced NET [93]. PFS at 6 months was 31% for 29 evaluable patients with pancreatic NET; 2 of these patients achieved a PR and 4 patients had SD exceeding 4 months.

#### Bevacizumab

Bevacizumab is a monoclonal antibody that binds to, and neutralizes, all biologically active forms of VEGF. In preclinical experiments, bevacizumab failed to inhibit growth of BON pancreatic NET cells in vitro, but reduced their angiogenic potential by blocking the cells' ability to stimulate endothelial cell tube formation and proliferation [94]. Treatment with bevacizumab impaired tumor growth in a xenograft model using BON cells.

In a phase II study of bevacizumab plus temozolomide in patients with advanced NET [95], 18 of 34 patients enrolled had pancreatic NET, of whom four (24%) achieved a PR and a further 12 (70%) exhibited SD. Two ongoing phase II trials are open to patients with advanced NET, including those of pancreatic origin [96, 97]. In a study of bevacizumab combined with the oxaliplatin-based chemotherapy regimen, FOLFOX (oxaliplatin, leucovorin, and 5-fluorouracil), 2 of 6 patients with pancreatic NET enrolled achieved a PR and 4 had SD

[98]. Since comparative trials of chemotherapy versus chemotherapy plus bevacizumab are lacking, and chemotherapeutic drugs alone may induce high remission rates in pancreatic NET (as has recently been shown by the use of capecitabine and temozolomide [99]), it remains an open question whether the addition of bevacizumab to systemic chemotherapy increases response rate or PFS.

#### *Inhibitors of Intracellular and Downstream Targets* Everolimus

Everolimus is an orally available derivative of rapamycin, which inhibits the activity of mTOR. Preclinical studies have shown the antiproliferative effects of everolimus in human pancreatic endocrine cells in vitro and in vivo [100, 101]. Application of everolimus was also associated with attenuated phosphorylation of all downstream targets of Akt, including TSC2, mTOR, and p70S6K [100], and with G0-/G1-phase arrest, as well as induction of apoptosis [101].

The combination of everolimus plus octreotide was investigated in a phase II study in advanced, low-to-intermediate grade NET [102]. Among 30 enrolled patients with pancreatic NET, 8 patients obtained a PR (27%) and 18 had SD (60%). Median PFS in this group was 50 weeks; median OS had not been reached. Another phase II study of everolimus in metastatic pancreatic NET after failure of chemotherapy has recently been published [103]. Patients with progressive disease who were not being treated with octreotide at study entry received single-agent everolimus (stratum 1;  $n = 115$ ), while patients who had been on octreotide LAR for at least 3 months, but also had evidence of progression, received everolimus plus octreotide (stratum 2;  $n = 45$ ). In stratum 1, 9.6% of patients achieved a PR and 67.8% had SD; in stratum 2, 4.4 and 80% of patients had a PR or SD, respectively, as their best response. Median PFS was 9.7 months and 16.7 months in strata 1 and 2, respectively. A phase III, randomized, double-blind trial of everolimus plus best supportive care versus placebo plus best supportive care in patients with progressive, well-differentiated, malignant pancreatic NET confirmed the activity of everolimus. Median PFS with everolimus was 11 months and with placebo was 4.6 months (hazard ratio = 0.35 [95% CI 0.27–0.45];  $p < 0.0001$ ) [104]. Estimates of the proportion of patients alive and progression-free at 18 months were 34% (95% CI 26–43) with everolimus, compared with 9% (95% CI 4–16) with placebo. Based on the results of this phase III study, regulatory submission for everolimus to treat patients with NET is underway.

**Table 4.** Summary of targeted agents in phase I or early phase II clinical development with potential for use in pancreatic neuroendocrine tumors

