Clinical Epigenetics of Neuroendocrine Tumors: The Road Ahead

Annamaria Colao¹, Filomena de Nigris², Roberta Modica³* and Claudio Napoli⁴

¹ Department of Clinical Medicine and Surgery, Unesco Chair Health Education and Sustainable Development, Federico II University of Naples, Naples, Italy, ² Department of Precision Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy, ³ Department of Clinical Medicine and Surgery, Federico II University of Naples, Naples, Italy, ⁴ Department of Advanced Medical and Surgical Sciences, University of Campania “Luigi Vanvitelli”, Naples, Italy

Neuroendocrine tumors, or NETs, are cancer originating in neuroendocrine cells. They are mostly found in the gastrointestinal tract or lungs. Functional NETs are characterized by signs and symptoms caused by the oversecretion of hormones and other substances, but most NETs are non-functioning and diagnosis in advanced stages is common. Thus, novel diagnostic and therapeutic strategies are warranted. Epigenetics may contribute to refining the diagnosis, as well as to identify targeted therapy interfering with epigenetic-sensitive pathways. The goal of this review was to discuss the recent advancement in the epigenetic characterization of NETs highlighting their role in clinical findings.

Keywords: epigenetics, neuroendocrine neoplasms, trials, biomarkers, neuroendocrine, neuroendocrine tumor

INTRODUCTION

Neuroendocrine neoplasms (NENs) are a heterogeneous group of malignancies originating from neuroendocrine cells diffuse throughout the body. The gastroenteropancreatic (GEP) tract and the bronchopulmonary system represent the main site of origin. NENs are mostly sporadic, but in 10–30% they can arise within the context of familial syndromes, mainly multiple endocrine neoplasia type 1 (MEN1) (1). Incidence and prevalence of NENs have markedly increased in the last decades, irrespective of stage and grade (2). Clinical presentation and prognosis of NENs may widely vary. NENs can be functional when they release biologically active hormones that cause distinct clinical syndromes or more often may be non-functional, thus diagnosed incidentally or due to mass effect. Delayed diagnosis is common, as well as the detection of metastases, mainly to the liver, already at diagnosis. Patients with localized disease have a better prognosis, with 5-year survival ranging from 78 to 93%, while in metastatic disease, the 5-year survival is worse (19–38%), although improved over the past years (3). The improvement of survival rates may be the consequence of the availability of effective therapies, as well as earlier and more accurate clinical and pathologic diagnoses with relative downstaging. NENs have usually an indolent course and patients need life-long therapy.

Abbreviations: CAPTEM, Capecitabine and temozolomide; cfDNA, Cell-free DNA; CIMP, CpG island methylator phenotype; CNV, Copy number variation; CTC, Circulating tumor cell; ctDNA, Circulating tumor DNA; ddPCR, Droplet digital PCR; GEP, Gastroenteropancreatic; MEN1, Multiple endocrine neoplasia type 1; NEN, Neuroendocrine neoplasm; NET, Neuroendocrine tumor; NEC, Neuroendocrine carcinoma; NF1, Neurofibromatosis type 1; PNET, Pancreatic neuroendocrine carcinoma; PNEC, Pancreatic neuroendocrine neoplasm; SI-NET, small intestinal neuroendocrine tumors; VHL, Von Hippel Lindau.
Notably, the landscape of the therapeutic options in NENs has considerably expanded in the last decades. The current systemic therapies for locally advanced or metastatic NENs include somatostatin analogs (SSAs), molecular targeted therapy with mTOR inhibitors (Everolimus), or anti-angiogenesis (Sunitinib), peptide receptor radionuclide therapy (PRRT) with either 90Ytrium (90Y) or 177Lutetium (177Lu) and chemotherapies with temozolomide, capcitabine or platinum-based regimens. These options can be used in sequence or association with surgery, locoregional treatments (e.g., radiofrequency ablation, cryoablation, chemoembolization, and radioembolization), and/or other drugs used as supportive therapies (e.g., telotristat, diazoxide and proton pump inhibitors) (4, 5). In this review we will focus on well or moderately differentiated neuroendocrine tumors (NETs), excluding neuroendocrine carcinomas (NEC) for their peculiar pathology and treatment.

