Models of Gastroenteropancreatic Neuroendocrine Neoplasms: Current Status and Future Directions

Katharina Detjen  Linda Hammerich  Burcin Özdirik  Münever Demir  Bertram Wiedenmann  Frank Tacke  Henning Jann  Christoph Roderburg

Department of Hepatology and Gastroenterology, Charité – University Medicine Berlin, Campus Virchow Klinikum and Charité Campus Mitte, Berlin, Germany

Abstract
Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are a rare, heterogeneous group of tumors that originate from the endocrine system of the gastrointestinal tract and pancreas. GEP-NENs are subdivided according to their differentiation into well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs). Since GEP-NENs represent rare diseases, only limited data from large prospective, randomized clinical trials are available, and recommendations for treatment of GEP-NEN are in part based on data from retrospective analyses or case series. In this context, tractable disease models that reflect the situation in humans and that allow to recapitulate the different clinical aspects and disease stages of GEP-NET or GEP-NEC are urgently needed. In this review, we highlight available data on mouse models for GEP-NEN. We discuss how these models reflect tumor biology of human disease and whether these models could serve as a tool for understanding the pathogenesis of GEP-NEN and for disease modeling and pharmacosensitivity assays, facilitating prediction of treatment response in patients. In addition, open issues applicable for future developments will be discussed.

Introduction
Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) represent a heterogeneous group of rare tumors that originate from the diffuse endocrine system of the gastrointestinal tract and pancreas. They are characterized by the expression of neuroendocrine markers including synaptophysin and chromogranin A. The incidence of neuroendocrine neoplasms (NENs) has significantly risen in the last 30 years, due to improved diagnostic techniques as well as awareness. The current age-adjusted incidence of GEP-NEN is estimated to be 6.98 cases per 100,000 persons for gastroenteropancreatic neuroendocrine tumors (GEP-NET) [1] and 0.4 cases per 100,000 persons for gastroenteropancreatic neuroendocrine carcinomas.
es per 100,000 persons for gastroenteropancreatic neuroendocrine carcinoma (GEP-NEC) [2] in the USA.

GEP-NENs demonstrate a variable clinical behavior depending on the primary location and the differentiation of the tumor. They are classified based on the Ki67 proliferative index: well-differentiated NET G1 displays a Ki67 index of <3% and NET G2 display a Ki67 index of 3–20%. G3 NENs with Ki67 fractions >20% are subclassified according to their morphological differentiation into GEP-NET G3 and undifferentiated GEP-NEC [3, 4]. Patients with well-differentiated NET G1 or G2 have an extraordinarily good prognosis [1], whereas survival in the G3 group ranges from several years in well-differentiated NET G3 to few month in poorly differentiated NEC with Ki67 ≥ 55% [3, 4].

Molecular Features of GEP-NET and GEP-NEC

Earlier information about the molecular underpinnings of NEN was mostly derived from observations in genetic syndromes. Hereditary GEP-NET syndromes include multiple endocrine neoplasia type 1 (MEN1) and type 4 (CDKN1B), tuberous sclerosis complex (TSC), von Hippel-Lindau (VHL), and neurofibromatosis type 1, which have been reviewed in detail elsewhere [5, 6]. Most importantly, MEN1 is an autosomal dominant syndrome, associated with pancreatic NETs (pNETs), which are caused by an inactivating mutation in MEN1, encoding for menin. Although its function is not yet fully clear, menin has been shown to act as a nuclear scaffold for transcription factors and chromatin modifying enzymes, thereby regulating pathways involved in growth and differentiation such as histone methylation, DNA damage repair, activity of the mTOR pathway, and inhibition of cell cycle progression [7].

Recently, several previously unknown hereditary NET syndromes have been identified, characterized by loss-of-function mutations in IPMK [8], MUTYH, and OGG1 [9], all affecting p53 function and/or DNA repair pathways. Furthermore, loss-of-function mutations in a subunit of the parietal cell proton pump (ATP4A) cause familial gastric NET [10].

Several of the genes that cause familial NET syndromes are now equally attributed to sporadic pancreatic NEN (pNEN) and small intestinal NEN (siNEN). MEN1 represents the most frequently affected gene in pNEN, followed by mutually exclusive mutations in death domain-associated protein (DAXX) or ATR-X (ATRX) [11, 12]. The latter 2 cooperate in deposition of histone H3.3 at telomeres, and mutations were found associated with alternative lengthening of telomeres [13] and chromosomal instability [14]. Either single or combined mutation of MEN1, DAXX, or ATRX in pNET is associated with features of islet α-cells and an adverse prognostic course [15]. Mutations within the cyclin-dependent kinase inhibitor CDKN1B, in DEPDC5 (suppressor of the mTOR-AKT pathway), PTEN, TSC1, and TSC2, were also recurrently present in NET [12, 16–18], implicating the mTOR network. Overall, however, the frequency of somatic mutations in NEN is low and epigenetic changes are increasingly recognized as drivers in NEN [5, 19, 20]. In pNET, such epigenetic features allow separation of 2 prognostic subgroups with distinct developmental features of α- or β-cell identity [21].

A distinctly different mutation profile characterizes GEP-NECs, which harbor mutations in the tumor suppressors tumor protein P53 (TP53) and retinoblastoma transcriptional corepressor 1 (RB1) in up to 70% of cases (reviewed in [5]). The almost universal inactivation of TP53 and RB1 is a well-documented feature of SCLC, which is perceived as closely related to GEP-NEC. The high rates of TP53 and RB mutations in NECs may help in differentiating NETs from NECs [22]. NECs furthermore share genetic features of adenocarcinomas from corresponding sites, for example, mutation of APC, BRAF, and K-RAS, and microsatellite instability [23, 24].

Models of GEP-NET and GEP-NEC

Advanced and faithful model systems have become a key to new insights into the molecular pathogenesis of rare tumor diseases. Indeed, progress in the generation of genetically modified organisms and the establishment of patient-derived xenograft (pdx) models improved our understanding of GEP-NEN. However, it also became clear that these experimental systems mostly reflect pNEN, do not fully recapitulate the mutation landscape, and only partially reflect the pathophysiology of human NENs. Here, we summarize current preclinical in vivo and in vitro models for GEP-NENs and discuss their advantages and limitations. We deliberately confined the in vivo part to mouse models and refer readers to earlier reviews for additional coverage of GEP-NEN in other model organisms such as rat or zebra fish [25].

Cell Lines and Cell Line-Derived Xenografts

The lack of large and representative panels of human NEN cell lines has long been a major obstacle in transla-
tional NEN research. The commonly used cell lines that originate from well-differentiated NET, that is, BON-1 [26], QGP-1 [27], and GOT-1 [28] were established decades ago and have proven to be valuable and easy to manipulate tools despite their much-discussed shortcomings (see Table 1 for available human NET cell lines). They are tumorigenic when implanted in nude mice and have been variably used in settings of subcutaneous, orthotopic, or metastatic tumor growth. In the latter application, xenografts have been helpful for studies of metastatic growth, which is poorly represented in most genetically modified mouse models of GEP-NET. CM insulinoma cells exhibit functional insulin secretion [29] but have not been reported to grow as xenografts. P-STS was first described in 2009 [30] and was later used as in vitro model for studying secretion in siNET. Several other cell lines have become available over time, but difficulties in maintaining the lines or their neuroendocrine identity have prevented broader use [30–32]. Excellent reviews on the origin and properties of GEP-NEN cell lines have been published [32, 33].

A recent promising addition to the repertoire of NET cell lines are NT-3 cells, which were established from a lymph node metastasis of a functional insulinoma obtained from a 33-year-old patient [34]. NT-3 cells form slow growing subcutaneous xenograft tumors, maintain the well-differentiated phenotype and functionality of the original tumor, and exhibit a Ki67 fraction of 15–25% akin to the human tissue. Another pNEN cell line as well as a siNET organoid line were recently announced by the NET research foundation and will be made available to all NEN researchers through the American Type Culture Collection (https://netrf.org/2020/03/11/net-cell-line/). Together with NT-3 and APL1 cells described in the “pdx, Organoid Cultures, and Primary Cultures” section, such new lines will offer valuable alternatives to existing BON and QGP1 models. The latter 2 have been fully sequenced, which revealed mutational profiles similar to pNEC rather than pNET, for example, TP53 and RAS mutations. Indeed, results from next-generation sequencing and transcriptome analyses have illustrated considerable differences between permanent NET cell lines and patients’ tumor tissues [35, 36], but also advocated cell lines as suitable tools, if they are validated in respect to clinical NEN samples [37]. Despite all shortcomings, established cell lines will remain valuable because they offer an unlimited and easy to manipulate source of material in a rare disease. In addition, established cell lines might be improved by genetic engineering based on our increasing knowledge of the genetic landscapes of NEN.

### Table 1. Overview of human GEP-NET models, indicating the site of origin of the parental human tumor and the source of the tissue used for initial implantation or culture

| Designation | Pdx | In vitro culture | Xenograft | Origin | Primary/metastasis | Reference |
|-------------|-----|-----------------|-----------|--------|-------------------|-----------|
| QGP-1a      | Cell line | + | Pancreas | Primary | Kaku et al. [27] |
| CM          | Cell line |      | Pancreas (insulinoma) | Metastasis (ascites) | Guel et al. [27] |
| BON         | Cell line | + | Pancreas | Metastasis (lymph node) | Evers et al. [26] |
| GOT1        | Cell line | + | Small intestine | Metastasis (liver) | Kölby et al. [28] |
| HuNET       | Cell line | Failed | Pancreas | Primary | Tillotson et al. [31] |
| P-STS       | Cell line | + | Small intestine | Metastasis (liver) | Pfrieger et al. [30] |
| APL1        | Cell line | + | Pancreas | Metastasis (liver) | Krampitz et al. [45] |
| NT-3        | Cell line | + | Pancreas | Metastasis (lymph node) | Benten et al. [34] |
| HNV PDX-PNET| Cell line | + | Pancreas (insulinoma) | Metastasis (liver) | Chamberlain et al. [46] |
| P0NETCL     | Cell line | | Rectum | Metastasis (liver) | Alvarez et al. [37] |

Models are organized chronologically. Reports of primary cultures are not included. GEP-NET, gastroenteropancreatic neuroendocrine tumors; pdx, patient-derived xenograft. a Publicly available at https://cellbank.nibiohn.go.jp/~cellbank/en/search_res_det.cgi?ID=2018.
mice, which prevents any studies of tumor-immune interactions or immunotherapies.

As results from larger series of systematic engraftment of NEN tissues are emerging, they emphasize the difficulty to obtain pdx from well-differentiated NEN. This is in line with the low number of permanent human NET cell lines that are available [32, 33], and with the comparatively late first reports of expandable organoid cultures of NET [42, 43]. Altogether, this attests to the need for an improved understanding of the microenvironment required for the propagation of these tumors (see Table 1 for overview of human GEP-NET models).

