

Summary of emerging personalized medicine in neuroendocrine tumors: are we on track?

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Abstract: Neuroendocrine tumors (NETs) comprise a heterogeneous group of malignancies, with differences in prognosis and effective therapies. Traditionally, NETs have been characterized by tumor grade, site of primary tumor, functional status, and presence of underlying familial syndrome. However, increased feasibility and utilization of next-generation sequencing and other methodologies have revealed new genomic and epigenetic aberrations. In the last decade, treatment options available for metastatic well-differentiated gastroenteropancreatic (GEP) NETs have expanded, with approval of antiangiogenic and mTOR-directed targeted therapies, and our armamentarium of active therapies is likely to further increase. Cytotoxic therapies also are an important option for pancreatic NETs, and MGMT promoter methylation and protein expression may be an important biomarker for efficacy of alkylating agents. Peptide receptor radioligand therapy is an emerging treatment that uses functional imaging to personalize dosimetry to the tumor and avoid nephrotoxicity. Nevertheless, there is a critical need for further biomarkers, particularly multianalyte biomarkers, to aid in prognostication and predict efficacy of therapies.

Keywords: Carcinoid tumor; neuroendocrine tumors (NETs); islet cell carcinoma; precision medicine

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Introduction

The FDA approval of the targeted therapies sunitinib and everolimus for advanced pancreatic neuroendocrine tumors (NETs) in 2011 was a reflection of our growing understanding of the biology and heterogeneity of NETs and their responses to various therapies (*Table 1*). Improvements in technology have continued to increase the feasibility and utilization of next-generation sequencing and other methodologies in NETs, revealing new genetic and biologic aberrations. These findings lead to testable hypotheses to determine relevant prognostic and ideally predictive biomarkers that may guide personalization of existing therapy and shed light on novel potential targets.

NETs have increased in incidence from the mid-1970s through the 2000s, with an incidence of 5.25/100,000 in

2004 (1). However, given prolonged overall survival for the majority of patients with NETs, the estimated prevalence of disease as of 2004 was 35/100,000 in the United States, outstripping the prevalence of esophageal cancer, gastric cancer, and pancreatic cancer (1). Encouragingly, survival of patients with metastatic NETs has improved in more modern treatment eras, with significant improvement in survival after 1988, likely attributable to the development and use of octreotide (1). Though unresectable metastatic NETs are invariably incurable malignancies, there is marked heterogeneity in the speed of progression and the responses to therapy. Classification of gastroenteropancreatic (GEP) NETs by histologic grade, primary site, and functional hormone secretion status provides some insight into this heterogeneity and is important to consider in determining an individual's sequence of therapies. We expect that

Table 1 United States Food and Drug Administration approval of agents for NETs within the last 10 years

Date of FDA approval	Drug	Indication
05/06/2011	Everolimus	Unresectable, locally advanced or metastatic, progressive pancreatic NET
05/20/2011	Sunitinib	Unresectable, locally advanced or metastatic, progressive pancreatic NET
12/16/2014	Lanreotide	Unresectable, well or moderately differentiated, locally advanced or metastatic GEP-NETs to improve progression-free survival
02/26/2016	Everolimus	Unresectable, well differentiated, locally advanced or metastatic, non-functional, progressive gastrointestinal or lung NET

FDA, Food and Drug Administration; GEP, gastroenteropancreatic; NETs, neuroendocrine tumors.

Table 2 WHO grading system of GEP NETs (2)

Differentiation	Grade	Proliferative rate
Well differentiated	G1 (low)	<2 mitoses/10 hpf and Ki-67 index <3%
	G2 (intermediate)	2–20 mitoses/10 hpf or Ki-67 index 3–20%
Poorly differentiated	G3 (high)	>20 mitoses/10 hpf or Ki-67 index >20%

GEP, gastroenteropancreatic; NETs, neuroendocrine tumors; hpf, high-power field.

molecular classification may in the future provide a more refined treatment algorithm.

Clinical classification of NETs

WHO grade

Pathologic assessment of WHO grade is critical in determining prognosis and thus serving as the most basic requirement for individualizing therapy of GEP NETs (Table 2) (2). Prognosis is dismal for patients with poorly differentiated disease, with median survival of 14 months with regional disease and only 5 months with distant metastatic disease in SEER data (1). Conversely, prognosis is markedly better for patients with well or moderately differentiated NETs, with median survival of 111 months with regional disease and 33 months even with distant metastatic disease in SEER data prior to 2004 (1). These survival rates have only improved with more modern treatment paradigms, as median survival in the PROMID study of octreotide was 84.7 months (3). However, even within well-differentiated NETs, there is significant heterogeneity of disease aggressiveness and prognosis. Stage-for-stage, grade 1 NETs had superior outcomes than grade 2 NETs in SEER analysis (1). Retrospective analysis of pathologic specimens from patients with metastatic GEP NETs also showed low-

grade NETs had superior survival than intermediate-grade NETs, with 5-year survival rate of 87% *vs.* 38%, and strong correlation between both Ki-67 index and mitotic rate with overall survival (4). Additional studies also demonstrated correlation between Ki-67 and overall survival for metastatic pancreatic and midgut NETs (5).