### EPIGENETIC MODIFICATIONS AND NEUROENDOCRINE TUMORS

Epigenetic changes, such as DNA methylation and histone modification, are critical for regulating genes and non-coding RNA expression. Genomic alterations and gene mutations which are involved in the pathogenesis of NETs, as MEN1, VHL-hypoxia-inducible factor, RASSF1A, have a consequence on the aberrant placement of epigenetic markers and related pathways (6–10).

Epigenetic mechanisms can modify gene expression altering DNA methylation status, histones post transcriptional modifications, and influencing the expression of non-coding RNAs. Hypermethylation of a promoter is a mechanism that determined gene silencing, while hypomethylation can lead to chromosomal instability and consequently influences gene expression (9, 10). Histone modifications involves the addition of methyl, acetyl, phosphorylation at different aminoacid residues of histone proteins. These modifications alter chromatin accessibility to transcription factors and lastly gene expression. MicroRNAs (miRNAs) and long noncoding RNAs are other layers of epigenetic regulation. They are small, or long sequences of non-coding RNAs regulating gene expression post-transcriptionally, considered to be a cancer-associated epigenetic mechanism (11).

### METHYLATION PATTERNS RELEVANCE IN THE PATHOGENESIS OF NETS AND CLINICAL FINDINGS

The pathogenesis of NETs is further to be elucidated, as in most other solid tumors. Nevertheless, epigenetic studies have improved our knowledge. Pancreatic neuroendocrine tumors (PNETs) account for 1 to 2% of all pancreatic tumors and most of them are sporadic and non-functioning, 5–7% arise within inherited syndromes, including MEN1, Von-Hippel Lindau (VHL) syndrome, neurofibromatosis type 1 (NF1), and tuberous sclerosis. The majority of familial PNETs are caused by germline inactivating mutations in the MEN1 gene, suggesting a key role in PNETs tumorigenesis. MEN1 gene encodes the transcription factor MENIN, ubiquitously expressed, and involved in many biological functions. MENIN, plays an essential role in chromatin remodeling and gene expression recruiting the H3K4me3 histone methyltransferase on mixed-lineage leukemia (MLL1) complex, regulating the expression of the cyclin-dependent kinase inhibitors, and influenced the epigenetic regulation of several genes (12). MEN1 mutations or loss of function deregulated cell growth in 75% cases of PNETs favoring hypermethylation of several tumor suppressor genes including RASSF1A (13), HIC-1, MLH1, CDKN2A, and MGMT (6, 7). Characteristics of the sporadic form of PNETs are mainly gene mutations in DAXX (death-domain-associated protein) or ATRX (alpha thalassemia/mental retardation syndrome X-linked) (12). Both DAXX and ATRX are chromatin remodelers and are involved in the incorporation of the histone variant H3.3 at the telomerases and pericentric heterochromatin necessary (14). Proteins loss, as well as mutations in DAXX or ATRX, are associated with chromosome instability (CIN), reduced genomic H3K9me, and aggressive PNET phenotype (12, 15). Increased risk of PNET was also associated with loss of chromosome 11q containing the genes Men1, but also DNA repair pathway genes as BRCA2 and ATM, and amplification region activating PIK3CA and mTOR pathway. In some cases associated with MENIN loss were also found mutation affecting VHL tumor suppressor gene that determined a constitutive hypoxia transcription factors (HIF) activation and uncontrolled angiogenesis (16, 17), suggesting that MENIN loss or mutation is a key initiator in PNET tumorigenesis (15, 18–21). In pulmonary NET in addition to MEN1 mutations affected also as histone lysine methyltransferase (SETDB1, SETDB2), histone acetylation modifiers (BRWD3 and HDAC5) and ATP-dependent chromatin remodeling SMARCA1 indicating a key pathogenic role (22). Genomic profile of small intestinal NET (SI-NET) identify two different groups, one characterized by loss of chromosome 18, and another one characterized by the presence of chromosome 18 but with clustered gains on chromosomes 4, 5, 7, 14, and 20 (23). Correlation of loss of chromosome 18 and RASSF1A promoter hypermethylated and hypo-methylation of long intergenic element 1 (LINE1) and ALU sequences was found in SI-NETs (24) although not associated with grade and tumor size (25).