In the largest series of pdx reported to date, 1 model was obtained from 106 engrafted GI NEN and CUPs [44]. Initial tumor formation was achieved in 7 cases, but 6 of these tumors failed subsequent reengraftment. The 1 remaining pdx originated from a metastatic tumor with a Ki67 fraction of 70–90% as well as a novel, possibly truncating TP53 mutation, both features suggestive of a biology more representative of an NEC.

A second study focused specifically on well-differentiated pancreatic NET [45]. One pdx model plus a corresponding cell line resulted from 39 engrafted samples. The tissue originated from a liver metastasis of a non-functioning G1 pNET. Both, pdx and APL1 cells derived from the pdx exhibit autocrine HGF-MET signaling, suggesting them as a useful pNET model for drugs targeted at this pathway. The authors furthermore identified CD47 as a feature of a pNET tumor stem cell population in this model.

Another pdx model of pNEN was derived from liver metastases of a malignant insulinoma [46]. Both, pdx and original tumor sustained mutations of MEN1, BRCA2, SETD2, and PTEN, rendering this pdx uniquely suitable for evaluation of mTOR pathway inhibitors. Indeed, everolimus and sapanisertib suppressed growth in this pdx model. Of interest sapanisertib reduced growth of tumors failed subsequent reengraftment. The 1 remaining pdx originated from a metastatic tumor with a Ki67 fraction of 70–90% as well as a novel, possibly truncating TP53 mutation, both features suggestive of a biology more representative of an NEC.

As results from larger series of systematic engraftment of NEN tissues are emerging, they emphasize the difficulty to obtain pdx from well-differentiated NEN. This is in line with the low number of permanent human NET cell lines that are available [32, 33], and with the comparatively late first reports of expandable organoid cultures of NET [42, 43]. Altogether, this attests to the need for an improved understanding of the microenvironment required for the propagation of these tumors (see Table 1 for overview of human GEP-NET models).

In the largest series of pdx reported to date, 1 model was obtained from 106 engrafted GI NEN and CUPs [44]. Initial tumor formation was achieved in 7 cases, but 6 of these tumors failed subsequent reengraftment. The 1 remaining pdx originated from a metastatic tumor with a Ki67 fraction of 70–90% as well as a novel, possibly truncating TP53 mutation, both features suggestive of a biology more representative of an NEC.

A second study focused specifically on well-differentiated pancreatic NET [45]. One pdx model plus a corresponding cell line resulted from 39 engrafted samples. The tissue originated from a liver metastasis of a non-functioning G1 pNET. Both, pdx and APL1 cells derived from the pdx exhibit autocrine HGF-MET signaling, suggesting them as a useful pNET model for drugs targeted at this pathway. The authors furthermore identified CD47 as a feature of a pNET tumor stem cell population in this model.

Another pdx model of pNEN was derived from liver metastases of a malignant insulinoma [46]. Both, pdx and original tumor sustained mutations of MEN1, BRCA2, SETD2, and PTEN, rendering this pdx uniquely suitable for evaluation of mTOR pathway inhibitors. Indeed, everolimus and sapanisertib suppressed growth in this pdx model. Of interest sapanisertib reduced growth of tumors failed subsequent reengraftment. The 1 remaining pdx originated from a metastatic tumor with a Ki67 fraction of 70–90% as well as a novel, possibly truncating TP53 mutation, both features suggestive of a biology more representative of an NEC.

A second study focused specifically on well-differentiated pancreatic NET [45]. One pdx model plus a corresponding cell line resulted from 39 engrafted samples. The tissue originated from a liver metastasis of a non-functioning G1 pNET. Both, pdx and APL1 cells derived from the pdx exhibit autocrine HGF-MET signaling, suggesting them as a useful pNET model for drugs targeted at this pathway. The authors furthermore identified CD47 as a feature of a pNET tumor stem cell population in this model.

Another pdx model of pNEN was derived from liver metastases of a malignant insulinoma [46]. Both, pdx and original tumor sustained mutations of MEN1, BRCA2, SETD2, and PTEN, rendering this pdx uniquely suitable for evaluation of mTOR pathway inhibitors. Indeed, everolimus and sapanisertib suppressed growth in this pdx model. Of interest sapanisertib reduced growth of tumors failed subsequent reengraftment. The 1 remaining pdx originated from a metastatic tumor with a Ki67 fraction of 70–90% as well as a novel, possibly truncating TP53 mutation, both features suggestive of a biology more representative of an NEC.

A second study focused specifically on well-differentiated pancreatic NET [45]. One pdx model plus a corresponding cell line resulted from 39 engrafted samples. The tissue originated from a liver metastasis of a non-functioning G1 pNET. Both, pdx and APL1 cells derived from the pdx exhibit autocrine HGF-MET signaling, suggesting them as a useful pNET model for drugs targeted at this pathway. The authors furthermore identified CD47 as a feature of a pNET tumor stem cell population in this model.

A second study focused specifically on well-differentiated pancreatic NET [45]. One pdx model plus a corresponding cell line resulted from 39 engrafted samples. The tissue originated from a liver metastasis of a non-functioning G1 pNET. Both, pdx and APL1 cells derived from the pdx exhibit autocrine HGF-MET signaling, suggesting them as a useful pNET model for drugs targeted at this pathway. The authors furthermore identified CD47 as a feature of a pNET tumor stem cell population in this model.
eral, proliferation is low or absent, making the amount of primary tissue a major limitation for all subsequent analyses. Depending on the donor material, primary cultures provide the option to study neuroendocrine secretion and thus offer unique tools for evaluating drugs aimed at hypersecretion. Data from up to 20 primary cultures characterized their responsiveness to somatostatin analogs [72], everolimus [71, 73], trametinib, and vorinostat [32], or enzastaurin [75]. Prolonged culture times were achieved with NEN primary cells in perfused poly-dimethylsiloxane bioreactors [76]. A most promising approach to in vitro drug screening of patient-derived pNET primary cultures was developed by combining a short- to mid-term culture approach with 3D culture conditions and optimized growth factor supply. Organoids formed, which faithfully reproduce proliferation rates and differentiation characteristics of the original tumor tissue, and differentially responded to established clinical drugs, for

Table 2. Overview of human GEP-NEC models, indicating the site of origin of the parental human tumor and the source of the tissue used for initial implantation or culture

| Designation | pdx In vitro culture | Xenograft origin | Primary/metastasis | Reported mutations | Cisplatin response | Reference |
|-------------|----------------------|------------------|--------------------|--------------------|-------------------|-----------|
| COLO 320    | Cell line + Colon    | Primary          | TP53 (COLO 320 DM) | 6.9 μM             | Quinn et al. [47] |
| LCC-18      | Cell line + Colon    | Primary          | na                 | na                 | Lundqvist et al. [48] |
| ECC18       | Cell line + Esophagus (sc) | Primary       | na | na | Fujiwara et al. [49] |
| TEG13       | + Esophagus (sc)     | Primary          | 99% inhibition     | Aizawa et al. [50] |
| TSG15       | + Stomach (sc)       | Primary          | 69% inhibition     | Aizawa et al. [50] |
| CT-nu-1 (cell line) | + Cell line       | Duodenum         | na                 | na | Konno et al. [51] |
| N-TAK-1     | + Cell line Rectum   | Metastasis (lymph node) | na | na | Tanaka et al. [52], Koizumi et al. [54] |
| NECS-P/NECS-L | Cell line Rectum    | Primary + metastasis (liver) | na | Takahashi et al. [53] |
| A99         | Cell line + Pancreas (sc) | Metastasis (liver) | TP53; KRAS | >10 μM | Yachida et al. [55] |
| NEC-DUE1    | Cell line + Gastric (lc) | Metastasis (liver) | >10 μM | Krieg et al. [56] |
| NEC-DUE2    | Cell line + Colon (lc) | Metastasis (lymph node) | >10 μM | Krieg et al. [56] |
| GA0087      | + Stomach           | Primary          | 64% inhibition     | Jiang et al. [57] |
| TYUC-1      | Cell line + Esophagus (sc) | 0.87 μM | Okumura et al. [58] |
| CRC14       | Organoid + Colon    | Primary          | TP53; BRAF; KRAS; APC; TCF7L2 | na | Fujii et al. [59] |
| CRC19       | Organoid Rectum     | Primary          | TP53               | na | Fujii et al. [59] |
| ANI-27S     | Spheroid CUP        | Metastasis (brain) | na | Iwata et al. [60] |
| NEC-DUE3    | Cell line + Anus (sc) | Metastasis (lymph node) | 1.0 μM | Dizdar et al. [62] |
| TCC-NECT-2  | Cell line + Duodenal | Metastasis (ascites) | BRAF; TP53 | 3.5 μM | Yanagihara et al. [63] |
| HROC57      | + Spheroid cell line Colon (lc) | Primary | BRAF | 2.1 μM | Gock et al. [64] |
| SS-2        | Cell line + Colon   | Primary          | 60% inhibition* | Shinji et al. [61] |

Models are organized in chronological order. If available, drug sensitivity to cisplatin is indicated. Concentrations refer to in vitro determination of IC50, % values indicate the percentage of tumor growth inhibition in vivo, except for the cell line SS-2, which was tested in vitro. GEP-NEC, gastroenteropancreatic neuroendocrine carcinoma; pdx, patient-derived xenograft. * Oxaliplatin (10 μM) was used instead of cisplatin for treatment of SS-2 cells. The CUP was negative for TTF and cdx2 markers in immunohistochemistry, suggesting potential pancreatic or non-gastroenteropancreatic origin.

Models of GEP-NEN
example, sunitinib, everolimus, and temozolomide [74]. So far, we still lack studies that connect such in vitro drug screening of primary cultures with clinical drug responses, but in principle, they offer the prospect of personalized in vitro drug trials.

Most recently, a large set of siNET organoid cultures and several pNET cultures were reported by the group of Hans Clevers. Different from the pNET short- to mid-term cultures mentioned above, these can be maintained and expanded in vitro, raising the hope that the organoid approach may finally provide the breakthrough in search for a preclinical tool to study siNET [43]. Organoid cultures from a pNET were also reported by Ichikawa et al. [42].

Organoid cultures of colorectal NEC were established as part of larger colorectal organoid collections [59, 77]. Both phenotypic and genotypic profiling indicated a high degree of similarity with the parental tumor tissue. Most importantly, patient’s drug responses in a clinical trial were matched by the drug responses in a companion trial on organoids [78].

Exciting data from a recent communication indicated that the use of tissue explants from GEP-NE N biopsies is feasible for detection of therapeutic vulnerabilities. As in pdx, such tissue explants retain the 3-dimensional architecture of the tissue and provide more faithful representation of stromal and extracellular matrix contributions. Tissue explants were successfully established from slices of 8 out of 14 liver biopsies, received drug treatment within 24 h, and were then subjected to RNA-seq and in silico modeling for drug sensitivity and mode of action [37].

