Neuroendocrine Neoplasia Management

New Approaches for Diagnosis and Treatment

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Italo Nosari, MD, born on June, 14, 1950, and passed away on March, 4, 2020.

This book on Neuroendocrine Neoplasia is dedicated to our friend and colleague Italo Nosari, endocrinologist, who brought a great personal contribution to the discipline and in particular to diabetology. He has held relevant roles inside the Association of Physicians Diabetologists (AMD) and the Italian Society of Diabetology (SID). He produced a lot of scientific papers published in international literature. He has always practiced his intense clinical activity over years with great passion, full dedication, and love for his patients, both pediatric and adults. He was a very active member of our Multidisciplinary Groups of Humanitas Gavazzeni showing in all circumstances availability, competence, and humanity. During the outbreak of COVID-19 in Bergamo, he generously offered his precious assistance in the Emergency Department of the Hospital without sparing himself. Unfortunately the viral infection took him away, while he was doing his mission, leaving everyone with an indelible memory.

Foreword

The book *Neuroendocrine Neoplasia Management*, edited by G. Beretta, A. Berruti, E. Bombardieri, N. Fazio, and O. Goletti, is a timely and comprehensive compilation of recent developments within the field of neuroendocrine tumors (NENs.)

Current book publications in endocrinology, oncology, surgery, and gastroenterology offer specific chapters on neuroendocrine tumors but only in a superficial manner. This book covers all the aspects of neuroendocrine tumor management including epidemiology, diagnosis, and treatment of various subtypes of NENs.

It will serve both as an excellent textbook for younger colleagues, medical students, and new beginners in the NEN field and as a refresher for more experienced colleagues. NENs is a complicated field that needs collaboration among many specialists, endocrinologists, oncologists, surgeons, radiologists, and gastroenterologists; therefore, this book will be an excellent guide in the management of NENs.

Neuroendocrine tumors is a rather young entity within the oncology field formulated by Italian and Scandinavian pathologists in the early 1970s, who also coined the term "Neuroendocrine Tumor" because of the characteristic picture of both neural and endocrine elements. The area started to mature with the development of specific radioimmunoassays for substances (biomarkers) secreted by the tumors. The diagnostic and therapeutic tools were rather sparse in the beginning, but during the 1980s and 1990s, the field started to grow exponentially thanks to new diagnostics and therapeutics (CgA, Ki-67, somatostatin scintigraphy, somatostatin analogs, IFNs, new chemotherapies).

The refined diagnostics showed differences in tumor biology and genetics, and new imaging procedures (PET/CT; PET/MR) gave improved staging procedures and a new classification system (WHO). This clearly demonstrated that the therapy had to be further developed, "one size did not fit all." Custom-made therapies have been developed including biotherapy, specific chemotherapies, targeted agents, and finally peptide receptor radio therapy (PRRT). Surgery has been more developed with tissue sparing procedures.

Instrumental works by Italian pathologists have given us insights into molecular genetics and oncology development which will in the future refine the management of NENs.

Looking at the authors list of this book, a number of them have made significant contributions to the NEN field, which further enhances the importance of this publication covering an earlier unmet need of summarizing the most recent development in the NEN area. This book should be in the bookshelf of every colleague working with NEN patients.

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Preface

Several books have been published on the subject "neuroendocrine tumors" in these years, because this topic is of great clinical importance in oncology and endocrinology. The reasons are to be found in their very interesting biology, the relevant epidemiology, the evolution in the diagnostic technologies, and the recent development of novel emerging and successful therapies that stimulated a lot of clinical trials. The agents targeting angiogenesis and/or PI3K/AKT/mTOR pathway, alone or in combination with analogues, have provided encouraging results in advanced disease.

In this book an original approach has been adopted, with the aim to provide a general update in prognosis, diagnosis, and therapy by covering the whole family of neuroendocrine tumors. As the title "Neuroendocrine Neoplasia (NEN)" suggests, all tumors from various organs and/or particular histology, MEN-related tumors, MiNEN, NEC, and Merkel's tumors have been included.

The structure of the book consists in a general part and in a part with specific chapters.

The general part of the book is focused on the history, the epidemiology, and the most important results and the fast developments in scientific and clinical knowledge in the field of diagnosis and therapy.

In particular the authors discuss the advances in genetic analysis and molecular biology, the endoscopic techniques combined with guided biopsy, the high-resolution imaging associated with endoscopy, the metabolic imaging from radioreceptor targeting, and the hybrid PET/CT and MRI instrumentation alone or in combination.

In the area of therapy, particular attention is paid to the emerging strategies of treatment, the surgery and minimally invasive surgery for both early stage and advanced diseases, and the loco-regional and systemic treatments including targeted therapy and/or biological therapies.

The second part of the book depicts the clinical management of the different groups of NEN from different anatomical origin and/or with particular histology and discusses some novel approaches of diagnosis and therapy, the spectrum of the current available options, and the most important results from the most successful clinical trials.

The structure and the content of this book follow the philosophy of the concept that is gaining increasing importance, i.e., that, among the big family of tumors taking origin from neuroendocrine cells, the traditional paradigm classifying neuroendocrine tumors as a single entity is no longer sufficient to

explain the differences often observed in the prognosis and tumor responsivity of the various groups of patients with "different neuroendocrine neoplasias."

The most distinguished experts in the field have been invited to contribute to this book. All authors worked together with great enthusiasm by integrating their different skills in a multidisciplinary collaboration, with the contribution of oncologists, endocrinologists, pathologists, nuclear physicians, surgeons, physicists, radio-pharmacists, gastroenterologists, and biologists. Thanks to their interactive work, they set up this informative publication that provides valuable insights for all professionals interested in the modern management of neuroendocrine neoplasias.

Bergamo, Italy — Giordano Beretta
Brescia, Italy — Alfredo Berruti
Bergamo, Italy — Emilio Bombardieri
Milan, Italy — Nicola Fazio
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Contents

Part I

Introduction

History of Neuroendocrine Neoplasia

1

Emilio Bajetta, Domenico De Toma, Adelmo Antonucci, Roberto Bajetta, and Monica Valente

1.1 Introduction

Neuroendocrine tumors are a group of malignant neoplasms that originate in neuroendocrine cells and can affect any part of the body. They are rare (≤5/100.000) and have been extremely difficult to discover and investigate; however, their incidence has risen in the last 20 years [1]. These tumors are nicknamed "zebras" due to their rarity, but despite their sporadic occurrence, physicians have been fascinated by their complexity and distinct clinical presentation. Carcinoid tumors are the most common endocrine tumors occurring in the gut. They may, however, develop in the bronchus, rectum, ovary, lung, and elsewhere. They grow slowly and are often clinically silent for many years before being recognized and metastasizing. The discovery of neuroendocrine tumors has been a challenge, first of all, for the pathologists with regard to diagnosis, as we can see following the different classifications that we have had over the past few years, and for the clinicians with regard to medical treatments.

1.2 Early History

The first pathological conditions defined as neuroendocrine were described in the Old Testament and in an Egyptian medical papyrus dating back to 1552 BC, known as the "Ebers Papyrus," in which cases of patients with disease conditions similar to acromegaly, gigantism, diabetes mellitus and neurofibromatosis type 1 were reported for the first time [2]. The Ebers papyrus is a handbook of Ancient Egyptian medicine and contains 879 individual texts in 110 columns, which cover nine medical topics. It is named after its discoverer, the Leipzig Egyptologist and novelist George Ebers, who purchased it from a Coptic antiquarian in Upper Egypt and transferred it to the University Library in 1873. The papyrus is currently kept at the Library of the University of Leipzig, in Germany. This is the first evidence of the existence of these diseases and several years were to elapse before neuroendocrine tumors began to be investigated and studied in a more

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systematic way. The first pathological description of these types of tumors was given by the German pathologist Theodor Langhans in 1867, when he described a carcinoid-like tumor at autopsy in a 50-year-old woman with tuberculosis [3]. He described a submucosal tumor that projected into the lumen of the small intestine, and he commented upon the very sharp borders without any evidence of peri-tumoral invasion. His report was principally a histological description of the tumor without discussion of growth and clinical behavior of this undocumented neoplasm. In 1888, the German pathologist Otto Lubarsch described two cases of ileal tumors during an autopsy examination [4]. In one case, the ileum contained numerous tubercular ulcers and nodules; in the second case, he described multiple small carcinomatous growths in the ileum, although he was initially reluctant to identify these lesions as carcinomas. Diarrhea was the main symptom in the latter patient, a possible manifestation of carcinoid syndrome, but he was unaware of a similar correlation with these types of tumors. After some scientific research, he was able to identify the records of 35 cases of intestinal carcinomas near the ileocecal valve and opined that in his estimation, several of these were not "true" carcinomas. In 1890, the British physician William Ransom was the third person to describe a case of a patient with a lesion similar to a carcinoid tumor with liver metastases. The patient, a 50-year-old woman, presented a pathological condition characterized by diarrhea, which had been persistent for more than 2 years, and wheezing upon eating. The autopsy revealed several small nodules in the ileum and in the liver (metastases) [5]. Despite these initial observations, a distinct pathological entity that united these pathological conditions had not yet been recognized. In 1895, a German pathologist, A. Notthafft, described three tumors of the upper ileum during an autopsy in a patient who had died of pneumonia [6]. These tumors had been uncharacteristically identified in the submucosa and histologically were not true carcinomas; he referred to them as "beginning carcinomas."

The existence of a group of gastrointestinal cells, different from the others due to their "yellow" chromate staining properties, was recognized for the first time in 1870 by the German physiologist Rudolf P. H. Heidenhain [7] and again, after a few years, followed by the Russian anatomist and histologist Nikolai K. Kultschitzky in 1897 [8]. In his paper "Zur Frage über den Ban des Darmkanals," he pointed out the differences between these cells and those that were "classical" mucus-secreting and absorbing mucosa cells. After this first description, these cells were variously called enterochromaffin cells, argentaffin cells, clear cells, enteroendocrine cells, and Kultschitzky cells [9]. The French surgeon Antonin Gosset and the French-Canadian pathologist Pierre Masson demonstrated the argentaffin-staining proprieties of carcinoid tumors, using silver impregnation techniques. They showed a silver-colored pattern and speculated on the etiology of a specific type of tumor from the enterochromaffin cells, Kultschitzky's cells, and of the intestinal mucosa [10, 11].

1.2.1 Carcinoid: The Origin of the Term

The word "carcinoid," from the German "karzinoid," was introduced by the German pathologist Siegfried Oberndorfer in 1907, to identify some gastrointestinal tumors that presented a prognosis and a more favorable clinical history than adenocarcinomatous lesions. He presented his discovery in his seminal paper "Karzinoide Tumoren des Dünndarms" in which he used the term "Karzinoide Tumoren" for these different types of benign gastrointestinal neoplasms [12].

All tumors described were located in the submucosa of the ileum, and the peculiarity was the discovery of multiple primary malignant tumors in the same organ. As a result of his observations, Oberndorfer identified five distinct characteristics of these tumors: (a) they were mostly small, patients commonly demonstrated multiple tumors; (b) the tumor cells were usually

surrounded by undifferentiated tissues, possibly demonstrating gland formation; (c) the tumors had not previously been described, and they had the potential to become invasive; (d) they did not metastasize; and (e) they apparently grew extremely slowly, achieving no substantial size and therefore appeared to have a harmless nature. The merit of Oberndorfer was to identify these tumors as actually true cancers, but without the tendency to grow rapidly and to metastasize, as in the case of carcinomas. For these reasons, he used the term "karzinoide" ("carcinoma-like") to describe these types of lesions more accurately. After some years, Oberdnorfer revised his initial observations about the benign behavior of these tumors in a manuscript, in which he described 36 carcinoid tumors of the appendix and small intestine, and he emphasized the possibility that they might exhibit malignant features and metastasize [13].

1.2.2 Carcinoid Classifications

In 1914, the surgeon Andre Gosset and the pathologist Pierre Masson hypothesized the origin of carcinoid tumors from the enterochromaffin cells of the gastrointestinal district, using silver impregnation techniques, and then, they demonstrated the argentaffin-staining properties of carcinoid tumors. Furthermore, the Austrian pathologist Friedrich Feyrter explained how the enterochromaffin cells were present not only in the digestive tract but also in many other anatomic districts, practically in all mucosal-lined organs of the body. Feyrter introduced the concept of the "diffuse" endocrine system on solid glands [14] and, subsequently, the concept of the "widespread neuroendocrine system" was developed. After that, another significant discovery was to distinguish between two different categories in this system: endocrine cells that discharged their hormonal content into the blood ("true" endocrine cells) and those that limited their action to a restricted anatomic field delimited by the dendrite-like prolongations present in those cells ("paracrine" cells) [9]. In 1952, the Italian pharmacologists Vittorio Erspamer and Biagio Asero identified serotonin (5-HT) as the main hormone produced by the enterochromaffin cells of the gastroenteropancreatic tract [15], and subsequently, its metabolite, urinary hydroxyindoleacetic acid (5-HIAA), was identified as another marker in cancer carcinoid patients. Only after some years, in the 1960s, was there the need to classify the tumors that develop from enterochromaffin cells in subtypes on the basis of their histological appearance and type of secretory product. In 1962, the British pathologist Elizabeth Williams classified the carcinoid tumors based on their origin from different embryonic segments of the gut, foregut, midgut, and hindgut [16]. This approach was a result of the work of the British pathologist Anthony Pearse, who developed the hypothesis of the "diffuse endocrine system," with cells located in different anatomical districts and different organs, but with a common embryological origin from the neural crest, a transient neural structure unique to vertebrates located on both sides of the embryonic neural plate, at the junction with the normal ectoderm.

The neural crest is composed of a pluripotent cell population, which migrates throughout the body during the normal embryonic development, and gives rise to different cell types, such as neurons, melanocytes, chromaffin cells of the adrenal medulla and extra-adrenal paraganglioma, and thyroid C cells. Pearse, then, established the APUD (Amine Precursor Uptake and Decarboxylation) concept [17]. Later, this gives rise to the terms "diffuse neuroendocrine system" (DNES) and "confined neuroendocrine system" (CNES) to identify those groups of cells capable of producing and releasing hormones, whether they are present in a widespread way in the body or confined to organs. DNES includes nerve and endocrine cells found in organs and tissues, CNES includes glandular tissue recognized by the traditional endocrinology [18, 19]. Almost half of these cells are in the gastroenteropancreatic (GEP) system where most neuroendocrine tumors occur (Table 1.1).

Regarding the hypersecretion of hormonal substances, some neuroendocrine tumors are biologically active called "biologically active neuroendocrine tumors" (BANTs) and others are biologically inactive called "biologically inactive neuroendocrine tumors" (BINTs). The BANTs, independently of the levels of hormonal substances present in the blood or of the immunopositivity identified in the tissue, present some symptoms and signs correlated to the effects of one of the hypersecreted hormones from which the syndrome takes its name. Otherwise, BINTs are not capable of secreting hormonal substances and they have no correlated syndromes so, since then, these tumors have been diagnosed via immunohistochemical investigation [19].

Over the years, there have been several classifications for neuroendocrine tumors. Since 1995, the Italian pathologist Carlo Capella suggested the term "neuroendocrine tumors" for all tumors relating to the digestive system instead of the term "carcinoid tumors" [20]. This classification was updated by another Italian pathologist Enrico Solcia and other expert pathologists in the first World Health Organization (WHO) classification in 2000 [21], in which the tumors were classified into: (a) well-differentiated endocrine tumors or a more aggressive grade with metastases, well-differentiated endocrine carcinomas; (b) poorly differentiated endocrine carcinomas; and (c) mixed exocrine-endocrine tumors.

In 2010, the WHO classification was updated [22] in the following categories, depending on mitotic counts and the Ki-67 labeling index:

a. well-differentiated neuroendocrine tumors G1;
b. well-differentiated neuroendocrine tumors G2;
c. neuroendocrine carcinomas; and.
d. mixed adeno-neuroendocrine carcinomas.

The WHO grading system was revised in 2017 [23], and in 2019 [24], a new subset of well-differentiated neuroendocrine neoplasms (NENs) has been recognized (Table 1.2).

Table 1.1 Neuroendocrine system (modified from Percopo V. Neuroendocrine tumors general aspects. In: GEP and multiple neuroendocrine tumors. Piccin 1996)

Diffuse neuroendocrine system (DNES)	Confined neuroendocrine system (CNES)
Gastroenteropancreatic apparatus	Pituitary
Respiratory apparatus	Thyroid and parathyroid
Urogenital apparatus	Hypothalamus
Kidney	Adrenals
Skin	Ganglia and paraganglia
Myocardium	Carotid body
Thymus	Pineal gland
Spleen	Placenta

1.2.3 Clinic History of Carcinoids

There were several symptoms and signs that were found in carcinoid diagnoses, such as flushing, diarrhea, edema, wheezing, now commonly referred to as the "carcinoid syndrome." The German pathologist, A. Schotle, was the first who described this condition in 1931, in a

Table 1.2 WHO 2019 Classification for neuroendocrine neoplasms of the gastrointestinal tract (modified from Nagtegaal ID et al. Histopathology 2020)

Terminology	Differentiation	Grade	Mitotic rate	Ki-67 index
NET, G1	Well differentiated	Low	<2	<3%
NET, G2		Intermediate	2–20	3–20%
NET, G3		High	>20	>20%
NEC, small-cell type (SCNEC)	Poorly differentiated	High	>20	>20%
NEC, large-cell type (LCNEC)			>20	>20%
MiNEN	Well/poorly differentiated	Variable	Variable	Variable

47-year-old male with an ileal carcinoid tumor, who complained of diarrhea, cough, lower extremity edema, and cardiac failure. Indeed, at autopsy, a hard thickening of the tricuspid valves and irregular endocardial thickening of the right atrium were evident, likely representing the first documentation of carcinoid heart disease [25]. In 1954, the Swede A. Thorson published the first series of patients presenting with pulmonary stenosis, tricuspid insufficiency, peripheral vasomotor symptoms, bronchoconstriction, and cyanosis in malignant carcinoid tumors of the small intestine with liver metastases and their symptomatology related to hypersecretion of 5-HT into the system circulation [26]. In the same year, B. Pernow and J. Waldenström described flushing, another sign of carcinoid syndrome [27], and in 1964, J. Oates demonstrated that some carcinoid tumors release kallikrein, which activates bradykinin, a potent vasodilator, and suggested that it might play a role in the flushing episodes so characteristic of the disease [28]. The role of 5-HT, as a plasma marker in carcinoid syndrome, and of 5-HIAA, the main 5-HT urine metabolite, was demonstrated by I. Page in 1954. Another sign we frequently observe in carcinoid tumors is fibrosis. In 1961, the American researcher, C. Moertel, first described the relationship between fibrosis and carcinoids, because these tumors stimulate fibroblastic reactions in the peritoneum, mesentery, and retroperitoneum, as well as in the lungs and cardiac valves [29]. In 1968, two physicians, Ladislav Krulich and Samuel McCann discovered an inhibitor of the growth hormone (GH) released from the pituitary gland [30], which attracted much attention because of its functional inhibitory role in the regulation of a wide variety of physiological functions such as the inhibition of both endocrine and exocrine secretion, cell proliferation, and survival. The dual actions of these products (inhibition of hormone release and cell growth) have made them ideal candidates for the treatment of neuroendocrine disorders. In 1973, a growth hormone inhibitor was isolated named somatostatin [31], and for this reason, the endocrinologist Roger Guillemin received the Nobel Prize for Physiology or Medicine in 1977. Some years after, a somatostatin analogue, octreotide acetate, was developed and used to control carcinoid syndrome, and its use was approved in Europe in 1988 and in the USA in 1989. After that, the FDA approved a new type of preparation, a longer acting octreotide acetate (octreotide long acting repeatable, LAR) following the publication of Joseph Rubin and colleagues, regarding the positive trial results of this drug [32]. Somatostatin compounds also played a role in the diagnostic phase when the Swiss pathologist Jean Claude Reubi and colleagues discovered different somatostatin receptor subtypes and the methods to detect or visualize them for the diagnosis of these types of tumors. OctreoScan® was the first product which became available in 1994, and more recently, 68Gallium (Ga)-DOTATOC and 68Ga-DOTATE were developed as PET (positron emission tomography) traces for somatostatin receptor imaging [33].

1.3 Conclusion

Neuroendocrine neoplasms are a rare family of tumors arising from various different epithelial cells with patterns of neuroendocrine differentiation. They share similar histopathological features, but, at the same time, these tumors vary greatly in their biological behavior and clinical characteristics. Although they are rare tumors, neuroendocrine neoplasms have a very long clinical history (Fig. 1.1) involving various medical figures, from surgeons to pathologists, oncologists, gastroenterologists, radiologists, nuclear physicians, and endocrinologists. Still today they are the subject of discussion and study. Proof of this is the continuous search to classify them in order to better diagnose and treat these rare and, at the same time, fascinating tumors.