In hereditary SI-NET causative role was attributed to germline mutations in IPMK (inositol polyphosphate multikinase) p53 activity and MutY DNA glycosylase genes (26) affecting the oxidative pathway. Above mentioned studies emerged that in SI-NET epigenetic machinery is not causative however the uncontrolled pathways of oxidative stress and genomic rearrangement activated several epigenetic modifications (27).
Illumina array (850k array) with the goal to find novel diagnostic markers. The study identified a distinct cluster of methylation genes associated with VHL, sporadic and MEN1-related PNETs, indicated that mutations in these genes influence the epigenetic pathway and clinical presentation of diseases (28, 29). Differential methylation patterns were also reported among GEP-NETs (24, 30). Indeed, the analysis conducted in 60 tumors selected a pool of 807 genes. These gene sets were able to distinguish NETs in functional GEP-NETs (insulinoma, gastrinoma) and non-function subtypes underlying the clinical and histological characteristics. Gastrinomas showed hypomethylation of genes including metalloproteinases (MMP1, MMP3, TIMP2, TIMP3), the serpin family (SERPINA5, SERPINB5), and oncogenes (IL2, MCF2, and MOS), whereas hypermethylation was reported for tumor suppressors (SMARCB1, CASP8, and NBL1) (24, 25, 30). Promoter hypermethylation of the IGF2 pathway was characteristic of insulinomas shedding a light on signaling responsible for their differentiation from a common origin (31). A study on SI-NET identified TCEB3C gene hypermethylation to be specific for this histology. Interestingly, treatment of SI-NET cell lines with the de-methylating agent decitabine and the histone methyltransferase inhibitor 3-deazaneaplaoncin A-induced TCEB3C re-expression, confirming an epigenetic regulation of this gene (32). Followed this stem study, Verdugo et al. and then Karpathakis et al. identified hyper-methylation of the gastric inhibitory polypeptide receptor (GIPR) as another specific marker of SI-NETs and reported hyper-methylation in several genes. They selected on chromosome 18 as laminin alpha 3 (LAMA3), serpin peptidase inhibitor clade B member 5 (SERPINB5), and factor receptor superfamily member 11a NFkB activator (RANK or TNSFRSF11A), suggesting that epigenetic silencing could be the possible second step in tumor development upon chromosome 18 loss (33–35).

## METHYLATION PATTERNS: RELEVANCE IN PROGNOSIS AND RESPONSE TO THERAPY

Since epigenetic changes play a key role in the progression of PNETs, finding the right position of epigenetic prognostic factor, is crucial (34). In particular, some epigenetic changes are correlated with DAXX or ATRX protein loss because this complex regulates H3K9me and influenced DNA methylase. Indeed, promoter hypermethylation of RASSF1A and p57Kip2 in PNEs was responsible for NAPI1.1 overexpression associated with the metastatic phenotype (35–38). Additionally, a peculiar group of PNETs named (CIMP) showed hypermethylation of CpG islands including tumor suppressor genes, such as RASSF1A, hMLH1, and hypomethylation of LINE-1 sequence. These peculiar epigenetic pathways were associated with poor prognosis and advanced stage of PNETs (39). While hypermethylation ofCDKN2A was associated with early tumor recurrence and poor outcomes of GEP-NETs (40). A general decrease in methylation levels was observed in SI-NET metastases compared to the primary tumors. In particular, differential methylation of AXL, CRMP1, FG5, CXXC5, and APOBEC3C genes were detected in primary tumors compared to metastases (34). However, no validation of these markers was reported in the study population. In a follow-up of primary SI-NET and liver metastases, it was selected a panel of epigenetically dysregulated genes that were progressively methylated or demethylated from the primary tumor to metastases (33, 41), suggesting their potential use as markers. Recently dysregulation of TET1/TET2 enzymes that catalyze DNA demethylation was observed in SI-NETs open a potential novel class of drug treatment (42, 43). Differential methylations of specific gene promoters were also associated with response to therapy. Table 1 shows the most representative observational studies involving epigenetic biomarkers. One example is O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme removing alkyl groups from an alkylguanine. Retrospective studies have found an association between methylation of MGMT and response to treatment with temozolomide (an alkylating agent) making it a promising marker (44–47) (Table 1). A prospective trial confirmed this correlation (48).