In summary, the development of model systems for culture or in vivo growth of primary NEN tissue is one of the most active and promising areas of NEN research. Combined with next-generation sequencing and the rapidly evolving powerful bioinformatics tools for data processing, these models offer unprecedented possibilities to discover clinically relevant tumor subgroups, identify drivers and vulnerabilities, and match them to therapy response in an experimental setting (please see Table 3 for a comparison of model systems). In view of shorter time frames, lower cost, the perspective to downscale the tissue requirement to biopsy size, and the relative ease of genetic manipulation, culture-based systems, such as organoids, are particularly attractive. Once validated in prospective settings, they could answer the urgent need for rational based, personalized therapy in a tumor entity that is perceived as uncommonly heterogeneous and unpredictable in its clinical course.

Genetically Modified Mouse Models of GEP-NE N

Genetically modified mice models (GEMMs) are commonly used to study human diseases and have been invaluable to oncological research, since tumors arise spontaneously in fully immunocompetent animals and develop metastases, which is an important feature of the human disease.

One drawback for developing accurate GEMMs of human sporadic NEN has been the lack of defining driver mutations. Available models mostly interrogated the role of mutations that cause familial NEN syndromes or else have addressed the roles of common oncogenes and tumor suppressors in the tissue-specific context of the neuroendocrine system. Additional NEN models became available as collateral gain from GEMM addressing islet cell biology in the context of diabetes research and, lastly, GEP-NE N emerged as pathologies in GEMMs, which were engineered as models of adenocarcinomas.

As data from sequencing efforts in large NEN cohorts emerge [11, 16, 21, 79, 80], these offer the prospect of more detailed blueprints for improved models. Despite such limitations, GEMM of GEP-NE N already proved useful for understanding NEN biology as well as for preclinical translational research, including drug trials. The most commonly used genetically modified mouse models for NENs are listed in online suppl. Table 1 (see www.karger.com/doi/10.1159/000509864 for all online suppl. material) and discussed in the following sections.

Men1 Deletion

MEN comprises a group of genetically determined syndromes associated with pathologically increased cell proliferation of endocrine organs. MEN syndrome type 1 (Wermer syndrome) develops when the MEN1 gene, a tumor suppressor gene, which is localized on the long arm of chromosome 11 (11q13), loses function, which in turn predisposes to tumors of the parathyroid glands, the pituitary gland, enteropancreatic endocrine cells, lung, and thymus. GEP-NE Ns in these patients are typically multifocal, evolve from hyperplastic precursor lesion, and occur mostly in the pancreas or at lower frequency in the duodenum and rarely in the small intestine. Though mostly indolent, they may progress to metastatic disease. About one-third of the GEP-NE Ns in MEN1 are functional, with gastrin and insulin representing the most prevalent secretion products. Thus, complications such as Zollinger-Ellison syndrome occur in MEN1 patients with gastrin-secreting tumors (reviewed in [81]). In rare cases, NECs develop in the thymus. MEN1 also constitutes the most frequently mutated gene in sporadic pNEN [11, 80,
Mice with homozygous deletion of MEN1 die at mid-gestation with multiple organ defects [83–85]. Heterozygous mice are however viable and develop a spectrum of tumors that is quite similar to the human syndrome with loss of the wild-type allele in the tumor tissues (reviewed in [86]). Tumor formation affects parathyroid glands, pancreatic islets, anterior pituitary, adrenal cortex, thyroides, lung (adenocarcinoma), and testes or ovaries. As seen in MEN1 patients, the pancreatic lesions in mice are multifocal and develop along a hyperplasia, adenoma, and carcinoma sequence. With respect to hormone expression, tumors in the pancreas were predominantly insulinomas, but glucagonomas, mixed hormone-producing tumors, and extrapancreatic, that is, duodenal gastrinomas were also reported, depending on the study [83, 85, 87, 88]. The frequency of glucagon-expressing tumors in mice with heterozygous menin deletion is influenced by the genetic background, as shown by backcrossing a model of MEN1 on a mixed 129S6/SvEv and C57BL/6 background to generate congenic C57BL/6 and 129S6/SvEv strain backgrounds. Glucagon-expressing tumors accounted for almost 30% of pNEN lesions in a 129S6/SvEv background but merely 2% of pNENs in C57BL/6 mice, suggesting the influence of genetic modifiers that impinge on tumor cell origin, plasticity, or tumor progress. Neither the overall incidence of pNEN nor the number of insulin-positive tumors differed between the 2 strains [89].

Besides global heterozygous menin deletion, different mouse models with cell type-specific homozygous menin deletion have been generated using the Cre/LoxP system: conditional deletion of Men1 within β-cells using the rat insulin promoter (RIP) to drive Cre expression generated insulinomas [90–92], which occurred at an earlier time point (5–12 months) than in mice with global heterozygous Men1 deletion. These β-cell-specific conditional menin knockouts are considered as suitable models closely reflecting insulinoma in humans. Mice with α-cell-specific loss of menin exhibit excessive α-cell hyperproliferation at very early time points, followed by formation of glucagonomas, insulinomas, and mixed tumors at 7

| Type of model           | Strength                                                                 | Disadvantages                                                                 |
|-------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| GEMM                    | Spontaneous tumor formation allows interrogation of all stages from initiation through metastatic progression | Time- and cost-intensive Epigenetic context of the introduced genetic alteration may differ from the human disease situation |
|                         | Reflects organ and host-specific tumor cell stroma interactions including immune cells | Variance in latency and incomplete penetrance complicate drug screening NEN phenotypes vary with mouse strains |
months of age [93]. Subsequently, these tumors evolve into insulinomas, attesting considerable plasticity to menin-deficient α-cells. A second model with α-cell specific deletion of menin also features insulinomas at 13–14 months, thereby corroborating the transdifferentiation process [94]. Conversely, glucagonomas were found in aging mice with β-cell-specific menin disruption, which displayed insulinomas at young age [95].

Altogether, these phenotypes indicate that loss of MEN1 permits islet cell proliferation, reprogramming to alternative islet cell fates and subsequent tumor formation. Hence, conditional MEN1-deficient mouse models should prove excellent models to experimentally address how menin loss affects differentiation trajectories and plasticity, how these mechanisms impinge on tumor formation, and whether they may be reverted by drugs.

Work on transdifferentiation of non-transformed islet cells identified α-cells as cell type with extraordinary plasticity [96], and α-cell features have been attributed to MEN1-mutant sporadic pNET [15]. Moreover, pNET tissues can be stratified by distinctive enhancer profiles, which correspond to transcriptional programs resembling α- and β-cells [21]. Importantly, the presence of DAXX or ATRX mutations had little impact on the outcome in β-like tumors but dramatically increased the risk of disease relapse in an α-like epigenetic background. Again, mechanistic studies in GEMM could leverage such observations for therapeutic strategies.

Though Men1 loss of function supported lineage plasticity of islet tumor cells, tumors across different MEN1-deficient mouse lines share functionality, a feature which is particularly remarkable in tumors that develop upon conditional, pdx1-driven knockout of Men1 in pancreatic precursor cells [97]. Mice undergo normal pancreatic development, but develop insulinomas within 10 months. In contrast, the exocrine pancreas remained unaffected, indicating a need for endocrine specific cofactors in tumors from MEN1-deficient cells.

Long latency, variable onset, mandatory functional absence of metastasis, and the occurrence of life-limiting pituitary tumors in mice with RIP-based conditional deletion of MEN1 in β-cells impede their use for modeling human sporadic pNET. Some of these limitations were addressed in modified MEN1-deficient mouse lines. Thus, Lines et al. [98] achieved temporal control of pancreatic insulinoma formation using tamoxifen-inducible Cre recombinase to abrogate MEN1, enabling studies on tumor initiation and in vivo drug assessment [99]. The latter suggested that pNEN growth can be restricted by targeting the binding of bro-
Models of GEP-NEN

ATP4A loss-of-function mutations impair the function of the parietal cell proton pump and hence result in achlorhydria, which in turn causes hypergastrinemia and leads to ECL cell hyperplasia [10]. Patients from affected families develop an atypical form of gastric NET. Mice carrying a knock-in of the corresponding ATP4A (R703C) mutation recapitulated the early onset gastric hypoacidity and hypergastrinemia. Consequently, the architecture of gastric glands deteriorated and severe hyperplasia, dysplasia, and glandular metaplasia occurred [108]. Unlike their human counterpart, the mice do not develop NEN. Nonetheless, they already provided valuable mechanistic clues regarding the relative functional contribution of achlorhydria and hypergastrinemia to the premalignant changes of the gastric glands. Thus, premalignant lesions were partly reversed by acidification of the drinking water, even though hypergastrinemia continued, pointing toward dominance of hypoacidity. ATP4A mice may therefore serve as valuable tools for prevention trials in conditions of achlorhydria and hypergastrinemia.

Vhl Deletion
VHL syndrome is an inherited disorder associated with highly vascularized tumors arising in multiple organs, including hemangioblastomas, clear cell renal cell carcinoma, pheochromocytoma/paraganglioma, serous cystadenomas, and pNEN. VHL is caused by mutations of the VHL tumor suppressor localized on the short arm of chromosome 3 (recently reviewed in [109]). While homozygous loss of Vhl in mice is lethal, Shen et al. [110] established 3 different cell-specific Vhl knockouts in the pancreas (GluCre, Rip-Cre, and Pdx1-Cre). It turned out that deletion of Vhl does not affect the function of the endocrine pancreas and loss of Vhl in differentiated α-cells or β-cells was not associated with pNET. In contrast, inactivation of Vhl in pancreatic progenitor cells caused early postnatal lethality, most likely due to impaired function of the exocrine pancreas. In surviving mice, the pancreas was largely replaced by fat at 16 months, but microcystic adenomas and islet hyperplasia characterized the remaining glandular tissue. The authors speculate that adenomas arise from the exocrine pancreatic compartment, for example, ductal or centroacinar cells, and concluded that these models will help to further define molecular mechanisms involved in VHL-associated pancreatic diseases [110].

Constitutively Active Akt
The phosphatidylinositol 3-kinase/Akt pathway is part of the mTOR network, which is frequently deregulated in pNEN [11, 80]. In line, sustained expression of dominant active myrAkt1 under an RIP promoter caused hypertrophy of β-cell mass and development of insulinomas at 12 months [111]. Insulinomas can be invasive and display a metastatic phenotype. Of note, Akt inhibitors were proposed as novel therapeutics for NET [112], emphasizing the translational value of such mouse models.

c-Myc Activation
Activation of c-Myc in adult β-cells induces proliferation but also leads to an excess of apoptosis, thereby preventing carcinogenesis. By crossing mice constitutively overexpressing the apoptosis inhibitor Bcl-xL and mice overexpressing an inducible variant of c-Myc under the RIP promoter (pIns-c-MycERTAM/RIP-Bcl-xL), Pelen garis et al. [113] presented a very interesting model of insulinoma. Upon induction of c-myc, these mice display rapid β-cell hyperplasia and unusually aggressive, poorly differentiated insulinomas develop even within 6 weeks. Lymphatic and hematogenous metastases follow at later times. When induction of c-Myc is stopped, the phenotype is completely reversible. Notably, this model only poorly reflects pathophysiological processes associated with insulinoma and/or PNET in humans. Nevertheless, the authors concluded that even highly complex tumors can be induced and maintained by only 2 interlocked molecular lesions [113].