Primary tumor site

The primary tumor site bears prognostic importance and may determine therapy options, particularly when determining pancreatic versus extrapancreatic primary. Among metastatic well-differentiated GEP NETs, those with duodenal and small bowel primaries have the best median survival (over 4.5 years), while pancreatic primaries confer median survival of 2 years and colorectal primaries have the worst survival at 5 months (Table 3) (1).

Traditionally, NETs were classified by the embryologic origin of their primary site as either foregut, midgut, or hindgut. Indeed, these classifications formerly defined inclusion criteria for specific clinical trials; for example, the PROMID study only enrolled patients with midgut NETs (6). While this classification was based on presumed differences in histologic structure, argentaffin staining patterns, association with carcinoid syndrome, and

Table 3 Median overall survival in months, by disease stage, of G1/G2 NETs based on SEER data from 1973 to 2004 (1)

Embryologic	Primary site	Localized	Regional	Distant
Foregut	Lung	227	154	16
	Thymus	110	68	40
	Gastric	154	71	13
	Pancreas	136	77	24
	Duodenum	107	101	57
Midgut	Small bowel	111	105	56
	Appendix	>360	>360	27
	Cecum	135	107	41
Hindgut	Colon	261	36	5
	Rectum	290	90	22

NETs, neuroendocrine tumors.

metastatic patterns (7), SEER data demonstrates marked heterogeneity in prognosis even within each embryologic grouping (Table 3). At present, the anatomic distinction that is most clinically important for metastatic NETs is pancreatic versus non-pancreatic primary. Pancreatic NETs are usually non-functional (90.8%), though non-functional tumors have inferior prognosis to functional tumors as they are usually discovered at a more advanced stage (8). Recent phase III clinical trials specifically enrolled either pancreatic or non-pancreatic NETs given the differential clinical and biologic characteristics of these distinct groups of tumors (9-11).

Functional syndromes

The presence of functional syndromes due to secretion of active hormones is an important consideration in treatment. Carcinoid syndrome, comprised classically of flushing, secretory diarrhea, bronchospasm, and right-sided cardiac valvular fibrosis, is attributed to excessive secretion of serotonin by neoplastic cells and is most frequently found in patients with well-differentiated midgut NETs with hepatic metastases (12). Additionally, functional pancreatic NETs can secrete insulin, glucagon, vasoactive intestinal peptide (VIP), gastrin, or somatostatin. For patients with functional syndromes, symptomatic control of the functional syndrome is an important goal of management. Somatostatin analogs (SSAs) are used to inhibit secretion of bioactive peptides and are a mainstay of symptomatic control for carcinoid

syndrome (13) and are also effective for VIPomas (14), glucagonomas (15), and somatostatinomas (16). The use of SSAs in insulinomas can paradoxically transiently aggravate hypoglycemia by suppressing counterregulatory glucagon and growth hormone secretion during fasting, causing symptomatic hypoglycemia, and thus should be used only with caution (17). Instead, diazoxide, which suppresses insulin release from pancreatic beta cells, is commonly used to control hypoglycemia in unresectable symptomatic insulinoma (18), and the use of SSAs is often reserved for cases refractory to diazoxide. Telotristat etiprate, an oral inhibitor of serotonin synthesis, significantly improved symptoms of refractory carcinoid syndrome and decreased urinary 5-HIAA levels in early phase clinical trials (19,20), and the randomized, double-blind, placebo-controlled phase III TELESTAR trial in patients with refractory carcinoid syndrome demonstrated that adding telotristat etiprate to SSA significantly improved symptoms of carcinoid syndrome (21).

Octreotide scintigraphy

An important additional imaging biomarker is positive uptake on octreotide-labeled nuclear imaging. Indium-111-pentetreotide (octreoscan) scintigraphy uses a radiolabeled SSA that can serve as a functional imaging biomarker for expression of somatostatin receptors overexpressed in the majority of GEP NETs (22), with 78% sensitivity to detect abdominal carcinoids and 67% sensitivity to detect pancreatic NETs (23), and frequently is able to detect metastatic tumors overlooked on conventional cross-sectional imaging (24,25).

Gallium-68-DOTA-peptide PET/CT has more recently been developed, using SSAs labelled with the positron-emitting ⁶⁸Ga isotope. A recent prospective study of imaging in patients with metastatic GEP NETs found ⁶⁸Ga-DOTATATE PET/CT imaging successfully identified 95.1% of lesions, compared to only 30.9% identified by ¹¹¹In-pentetreotide imaging (26). These results may result in more widespread use of ⁶⁸Ga-DOTA-peptide PET imaging in the U.S. Octreotide-targeted therapies such as peptide receptor radiotherapy (PRRT) routinely require positive functional somatostatin receptor imaging such as ¹¹¹In-pentetreotide or ⁶⁸Ga-DOTA-peptide imaging to serve as a surrogate biomarker indicating sufficiently high somatostatin receptor expression (27). Indeed, in an early study of PRRT, higher rates of tumor remission were observed in patients with greater uptake on baseline ¹¹¹In-

