REVIEW

Neuroendocrine neoplasms of lung, pancreas and gut: a morphology-based comparison

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Abstract

The bronchopulmonary (BP) and gastroenteropancreatic (GEP) organ systems harbor the majority of the neuroendocrine neoplasms (NENs) of the body, comprising 20 and 70% of all NENs, respectively. Common to both NEN groups is a classification distinguishing between well- and poorly differentiated NENs associated with distinct genetic profiles. Differences between the two groups concern the reciprocal prevalence of well and poorly differentiated neoplasms, the application of a Ki67-based grading, the variety of histological patterns, the diversity of hormone expression and associated syndromes, the variable involvement in hereditary tumor syndromes, and the peculiarities of genetic changes. This review focuses on a detailed comparison of BP-NENs with GEP-NENs with the aim of highlighting and discussing the most obvious differences. Despite obvious differences, the principle therapeutical options are still the same for both NEN groups, but with further progress in genetics, more targeted therapy strategies can be expected in future.

Key Words

- ▶ neuroendocrine neoplasm
- ▶ lung
- ▶ digestive system
- ▶ histopathology
- ▶ genetics

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Introduction

Neuroendocrine neoplasms (NENs) arise from various anatomical sites in the body but share basic features. NENs consist of two main families, well-differentiated NENs, also called neuroendocrine tumors (NETs) or carcinoids, and poorly differentiated NENs called neuroendocrine carcinomas (NECs) (Capella *et al.* 1995, Klöppel *et al.* 2017a, Rindi *et al.* 2018b). In fact, the similarities between the site-specific NEN entities are so large that a concept has been designed to include all NENs into a general classification (Rindi *et al.* 2018b).

The most important site-specific NENs are the NENs from the bronchopulmonary (BP-) and the gastroenteropancreatic (GEP) organs, as they account for over 90% of all NENs of the body (Dasari *et al.* 2018). In these sites, NECs of the BP-system are approximately 20 times more frequent than NECs of the GEP-system

(8.36 vs 0.4 per 100,000) (Dasari *et al.* 2018). BP-NECs are also much more frequent than their well-differentiated counterparts, the BP-NETs, with a ratio of approx. 7 to 1 (Gustafsson *et al.* 2008). The opposite is the case in the GEP-system. Here are NETs much more prevalent than NECs (ratio 5–10:1), with one notable exception, the esophagus, where almost no NETs occur (Klimstra *et al.* 2019). A reason for this conspicuous difference in prevalence between NETs and NECs in the BP- and GEP-systems is probably the impact of environmental cancerogenic factors. Smoking is well known as the major etiological factor in BP-NECs, making these neoplasms to the most frequent NECs in the body (Dasari *et al.* 2018). In the GEP-system, where smoking does not play a dominant role, NECs, like the conventional carcinomas in these localizations,

are more associated with other environmental factors such as diet.

Other differences between the two NEN entities regard terminology, classification related grading, variability of histological patterns, variegated hormone and transcription factor expression, site-specific occurrence of functioning syndromes, occurrence of precursor lesions, involvement in hereditary diseases and complexity of the genetic landscape.

Terminology, classification and grading

Carcinoid is the term traditionally applied to the NETs of the stomach, intestine and lung, since it was coined by Oberndorfer in 1907 in his seminal work on the NETs of the ileum, and defined by Hamperl in 1937 for the NETs of the lung (Hamperl 1937). Islet cell tumor is the term that denotes the NETs of the pancreas because of the resemblance of their cells with those of the islets. In 1995, it was suggested to call the islet cell tumor and the carcinoid of the stomach, intestine and lung 'neuroendocrine tumor' in order to make the terminology of all these neoplasms comparable (Capella *et al.* 1995). In 2000, the WHO classification of digestive system tumors took over the term neuroendocrine tumor (Solcia *et al.* 2000), while the 2004 WHO classification of lung tumors retained the term 'carcinoid' (Travis *et al.* 2004). This has resulted in the current situation that we are dealing with 'carcinoid' in the lung and 'NET' in the digestive system (Brambilla *et al.* 2015, Klimstra *et al.* 2019) (Table 1). However, since the NENs of BP- and GEP-systems are similar in terms of morphology, molecular characteristics and clinical behavior, the term BP-carcinoid could be replaced by the term BP-NET (Rindi *et al.* 2018b, Kasajima *et al.* 2019, Oka *et al.* 2020). The term neuroendocrine carcinoma can

be retained for the poorly differentiated BP-NENs, with the small cell subtype equaling 'small cell lung carcinoma (SCLC)' and the large cell subtype equaling 'large cell neuroendocrine carcinoma (LCNEC)'. In the following, we will use this adapted terminology.

In 1972, Arrigoni described and defined typical and atypical carcinoid of the lung and correlated these two subtypes to prognosis (Arrigoni *et al.* 1972). The features that were found to separate two carcinoid subtypes included mitotic count and intratumoral necrosis. In 1998, Travis *et al.* defined typical carcinoids (TCs) by a mitotic count less than 2 mitoses per 2 mm² and absence of necrosis, while atypical carcinoids (ACs) were characterized by 2–10 mitoses per 2 mm² and/or foci of necrosis (Travis *et al.* 1998). The two carcinoid subtypes differed in prognosis from each other, and also from SCLC and LCNEC (Travis *et al.* 1998). The carcinoid definition was included into the 2004 WHO classification of the BP-system (Travis *et al.* 2004). In GEP-NETs, such a correlation between morphology and prognosis was only shown in the years following the publication of the 2000 WHO classification of endocrine tumors, which included GEP-NETs (Solcia *et al.* 2000). In GEP-NETs, the prognostic stratification was based on the proliferative activity of the neoplasms, using mitotic count and/or the nuclear labeling of the tumor cells with the proliferation marker Ki67 (Klöppel & La Rosa 2018). In 2010, the WHO classification graded GEP-NETs with a mitotic count of <2/2 mm² and Ki67 of <2% as NET G1, and GEP-NETs with a mitotic count of 2–20/2 mm² and Ki67 of 2–20% as NET G2 (Bosman *et al.* 2010). NECs were defined in this grading system as neoplasms with mitotic count above 20/2 mm² and/or Ki67 index above 20% (Bosman *et al.* 2010). When these three groups were correlated to survival, a clear-cut prognostic significance became apparent (Pape *et al.* 2008, Jann *et al.* 2011).

Table 1 WHO classifications of neuroendocrine neoplasms of bronchopulmonary (BP) and gastroenteropancreatic (GEP) neuroendocrine neoplasms (BP-NEN WHO 2015 and GEP-NEN WHO 2019).