Disruption of Glucagon Signaling
GEMMs with alterations in glucagon signaling were originally generated to assess the potential benefits of glucagon ablation in diabetes [114, 115]. Knockout of the glucagon receptor (GCGR) [114, 116], deletion of pro-hormone convertase 2 (PC2) [117], and replacement of glucagon by a glucagon/green fluorescent protein (GFP) knock-in allele [118] were strategies pursued for disruption of glucagon signaling. All strategies produced islet hyperplasia due to a dramatic increase of glucagon-positive cells. Hyperplastic islets frequently contained minor populations of cells expressing somatostatin or pancreatic polypeptide, multihormonal cells coexpressing insulin and glucagon [114, 116–119], or coexpression of markers for mature and precursor cells (glucagon and pdx1, respectively), suggestive of relaxed lineage commitment. Glucagon expression was moreover noted in scattered cells of pancreatic ducts adjacent to hyperplastic islets [114, 116, 117], suggesting that putative stem cells in the ductal compartment are recruited to increase α-cell mass, as has been shown in models of β-cell regeneration following injury [120, 121]. Hyperplastic changes were reversed by glucagon substitution in PC2 mice, in line.
with an adaptive function. Left untreated, hyperplastic islets of PC2 knockout mice progressed to dysplastic lesions, adenomas, and invasive carcinomas with occasional areas of anaplastic cells. Similarly, glucagon receptor knockout mice developed by Yu et al. [116] featured dysplastic lesions and finally overt NEN in 10- to 12-month-old mice, with loss of hormone expression in a portion of the tumors. Metastases were absent [114, 117] or rare (1/14 mice [116]). Macroscopic metastases occurred at higher frequencies in mice with replacement of proglucagon [118]. Screening of these mice for micrometastases based on GFP expression documented full penetrance of liver involvement and lung lesions in >90% of mice. Intriguingly, tumor growth remained dependent on endocrine factors produced specifically in the glucagon/GFP mice, as allograft transplanted tumors proliferated in these mice, but not in proglucagon proficient wild-type mice [118].

As tools for studying NEN, mice with disrupted glucagon signaling offer an ideally suited model for Mahvash disease, a rare familial pNET syndrome caused by biallelic inactivating mutations of the glucagon receptor gene. The models also have benefits for studying sporadic pNEN, because invasive carcinomas arise without need for an artificial oncogene such as simian virus 40 (SV40). However, the profound metabolic changes that initiate these pNENs lack a tangible correlate in sporadic NEN and - apart from an implication of menin loss - the transformation process remains undefined. Identification of participating mechanisms should be feasible and informative for sporadic pNEN and future model use, which offers the critical benefit of producing metastatic disease.

Another interesting feature of these NEN models is the implication of ductal cells with putative stem cell characteristics. The existence of adult pancreatic stem cells in humans has been a matter of debate and, consequently, there are no data to support development of pNEN from a ductal stem cell compartment in humans at this time. Recently, however, bona fide stem cells residing in pancreatic ducts have been reported [122, 123]. Thus, their role in pancreatic endocrine adaptation and transformation will hopefully be elucidated in the near future.

Finally, the relaxed lineage commitment of the islet tumors with coexpression of α- and β-lineage markers is a feature of some sporadic pNEN as well as aging normal pancreatic islet cells [21, 124]. Similarly, the nonfunctioning tumors that occasionally arose have a counterpart in human sporadic NEN. Models with disrupted glucagon action may thus be suitable to address the role of lineage plasticity and dedifferentiation in pNEN.
ingly, almost one-third of these mice develop pNENs as part of a more diverse spectrum of tumors including mesenchymous, pituitary, and Leydig cell tumors.

Overexpression of polyoma middle T (PyMT) has been used as a surrogate for activation of oncogenic signaling pathways (e.g., mTOR, MAPK, or HIPPO signaling). When conditionally expressed in β-cells, PyMT causes hyperplasia. An additional loss of p53 and p16/p19 in β-cells individually and cooperatively induced pNETs. If conditional expression of PyMT is directed to pancreatic precursors via control by the pdx1 promoter [131], additional acinar cell carcinomas arise, albeit at low frequency (10%). Deletion of p53 in the progenitor model induced full penetrance of metastatic acinar cell carcinoma, a phenotype that remained unchanged by co-deletion of p16/p19 [132]. Much in contrast, long latency pNET developed upon exclusive deletion of p16/p19 in pdx1 progenitor cells. Together, these findings supported a causal role of p16/p19 in pNEN, at least in a background of PyMT. In earlier models, the TVA receptor for avian leukemia sarcoma virus subgroup A under the control of the elastase promoter was used for delivery of avian retrovirus-bearing PyMT or c-Myc to acinar cells in newborn mice. Of note, amphicrine phenotypes with coexpression of chymotrypsin and synaptophysin emerged upon postnatal acinar cell-specific PyMT expression in a background of either p53 or p16/p19 deficiency [133, 134], supporting a permissive role for both tumor suppressors in the acquisition of NE features in otherwise acinar tumors. By comparison, the virus delivery of c-Myc to the acinar cell compartment of p16/19-deficient mice led to the exclusive development of well-differentiated insulinomas [133], a finding that further supports the contribution of p16/19 inactivation for neuroendocrine differentiation and furthermore an oncogenic function of c-Myc that either depends on or causes endocrine lineage differentiation.

A permissive role of p53 deficiency in neuroendocrine differentiation is also suggested from models testing the cooperative action of p53 loss and chronic inflammation in the pancreas. More specifically, conditional expression of cyclooxygenase-2 or IkB kinase-2 (to induce chronic inflammation) as well as conditional loss of p53 were driven by the elastase promoter and were therefore restricted to acinar cells [135]. Despite this acinar origin, mixed carcinomas of different histologic subtypes emerged, including acinar cell carcinoma, ductal adenocarcinoma, poorly differentiated tumors with sarcomatoid features, and NEC, all of which retained wild-type K-ras. This suggests that NEC may arise from dedifferen-

Human Papillomavirus

High-risk human papillomavirus (HR-HPV) was recently found in anorectal NEC without mutations in TP53 or RB1, suggesting HR-HPV infection as an alternative mechanism of E2F deregulation in NEC [141]. In line with such a mechanism, gastric NEC with full penetrance in mice with expression of HPV-16 early region under the control of the bovine keratin 6 promoter [142].

Altogether, most of the GEMMs described above give rise to pNET, produce functioning tumors within 6 through >12 months, and lack metastasis. Thus, there remains an unmet need for models of nonfunctional, well...
differentiated but metastatic pNET as well as for midgut tumors. Human sporadic pNETs segregate into subgroups characterized by differentiation features of either α- or β-islet cells, and a recurrent theme is a more adverse prognosis in patients with α-like features. This raises the question whether targeting of transgenes to α-cells might yield more aggressive models than use of the RIP system. Another layer of complexity relates to the timing of transgene expression. Thus, sporadic MEN1 mutations in humans were largely confined to α-like pNET, but a cohort of patients with MEN1 syndrome exhibited no enrichment of α-like tumors. Perhaps, inducible strategies in adult α-cells and/or combination with DAXX/ATRX inactivation will be required for more metastatic models. A mouse line that allows tamoxifen-inducible Cre expression in α-cells and enteroendocrine L-cells without disrupting proglucagon gene expression has recently become available (Ggc-CreER<sup>+</sup>[143]).

Present models with full penetrance have already been used for drug trials [97, 99, 100], and these settings offer the unique opportunity to address the role of the immune system in the development of well-differentiated pNETs and their response to treatments. This applies even more to GEMMs representing NEC, which also allow the study of metastatic disease [144] along physiologic routes in an immune competent setting, a clear advantage when compared to injection of permanent cell lines in xenograft metastasis models. Apart from such emerging translational applications, GEMMs that model the genetic landscape of GEP-NENs will remain invaluable tools for understanding their pathobiology.

### SV40-Based Transgenic Mouse Models

Preceding the GEMMs with alterations in NEN-related genes, transgenic mice with overexpression of the SV40-Tag under control of endocrine-specific or epithelial promoters have provided a range of models for NEN.

### SV40-Tag Expression Controlled by Endocrine Promoters

The RIP-Tag mouse model, in which the RIP drives the expression of Tag specifically in islet cells and β-cells become highly proliferative, represents one of the best-studied models in the context of GEP-NEN [145–147]. While several different lines were tested over the years, the RIP1-Tag2 and RIP1-Tag5 lines are most commonly used [145, 147–150]. RIP1-Tag2 mice feature early expression of Tag with β-cell hyperplasia occurring at 8 weeks and die prematurely at ≈9–12 weeks due to hypoglycemia. Mice fed with a high-carbohydrate diet survive longer and develop multiple insulinomas at approximately 10 weeks, which progress to highly invasive islet cell carcinomas [145]. Tumors in RIP1-Tag2 mice display an aggressive phenotype with heavy tumor load, but few lymphatic metastases and rare hepatic (micro)metastases [118, 151]. Though SV40-Tag expression is detected in all islets of RIP1-Tag2 mice, <2% of islets progress to islet carcinomas, suggesting the contribution of additional transforming events. At the mechanistic level, the SV40 Tag viral oncoprotein acts via inactivation of p53 and Rb. RIP1-Tag2 mice exhibit residual Rb activity, though, since reduced latency and increased insulinoma burden were found upon added conditional deletion of Rb [152].

RIP1-Tag mice constitute one of the pioneer transgenic models of cancer with impact far beyond the biology of NEN. They have been instrumental for developing concepts of tumor angiogenesis and for translating them into clinical therapies [146]. Data from RIP1-Tag2 mice also implicated the innate immune system in the progression of pNEN, as depletion of tumor-associated macrophages prevented angiogenesis and transformation of hyperplastic islet into invasive carcinoma [153]. RIP1-Tag2 mice also develop poorly differentiated NEC, although at much lower frequencies than insulinomas [154].

RIP1-Tag2 mice represent an excellent model for studying highly vascular malignant insulinomas. Their well-defined stepwise progression with concomitant neoangiogenesis uniquely qualified RIP1-Tag2 mice for use in preclinical intervention trials [146]. Thus, results from drug trials with everolimus and sunitinib in RIP1-Tag2 mice anticipated the positive outcome of subsequent clinical trials in sporadic NEN [155–159] even though sporadic human tumors lack an obvious correlate to the well-defined multistep sequence of tumor initiation and progression in RIP1-Tag2 mice. Possibly, an accurate representation of the tumor microenvironment of sporadic (p)NET in RIP1-Tag2 is the determinant that accounts for the good predictive capacity of preclinical trials in mice. Indeed, work on the RIP1-Tag2 model has highlighted the importance of the tumor microenvironment for mechanisms of resistance to antiangiogenic therapies and for strategies to circumvent resistance (reviewed in [160–162]). The latter include combined therapies with additional targeting of c-met [163], PDL-1 [164], or mTOR [165], suggesting such combinations might also benefit patients with pNET.