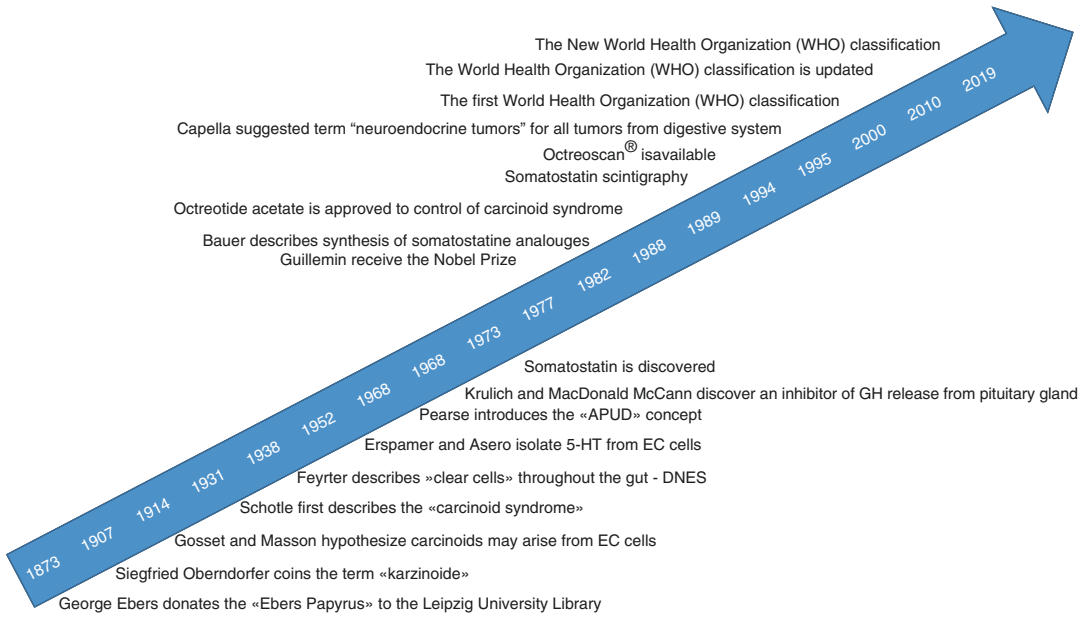

Fig. 1.1 The timeline of neuroendocrine neoplasms (adapted from Modlin IM et al. A century of advances in neuroendocrine tumor biology and treatment: a tribute to Siegfried Oberndorfer. Felsenstein CCCP 2007)

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2 Epidemiology of Neuroendocrine Neoplasms

Annalisa Trama

Neuroendocrine neoplasms are rare cancers [1]; thus, their epidemiology is best studied in large, population-based cancer registries (CRs).

CRs are a crucial source of data on the number of new cancer cases ("incidence"), cancer-related deaths ("mortality"), individuals living with cancer ("prevalence"), as well as cancer "survival" rates. CRs register all cancers, therefore also the rare ones. The *International Agency for Research on Cancer* (IARC) promotes collaboration among CRs, defines data collection standards and provides training for CR personnel. As a result, from the end of the 1960s, CRs have contributed data to *Cancer Incidence in Five Continents* and to other collaborative projects. These collaborations have contributed to set common criteria and rules to improve the quality and comparability of data among CRs. However, the quality of a CR inevitably depends on the local healthcare environment and the available sources of information. For a CR to function, it needs to define a catchment area and to have access to reliable population statistical data, medical data from hospitals, death certificates, etc. [2]. Quality of care is also relevant to quality of CRs. For example, inappropriate pathological diagnoses will result in misclassification in CRs. Rare cancers are particularly exposed to discrepancies in quality of care, with some of them (e.g. sarcomas, neuroendocrine neoplasms) being especially affected in comparison to others (e.g. squamous cell head and neck carcinomas). Misclassification at registration may also happen when (a) information source is correct and complete, but registration is wrong and (b) classifications are ambiguous, obsolete terms are used and entities lack proper codes. In addition, problems in classification may be caused by delays between description of new entities and updates of the *WHO Classification of Tumours* series, the so-called "blue books" (https://whobluebooks.iarc.fr/), and between changes thereof and updates of International Classifications of Disease for Oncology (ICD-O) which is used by CRs [3].

This chapter describes the epidemiology of neuroendocrine neoplasms based on population-based CRs. It should be kept in mind that (1) registration is based on the ICD-O code, and despite a third revision in 2013, a significant number of neuroendocrine neoplasms are still difficult to classify and (2) CRs register only malignant tumours. Thus, previous and current estimates may suffer of a certain degree of underestimation. Most of the papers considered in this chapter identified the neuroendocrine neoplasms using the ICD-O3 codes as follows: neuroendocrine tumours included islet cell carcinoma (8150), insulinoma (8151), glucagonoma (8152), gastrinoma (8153), mixed islet cell/exocrine adenocarcinoma (8154), vipoma

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(8155), somatostatinoma (8156), enteroglucagonoma (8157), carcinoids (8240), enterochromaffin cell carcinoid (8241), enterochromaffin-like cell tumours (8242), goblet cell carcinoid (8243), composite carcinoid (8244), adenocarcinoid (8245) and atypical carcinoid (8249). Small-cell and large-cell neuroendocrine carcinomas were also considered in different ways, e.g. Korse et al. [4] combined large-cell neuroendocrine carcinoma (8013) and neuroendocrine carcinoma (8246) as G3-large-cell neuroendocrine carcinoma [G3-LCNEC] and named small-cell neuroendocrine carcinoma as G3-small-cell neuroendocrine carcinoma [G3-SCNEC]; Leoncini et al. [5] grouped large-cell neuroendocrine carcinoma (8013), small-cell carcinoma (8041) and neuroendocrine carcinoma (8246) as high-grade neuroendocrine neoplasms; Dasari et al. [6] described neuroendocrine neoplasms different aggressiveness using the grading (G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated and G4, undifferentiated or anaplastic); Boyar Cetinkaya et al. [7] combined small-cell and large-cell neuroendocrine neoplasms in the group of highly aggressive neuroendocrine neoplasms. Data on mixed neuroendocrine/non-neuroendocrine phenotype are not available as individual grouping.

2.1 Incidence Rate

The overall neuroendocrine neoplasms crude incidence rate was 5/100,000 [8] and 3.5/100,000 (www.rarecarenet.eu) in the USA (2000–2004) and in Europe (2000–2007), respectively. However, studies show geographical and racial differences with annual incidence rates varying from around one to five x 100,000 across European countries (www.rarecarenet.eu), Australia [9, 10] and Asian countries [11]. Regarding race, African Americans seem to have a higher incidence rate compared to white and Asian Pacific Islanders [6, 8, 12]. Tsai et al. [13] confirmed that the Asian population had a lower incidence rate than whites and African Americans, supporting a role for genetic factors. However, the higher incidence rate of neuroendocrine neoplasms among Asian-Americans compared to Asians in Asia suggests that environmental factors may also be important in the neuroendocrine neoplasms development. Further studies are needed to understand whether these differences are due to underlying biologic factors, unknown risk factors, healthcare patterns and/or data capture by CRs.

2.1.1 Neuroendocrine Neoplasms by Age and Gender

Neuroendocrine neoplasms present a slightly higher male predominance (www.rarecarenet.eu). Incidence increases with age and it is highest in patients of 65 years or older in both, males and females ([6, 14]; www.rarecarenet.eu) (Fig. 2.1).

2.1.2 Neuroendocrine Neoplasms by Site

Neuroendocrine neoplasms site distribution may differ across population especially comparing western and eastern countries; however, the most common sites of neuroendocrine neoplasms diagnoses are lung and gastrointestinal pancreatic (GEP) sites everywhere. In USA, in 2000–2012, the neuroendocrine neoplasms incidence was 1.49, 3.56 and 0.84/100,000 in the lung, GEP sites and unknown primary site of origin, respectively. Within the GEP sites, the most common site was the small intestine (1.05/100,000) followed by the rectum (1.04/100,000) and pancreas (0.48/100,000) [6]. In Europe, neuroendocrine neoplasms distribution by site was similar but, within GEP sites, most common sites were small intestine, stomach and pancreas [14]. However, the population from Taiwan presented very few cases of neuroendocrine neoplasms in the small intestine and a high proportion of cases in the rectum [13].

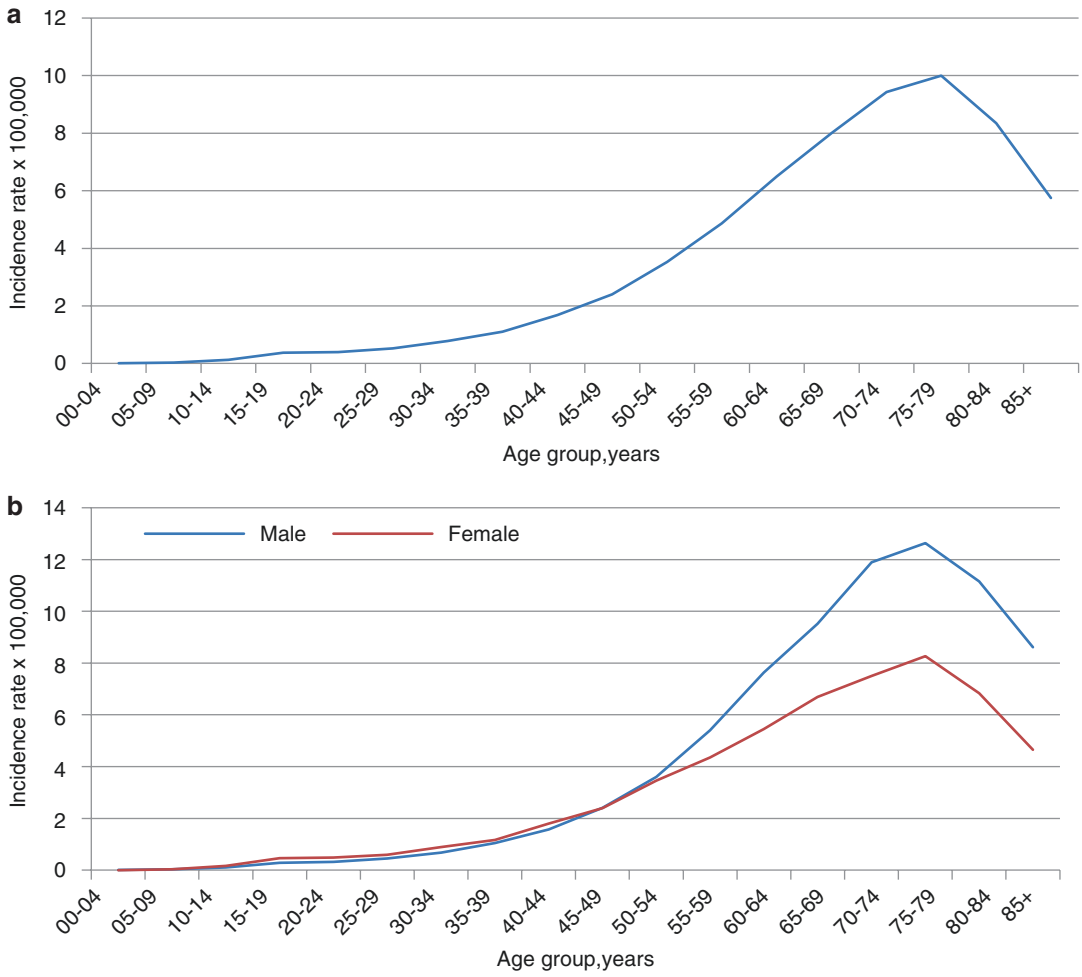

Fig. 2.1 Age-specific incidence rate of neuroendocrine neoplasms overall (**a**) and by gender (**b**), any site, Europe 2000–2007. Source: adapted from www.rarecarenet.eu

2.1.3 Neuroendocrine Neoplasms by Stage

The stage at diagnosis differs when series from several countries are compared. In the USA, in 2000–2012, of 53,465 neuroendocrine neoplasms with a known stage, 52% were localized, 20% were regional and 28% were distant at the time of diagnosis [6]. A comparison between Norway and USA in the years 1993–2004 showed an overall proportion of localized neuroendocrine neoplasms disease lower in Norway (27%) compared with the USA (40–46%), a proportion of regional disease higher in the Norway (39%) compared with the SEER (17–20%) and a similar distribution of distant disease in both populations (18–22%) [12]. In the Tuscan CR (Italy), from 1985 to 2005, a higher number of neuroendocrine neoplasms were diagnosed at regional stage (incidence rate 0.3/00,000) than at localized (0.2/100,000) or at distant stage (0.2/100,000) [15]. In Iceland, data are available for GEP neuroendocrine neoplasms only and, in the years 1985–2014, showed 65% of GEP neuroendocrine neoplasms confined to their organ of origin at the time of

diagnosis [16]. The differences in time periods covered, neuroendocrine neoplasms site included, may partially explain these discrepancies, but differences in healthcare and screening organizations could also impact on stage at diagnosis.

Finally, the latest data from the USA showed in 2000–2012 that of 45,318 neuroendocrine neoplasms with a known grade, 51% were G1, 16% were G2, and 33% were G3 and G4 [6].

2.2 Incidence Trends

The incidence rate of neuroendocrine neoplasms has shown a significant increase over time, overall and for all neuroendocrine neoplasms common cancer sites, across populations (Australia, Canada, Denmark, Italy, Netherlands, Norway, Switzerland, Taiwan, USA) with different magnitude of change [5, 9, 10, 13].

In Italy, Caldarella et al. [15] reported an increase in incidence from 0.5/100,000 in 1985 to 1.9/100,000 in 2005. By behaviour, incidence rate for uncertain behaviour neuroendocrine neoplasms increased from 0 to 0.3/100,000; however, malignant neuroendocrine neoplasms incidence rate also increased. In the Netherlands, the incidence rate of neuroendocrine neoplasms increased from 2.1/100,000 in 1990 to 4.9/100,000 in 2010. The incidence of well-differentiated, low-grade neuroendocrine neoplasms showed a moderate increase from 2.0/100,000 to 3.0/100,000; the incidence of well-differentiated, intermediate grade or atypical carcinoid increased from 0.01/100,000 to 0.2/100,000 in 2010. The largest increase in incidence was observed in poorly differentiated large-cell neuroendocrine carcinoma from 0.01/100,000 in 1990 to 1.8/100,000 in 2010 [4]. In the USA, incidence of neuroendocrine neoplasms was 1.09/100,000 in 1973 and increased to 6.98/100,000 by 2012. The increase occurred across all sites, stages and grades although the most dramatic rise was noted in patients 65 years or older; in the stomach, in G1 neuroendocrine neoplasms and, among the stage groups, in localized neuroendocrine neoplasms [6]. In Canada, the incidence neuroendocrine neoplasms increased from 2.48/100,000 in 1994 to 5.86/100,000 in 2009. The proportion of patients presenting with metastatic disease at the time of diagnosis decreased from 29% in 1994 to 13% in 2009. However, because incidence of all neuroendocrine neoplasms increased, the incidence of metastatic neuroendocrine neoplasms at presentation remained stable [17]. Finally, in the USA, the overall incidence rate of low-grade neuroendocrine neoplasms increased from 1.09/100,000 in 1973 to 3.51/100,000 in 2012 (3.2-fold increase); the overall incidence rate of high-grade neuroendocrine neoplasms increased from 2.54/100,000 to 10.52/100,000 (4.1-fold increase) [5].

The observed trends have been explained by an increased diagnosis of asymptomatic, early-stage disease due to an increased use of endoscopic and imaging procedures in clinical practice as well as to an increased recognition and widespread adoption of the formalization of the nomenclature, grading and staging of these tumours. However, neuroendocrine neoplasms overall are stably increasing independently of grade. This raises the hypothesis that neuroendocrine neoplasia (NENs) share susceptibility factors independently of cancer grade. Anyway, we are still dealing with a poorly understood phenomenon that will need further investigations to answer the rising demand for cure and prevention for this group of neoplasms [5].

2.3 Neuroendocrine Neoplasms Prevalence

The prevalence is a measure of the cancer burden because it counts the number of patients alive at a certain date, in a defined population, who have been diagnosed with a given cancer. Limited-duration prevalence limits the number of patients to those diagnosed with cancer within a fixed time in the past (i.e. 2, 5 or 20 years) of a prevalence index date. Complete prevalence count/proportion includes all previously diagnosed

patients alive at the prevalence index date, regardless of how long ago the diagnosis was given.

In the USA, based on the 20-year limited duration prevalence, at 1 January 2014, 171,321 neuroendocrine neoplasms patients were estimated to be alive (prevalent) [6]. In Europe, at 1 January 2008, the number of neuroendocrine neoplasms prevalent patients was 117,237 (www.rarecarenet.eu).

This "high" prevalence may seem to be in contrasts with the low incidence of neuroendocrine neoplasms. Prevalence includes patients irrespective of whether they are under treatment or considered cured; thus, it is a composite of the incidence and survival rates. Thus, neuroendocrine neoplasms prevalence can be explained by the overall favourable prognosis of most neuroendocrine neoplasms. The rising incidence, and likely identification of tumours at earlier stages, will lead to an increase in the prevalence of neuroendocrine neoplasms, and clinicians should be encouraged to become familiar with this particular type of cancer [18].

2.4 Neuroendocrine Neoplasms Survival

Data on neuroendocrine neoplasms survival coming from CRs should be read considering that CRs collect data on malignant tumours only. In addition, it is very difficult to accurately compare results from different countries (Table 2.1). Anyway, five-year survival overall seems to be around 50–60% with differences across anatomical sites, grading and stage. Among neuroendocrine neoplasms, common sites, colon, appendix and small intestine, are those with highest survival whereas lung and pancreas are those with the lowest survival. Higher survival has been reported in the USA as is the case for most common adult cancers. Survival rates are also higher in hospital-based series due to patient selection [19]. Independent predictors of neuroendocrine neoplasms survival included older age, male sex, low socioeconomic status, rural, advanced stage and neuroendocrine neoplasms primary tumour sites.

Increasing survival over time has been reported in several studies across populations. However, few reports on survival trends by neuroendocrine neoplasms aggressiveness are available to properly disentangle the reasons of survival changes over time. In The Netherlands, it was observed an on-going improvement in survival with well-differentiated neuroendocrine neoplasms, mainly in patients with neuroendocrine neoplasms of grade 1 and metastatic disease, and the authors suggested that the introduction of somatostatin analogues and their long-acting forms may explain this change in survival over time [4]. In Norway, improved survival was observed in both low/intermediate and highly aggressive neuroendocrine neoplasms after year 2000, regardless of tumour stage, gender and age group (period analyzed 1993–2015) [7]. In the USA, compared with 2000–2004, patients who received a diagnosis between 2005 and 2008 had a 17% lower risk of death and those diagnosed in 2009–2012 had a 21%. To evaluate the effect of the evolution of systemic therapies on survival, overall survival trends of distant stage neuroendocrine neoplasms and of distant gastrointestinal and distant pancreatic neuroendocrine neoplasms were evaluated. An improvement in overall survival in all distant neuroendocrine neoplasms over time was observed. The improvement in survival was more pronounced in the subgroup with distant gastrointestinal neuroendocrine neoplasms but the subgroup with distant pancreatic neuroendocrine neoplasms saw the biggest improvements [6].

The improvements in survival can be driven by changes in the incidence previously discussed, including a higher proportion of more indolent neuroendocrine neoplasms, stage migration due to improvements in diagnostic techniques, adoption of standardized staging and pathology guidelines. However, it seems that improvements in the management of neuroendocrine neoplasms, including development of Octreoscans in the late 1980s, may also have contributed to the survival improvement [6].