Differentiation	BP-NEN				GEP-NEN		
	Terminology	Mitoses per 2 mm ²	Necrosis	Ki67 index ^a	Terminology	Mitoses per 2 mm ²	Ki67 index
Well-differentiated	TC	0–1	No	up to 5%	NET G1	<2	<3%
	AC	2–10	Focal, if any	up to 20%	NET G2	2–20	3–20%
Poorly differentiated	LCNEC	>10	Yes	40–80%	NET G3	>20	>20%
	SCLC	>10	Yes	50–100%	Large cell type (LCNEC)	>20	>20%
Mixed NE and non-NE neoplasms	Combined SCLC / LCNEC ^b				Small cell type (SCNEC)	>20	>20%
					MinEN ^c		

^aNot included in criteria; ^bAmount of the non-NEN components is not defined; ^cEach component accounts for at least 30% of total tumor cell population. TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; NE, neuroendocrine; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; MinEN, mixed neuroendocrine and non-neuroendocrine neoplasm.

In 2014, Rindi *et al.* proposed a prognosis-relevant three-tiered grading of BP-NENs on the basis of three variables: mitotic count, Ki67 index and necrosis (Rindi *et al.* 2014). This grading, however, was not included into the WHO classification of the BP-system tumors published in 2015 (Brambilla *et al.* 2015).

In the years following the release of the 2010 WHO classification of digestive system tumors, it was increasingly recognized, especially in pancreatic NETs (PanNETs), that some NETs presented with a Ki67 index greater than 20% that qualifies such a tumor for a NEC, when the 2010 WHO classification was applied. However, careful analysis of such tumors revealed that they had retained their typical well-differentiated histology and functional capacity and did not transform into NECs. For the PanNETs, it was thus concluded that they can overlap in their proliferative activity with PanNECs, but still belong to the NET category (Klöppel *et al.* 2017a). The WHO classification from 2017 for endocrine organ tumors, including PanNENs, added, therefore, a PanNET G3 category, defined by a Ki67 index exceeding 20%, to the existing PanNEN categories (Klöppel *et al.* 2017a). The WHO 2019 classification for GEP-NENs finally adapted the NET G3 category for all NENs of the digestive system (Klimstra *et al.* 2019).

In the BP-system, a number of studies focused on a prognostic segregation using the proliferation marker Ki67. Although most of the studies found a correlation between defined Ki67 index groups and prognosis, they are difficult to compare because of the use of different cut-off values (Volante *et al.* 2015, Naheed *et al.* 2019), and the 2015 WHO classification did not use the data from these studies to develop a detailed grading system comparable to that of the digestive system NENs. Recently, others and we identified a small group of BP-NENs with well-differentiated morphology, a mitotic count of more than 10 per 2 mm² and/or a Ki67 index greater than 20% (Quinn *et al.* 2017, Kasajima *et al.* 2019, Oka *et al.* 2020, Rekhtman *et al.* 2019, Cros *et al.* 2020). Interestingly a similar group was recently described in thymic NENs (Dinter *et al.* 2019). In our study on 393 BP-NENs from Japan and Germany, we applied the 2019 WHO classification of digestive system tumors and found that BP-NETs G1 largely corresponded to TCs, while ACs corresponded to BP-NETs G2, and a few ACs and LCNECs corresponded to BP-NET G3 (Oka *et al.* 2020). The prevalence of primary BP-NETs G3 in our total cohort of BP-NENs was 4% (Kasajima *et al.* 2019, Oka *et al.* 2020). This NET G3 prevalence matches that of thymic NENs (5%) (Dinter *et al.* 2019), but is slightly higher than that

reported for the pancreas (2%) (Rindi *et al.* 2018a). The percentage of BP-NETs G3, however, can increase to up to 27 % in the course of the disease, as has recently been reported for Stage IV metastatic BP-NETs (Rekhtman *et al.* 2019). The outcome of the BP-NET G3 patients in our study was significantly worse than that of BP-NET G1/G2 patients but statistically not different from that of BP-NEC patients. After a course of 5 years, BP-NET G3 patients showed a rapid worsening in survival (Oka *et al.* 2019), similar to the clinical outcome reported in a large series of PanNETs (Rindi *et al.* 2018a).

Taken together, all the studies clearly show that BP-NENs, like GEP-NENs, can be effectively classified into prognosis relevant groups, using the Ki67 based grading system of GEP-NENs. It is particularly possible to sort out the special group of NETs G3, which morphologically, and as will be shown later, genetically relate to the well-differentiated NEN category, although clinically they behave like poorly differentiated NENs. This intriguing group of NENs that so far only includes a relatively small number of cases has to be further investigated in larger series, considering morphology, genetics and biology. In addition, the BP-NET G3 histology needs a more refined definition.

The GEP-NEN 2019 WHO classification also includes the category of mixed neuroendocrine non-neuroendocrine neoplasms (MiNENs), formerly referred to as mixed adenoneuroendocrine carcinomas (MANECs) (Table 1). MiNEN is a conceptional approach to a group of tumors that is not really an entity but consists of neoplasms, which, although mostly monoclonal, show a variable mixture of non-neuroendocrine cell elements (i.e. adenocarcinoma or squamous cell carcinoma) with usually poorly differentiated neuroendocrine cells (Klöppel *et al.* 2017a, Klimstra *et al.* 2019). The WHO classification of digestive system tumors defines MiNEN as neoplasm, in which each component accounts for more than 30% of the tumor cell population (Klimstra *et al.* 2019). In the BP-system, mixed neoplasms corresponding to MiNEN also exist, but are called combined SCLC or LCNEC. However, the definition of these combined neoplasms is not as clear as that of MiNEN, because of the largely lacking quantification of the different components. Only in the SCLC that is combined with LCNEC, it is stated that the LCNEC component should exceed 10% of the tumor cell population (Brambilla *et al.* 2015). No quantification is recommended, if SCLC or LCNEC is combined with an adenocarcinoma, a squamous cell carcinoma, a giant cell carcinoma or a spindle cell carcinoma component (Brambilla *et al.* 2015). MiNENs and combined SCLC/LCNECs have received

increased attention, due to their more frequent detection by the common use of immunohistochemical markers (La Rosa *et al.* 2016, 2018), and are currently under discussion concerning their biological and prognostic features as well as special treatment (Qin & Lu 2018, Frizziero *et al.* 2019, Zhang *et al.* 2020).

Histology and hormone production

In general, all NETs and NECs of the body have similar histology, but among the NETs there are some organ-specific growth patterns. Common to BP-NETs and GEP-NETs are solid and trabecular patterns, but in the digestive tract system, in contrast to the lung, these two basic patterns may show very specific variations, particularly in the pancreas, the periampullary duodenum, the ileum, the appendix and the rectum (Table 2). In the pancreas, a trabecular pattern connected with amyloid deposition is associated with insulin expression. Reticulated trabecular pattern and cystic changes are often observed in PanNETs with glucagon expression (Konukiewicz *et al.* 2011). Paraganglioma-like solid patterns are linked to the expression of somatostatin (Garbrecht *et al.* 2008, Konukiewicz *et al.* 2020). Solid architecture and sclerotic stroma are indicative of serotonin expression (McCall *et al.* 2012). In the duodenum, the periampullary NETs with glandular pattern and psammoma bodies express almost exclusively somatostatin (Garbrecht *et al.* 2008). In the ileum and the appendix, NETs with a solid and

cribriform pattern and peripheral palisading usually label for serotonin. In the rectum, a solid glandular pattern associates with glucagon and pancreatic polypeptide (PP) expression. Moreover, the rectal NETs express the transcription factor islet 1 (ISL1) (Agaimy *et al.* 2013), relating these NETs closely to PanNETs. All these observations demonstrate that certain neuroendocrine cell types are associated with typical architectural features of the respective tumors. The mechanisms behind these functional-structural relationships are so far not known, but it is conceivable that certain cell type specific genetic changes determine the structure of the tumors.