In RIP1-Tag5 mice, T antigen expression is observed somewhat later at 10 weeks and insulinoma develop by 25 weeks of age. Of note, RIP1-Tag5 mice display a strong activation of the immune system against the T antigen;
thus, many experts have proposed these mice as a model to study the role of immune-related processes in the development of NEN [148, 149].

Progression of insulinomas to invasive carcinomas varied with different genetic backgrounds of RIP1-Tag2 mice [166]. Following up on genetic linkage analysis, the anaplastic lymphoma kinase (ALK) emerged as a driver of invasiveness in the C57BL/6 background when compared to C3HeB/Fe mice.

Crossing mice from a C57BL/6 to other inbred mice strains also yielded variants of the RIP1-Tag2 model, which more closely match the biology of human sporadic NENs. Thus, nonfunctioning pNENs with hepatic metastases develop in an AB6 [167] or B6A [168] genetic background. Despite the high similarity of the AB6 and BA6 genetic background, phenotypes of the 2 strains differed: RIP1-Tag2 mice exhibit reduced pancreatic tumor load and metastases as well as de novo occurrence of siNEN specifically in the BA6 background, implicating imprinted genes in these phenotypic traits. Fittingly, a loss of imprinting with increased expression of the IGF2 gene was found in human siNET tissue samples. Moreover, decreased gene dosage of the IGF2 inhibitory interaction partner IGFBP1 in RIP1-Tag2/IGfbp1<sup>+/−</sup> mice recapitulated the development of siNEN lesions, and phenocopied the reduction of pancreatic and hepatic tumor load observed in the B6A background [168].

Different genetic backgrounds can hence be exploited to better understand and modify the spectrum and behavior of NEN. Such modifications may answer specific needs in preclinical research, for example, representation of hepatic metastasis or midgut NET.

As described above, RIP1-Tag2 mice do not regularly develop tumors in the small intestine in a C57BL/6 background, although occasional lesions have been reported [169]. However, small intestinal cancers with almost complete penetrance were obtained upon combined expression of SV40-Tag with polyoma small T [169, 170]. Secretin production as well as early invasive and metastatic growth were characteristics of these small intestinal carcinomas. The permanent murine STC1 cell line was derived from these mice and has been used to model siNEN in vitro.

In several other experimental approaches, combined intestinal and pancreatic tumors developed due to control of SV40-Tag expression by promoters that were active in both enteroendocrine and islet cells. For instance, mice with Tag expression driven by the secretin promoter exhibited pancreatic insulinomas that resembled the tumors of RIP-Tag mice. In addition, NET with glucagon or secretin expression formed in the proximal colon. In contrast to these well-differentiated tumors, lesions in the small intestine were less differentiated as judged by immunoreactivity to neuron-specific enolase and the neural marker PGP9.5 but not enteroendocrine hormones. Finally, lymphoid-like neoplasias devoid of neural or endocrine markers occurred in liver, spleen, and thymus, suggestive of a non-enteroendocrine origin [171].

Different portions of the rat preproglucagon promoter were employed to direct SV40-Tag to islets and enteroendocrine cells. In their initial study, Efrat et al. [172] used an 850-bp fragment, which drove expression of the Tag in α-cells of the endocrine pancreas and distinct neuron populations. Mice revealed stepwise progression from α-cell hyperplasia to dysplastic lesions and glucagonomas, including invasive and metastatic tumors. Abundant expression of neuroendocrine markers characterized benign lesions, but not an invasive carcinoma with concurrent metastasis that had formed [173], a finding that is reminiscent of the poorly differentiated islet carcinomas arising at minor frequency in RIP1-Tag2 mice [154].

A different tumor spectrum developed when SV40 expression was driven from a larger, 2.5-kb fragment of the preproglucagon promoter [174]. Intestinal tumors dominated the phenotype of these GluTag-Y mice, which suffered from invasive and metastatic, often, plurihormonal carcinomas, which became apparent by 4 weeks of age. Body weight was reduced to almost half compared to littermate controls and transgenic mice succumbed to premature death between 4 and 12 weeks. In the pancreas, hyperplastic islets composed of large pleomorphic cells had developed by 4 weeks and progressed to tumors in mice surviving the subsequent weeks [174, 175]. Despite expression of SV40-Tag in neuroendocrine cells of the small intestine and stomach, neither hyperplastic nor neoplastic lesion occurred there. Pancreatic islet tumors with similarity to RIP1-Tag2 mice plus pituitary tumors were observed in mice with SV40-Tag expression controlled by the vasopressin promoter [176].

Taken together, most phenotypes of mice with targeting of SV40-Tag to enteroendocrine or islet cells are remarkable for their diversity with respect to differentiation, functionality, and aggressiveness, both within 1 line and in between lines. Furthermore, closely related enteroendocrine cells apparently exhibited inherent susceptibility or resistance to the same oncogenic challenge. In retrospect, these diverse phenotypes may be in part explained by expression of SV40-Tag at different stages of lineage commitment, either en route to enteroendocrine differentiation from intestinal stem cells or during development.
of the pancreas. Combined with these specific contexts of oncogene induction, SV40-Tag expression itself in turn likely affected lineage specific differentiation programs, thereby further supporting tumor heterogeneity.

SV40-Tag Expression Controlled by Non-Endocrine Promoters

Interestingly, NENs have been observed in murine models that were initially developed for other purposes and used non-neuroendocrine promoters for targeting of SV40-Tag to specific gastroenteropancreatic tissues. Insulinomas were described along with different hepatobiliary tumors in transgenic mice expressing the SV40-Tag under a human gastrin promoter [177] or with brain tumors when using the Moloney murine sarcoma virus promoter [178].

Similarly, invasive acinar carcinoma, D-cell hyperplasia, and (less frequently) insulinomas were observed when expressing SV40 T antigen under a rat elastase 1 promoter [179], while insulinomas developed along with hepatocellular carcinoma (HCC) in transgenic mice overexpressing the large and small SV40 T antigens under a murine metallothionein-I promoter [180]. A combination of HCC and islet cell carcinoma was also seen when SV40-Tag was controlled by the L-type pyruvate kinase (L-PK) promoter. Islet cell carcinomas but not HCC lesions were diet dependent in this model, in line with regulation of the L-PK promoter by insulin and glucagon [181].

Poorly differentiated carcinomas were initiated by targeting SV40-Tag to different intestinal epithelia: Syder et al. [182] placed the SV40-Tag under conditional control of Atp4b (noncatalytic β-subunit of H, K-ATPase) and thereby directed it to committed non-neuroendocrine parietal cell precursors. They observed small cell cancers in over half of these mice by 48 weeks with metastatic spread to lymph nodes or liver [182]. Transcriptomes of the invasive cancer cells documented an induction of neurogenic transcription factors on the one hand and of transcription factors that maintain an undifferentiated state, such as Sox2 or Hey, on the other hand, when compared to non-transformed pre-parietal cells.

A second model with full penetrance of NEC utilized the intestinal trefoil factor promoter for SV40-Tag expression in colonic goblet cells [183]. Instead of mucinous adenocarcinoma, multifocal, rapidly dividing aggressive small cell cancer of the proximal colon caused premature death at 10–12 weeks of age. Transgene expression was also seen in conjunction with enlarged and dysplastic villi in the duodenum, but none of these progressed to tumor formation.

As above, the neuroendocrine phenotype came as an unexpected finding in multifocal, morphologically undifferentiated gastric carcinomas induced by SV40-Tag under control of the carcinoembryonic antigen (CEA) minimal promoter. Carcinomas progressed rapidly and ultimately caused premature death around 115 days [184]. Transcriptome analyses of tumors from these CEA424-SV40-Tag mice confirmed the expression of neurogenic transcription factors and marker genes, with partial overlap to published signatures from NEN in ATP4B mutant mice. Follow-up studies on an NEC cell line generated from a CEA424-SV40-Tag tumor indicated that the neuroendocrine signature required continued expression of the SV40-Tag. This finding suggested that the neuroendocrine phenotype resides in the mechanisms of transformation rather than in a preexistent neuroendocrine commitment of the cell of origin. By means of inducible lineage tracing of LGR5-positive adult stem cells in the CEA424-SV40-Tag model, the origin of the neuroendocrine carcinomas was more directly addressed. Results clearly separated SV40-Tag and LGR5 expression, respectively, consistent with long-lived pre-enteroendocrine precursor cells or reserve stem cells as putative tumor originating cells [185].

While the above models exhibit dominant NE differentiation, carcinomas with either epithelial or NE marker expression were observed in aging Vil-Cre-ER(T2) × LoxP-Tag mice [186]. In the absence of tamoxifen, Tag expression depended on stochastic activation of Cre recombinase in intestinal (stem) cells with active villin promoter, resulting in long latency of tumor formation and concurrent host immune response. Single tumor lesions with either glandular differentiation or undifferentiated morphology and synaptophysin expression developed. Both morphologies were observed either separate or mixed within 1 lesion, suggesting either parallel development from a common precursor or transdifferentiation between the 2 phenotypes. Based on the above features, Vil-Cre-ER(T2) × LoxP-Tag mice would be uniquely suited to model NEC and mixed neuroendocrine neoplasias. Unfortunately, the long and variably latency period in the range of 20 months counterbalances these advantages.

Conclusion

Recent advances in the molecular characterization of NEN identified novel molecular subtypes that might explain the different clinical characteristics of NEN, their distinct sensitivities to treatment, and the variable pa-
Models of GEP-NEN

**References**

1. Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. *JAMA Oncol.* 2017 Oct 1;3(10):1335–42.

2. Dasari A, Mehta K, Byers LA, Sorbye H, Yao JC. Comparative study of lung and extrapulmonary poorly differentiated neuroendocrine carcinomas: a SEER database analysis of 162,983 cases. *Cancer.* 2018 Feb 15;124(4):807–15.

3. Milione M, Maisonneuve P, Spada F, Pellegrielli A, Spaggiari P, Albarello L, et al. The clinicopathological heterogeneity of grade 3 gastroenteropancreatic neuroendocrine neoplasms: morphological differentiation and proliferation identify different prognostic categories. *Neuroendocrinology.* 2017;104(1):85–93.

4. Sorbye H, Welin S, Langer SW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol.* 2013;24(1):152–60.

5. Mafficini A, Scarpa A. Genetics and epigenetics of gastroenteropancreatic neuroendocrine neoplasms. *Endocr Rev.* 2019 Apr 1;40(2):306–36.

6. Crona J, Skogseid B. GEP-NETS UPDATE: genetics of neuroendocrine tumors. *Eur J Endocrinol.* 2016 Jun;174(6):R275–90.

7. Drejerink KMA, Timmers HTM, Brown M. Twenty years of menin: emerging opportunities for restoration of transcriptional regulation in MEN1. *Endocr Relat Cancer.* 2017 Oct;24(10):T135–145.