Table 2.1 Characteristics of the studies with data on neuroendocrine neoplasms survival together with survival information

Study	Period	Population	Neuroendocrine neoplasms included	Survival
Dasari A et al.	1973–2012	USA	Islet cell carcinoma (ICD-O3 8150), insulinoma (ICD-O3 8151), glucagonoma (ICD-O3 8152), gastrinoma (ICD-O3 8153), mixed islet cell/exocrine adenocarcinoma (ICD-O3 8154), vipoma (ICD-O3 8155), somatostatinoma (ICD-O3 8156), enteroglucagonoma (ICD-O3 8157), carcinoids (ICD-O3 8240), enterochromaffin cell carcinoid (ICD-O3 8241), enterochromaffin-like cell tumours (ICD-O3 8242), goblet cell carcinoid (ICD-O3 8243), composite carcinoid (ICD-O3 8244), adenocarcinoid (ICD-O3 8245), neuroendocrine carcinoma (ICD-O3 8246) and atypical carcinoid (ICD-O3 8249)	*Median overall survival (OS) time of 30 years* All = 9.3 years Localized = >30 years; Regional = 10.2 years; Distant = 12 months G1 = 16.2 years; G2 = 8.3 years; G3–G4 = 10 months Rectum = 24.6 years; Appendix = >30.0 years; Pancreatic = 3.6 years; Lung = 5.5 years
Luke C et al.	1980–2006	Australia	ICD-O-3 histology codes of 8150–8157, 8240–8246 or 8249	*Disease-specific survival* All sites combined survival 5 years = 68.5%; 10 years = 60.6%;15 years = 55.9%; 20 years = 49.9% *5-year survivals* Appendix = 93.8%; Rectum = 85.8%; Unknown primary site = 27.8%; Pancreas = 42.4%
Boyar Cetinkaya et al.	1993–2015	Norway	62 ICD-O morphology codes	*5-year relative survival* Low/intermediate aggressive neuroendocrine neoplasms = 64.8% high aggressive neuroendocrine neoplasms = 8.4%
Gudmundsdottir H et al.	1985–2014	Iceland	GEP only (morphology codes not available)	*5-year overall survival* All GEP sites = 75% Localized = 89%; Regional = 70%; Distant = 37%
Maarten van der Zwan JM et al.	2000–2002	Europe	Carcinoids (8240–8246); atypical carcinoids (8249); GEP well-differentiated endocrine carcinoma non-functioning (8240–8246, 8249, 8150); GEP well-differentiated endocrine carcinomas functioning (8151–8153,8155–8157); poorly differentiated endocrine carcinomas (8013, 8041–8045 all sites, but skin and thyroid); mixed endocrine–exocrine carcinomas (8154); neuroendocrine neoplasms of thyroid gland (8013, 8041–8045,8510, 8345–8347) and skin (8041–8044; 8240–824)	*5-year relative survival* All sites = 50% Thyroid gland = 82%; Well-differentiated endocrine carcinoma pancreas digest organs (non-functioning) = 64%; Mixed endocrine–exocrine carcinoma = 62%; Skin = 58%; Carcinoids = 30% Poorly diff endocrine carcinoma = 12%
Tsai HJ et al.	1996–2008	Taiwan	ICD-O3 8240–8249, 8013 (large-cell neuroendocrine carcinoma), and 8574 (adenocarcinoma with neuroendocrine differentiation)	*5-year observed overall survival* All sites = 50.4%; Rectum = 80.9%; Appendix = 75.7%; Lung = 34%; Pancreas = 30%
Hallet J et al.	1994–2009	Canada	ICD-O3 8240, 8241, 8242, 8243, 8245, 8246, 8249, 8150, 8152, 8153, 8154, 8155, 8156 and 8157	*5-year overall survival* All sites = 61%; Rectum = 87%; Small Intestine = 73%; Pancreas = 49%

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Part II

Diagnosis

New Concepts in Pathology

3

Massimo Milione, Laura Cattaneo,
and Alessandro Mangogna

3.1 Classifications at Present

3.1.1 Gastroenteropancreatic Neuroendocrine Neoplasms (GEP-NENs): World Health Organization (WHO) 2019 Rules (Fig. 3.1a)

The highest percentage of neuroendocrine neoplasms (NENs) arise in gastroenteropancreatic (GEP) system [1]. GEP-NENs represent a heterogeneous tumor group described by variable biological and clinical characteristics. Histological grading drives GEP-NEN's clinical outcome and therapeutic strategy.

GEP-NENs grading is given by their morphological features and proliferative activity evaluation. In contrast to ordinary carcinomas where "grade (G)" represents the histological parameter based on histologic resemblance between neoplastic cells and their normal counterpart, GEP-NENs grading has to be considered properly a prognostic parameter; when G increases, GEP-NENs patients clinical outcome became poorer. Since 2010, WHO classifications defined rigid rules to define the GEP-NENs grading system [2].

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3.1.1.1 Current WHO 2019 Classification Classes

GEP-NENs 2019 WHO classification (hereinafter called simply WHO 2019) defines the following prognostic categories, since 2017 associated only to pancreatic NENs [3, 4] (Fig. 3.1a):

A. Well-Differentiated Neuroendocrine Tumor (NET).
   - NET G1: well-differentiated neuroendocrine tumor, Ki-67 index <3%, and/or mitotic count <2/2 mm^2 or 10 higher power fields (HPF);
   - NET G2: well-differentiated neuroendocrine tumor, Ki-67 index 3–20%, and/or mitotic count 2–20/2 mm^2 or 10 HPF;
   - NET G3: well-differentiated neuroendocrine tumor, Ki-67 index >20%, and/or mitotic count >20/2 mm^2 or 10 HPF.

B. Poorly Differentiated Neuroendocrine Carcinoma (NEC).

   Poorly differentiated neuroendocrine carcinoma, Ki-67 index >20%, and/or mitotic count >20/ 2 mm^2 or 10 HPF. Further distinguished in:
   - Large cells NEC;
   - Small cells NEC.

   The importance of separating NEC according to the neoplastic cells size features takes origin from bronchopulmonary NEC and so we will discuss it in paragraph 2.

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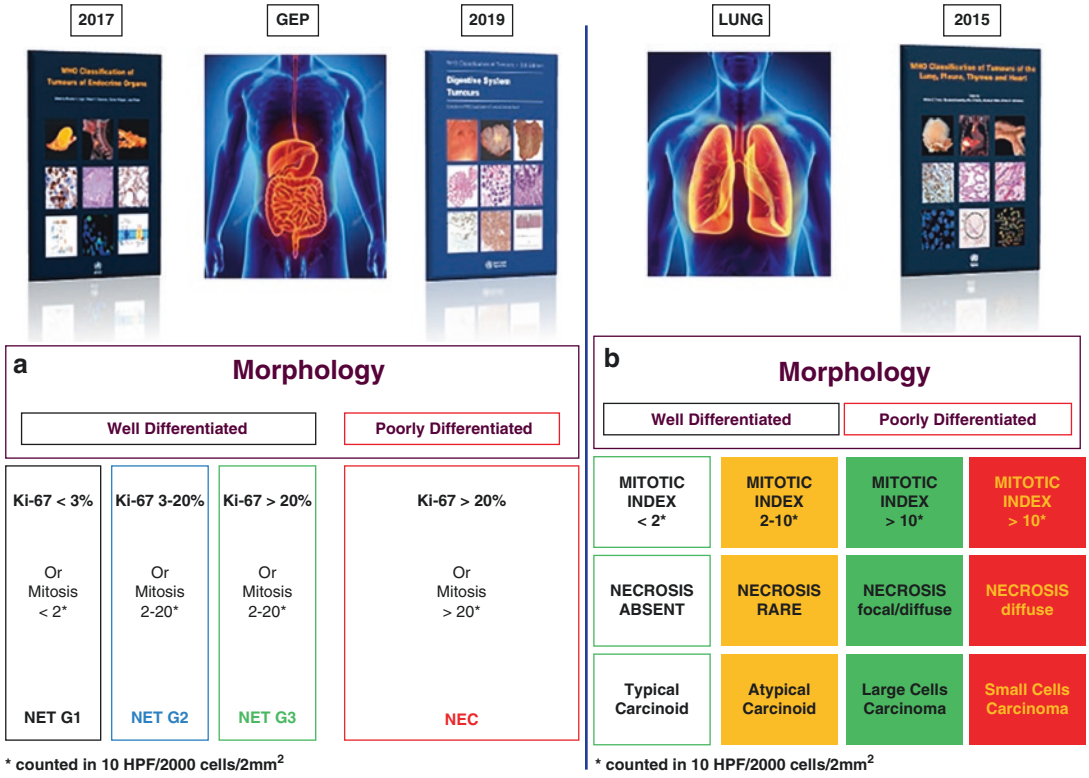

Fig. 3.1 (**a**) The World Health Organization (WHO) 2019 classification distinguishes gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NENs) on the basis of morphological aspects (well differentiated and poorly differentiated) and the cyto-proliferative activity of the tumor, expressed as grading (G). The G is based on the proliferative index of the tumor (number of mitoses on 10 high-magnification fields—HPF, High Power Field, with a minimum magnification of 40×) or as a value of Ki-67 (immunohistochemical parameter obtained by measuring the percentage of MIB-1 antibody positive cells out of 2000 cells, evaluated in the area of greatest nuclear labeling). Based on the assessment of the mitotic count and the proliferation index with Ki-67, the G of the GEP-NENs is defined: neuroendocrine tumor (NET) G1, NET G2, NET G3, and neuroendocrine carcinoma (NEC). The proposed cut-off to distinguish NET G1 from NET G2 is 2 mitosis/10 HPF and 3% Ki-67 index. The category of NET G3, characterized by well-differentiated neoplasms but with a Ki-67 proliferative index >20%, includes NENs characterized by high proliferative activity, but well-differentiated morphology, typical of NETs. Finally, a mitotic count >20/10 HPF and a Ki-67 index >20%, but with poorly differentiated morphology, define the NECs. The aforementioned principles, initially proposed in the WHO 2010 classification, were partially modified in the WHO 2017 classification which concerned only the pancreatic site (pancreatic NENs). The result of the changes made to the WHO 2010 classification in the 2017 version for the pancreatic site alone has been condensed, incorporated, and extended to the entire GEP system in the WHO 2019 classification. (**b**) The terminology to be used to describe lung NENs (LU-NENs) is that contained in the WHO classification, 2015 edition, which identifies four morphological variants: typical carcinoid (CT), atypical carcinoid (CA), large cell neuroendocrine carcinoma (LCNEC), and small cell lung carcinoma (SCLC). CT and CA have well-differentiated morphology, whereas LCNEC and SCLC poorly differentiated. Based on the assessment of the mitotic count and presence/absence of necrosis, the G of the LU-NENs is defined: CT, CA, LCNEC, and SCLC. The proposed cut-off to distinguish CT from AC is <2 mitosis/10 HPF and absence of necrosis. The category of poorly differentiated is defined by a mitotic count >10/10 HPF and presence of necrosis. Cytological features such as cell size, nuclear morphology, and architecture are additional characteristics useful to distinguish between LCNEC and SCLC

C. Mixed Neuroendocrine–Non-neuroendocrine Neoplasm (MiNEN).

The coexistence of neuroendocrine and non-neuroendocrine components in the same neoplasm is a rare but a well-known phenomenon in the digestive system.

WHO 2019 described this phenomenon as "mixed neuroendocrine–non-neuroendocrine neoplasm (MiNEN)" using the same term just proposed by 2017 WHO pancreatic neoplasm classifications [5]. In more details, MiNENs represent mixed neoplasms composed by the association between well or poorly neuroendocrine and other (non-neuroendocrine) neoplasms only when each counterpart covers at least 30% (≥30%) within the whole neoplasm [6, 7]. MiNENs enclose the previous mixed neoplasm categories: mixed adeno-neuroendocrine tumor (MANET) [8] and mixed adeno-neuroendocrine carcinoma (MANEC) [2, 9, 10].

MANET and MANEC are discussed extensively in further paragraph "From MANEC to MiNEN."

3.1.1.2 GEP-NENs Morphological Examination Rules

NETs (Fig. 3.2a) are composed by neoplastic cells, uniform in size and features, arranged in trabecular, organoid, gyriform, or ribbon architecture, and cytoplasm is intensively and diffusely (100% of neoplastic cells) stained by general neuroendocrine markers [Synaptophysin (Syn) and Chromogranin A (CgA)] because it is

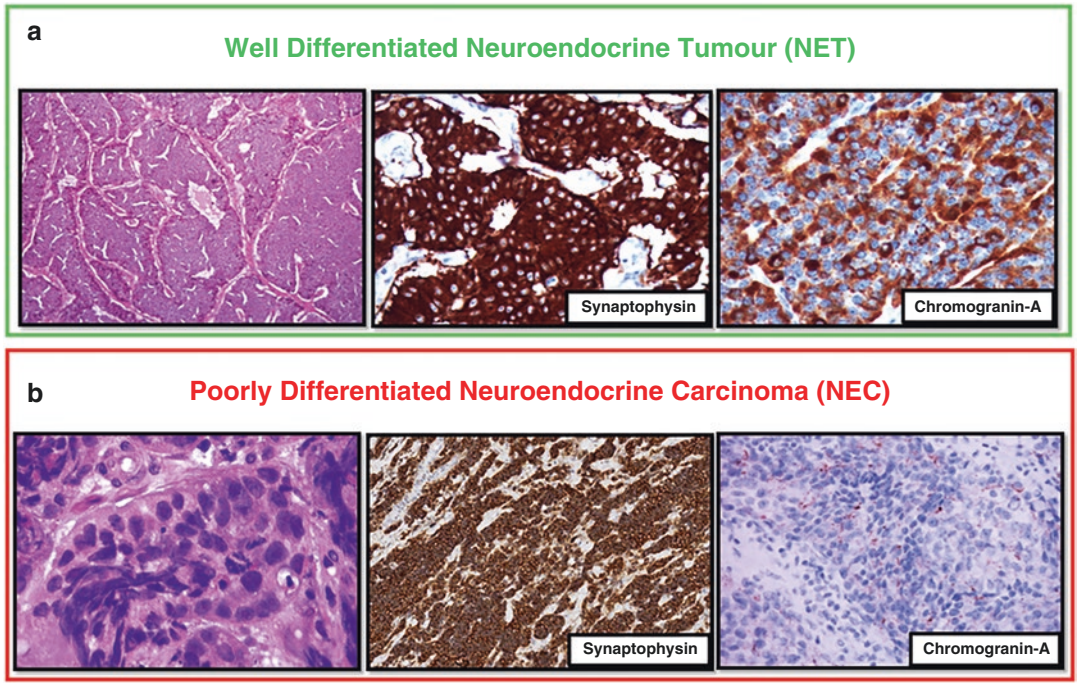

Fig. 3.2 The identification of the neuroendocrine phenotype involves the use of immunohistochemical markers capable of defining the neuroendocrine nature of the neoplasm: Chromogranin A (CgA) and Synaptophysin (Syn). (**a**) NETs show intense positivity for Syn and CgA. (**b**) NECs preserve positivity for Syn but may show reduced expression of CgA

Table 3.1 Morphological features of NENs [11–17]

Features	WD (NET)	PD large cells (NEC)	PD small cells (NEC)
A. NEN's architectural features			
– Typical architecture with an organoid growth pattern	Present	Absent	Absent
– Nodular or solid architecture with rosette formation or palisading	Absent	Present	Absent
B. NEN's cytological features			
– Low nuclear-to-cytoplasmic ratio	Present	Present	Absent
– High nuclear-to-cytoplasmic ratio	Absent	Absent	Present
– Abundant eosinophilic or amphophilic cytoplasm	Present	Absent	Absent
– Large amounts of cytoplasm often basophilic	Absent	Present	Absent
– Ovoid nuclei and/or salt and pepper chromatin	Present	Absent	Absent
– Nuclear atypia	Rare	Present	Present
– Nuclear pleomorphism	Rare	Present	Absent
– Obvious nucleoli	Absent	Present	Absent
– Large-size tumor cells*	Absent	Present	Absent
C. Tumor necrosis	Rare	Present	Present
D. Small-cell typical features as definite for LU-NECs	Absent	Absent	Present

Note. *NEN* neuroendocrine neoplasm, *WD* well differentiated, *NET* neuroendocrine tumor, *PD* poorly differentiated, *NEC* neuroendocrine carcinoma, *LU-NEC* lung neuroendocrine carcinoma. * by convention larger than three lymphocytes. Table modified by Fazio N, Milione M. Heterogeneity of grade 3 gastroenteropancreatic neuroendocrine carcinomas: New insights and treatment implications. Cancer treatment reviews. 2016; 50:61–7 (courtesy of Elsevier) [12]

rich in secretory granules. Nuclear chromatin is regular with inconspicuous nucleoli, without atypia. Mitoses are uncommon or at least rare.

NEC's cells (Fig. 3.2b), if small cell (SC) or if large cell (LC) (see Table 3.1), arranged in solid growth pattern, show pleomorphic and highly atypical nuclei rich in mitotic figures intermingled by abundant nonischemic necrosis that may be focal (punctate or spot) or diffuse (geographic or map). Syn and CgA positivity confirmed at immunohistochemistry (IHC) analysis are mandatory; even if Syn staining has to be maintained in whole neoplasm, CgA expression usually in the highest grade tumors. Criteria for distinguishing SC from LC and NETs from NECs are deeply listed in Table 3.1 [11, 12, 18–20].

3.1.1.3 *Proliferative Indices*: Mitotic Index (MI) and Ki-67 Labeling Index (Ki-67 LI)

- Mitotic index (MI): Understood as the number of mitoses on 10 high-magnification fields [high power field (HPF), with minimum magnification 40×] corresponding to a tumoral area of 2 mm^2 [2].
- Ki-67 labeling index (LI): An immunohistochemical parameter obtained by measuring the percentage of Ki-67 (MIB-1 antibody) nuclear positivity in tumoral cells out of 500–2000 cells (corresponding to a tumoral area of 2 mm^2), evaluated in the area of greatest nuclear marking, the so called hot spot [2].

Some tips and tricks are useful to properly define the tumoral area where proliferative indices will be evaluated. The aforesaid 2 mm^2 has to be searched in the so-called specimen's "hot-spots" in depth areas where at panoramic (larger microscopic fields) observation the higher proportion of stained nuclei and/or mitotic figures could be detected. According to WHO classification since 2010, the aforesaid 2 mm^2 areas could be properly covered by 10 high power optical microscopic field (HPF) at 40× magnification considering that each HPF could be sized at 0.5 mm [2]. Of note, HPF real size in the current microscopes available is not uniform covering a range between 0.096 and 0.31 mm^2. As concluding remark considering 10 HPF, according to WHO, could not be precisely reproducible in daily practice, otherwise could be better to consider HPF final number according to the specific microscope considering that each manufacturer should indicate the HPF size in mm^2 [21].

MI is the quantitative expression of M phase's cell cycle. The M phase is the shortest of the cell cycle and is therefore very fleeting. As a result, MI underestimates proliferating cells; otherwise Ki-67, a nuclear antigen expressed in proliferative cells (both S and M phases), has been proven as the powerful independent tool in predicting NENs clinical outcome [22–28].

3.1.2 Lung Neuroendocrine Neoplasms (LU-NENs) WHO 2015 Rules (Fig. 3.1b)

Lung neuroendocrine neoplasms (LU-NENs) represent a heterogeneous group of tumors showing different morphological features and clinical aggressiveness. According to 2015 WHO classification, LU-NENs are distinguished in four morphological and prognostic categories namely typical carcinoid (TC) and atypical carcinoid (AC), well-differentiated NENs, respectively, large-cell neuroendocrine carcinoma (LCNEC) and small-cell lung carcinoma (SCLC), still called microcitoma (Fig. 3.1b), poorly differentiated NENs, respectively [29, 30]. LU-NENs classification, similarly to GEP-NENs, considered as main skill the distinction between well or poorly differentiated NENs, using tumoral cells proliferation, exclusively identified by mitotic index, and necrosis assessment (Fig. 3.1b).

Different past terminologies are not recommended, deeply "carcinoma" and/or "malignant carcinoid," to collectively indicate TC and AC have to be carefully avoided because they could lead to inappropriate treatments [30].