In contrast to the GEP-NETs with a spectrum of distinctive structural and functional features, the BP-NETs are rather simple. None of the commonly expressed hormones, that is, gastrin-related peptide (GRP), serotonin, calcitonin and/or adrenocorticotropin (ACTH) (Bonato *et al.* 1992), is combined with any particular histological pattern. Even the most conspicuous pattern, the solid-spindle cell pattern, shows neither special hormone expression, nor a clear site-specific relationship (Tsuta *et al.* 2011). It seems, therefore, that the hormonal status of BP-NET cells does not have a major impact on the architectural composition of the tumors.

Immunohistochemical markers other than hormones

Apart from the hormones that are expressed in BP- and GEP-NENs, there are other immunohistochemical

Table 2 Association between tumor site, histological pattern, and hormone expression in bronchopulmonary and gastroenteropancreatic neuroendocrine neoplasms (BP-NENs and GEP-NENs).

	Type	Site	Morphology	Hormone
BP-NENs	TC	Central	Trabecular > solid	Various hormones ^a
	AC	Peripheral	Solid and spindle cell > trabecular	Various hormones ^a
	NEC	Peripheral	Large cell > small cell	–
GEP-NENs	NET	Pancreas	Solid, trabecular	Various hormones ^b
	NET	Pancreas	Trabecular, amyloid deposition	Insulin
	NET	Pancreas	Reticulated-trabecular, cystic	Glucagon
	NET	Pancreas	Solid, paraganglioma-like	Somatostatin
	NET	Pancreas	Solid, sclerosing	Serotonin
	NET	Ileum	Solid, cribriform, palisading	Serotonin
	NET	Appendix	Solid, cribriform, palisading	Serotonin
	NET	Appendix	Tubular	Glucagon
	NET	Stomach	Solid, glandular	ECL-histamine
	NET	Duodenum	Solid, glandular	Gastrin
	NET	Duodenum	Glandular, psammoma body	Somatostatin
	NET	Rectum	Solid, glandular	Glucagon, PP
	NEC	No preferential site	Large cell >> small cell	Unusually, no hormone

^aACTH, GRP, calcitonin, serotonin are slightly more common in the periphery than in the hilar lesion; ^bInsulin, PP, somatostatin, gastrin, VIP, ACTH.

AC, atypical carcinoid; ACTH, adrenocorticotropin; ECL, enterochromaffin-like cell; GRP, gastrin-related peptide; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; PP, pancreatic polypeptide; TC, typical carcinoid.

markers that play a role in diagnosing, characterizing and identifying these neoplasms (Table 3). Of significance for the differential diagnosis are CK7, TTF-1, ISL1 and CDX2. Somatostatin receptor 2 (SST2), on the other hand, is a molecular marker of significant diagnostic and therapeutic importance.

Cytokeratin and neuroendocrine markers

Expression of CK18 is observed in BP- and GEP-NENs and proves the epithelial nature of these tumors. Interestingly, CK7, a cytokeratin usually seen in adenocarcinomas, is expressed in 60% of BP-NETs, whereas it is negative or very occasionally positive in GEP-NETs (Chan *et al.* 2012). NECs, which all express CK18, though to a variable extent, often exhibit a conspicuous dot-like staining pattern.

For the proof of neuroendocrine differentiation of the neoplasms labeling with synaptophysin (SYN) and chromogranin A (CgA) is required in GEP-NENs (Klimstra *et al.* 2019). The expression of CD56, a cell membrane-bound glycoprotein, can be a helpful adjunct in case of weak SYN and CgA stainings. However, positivity of CD56 that is not accompanied by SYN and CgA expression, does not prove the neuroendocrine nature of the tumor (Klöppel *et al.* 2017a), since it is expressed not only in NENs but also in various

other neoplasms, including basaloid squamous cell carcinoma, Ewing sarcoma, synovial sarcoma, chondrosarcoma or poorly differentiated neoplasms. Therefore, the statement in the WHO 2015 classification that CD56 positivity alone is enough to prove the neuroendocrine nature of BP-NENs (Brambilla *et al.* 2015) has, therefore, to be reconsidered in future classification of these tumors. Insulin-associated protein 1 (INSM1) has been proposed as a new neuroendocrine marker with high sensitivity (over 90% in both NETs and NECs), but needs to be further studied (Fujino *et al.* 2015, Rooper *et al.* 2017).

Transcription factors

TTF-1 is expressed in type II alveolar cells, Clara cells, and neuroendocrine cells in the peripheral airways of the lung. In BP-NETs, TTF-1 expression is more often seen in peripheral tumors (73%) than in central tumors (23%) (La Rosa *et al.* 2010). In GEP-NETs, and also in thymic NETs, TTF-1 is mostly negative (Chan *et al.* 2012). Thyroid medullary carcinoma and larynx NETs strongly express TTF-1, but can be differentiated from BP-NETs by the co-expression of CEA and calcitonin. In BP-NECs, TTF-1 is expressed in 84% of SCLCs, 77% of LCNECs (La Rosa *et al.* 2010). However, it is also expressed in GEP-NECs (up to 15%) (La Rosa *et al.* 2010) and other

Table 3 Immunohistochemical markers of bronchopulmonary and gastroenteropancreatic neuroendocrine neoplasms (BP-NENs and GEP-NENs).

		BP-NENs		GEP-NENs	
		NET (TC/AC)	NEC (LCNEC/SCLC)	NET	NEC
Neuroendocrine markers	CgA	Diffuse	Focal	Diffuse ^a	Focal
	SYN	Diffuse	Variable	Diffuse	Variable
Cytokeratins	CK7	60%	Focal	Neg	Neg
	CK20	Neg	0–5%	10–30%	Focal
	CK8/18	Diffuse	Focal	Diffuse, strong	Focal
Cell cycle markers	p53/Rb1	Normal	Overexpression/loss	Normal	Overexpression/loss
Transcription factors	TTF1	30–50%	30–80%	Neg	Focal, weak
	ISL1	Neg	80–100%	80% ^b	Neg
	PDX1	10%	–	60% ^c	–
	PAX8	8%	40% (weak)	80% ^d	Weak
	CDX2	Neg	0–25%	Diffuse ^e	50–80%
				Diffuse ^f	5%
Peptide hormones	Serotonin	30–60%	–	Rare	Rare
	Calcitonin	5–40%	20%	Rare	Rare
	ACTH	25%	5%	Rare	Neg
	GRP	40–80%	Neg	Neg	–
	PTHrP	<1%	Rare	Neg	Neg
Receptor marker	SST2A	30–100%	50–70%	70–100%	<10%

^aApart from rectal NET; ^bPancreas, duodenum and rectum; ^cPancreas, stomach and appendix; ^dPancreas and rectum; ^eGI tract; ^fmidgut.