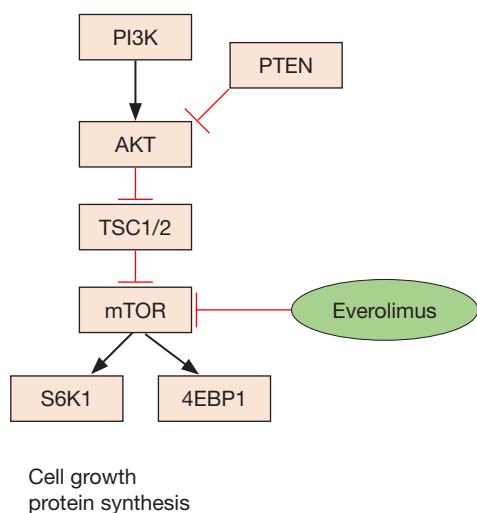


Figure 1 The PI3K/AKT/mTOR pathway is a critical pro-survival cell signaling pathway within NETs that mediates cell growth and peptide translation. Targeted mTOR inhibiting therapies such as everolimus have demonstrated efficacy in pancreatic and extrapancreatic well-differentiated NETs. NETs, neuroendocrine tumors.

pentetreotide imaging (28). Thus, radiolabeled somatostatin imaging serves as an example of noninvasive imaging serving as a predictive biomarker.

Inherited syndromes

Finally, NETs arising in the context of a familial inherited syndrome have distinct underlying pathobiology and differential risk for development of second primary NETs than sporadic NETs. In multiple endocrine neoplasia-1 (MEN-1) syndrome, germline inactivating mutations of the *MEN1* tumor suppressor promote development of parathyroid hyperplasia, pituitary adenomas, and pancreatic NETs. *MEN1* encodes menin, a nuclear scaffolding protein that interacts with many partners, including transcription factors, cytoskeletal proteins, and DNA repair proteins, to effect cell growth and alter transcriptional programs (29). Generally, treatment of metastatic MEN-1-associated pancreatic NETs is not different than for sporadic pancreatic NETs, though MEN1-associated pancreatic NETs may be slower growing than sporadic pancreatic NETs (30,31).

NET biology and genetics

NETs are believed to derive from enterochromaffin cells that originate embryologically within the neural crest and disperse

throughout the respiratory and gastrointestinal mucosal tracts (32,33). NETs overexpress somatostatin receptors, a family of G protein coupled receptors that bind the somatotropin-release inhibiting factors with nanomolar affinity. There are five subtypes of somatostatin receptor, *sst*₁₋₅, which are typically all expressed in normal pancreatic islets. Ligand binding triggers intracellular signal transduction changes, inhibiting activity of adenyl cyclase. Ultimately, somatostatin receptor activation inhibits hormone secretion and can inhibit proliferation and induce apoptosis (34). NETs broadly express the somatostatin receptors, particularly *sst*₂ (35). The SSAs octreotide and lanreotide both are agonists that bind *sst*₂ with high affinity, and also bind *sst*₃ and *sst*₅ with intermediate affinity (34).

Pancreatic NETs

Several lines of evidence point toward the AKT-mTOR pathway as a key driver of pancreatic NETs. Gene expression microarrays of pancreatic NETs demonstrated activation of the AKT-mTOR pathway in both insulinomas and non-functional pancreatic NETs, along with downregulation of *TSC2*, an inhibitor of the AKT-mTOR pathway (Figure 1). Altered protein levels of *TSC2*, *PTEN*, or both have been found in 85% of primary tumors, and none of 20 patients whose primary tumors had normal levels of both *TSC2* and *PTEN* proteins developed metastatic disease, while 8/25 of those whose tumors had low levels of *TSC2* and *PTEN* did develop metastases (36). Thus, activation of the AKT-mTOR pathway was thought to be a potentially prognostic, targetable aberration in pancreatic NETs.

Epigenetic dysregulation is increasingly appreciated as a major regulator of pancreatic NET biology. Indeed, hypermethylation, which is commonly associated with gene silencing, has been described in 87% of pancreatic NETs, with the most common hypermethylated genes including *RASSF1A* (75%), *CDKN2A* (40%), *MGMT* (40%), *RARB* (25%), and *MLH1* (23%) (37). These are potentially clinically significant, as *CDKN2A* encodes the p16^{INK4A} cyclin dependent kinase inhibitor, *MGMT* impacts efficacy of alkylating chemotherapy agents, and hMLH1 loss causes microsatellite instability.