Among the main well and poorly differentiated LU-NENs classes, differential diagnosis is based on the presence/absence of necrosis and the mitotic index (MI) per 2 mm^2 (Fig. 3.3). In

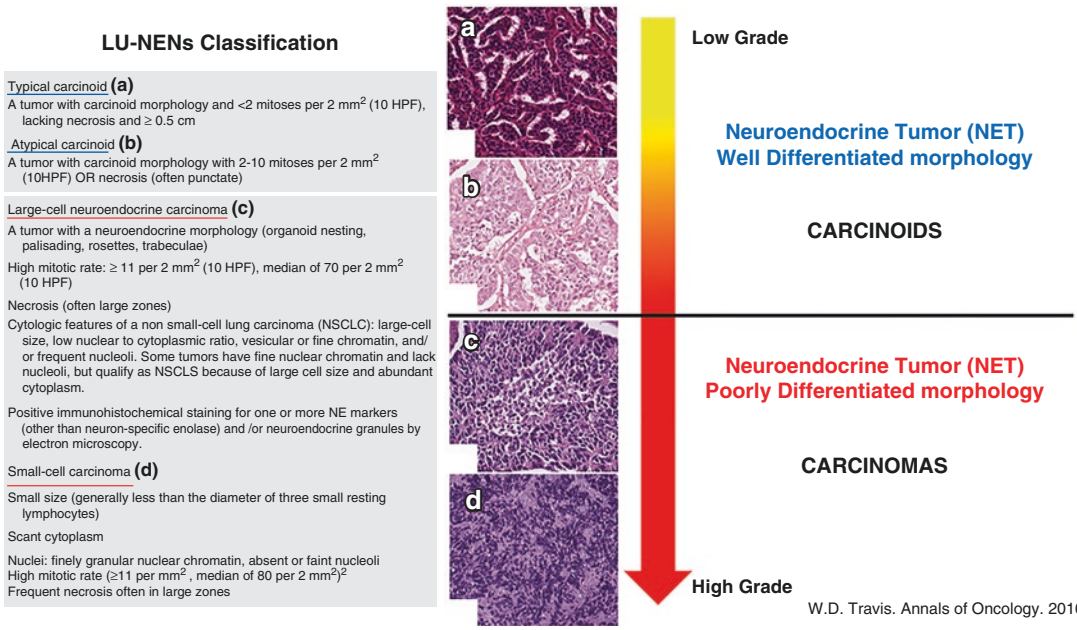

Fig. 3.3 Criteria for diagnosis of lung neuroendocrine neoplasms (LU-NENs). Terminologies used in the past are not recommended, indeed should be carefully avoided, in particular the use of the term "carcinoma" to collectively indicate typical carcinoid (CT) and atypical carcinoid (CA) or that of "malignant carcinoid," because they could lead to inappropriate therapeutic treatments or not be consistent with the classification criteria. Based on the morphological and immunophenotypic similarities between the neoplastic cells of CT (**A**) and CA (**B**) and the normal cellular counterpart of the diffuse NE system of the respiratory system, they represent a group of well-differentiated tumors (NETs) as opposed to large cell neuroendocrine carcinoma (**C**) and small cell carcinoma (**D**) which are poorly differentiated carcinomas

more details, TC does not show necrosis and MI is <2 mitosis per 2 mm^2, while AC group shows necrosis, even if focal and/or MI between 2 and 10 per 2 mm^2 finally poorly differentiated carcinomas must have >10 mitosis per 2 mm^2 and extensive necrotic areas [30]. Cytological features such as cell size, nuclear morphology, and architecture are additional characteristics useful to distinguish between LCNEC and SCLC, but not between AC and TC that, according to their common well-differentiated morphology, always share similar cyto-architectural features [30]. LU-NENs, confirmation is given by immunohistochemical markers such as CgA, Syn, and NCAM/CD56 [30]. WHO indicated CgA and Syn as reliable neuroendocrine markers (Fig. 3.4), NCAM/CD56, cell adhesion molecule, as helpful but not mandatory marker [29] and neuron-specific enolase, has been not recommended, because it lacked reproducibility [30].

LU-NENs WHO classification considered Ki-67 LI (%) (expressed as the percentual of positive tumor cells) only as additional parameter, in contrast European Neuroendocrine Tumor Society (ENETS) required Ki-67 for adequate diagnostic and prognostic LU-NENs assessment [31]. Deeply according to WHO, the main diagnostic Ki-67 role is still limited to: (1) distinguish the TC and AC from the high-grade LCNEC and SCLC [32], with a practical cutoff point of 25% to operate this distinction [29, 32]; (2) distinguish TC and AC from poorly differentiated NECs, in particular SCLC, in limited diagnostic material (cytology and biopsies) [33].

Even if Ki-67 role in LU-NENs prognostic stratification remains controversial, an intriguing proposal for LU-NENs "classification" has recently advanced. It has been based on the integration of three parameters: (1) Ki-67 labeling index evaluation, (2) necrosis assessment, and (3) MI, each of these categorized by three different "cutoffs" leading to the identification of three LU-NENs prognostic categories (G1, G2, and G3). These levels are indicated as follows: level 1 (G1) (2 mitoses, Ki-67 <4%, tumor necrosis absent), level 2 (G2) (>2–47 mitoses, Ki-67 4–25, tumor necrosis <10%), and level 3 (G3) (>47 mitoses, Ki-67 >25, tumor necrosis >10%)

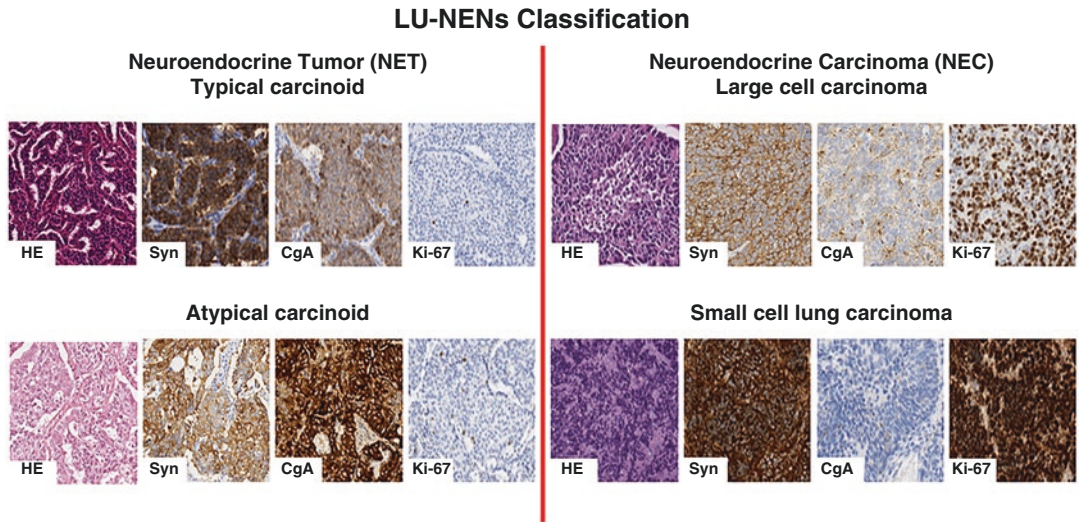

Fig. 3.4 Immunohistochemical criteria for the diagnosis of lung neuroendocrine neoplasms (LU-NENs). Chromogranin A (CgA) and synaptophysin (Syn) are the most helpful neuroendocrine lung tumors immunohistochemical markers. A low proliferation rate is seen in typical carcinoid by Ki-67 staining compared with atypical carcinoid where it is usually between 5% and 20%. Large cell neuroendocrine carcinoma and small cell carcinoma have very high proliferation rates (Ki-67 proliferative index >20%) and CgA weakly stains the tumor cells

[34, 35]. In depth, G1 (well-differentiated, low-grade) tumors are those that show at least two of three parameters at level 1, G2 (well-differentiated, intermediate-grade) tumors are identified by two of three parameters at level 2, and finally G3 (poorly differentiated, high-grade) tumors are characterized by at least two out of three parameters at level 3. Thus, tumors G1 include all TC and a fraction of AC, G2 tumors include most of AC but also some SCLC and LCNEC, and G3 tumors add up most of SCLC and LCNEC but even a small fraction of AC. This subdivision is in line with the literature data that see AC and LCNEC as somewhat heterogeneous categories of tumors from the behavioral point of view and a fraction of SCLC characterized by long survival. However, this proposal must be confirmed by independent and prospective validation studies [35, 36].

In conclusion even if the WHO includes only MI and assessment of necrosis [30], the ENETS in consensus statement on best practices for pulmonary neuroendocrine tumors noted that tumor grading based on a combination of Ki-67, mitotic rate, and necrosis may be of clinical importance but lacks validation [31]. According to recent evidences, a major role for Ki-67 also in LU-NENs classifications is requested [29, 37].

3.1.3 TNM in GEP- and LU-NENs

NENs are malignant tumors and consequently should be staged according to a site-specific staging system (TNM). NENs spread equally via blood or lymphatic system, and metastasis represents the most important prognostic determinant after grading. Lymph nodes represent the main GEP-NENs metastatic site followed by liver, lung, peritoneum, and pancreas.

At present, two different TNM systems work as NENs staging systems in more details:

1. The Union for International Cancer Control and the American Joint Committee on Cancer (UICC/AJCC) have published TNM classification (eighth edition) that have been applied to well-differentiated NETs (Grade 1 and 2) of the gastrointestinal tract, including the pancreas. Whereas, poorly differentiated (Grade 3) NECs are excluded and should be classified according to criteria for classifying carcinomas at the respective site [20, 38].
2. Based on clinical, surgical, and imaging data, European Neuroendocrine Tumor Society (ENETS) has published TNM staging recommendations that have been applied to all grades of NENs [39, 40]. Information on the presence or absence of metastasis has to be available as a minimum requirement for ENETS staging [39, 40].

ENETS staging system introduced different staging rules in comparison to UICC/AJCC (eighth edition) in gastric, appendicular, and pancreatic locations [40, 41] staging of pancreatic NENs (PanNENs) according to ENETS staging stratify better than UICC/AJCC into prognostically significant groups, whereas UICC/AJCC has been proved better in appendix NEN's patients clinical outcome prediction [42]. It is advisable to apply both schemes.

ENETS system applies to gastrointestinal NENs of foregut, midgut, and hindgut. Foregut NETs are further divided into three groups: (a) gastric NENs; (b) duodenum, ampulla, and proximal jejunum NENs; and (c) PanNENs. Midgut and hindgut NENs category include lower jejunum/ileum, appendix, and colon/rectum. ENETS staging criteria use stage 0 only for the stomach because this is the only anatomic site where T is defined. Stage I includes the T1 NENs with limited growth. Stage II applies to the tumors larger in size or more invasive, either T2 or T3, but always in the absence of metastasis. Stage III includes tumors with invasion into surrounding structures but without lymph node metastasis (stage IIIA) and tumors of any AJCC T stages (T1, T2, T3, and T4) in the presence of regional node metastasis (stage IIIB). Stage IV includes tumors of any AJCC T stage and

of any AJCC N stage (N0 or N1) with the presence of distal metastasis (M1) at the time of initial diagnosis [39, 40].

LU-NENs staging currently is represented by the TNM UICC/AJCC system, eighth edition, and should be classified according to criteria for carcinoma of the lung [38]. For carcinoids, however, the descriptive categories and the impact of multicentricity will have to be better defined, to better adhere to the biological reality of these neoplasms. For example, many multiple carcinoids, TC or AC, are multicentric synchronous primitive neoplasms rather than intrapulmonary metastases, especially if born in diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. For SCLCs, the use of terms such as "extended disease" and "limited disease" is discouraged, the latter being, in turn, diversifiable into subgroups with different prognosis according to the TNM system [43, 44].

3.2 Moving to 2017/2019 GEP Classifications

3.2.1 NET G3 History (Fig. 3.5)

The term well-differentiated neuroendocrine tumor (NET) G3 is a neologism used for the first time by a French group [13], and then later also by other authors [14, 45, 46], that means GEP-NEN with a well-differentiated (WD) morphology and a Ki-67 higher than 20%. NETs G3, until 2017 WHO classification was enclosed in NEC G3 covered 10–20% of all GEP NECs [12, 14, 46]. Compared to the latter, NET G3 patients were younger, the primary tumor site was espe-

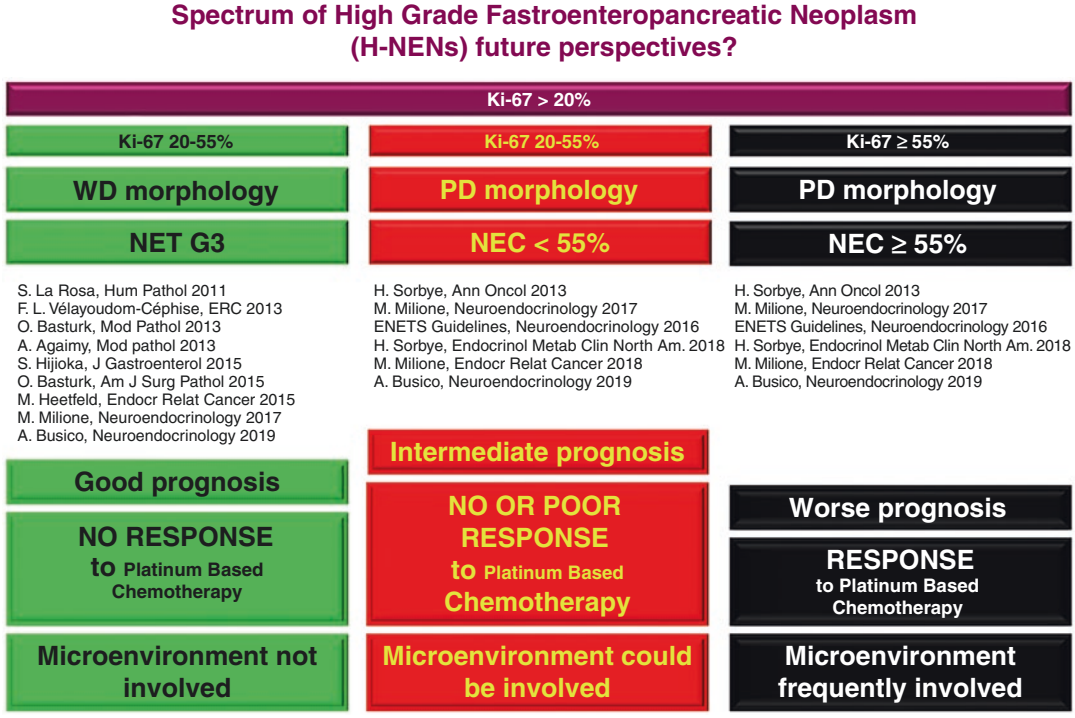

Fig. 3.5 Classification of high-grade gastro-entero-pancreatic neuroendocrine neoplasms (H-NENs) according to scientific literature. The prognostic categories neuroendocrine tumor (NET) G3 and neuroendocrine carcinoma (NEC) <55% and NEC ≥55% are defined by the contextual application of the morphological characterization and the quantitative evaluation of the proliferation, mainly through Ki-67, of the neoplastic cells. Therefore, the three categories of H-NENs show important differences between them both in prognostic terms and in terms of therapeutic approach

cially the pancreas, and the overall survival was significantly better than the NECs [14, 47]. When gastrointestinal (GI) NETs G3 were analyzed separately, as it happened for pancreatic (Pan) NETs G3, a great difference was detected with the respective NECs (both GI and Pan); it came to light that colorectal locations, such as the pancreas, have a poor prognosis [12, 14–17].

Mitotic index (MI) was considered inferior to Ki-67 LI to define NET G3 [12]. A possible explanation is that mitoses are related to a shorter phase (M phase) of the cell cycle than the Ki-67 antigen, present in the nucleus in all phases of the cell cycle, while it is absent in phase G_0 (growth).

Although all authors agree that NET G3 Ki-67 should be above 20%, a precise upper limit was not defined, with variable reported values: 55% in Italian study, 60% in French, and 70% in the European study [12]. In the studies that compare GEP-NET G3 to NEC, median Ki-67 (range) was 30 [13–17, 26–70] in NET G3 compared to 80 (25–100) in NEC [14, 46, 48]. A possible explanation could be related to the absence of reproducibility of study method: only Italian group selected case after a centralized pathologist revision, only the Nordic Group and Italian studies performing a specific analysis to find a threshold for Ki-67, showed that the 55% threshold works well from the prognostic point of view [14, 48], otherwise other groups reported descriptive statistics to evaluate of Ki-67 identified WD NETs with Ki-67 > 55%. Probably, in these rare conditions, WD NETs could have a component mixed of poorly differentiated (PD) NECs, and the Ki-67 could be evaluated as the median of the whole neoplasm, resulting in a Ki-67 >55% [49]. Therefore, in these particular cases, it would be more appropriate to evaluate Ki-67 separately in WD and PD areas, assigning two different values [12].

Over the years, given the previous WHO 2010 classification, NET G3 patients have been treated similarly to NEC even in the absence of sufficient information that these are clinically very different malignancies. Several retrospective studies showed that NETs G3 are less sensitive to platinum-based chemotherapy, cisplatin or carboplatin plus etoposide, than NEC. This is probably due to the fact that prognosis NET G3 is more related to the WD morphology of the tumor rather than the Ki-67 value, considering NETs G3 closer to NETs G2 than to NECs. In addition, positive responses to chemotherapy have been reported in patients NET G3 undergoing a combination of oxaliplatin with fluoropyrimidines, based on clinical evidence that oxaliplatin shows synergy with chemotherapeutic treatments potentially active both in G2 and in G3 NENs [50–53]. However, even if much rare, NETs G3 with Ki-67 index above 55% should be evaluated with particular consideration in terms of progression, prognosis, and behavior versus the traditional protocol, [54] and cisplatin or carboplatin plus etoposide regimen may be considered.

In clinical practice, identifying a PanNET G3 is perhaps more important of a GI NET G3, since molecular targeted agents, sunitinib and everolimus, are approved in well/moderately differentiated PanNET regardless of Ki-67 value.

Regarding the use of treatment with immune checkpoint inhibitors targeting PD-L1 or PD-1, NETs G3 have a cold immune microenvironment with few tumor infiltrating lymphocytes and the lower expression of PD-L1 compared to NECs. Likewise, NET G3s have a lower mutation burden than NECs, not making so them a potential target for immune checkpoint inhibitors [26, 55–57].

As concluding remark, it is important for clinicians that pathologists report both information on tumor morphology and the value of Ki-67.

3.2.2 Ki-67 New CutOff (Fig. 3.5)

Only about 5% of all GEP-NENs belongs to the G3 category [42], anyhow the distinction between NET G3 and NEC G3 is clinically and prognostically significant, and it is associated with adverse prognosis, good response to platinum-based chemotherapy (PBC), and insufficient response to temozolomide chemotherapy [12–15, 28, 45–47, 58–60]. Since 2011, several studies specifically investigating large series of GEP-NENs G3 have been published, including about 800 patients [12].

Category G3 with Ki-67 >20% is extremely heterogeneous and the broad interval (Ki-67 between 21% and 100%) include a spectrum of different neoplasms, with several responses to therapy [12–15, 28, 45–47, 58–60]. Median Ki-67 in poorly differentiated (PD) was 80% (range 25–100) and in well-differentiated (WD) 30% (range 21–70). Hence the ENETS, in the context of G3 category, proposed that the division of high-grade NENs (H-NENs) into three categories (NET G3, NEC <55%, and NEC ≥55%) in addition to a prognostic value also has therapeutic implications [42]. On the one hand, the prognostic role of the 55% threshold (Ki-67 value) into NECs has been already validated in different studies, and it has also included into ENETS guidelines since 2016. From these evidences, we can consider the spectrum of high-grade NENs (H-NENs) in three prognostic categories NET G3, NEC <55%, and NEC ≥55%, respectively, with good, intermediate, and worst prognosis [14, 24, 28, 42, 48, 49, 61]. In fact, from the study of several cohorts of patients with high-grade and PD lesions (PD NECs), a remarkable heterogeneity emerged from a molecular, prognostic, and therapeutic response point of view. For example, an Italian study has shown that for these lesions (PD NECs), the 55% threshold for the fraction of Ki-67-positive tumor cells (Ki-67 value or Ki-67 labeling index) allows a better stratification of the prognosis estimate; in particular, NECs with Ki-67 value <55% (NEC <55%) were associated with a median overall survival (mOS) of 12.9 months, whereas NECs with Ki-67 value ≥55% (NEC ≥55%) an mOS of 5.3 months [14]. On the other hand, for NET G3, a therapeutic approach similar to that used for category G2 is often considered, debating biological therapies, chemotherapy, peptide receptor radionuclide therapy, and liver-directed treatments within a multidisciplinary team. In GEP-NENs G3, chemotherapy represents the most common therapeutic approach. Although these neoplasms appear relatively chemosensitive, their prognosis is poor. The most frequently proposed therapy is PBC possibly combined with etoposide. It has been seen that an alkylating-based or oxaliplatin-based chemotherapy can be considered in NECs <55%, whereas therapies represented by PBC combined with etoposide in NECs ≥55% [13, 14, 46, 48, 61, 62].

From a molecular point of view, it is impressive observing that gene mutations were significantly enhanced in NECs ≥55%, involving *TP53*, *KRAS,* and *BRAF* genes [28]. In the context of H-NENs, the distinction between NET and NEC is also important for the study of the tumor microenvironment and the possible use of immune checkpoint inhibitors. We know that expression of PD-L1, tumor-infiltrating lymphocytes (TILs), tumor mutation burden (TBM), and neoantigen load can predict response to immune checkpoint blockade. Having said that, WD NETs do not seem like good candidates for immunotherapy, at least theoretically. Indeed, NETs G1/G2 have a cold immune microenvironment with few TILs and heterogeneous expression of PD-L1, whereas NECs have hot immune microenvironment with abundant TILs, a greater expression of PD-L1 and a high TBM. Moreover, applying the 55% threshold (Ki-67 value) into PD H-NENs, NECs ≥55% present an extensive mutational load, a dense immune infiltration and a higher expression of PD-L1 than NECs <55%. Therefore, due to their immune background, NECs ≥55% seem to represent excellent candidates for immunotherapy [26, 28, 55–57, 63].