AC, atypical carcinoid; ACTH, adrenocorticotropin; BP, bronchopulmonary; CDX2, homeobox gene; CgA, chromogranin A; CK, cytokeratin; GI, gastrointestinal; GRP, gastrin related peptide; ISL1, islet gene enhancer protein 1; LCNEC, large cell neuroendocrine carcinoma; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; PAX8, paired box gene 8; PDX1, pancreatic and duodenal homeobox 1; PTHrP, paratholmon-related protein; Rb1, retinoblastoma 1; SCLC, small cell lung carcinoma; SST2A, somatostatin receptor 2A; SYN, synaptophysin; TC, typical carcinoid; TTF1, thyroid transcription factor.

extrapulmonary NECs, such as larynx, urinary bladder, prostate and uterine cervix NECs (La Rosa *et al.* 2010).

Nuclear orthopedia homeobox (OTP) is a recently introduced transcription factor as highly specific marker for BP-NETs and is only occasionally expressed in NECs and NENs of other organs (Papaxoinis *et al.* 2017, Moonen *et al.* 2019).

ISL1 plays a crucial role in embryogenesis and differentiation of pancreatic beta cells and is expressed in almost 90% of PanNETs (including duodenal and ampullary NETs) but negative in BP-NETs (Agaimy *et al.* 2013). Therefore it is useful to distinguish BP- from GEP-NETs, particularly in case of a NET liver metastasis with unknown primary. In PanNETs, ISL1 is usually negative, while it is frequently positive in BP-NETs and medullary thyroid carcinomas (Agaimy *et al.* 2013).

Pancreatico-duodenal homeobox 1 gene product (PDX-1) is also an essential transcription factor in embryonic development of beta cell and intestinal neuroendocrine cells. PDX-1 is frequently expressed in insulin-producing PanNETs, also in duodenal and ileal NETs, but not in BP-NETs (Hermann *et al.* 2011, Yang *et al.* 2018).

CDX2 (Homeobox protein) regulates the development of intestinal epithelial cells. CDX2 is expressed in small and large intestine NETs and in few PanNETs, but constantly negative in BP-NETs (Chan *et al.* 2012). In BP-NETs and also GEP-NETs, CDX2 may be occasionally expressed (Bari *et al.* 2014, Lee *et al.* 2018).

Somatostatin receptors

SST2A labeling is strongly correlated to somatostatin receptor radio diagnosis and therapy. Interestingly, SST2A is expressed in almost all GEP-NETs, excluding insulinomas (where it is only positive in 50% of the cases), while it is only seen in about 50% of BP-NETs (Righi *et al.* 2010, Kaemmerer *et al.* 2015, Kasajima *et al.* 2019). In BP-NETs, SST2A labels 35–60% of the tumors (Righi *et al.* 2010, Kaemmerer *et al.* 2015, Kasajima *et al.* 2019, Oka *et al.* 2019), while in GEP-NETs, it is only positive in about 10% (Konukiewicz *et al.* 2017). The reason for this differential expression of SST2A in BP- and GEP-NENs is not known so far. The fact that SST2A also labels about 35–60% of BP-NETs offers an interesting treatment option for these neoplasms, although PRRT efficacy in these tumors has been questioned (Sollini *et al.* 2013). SST5 shows also a high affinity to somatostatin analogs and its expression is observed in 30–50% of BP-NETs (Vesterinen *et al.* 2019).

Functioning syndromes

Fifteen peptide hormone-producing neuroendocrine cells are found in the GEP-organs. They are involved in endocrine regulation, enzyme secretion or intestinal motility. Of the 15 peptide hormones, only 7 (insulin, glucagon, somatostatin, PP, gastrin, serotonin, histamine) show a significant production in GEP-NET cells. The normal lung contains GRP and calcitonin-producing cells (Tsutsumi 1989), and these cells are also frequently found in BP-NENs (Bonato *et al.* 1992). Hormones that are ectopic to the lung and/or the GEP-systems, but may occur in respective NETs are ACTH, vasoactive intestinal peptide (VIP), growth hormone-releasing hormone (GHRH), serotonin (only in the lung), antidiuretic hormone (ADH) and parathormon-related protein (PTHrP). Most important is ACTH, as it is frequently expressed in BP-NETs (25%) (La Rosa *et al.* 2019), and is associated with Cushing syndrome in up to 5% of these tumors. It has no prognostic impact compared to non-ACTH producing BP-NETs (La Rosa *et al.* 2019). The reason for the common expression of ACTH in BP-NET cells is not known. In the GEP system, ACTH producing NETs are rare but occur most frequently in the pancreas (Maragliano *et al.* 2015). These ACTH producing PanNETs are often metastasizing and multihormonal. In the clinical course of the disease, the patients may present with Zollinger-Ellison syndrome or hyperinsulinemic hypoglycemia, before Cushing syndrome becomes overt (Maragliano *et al.* 2015).

The NENs associated with hormone production and hormonal syndromes are almost exclusively NETs. There are a number of reports in the literature, which associate SCLC with hormone production and syndromes, notably ACTH and Cushing syndrome, as well as ADH and syndrome of inappropriate ADH secretion (Seute *et al.* 2004). However, the presented evidence for ACTH or ADH production and small cell morphology is often scarce or completely missing.

Functioning tumors are much more common among GEP-NETs than BP-NETs (Gustafsson *et al.* 2008, Pape *et al.* 2008, Jann *et al.* 2011) (Table 4). In the GEP-system most functioning tumors derive from orthotopic cells, that is, serotonin cells in the ileum, gastrin cells in the duodenum, and insulin and glucagon cells in the pancreas. Most frequent is the carcinoid syndrome that is found in 28% of GEP-NETs (Pape *et al.* 2008, Jann *et al.* 2011) and is most commonly associated with NETs in the ileum and only rarely with NETs in the pancreas and the lung (La Rosa *et al.* 2011, McCall *et al.* 2012, Halperin *et al.* 2017). Since the functionally active metabolites of serotonin are

mainly produced in the liver, the carcinoid syndrome typically occurs after intensive metastasis to the liver (Halperin *et al.* 2017). BP-NETs are positive for serotonin in 30–60% of the cases (Bonato *et al.* 1992, Vesterinen *et al.* 2018), but rarely associate with a carcinoid syndrome (Halperin *et al.* 2017) (Table 4). Serotonin expressing ileal and pancreatic NETs show an intensive sclerotic stroma, which is probably the result of the promoting effects of serotonin on fibroblast growth factor (McCall *et al.* 2012). Because in the pancreas the serotonin positive PanNETs often locate adjacent to the main pancreatic duct, these tumors cause duct stenosis with upstream dilatation

Table 4 Functioning disease associated bronchopulmonary and gastroenteropancreatic neuroendocrine tumors (BP-NETs and GEP-NETs).