Genomic sequencing studies of pancreatic NETs also demonstrate aberrations within the AKT-mTOR pathway along with additional novel mutations. A seminal study of 68 sporadic pancreatic NETs revealed recurrent mutations in *MEN1* (44%), *DAXX* or *ATRX* (43%), and genes in

the mTOR pathway (15%) (38). The high frequency of mutations of *MEN1* even in sporadic pancreatic NETs underscores the importance of *MEN1* as an important common driver mutation in pancreatic NETs. Inactivating *DAXX* and *ATRX* mutations appear to be mutually exclusive. *DAXX* and *ATRX* encode interacting proteins that are involved in the alternative lengthening of telomeres phenotype (39), and *DAXX* or *ATRX* protein loss also are associated with chromosomal instability (40). Studies conflict on whether the presence of *DAXX/ATRX* mutations confers a favorable (38) or unfavorable prognosis (40). Mutations within the mTOR pathway included *PTEN* (5/68 cases), *TSC2* (6/68), and *PIK3CA* (1/68) (38). The prognostic or predictive effect of these mutations on therapies used for pancreatic NETs remains unclear, and future work will almost certainly focus on associating genotype with prognosis or outcome with specific therapies.

Small bowel NETs

Consistent with the clinical differences in these entities, small intestinal NETs appear to have a distinct biology and mutational landscape compared with pancreatic NETs. Whole exome sequencing on 48 small bowel NET samples showed there were few recurrent mutations identified, though copy number variation (CNV) aberrations were detected. Like pancreatic tumors, 29% of small bowel NETs had genetic alterations in the PI3K-AKT-mTOR pathway, including amplification of *AKT1* or *AKT2* in 13 cases (27%) and amplification of *MTOR* in 4 cases. Unlike the pancreatic NETs, *SRC* was amplified in 11/48 cases (23%), and mutation or deletion in *SMAD2* or *SMAD4* was found in 22/48 cases (46%) (41). Additionally, recurrent somatic mutations and deletions in *CDKN1B* were found in 14/180 (8%) small intestinal NETs. *CDKN1B* encodes p27, a cyclin-dependent kinase inhibitor that binds to and inhibits CDK2 and CDK4 (42). As such, inhibition of AKT-mTOR pathway components, *SRC*, or cell cycle pathway components is a rational consideration.

Rather than harboring significant mutational burdens, small intestinal NETs were recently found to have marked epigenetic dysregulation. A comprehensive genomic, epigenetic, and gene expression profiling study of 97 small intestinal NETs revealed three subtypes of disease with markedly different prognoses. The largest group (55%) was characterized by loss of heterozygosity of chromosome 18, CpG island methylator phenotype (CIMP) negative status, higher proportion of *CDKN1B* mutations, and

superior progression-free survival (PFS) after resection of localized tumors. Another subgroup (19%) had no CNV, high rate of CIMP positivity, and intermediate PFS after resection of localized tumors. A third subgroup (26%) had multiple CNV aberrations including several chromosomal amplifications, and worst PFS after resection of localized tumors. These findings may motivate personalization of therapeutic strategies based on risk stratification after resection and may provide rationale for clinical trials that modulate epigenetics, such as hypomethylating agents (43).

Additionally, a study of IHC expression of several PI3K pathway components in archived NETs, the majority of which had small intestine primary, showed correlation between expression of *PIK3CA*, *MTOR*, and phospho-*EIF4EBP1* and high Ki-67. Notably, high expression of *MTOR* or its downstream activated targets phospho-*RPS6KB1*, phospho-*RPS6*, or phospho-*EIF4EBP1* was associated with inferior outcomes (44). Whether treatment of these tumors with mTOR inhibitors will provide greater benefit compared to those without abnormalities of the PI3K-mTOR axis remains to be determined.

Systemic therapies to slow progression of GEP NETs

SSAs

While SSAs are used to control carcinoid syndrome and other syndromes due to release of bioactive amines from functional NETs, their ability to slow the growth of advanced NETs is also now established. In the randomized, double-blind, placebo-controlled PROMID clinical trial, octreotide LAR prolonged PFS compared to placebo in 85 patients with metastatic well-differentiated midgut NETs (PFS 14.3 vs. 6 mo; HR 0.34, 95% CI: 0.20–0.59). In subgroup analysis, there was no significant difference in benefit based on presence of carcinoid syndrome, chromogranin A (CgA) elevation, or age (6). There was no evidence of benefit in OS, though the majority of patients on the placebo arm crossed over, confounding results (3). Notably, essentially all patients enrolled on PROMID had Ki-67 <2%, and thus results from the study could only reliably be generalized to G1 midgut NETs.

The subsequent CLARINET study was a larger randomized, double-blind, placebo-controlled phase III study of 204 patients with well or moderately differentiated, nonfunctional pancreatic, midgut, or hindgut NETs who were randomized to receive either lanreotide or placebo,

and confirmed the findings of PROMID. Treatment with lanreotide significantly improved PFS (HR 0.47, 95% CI: 0.30–0.73), and subgroup analysis showed nearly all subgroups demonstrated significant or strong trend toward improved PFS with lanreotide, including midgut and pancreas primaries, grade 1 and 2 (all had Ki-67 <10%), and hepatic tumor volume $\leq 25\%$ or $>25\%$ (45).