In conclusion, based on growing evidence, the Ki-67 proliferative index should be used together with morphology, to define the following five categories: NET G1 (Ki-67 <3%, WD), NET G2 (Ki-67 3–20%, WD), NET G3 (Ki-67 21–54%, WD), NEC G3 (Ki-67 21–54%, PD), and NEC G4 (Ki-67 ≥55%, PD) [12].

However, part of the scientific community does not fully agree on the 55% threshold [64]. The latter consider Ki-67 thresholds intrinsically arbitrary and not necessarily generalizable between tumor types (i.e., NET versus NEC, various sites of origin) and outcomes (as prognosis or prediction). In particular, they claim that an absolute Ki-67 threshold is not applicable to the distinction between NET G3 and NEC. Therefore, further studies are needed to validate this threshold.

3.2.3 From MANEC to MiNEN (Figs. 3.6 and 3.7)

Mixed neuroendocrine–non-neuroendocrine neoplasms (MiNENs) are defined by the coexistence in the same neoplasm of two different oncotypes: the neuroendocrine type plus the non-neuroendocrine type, variously associated from a qualitative and quantitative point of view [3, 4, 6, 8].

Quantitative Association The 2019 WHO classification has maintained the "magic" number of 30%, that it is an arbitrarily defined percentage but on which there is wide agreement in the scientific world [3]. A neoplasm is considered mixed if at least one of the two "neuroendocrine and non-neuroendocrine" components is represented in at least 30% of the entire tumor [2].

Given the importance of the number 30%, when the presence of a mixed neoplasm on a histological section is suspected, it is necessary to ensure that the whole neoplasm has been sampled in such a way that this assessment is effectively applied to the entire neoplastic extension, the partial evaluation of the neoplasm risks underestimating and/or overestimating the real extension of the two components [4, 9].

There are mixed neoplasms from a morphological and biological point of view which however do not respond to the 30% cutoff on which currently there are no classification criteria, and they are generally described as adenocarcinomas with neuroendocrine differentiation (NED) or neuroendocrine carcinomas with focal glandular differentiation [65–69]. The standard of >2% cut is according to the 1% neuroendocrine cell in normal mucous [66, 70]. These latter conditions are rare, or perhaps not yet studied and understood, and there are no targeted studies so there is no scientifically validated information on the prognostic weight of the presence of the mixed component in these cases compared to the same pure tumor.

The presence of neuroendocrine cells within colorectal adenocarcinomas (CR-ADCs) has been documented and therefore it is not an

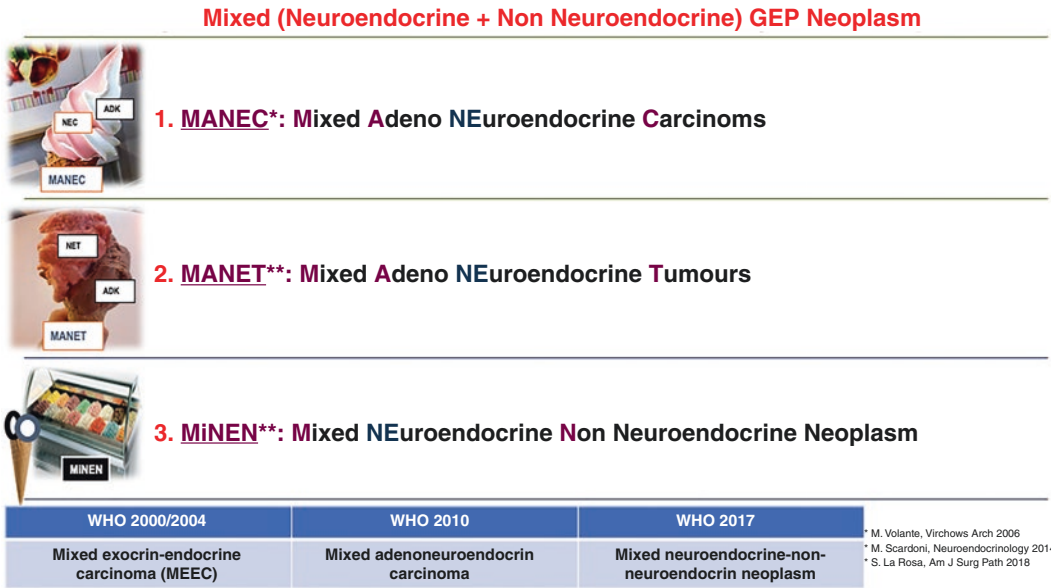

Fig. 3.6 The spectrum of mixed (neuroendocrine non-neuroendocrine) neuroendocrine neoplasms (MiNENs) in real life. Mixed adeno-neuroendocrine tumor (MANET) and mixed adeno-neuroendocrine carcinoma (MANEC) converge in the new definition of MiNENs

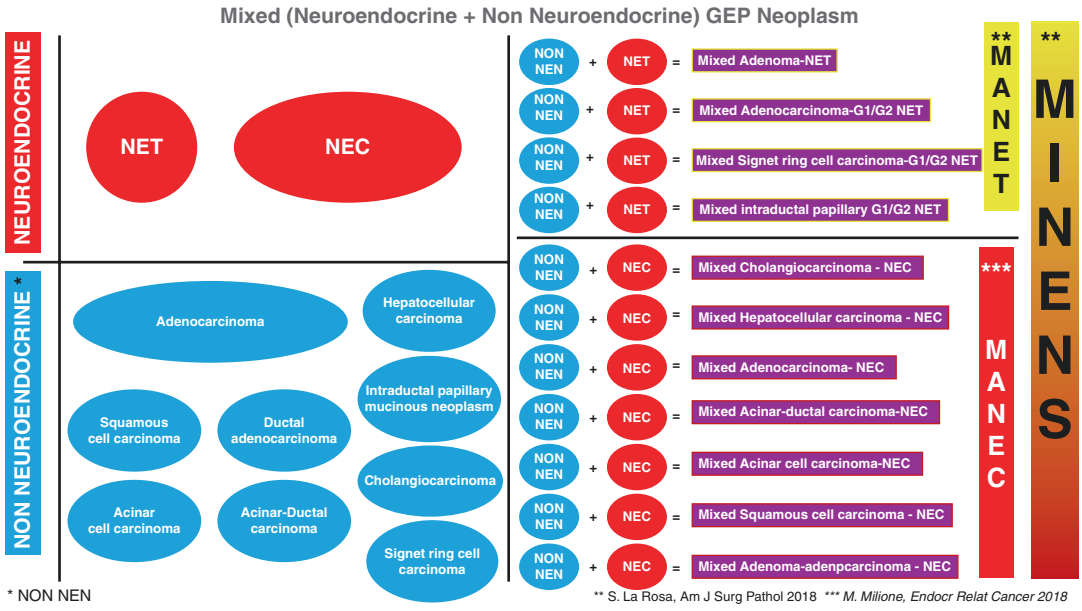

Fig. 3.7 Analogous view for mixed (neuroendocrine non-neuroendocrine) neuroendocrine neoplasms (MiNENs). The classification of gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NENs) according to WHO 2019 includes all mixed neuroendocrine and non-neuroendocrine neoplasms in the new "mixed neuroendocrine non-neuroendocrine neoplasm" (MiNEN) category. MiNEN combines the category of mixed adeno-neuroendocrine carcinoma (MANEC) and the categories of the very rare mixed adeno-neuroendocrine tumor (MANET). MiNEN always consists of invasive neoplasms; on the contrary, the association of "adenoma" and "neuroendocrine carcinoma" (NEC) will not produce either MiNEN or MANEC but simply NEC and adenoma

unexpected data [71, 72]. In fact, up to 41% of CR-ADCs can have immunohistochemically detectable neuroendocrine cells and the frequency of these neuroendocrine cells appears to be dependent on the method of determination [73–77]. It has also been seen that poorly differentiated CR-ADC appeared to have more frequent NED [7, 78, 79]. In these poorly differentiated ADCs, neuroendocrine cells are poorly differentiated (presenting as an oval, round, or irregular shape without polarizations) and similar to the adjacent non-neuroendocrine tumor cells; by contrast well-differentiated ADCs include well-differentiated neuroendocrine cells (presenting as a pyramid or bar shaped with the apex pointing to the cavity of the gland) [74].

Qualitative Association This represents the real revolution or novelty of the 2019 WHO classification, which has determined the new diagnostic category (MiNEN), previously adopted in 2017 for mixed neuroendocrine neoplasm of the pancreas [3, 4, 7].

MiNEN overcomes a problem of clinical and nosological practice represented by the previous definition of mixed adeno-neuroendocrine carcinoma (MANEC), which is a neoplasm that showed both glandular and neuroendocrine differentiation, thus overcoming the problem of neoplastic differentiation [2, 8–10].

With the term "MANEC," it was assumed that the two components were both carcinomatous and in particular glandular (adenocarcinoma) and neuroendocrine (neuroendocrine carcinoma) [8–10, 24].

If the non-neuroendocrine component is restricted to a precursor lesion such as a noninvasive carcinoma (i.e., adenoma) the neoplasm has to be considered pure NEC [24].

Neoplasm previously treated with neoadjuvant therapy should not be considered MiNEN

even if the mixed nature of the neoplasm was defined on different specimen obtained before the aforementioned treatment [24, 80, 81].

The new definition "MiNEN" goes further and widens the definition of the two components: neuroendocrine and non-neuroendocrine. On the one hand, it expands the spectrum of non-neuroendocrine lesions, including non-glandular histotypes (for example, squamous cell carcinomas or acinar cell carcinomas for pancreas) and also non-carcinomatous lesions (for example, adenomas). On the other hand, the term MiNEN includes also well-differentiated neuroendocrine neoplasms. Therefore, regardless of the type of non-neuroendocrine lesion, there will be two categories of mixed neoplasms: mixed neoplasms with well-differentiated neuroendocrine components (mixed adeno-neuroendocrine tumor, MANET) and mixed neoplasms with neuroendocrine carcinoma (mixed adeno-neuroendocrine carcinoma, MANEC). In conclusion, the sum of MANET plus MANEC defines the great MiNEN galaxy [2–4, 6–10, 24].

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4 Molecular Biology of Neuroendocrine Tumors

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4.1 Introduction

Neuroendocrine tumors (NETs) are diagnostically challenging tumors, as they comprehend a heterogeneous group of epithelial neoplasms with neuroendocrine differentiation [1, 2]. They are molecularly different from neuroendocrine carcinomas (NECs), as they rarely display inactivation of *RB1* and *TP53*, which instead are characteristic driver events in NECs [3, 4].

In the past, medical treatment has been mostly based on chemotherapy and has not taken into consideration varying tumor biology. In fact, the molecular study of NETs has been significantly limited for many years, due in large part to their relative scarcity. As a result, the knowledge on their cellular and molecular biology was significantly limited in comparison to that of other more common cancers [5, 6]. This limitation has been counteracted in recent years by a steady increase in the prevalence of these tumors, deriving from both longer survival of patients compared to other neoplasms and increased diagnosis rates thanks to improved imaging techniques [7, 8]. Additionally, the clinical-therapeutic management of NET patients considerably suffered by the lack of universally accepted standards for the disease, including both a diagnostic nomenclature and a staging system [1]. This significantly limited the conduction of appropriate clinical trials, and thus survival rates remained virtually unchanged for decades.

While efforts on classification and staging have provided a better grouping of NETs according to clinicopathologic and histologic features [4, 9, 10], recent genomic and epigenomic findings have significantly expanded our knowledge of NETs molecular landscape, allowing finer patient stratification and improved targeted therapies to achieve personalized patient care [6, 11].

4.2 The Lesson from Hereditary Syndromes

While most NETs are sporadic, a small fraction arises in a variety of inherited cancer-predisposition syndromes [12]. Similar to other types of cancer, the first clues about NETs molecular tumorigenesis came from genetic alterations associated with these hereditary syndromes (Fig. 4.1). While the detail of NETs arising from genetic syndromes will be treated in part V of this

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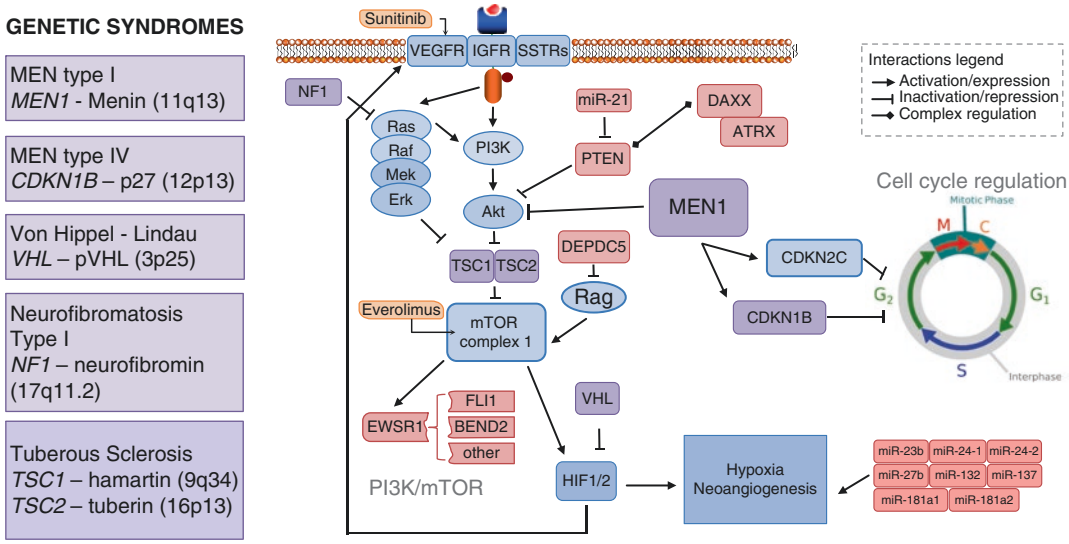

Fig. 4.1 In NETs, the study of inherited syndromes allowed the identification of the common deregulation of genes involved in the mTOR pathway and in cell cycle progression (purple boxes). As a master regulator of different cell functions, mTOR activation is subjected to tight and coordinated regulations through diverse positive and feedback regulatory loops. The more recent "next-generation sequencing" effort identified additional deregulation of the pathway through mutation of other mTOR inhibitors like *PTEN* and *DEPDC5* in pancreatic sporadic tumors. The upregulation of miR-21 also plays a role inhibiting *PTEN*. *ATRX* and *DAXX* were the first chromatin remodeling genes after *MEN1* to be identified as mutated in PanNETs, followed by many others in all NETs subtypes. The hypoxia-related genes have been recently implicated in a subgroup of PanNETs prone to metastasize, also in association with an miRNA signature. Approved targeted therapies for NETs are illustrated by orange boxes

book, a summary of their driver genetic alterations and their contribution to the research on sporadic NETs biology is presented here.

The most common NET-related inherited syndrome is the multiple endocrine neoplasia type I (MEN1; OMIM 131100). MEN1 is an autosomal dominant cancer susceptibility syndrome caused by inactivating mutations in the *MEN1* gene [13]. MEN1 patients feature a greatly elevated prevalence of various endocrine tumors, including pituitary tumors, parathyroid tumors, and pancreatic NETs (PanNETs). By the clinical standpoint, MEN1 patients harbor multiple small (<0.5 cm) pancreatic neuroendocrine microadenomas, which are thought to be precursor lesions to PanNETs. These data supported an early involvement of the *MEN1* tumor suppressor gene in PanNET tumorigenesis [14–16].

Menin, the protein encoded by the *MEN1* gene, is a ubiquitously expressed nuclear protein. It has been demonstrated to interact with a SET1-like histone methyltransferase (HMT) complex containing the MLL (KMT2A) protein. MLL specifically methylates lysine 4 of histone 3 (H3K4), an epigenetic modification associated with activation of genes transcription [17]. In normal pancreatic islet cells, menin was shown to negatively regulate islet cell growth by fostering the expression of two key negative regulators of the cell cycle: p18 (*CDKN2C*) and p27 (*CDKN1B*). In this context, menin was shown to target the HMT complex to the promoters of the two genes [18]. Menin may also affect the cell cycle via the PI3K/Akt/mTOR signaling pathway by inhibiting the activity of the key serine/threonine kinase Akt1 [19].

In addition to its role in regulation of cell proliferation, menin is involved in the response to DNA damage, which triggers its phosphorylation. This event results in transcriptional activation of several genes whose protein products are involved in the homologous recombination pathway of DNA repair (e.g., *BRCA1* and *RAD51*) [20]. Conversely, depletion of menin leads to

increased use of nonhomologous end joining DNA repair activity, a more error-prone repair pathway [21].

Taken together, the above results suggest that loss of menin leads to the deregulation of cell growth control and to genomic instability, creating an ideal setting for malignant transformation to begin. In line with this concept, *MEN1* alteration has been reported also in a large fraction of sporadic NETs, where mutations or chromosomal deletions involving the *MEN1* locus at 11q13 have been observed in up to 70% of cases [22–31]. Additionally, in vivo studies demonstrated that *MEN1* deficiency leads to pancreatic islet cell hyperplasia and neuroendocrine tumors due to the disrupted expression of p18 and p27 [32]. Weak or negative nuclear expression of menin has been reported in over 70% of sporadic PanNETs and lung NETs by immunostaining, while only about 30% of cases had *MEN1* gene mutations, most of which were inactivating [22, 33]. In patients with lung carcinoids, the occurrence of *MEN1* mutation or loss was associated with shorter overall survival. Correspondingly, low *MEN1* mRNA levels correlated with the presence of distant metastasis and shorter survival [34].

A second autosomal dominant hereditary syndrome is multiple endocrine neoplasia type 2 (MEN2). MEN2 is associated with the occurrence of NETs in the thyroid, parathyroid, and adrenal glands [35]. Medullary thyroid carcinoma (MTC) is the most common tumor in these patients, and 25% of MTC cases are hereditary. By the clinical standpoint, MEN2 is subclassified into three different syndromes, MEN2A, MEN2B, and familial medullary thyroid cancer. However, all of them are caused by mutations of the *RET* oncogene. *RET* encodes a receptor tyrosine kinase that normally binds a family of ligands including glial-derived neurotrophic factor. It is thought to provide growth and survival signaling via the RAF-MEK-ERK and PI3K/Akt/mTOR pathways [35]. Therefore, activating mutations of *RET* can confer ligand-independent growth and resistance to apoptotic stimuli. Although *RET* alteration is infrequent in sporadic NETs, the pathways it is involved in have been shown to be altered in a consistent fraction of NETs as detailed in Sect. 4.3 and 4.6.

A recent addition to the MEN spectrum, MEN type IV (MEN4; OMIM 610755) has its onset in the third decade of life with the appearance of parathyroid and pituitary tumors. Gastric, pancreatic, and bronchial NETs or gastrinomas may also occur, at a lower frequency [36]. The syndrome was discovered in rats lacking mutations in *Men1* and *Ret*, and it was initially named MENX due to its unknown driver gene. It was later associated with inactivating germline mutations of the *Cdkn1b* gene, which were confirmed to correlate with the disease also in humans [37]. *CDKN1B* encodes p27, a cyclin-dependent kinase inhibitor which is a master regulator of the cell cycle. Heterozygous mutation of *CKDN1B* was associated to lack of p27 in the affected tissues due to haploinsufficiency, that is the inability of a gene to preserve its function even in case of heterozygous inactivation [37–39]. Moreover, *MEN1* mutations were associated with the disrupted expression of p27. Overall, these data support the involvement of p27 in the early phases of NET development [40].

Other non-MEN syndromes feature an elevated rate of NETs, like the von Hippel-Lindau disease (VHL; OMIM 193300). This autosomal dominant predisposition syndrome is caused by mutation of the *VHL* tumor suppressor gene located at 3p25; nonfunctional PanNETs arise in 5–17% of patients [41]. Compared to *MEN1*, *VHL* gene is infrequently mutated in sporadic tumors. However, the *VHL* gene may be inactivated by different mechanisms: promoter hypermethylation or deletion have been reported in up to 25% of PanNETs [42]. The *VHL* gene product is involved in the oxygen-regulated degradation of hypoxia-inducible factor alpha (HIF1α). This transcription factor is activated by the PI3K/mTOR pathway and regulates gene expression in response to low oxygen conditions. Tumors harboring *VHL* gene inactivation display elevated expression of HIF1α downstream genes like vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) [42]. These, in turn, may activate a

feed-forward signaling loop with the PI3K/mTOR pathway itself [43].