8. Sei Y, Zhao X, Forbes J, Szymczak S, Li Q, Trivedi A, et al. A hereditary form of small intestinal carcinoid associated with a germline mutation in inositol polyphosphate multikinase. *Gastroenterology.* 2015 Jul;149(1):67–78.

9. Dumanski JP, Raci C, Björklund P, Davies H, Ali AS, Grönberg M, et al. A MUTHY germ-line mutation is associated with small intestinal neuroendocrine tumors. *Endocr Relat Cancer.* 2017 Aug;24(8):427–43.

10. Calvete O, Reyes J, Zúñiga S, Paumard-Hernández B, Fernández V, Bujanda L, et al. Exome sequencing identifies ATP4A gene as responsible of an atypical familial type I gastrectomy syndrome. *Hum Mol Genet.* 2015 May 15;24(10):2914–22.

11. Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science.* 2011 Mar 4;331(6021):1199–203.

12. Scarpa A, Chang DK, Nones K, Corbo V, Patch AM, Bailey P, et al. Whole-genome landscape of pancreatic neuroendocrine tumors. *Nature.* 2017 Feb 15;543(7643):65.

13. Heaphy CM, de Wilde RF, Jiao Y, Klein AP, Edil BH, Shi C, et al. Altered telomeres in tumors with ATRX and DAXX mutations. *Science.* 2011 Jul 22;333(6041):425.

14. Marinoni I, Kurrer AS, Vassella E, Dettmer M, Rudolph T, Banz V, et al. Loss of DAXX and ATRX are associated with chromosome instability and reduced survival of patients with pancreatic neuroendocrine tumors. *Gastroenterology.* 2014 Feb;146(2):453–60.e5.

15. Chan CS, Laddha SV, Lewis PW, Koletsky MS, Robzyk K, Da Silva E, et al. ATRX, DAXX or MEN1 mutant pancreatic neuroendocrine tumors are a distinct alpha-cell signature subgroup. *Nat Commun.* 2018 Oct 12;9(1):4158.

16. Alvarez MJ, Subramaniam PS, Tang LH, Grunn A, Aburi M, Riechkof G, et al. A precision oncology approach to the pharmacological targeting of mechanistic dependencies in neuroendocrine tumors. *Nat Genet.* 2018 Jul;50(7):79–89.

17. Banck MS, Kanwar R, Kulkarni AA, Boora GK, Metge F, Kipp BR, et al. The genomic landscape of small intestine neuroendocrine tumors. *J Clin Invest.* 2013 Jun;123(6):2502–8.

18. Francis JM, Kiezun A, Ramos AH, Serra S, Pedamalli CS, Qian ZR, et al. Somatic mutation of CDKN1B in small intestine neuroendocrine tumors. *Nat Genet.* 2013 Dec;45(12):1483–6.

19. Karpathakis A, Dibra H, Pipinikas C, Feber A, Morris T, Francis J, et al. Progressive epigenetic dysregulation in neuroendocrine tumour liver metastases. *Endocr Relat Cancer.* 2017 Feb;24(2):L21–25.

20. Karpathakis A, Dibra H, Pipinikas C, Feber A, Morris T, Francis J, et al. Prognostic impact of novel molecular subtypes of small intestinal neuroendocrine tumor. *Clin Cancer Res.* 2016 Jan 1;22(1):250–8.

21. Cea JS, Drier Y, Drejerink KMA, Brosens LAA, Deshpande V, Epstein CB, et al. Enhancer signatures stratify and predict outcomes of non-functional pancreatic neuroendocrine tumors. *Nat Med.* 2019;25(8):1260–5.

22. Tang LH, Basturk O, Sue JJ, Klimstra DS. A practical approach to the classification of WHO grade 3 (G3) well-differentiated neuroendocrine tumor (WD-NET) and poorly differentiated neuroendocrine carcinoma (PD-NEC) of the pancreas. *Am J Surg Pathol.* 2016 Sep;40(9):1192–202.

23. Konukiewitz B, Jesinghaus M, Steiger K, Schlüter AM, Kasajima A, Sipos B, et al. Pancreatic neuroendocrine carcinomas reveal a closer relationship to ductal adenocarcinomas than to neuroendocrine tumors G3. *Hum Pathol.* 2018 Jul;77:70–9.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

**Funding Sources**

Supported by a grant from the WILL FOUNDATION, Berlin, to B.W., a gift by G. Speidel to B.W., and a generous donation in memoriam “Sören Piepers” by Dr. M.G. to B.W.

**Author Contributions**

All authors were involved in writing and designing the manuscript. All authors have read and approved the manuscript.
24. Weischnke C, Schaff CW, Yang HM, Vieth M, Veits L, Geddert H, et al. In-depth mutational analyses of colorectal neuroendocrine carcinoma with adenoma or adenocarcinoma components. *Mod Pathol.* 2017 Jan;30(1):95–103.
25. Kawasaki K, Fujii M, Sato T. Gastroentero-pancreatic neuroendocrine neoplasms: genes, therapies and models. *Dis Model Mech.* 2018; 11(2):029595.
26. Evers BM, Townsend CM Jr, Allen E, Hurburt SC, Kim SW, et al. Establishment and characterization of a human carcinoid in nude mice and effect of various agents on tumor growth. *Gastroenterology.* 1991;101(2):303–11.
27. Kaku M, Nishiyama T, Yagawa K, Abe M. Establishment of a carcinoidoembryonic antigen-producing cell line from human pancreatic carcinoma. *Gan.* 1980;71(5):596–601.
28. Kölby L, Bernhardt P, Ahlman H, Wängberg B, Johanson V, Wigander A, et al. A transplanted human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. *Am J Pathol.* 2001;158(2):745–55.
29. Gueli N, Toto A, Palmieri G, Carmenini G, Delpino A, Ferrini U. In vitro growth of a cell line originated from a human insulinoma. *J Exp Clin Cancer Res.* 1987 Jan 01;6:281–5.
30. Pfirnager R, Bemal A, Höger H, Beham A, Ingolic E, Stelzer I, et al. Establishment and characterization of three novel cell lines – P-STS, L-STS, H-STS – derived from a human metastatic midgut carcinoid. *Anticancer Res.* 2009 Jun;29(6):1951–61.
31. Tillotson LG, Lodestro C, Höcker M, Wiedenmann B, Newcomer CE, Reid LM. Isolation, maintenance, and characterization of human pancreatic islet tumor cells expressing vasoactive intestinal peptide. *Pancreas.* 2001;22(1):91–8.
32. Hofving T, Arvidsson Y, Almobarak B, Inge Evers BM, Townsend CM Jr, Upp JR, Allen E, Grozinsky-Glasberg S, Shimon I, Rubinfeld Kawasaki K, Fujii M, Sato T. Gastroentero-pancreatic neuroendocrine tumors: genes, therapies and models. *Dis Model Mech.* 2018; 11(2):029595.
33. Grozinsky-Glasberg S, Shimon I, Rubinfeld Kölby L, Bernhardt P, Ahlman H, Wängberg B, Johanson V, Wigander A, et al. A transplanted human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. *Am J Pathol.* 2001;158(2):745–55.
34. Benten D, Behrang Y, Unrau L, Weissmann Gueli N, Toto A, Palmieri G, Carmenini G, Delpino A, Ferrini U. In vitro growth of a cell line originated from a human insulinoma. *J Exp Clin Cancer Res.* 1987 Jan 01;6:281–5.
35. Pfirnager R, Bemal A, Höger H, Beham A, Ingolic E, Stelzer I, et al. Establishment and characterization of three novel cell lines – P-STS, L-STS, H-STS – derived from a human metastatic midgut carcinoid. *Anticancer Res.* 2009 Jun;29(6):1951–61.
36. Tillotson LG, Lodestro C, Höcker M, Wiedenmann B, Newcomer CE, Reid LM. Isolation, maintenance, and characterization of human pancreatic islet tumor cells expressing vasoactive intestinal peptide. *Pancreas.* 2001;22(1):91–8.
37. Hofving T, Arvidsson Y, Almobarak B, Inge Evers BM, Townsend CM Jr, Upp JR, Allen E, Grozinsky-Glasberg S, Shimon I, Rubinfeld Kawasaki K, Fujii M, Sato T. Gastroentero-pancreatic neuroendocrine tumors: genes, therapies and models. *Dis Model Mech.* 2018; 11(2):029595.
38. Kobayashi, Hwangberg L, Bernhardt P, Ahlman H, Wängberg B, Johanson V, Wigander A, et al. A transplanted human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. *Am J Pathol.* 2001;158(2):745–55.
39. Gueli N, Toto A, Palmieri G, Carmenini G, Delpino A, Ferrini U. In vitro growth of a cell line originated from a human insulinoma. *J Exp Clin Cancer Res.* 1987 Jan 01;6:281–5.
40. Pfirnager R, Bemal A, Höger H, Beham A, Ingolic E, Stelzer I, et al. Establishment and characterization of three novel cell lines – P-STS, L-STS, H-STS – derived from a human metastatic midgut carcinoid. *Anticancer Res.* 2009 Jun;29(6):1951–61.
41. Kobayashi, Hwangberg L, Bernhardt P, Ahlman H, Wängberg B, Johanson V, Wigander A, et al. A transplanted human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. *Am J Pathol.* 2001;158(2):745–55.
42. Gueli N, Toto A, Palmieri G, Carmenini G, Delpino A, Ferrini U. In vitro growth of a cell line originated from a human insulinoma. *J Exp Clin Cancer Res.* 1987 Jan 01;6:281–5.
Models of GEP-NEN

62 Dizdar L, Drusenheimer J, Werner TA, Möhlendick B, Schütte SC, Esposito I, et al. Establishment and characterization of a novel cell line derived from a small cell neuroendocrine carcinoma of the anal canal. Neuroendocrinology. 2018;107(3):246–56.

63 Yanagihara K, Kubo T, Mihara K, Kuwata T, Ochiai A, Seyama T, et al. Establishment of a novel cell line from a rare human duodenal poorly differentiated neuroendocrine carcinoma. Oncotarget. 2018 Nov 23;9(92):36503–14.

64 Gock M, Mullins CS, Harnack C, Prall F, Ramsey R, Götler A, et al. Establishment, functional and genetic characterization of a colon derived large cell neuroendocrine carcinoma cell line. World J Gastroenterol. 2018 Sep 7;24(33):3749–59.

65 Yachida S, Vakiani E, White CM, Zhong Y, Ohmoto A, Suzuki M, Takai E, Rokutan H, Mullins CS, Micheel B, Matschos S, Leuchter M, Mohamed A, Blanchard MP, Albertelli M, Barbieri F, et al. Anti-proliferative and anti-secretory effects of everolimus on human pancreatic neuroendocrine tumors primary cultures: is there any benefit from combination with somatostatin analogs? Oncotarget. 2017 Jun 20;8(25):41044–63.