## MiRNAs Relevance in Differential Diagnosis and Prognosis

MicroRNAs (miRNAs) are small (19–24 nt) regulatory RNA molecules that can also be used to classify cancer because of their abundance, cell-type, and disease-stage specificity which support their possible use to predict clinical outcomes and differential diagnosis. Multiple miRNA profiling studies have been performed on NET pathological types using different RNA isolation, detection, and analysis methods. Although these differences complicate inter-study comparisons, miRNAs still hold much promise as markers. A set of 10 miRNAs (miR-99a, 99b, 100, 125a, 125b-1, 125b-2, 129-2, 130a, 132, and 342) was selected as a potential tool to differentiate pancreatic NEN from pancreatic acinar cell carcinoma (49), while miR-21a was selected as potential biomarker for GEP-NETs (50). Moreover, in another study in insulinomas, miR-204 was the unique miRNA selectively overexpressed while miR-186 showed significantly downregulated in 39 colorectal NET patients (51).

Different sets of miRNAs were identified as predictors of metastases on the base of tissue used as control. Overexpression of miR-21, involved in the regulation of the PI3K/Akt/mTOR pathway, and the Ki-67 proliferation index was significantly associated with liver metastases when pancreatic normal tissue was used as control (52). In contrast proliferation index Ki-67, miR-642, and miR-210 were correlated with metastases of PNETs when pancreatic islets were used as control (49). These data suggest that reference tissue influences the selection of markers. From the comparison of primary tumor and metastasis and then validation in 37 patients, the miRNA-196a was found significantly associated with tumor grade and recurrence (53).

A different approach is the NETest algorithm for the prediction of the clinical status of NETs (54, 55). The test is PCR-based
measuring 51 individual circulating genes in 1 ml of blood. An algorithmic analysis provides a numeric score of disease status. It can define the completeness of surgical resection, identify residual disease, monitor disease progression, and determine the efficacy of treatment (56–58). NETest was used to evaluate the alteration in genes during the treatment with SSA, PRRT, and following surgery (56–58). Interestingly, the NETtest was successfully used to evaluate efficacy and response to PRRT in metastatic NETs (63) (Table 1).

Several miRNAs were also associated with tumor progression of SI-NETs. In the miRNAs study performed by Heverhagen (64), the most promising diagnostic miRNA-biomarker was miR-7-5p higher in pathological tissue compared to control and selected samples from any kind of treatment. Furthermore, miR-885-5p predicted NETs (65). In a Dutch cohort of GEP-NET patients, the NETest had good sensitivity but the specificity was relatively low. Thus, NETest would be less suited for screening but could be valuable for the detection of residual disease after therapy (62).
status or tumor stage (71). In another study, 4 differentially expressed miRNAs (miR-21-5p, miR-22-3p, and miR-150-5p) reached a statistical significance (72) underlying the need to add tissue markers, to discriminate NETs and to confirm the findings in annotated sample sets. Two miRNA profiling studies conducted on SL-NETs (66, 69, 73), compared metastatic tumors to primary malignancy, merging the data from both studies (metastasis vs primary) downregulation of miR-133a and upregulation of miR-183 were associated with poor prognosis and the spread of malignancy.

ROLE OF LONG NON-CODING RNAS IN NENS CLINICAL FINDINGS

Long non-coding RNAs (lncRNAs) are non-protein coding RNA transcripts longer than 200 nucleotides that exert multiple types of regulatory functions of all known cellular processes. Increasing evidence supports the role of lncRNAs in NENS development and progression with different mechanisms. In PNETs, tumor hypermethylation and silencing of long noncoding MEG3, determined activation of miR183/BRI3 axis, and cell proliferation due to c-MET oncogene activation (73). The reactivation of MEG3 by demethylating agents suppresses c-MET dependent cell proliferation suggesting that epigenetic targeting of MEG3 may represent an interesting approach in MEN1-PNETs treatment (59).