Two other inherited autosomal dominant cancer-predisposition syndromes that develop NETs, albeit infrequently, are neurofibromatosis type I (NF1; OMIM 162200) and tuberous sclerosis (TS; OMIM191100). NF1 is caused by a germline mutation of the *NF1* gene at 17q11.2, which encodes the protein neurofibromin. This protein is a negative regulator of both the Ras and the mTOR signal transduction pathways [44]. About 10% of NF1 patients develop a duodenal or periampullary NET, usually a somatostatinoma [45]. Tuberous sclerosis is caused by mutations in one of two different genes: *TSC1* located at 9q34 and *TSC2* located at 16p13.3, which encode for the proteins hamartin and tuberin, respectively. Despite a rare occurrence of NETs in the frame of a TS syndrome [46], the *TSC1*/*TSC2* genes have an important role in PanNET pathogenesis. They suppress mTOR signaling, and the activity of *TSC2* is sustained by NF1 which inhibits its degradation (Fig. 4.1) [44]. *TSC2* expression is downregulated in 35% of sporadic PanNETs, and *TSC2* gene is mutated in 8.8% of sporadic PanNETs [26, 47]. These data further support the importance of this pathway's deregulation in NETs.

4.3 Next-Generation Sequencing Era: The Discovery of Novel Cancer Pathways

Although genetic syndromes provided the first clues on NETs molecular alterations, research on sporadic tumors was limited for many years. The main reasons were the low incidence of these tumors and the high amount of tissue needed for low-throughput biomolecular analyses. The advent of massively parallel high-throughput sequencing, also known as "next-generation sequencing" (NGS), has revolutionized the field of cancer genetics over the past decade. This technology allows for the unbiased identification of genetic alterations across the whole genome or exome (the protein-coding part of the genome), at the single-nucleotide level [48, 49]. The recognition of peculiar mutational landscapes and driver mutations in specific tumors has provided a compelling rationale for the introduction of novel targeted therapies [6].

The first NET whole-exome study was published by Jiao et al. in 2011 and focused on PanNETs [26]. The average mutation rate was 16 mutations per tumor, significantly lower compared to that previously reported for pancreatic ductal adenocarcinoma (i.e., 66 mutations per tumor) [50]. Furthermore, the most frequently mutated genes in pancreatic adenocarcinoma (*CDKN2A*, *KRAS*, *TP53*, *TGFBR1*, *SMAD3*, and *SMAD4*) were never or rarely mutated (*TP53*; 5%) in PanNETs. As anticipated from prior studies, *MEN1* was frequently mutated (44%). Genes coding for members of the mTOR signaling pathway were also mutated in a significant fraction of cases. These included inactivating mutations in *TSC2* (8.8%) and *PTEN* (7.3%), plus a single case displaying an activating mutation in *PIK3CA* [26].

Two novel genes were implicated in NET carcinogenesis: *ATRX* (alpha-thalassemia/mental retardation syndrome, X-linked; OMIM 300032; located on Xq21.1) and *DAXX* (death domain-associated protein; located on 6p21.3). A total of 43% of tumors harbored a mutation in either *ATRX* or *DAXX* (18% and 25%, respectively). Mutations in *ATRX* or *DAXX* were mutually exclusive, implying that *ATRX* and *DAXX* are complementary players in a common pathway whose disruption is relevant to the pathogenesis of PanNETs. In 53% of cases with either *ATRX* or *DAXX* mutations, *MEN1* mutation was detected as well [26].

ATRX is a large nuclear member of the SWI/SNF family of chromatin remodeling proteins [51]. Germline mutations in the *ATRX* gene cause the rare syndrome ATRX, which features alpha-thalassemia plus impaired intellectual development [52]. However, inherited syndromic *ATRX* mutations are typically hypomorphic missense mutations showing no association with elevated tumor risk. Conversely, somatic mutations detected in sporadic tumors tend to be nonsense mutations or insertions/deletions which lead to loss of protein expression [26, 52, 53]. This is in

keeping with the observation that engineered *ATRX* loss in the mouse is embryonic lethal. Thus, germline inactivating mutations in humans are also likely to be lethal and, as such, are not observed.

DAXX is also a nuclear protein involved in the regulation of chromatin structure and gene transcription. DAXX contains a SUMO-recognition motif, which allows it to bind many sumoylated proteins, including several transcription factors [54, 55]. As suggested by mutational data, DAXX binds the histone variant H3.3 and physically interacts with ATRX to form a histone chaperone-chromatin remodeling complex [56]. This complex deposits H3.3 at the upstream regions of several genes, at pericentromeric regions and at the telomeres [56, 57].

Heaphy and colleagues demonstrated that *ATRX* or *DAXX* mutation, or loss of protein expression, were perfectly correlated with the telomerase-independent mechanism called "alternative lengthening of telomeres" (ALT) [53]. At the cellular level, ALT is revealed by abnormally enlarged promyelocytic nuclear bodies containing large amounts of telomeric DNA repeats, detectable in tissue specimens by telomere-specific FISH analysis [58]. ALT or *DAXX*/*ATRX* mutation is observed in approximately half of PanNET cases, whereas it has only rarely been observed in other NETs, including 5 of 107 cases of gastrointestinal NETs and 1 of 95 lung carcinoids [53, 59, 60].

De Wilde and colleagues evaluated the timing of *ATRX* and *DAXX* mutations during PanNET tumorigenesis and progression by assessing ATRX or DAXX nuclear protein expression and ALT status in tissue samples from a cohort of MEN1 patients [61]. Loss of ATRX or DAXX and ALT-positivity was found exclusively in PanNETs and not in neuroendocrine microadenomas. In addition, these abnormalities correlated with higher tumor grade (G2 versus G1), and tumor diameter >3 cm, suggesting that *ATRX* and *DAXX* mutations are relatively late events in PanNET tumorigenesis [61]. Moreover, loss of ATRX or DAXX immunolabeling was associated with chromosomal instability, earlier recurrence, and poorer prognosis in 149 PanNETs [62].

A recent whole-genome analysis of 98 PanNETs has further expanded and integrated the information from previous literature [31]. The overall scenario relative to *ATRX/DAXX* alterations, *MEN1* mutation/loss, and ALT was confirmed. Patients bearing *ATRX/DAXX* mutation showed a poorer prognosis. Moreover, an integrative analysis showed that a recurrent pattern of chromosome losses was associated with ALT and *ATRX/DAXX* alterations in one-third of cases. On the other hand, cases with shorter telomeres and no *ATRX/DAXX* alterations showed a higher frequency of genomic rearrangements, including chromothripsis and/or EWSR1 gene fusions. The latter are recurrent events in Ewing sarcoma but were not previously reported in PanNETs. The fusion transcript is a downstream effector of the PI3K/mTOR pathway [63]. Other rearrangements and somatic mutations inactivated genes belonging to the mTOR pathway (*PTEN*, *TSC1/2*, *DEPDC5*), the cell cycle checkpoints (*CDKN2A*, *CDKN1A*, *CDKN1C*), SWI/SNF chromatin remodeling (*ARID2, SMARCA4*), and histone methylases (*SETD2*, *KMT2C*).

Five mutational signatures were detected, two of which led to further insights: a novel signature associated with biallelic inactivation of *MUTYH* due to germline mutation and somatic LOH in five cases, and a BRCA signature associated with the biallelic inactivation of *BRCA2* due to germline mutation and somatic LOH in one case [31]. *MUTYH* is involved in base excision repair, and its germline mutation predisposes to hereditary polyposis [64]. *BRCA2* is part of the DNA double-strand break repair machinery, and its germline mutation entails high risk of breast and ovarian carcinoma [65]. This finding suggested that several "sporadic" PanNETs may harbor germline driver events and prompted a search throughout the whole cohort. Detected germline mutations with somatic LOH included six in *MEN1*, one in *VHL*, and one in *CDKN1B*. Moreover, four cases displayed germline mutation and somatic LOH of *CHEK2*, which is activated upon DNA double-strand breaks and regulates the cell cycle by interacting with BRCA1 and p53. Its germline inactivation was previously associated with Li–Fraumeni

syndrome and familial breast cancer, but not with NETs [65]. Most cases with *CHEK2* or *MUTYH* mutations were wild type for *MEN1,* suggesting a MEN1-independent oncogenesis.

Chromosomal copy number variations (CNVs) included recurrent loss of chromosomes 1, 3, 6, 10q, and 11 and gain of chromosomes 4, 5, 7, 12q, 14, 17, 19, and 20 [31]. These CNVs clustered in four groups (Fig. 4.2): most losses were featured in the "recurrent pattern of chromosomal loss" group that associated with ALT and *MEN1/DAXX/ATRX* mutation, or in a second group characterized by loss of chromosome 11. The third group comprised polyploid tumors, while the last featured a recurrent pattern of chromosomal gains and was later associated with higher risk of metastasis [66].

RNA sequencing was also performed on 30 cases. Differentially expressed genes clustered in three groups [31], one of which had overlapping features with a previously described metastasis-prone group of PanNETs [67]. Enrichment analysis of this subgroup showed an overrepresentation of genes controlled by the HIF1/2 factors and the consequent deregulation of glucose metabolism [68]. In both works, this "HIF signature" was not

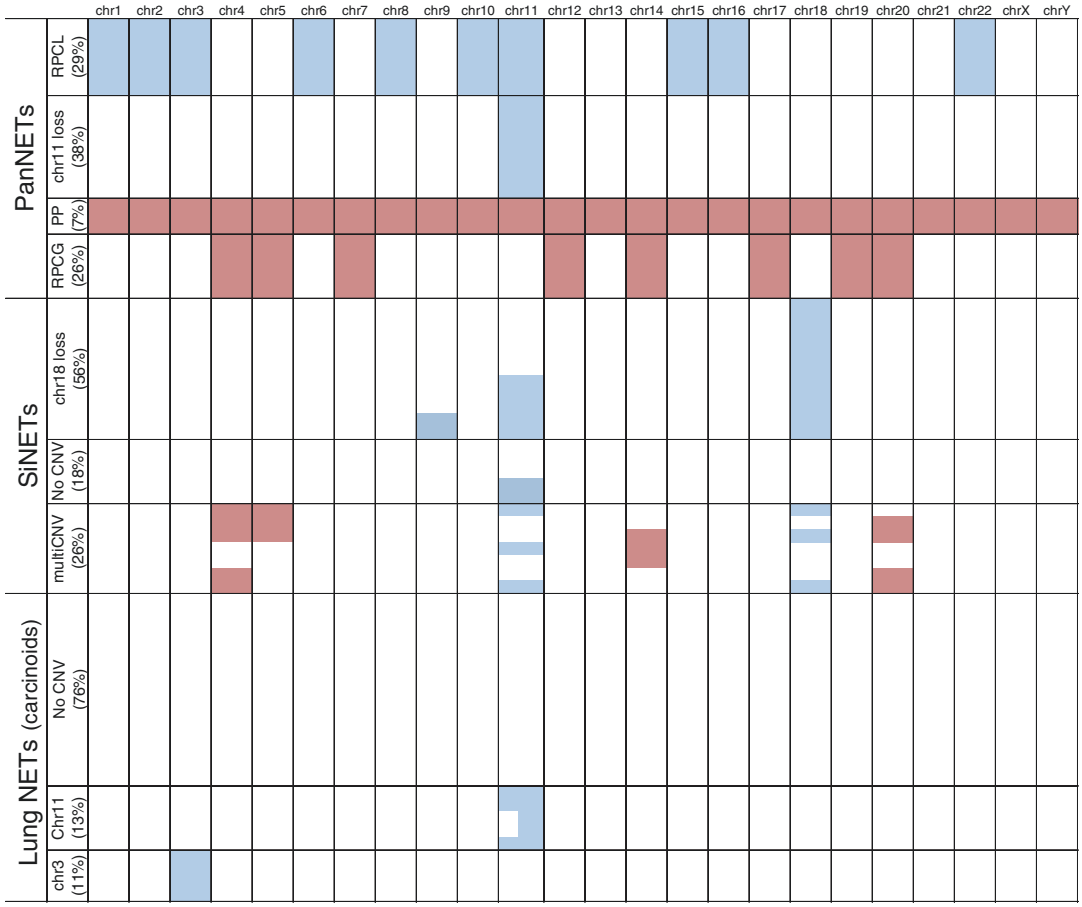

Fig. 4.2 Subtypes of PanNETs, SiNETs, and lung NETs according to chromosomal alterations. Four subgroups have been identified in PanNETs and three in SiNETs. The majority of lung NETs present no recurrent alterations, while two smaller groups feature loss of chromosome 3 or chromosome 11/11q. Copy gains are shown in red, losses in blue. In each subgroup of tumors, cases with an identical CNV pattern are represented by individual rows, and the height of the row is proportional to the fraction of cases harboring that CNV pattern. *Chr* chromosome, *multiCNV* multiple CNV, *PP* polyploid, *RPCG* recurrent pattern of chromosomal gains, *RPCL* recurrent pattern of chromosomal loss. Adapted from Mafficini and Scarpa, Endocrine reviews (2019) [10]

associated with a defined mutational pattern. It is thus still unclear to which extent is it a consequence of the neoplastic process or an adaptation to a hypoxic environment.

Loss of *ATRX/DAXX/MEN1* was associated to longer overall survival in the first study by Jiao et al. [26]. However, only univariate analysis was performed, and subsequent studies reported contrasting results. In particular, Marinoni et al. and Scarpa et al. reported *ATRX/DAXX* loss or mutation to be associated with worse outcome and chromosomal instability [31, 62]. A third study by Chan et al. also reported poorer prognosis for *ATRX/DAXX/MEN1* (ADM) mutant PanNETs and performed differential expression of ADM mutant vs. ADM wild-type PanNETs on 47 cases. This analysis demonstrated enriched expression of pancreatic alpha cell-specific genes (*ARX*) and reduced expression of beta cell-specific genes (*PDX1*) in this group of tumors. Since *ATRX/DAXX* mutation is considered a late event in PanNET oncogenesis, these data suggest that their mutation might preferentially affect alpha-like cells undergoing neoplastic transformation [69]. A second work by Cejas et al. further explored this topic by chromatin immunoprecipitation sequencing on 21 PanNETs. Results showed the existence of three groups of tumors according to the different H3K acetylation/expression of alpha and beta cell-specific genes, including *ARX* and *PDX1,* respectively. Alpha cell-like tumors expressed *ARX* but not *PDX1*; beta cell-like tumors expressed *PDX1* but not *ARX*; a third group expressed both at a similar level [70]. In the same work, immunolabeling for ARX and PDX1 was evaluated in 103 PanNETs with clinical follow-up, and ALT was determined as a proxy for *ATRX/DAXX* mutation. ALT was strongly associated, although not exclusive, to ARX+ tumors, and all ALT+ARX+ tumors had disease recurrence, whereas only 9% of ALT-ARX+ cases experienced disease recurrence and only one PDX1+ case recurred [70]. Overall, the two works complement each other in showing that the vast majority of PanNETs exhibit either alpha or beta cells expression and that ALT (or *ATRX/DAXX* mutation) is enriched and associates with aggressive behavior specifically in tumors with an alpha cell-like expression profile.

Functioning and nonfunctioning PanNETs exhibit a certain overlap of molecular features, with a notable exception: insulinoma, which is set apart in terms of gene expression [47]. This is mirrored by recent mutational data, as whole-exome sequencing showed rare (2%) *MEN1* mutations and recurrent mutation of the *YY1* (Yin Yang 1) gene. Prevalence ranged from 10% to 30% in different publications and was higher in Asian patients [71–74]. Mutation was associated with a late onset of the tumor and deregulated transcriptional activity of the YY protein. Although these findings require further investigation, YY is a target of mTORC1 and inhibitors like everolimus have been suggested already as a potential therapeutic option [71]. In keeping with their genetic landscape, insulinomas have been recently shown to be PDX1+/ARX- at immunolabeling, as expected for beta-cell like tumors [70].

A small proportion of pancreatic neuroendocrine neoplasms are classified as poorly differentiated, high-grade pancreatic neuroendocrine carcinomas (PanNECs). As outlined at the beginning of this chapter, their mutational landscape is different from that of both PanNETs and pancreatic adenocarcinoma [3]. *KRAS* and *CDKN2A*, which are commonly mutated in adenocarcinomas, and *MEN1*, *ATRX*, *DAXX*, and mTOR pathway genes, frequently mutated in PanNETs, are infrequently or never altered in PanNECs. On the other hand, mutations in *TP53* and *RB1* are very common in this group of tumors. *TP53* is rarely mutated in well-differentiated PanNETs; its pathway, however, has been suggested to be deregulated by frequent copy gain of p53-related genes, such as *MDM2* (22%), *MDM4* (30%), and *WIP1* (51%) [75]. Similarly, 80% of PanNETs show an unchanged sequence of the *RB1* gene but display genetic alterations of other components of the Rb pathway, such as cyclin-dependent protein kinase 4 and 6 and cyclin D [76]. However, the involvement of P53 and RB pathways in PanNETs was not evident from whole-genome analysis [31].

The genomic landscape of small intestinal NETs (SiNETs) has been recently investigated [77–79]. The group led by Matthew Ames [77] performed an integrative analysis of somatic mutations and copy number variations of 48 SiNETs, reporting genetic alterations of different members of the PI3K/Akt/mTOR pathway in 30% of the samples and the loss of *SMAD* genes in 45% of samples. The average mutation rate was low (0.77 mutations/megabase), comparable to that reported for PanNETs (0.82 mutations/megabase) [26]. A positive correlation was observed between primary tumors with higher mutation rate and the presence of liver metastases. Mutations were identified in several cancer-related genes (e.g., *BRAF*, *EZH2*, *FANCD2*, *FGFR2*, *MEN1*, *VHL*) but no recurrent driver emerged. Notably, genes which are frequently mutated in PanNETs, PanNECs, and pancreatic adenocarcinomas were wild type in this cohort. The alteration of the above cited pathways was thus mainly driven by copy number alterations, including recurrent loss of chromosome 11 and 18, and gain of chromosome 4, 5, 14, and 20 (Fig. 4.2). Concomitantly, another group identified the haploinsufficient gene *CDKN1B* as recurrently inactivated by mutation or copy loss in 11 of 50 SiNETs [78]. This gene is frequently inactivated in many forms of cancer and is the cause of the MEN4 genetic syndrome [37–39, 80]. The results of both these studies were confirmed by independent investigations, which also showed the existence of three groups of SiNETs based on clustering of chromosomal alterations [79, 81]. Additionally, cases with copy gains were associated to poorer prognosis [79, 81]. Recurrent mutation and copy loss of another haploinsufficient gene, namely *APC,* has been reported by Bottarelli et al. in 7 and 4 of 30 SiNETs, respectively and confirmed by Simbolo et al. in 4 and 2 of 52 cases, respectively [81, 82]. However, this finding still requires investigation to clarify the role of *APC* in the subset of SiNETs which feature its alteration.

Whole-exome sequencing of 17 sporadic MTCs showed that approximately 90% of tumors had mutually exclusive mutations in *RET*, *HRAS*, and *KRAS* genes, suggesting that RET and RAS are the predominant driver pathways in MTC [35]. Few other mutations were observed, and no further driver event [35].

Pulmonary carcinoids were recently investigated by whole genome/exome and transcriptome sequencing, including gene copy number analysis [33, 60, 83]. Fernandez-Cuesta et al. demonstrated frequent mutations in chromatin remodeling genes; covalent histone modifiers and subunits of the SWI/SNF complex were mutated in 40% and 22% of the cases, respectively, with *MEN1*, *PSIP1*, and *ARID1A* being recurrently affected [83]. In contrast to small-cell lung cancer and large-cell neuroendocrine lung tumors, *TP53* and *RB1* mutations were rare events. The involvement of chromatin remodeling and histone modifiers was further confirmed by Simbolo et al. in a comparison between typical and atypical carcinoids, large-cell and small-cell carcinomas of the lung by whole exome and targeted sequencing [60]. Moreover, this work showed that some carcinoids display features of the more aggressive carcinomas, leading to hypothesize that the latter may evolve from carcinoids [84]. A similar concept was expressed by parallel works comparing large- and small-cell carcinomas of the lung, which demonstrated the existence of large-cell carcinomas with milder aggressiveness, or "carcinoid-like" carcinomas [85, 86]. This concept was further explored by a recent integrative genetic/transcriptomic study of carcinoids and large-cell carcinomas of the lung. Clustering analysis of 35 atypical carcinoids and 32 large-cell carcinomas showed the existence of three groups, comprising one group dominated by carcinoids, one by carcinomas, and a mixed group of carcinoids and carcinomas sharing transcriptional features [33]. The existence of this mixed group was further explored by Alcala et al., who performed machine learning studies on 257 lung neuroendocrine neoplasms and again identified a group of "supra-carcinoids." These tumors, although morphologically classified as atypical carcinoids, featured a shorter overall survival and enriched expression of immune checkpoint genes, including *CD274* (PD-L1) [87].