66 April-Monn SL, Wiedmer T, Magdalena S, Rauber K, Boeck I, et al. A growth model of neuroendocrine tumor surrogates and the efficacy of a novel somatostatin-receptor-guided anti-body-drug conjugate: perspectives on clinical response? Surgery. 2020;167(1):197–203.

67 Mole D, Gagliano T, Gentilin E, Tagliati F, Pasquali C, Ambrosio MR, et al. Targeting protein kinase C by enzastaurin restrains proliferation and secretion in human pancreatic endocrine tumors. Endocr Relat Cancer. 2011 Aug;18(4):439–50.

68 Herring B, Whitt J, Aweda T, Ou J, Guenter R, Lapi S, et al. A growth model of neuroendocrine tumor surrogates and the efficacy of a novel somatostatin-receptor-guided anti-body-drug conjugate: perspectives on clinical response? Surgery. 2020;167(1):197–203.

69 Dizdar L, Werner TA, Drusenheimer JC, Mohlendick B, Raba K, Boeck I, et al. BRAF(V600E) mutation: a promising target for basic and preclinical studies. Int J Endocrinol. 2014 Apr;2014:6075–85.

70 Bertolino P, Tong WM, Calendo D, Wang ZQ, Zhang CX. Heterogeneous Men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1. Mol Endocrinol. 2003 Sep;17(9):1880–92.

71 Harding B, Lemos MC, Reed AA, Walls GV, Jeyabal J, Bowl MR, et al. Multiple endocrine neoplasia type 1 knockout mice develop parathyroid, pancreatic, pituitary and adrenal tumours with hypercalcaemia, hypophysiatena and hypercorticotremaea. Endocr Relat Cancer. 2009 Dec;16(4):1313–27.

72 Lines KE, Javid M, Reed AAC, Walls GV, Stevenson M, Simon M, et al. Genetic background influences tumour development in heterozygous Men1 knockout mice. Endocr Connect. 2020 May;9(5):426–37.

73 Mohamed A, Romano D, Saveana A, Roche C, Albertelli M, Barbieri F, et al. Anti-proliferative and anti-secretory effects of everolimus on human pancreatic neuroendocrine tumors primary cultures: is there any benefit from combination with somatostatin analogs? Oncotarget. 2017 Jun 20;8(25):41044–63.

74 Crabtree JS, Scacheri PC, Ward JM, Garrett-Beal L, Emmert-Buck MR, Edgomon KA, et al. A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tu- mors. Proc Natl Acad Sci U S A. 2001 Jan 30;98(3):1118–23.

75 Agarwal SK. Exploring the tumors of multiple endocrine neoplasia type 1 in mouse models for basic and preclinical studies. Int J Endocr Oncol. 2014(12):153–61.

76 Mohamed A, Romano D, Saveana A, Roche C, Albertelli M, Barbieri F, et al. Anti-proliferative and anti-secretory effects of everolimus on human pancreatic neuroendocrine tumors primary cultures: is there any benefit from combination with somatostatin analogs? Oncotarget. 2017 Jun 20;8(25):41044–63.

77 April-Monn SL, Wiedmer T, Magdalena S, Rauber K, Boeck I, et al. A growth model of neuroendocrine tumor surrogates and the efficacy of a novel somatostatin-receptor-guided anti-body-drug conjugate: perspectives on clinical response? Surgery. 2020;167(1):197–203.

78 Bertolino P, Tong WM, Calendo D, Wang ZQ, Zhang CX. Heterogeneous Men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1. Mol Endocrinol. 2003 Sep;17(9):1880–92.

79 Harding B, Lemos MC, Reed AA, Walls GV, Jeyabal J, Bowl MR, et al. Multiple endocrine neoplasia type 1 knockout mice develop parathyroid, pancreatic, pituitary and adrenal tumours with hypercalcaemia, hypophysiatena and hypercorticotremaea. Endocr Relat Cancer. 2009 Dec;16(4):1313–27.

80 Lines KE, Javid M, Reed AAC, Walls GV, Stevenson M, Simon M, et al. Genetic background influences tumour development in heterozygous Men1 knockout mice. Endocr Connect. 2020 May;9(5):426–37.

81 Liu J, Herrera PL, Carreira C, Bonnivion R, Seigne C, Calender A, et al. Alpha cell-specific Men1 ablation triggers the transdifferentiation of glucagon-expressing cells and insulina- noma development. Gastroenterology. 2010 May;138(5):1954–65.

82 Shen HC, Ylalva K, Pechhold K, Wilson A, Adem A, Hewitt SM, et al. Multiple endocrine neoplasia type 1 deletion in pancreatic alpha-cells leads to development of insulinomas in mice. Endocrinology. 2010 Aug;151(8):4024–30.

83 Li F, Su Y, Cheng Y, Jiang X, Peng Y, Li Y, et al. Conditional deletion of Men1 in the pancreatic β-cell leads to glucagon-expressing tu- mor development. Endocrinology. 2015 Jan;156(1):48–57.

84 Bramswig NC, Everett LJ, Schug J, Dorrell C, Liu C, Luo Y, et al. Epigenomic plasticity enables human pancreatic α to β cell reprogram- ming. J Clin Invest. 2013 Mar;123(3):1275–84.
97 Shen HC, He M, Powell A, Adem A, Lorang D, Heller C, et al. Recapitulation of pancreatic neuroendocrine tumors in human multiple endocrine neoplasia type 1 syndrome via Pdx1-directed inactivation of Men1. Cancer Res. 2009 Mar 1;69(5):1858–66.
98 Lines KE, Ves Nunes RP, Frost M, Yates CJ, Stevenson M, Thakker RV. A MEN1 pancreatic neuroendocrine tumour mouse model under temporal control. Endocr Connect. 2017 May;6(4):232–42.
99 Veniaminova NA, Hayes MM, Varney JM, Bottino R, Kim SK, et al. Single-cell analysis of human pancreas reveals transcriptional signatures of aging and somatic mutation patterns. Cell. 2017 Oct 5;171(2):321–e14.
100 Loomans CJM, Williams Giuliani N, Balak J, Ringnalda F, van Gurp L, Huch M, et al. Expansion of adult human pancreatic tissue organoids harboring progenitor cells with endocrine differentiation potential. Stem Cell Rep. 2018 Mar 13;10(3):712–24.
101 Enge M, Arda HE, Mignardi M, Beausang J, Bottino R, Kim SK, et al. Cooperative tumorigenic effects of germline mutations in Rb and p53. Nat Genet. 1994 Aug;7(4):480–4.
102 Gelling RW, Du XQ, Dichmann DS, Romer A, Aye T, Toschi E, et al. Transdifferentiation of pancreatic ductal cells to endocrine beta-cells. Int J Exp Pathol. 2014 Feb;95(1):29–48.
103 weekends 11–22.
104 Loffler KA, Biondi CA, Gartside MG, Malumbres M, et al. Wide specificity of Myc oncogene-dependent alpha cell hyperplasia in glucagon receptor knockout mice. Proc Natl Acad Sci U S A. 2003 Feb 4;100(3):1438–43.
105 Parker JC, Andrews KM, Allen MR, Stock JL, McNish JD. Glycemic control in mice with targeted disruption of the glucagon receptor gene. Biochem Biophys Res Commun. 2002 Jan 18;290(2):839–43.
106 Yu R, Dhall D, Nissen NN, Zhou C, Ren SG. Pancreatic neuroendocrine tumors in mice deficient in Men1 and Rb1 knockout mice. Oncogene. 2007 Jun 7;26(27):4009–17.
107 Veniaminova NA, Hayes MM, Varney JM, Merchant JL. Conditional deletion of Men1 and sox15 in the adult human exocrine pancreas are associated with endocrine differentiation. J Biol Chem. 1990 Nov 15;265(32):19916–22.
108 Nordstrom-O’Brien M, van der Luit JB, van Rooijen E, van den Ouweland AM, Ma-joor-Krakauer DF, Lolkema MP, et al. Genetic analysis of von Hippel-Lindau disease. Hum Mutat. 2010 May;31(5):521–37.
109 Shen HC, Adem A, Ylaya K, Wilson A, He M, Lorang D, et al. Deciphering von Hippel-Lindau (VHL/Vhl)-associated pancreatic manifestions by inactivating Vhl in specific pancreatic cell populations. PLoS One. 2009;4(4):e4697.
110 Brooks KB, Kistler J, Veenstra R, Silliman R, et al. Constitutively active Akt1 expression in mouse pancreas requires S6 kinase 1 for insulinoma formation. J Clin Invest. 2008 Nov;118(11):3629–38.
111 Wolin EM. PI3K/Akt/mTOR pathway inhibitors represent po-tential drugs for treating pancreatic and bronchial neuroendocrine tumors. Oncogene. 2017 May 15;36(5):e332.
112 Wolin EM. PI3K/Akt/mTOR pathway inhibitors represent po-tential drugs for treating pancreatic and bronchial neuroendocrine tumors. Oncogene. 2017 May 15;36(5):e332.
113 Lewis BC, Klimstra DS, Varmus HE. The c-myc and PyMT oncogenes induce different tumor types in a somatic mouse model for pancreatic cancer. Genes Dev. 2003 Dec 15;17(24):3127–38.
Models of GEP-NEN

134 Morton JP, Klimstra DS, Mengeau ME, Lewis BC. Trp53 deletion stimulates the formation of metastatic pancreatic tumors. Am J Pathol. 2008 Apr;172(4):1081–7.

135 Swidnicka-Siergiejko AK, Gomez-Chou SB, Cruz-Monserrate Z, Deng D, Liu Y, Huang H, et al. Chronic inflammation initiates multiple forms of K-Ras-independent mouse pancreatic cancer in the absence of TP53. Oncogene. 2017 Jun 1;36(22):3149–58.

136 Gidekel Friedlander SY, Chu GC, Snyder EL, Giriunis N, Dilubis C, Crowley D, et al. Context-dependent transformation of adult pancreatic cells by oncogenic K-Ras. Cancer Cell. 2009 Nov 6;16(5):379–89.

137 Shamir ER, Devine WP, Pekmezci M, Umetsu SE, Krings G, Federman S, et al. Identification of high-risk human papillomavirus and Rb/E2F pathway genomic alterations in mutually exclusive subsets of colorectal neuroendocrine carcinoma. Mod Pathol. 2019;32(2):290–305.

138 Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, et al. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature. 2003 Jul 31;424(6984):561–5.

139 Pei KH, Bai F, Li Z, Smith MD, Whitewolf G, Jin R, et al. Cytoplastmic CUL9/PARC ubiquitin ligase is a tumor suppressor and promotes p53-dependent apoptosis. Cancer Res. 2011 Apr 15;71(8):2969–77.

140 Parisi T, Bronson RT, Lees JA. Inactivation of the retinoblastoma gene yields a mouse model of malignant colorectal cancer. Oncogene. 2015 Nov 26;34(48):5890–9.