	Syndrome	Prevalence (%) and type	Hormone
BP-NET ^a	Functioning: non-functioning (100%)	5%:95% ^a	
	Cushing syndrome	1–4.3% ^{a,b,c}	ACTH
	Carcinoid syndrome	1.6–8% ^{a,d,e}	Serotonin
	Acromegaly	Rare	GHRH
	Hypercalcemia	Rare	PTHrP
	Hyponatremia	Rare	ADH
	No defined syndrome	Common	Calcitonin
	No defined syndrome	Common	GRP
GEP-NET	Functioning: non- functioning (100%)	35%:65% ^f	Others ^h
	Carcinoid syndrome	28% SI-NET ^{f,i}	Serotonin
	Other syndromes	7% ^f	
	Insulinoma	PanNET	Insulin
	ZES	Duo- and PanNET	Gastrin
	Glucagonoma	PanNET	Glucagon
	Verner-Morrison syndrome	PanNET	VIP
	Cushing syndrome	Pan and GI-NET	ACTH
	Acromegaly	PanNET	GHRH
	Hypercalcemia	PanNET	PTHrP
	No defined syndrome	PanNET	Calcitonin
	No defined syndrome	Pan- and GI-NET	Others ^j

^aGustafsson *et al.* 2008; ^bGranberg *et al.* 2000; ^cLa Rosa *et al.* 2019; ^dDaddi *et al.* 2014; ^eHalperin *et al.* 2017; ^fPape *et al.* 2008 & Jann *et al.* 2011; ^gIn the literature, SCLC is often mentioned as Cushing syndrome causing primary tumor, but morphological and immunohistochemical evidence is rare/not existent; ^hPancreatic polypeptide (PP), human chorionadotrophic hormone beta, enkephalin; ⁱIncludes 3% PanNETs; ^jSomatostatin, PP and cholecystokinin in PanNET, histamin in gastric NET.

ACTH, adrenocorticotropin; ADH, antidiuretic hormone; Duo-NET, duodenal NET; GHRH, growth hormone-releasing hormone; GI-NET, gastrointestinal NET; GRP, gastrin-related peptide; NET, neuroendocrine tumor; PanNET, pancreatic NET; PTHrP, parathormon-related protein; SI-NET, small intestinal NET; VIP, vasoactive intestinal peptide.

(McCall *et al.* 2012). Appendiceal NETs also produce serotonin, but there is no convincing report associating these NETs with a carcinoid syndrome, most likely because they almost never metastasize to the liver.

GRP expressing NETs are exclusively found in the lung and are not associated with any defined hormonal syndrome. Calcitonin is not only expressed in the lung, where it is found in 5–40% of BP-NETs (Tsutsumi 1989, Nozieres *et al.* 2016, Vesterinen *et al.* 2018), but also in about 10% of PanNETs, where it often occurs together with other hormones (Uccella *et al.* 2017). Calcitonin is secreted into the circulation but a well-defined hormonal syndrome is missing, although some of the patients may present with diarrhea, and/or hypercalcemia or have carcinoid syndrome or Cushing syndrome (Nozieres *et al.* 2016).

PTHrP expression is detected in various types of lung carcinomas, especially in lung squamous cell carcinoma. Hypercalcemia due to PTHrP production in BP-NETs is very rare, reported in less than 1% of the patients, and exceedingly seldom in GEP-NETs (Daskalakis *et al.* 2019). Also very rare is acromegaly caused by ectopic GHRH but may occur in both BP- and GEP-NETs (Daddi *et al.* 2014) (Table 4).

Precursor lesions and hereditary tumor syndromes

Precursor lesions to NENs have been observed in diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH) of the lung and in forerunners to multiple endocrine neoplasia type 1 (MEN1) associated NETs of the lungs, pancreas, duodenum and stomach, as well as in the stomach with chronic atrophic gastritis. Precursor lesions to VHL associated NETs are restricted to the pancreas and have never been observed in the lung.

DIPNECH is a complex disease. It is defined by multifocal neuroendocrine cell hyperplasia in the bronchiolar epithelium, by the occurrence of tumorlets (small endocrine cell foci < 0.5 cm) and/or BP-NETs around a terminal bronchiole, and by chronic obstructive changes of the bronchioles (Brambilla *et al.* 2015). The patients present obstructive bronchiolitis with asthma-like symptoms and chronic functional lung impairment (Mengoli *et al.* 2018). DIPNECH BP-NETs were thought to be indolent but recently a metastatic rate of 13% has been reported in 46 patients (Hayes *et al.* 2018). Cause and pathogenesis of the disease are not known. DIPNECH frequently expresses peptide hormones, including GRP,

calcitonin and occasionally ACTH (Mengoli *et al.* 2018). It is not associated with any hormone-related symptom. So far, no familial background has been observed in DIPNECH patients, but the disease may be, though very rarely, associated with MEN1 (Bartsch *et al.* 2016). DIPNECH has to be distinguished from neuroendocrine cell hyperplasia that may occur adjacent to various lung tumors in up to 30% (Rizvi *et al.* 2009).

In the GEP organs, NET precursor lesions are seen in the stomach, the duodenum and pancreas. They are mostly associated with MEN1, with one notable exception. This is the occurrence of multiple enterochromaffin cell like (ECL)-cell NETs in the stomach in the presence of chronic atrophic corpus gastritis with intestinal metaplasia. The ECL-cell hyperplasia is induced and stimulated by the hypergastrinemia that follows the disappearance of parietal cells in the development of autoimmune chronic atrophic gastritis. The tumors are usually indolent and can be controlled by endoscopic removal.

The precursor lesions seen in MEN1 patients with BP-NETs were described as linear and micronodular neuroendocrine cell hyperplasia in subsegmental bronchi (Bartsch *et al.* 2016). BP-NETs manifest in about 3–13% of the MEN1 patients, a frequency that is much lower than that observed in GEP-organs affected by MEN1 tumors (50–70%) (Bartsch *et al.* 2016, Klöppel *et al.* 2017a). So far, all MEN1-associated BP-NETs were found to be hormonally inactive (Bartsch *et al.* 2016). Hormonal syndromes, in contrast, occur frequently in MEN1-associated GEP-NETs, duodenal gastrinomas and pancreatic insulinomas being observed in up to 50 and 35% of the patients, respectively (Klöppel *et al.* 2017a). These tumors are preceded by microadenomas that in the pancreas, most often express glucagon (Perren *et al.* 2007) and/or PP, and in the duodenum gastrin or somatostatin (Anlauf *et al.* 2006). BP- and PanNETs arising in MEN1 patients are usually low grade (mostly G1) and the patients show a 10-year survival of over 70% (de Laat *et al.* 2014). Aggressive NETs, as they occur in the thymus with a reported 10-year survival of 25%, are not seen in the BP- and GEP-system (de Laat *et al.* 2014).