4.4 Epigenetic Changes in NETs

The main epigenetic events by which the genome is regulated include DNA methylation and chromatin modification, especially via histone methylation or acetylation. The advent of molecular techniques allowing genome-wide assessment of epigenetic changes, such as chromatin immunoprecipitation sequencing (ChIP-Seq) and genome-wide DNA methylation analysis, has pushed the exploration of epigenetic abnormalities, which are widespread in cancer, cooperate with genetic lesions, and often originate from them [88].

DNA methylation has been reported at different levels in a variety of NETs [10, 89]. Moreover, the known alteration of *MEN1* and of several genes belonging to the SWI/SNF or to the histone methyltransferase families further supports the idea that epigenetic changes may be central to NETs biology.

Choi et al. performed global analysis of the methylation status of LINE-1 and Alu repeat sequences that are widely distributed throughout the genome. These sequences were hypomethylated in most cases compared to adjacent normal tissue. LINE-1 hypomethylation was stronger in ileal compared to pancreatic and pulmonary NETs and correlated with lymph node metastasis. A second study on 56 PanNETs found strong hypomethylation of LINE-1 in 12 cases, and correlation of this feature with advanced stage and poor prognosis [90, 91].

Another phenomenon involving widespread DNA methylation is CpG island methylator phenotype (CIMP). CIMP-positive tumors display simultaneous abnormal methylation of multiple CpG islands, including several associated with known tumor suppressor genes. In a large series of gastroenteropancreatic NETs, CIMP prevalence was 70%, ranging from 50% in gastrinomas to 100% in VIPomas and glucagonomas [92]. CIMP has also been found in over half of colorectal neuroendocrine carcinomas, where it correlated with microsatellite instability [93, 94].

Two recent publications by Karpathakis et al. focused on global methylation profiling of SiNETs. The first study showed the existence of three groups of tumors, with different prognosis, defined by both copy number alterations and methylation profiles. Enrichment analysis reported prominent involvement of the MAPK, mTOR, and Wnt pathways [79]. The list of top differentially methylated genes was used in a follow-up study to compare the methylation and expression profiles of 49 primary SiNETs vs. 20 liver metastases. The analysis showed progressive differential methylation in 67% of genes and differential expression in 51% [95]. These studies are a clear example of the above cited synergy between genetic (CNV) and epigenetic (methylation) alterations.

As for individual genes, three have been demonstrated to be highly methylated in NETs: *MGMT*, *RASSF1A*, and *CDKN2A*. *MGMT* expression is commonly deficient in PanNETs (up to 51% of cases) and, more importantly, its status can be used to predict response to alkylating agents. On the other hand, *MGMT* is usually expressed in most G1 gastroenteropancreatic NETs, which may explain much of the differential sensitivity to temozolomide-based therapies between these two tumor types [96, 97].

The *RASSF1A* tumor suppressor gene, located on 3p21, is rarely mutated in cancer; however, it is frequently silenced via promoter hypermethylation [98]. In PanNETs, *RASSF1A* promoter is reportedly methylated in 75–83% of lesions, with an associated reduction of *RASSF1A* mRNA expression, increased tumor size, and presence of metastases [96, 99–101]. However, *RASSF1A* methylation is also observed in a significant fraction of tumor-adjacent normal pancreas tissue, which strongly affects its specificity as potential tumor biomarker [96, 99–101].

The *CDKN2A* gene codes for the p16 tumor suppressor protein, a member of the Rb cell cycle regulatory pathway that regulates entry into S-phase and other cellular processes. This pathway is thought to be disrupted in practically all human cancers, although through different mechanisms. *CDKN2A* has been reported to be methylated in 10–58% of PanNETs and up to 44% of SiNETs; methylation was also associated with presence of metastasis in PanNETs and with poorer prognosis in SiNETs [76, 96, 102]. Unlike

RASSF1A, methylation of the *CDKN2A* displayed good specificity for tumor tissue vs. adjacent normal tissue [76, 96, 102].

PDX1 and *ARX* are two homeobox genes involved in pancreatic islets development. PDX1 is expressed early during pancreatic differentiation: its expression is maintained by beta cells and required for their development and to ensure glucose homeostasis [103]. ARX is also expressed in the developing pancreas, including the forming islets of Langerhans, and its knockdown completely abolishes the formation of beta cells [104]. As part of the above-described subtyping of PanNETs into alpha cell-like and beta cell-like tumors, *PDX1* was recently shown to be methylated and its expression specifically downregulated in a subset of PanNETs, featuring an alpha cell-like expression profile [69]. These tumors featured histone acetylation and expression of the *ARX locus*, which however was not differentially methylated across alpha cell-like and beta cell-like PanNETs [70]. Thus, specific methylation of *PDX1* seems to be an important event in the genesis of alpha cell-like PanNETs, in the context of intact *ARX* expression.

4.5 microRNA Deregulation in NETs

In contrast to many other tumor types, little is still known about microRNA (miRNA) expression patterns in NETs. However, a significant number of oncogenic and suppressor miRNAs have been identified so far, also supporting the possible use of specific miRNAs signatures to predict clinical outcome in NETs [105–108].

Of interest, unlike most mRNAs, miRNAs are long-living *in vivo* and very stable *in vitro*. This structural solidity is fundamental to miRNAs analysis in FFPE samples, in which they can also be investigated at the level of individual cells by applying in situ hybridization (ISH) techniques [109]. Another important point is the evidence for the applicability of miRNAs as noninvasive biomarkers, as they are easily and reproducibly detectable in all body fluids [109].

Roldo and colleagues investigated global microRNA expression signatures of normal pancreas, Langerhans' islets, PanNETs, and pancreatic acinar carcinomas [105]. The overexpression of miR-103 and miR-107, associated with lack of expression of miR-155, significantly discriminated tumor from normal samples. Moreover, a set of 10 miRNAs (miR-125a, miR-99a, miR-99b, miR-125b-1, miR-342, miR-130a, miR-132, miR-129-2, miR-125b-2) distinguished PanNETs from acinar tumors. This specific miRNA signature is possibly associated with either endocrine differentiation or tumorigenesis. Among PanNETs, miR-204 was primarily expressed in insulinomas and correlated with immunohistochemical expression of insulin. On the other hand, miR-21 overexpression was associated with higher Ki67 proliferation index and the presence of liver metastases [105]. *PTEN* is one of miR-21 targets, which adds another layer of complexity to the involvement of the mTOR pathway in NET pathogenesis.

A second study evaluated 37 PanNETs against nonneoplastic pancreas and pancreatic islets [110]. The profiles of differentially expressed miRNAs in PanNETs obtained using either nonneoplastic pancreas or pancreatic islets were radically different, showing that the choice of the control tissue plays a critical role and may account for a large portion of the variability between different studies. miR-193b was overexpressed in PanNETs compared to pancreatic islets and in sera of PanNET patients compared with healthy subjects, whereas miR-642 correlated with Ki-67 and miR-210 with the presence of metastasis [110]. Further confirming miRNAs' stability in body fluids, a nine miRNA expression pattern (miR-24, miR-30a-3p, miR-18a, miR-92a, miR-342-3p, miR-99b, miR-106b, miR-142-3p, and miR-532-3p) derived from the analysis of selected miRNAs in cyst fluid samples, successfully discriminated cystic forms of PanNETs from other pancreatic cystic lesions [111]. Another study assessed the prognostic value of eight candidate miRNAs on 37 PanNETs; overexpression of miR-196a emerged as an independent predictor of earlier recurrence, also

associated with higher stage, grade, and lymphatic vessel invasion at diagnosis [112].

Two relatively recent miRNA expression profile studies found evidence of miRNA deregulation in SiNET progression [106, 107]. Ruebel and colleagues from Mayo Clinic reported the association of miR-133a downregulation with progression to metastatic carcinoid tumor, which was validated in an independent cohort. This report suggests that miR-133a may have an important role in ileal NET development and progression and could be useful for diagnostic and/or prognostic stratification [106]. The same group in collaboration with the Uppsala University investigated miRNA expression in a new set of SiNETs with matched metastases. Nine miRNAs were significantly deregulated during tumor progression: five (miR-96, miR-182, miR-183, miR-196, and miR-200) were upregulated, whereas four (miR-31, miR-129-5p, miR-133a, and miR-215) were downregulated. This work provided further validation for miR-133a and for miR-183, which was reportedly upregulated in the first study but lacked validation [107].

A third report by Miller et al. analyzed 28 SiNETs and matched normal tissue (n = 14), lymph node metastases (n = 24), and liver metastases (n = 15) [113]. The analysis identified 39 differentially expressed miRNAs in neoplastic tissues compared to normal samples, with good overlap between primary tumors and metastases. Results substantially confirmed the previous landscape and added the upregulation of miR-204, -7-5p, -375 and the downregulation miR-1 and miR-143-3p. Downregulation of miR-1 was expected from previous data, as miR-1 and miR-133a are transcribed as a single cistron [113]. Upregulation of a set of miRNAs (miR-96, -182, -196a, and -200a) was also detected in the sera of SiNET patients upon treatment with somatostatin analogs, again suggesting these molecules as possible circulating markers [114].

MiRNAs have been also demonstrated to be significantly deregulated in MTCs, with miR-224 upregulation associated with a better outcome [115]. In lung NETs, members of the miR-29 family seem to have a significant role in the carcinogenetic process [116].

4.6 From Single Gene Alteration to Signaling Pathway Perturbations

In recent years, much attention has focused on identifying key cellular signal transduction pathways that are abnormally activated or deactivated in cancer cells [10, 117]. Such pathways regulate cancer-relevant cellular processes, such as cell growth, cell division, and cell survival. Typically, these pathways involve cascades of cytoplasmic kinases that ultimately impinge on gene transcription. These kinases are attractive targets for drug development; thus, there is keen interest in elucidating the specific pathways altered in each tumor to identify relevant targets and predict treatment responses.

Upon sequencing of 35 cancer-related kinase genes in a series of 36 primary PanNETs, only three mutations were detected—one in *KIT* and two in *ATM*—indicating a low mutation rate for these genes in PanNETs [118]. Further mutations were identified in PanNET-derived cell lines (QGP1, CM, and BON) in the *FGFR3*, *VEGFR1*, and *PIK3CA* genes. Of interest, the membranous immunohistochemical expression of c-Kit was associated with shorter patient survival [118].

The PI3K/Akt/mTOR signaling pathway was found to be altered in most NET studies [26, 31, 47, 119–121]. This pathway regulates several cellular processes, including cell growth, proliferation, anabolic metabolism, and apoptosis [122]. The RADIANT trials showed that the mTOR inhibitor everolimus was effective in the treatment of patients with advanced disease, which led to its approval as targeted treatment for NETs [6, 123]. However, further studies will be necessary to characterize the molecular bases and find how to overcome the acquisition of therapy resistance. This latter issue was recently investigated by using the combination of molecular drugs (e.g., everolimus and PI3K inhibitors) with promising results [124].

Somatostatin and its synthetic analogs (e.g., lanreotide, octreotide) act through a family of 5 G-protein–coupled receptors termed sst1–sst5 to exert a variety of functions, including

inhibition of endocrine and exocrine secretions and of tumor cell growth [125]. Somatostatin analogs have been successfully implemented into clinical practice; however, patients may develop resistance to treatment over time [126]. This has been partially explained by the recent finding of novel truncated sst5 receptor variants in humans [127]. One variant, sst5TMD4, which is barely expressed in normal human tissues, shows a marked upregulation in tumors, where it seems to entail pathologically relevant functions. Thus, for example, expression of sst5TMD4 in pituitary adenomas causing acromegaly is related to the reduced ability of octreotide at normalizing hormone secretion in poorly responsive tumors [127].

A pathogenic role for SRC family nonreceptor tyrosine kinases has been suggested in NETs. In a gene expression profiling study of advanced PanNETs, Capurso and colleagues identified the SRC-related kinase LCK as one of the genes overexpressed in these cancers [128]. An increased copy number of the *SRC* gene as well as an increased SRC expression has been observed in 23% of SiNETs and has been reported by several studies [77, 81, 129]. Of interest, a potential link between SRC and mTOR pathway activation has been identified by immunohistochemical studies, and the concomitant inhibition of the two pathways is more active in impairing cell growth than the use of single agents [130, 131]. Notably, whereas treatment with mTOR inhibitors triggered the activation of pro-survival feedback dependent on PI3K/Akt signaling, the simultaneous inhibition of both pathways blocked this escape signal.

The VEGF signaling pathway is also deregulated in NETs [132]. NETs are highly vascularized tumors, and a link between the VEGF pathway and PanNETs was recognized in the RIP-Tag transgenic mouse model of PanNET. Moreover, strong mRNA expression of *VEGFA* and its encoded protein's receptors (*VEGFR1* and *VEGFR2*) was observed in tumors of VHL patients [133]. Several VEGF pathway inhibitors including the VEGF inhibitor bevacizumab, the VEGF receptor inhibitors pazopanib, and sorafenib, and the multi-kinase inhibitor sunitinib have shown clinical activity [6, 134]. In particular, sunitinib was recently approved for the treatment of progressive well-differentiated locally advanced or metastatic PanNETs [135]. Another multi-tyrosine kinase inhibitor, Lenvatinib, was initially approved for the treatment of thyroid cancer and is currently being tested also on gastroenteropancreatic NETs [136]. Lenvatinib targets include RET, KIT, PDGFR, VEGFR1-3, and FGFR1-4, and a recent phase II clinical study on advanced gastroenteropancreatic NETs showed promising results in 31 of 111 patients [137].

Activation of the epidermal growth factor receptor (EGFR) may also play a role in gastrointestinal NETs [138]. In an immunohistochemical study, Shah and colleagues found evidence for activated EGFR in 63% of 89 gastrointestinal NET samples as well as activation of Erk and Akt proteins, the downstream targets of activated EGFR [139]. *EGFR* and *ERBB2* amplification have also been recurrently reported in 8–10% of SiNETs [77, 79, 81, 95].

While clinical and functional studies are exploring the mechanism of action of new drugs and combination therapies, genomics studies are focusing on deep sequencing of tumors to allow detection of intra-tumor heterogeneity and to anticipate the emergence of resistant clones [140].

4.7 Conclusions

NETs are a challenging group of heterogeneous cancers whose clinical course is often difficult to predict. A limited number of useful clinicopathological prognostic indicators are currently available for these tumors, including tumor grade and the presence of metastases.

The data generated by high-throughput studies in the last decade provided an important background to understand the biology of various NET subtypes, improving patient stratification and therapeutic decision-making. In addition, several novel targetable pathways have been identified, opening the possibility of tailoring treatment to different subtypes of tumors.

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Potential Competing Interests The authors have no competing interests to declare.

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5 Circulating Biochemical Markers of Gastro-Entero-Pancreatic (GEP) Neuroendocrine Neoplasms (NENs)

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5.1 Introduction

Neuroendocrine neoplasms (NENs) are a heterogeneous group of rare malignancies which represent a true challenge for clinicians at all stages of the disease, from diagnosis to treatment. The term "neuroendocrine" adequately describes the cell features, characterized by the presence of dense-core granules, similar to those found in serotonergic neurons, which is the reason for the "neuro" term, whereas "endocrine" refers to the secretive properties of these tumors. NENs arise from neuroendocrine cells which are derived from the diffuse endocrine system and represent approximately 2% of all malignant tumors of the gastro-entero-pancreatic (GEP) system [1]. Their incidence and prevalence have been increasing over the past years partly due to increased awareness and improvements in instrumental diagnostic techniques. NENs are usually divided into functioning and nonfunctioning forms. Functioning tumors usually synthesize, store, and secrete peptides and neuroamines that can cause distinct clinical syndromes, while nonfunctioning forms are clinically silent, being lately diagnosed once metastatic with mass effects [2].

Management of NEN represents a clinical challenge because of its late presentation, scarcity of standardized treatment options, and limitations in present imaging modalities and biomarkers to guide management. Biochemical markers are evaluated in the blood, urine, or other body fluids and are usually elevated in the presence of a tumor [3]. Of note, the beginning of the diagnostic process of NENs is often based on the measurement of circulating markers, before planning expensive and invasive diagnostic tests [4, 5]; however up to 60–80% of NENs are metastatic at diagnosis, which highlights the frequent failure to identify symptoms or to establish a biochemical diagnosis [2]. Furthermore, the majority of available markers, which can be divided into general and specific biomarkers, lack sensitivity and/or specificity and are often not helpful in the diagnostic process. A multinational consensus meeting of multidisciplinary experts in NENs assessed the use of current biomarkers and defined the perquisites for novel biomarkers via the Delphi method. Consensus (at >75%) was achieved for 88 (82%) of 107 assessment questions. The panel concluded that circulating

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multianalyte biomarkers provide the highest sensitivity and specificity necessary for minimum disease detection and that this type of biomarker had sufficient information to predict treatment effectiveness and prognosis. The panel also concluded that no monoanalyte biomarker of NENs has yet fulfilled these criteria and there is insufficient information to support the clinical use of miRNA or circulating tumor cells as useful prognostic markers for this disease.

The identification of biomarkers of both diagnostic and prognostic value for NENs is urgently needed to improve patient management and tailor the therapeutic approach for each patient [6].

5.2 Specific Biomarkers

Specific biomarkers are secreted by specialized neuroendocrine cells by functioning NEN and are responsible for specific GEP-NEN associated clinical syndrome. Specific markers include 5-hydroxyindole acetic acid (5-HIAA), insulin, gastrin, vasoactive intestinal peptide (VIP), glucagon, growth hormone-releasing hormone (GHRH), calcitonin, adrenocorticotrophic hormone (ACTH), and corticotropin-releasing hormone (CRH). The main features of the established functioning neuroendocrine syndromes have been reported in Table 5.1. Also, several other biologically active substances may be released from NENs such as bradykinin, substance P, neurotensin, human chorionic gonadotropin, neuropeptide K, and neuropeptide L.

5.2.1 5-Hydroxyindole Acetic Acid (5-HIAA)

5-hydroxyindole acetic acid (5-HIAA) is the urinary metabolite of serotonin or 5-hydroxytryptamine (5-HT) a peptide mainly synthesized and stored in the enterochromaffin cells of the gastrointestinal (GI) tract (80% of total body serotonin) [7], as well as in the serotoninergic neurons of the central nervous system [8] and the platelets. Serotonin is involved in different biological functions including vasoconstriction, neurotransmission, regulation of sleep, appetite, and gastrointestinal motility [9].

Hypersecretion of 5-HT and other biologically active amines (such as tachykinins, prostaglandins, and bradykinins) is usually observed in the presence of a metastatic small intestine NEN and results in a typical carcinoid syndrome. Elevated 5-HIAA levels in the urine are highly suggestive of an ileal NEN (approximately 75% of midgut NENs are associated with a positive urinary 5-HIAA test), although some NENs found in the lung and pancreas also secrete serotonin [9, 10]. The typical presentation is characterized by flushing, diarrhea, and abdominal pain. Less frequent symptoms are bronchospasm, headache, hypotension, lacrimation, profuse sweating, and cutaneous manifestations pellagra-like due to lack of niacin (Fig. 5.1) [5, 11, 12]. In about 10–20% of patients, carcinoid syndrome may lead to carcinoid heart disease in which cardiac fibrosis and thickening of the heart valves result in right heart failure [13]. The presence of heart disease confers a significantly worse prognosis, thus initial screening and multidisciplinary assessment are essential for both controlling carcinoid and cardiovascular symptoms and determining a strategy for medical and surgical management [14].