Everolimus in pancreatic NETs

Given the evidence for mutations in and activation of the AKT-mTOR pathway in sporadic NETs, a series of clinical trials evaluated the efficacy of everolimus (RAD001) in well-differentiated NETs. An early single-arm study at MD Anderson Cancer Center of everolimus combined with octreotide LAR in 30 pancreatic and 30 non-pancreatic well-differentiated NET patients, found median PFS of 60 weeks and overall response rate of 20%, with decrease in Ki-67 index detected in 2-week on-treatment biopsies in 5/7 patients (46). The subsequent multinational single-arm phase II RADIANT-1 study of everolimus among patients with pancreatic NETs who failed prior cytotoxic chemotherapy found median PFS with everolimus ranging from 9.7 to 16.7 months among subgroups (47). The confirmatory randomized phase III RADIANT-3 trial in 410 patients with well-differentiated pancreatic NETs that had progressed within the prior 12 months verified there was indeed improvement in the PFS primary endpoint with everolimus compared to placebo (11.0 mo with everolimus *vs.* 4.6 mo with placebo; HR 0.35, 95% CI: 0.27–0.45). Though there was no significant difference in response rates (5% *vs.* 2%), 64% of patients receiving everolimus had at least some tumor shrinkage compared to 21% of patients receiving placebo. Notably, patients randomized to the placebo arm were allowed to crossover, and 73% did in fact crossover, confounding overall survival (9). The positive results of the RADIANT-3 study motivated FDA approval of everolimus for well-differentiated pancreatic NETs.

Notably, the PFS benefit on treatment with everolimus was present for all clinically defined subgroups in RADIANT-3, including both well and moderately differentiated NETs, prior SSA therapy, and prior chemotherapy (9,48). In the RADIANT-1 and MD Anderson studies, elevated levels of CgA and neuron-specific enolase (NSE), markers likely associated with higher disease burden, were each associated with inferior median PFS. Moreover, patients who had a response in their CgA or NSE levels, defined as 30% decrease

compared to baseline at week 4, were more likely to experience some degree of tumor shrinkage (49). Thus, the baseline level of CgA or NSE appears to be a prognostic biomarker in pancreatic NETs, and early CgA or NSE response is associated with radiographic tumor shrinkage and may be a predictor of treatment outcome. Pharmacodynamic studies from the MD Anderson trial using 17 paired pretreatment and 2-weeks on-treatment tumor biopsies found significant decrease in phosphorylated-S6 on-treatment, demonstrating on-target effect of everolimus. Additionally, samples with greater feedback activation of AKT, demonstrated by greater phospho-AKT S473 levels, actually had greater degree of tumor response to everolimus (50). Thus, feedback activation of AKT, at least after two weeks of treatment, actually appears to be a biomarker of greater efficacy of everolimus rather than a mechanism of resistance as has been described in other settings (51). Finally, treatment with everolimus was associated with decrease in detectable VEGF pathway components, such as soluble VEGF receptor 2 and placental growth factor, though it remains unclear if these changes have prognostic or predictive implications (52).

Everolimus in non-pancreatic NETs

Everolimus also appears effective in non-pancreatic NETs. In the randomized, double-blind, placebo-controlled RADIANT-2 phase III clinical trial, 429 patients with well-differentiated NETs with carcinoid syndrome that progressed within the past 12 months were randomized to receive either everolimus or placebo in combination with octreotide LAR. Median PFS was 16.4 months in the everolimus arm and 11.3 months in the placebo arm (HR 0.77, 95% CI: 0.59–1.00) (53). Subsequently, in the randomized, double-blind, placebo-controlled RADIANT-4 phase III trial, 302 patients with well-differentiated, non-functional, progressive lung or GI NETs were randomized to receive either everolimus or placebo. Median PFS was 11.0 months in the everolimus arm and 3.9 months in the placebo arm (HR 0.48, 95% CI: 0.35–0.67) (10). This data recently culminated in FDA approval of everolimus for gastrointestinal and lung NETs.

Subgroup analysis of RADIANT-2 showed benefit with everolimus in all prespecified subgroups, independent of tumor grade, primary tumor site (53), prior SSA use (54), colorectal primaries (55), and lung primaries (56). Notably, preclinical data had suggested that the presence of the *FGFR4*-G388R

single-nucleotide polymorphism may be associated with more aggressive NET biology and decreased susceptibility to everolimus (57). *FGFR4* interacts with the adhesion molecule N-cadherin, which is related to tumor invasion and metastasis and activates downstream signaling cascades including the PI3K-AKT-mTOR pathway (57). However, in retrospective clinical data, the presence of the *FGFR4* polymorphism was not associated with PFS or OS (58).

Sunitinib and anti-angiogenics

NETs have been well recognized to be particularly vascular tumors, and IHC shows increased expression of vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) subtypes in both pancreatic and non-pancreatic NETs (59). Sunitinib malate is a multitarget tyrosine kinase inhibitor that can inhibit several proangiogenic kinases including VEGFR1, VEGFR2, PDGFR α , and PDGFR β . The efficacy of sunitinib in extending PFS in treatment of pancreatic NETs was confirmed in a placebo-controlled, randomized, double-blind phase III clinical trial comparing sunitinib to placebo. In the study, 171 patients with well-differentiated pancreatic NETs were randomized to receive sunitinib or placebo, and the study was halted after interim analysis after 154 patients' data was analyzed due to increased deaths and serious adverse events in the placebo arm. The primary endpoint of PFS was superior in patients who received sunitinib (11.4 *vs.* 5.5 months with placebo, HR 0.42, 95% CI: 0.26–0.66). ORR with sunitinib was 9.3%, including two complete responses (11). This study resulted in FDA approval for sunitinib in pancreatic NETs.

