Updates in Neuroendocrine Tumor Genomic Studies and Systemic Therapies

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Abstract

Neuroendocrine tumors (NETs) are a mysterious group of malignancies that can form throughout the diffuse neuroendocrine system and produce biological effects distant from their primary location. They have an increasing incidence and can also behave more aggressively than previously recognized. These heterogeneous features have interfered with a more fundamental understanding of tumor biology. Over the past several years, systematic genetic surveys NETs from the major sites of origin have revealed important genomic changes, with potential driver mutations, that have an impact on prognosis and clinical development of targeted therapies. We will take a focused approach in reviewing this data and its impact on clinical medicine.

Keywords: Neuroendocrine tumor; Carcinoid; Small molecule targeted therapy; PRRT; NTRK fusion

Introduction

Neuroendocrine tumors (NETs) originate from the enterochromaffin cells of the neuroendocrine system located throughout the body. NETs are rare but have increasing incidence and prevalence [1]. The annual age-adjusted incidence of NETs in the United States was 6.98 per 100,000 persons in 2012, compared to 1.09 per 100,000 persons in 1973 [2]. Historically, NETs have been perceived as an indolent disease however they can behave aggressively and their heterogeneity has been appreciated more lately [3,4]. In this article, we summarize current molecular and genetic data of NETs, provide a concise review on how approved treatments interact with these pathways, and highlight emerging small molecule targeted therapies.

NETs are currently classified by primary location, stage, and histologic grade. Foregut NETs include tumors of lung, bronchi, gastric, duodenal, and pancreatic origins. Medullary NETs cover ileum to ascending colon. Lastly, hindgut NETs are composed of distal large bowel and rectum primary sites. The TNM staging system is used in NETs [5]. Present World Health Organization (WHO) grading system of NETs is based on two proliferation markers, Ki-67 score and mitotic index (MI). WHO 2010 classification of digestive NETs defines both grade 1 (Ki-67 < 2%, MI<2/10 per high-power filed, HPF) and grade 2 (Ki-67 2-20%, MI 2-20/10 HPF) tumors as well-differentiated neuroendocrine tumors, whereas grade 3 (Ki-67 > 20%, MI > 20/10 HPF) neuroendocrine carcinoma (NEC) are poorly-differentiated [6]. In 2017, WHO updated its grading system of pancreatic NETs to broaden grade 1 tumor to include Ki-67 <3% [7]. A second change in WHO 2017 classification was to subdivde grade 3 cancers (Ki-67 >20%) to well-differentiated NET G3 and poorly differentiated NEC. When compared to NET G3, NEC G3 is rarely involved in hereditary syndromes, has a poor prognosis, but is more responsive to platinum agents [8]. A recent study by French researchers suggests that perhaps using 5% Ki-67 cut-off to differentiate grade 1 and grade 2 tumors can provide more accurate risk stratification [9].

Literature Review

Part I – Molecular genetic updates

In the past decade, molecular analysis has assumed a critical role in understanding NETs and in uncovering potential treatment targets. Despite the majority of NETs being sporadic, there are hereditary syndromes associated with NETs, including multiple endocrine neoplasia type 1 (MEN 1), von Hippel-Lindau syndrome, neurofibromatosis type 1, and tuberous sclerosis complex [10-13]. All four diseases have autosomal-dominant inheritance and are caused by the tumor suppression genes, MEN1, VHL, NF1, and TSC1/TSC2, respectively. Whole-genome landscape analysis of pancreatic NETs was published in Nature in 2017 [14]. In this pivotal study, genome sequencing was performed on 102 cases of sporadic pancreatic NETs and noted a higher than expected prevalence of germline mutations in patients with presumed clinically sporadic pancreatic NETs as 17% of patients studied had germline mutations such in MUTYH, CHEK2, BRCA2, MEN1 and VHL. This result reinforces the role of genetic changes and potential driver mutations in the pathogenesis and treatments for NETs.

Four common pathways of somatic mutations have been identified in pancreatic NETs. These include chromatin remodeling, DNA damage repair, mTOR (mammalian target of rapamycin) signaling activation, and telomere maintenance (Table 1) [15]. Nearly half (43%) of pancreatic NETs have altered telomeres associated with inactivating mutations in the ATRX (ATRX chromatin remodeler) or DAXX (death-domain associated protein) genes [16]. The exact prognostic value of these mutations is unclear [17]. Mutations in the mTOR pathway were found in 14% of pancreatic NETs. Retrospective and observation studies have shown abnormal mTOR pathway in small intestinal NETs along with other primary sites. High immunohistochemical expression of mTOR or its downstream targets is associated with adverse clinical outcomes [18]. The product of PTEN (phosphatase and tensin homolog) gene normally inhibits the P13K-AKT-mTOR pathway. Loss of PTEN results in increased phosphorylated AKT expression and is associated with metastatic potential of low-grade NETs [19]. Similarly, low-grade lung NETs often have high PTEN expression and is associated with markedly better prognosis than their gastroenteropancreatic counterparts.

Targeted next-generation sequencing of NETs from a variety

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I and II studies have been identified a more effective radionuclide (177Lu-
activity, providing support for the idea of NET heterogeneity [20].