Moreover, downregulation of noncoding MEG3 and HOX genes has been associated with the development of non-functional pituitary adenomas and parathyroid tumors, respectively (74). Two other lncRNAs are implicated in the pathogenesis of PNENs, the HOX antisense intergenic RNA chromatin-modifier (HOTAIR) and the metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (75). HOTAIR reprograms neuroendocrine differentiation of prostate cancer (76), and its overexpression increases H3K27me and metastatic potential of breast cancer cells (77). Evidence supports the hypothesis that both lncRNAs through epigenetic modification activate downstream pathways Wnt/β-catenin (78) and ERK/MAPK (79) promoting epithelial-mesenchymal transition (EMT). In contrast, the upregulation of both lncRNAs in primary GEP-NETs was associated with less aggressive disease (80), as well as IncRNA, telomeric repeat-containing RNA (TERRA), is necessary to maintain genome integrity (81).

EPIGENETIC MODIFICATIONS ASSESSED IN LIQUID BIOPSIES AS PROGNOSTIC MARKERS

Unlike traditional tissue biopsies, liquid biopsies are faster, less invasive, have the potential to reflect all metastatic sites (i.e. tumor heterogeneity), and can indicate therapeutic response or progression through serial sampling. By considering the potential of genomic analysis, liquid biopsies offer a facilitated means of detecting genomic alterations and can be easily repeated over time. Moreover, cancer-specific circulating DNA (ctDNA) methylation can be used to measure circulating tumor DNA, as well as reveal the methylation patterns in the tumor (10).

In metastatic PNET patients, free circulating DNA carrying oncogenic mutations or methylation have been identified by mutation-specific droplet digital PCR (ddPCR) (82). In particular in a prospective trial (“MGMT-NET”), MGMT hypermethylation was also detectable in ctDNA instead of tissue (83, 84).

The Phase II PAZONET study is evaluating the epigenome modification in circulating tumor cells (CTCs), as potential biomarkers of response to therapy. The same goal was also assessed during SSA treatment in association with PRRT (85–87). This novel approach indicates that epigenetic profiling can identify serum biomarkers with prognostic potential (10).

EPIGENETIC TARGETED AGENTS AND CLINICAL TRIALS

Several clinical studies reported disease control targeting the somatostatin receptor (SSR), overexpressed in 70% of GEP-NETs, supporting the efficacy of both the available SSA octreotide and lanreotide (88–90). To improve the efficacy and adverse metastatic phenotype, several ongoing trials are evaluating other targets as an inhibitor of angiogenesis, immunotherapy, or combinations of them (Table 2 and Figure 1).

Epigenetics represents a very promising tool in cancer treatment because it can be reverted and epigenetic drugs are in use for the treatment of several cancer types (10, 91). In vitro studies have already tested DNA methyltransferase inhibitors (DNMTs) because of MEN1 loss increase DNA hypermethylation (92). Promising results in PNET and small intestine cell lines were obtained using inhibitors of DNA methylases and HDAC to reduce cell viability and restoring gene expression (93–97). Interestingly, decitabine increased the expression of SSTR2 and the Ga-DOTATOC uptake also in BON1 tumor-bearing mice, indicating a possible therapy implication (98). However, decitabine has not yet been trialed in humans mainly because this agent targeted the whole methylated genome. Panobinostat, a histone deacetylase inhibitor (HDACi), was used in a completed phase II trial for the treatment of low-grade NENs. Patients showed a high stable disease with the median progression-free survival (PFS) of 9.9 months, and the median overall survival was 47.3 months. However, the low response rates, limited further investigation (99). Inhibitors of the Bromo and extra terminal domain (BET) protein family, epigenetic readers of histone code, have also tested in experimental models (100). Of particular interest is Rx-001 which acts by blocking both DNMT and HDACs, activity. It showed to induce global epigenetic changes in tumors favoring infiltration of T cells, this histology was correlated with clinical benefit and sensitize tumor microenvironment to chemotherapy (101).