Agent	Target	Phase	Population	Reference/NCT identifier
Pasireotide	SSTR1, SSTR2, SSTR3 and SSTR5	Phase I, monotherapy Phase II, monotherapy Phase I, in combination with everolimus	Mixed NET, including pancreatic Metastatic NET Gastrointestinal or pancreatic NET	NCT00958841 NCT01253161 Chan [134], 2010 (NCT00804336)
<sup>90</sup> Y-DOTATOC	SSTR	Phase I Phase II	Malignancies expressing somatostatin receptors Mixed NET	NCT00006368 Waldherr et al. [81], 2001 NCT00978211
<sup>177</sup> Lu-DOTATOC	SSTR	Phase II	Mixed NET	NCT00978211
AMG 479	IGF-1R	Phase I Phase II	Solid malignancies/NHL Gastrointestinal or pancreatic NET	Tolcher [126], 2009 NCT01024387
MK-0646 (dalotuzumab)	IGF-1R	Phase I Phase II	Solid malignancies Gastrointestinal or pancreatic NET	Atzori [127], 2008; Hidalgo [128], 2008; Reidy et al. [129], 2010 (NCT00610129)
R1507	IGF-1R	Phase I Phase I/II	Solid malignancies Phase I: advanced solid tumors; Phase II: RCC or pancreatic NET	Rodon [130], 2007 NCT00985374
MK-2206	Allosteric Akt inhibitor	Phase I Phase II	Solid tumors Gastrointestinal or pancreatic NET	Tolcher et al. [131], 2009 NCT01169649
Romidepsin (FR901288/ depsipeptide)	Histone deacetylase	Phase I Phase I/II Phase II	Thyroid and other advanced cancers Solid malignancies Gastrointestinal or pancreatic NET	Piekarz [132], 2008 NCT00379639 NCT00084461
AZD0530	scr/abl	Phase I, monotherapy Phase I, in combination with cediranib (AZD2171)	Solid malignancies Solid malignancies	NCT00704366 Trarbach [133], 2008

RCC = Renal cell carcinoma.

### Temsirolimus

Like everolimus, temsirolimus is a rapamycin derivative with specific activity against mTOR. To date, a single phase II trial of temsirolimus in advanced NET has been published [105]. Among 15 patients with advanced pancreatic NET and 21 patients with gastrointestinal tumors enrolled, 1 patient with each tumor type achieved a PR (ORR, 6.7 and 4.8%, respectively). The overall median TTP was 6.0 months, and one-year PFS was 40.1%. Median OS had not been reached.

### Dual Therapeutic Targeting

Combined targeting of mTOR and EGFR signaling pathways has shown potential clinical benefit in the RIP1-Tag2 model, compared with inhibition of either pathway in monotherapy [106]. A phase II trial is ongoing to evaluate the efficacy of this treatment regimen in human NET (NCT00843531).

### Agents in Early Clinical Development

A number of agents directed at molecular targets that appear relevant in the pathology of pancreatic NET (as discussed above) are currently in phase I or early phase II clinical development (table 4); these include, in particular, agents directed at SSTRs and insulin-like growth factor type 1 receptor (IGF-1R), as well as an inhibitor of histone deacetylase.

In summary, pancreatic NET are a diverse group of tumors which vary in their degree of malignancy and functionality. Before the development of targeted agents, several systemic options were available to control tumor growth and improve patients' quality of life, including interferon therapy, somatostatin analogs and cytotoxic chemotherapy. While chemotherapy may induce significant tumor shrinkage, these effects are transient. Researchers have therefore investigated molecular targets that offer scope for new therapeutic approaches that could significantly improve management of patients with pancreatic NET. We have undertaken an extensive review

of published literature on targets specific to pancreatic NET, and have summarized the available clinical data for agents directed at some of these targets.

Preclinical experiments have demonstrated the relevance of angiogenesis in the development of pancreatic NET. Several RTKs with both angiogenic and direct roles in supporting tumor growth are expressed to varying degrees in human pancreatic NET biopsies, although interpretation of the published literature is complicated by differences in technique. In the clinic, preliminary activity in pancreatic NET has been reported with several RTK inhibitors. Most notably, the multitargeted RTK inhibitor, sunitinib, has recently demonstrated superiority over placebo in a randomized phase III study and has received approval by the European Commission for treating patients with pancreatic NET.

The PI3K/Akt/mTOR pathway, with its critical role in regulating cell growth and apoptosis, is also implicated in the growth of pancreatic NET. The mTOR inhibitor, everolimus, has demonstrated activity after failure of chemotherapy in a large phase II trial and superiority over placebo in a placebo-controlled phase III trial. Everolimus and sunitinib are the two most advanced of all molecular

targeted drugs in the field of NET and can be considered similarly effective in pancreatic NET with respect to PFS.

In conclusion, several targeted agents have potentially useful activity in pancreatic NET. More randomized, carefully designed studies are urgently needed to assess the real benefits of these agents, either alone or in combination. The rarity of pancreatic NET means that enrollment is likely to be slow, and cooperative, multicenter studies will be needed to progress development in a timely fashion.

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