Renal NETs are rare with only approximately 5% of all NETs, and fewer than 10 cases have been published. The genetics of renal NETs remain largely unexplored, with only a handful of studies reporting the genetic landscape of this tumor type. In a study of nine primary renal well-differentiated NETs, all cases had mutations in various genes, including PIK3R2, BCR, and MYC [21]. These findings suggest that renal NETs may have a distinct genetic profile compared to other NET subtypes.

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In a study of 9 cases with an average of 5 variants per sample. Repeated mutations included CHD1, TET2, AKT3, ROS1, PIK3R2, BCR, and MYC. Renal NETs are rare with only approximately one hundred case reports published, but this reflects the challenges of defining the genetics of NETs.

**Part II – Treatment development**

**Somatostatin analogues:** Well-differentiated NETs are known to overexpress somatostatin receptors, especially somatostatin receptor type 2. The somatostatin analogue octreotide has been FDA approved for symptom control in carcinoid syndrome since the 1980s. Somatostatin was first identified and isolated from hypothalamic extracts in 1973 at the Salk Institute [23]. Therapeutic usage of somatostatin was limited due to its short half-life that required continuous intravenous infusion. Long-acting somatostatin analogue SMS 201-995, later known as octreotide, was therefore developed by Sandoz. In 1986, Mayo Clinic was the first to report octreotide effectiveness in carcinoid syndrome symptoms control among a group of 25 patients [24]. The antitumor effect of octreotide was long suspected and eventually confirmed by a phase III double-blind controlled trial (PROMID study; NCT00178737) published in 2009 with an improved median time to tumor progression when compared with placebo (14.3 months vs 6 months, p<0.000072) [25]. However, long term follow up on overall survival was not statically different and was thought related to crossover of the majority of placebo patients to octreotide treatment in this small cohort of 85 patients [26]. In 2014, interim results of the CLARINET trial (NCT00353496) was published. This double-blind, placebo-controlled, multinational Phase III trial of somatostatin analogue lanreotide enrolled 204 patients with progressive, advanced, well-to-moderately differentiated, grade 1-2, nonfunctioning, somatostatin receptor-positive NETs. Lanreotide was found to significantly prolong median progression-free survival (PFS) in patients with gastroenteropancreatic NETs when compared to placebo (median not reached vs 18 months, p<0.001) [27]. Subsequently, lanreotide was approved by the FDA for gastroenteropancreatic NETs based on the improved PFS endpoint, whereas octreotide only has labeling approved for symptomatic control.

**Peptide receptor radionuclide therapy (PRRT):** The NETTER-1 trial (NCT01578239) was an international open-labeled Phase III study that evaluated Lutetium-177(177Lu)-Dotatate in well-differentiated metastatic midgut NETs. The 177Lu-Dotatate radioisotope emits β particles with 2mm range and a short half-life of 6 hours. Linking 177Lu-Dotatate to a somatostatin analogue, a type of PRRT, enables precise delivery of radioactive therapy to tumor sites throughout the body. Since the initial report of PRRT usage, a number of Phase I and II studies have been identified a more effective radionuclide (177Lu)

vs 111In and 90Y) and more specific peptide (octreotate vs octreotide) approaches, paving the road for the high-quality, prospective NETTER-1 trial [28-31]. Patients in the 177Lu-Dotatate group (n=116) had a response rate of 18% compared to 3% in the control group using octreotide alone (p=0.0001). The primary endpoint of this trial was PFS, and although the median PFS had not yet been reached, the estimated rate of PFS at 20 months was 65.2% in the 177Lu-Dotatate group and 10.8% in the control group (p=0.004). Study follow-up is still ongoing and more mature survival outcomes are awaited.

**mTOR kinase inhibitors:** Yao and colleagues first reported antitumor activity of everolimus in pancreatic NETs in 2008 [32]. The subsequent open-labeled phase III RADIANT-3 study of 410 patients with advanced pancreatic NETs met its primary endpoint of improved median PFS (11.0 months vs 4.6 months, p<0.0001) using everolimus compared to placebo [33]. Everolimus was associated with a median OS of 44 months, translating to a 6.5 months of survival benefit over placebo, however it did not reach statistical significance (p=0.30) likely due to significant crossover of patients in this study [34]. The RADIANT-2 study was a double-blinded phase III trial that enrolled patients with carcinoid syndrome from advanced NETs of various primary sites. In this study, everolimus plus octreotide improved median PFS by 5.1 months compared with placebo plus octreotide [35]. The P-value (0.026) was marginally above the pre-specified threshold for statistical significance (P <= 0.0246). Even though this study did not meet its primary endpoint, it suggested a potential antitumor effect of everolimus in non-pancreatic NETs. The follow-up Phase III RADIANT-4 study noted that everolimus monotherapy had significant improvement in median PFS (11 months vs 3.9 months in placebo group, p<0.0001) in advanced, non-functional NETs of lung and gastrointestinal tract [36]. The data from RADIANT-3 and RADIANT-4 studies support the use of everolimus in a broader range of NETs. Another mTOR inhibitor that has been studied in NETs is temsirolimus. In a single-arm phase II study (NCT01010126) of pancreatic NETs, temsirolimus and vascular endothelial growth factor antibody bevacizumab combination demonstrated an encouraging response rate of 41% with relatively tolerable side effect profile [37].