Pazopanib has been investigated in several single-arm phase II studies in pancreatic and extrapancreatic NETs, with PFS ranging from 9.1 to 14.4 months (60–62). A placebo-controlled randomized phase II clinical trial, A021202, is ongoing to evaluate the efficacy of pazopanib in progressive well-differentiated non-pancreatic NETs (NCT01841736).

As is the case in nearly all tumor types, correlative studies to identify prognostic and potentially predictive biomarkers of outcome with treatment with VEGFR TKIs are needed. Plasma cytokine analysis from the phase II trial of sunitinib in pancreatic and non-pancreatic NETs (63) revealed that after 28 days of sunitinib treatment, there was a significant increase in VEGF, IL-8, and stromal cell-derived factor-1 α (SDF-1 α) levels, and a significant decrease in sVEGFR-2 and sVEGFR-3 levels. High baseline SDF-1 α level was associated with inferior PFS and OS in pancreatic and non-pancreatic NETs,

and high baseline IL-8 and sVEGFR3 were also associated with inferior PFS and OS in non-pancreatic NETs (64). Notably, SDF-1 α , or CXCL12, is a chemokine that binds its receptor CXCR4 to promote vascular endothelial cell migration during neovascularization and also promotes leukocyte chemotaxis (65). In another phase II study of pazopanib in patients with pancreatic or non-pancreatic well-differentiated NETs, there were non-significant trends toward improved PFS in patients with no baseline circulating tumor cells, lower soluble-VEGFR2 levels, absence of the *VEGFR3* rs307821 (R1324L) polymorphism, and absence of the *VEGFR3* rs307826 (T494A) polymorphism (61). The results were not significant, though could be underpowered. Nevertheless, these results would be at best prognostic rather than predictive, and further study in well-powered prospective studies would be required.

Selection of targeted therapy

At present, in pancreatic NET patients, generally selection of everolimus or sunitinib for initial systemic therapy is guided by the toxicity profiles of the drugs rather than by biologic susceptibility of an individual's tumor to the drug, other than metastatic insulinomas, in which case everolimus is recommended to better control hypoglycemia (66–68). A prospective open-label phase II clinical trial is planned for well-differentiated pancreatic or non-pancreatic NETs by the National Cancer Institute in which patients will have tumor genotyping performed and be assigned to receive either sunitinib or everolimus based on the germline or somatic mutations present. Patients with mutations in *MEN1*, *PDGFR*, *KIT*, or *FLT3* would be assigned to receive sunitinib, while patients with mutations in *NF1*, *PTEN*, *PI3K*, *AKT*, *MTOR*, *VHL*, or *TP53* will be assigned to receive everolimus. Those with multiple of the above mutations or those with no mutations will be assigned to receive sunitinib. Patients will crossover to the other drug upon first progression (69).

Cytotoxic therapies for pancreatic NETs

While everolimus and sunitinib improve PFS, they have low response rates, and several consensus guidelines recommend or suggest the use of cytotoxic chemotherapy for patients with bulky or rapidly progressive metastatic pancreatic NETs (30,70,71). It should be noted, however, that cytotoxic chemotherapies have never been shown

definitively to improve survival.

Streptozocin is an alkylating agent approved by the U.S. FDA in 1982 for treatment of metastatic pancreatic NETs. Streptozocin is an analog of glucose and undergoes cell transport via the glucose transporter GLUT2 (72), which is expressed in essentially all pancreatic NETs (73). The efficacy of streptozocin-based combination chemotherapy regimens in treatment of pancreatic NETs was established in early clinical trials (74,75), and further retrospective studies using modern response criteria demonstrated response rate of 35–40% (76–78).

Temozolomide-based regimens also demonstrate efficacy on retrospective trials, with capecitabine/temozolomide having 70% response rate among pancreatic NET patients in a retrospective study of 30 patients (79). Prognostic or predictive biomarkers or characteristics for efficacy of alkylating agents remain under investigation. A retrospective study of patients who received streptozocin/5-fluorouracil/doxorubicin found that patients who had positive octreoscan and who experienced biochemical response were more likely to have an objective response (80).