Novel frontier in solid tumor treatment is evaluating a combination of immunotherapy with epigenetic drugs, mainly
| Hystology                                         | Drugs and targets                                      | Phase     | NIH Clinical Trial     | End points                                                                 | n. Patients |
|--------------------------------------------------|-------------------------------------------------------|-----------|------------------------|-----------------------------------------------------------------------------|-------------|
| **Monotherapy**                                   |                                                       |           |                        |                                                                             |             |
| Solid tumor including adenocarcinoma gastric cancer | Drug: MLN8237 target aurora kinase                     | Phase I/II| EudraCT: 2008-006981-27 (2011 completed) | Safety, tolerability, and efficacy                                          | 273         |
| Advanced Neuroendocrine Cancer                   | Drug: pazopanib target antiangiogenesis                | Phase II  | NCT00454363            | Disease progression laboratory biomarker                                    | 52          |
| Low grade neuroendocrine tumor                   | Drug: panatinostat target HDAcis                       | Phase II  | NCT00985946            | Response to therapy                                                         | 15          |
| Gastro-enteropancreatic metastatic Neuroendocrine Tumor | Drug: Farnentinb target c-Kit, VEGFR2, PDGFR, VEGFR3, Fit1 and Fit3 | Phase II  | NCT01994213            | Efficacy and molecular testing including evaluation of DNA mutation, and immunohistochemistry | 53          |
| First-line treatment in newly-diagnosed patients with Advanced GI Neuroendocrine Tumor G3 | Drug: everolimus, target: mTor | Phase II  | NCT01648465 | Efficacy as first line                                                      | 25          |
| Gastroenteropancreatic Neuroendocrine Tumor G3    | Drug: Anlotinib target: tyrosin kinase inhibitor VEGFR2, PDGFR, VEGFR3, Fit1 and Fit3 | Phase II  | NCT0278844             | Clinical and molecular data of disease progression Safety                    | 60          |
| A Safety and Tolerability Study of INCAGN02385 in Select Advanced Malignancies | Biological: INCAGN02385 target LAG3 | Phase I   | NCT03538028             | Efficacy as first line                                                      | 395         |
| In Patients With Advanced Neuroendocrine Tumors After Progression on Everolimus (CABINET) | Cabozantinib S-malate, target: inhibitor tyrosine kinase VEGFR2 RET MET AXL | Phase III | NCT03375320             | Safety                                                                      | 54          |
| Treatment of Advanced Adult Solid Tumors including gastric and neuroendocrine | Drug: VMD-928 Capsules target tyrosine kinase | Phase I   | NCT03556228             | Safety                                                                      | 41          |
| Select Advanced Malignancies and Neuroendocrine Tumor | Drug: INCAGN02390 target: antagonize the TIM-3 pathway | Phase I   | NCT02652077             | Safety                                                                      | 6,245       |
| Refractory Solid Tumors, Esophageal Carcinoma Gastric (The MATCH Screening Trial) | Drug: Crizotinib Inhibitor of ALK and ROS1 | Phase II  | NCT02465060             | End-of-treatment biopsy and collection of blood samples for research purposes |             |
| Study of CVM-1118 for Patients With Advanced Neuroendocrine Tumors | Drug: CVM-1118 inhibitor of vasculogenic mimicry | Phase II  | NCT038600233            | Efficacy                                                                    | 30          |
| Unresectable Gastroenteropancreatic Neuroendocrine Tumors (GEP NETs) | Drug: Abemaciclib, target: CDK4/6 inhibitors | Phase II  | NCT03891784             | Disease progression                                                         | 37          |
| Patients With Grade 2 and Grade 3 Advanced GEP-NET (NETTER-2) | Drug: Lutathera Drug: long-acting octreotide target receptor somatostatin | Phase III | NCT03972488             | Efficacy of treatment                                                       | 222         |
| Observational Study Following Neuroendocrine tumor | FT500 Cellular Immunotherapy Alogeneic natural killer (NK) cells | Phase I   | NCT04106167             | Safety                                                                      | 76          |
| Malignant Esophagogastric Neoplasm MAGE-A4^+^+^+ for Multi-Tumor | Autologous genetically modified MAGE-A4^+^+^+ cells in subjects who have the appropriate HLA-A2 tissue marker | Phase I   | NCT03132922             | Safety                                                                      | 42          |
| Gastric cancer                                    | Drugs: Atezolizumab target: PD-L1 immune-checkpoint | Phase II  | EduaCT: 2015-000269-30 (2015-ongoing) | Tolerability and efficacy                                                   | 725         |
| Pancreatic neuroendocrine tumor                   | Drug: sunitinib target: tyrosine kinases               | Phase II  | EduaCT: 2012-000425-45 (2012-ongoing) | Effects of morning vs evening dosing on the pharmacokinetics and pharmacodynamics of sunitinib | 18          |
| Combination therapy                               | Drug: cyclophosphamide chemotherapy                  | Phase I/II| NCT00553683             | Safety and efficacy                                                         | 50          |
| Patients with unresectable, Neuroendocrine Tumor Metastatic Liver Cancer | Drug: poly-IL-15C immuno-stimulatory agent Radiation | Phase I   | NCT01263353             | Safety                                                                      | 38          |
| Advanced Metastatic NETs (COOPERATE-1)            | Pasireotide target: somatostatin receptor Drug: Everolimus Targets: mTor | Phase I   | NCT01263353             | Safety                                                                      |             |