**Multitargeted tyrosine kinase inhibitors (MTKIs):** Sunitinib exhibits antitumor and antiangiogenic activities by blocking all three vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors (PDGFRα and PDGFRβ), stem cell factor receptor KIT, and fetal liver tyrosine kinase receptor 3 (FLK3) [38]. When studied against placebo in a double-blind phase III trial in patients with advanced well-differentiated pancreatic NETs, sunitinib was associated with statistically significant median PFS improvement compared to placebo (11.4 months vs 5.5 months, p<0.001) [39]. The response rate was 9% compared to 0% in placebo group. A number of MTKIs, including axitinib, lenvatinib, pazopanib, and suratinib, have also shown potential efficacy in NETs in Phase II studies [40-43]. Levatinib produced radiological response in pretreated patients including those progressed on other TKIs. Phase III trials are ongoing for cabozantinib (NCT03375320) and surfnatinib (NCT02589821 and NCT02588170).

**Tropomyosin receptor kinase (TRK) inhibitors:** Entrectinib (RXDX-101), an oral tyrosine kinase inhibitor of TRK A/B/C, C-ros oncogene 1 (ROS1) proteins, and anaplastic lymphoma kinase (ALK), was reported to have significant activity against a newly identified driver mutation in NET. The TRK receptor family are expressed in neuronal tissue and regulate nervous system development and function by activating neurotrophins [44,45]. It is composed of 3 transmembrane
proteins referred to as Trk A, Trk B, and Trk C which are coded by the NTRK1, NTRK2 and NTRK3 genes, accordingly. Fusion of Trk with other genes leads to a constitutively activated kinase triggering uncontrolled downstream proliferation. There are a wide variety of NTRK gene fusion partners however they all share similar tyrosine kinase domains of Trk protein and can be targeted by a tyrosine kinase inhibitor [46]. NTRK fusions were identified in approximately 0.3% of NETs, across all subtypes [47]. Entrectinib produced rapid, profound, and protracted tumor response in a patient with a small intestinal NET [48]. The U.S. Food and Drug Administration (FDA) has granted Priority Review for entrectinib in treatment of NTRK fusion-positive advanced solid tumors and is expected to have a decision regarding approval by August 2019.

Alkylating agents: Streptozocin (STZ) was approved in pancreatic NETs in 1982 and STZ based chemotherapy is still the standard of care for advanced pancreatic NETs in the European Union. Oral chemotherapy combination capetibane-temozolomide has also demonstrated effectiveness in metastatic well-differentiated NETs, but now often reserved for intermediate to high grade NETs due to the effectiveness of somatostatin analogues and small targeted therapies [49,50]. The SEQTOR study (NCT02246127) is a phase III trial current recruiting in hope to answer what is the best therapy sequence for advanced pancreatic NETs, whether everolimus followed by STZ-based chemotherapy combination capecitabine-temozolomide has also demonstrated effectiveness in metastatic well-differentiated NETs, but now often reserved for intermediate to high grade NETs due to the effectiveness of somatostatin analogues and small targeted therapies [49,50]. The SEQTOR study (NCT02246127) is a phase III trial current recruiting in hope to answer what is the best therapy sequence for advanced pancreatic NETs, whether everolimus followed by STZ-based chemotherapy combination capecitabine-temozolomide has also demonstrated effectiveness in metastatic well-differentiated NETs, but now often reserved for intermediate to high grade NETs due to the effectiveness of somatostatin analogues and small targeted therapies [49,50].

Immunotherapy: At the moment, there is minimal data supporting usage of checkpoint inhibitor immunotherapy in well-differentiated NETs [51]. Phase 2 studies are ongoing evaluating safety and efficiency of nivolumab, nivolumab/ipilimumab, and pembrolizumab in NETs [51]. Phase 2 studies are ongoing evaluating safety and efficiency of nivolumab, nivolumab/ipilimumab, and pembrolizumab in NETs [51].

Pathogenic pathways Associated mutations

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<tr>
<th>Chromatin remodeling</th>
<th>SETD2, MLL3</th>
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<tr>
<td>Altered telomere length</td>
<td>DAXX, ATRX</td>
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<tr>
<td>mTOR signaling</td>
<td>MEN1, PTEN, TSC1, TSC2, DEPDC5, P38 AKT → mTOR → cell growth proliferation</td>
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<tr>
<td>DNA damage repair</td>
<td>MUTYH, CHEK2, BRCA2</td>
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**Table 1:** Main pathways and their associated mutations in pancreatic NETs. MEN1 encodes menin which is a histone modifier and its inactivation leads to widespread transcriptional dysregulations that affect all four processes. Inactivating mutation in apoptotic regulator DAXX or chromatin modifier ATRX promotes alternative lengthening of telomeres and chromosomal instability. Loss of suppressive function of PTEN, TSC1, TSC2, or DEPDC5 results in overactivation of mTOR pathway.

**References**