Promoter methylation and gene silencing of the O6-methylguanine DNA MGMT gene is a likely biomarker of susceptibility to temozolomide or other alkylating agents (81), and MGMT was one of the most commonly hypermethylated genes noted in a study of pancreatic NETs (37). Alkylating agents induce DNA damage by covalently attaching alkyl or methyl groups to guanine residues, causing interstrand crosslinks and ultimately inducing cell death. MGMT repairs this DNA damage by removing the aberrant methyl groups, impairing the efficacy of alkylator chemotherapy (82). The presence of MGMT promoter methylation and gene silencing is a predictive biomarker for improved outcomes on treatment with temozolomide in glioblastoma (83,84). Indeed, a retrospective study of MGMT expression by IHC in archival pancreatic and non-pancreatic NET samples showed that 51% of pancreatic NETs were deficient in MGMT, while 0% of the non-pancreatic NETs were deficient in MGMT. An independent cohort of patients treated with temozolomide-based chemotherapy indeed demonstrated superior response rate in pancreatic NET patients (34%) compared to non-pancreatic NET patients (2%). Twenty-one of these 101 temozolomide-treated patients had their tumors tested for MGMT expression, and 4/5 pancreatic NET patients with deficient MGMT had response, while 0/3 pancreatic NET patients with intact MGMT had response. Conversely, 13 patients with non-pancreatic NETs all had intact MGMT,

and none had a response (85). Similarly, 12/99 pancreatic and non-pancreatic NET samples had MGMT promoter methylation when detected by methyl-specific PCR and 24/99 samples had promoter methylation when detected by pyrosequencing, while 29/89 (33%) of samples had MGMT loss by IHC. PFS since the start of alkylator chemotherapy, including temozolomide, dacarbazine, or streptozocin-based regimens, was superior in patients with MGMT protein loss or methylation by pyrosequencing, though was not associated with OS (86). Thus, the superior response rate of alkylating agents in pancreatic NETs may be accentuated by MGMT promoter methylation and protein deficiency.

Cytotoxic chemotherapy has been thought to have low response rates in non-pancreatic NETs. Streptozocin/5-FU and doxorubicin/5-FU regimens both had response rates of 16%, though streptozocin/5-FU had superior PFS (87). A retrospective study of capecitabine/temozolomide in non-pancreatic NETs showed 2/4 patients had response (88), and similarly a phase II prospective study showed 5/12 non-pancreatic NET patients had a response (89). Generally, however, cytotoxic chemotherapy is only considered for non-pancreatic NETs for patients without other therapeutic options, and its efficacy remains unproven (30).

PRRT

The use of ⁹⁰Y- or ¹⁷⁷Lu-radiolabeled octreotide analogs, though considered investigational in the United States, is standard in Europe and incorporated into European Society of Medical Oncology (ESMO) clinical practice guidelines (30,90). Several single-arm trials have been completed. A single-center single-arm study of ⁹⁰Y-DOTATOC in 1,109 patients with positive octreoscan and progressive metastatic NET, showed 29.7% clinical response rate, including 23–38% for well-differentiated non-pancreatic NETs depending on primary site and 29% for nonfunctioning pancreatic NETs. Greater tumor radiopeptide uptake was significantly associated with superior overall survival. Kidney injury was dose-limiting, with 9.2% rate of grade 4–5 permanent nephrotoxicity (91). An independent single-arm study of ¹⁷⁷Lu-DOTATATE was performed in 458 patients with metastatic well-differentiated GEP NETs and positive octreoscan, 310 of whom were evaluable. A total of 29% of patients had response, including 22% of patients with well-differentiated non-pancreatic GEP NETs, 42% of patients with non-functional pancreatic NETs, 5/12 patients with gastrinoma, and 3/5 patients with insulinoma. Median PFS was 33 months and median OS was 46 months (92). In

a previously published subset of these patients, the degree of tumor uptake on octreoscan was significantly associated with the proportion of patients who had tumor shrinkage (28).

While the intensity of uptake on baseline octreoscan is associated with response rate, it remains unclear if uptake on ^{68}Ga -DOTA-peptide PET imaging is similarly associated. About 27/32 patients with no uptake and 14/16 patients with only faint uptake seen on octreoscan had positive ^{68}Ga -DOTATATE PET/CT, allowing 20 of the patients to proceed to PRRT (93). However, there are no well-validated cutoff criteria for PRRT using baseline ^{68}Ga -DOTA-peptide PET scanning (94). The issue is further complicated because of the use of multiple SSA peptides for imaging, including ^{68}Ga -DOTATATE, ^{68}Ga -DOTATOC, and ^{68}Ga -DOTANOC. Each of these agents has a different specificity among the 5 subtypes of somatostatin receptors: ^{68}Ga -DOTANOC binds $\text{sst}_{2,3,5}$, while ^{68}Ga -DOTATATE and ^{68}Ga -DOTATOC only bind sst_2 (95,96).

To personalize therapy with PRRT, individualizing dosimetry to maximize effect while minimizing toxicity, particularly renal toxicity, is important. There is a significant association between severe nephrotoxicity and renal uptake on baseline somatostatin scintigraphy, especially with ^{90}Y -DOTATOC (91). Personalizing dosing based on calculation of renal biological expected dose (BED) using ^{111}In -pentetreotide with concurrent amino acid infusion could be performed, and if the renal BED after four cycles exceeded 37 Gy, subsequent cycles were dose-reduced or cancelled, thus preventing the development of long-term renal failure at 15 months after treatment with ^{90}Y -DOTATOC (97). Indeed, dosimetry to various organs may dynamically evolve over the course of subsequent cycles of PRRT, with a case report of a patient having a marked increase in plasma half-life of ^{111}In -octreotide after her first cycle of treatment with ^{90}Y -DOTATOC resulting in an increase in BED to kidneys and bone marrow, indicating the importance of serial reassessment of dosimetry (98).