(Continued)
because some immunosuppressive cancer antigens are regulated by acetylation of their genomic regulative element.

Some trials are testing a combination of agonists of TNF and immunotherapy via checkpoint inhibition (NCT04198766) or antibody with double specificities against PD-L1 and CTLA-4 (NCT03517488). However major interest gained depleting tryptophan enzymes as indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase (ICI). This because tryptophan is able to induce immune suppression within the cancer microenvironment. In tumor cells and nude mice have already targeted tryptophan. The authors by specific inhibitors or by preventing tryptophan promotor acetylation using histone deacetylase inhibitors as BET reported the reduction of immunosuppressive protein expression (10, 102) suggesting a novel therapeutic approach.

**CONCLUSIONS AND THE WAY FORWARD**

The development of high-throughput techniques and larger datasets (i.e. The Cancer Genome Atlas) have accelerated research even in the field of NENs. Some pioneer studies have used an integrative approach in GEP-NETs (103). EWAS showed that these epigenome profiles can distinguish subtypes with different clinical features (Figure 2). The development of the NETest and liquid biopsy, as well as organoids (104), can be used to predict response to therapy and during the clinical follow-up, although not routinely used.
FIGURE 1 | A list of epigenetic agents useful in the therapy of NETs.

Signaling pathway inhibitors
- mTOR (Everolimus)
- Hedgehog (Sonidegib)
- CD117 (Regorafenib)
- Somatostatin (Octreotide, Lanreotide)

Immune modulators
- VEGF
- SO-C101
- polyICLC

Epigenetic drugs (erasers)
- DNA demethylation agents (Decitabine)
- HDACs inhibitors (Panabonstat)

Anti angiogenic drugs
- (Pazopanib, CVM-1111E)
- Tyrosine Kinase (Sorafatin)

Immune checkpoint inhibitors
- anti-PD-1 (Nivolumab)
- anti-PD-1 (Pembrolizumab)
- anti-CTLA-4 (Ipilimumab)
- anti-PD-L1 (Avelumab)

FIGURE 2 | Major epigenetic pathways involved in NETs. IGF1R, insulin growth factor 1 receptor; FGFR, fibroblast growth factor receptor; SCF, colony stimulation factor; c-KIT, c-Kit proto-oncogene; PI3K, phosphatidylinositol 3-kinase; PTEN, Phosphatase and tensin homolog; PDK1/2, protein 3-phosphoinositide-dependent protein kinase-1; TSC1/2, Tuberous sclerosis 1/2; RheB, Ras homolog enriched in brain; HIF, hypoxia factor; RheB, Ras homolog enriched in brain; VHL, Von Hippel-Lindau; DEPDC5, DEP domain containing 5; NPRL3, neuropilin 3.
Recently, it was proposed a bioresponsive drug-delivery depot for a combination of epigenetic modulation and immune checkpoint blockade (10). From the analysis of the clinical trials reported in Table 1 and Table 2, it emerges that the evaluation of the epigenetic pathway as a biomarker of response is of most interest in many studies, involving different kinds of therapies, even in combination (10). NCT03475953 and NCT03841110 ongoing trials are evaluating the therapeutic potential of the combination of direct drugs against tyrosine kinases and immune response pathways such as PD-1/PD-L1 and the opportunity to select from patients’ blood epigenetic biomarkers. The major challenge will now be to clinically validate such epigenetic biomarkers, within clinical trials for therapeutics in the new light of precision medicine, as well as network medicine (104, 106–108).

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AUTHOR CONTRIBUTIONS

AC, FN, and CN contributed to conception and design of the study. FN, RM and CN analyzed the data and wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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