141 Shamir ER, Devine WP, Pekmezci M, Umetsu SE, Krings G, Federman S, et al. Identification of high-risk human papillomavirus and Rb/E2F pathway genomic alterations in mutually exclusive subsets of colorectal neuroendocrine carcinoma. Mod Pathol. 2019;32(2):290–305.

142 Searle PF, Thomas DP, Faulkner KB, Tinsley JM. Stomach cancer in transgenic mice expressing human papillomavirus type 16 early region genes from a keratin promoter. J Gen Virol. 1994 May;75(Pt 5):1125–37.

143 Ackermann AM, Zhang J, Heller A, Briker A, Kaestner KH. High-fidelity glucagon-CreER mouse line generated by CRISPR-Cas9 assisted gene targeting. Mol Metab. 2017 Mar;6(3):236–44.

144 Gräfin St. Jones CA, Sexton S, LeVea CM, Caraker SM, Hajduczok G, et al. Conditional deletion of p53 and Rb in the rein-expressing compartment of the pancreas leads to a highly penetrant metastatic pancreatic neuroendocrine carcinoma. Oncogene. 2013 Dec 20;32(30):5706.

145 Hanahan D. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. Nature. 1985 May 9–15;315(6015):115–22.

146 Tuveson D, Hanahan D. Translational medicine: cancer lessons from mice to humans. Nature. 2011 Mar 17;471(7338):316–7.

147 Singh M, Couto SS, Forrest WF, Lima A, Cheng JH, Molina R, et al. Anti-VEGF antibody therapy does not promote metastasis in genetically engineered mouse tumour models. J Pathol. 2012 Aug;227(4):417–30.

148 Adams TE, Alpert S, Hanahan D. Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. Nature. 1987 Jan 15;325(6101):223–8.

149 Onrust SV, Hartl PM, Rosen SD, Hanahan D. Modulation of L-selectin ligand expression during an immune response accompanying tumorigenesis in transgenic mice. J Clin Invest. 1996 Jan 1;97(1):54–64.

150 Pazei-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009 Mar 3;15(3):220–31.

151 Casanovas O, Hager JH, Chun MG, Hanahan D. Incomplete inhibition of the Rb tumor suppressor pathway in the context of inactivated p53 is sufficient for pancreatic islet tumorigenesis. Oncogene. 2005 Oct 6;24(44):6597–604.

152 Krug S, Abbassi R, Griesmann H, Sipos B, Wiese D, Rixin P, et al. Therapeutic targeting of tumor-associated macrophages in pancreatic neuroendocrine tumours. Int J Cancer. 2018 Oct 1;143(7):1806–16.

153 Hunter KE, Quick ML, Sadanandam A, Hanahan D, Joyce JA. Identification and characterization of poorly differentiated invasive carcinomas in a mouse model of pancreatic neuroendocrine tumor progression. Cancer Cell. 2009 Sep 15;16(3):270–87.

154 Raymond E, Dahan L, Raoul JL, Bang YJ, Bautch VL. Early invasiveness characterizes metastatic colorectal neuroendocrine tumors. Int J Cancer. 2011 Mar 15;128(6):1245–56.

155 Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of anti-PD-L1 therapy stimulates tumor immunity through HERV activation. Cancer Res. 2017 Apr 12;77(7):2207–16.

156 Raymond E, Dahan L, Raoul JL, Bang YJ, Bautch VL. Early invasiveness characterizes metastatic colorectal neuroendocrine tumors. Int J Cancer. 2011 Mar 15;128(6):1245–56.

157 Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of anti-PD-L1 therapy stimulates tumor immunity through HERV activation. Cancer Res. 2017 Apr 12;77(7):2207–16.

158 Jaeckel J, Shah MH, Ito T, Bohas CL, Wollin EM, Van Cutsem E, et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med. 2011 Feb 10;364(6):514–23.

159 Chiu CW, Nozawa H, Hanahan D. Survival benefit with proapoptotic molecular and pathologic responses from dual targeting of mammalian target of rapamycin and epidermal growth factor receptor in a preclinical model of pancreatic neuroendocrine carcinogenesis. J Clin Oncol. 2010 Oct;28(29):4425–33.

160 Mazzone M, Bergers G. Regulation of blood and lymphatic vessels by immune cells in tumors and metastasis. Annu Rev Physiol. 2019 Feb 10;81:535–60.

161 Jayson GC, Kerbel R, Ellis LM, Harris AL. Antiangiogenic therapy in oncology: current status and future directions. Lancet. 2016 Jul 30;388(10043):518–29.

162 Sennino B, Ishiguro-Oonuma T, Wei Y, Naylor RM, Williamson CW, Bhagwandin V, et al. Suppression of tumor invasion and metastasis by concurrent inhibition of c-Met and VEGF signaling in pancreatic neuroendocrine tumors. Cancer Discov. 2012 Mar;2(3):270–87.

163 Allen E, Jabouille A, Rivera LB, Lodewijckx I, Missiaen R, Steri V, et al. Combined antiangiogenic and anti-PD-L1 therapy stimulates tumor immunity through HERV activation. Sci Transl Med. 2017 Apr 12;9(385):9.

164 Allen E, Mvielle P, Warren CM, Saghafinia S, Li L, Peng MW, et al. Metabolic symbiosis enables adaptive resistance to anti-angiogenic therapy that is dependent on mTOR signaling. Cell Rep. 2016 May 10;15(6):1144–60.

165 Chun MG, Mao JH, Chiu CW, Balmain A, Hanahan D. Polyomavirus genetic control of tumor invasion in a mouse model of pancreatic neuroendocrine carcinogenesis. Proc Natl Acad Sci U S A. 2010 Oct 5;107(40):17268–73.

166 Kobayashi S, Contractor T, Vosburgh E, Du YN, Tang LH, Clausen R, et al. Alleles of Insm1 determine whether RIP1-Tag2 mice produce insulinomas or nonfunctioning pancreatic neuroendocrine tumors. Oncogene. 2019 Feb 22;38(3):16.

167 Contractor T, Clausen R, Harris G, Rosenfeld J, Carpizo D, Tang L, et al. IGFE2 drives formation of ileal neuroendocrine tumors in patients and mice. Endocr Relat Cancer. 2020 Jan 1;ERC-19-0505.R1.

168 Grant SG, Seidman I, Hanahan D, Bautch VL, Early invasiveness characterizes metastatic carcinoid tumors in transgenic mice. Cancer Res. 1991 Sep 15;51(18):4917–23.

169 Rindi G, Grant SG, Yangou Y, Ghatei MA, Bloom SR, Bautch VL, et al. Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Heterogeneity of hormone expression. Am J Pathol. 1990 Jun;136(6):1349–63.

DOI: 10.1159/000509864
171 Lopez MJ, Upchurch BH, Rindi G, Leiter AB. Studies in transgenic mice reveal potential relationships between secretin-producing cells and other endocrine cell types. J Biol Chem. 1995 Jan 13;270(2):885–91.

172 Efrat S, Teitelman G, Anwar M, Ruggiero D, Hanahan D. Glucagon gene regulatory region directs oncogene expression to neurons and pancreatic alpha cells. Neuron. 1988 Sep;1(7):605–13.

173 Rindi G, Efrat S, Ghatei MA, Bloom SR, Solcia E, Polak JM. Glucagonomas of transgenic mice express a wide range of general neuroendocrine markers and bioactive peptides. Virchows Arch A Pathol Anat Histopathol. 1991;419(2):115–29.

174 Lee YC, Asa SL, Drucker DJ. Glucagon gene 5′-flanking sequences direct expression of simian virus 40 large T antigen to the intestine, producing carcinoma of the large bowel in transgenic mice. J Biol Chem. 1992 May 25;267(15):10705–8.

175 Asa SL, Lee YC, Drucker DJ. Development of colonic and pancreatic endocrine tumours in mice expressing a glucagon-SV40 T antigen transgene. Virchows Arch. 1996 Mar;427(6):595–606.

176 Murphy D, Bishop A, Rindi G, Murphy MN, Stamp GW, Hanson J, et al. Mice transgenic for a vasopressin-SV40 hybrid oncogene develop tumors of the endocrine pancreas and the anterior pituitary. A possible model for human multiple endocrine neoplasia type I. Am J Pathol. 1987 Dec;129(3):552–66.

177 Montag AG, Oka T, Baek KH, Choi CS, Jay G, Agarwal K. Tumors in hepatobiliary tract and pancreatic islet tissues of transgenic mice harboring gastrin simian virus 40 large tumor antigen fusion gene. Proc Natl Acad Sci U S A. 1993 Jul 15;90(14):6696–700.

178 Theuring F, Götz W, Balling R, Korf HW, Schulze F, Herken R, et al. Tumorigenesis and eye abnormalities in transgenic mice expressing MSV-SV40 large T-antigen. Oncogene. 1990 Feb;5(2):225–32.

179 Bell RH Jr, Memoli VA, Longnecker DS. Hyperplasia and tumors of the islets of Langerhans in mice bearing an elastase 1-SV40 T-antigen fusion gene. Carcinogenesis. 1990 Aug;11(8):1393–8.

180 Dyer KR, Messing A. Peripheral neuropathy associated with functional islet cell adenomas in SV40 transgenic mice. J Neuropathol Exp Neurol. 1989 Jul;48(4):399–412.

181 Cartier N, Miquerol L, Tulliez M, Lepeit N, Levrat F, Grimmer G, et al. Diet-dependent carcinogenesis of pancreatic islets and liver in transgenic mice expressing oncogenes under the control of the L-type pyruvate kinase gene promoter. Oncogene. 1992 Jul;7(7):1413–22.

182 Syder AJ, Karam SM, Mills JC, Ippolito JE, Ansari HR, Farook V, et al. A transgenic mouse model of metastatic carcinoma involving transdifferentiation of a gastric epithelial lineage progenitor to a neuroendocrine phenotype. Proc Natl Acad Sci U S A. 2004 Mar;101(13):4471–6.

183 Gum JR Jr, Hicks JW, Crawley SC, Yang SC, Borowsky AD, Dahl CM, et al. Mice expressing SV40 T antigen directed by the intestinal trefoil factor promoter develop tumors resembling human small cell carcinoma of the colon. Mol Cancer Res. 2004 Sep;2(9):504–13.

184 Ihler F, Vetter EV, Pan J, Kammerer R, Debey-Pascher S, Schulzle JL, et al. Expression of a neuroendocrine gene signature in gastric tumor cells from CEA 424-SV40 large T antigen-transgenic mice depends on SV40 large T antigen. PLoS One. 2012;7(1):e29846.

185 Vetter E, Kronast M, Tölge M, Zimmermann W. Lgr5-expressing stem cells are not the cells of origin of pyloric neuroendocrine carcinomas in mice. J Pathol. 2016 Jan;238(1):42–51.

186 Czech M, Loddenkemper C, Shalapour S, Schon C, Robine S, Goldscheid E, et al. The immune response to sporadic colorectal cancer in a novel mouse model. Oncogene. 2010 Dec 16;29(50):6591–602.