The multicenter, randomized phase III NETTER-1 trial compared treatment with ^{177}Lu -DOTATATE combined with SSAs compared to octreotide LAR 60 mg in patients with unresectable, advanced, progressive well-differentiated midgut NETs with positive ^{111}In -octreotide scintigraphy. The study enrolled 229 patients, with marked improvement in PFS [median estimated 40 months with ^{177}Lu -DOTATATE *vs.* 8.4 months with high-dose octreotide, HR 0.21 (0.129–0.338) and overall response rate (18% *vs.* 3%, $P=0.0008$) (99). Subgroup analyses are pending, but this comprises the first randomized,

controlled trial demonstrating the efficacy of PRRT. Notably, NETTER-1 did not include pancreatic or other foregut NETs, but as described above, previous single-arm studies showed efficacy of PRRT in both functional and nonfunctional pancreatic NETs, with even higher response rates than in small intestinal NETs (92). Hopefully, the results of NETTER-1 will make PRRT more accessible for North American patients with metastatic NETs.

Immunotherapy

Historically, interferon- α has been used in therapy of carcinoid tumors, since an early study of treatment with leukocyte interferon in 9 patients with small intestinal carcinoid tumors showed improvement in the symptoms of carcinoid syndrome and decreased in urinary 5-HIAA levels (100). However, the data is unclear on the true efficacy of interferon- α , as randomized studies have been small and likely underpowered. One study showed that combination octreotide and interferon- α decreased risk of progression compared to octreotide alone (HR 0.28, 95% CI: 0.16–0.45) (101), but other studies found that adding interferon- α to SSA did not improve 12-month disease stability rate (102) or time to treatment failure (103). Based on these studies, some authors argue that there is at least a trend toward improved survival with combination interferon- α and SSA compared to SSA alone (104). At present, consensus guidelines state interferon alfa-2b may be considered for unresectable or metastatic GI tract, lung, or thymus NETs (30).

With the discovery of the efficacy of immune checkpoint inhibitors, such as CTLA-4 inhibitors, PD-1 inhibitors, and PDL-1 inhibitors in other malignancies, there has been consideration of investigating these therapies in NETs. Retrospective analysis of a series of archival well-differentiated pancreatic NETs found that 68% of NETs had T-cell infiltration, while 34% of NET samples had infiltrating FoxP3+ regulatory T cells (Tregs). Among the subset of intermediate grade NET samples, the extent of T cell infiltrate was significantly associated with recurrence. Within the subset of NET samples obtained from hepatic metastasectomy, the degree of FoxP3+ T cell infiltration was inversely associated with overall survival (105). Additionally, studies have found varying rates of microsatellite instability (MSI-high) in well-differentiated NETs, with 0–10% of pancreatic NETs (106,107) and 33% of insulinomas MSI-high, usually driven by MLH1 promoter methylation and loss of heterozygosity (108). Immune checkpoint inhibitors appear effective in MSI-high tumors, as the PD-1 inhibitor

pembrolizumab yielded 50% response rate in MSI-high metastatic non-colorectal cancers (109,110). Given that MLH1 hypermethylation was one of the most commonly hypermethylated genes in pancreatic NETs (37), further study of immune checkpoint inhibitors in metastatic NETs, especially pancreatic NETs, is rational. Notably, essentially 0% of small bowel NETs were MSI-high (106,111). Nevertheless, the phase I study of pembrolizumab included a patient with carcinoid tumor with 19 weeks of stable disease and a patient with pancreatic NET who had 11 weeks of stable disease (112). Studies using immune checkpoint inhibitors in pancreatic and non-pancreatic NET patients are planned, including pembrolizumab in well and moderately-differentiated pancreatic, small intestinal, appendiceal, and colorectal NETs (NCT02628067).

Conclusions

Our understanding of the genomic and epigenetic underpinnings of pancreatic and extrapancreatic NETs has grown significantly in the last decade. The positive phase III clinical trials for everolimus and sunitinib in metastatic pancreatic NETs confirmed the biologically driven hypothesis that targeting mTOR signaling and angiogenic pathways results in clinically meaningful tumor growth suppression. With the positive RADIANT-4 and NETTER-1 trials, our therapy options against extrapancreatic NETs also continue to grow. While we can personalize therapy of metastatic GEP NETs by clinical characteristics, by biochemical response, and by somatostatin based imaging, there is a critical need for improved biomarkers. A recent National Cancer Institute summit concluded that monoanalyte biomarkers have generally been lacking in sufficient prognostic or predictive value, and instead multianalyte biomarkers, particularly incorporating genomics, should be further developed (113). We need to prospectively assess the predictive role that underlying genomic mutations, especially in pancreatic NETs, has in guiding response to targeted therapies. The potential of achieving durable disease control and response with immunotherapy is also exciting, and development of clinical trials to assess the efficacy of immune checkpoint therapy is ongoing.

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Footnote

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