Patients with VHL may develop PanNETs, that often show clear/lipid-rich cells, and have microadenomas in about 70% of the cases (Perigny *et al.* 2009). Neurofibromatosis 1 (NF1) patients may show periampullary NETs with somatostatin expression and psammoma body manifestation (Garbrecht *et al.* 2008). No manifestation of NF1 has been so far reported in the lung.

Genetic alterations

Recent studies, using whole-genome or targeted gene sequencing, have tremendously increased our understanding of the main genetic alterations in BP- and GEP-NENs (Table 5). In GEP-NENs, the data so far available concern mainly PanNENs and, to a small extent, ileal NENs. Ileal NENs differ distinctly in their genetic profile from that of BP-NENs and PanNENs, and are therefore separately discussed (see subsequently), while BP-NENs and PanNENs share a number of genetic features.

Common to both BP- and PanNENs is the morphologic and genetic dichotomy characterized by frequent *MEN1* mutations in NETs as opposed to frequent *TP53* and *RB1* mutations in NECs (George *et al.* 2015, Rekhtman *et al.* 2016, Scarpa *et al.* 2017, Simbolo *et al.* 2017). This genetic dichotomy is also shared with a number of other NENs of the body (Klöppel 2017). Also common to NETs and NECs of the lung and pancreas are the frequent alterations in chromatin remodeling genes (Scarpa *et al.* 2017, Simbolo *et al.* 2017, Asiedu *et al.* 2018).

Differences between BP- and PanNENs concern the frequency of *MEN1* alterations (BP-NETs 11–18% vs PanNETs 36–44%), and the near absence of *DAXX*/*ATRX* mutations in BP-NETs (less than 1%) as opposed to PanNETs (25–43%) (Jiao *et al.* 2011, Fernandez-Cuesta *et al.* 2014, Swarts *et al.* 2014, Scarpa *et al.* 2017, Simbolo *et al.* 2017, Chan *et al.* 2018, Alcalá *et al.* 2019) (Table 5). In BP-NETs, *MEN1* alterations are somewhat more common in ACs than in TCs (Swarts *et al.* 2014, Simbolo *et al.* 2017, Alcalá *et al.* 2019).

The reason why the *MEN1* gene is more frequently affected in PanNETs than in BP-NETs is not known so far. It might, however, be an indication that the neuroendocrine cells of the lung differ in their biology from pancreatic neuroendocrine cells. This might also be the reason of the different frequency of BP- and PanNETs in MEN1 patients. While PanNETs occur in about 50–70% of MEN1 patients, BP-NETs are present in less than 10% (Goudet *et al.* 2011, Bartsch *et al.* 2016). In PanNETs, *MEN1* mutations are detected in the early phase of the tumor development, since loss of *menin*, that indicates *MEN1* mutation, is frequently observed in microadenomas of the pancreas (Hackeng *et al.* 2016). In the MEN1-pancreas the development of microadenomas is characterized by biallelic inactivation of the *MEN1* gene. The latter observations together with the frequency of *MEN1* mutations in PanNETs suggest that the *MEN1* gene most likely acts as a driver gene in PanNETs,

Table 5 Comparison of common genetic alterations in bronchopulmonary and pancreatic neuroendocrine neoplasms (BP-NENs and PanNENs).

	Gene	BP-NENs	PanNENs
		Prevalence (%)	Prevalence (%)
NET	<i>MEN1</i>	11–18% ^{a,b,c,l}	37–44% ^{e,f,g}
	<i>DAXX/ATRX</i>	<1% ^a	25–43% ^{e,f,g,h,i}
	Other chromatin-remodelling genes ^o	46% ^{a,d}	50% ^e
	mTOR signaling genes	2% ^a	12–15% ^{e,f,g,i}
	<i>TP53/Rb1</i>	<10% ^a	<3% ^{e,f,g}
NEC	<i>TP53</i>	70–90% ^{a,m}	70–90% ^{i,j}
	<i>Rb1/p16</i>	70% ^a	50–60% ^{i,j}
	Other chromatin-remodelling genes ^o	55% ^a	NA
	<i>KRAS</i>	10% ^{a,n}	30–40% ^{i,j}
	mTOR signaling genes	12% ^a	NA
	<i>MEN1</i>	<1% ^a	0% ^j
	<i>DAXX/ATRX</i>	<1% ^a	0% ^k

^aSimbolo *et al.* 2017; ^bSwarts *et al.* 2014; ^cAlcala *et al.* 2019; ^dFernandez-Cuesta *et al.* 2014; ^eScarpa *et al.* 2017; ^fChan *et al.* 2018; ^gJiao *et al.* 2011; ^hSinghi *et al.* 2017; ⁱYachida *et al.* 2012; ^jKonukiewicz *et al.* 2018; ^kKonukiewicz *et al.* 2017; ^lAssociated with high grade tumor; ^mA higher prevalence of *TP53/Rb1* biallelic mutation in small cell lung carcinoma than in large cell neuroendocrine carcinoma (LCNEC); ⁿDetected in LCNEC; ^oIncludes ARID family in BP and PanNET. EIF1AX and KMT2 family in BP-NEN, STED2 and MLL3 in PanNET.

NA, no data available; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor.

while it seems to play no significant role in the development of most BP-NETs.

In PanNETs, *MEN1*, in cooperation with *DAXX/ATRX*, is probably also involved in chromosome maintenance. PanNETs with mutated *MEN1* and *DAXX/ATRX* are strongly associated with alternative lengthening of telomeres (ALT) and chromosomal instability (Marinoni *et al.* 2014, Scarpa *et al.* 2017). This seems not to be the case in BP-NETs, although data correlating *MEN1* mutations in TCs and ACs with ALT are lacking (Zaffaroni *et al.* 2005, Nishio *et al.* 2007). It was, however, shown that ALT may occur in BP-NECs but not in BP-NETs (Zaffaroni *et al.* 2005, Nishio *et al.* 2007).

In PanNETs, the *MEN1* mutation in concert with *DAXX/ATRX* mutations is associated with a PanNET phenotype characterized by an alpha-cell lineage signature (Chan *et al.* 2018). In this context, it is of interest that glucagon, together with PP and somatostatin, belongs to the most frequently expressed hormones in sporadic PanNETs (Konukiewicz *et al.* 2020). It is also the most frequent hormone in *MEN1* and non-*MEN1* microadenomas (Perren *et al.* 2007, Hermann *et al.* 2011, Hackeng *et al.* 2016).

In the group of poorly differentiated NENs, BP-NECs and GEP-NECs frequently share *TP53* and *RB1* mutations. In BP-NECs, however, *RB1* mutations are much more frequent than in GEP-NECs, while *TP53* mutations dominate in GEP-NECs (Jesinghaus *et al.* 2017, Konukiewicz *et al.* 2017, Simbolo *et al.* 2017, Konukiewicz *et al.* 2018). Recently, the genetic profiles

of the two subtypes of BP-NECs, small cell and large cell NEC, were compared with each other and also with that of BP-NETs, a study which is so far missing in GEP-NECs, simply because the number of NECs, particular of small cell NECs, is much greater in the BP-system than in the GEP-system. SCLCs, which are morphologically rather homogeneous, are characterized by both *TP53* and *RB1* alterations in over 90% of the cases. LCNECs, in contrast, show heterogeneous phenotypical features and are also genotypically heterogeneous (George *et al.* 2015, 2018, Rekhtman *et al.* 2016, Derks *et al.* 2018b). About 40% of LCNECs share the genetic profile with SCLCs (*TP53/RB1* co-alteration, *MYCL1* and *SOX2* amplification) and show in 72% SCLC-like morphology (Rekhtman *et al.* 2016). Rekhtman *et al.* called these neoplasms SCLC-type LCNECs (Rekhtman *et al.* 2016). George *et al.* studied BP-NECs and called LCNECs with genetic similarity to SCLCs type II LCNECs, emphasizing that in addition to *TP53/RB1* co-alterations the type II LCNECs are characterized by elevated expression of genes associated with active Notch signaling (George *et al.* 2018). The other fraction of the LCNECs mostly lack *RB1* mutation and frequently share gene abnormalities of *STK11*, *KRAS*, *KEAP1* and *NKX-2*, which are observed in non-small cell lung carcinomas (NSCLCs), particularly of adenocarcinoma type (Rekhtman *et al.* 2016, 2018, Derks *et al.* 2018b, George *et al.* 2018). These LCNECs often present an 'NSCLC-spectrum' morphology (Rekhtman *et al.* 2016, 2018). It is likely that the LCNECs with NSCLC-spectrum morphology relate to the group of combined LCNECs

(see terminology and grading), which are phenotypically defined by a neuroendocrine carcinoma admixed most frequently with an adenocarcinoma or squamous cell carcinoma. Miyoshi *et al.* found *KRAS* mutation in 1 of 10 combined LCNECs in both the neuroendocrine and the adenocarcinoma component (Miyoshi *et al.* 2017). Rekhtman stained 112 LCNECs with napsin A, an adenocarcinoma marker, and found napsin A positivity in 17 of them. Molecular analysis of 14 out of 17 tumors revealed mutations of *KRAS*, *STK11* or both in 36, 29 and 14% of the tumors, respectively (Rekhtman *et al.* 2018). These observations correspond to data in MiNENs and large cell NECs of the colon and the pancreas, which frequently were found to have gene alterations of the respective conventional adenocarcinomas such as *KRAS* (22–42%), *BRAF* (8–33%) and *APC* (8–30%) (Jesinghaus *et al.* 2017, Konukiewitz *et al.* 2018). MiNENs and combined BP-NENs also showed monoclonality, when exocrine and endocrine components could be separately examined (Jesinghaus *et al.* 2017, Miyoshi *et al.* 2017). The genetic relationship of MiNEN and combined LCNEC suggest that a fraction of conventional adenocarcinomas of the lung, colon and pancreas may undergo neuroendocrine differentiation. In this context, it is also interesting that 3–10% of lung adenocarcinoma patients, who show *T790M* mutation after acquired resistance to EGFR tyrosine kinase inhibitor treatment, develop BP-NECs of the small cell type (Derks *et al.* 2018a).

Many NETs and NECs are clearly separated by their morphology and also by their molecular alterations, as has been demonstrated in Asiedu's transcriptomic analysis (Asiedu *et al.* 2018). However, some rare cases of NET G3 seem to progress to a NEC-like NEN, driven by a *TP53* mutation. In the pancreas, this was observed in one out of nine NETs G3 (Konukiewitz *et al.* 2018). In the lung, Simbolo identified three BP-NEN clusters using next-generation sequencing. The first cluster included ACs characterized by *TP53*^{WT}, *RB1*^{WT}, and *MEN1*^{mut}. The second cluster included LCNECs showing *TP53*^{mut}, *RB1*^{mut} and *MEN1*^{wt} profile. The third cluster included ACs and LCNECs with *TP53*^{mut} in 41%, *MEN1*^{mut} in 23% and *RB1*^{mut} in 18% (Simbolo *et al.* 2019). The patients in the latter cluster with tumors genetically in-between ACs and LCNECs also had a clinical course in-between the other clusters (Simbolo *et al.* 2019). These tumors appear to be similar to 6 BP-NETs reported by Alcala *et al.* and called 'supra-carcinoid' (Alcala *et al.* 2019) and 3 (either with *TP53* or *Rb1* mutation) of the 11 BP-NETs reported by Cros *et al.* as high-grade pulmonary carcinoid (Cros *et al.* 2020). In two of the three studies discussed previously, it is difficult to relate the genetic subclassification

to the morphological features of the respective neoplasms, since no subtle description is provided (Alcala *et al.* 2019, Simbolo *et al.* 2019). In the study by Cros, at least 70% of the neoplasms appeared to have a high cellularity, diffuse pattern and slightly pleomorphic nuclei (Cros *et al.* 2020), making them comparable to the BP-NETs G3 described by us (Kasajima *et al.* 2019). It seems that also some of the BP-NENs assembled in the complex study of Pelosi *et al.* belong to the previously discussed NET subgroup that genetically acquires a NEC-like profile in the course of the disease (Pelosi *et al.* 2018). Since evidence of a clear-cut morphological transition from so-called well-differentiated BP-NENs to poorly differentiated BP-NENs is so far lacking, the NET to NEC transformation hypothesis needs to be further studied in a phenotypically and genotypically integrated analysis.

As the genetic features of BP-NENs underpin the classification of BP-NENs, they are summarized in Fig. 1.

Within the genetic landscape of GEP-NETs, the genetic profile of the NETs of the ileum is unique and also not comparable to that of the BP-NETs. In ileal NETs, somatic mutations are rare and mainly concern the *CDKN1B* gene. Driver mutations are missing (Karpathakis *et al.* 2016). Frequent, however, are chromosome 18 deletions seen in 60–90% of the cases, and epigenetic changes (Karpathakis *et al.* 2016). Subtyping the molecular profile of ileal NETs revealed three prognostically different subgroups (Karpathakis *et al.* 2016). Favorable prognosis was associated with chromosome 18 loss and CpG islet methylator phenotype negativity and *CDKN1B* mutation. CpG islet methylator phenotype positivity and no whole-arm copy-number variation were related to intermediate prognosis, while multiple whole-arm copy-number variation correlated with poor prognosis. The special constellation of the ileal NETs is complemented by the near absence of ileal NECs, a feature that they share with appendiceal NENs.

Molecular therapeutical targets

The major morphological, endocrinological and molecular similarities between BP- and GEP-NENs explain their comparable response to a common multi-disciplinary therapy, including everolimus, somatostatin analogues, chemotherapeutics and peptide receptor radionuclides (PRRT) (Pavel *et al.* 2016, Yao *et al.* 2016). With respect to PRRT using ¹⁷⁷Lu-DOTATATE, which seems to be the most favorable treatment in metastasized or inoperable GEP- and BP-NETs that express SSTs (Brabander *et al.* 2017).

Figure 1

WHO 2015 classification of bronchopulmonary neuroendocrine neoplasms (BP-NENs) related to the main genetic changes in carcinoids and neuroendocrine carcinomas (NEC), and compared to WHO 2019 classification of gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs). TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; NET, neuroendocrine tumor; MiNEN, mixed neuroendocrine non-neuroendocrine neoplasm. *ARID, EIF1AX and KMT2 family. Data extracted from: Rekhtman *et al.* (2016), Simbolo *et al.* (2017), Derks *et al.* (2018a,b), George *et al.* (2018), Rekhtman *et al.* (2018), Alcala *et al.* (2019) & Simbolo *et al.* (2019).

It was shown that the overall survival was shorter in BP-NETs (52 months) compared to Pan- and Midgut-NETs (71 and 60 months) (Brabander *et al.* 2017). A better overall survival in Pan- and Midgut-NENs compared to BP-NENs was also shown in the study by Baum *et al.* (2018), although the survival difference was not as significant as in the aforementioned study, probably because Baum's study included NENs of all grades and not only NETs (Naraev *et al.* 2019). Why BP-NETs and GEP-NETs obviously have different PRRT response rates, is not known so far.

Approved drugs targeted to specific molecular structures are so far few and concentrate on everolimus, an inhibitor of the *mTOR/AKT/PIK3CA* signaling pathway (Derks *et al.* 2018a). The disadvantage of this inhibitor, however, is that biomarkers predicting its response have not yet been established. Moreover, the *mTOR/AKT/PIK3CA* gene alterations were only found in a rather small number of patients, accounting for 2–12% and 15% BP- and PanNET patients, respectively (Scarpa *et al.* 2017, Simbolo *et al.* 2017).

Agents specifically targeted to *EGFR*, *KRAS*, *BRAF* and other adenocarcinoma related gene targets (i.e. *EML4/ALK*, *ROS1*) (see also Derks *et al.* 2018a) are of special interest for the treatment of MiNENs, because molecular studies in MiNENs and conventional adenocarcinomas of the colon revealed a close genetic relationship between these tumors (Jesinghaus *et al.* 2017). A similar genetic relationship was also found in the BP-system between LCNECs and adenocarcinomas as well as squamous cell carcinomas (Rekhtman *et al.* 2016).

In SCLCs, *DLL3*, which is an inhibitory ligand of the *NOTCH* pathway, was found to be expressed. Treatment with a *DLL3*-targeted antibody conjugated to a DNA-damaging pyrrolizidine dimer doxorubicin induced durable regression in patient-derived xenograft tumors (Derks *et al.* 2018a), but phase III clinical trials (NCT03061812, NCT03033511) failed to show any survival benefit for the patients. Currently *DLL3* targeting chimeric antigen receptor T cell therapy (CAR-T) AMG 119 (NCT03392064) is tested (Isobe *et al.* 2020).

Prognostic molecular factors

A wide-range of prognostic molecular factors has been reported in BP-NENs and GEP-NENs. The most important molecular prognostic factors are listed in Table 6.

Conclusions

BP-NENs and GEP-NENs have a common phenotype and genotype, however, with some notable exceptions. Common to both is a general morphological and genetical dichotomy in well- and poorly differentiated neoplasms, whose proliferative activity can be well depicted in a Ki67-based grading system with strong prognostic implications. This grading system is well established for the stratifications of GEP-NENs, while it has to be fully validated in BP-NENs. This also applies to

Table 6 Dismal molecular prognostic factors in bronchopulmonary and gastroenteropancreatic neuroendocrine neoplasms (BP-NENs and GEP-NENs).

	Prognostic factors	Study cohort	Reference
BP-NENs	<i>MEN1</i> deletion	55 NETs (stage not given)	Swarts <i>et al.</i> 2014
	Low CD44, OTP expression	108 NETs (55% pT1, 75% pN0)	Papaxoinis <i>et al.</i> 2017
	<i>TERT</i> gain	88 NETs (72% Stage I)	Simbolo <i>et al.</i> 2017
	<i>Rb1</i> mutation	60 NECs (22% Stage I)	
GEP-NENs	PTEN loss	160 PanNETs (25% Stage I)	Estrella <i>et al.</i> 2014
	PgR loss		
	Aneuploidy	27 PanNETs (stage not given)	Donow <i>et al.</i> 1991
	CK19 expression	216 PanNETs (surgically resected; stage not given)	Schmitt <i>et al.</i> 2007
	KIT expression	97 PanNETs (surgically resected; 50% N0M0)	Zhang <i>et al.</i> 2009
	Loss of heterozygosity at <i>1p, 3p, 6q, 17p, 22q</i> and <i>X</i>	34 PanNETs (stage not given)	Pizzi <i>et al.</i> 2002
	Low DAXX/ATRX Expression. ALT activation	321 PanNETs (36% pT1, 63% pN0)	Singhi <i>et al.</i> 2017
		243 PanNETs (67% M0)	Marinoni <i>et al.</i> 2014
		97 SI-NETs (25% M1)	Karpathakis <i>et al.</i> 2016
	CpG islet methylator phenotype positivity and multiple whole-arm copy-number variation		

BP-NEN, bronchopulmonary neuroendocrine neoplasm; GEP-NEN, gastroenteropancreatic neuroendocrine neoplasm; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; OTP, orthopedia homebox; PanNET, pancreatic neuroendocrine tumor; PgR, progesteron receptor; SI-NET, small intestine neuroendocrine tumor.

the new category of NET G3 identified in the GEP-system. Whether all NETs G3 remain genetically different from NECs or evolve in some cases to NECs by additional genetic changes, such as *TP53* mutations, has to be clarified in future studies. Differences between BP-NETs and GEP-NETs mainly concern the hormonal composition of the tumors and the pathogenetic role of the *MEN1* gene. Other differences focus on the large cell subgroup of NECs and its heterogeneous genetic profile, which partly shows a site-specific relationship to the main genetic abnormalities of the respective conventional carcinomas. Future classifications of BP- and GEP-NENs should consider the great commonalities between the two most important NEN groups of the body regarding terminology and grading but also allow to categorize the neoplasms according to the most obvious and therapy relevant site-specific differences.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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