While 5-HT measurement is not recommended due to fluctuations in secretion as well as wide interindividual variations, the urinary 24-h measurement of 5-HIAA is a useful specific marker for 5-HT secreting NENs. Samples should be collected for 24 h using plastic jars shielded from light and prefilled with an acidic additive to keep pH below 3 (to ensure sterility and stability) [15]. Reliable methods for 5-HIAA determination are high-performance liquid chromatography (HPLC), automated assays, and mass spectrometry [16]. 5-HIAA presents a high intraindividual variability, thus a mean of two consecutive 24-h collections should be taken as reference [9]. The overall sensitivity and specificity of urinary 5-HIAA in the presence of the carcinoid syndrome are up to 90% [17]. However, as for most biomarkers, 5-HIAA presents false-positive and false-negative results. 5-HIAA levels

Table 5.1 Main epidemiological and biochemical features of the established functioning neuroendocrine syndromes in adults

Tumor	Markers	Tumor location	Incidence (cases/1000000/year)	Clinical syndrome
Carcinoid	Urinary 5-HIAA, serum 5-HIAA (less reliable)	Small intestine (60%) Colon-rectum (10%) Lung (30%) Pancreas (<1%)	3–11	Carcinoid syndrome
Insulinoma	Insulin (72 h fasting), C-peptide, proinsulin glucagon stimulation test	Pancreas (>99%)	1–3	Hypoglycemic symptoms
Gastrinoma	Gastrin, secretin stimulation test, calcium stimulation test, glucagon stimulation test	Duodenum (65%) Pancreas (30%) Other sites (5%)	0.5–2	Recurrent peptic ulcers Gastroesophageal reflux Diarrhea
VIPoma	VIP	Pancreas (90%) Other sites (10%)	0.05–2.0	Watery diarrhea Hypokalemia Achlorhydria
Glucagonoma	Glucagon	Pancreas (100%)	0.01–0.1	Necrolytic migratory erythema Diabetes mellitus Muscle wasting Weight loss
Somatostatinoma	Somatostatin	Pancreas (55%) Duodenum/small intestine (44%)	0.04	Diabetes mellitus Diarrhea cholelithiasis Weight loss Hypochlorhydria
ACTHoma	ACTH, cortisol	Lung (35%) Pancreas (25%) Thyroid (20%) Pheochromocytoma (10%) Other sites (10%)	Rare	Cushing's syndrome
CRHoma	CRH ACTH Cortisol	Thyroid (33%) Pheochromocytoma (19%) Lung (10%) Small intestine (5%)	Rare	Cushing's syndrome
GRHoma	GRH	Lung (54%) Pancreas (30%) Small intestine (7%) Other sites (13%)	Rare	Acromegaly
Calcitoninoma	Calcitonin	Pancreas Lung Pheochromocytomas other sites	Rare	Diarrhea

5-HIAA 5-Hydroxyindole acetic acid, *GRH* growth hormone-releasing hormone, *ACTH* adrenocorticotropic hormone, *CRH* corticotropin-releasing hormone, *VIP* vasoactive intestinal peptide

depend on tumor burden and can be normal in nonmetastatic patients [16]. NEN localization also influences urinary 5-HIAA levels; the sensitivity is lower in patients with fore- and hindgut NENs due to less serotonin production from these tumors than midgut forms. Moreover, renal failure and/or hemodialysis could result in falsely low 5-HIAA levels [10]. Somatostatin analogs are known to decrease levels of 5-HIAA, similarly other medications such as levodopa, methyldopa,

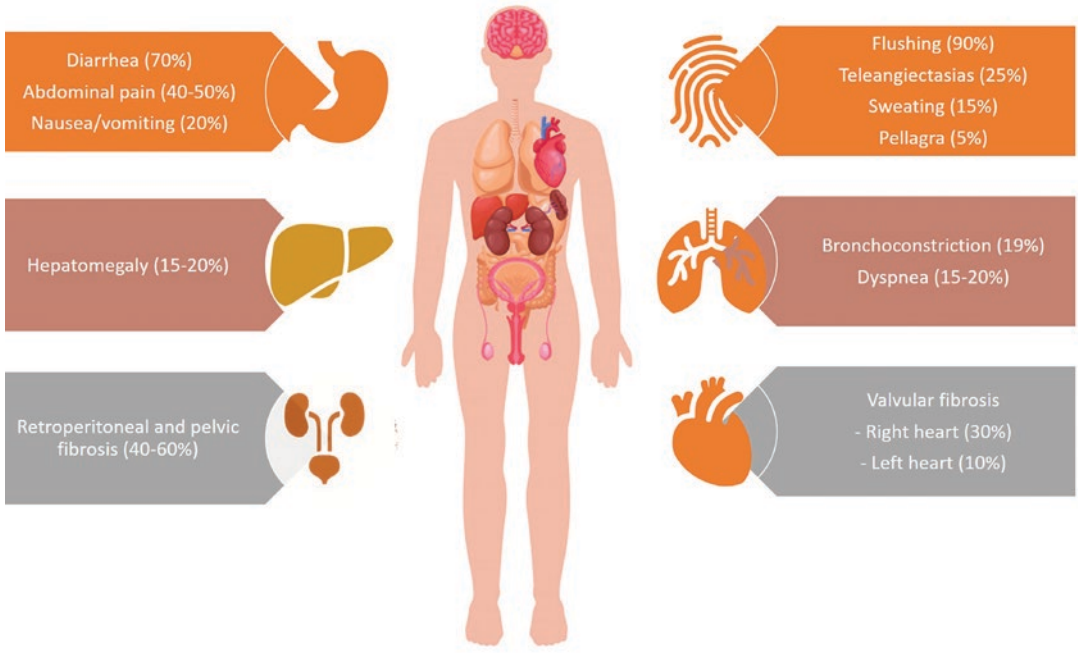

Fig. 5.1 Main manifestations of carcinoid syndrome and their relative frequencies

acetylsalicylic acid, adrenocorticotrophic hormone (ACTH), and phenothiazines may give false-negative results [18].

False-positive results can be observed in the presence of malabsorptive condition (i.e., celiac disease, tropical sprue, Whipple disease, intestinal stasis, and cystic fibrosis) or due to consumption of tryptophan/serotonin-rich food collection (i.e., tomatoes, plums, pineapples, bananas, eggplants, avocados, and walnuts) [18]. A three-day diet free of food rich in tryptophan/serotonin is advised to avoid false-positive results [9, 19].

A prognostic value of 5-HIAA in patients with carcinoid syndrome has been proposed. Different studies reported high 5-HIAA levels to be associated with a worse prognosis [20, 21], as well as a shorter 5-HIAA doubling time [20]. Moreover, a strong correlation between 5-HIAA circulating levels and carcinoid heart disease onset and progression has been observed.

5.2.2 Insulin

Insulin is a polypeptide composed of 51 amino acids produced in the pancreatic islets of Langerhans from β cells. The active form of insulin is synthesized from the proinsulin precursor molecule and consists of two peptide chains, the A-chain and B-chain [22]. Insulin plays a key role in energy balance and glucose metabolism mainly reducing blood glucose levels, by increasing glycogen synthesis and promoting the storage of glucose in the liver (and muscle) cells.

Thus, an inappropriate secretion of insulin, observed in presence of insulin-producing tumors or insulinomas, results in hypoglycemia. The low level of blood glucose accounts for the typical clinical features including both adrenergic activation (palpations, sweating, pallor, anxiety) and neuroglycopenic symptoms (personality changes and loss of consciousness) [9]. Insulinomas arise almost exclusively from the

pancreas and represent the most common pancreatic functioning NENs [9]. Insulinomas are usually present as small, hyper-vascularized neoplasms and may occur sporadically in up to 90% of cases or as part of multiple endocrine neoplasia type 1 (MEN1) syndrome in about 10% of the cases [23].

Insulinoma should be suspected in the presence of the Whipple's triad: symptomatic episodes of hypoglycemia, demonstration of serum glucose level <2.5 mmol/l (45 mg/dl), and relief of symptoms following glucose administration [24]. The biochemical diagnosis requires the presence of hypoglycemia <2.5 mmol/l (45 mg/dl) along with evidence of inappropriately increased insulin levels (>6 U/L) and C-peptide and proinsulin, which can be demonstrated in blood samples. Of note, insulin concentrations may be within the reference range; however, insulin is inappropriately high for the blood glucose level. In the presence of an episode of spontaneous severe hypoglycemia with hyperinsulinism, the simultaneous measurement of serum C-peptide and beta-hydroxybutyrate is appropriate [17]. The gold standard for the diagnosis is a 72-h fasting test and it attests autonomous insulin secretion and the failure of appropriate insulin suppression in the presence of hypoglycemia. The test requires the hospitalization and placement of an intravenous line as the patient undergoes a blood sampling for serum glucose and insulin every 6 h, or whenever symptoms of hypoglycemia occur. The test is suspended when plasma glucose falls below the threshold of 55 mg/dL, and the patient develops symptoms of hypoglycemia. Hypoglycemia develops within 12 h in 30% of patients, in 90% within 48 h, and approaches 100% within 72 h [9]. If the 72-h fasting test is not conclusive despite a strong clinical suspicion, a glucagon stimulation test can be performed immediately after the 72-fasting test: glucagon 1 mg is administered intramuscularly with a consequent increase in serum glucose levels that demonstrates adequate glycogen stores and is usually observed in patients with insulinoma.

The differential diagnosis of insulinoma includes abuse of insulin, sulphonylurea, or related insulin secretagogues and the use of hypoglycemic medications in the setting of renal impairment [9, 23].

5.2.3 Gastrin

Gastrin is an aminoacidic peptide physiologically involved in the stimulation of gastric acid (HCl) secretion and gastrointestinal motility. Gastrin is synthesized by G cells in the gastric antrum, duodenum, and the pancreas as a large precursor, progastrin. After cleavage and processing, progastrin is metabolized in several biologically active peptides including gastrin 34, gastrin 17, and C-terminally extended gastrins [25]. The release of gastrin is stimulated by food and inhibited by a low gastric pH. Gastrin binds to the cholecystokinin-2 receptor regulating the meal-stimulated gastric acid secretion. Besides, it plays important roles in epithelial cell proliferation in the gastrointestinal tract [25].

Gastrin-producing tumors, named gastrinomas, are the second most common functioning NENs. They usually arise in the duodenum (50–70% of cases) or the pancreas (20–40%) in a small portion called the gastrinoma triangle [26]. Gastrinomas can be sporadic or occur as part of MEN1 syndrome (approximately 25–35%). Thus, in case of gastrinoma diagnosis, screening for MEN1 is, therefore, advisable [16].

Hypersecretion of gastrin from gastrinoma leads to Zollinger-Ellison syndrome (ZES) characterized by increased gastric acid production from fundic parietal cells [27]. The excess in gastric acid secretion causes severe recurrent peptic ulcer disease and inactivates pancreatic digestive enzymes with consequent fat malabsorption and diarrhea. The inhibition of absorption of sodium and water by the small intestine results in a secretory diarrhea [28]. Malabsorption

and weight loss may occur in patients with long-standing untreated disease [29]. The diagnosis of gastrinoma is usually delayed of an average of 8 years from the start of symptoms to diagnosis, this is mostly due to the widespread use of proton pump inhibitors (PPIs) which can conceal ZES symptoms [30, 31].

Refractory gastric hyperacidemia, recurrence of ulcers despite maximal medical therapy, and presence of large, multiple ulcers should arise a high level of suspicion. Measurement of fasting serum gastrin is suggested to diagnose ZES: high gastrin levels (often ten times the upper normal value) and low gastric pH are required to perform diagnosis [8, 32]. However, 50–60% of patients with ZES have serum gastrin concentrations less than ten times the upper normal value (generally between 150 and 1000 pg/mL), so fasting gastrin alone is not adequate for a conclusive diagnosis of ZES. Moreover, hypergastrinemia may be observed in other conditions than ZES such as hypochlorhydric conditions (PPIs use, chronic atrophic autoimmune gastritis), antral G cell hyperplasia, gastrojejunostomy, hypercalcemia, and chronic renal impairment (Table 5.2). PPIs should be discontinued 2 weeks before serum gastrin evaluation, a switch to high doses of H2 blockers is recommended in order to prevent peptic complications [16, 33, 34].

Provocative tests can be used for gastrinoma diagnoses when serum fasting gastrin is mildly increased, or in patients undergoing PPIs treatment [9]. The secretin stimulation test is the most used provocative test for the diagnosis of gastrinomas having high sensitivity and specificity (94% and 100%, respectively) and can differentiate patients with gastrinomas from those with hypergastrinemia from different causes. The test consists of the administration of secretin (2 U/kg body weight) by intravenous bolus; serum gastrin is measured at baseline (15 and 1 min before the test) and then 2, 5, 10, 15, 20, and 30 min after secretin administration. An increase of ≥120 pg/mL at any time during the test confirms the diagnosis [16, 17] (Fig. 5.2). Additional stimulation tests can be considered in the case of an inconclusive secretin test. The calcium stimulation test is the most used in the presence of high clinical suspicion for ZES with a negative secretin test [35]. Serum gastrin is assessed every 30 min after the administration of calcium gluconate (5 mg/kg) over 3 h. An increase in serum gastrin >20% from baseline, usually with gastrin above 300 pg/mL, is conclusive for diagnosis. Additionally, the glucagon test is used for the diagnosis of gastrinomas. Glucagon is infused at 20 μg/kg/h for 30 min; an increase over the baseline within 10 min in presence of circulating gastrin over 200 pg/mL is suggestive for gastrinoma [36]. The glucagon test can also be used postoperatively, as a measure of surgical efficacy: a negative response representing a sign of adequate tumor removal and being associated with a decreased chance of recurrence [37]. Finally, the basal acid output

Table 5.2 Conditions associated with hypergastrinemia in relation to gastric acid secretion

Decreased acid secretion (pH > 5)	Normal or increased acid secretion (pH < 5)
Chronic autoimmune atrophic gastritis	Gastrin secreting tumor
Proton pump inhibitors/H-2 blockers intake	Antral G cell hyperplasia
H. pylori infection	Duodenal ulcer
Vagotomy	Retained antrum syndrome
Gastric cancer without the involvement of the gastric antrum	Pyloric stenosis
	Hypercalcemia
	Massive bowel resection
	Chronic renal impairment

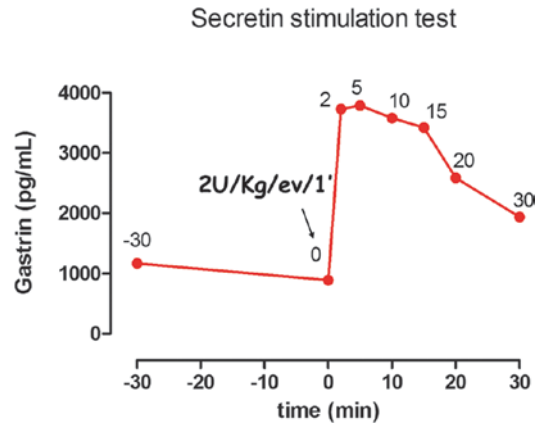

Fig. 5.2 Positive secretin stimulation test in a patient affected by gastrin secreting tumors. Plasma gastrin levels were measured at −30 and 0 time, and 2, 5, 10, 15, 20, and 30 min after intravenous secretin infusion

(BAO) can support the diagnosis of ZES: a BAO >15 mmol/h is suggestive for this diagnosis [9].

5.2.4 Vasoactive Intestinal Peptide (VIP)

Vasoactive intestinal peptide (VIP) is a neurotransmitter, belonging to the secretin-glucagon family, composed of 28 amino acids. VIP physiologically acts as a neuromodulator and not as a hormone. VIP is released from neurons and peripheral ganglia in several tissues throughout the GI tract, in the urogenital system, respiratory tract, blood vessel, and in the central nervous system in the suprachiasmatic nuclei of the hypothalamus. On the digestive system, VIP has several effects: vasodilatation, smooth muscle regulation, stimulation of water and electrolyte secretion from the GI tract, inhibition of gastric acid secretion, and increase of blood flow in the GI tract. These effects work together to increase GI motility. Moreover, VIP promotes insulin and glucagon secretion [16].

VIP secreting tumors, namely VIPomas, are rare tumors occurring both in children and adults, with an incidence ranging from 0.05% to 2.0% [38]. In adults, they are mostly located in the pancreatic tail [39], while a small proportion of VIP secreting tumors has been reported in association to colorectal cancer, lung cancer, pheochromocytoma, neurofibroma, and ganglioneuroblastoma. The majority of VIPomas present as isolated tumors, but in about 5% of patients, they are part of the MEN1 syndrome [4]. More than 50% of VIPomas have metastasized by the time of diagnosis. In children, VIPomas are typically diagnosed at 2–4 years and typically occur in ganglioneuroma and neuroblastoma [40].

Due to VIP effects as a potent stimulator of intestinal secretion and inhibitor of gastric acid secretion, VIPoma is characterized by watery diarrhea, hypokalemia, and achlorhydria (hence WDHA syndrome or pancreatic cholera syndrome, also called Verner Morrison syndrome). In the WDHA syndrome, the watery diarrhea is chronic with a fasting stool volume from 750 to 1000 mL/day, resulting in dehydration, hypokalemia, achlorhydria, acidosis, hyperglycemia, and vasodilation. Watery diarrhea may be intermittent at the onset, but it can rapidly escalate and reach a volume of 15–20 L per day, causing profound fluid and electrolyte imbalance. Hypokalemic acidosis is due to bicarbonate and potassium loss across the bowel mucosa; it may provoke asthenia and tetanic contraction. Gastric achlorhydria occurs in 50% of patients only, while hypochlorhydria is usually present. Abdominal pain and weight loss are also common features. Vasodilation causing flushing and hypotension mimics the classical midgut carcinoid syndrome. Finally, hypercalcemia can be observed due to VIP direct action on bone metabolism [23].

In physiological conditions, VIP circulates in low quantities, so even increases of 20–50% can be significant, therefore it has a high specificity; however, data on its sensitivity are lacking.

5.2.5 Glucagon

Glucagon is a 29-amino acid peptide hormone secreted by pancreatic α cells and from the L cells in the intestinal mucosa. In the pancreas, proglucagon is processed to produce glucagon, glicentin-related peptide, intervening peptide, and the major glucagon fragment. Intestinal proglucagon undergoes alternative posttranslational processing that generates glicentin, glucagon-like peptide 1 (GLP1), and glucagon-like peptide 2 (GLP2) [4]. Glucagon's main action is to raise blood glucose levels, stimulating glycogenolysis and gluconeogenesis, with an opposite action compared to insulin. Glucagon is released in response to hypoglycemia, amino acid ingestions, increased catecholamines, and ghrelin. On the other hand, glucagon is inhibited by hyperglycemia, insulin, somatostatin, and GLP-1 [41, 42].

Glucagon secreting tumors, named glucagonomas, are rare tumors with an annual incidence ranging from 0.01 to 0.1 per 100,000 [43]. They typically arise from the tail or the body of the pancreas due to the high prevalence of alpha

cells in this area. More than 50% are metastatic at the time of diagnosis.

Excessive secretion of glucagon from the tumor causes a clinical syndrome called "4D syndrome," consisting of dermatosis (necrolytic migratory erythema), diabetes, deep vein thrombosis, and depression. Weight loss, diarrhea, and mucosal abnormalities (i.e., stomatitis, cheilitis, and glossitis) may also be observed [44–46]. Necrolytic migratory erythema, which is present in up to 90% of the patients, usually appears as an itchy rash on the perineum, thighs, and distal extremities prone to secondary infections. The pathophysiology of this dermatological manifestation has not been clarified, but it is thought to be secondary to a combination of poor nutrition, low zinc, and amino acid levels.

Elevated plasma glucagon levels, above 500 pg/ml (normal value <150 pg/mL), are usually observed only in the presence of glucagonomas [47]. Also, glicentin could be measured resulting markedly increased.

Mild elevation in glucagon levels can be observed in different conditions, such as cirrhosis, untreated diabetes mellitus, prolonged fasting, sepsis, burns, and Cushing's syndrome [17].

5.2.6 Somatostatin

Somatostatin is a peptide hormone secreted from the delta cells of the pancreas, the gastric antral D cells, and the APUD (Amine Precursor Uptake and Decarboxylation) cells [48]. Somatostatin acts on the anterior pituitary inhibiting the release of growth hormone (GH) and thyroid-stimulating hormone, adrenocorticotropic hormone (ACTH), and prolactin [49]. In the neuro GI system, somatostatin suppresses the secretion of several gastrointestinal and pancreatic hormones such as pancreatic polypeptide (PP), glucagon, cholecystokinin, gastrin, secretin, cholecystokinin, VIP, gastric inhibitory polypeptide, motilin, and neurotensin [49]. In addition, somatostatin has a direct inhibitory effect on gastric acid secretion and reduces smooth muscle contractions and bowel motility [4].

Somatostatinoma are rare neoplasms with an incidence of 1 in 40 million individuals [50]. They are localized in the pancreas in up to 70% of the cases, while other common sites include duodenum (19%), ampulla of Vater (3%), and small bowel (3%) [49, 51]. Reports exist of rare instances of extra-GI primaries [52]. Somatostatinoma can be sporadic or may occur in association with familial syndromes such as MEN1 (40 to 50% of cases), neurofibromatosis type 1, and Von Hippel-Lindau syndrome. The most common manifestations include cholelithiasis, which is present in almost 70% of the cases, and diabetes mellitus in 60% of the cases [51]. Rarely, somatostatinomas manifest as a triad of diabetes mellitus, cholelithiasis, and steatorrhea referred to as inhibitory syndrome due to the suppression of insulin, cholecystokinin, and pancreatic exocrine enzymes, respectively. Moreover, hypochlorhydria can be observed due to the inhibition of gastrin secretion [48, 49]. However, in most cases, somatostatinoma is detected in an advanced stage in presence of mass effect or in presence of metastases with clinical manifestations.

Somatostatinomas usually present elevated fasting serum somatostatin levels (greater than 14 mmol/l) [51]. However, serum somatostatin levels have been reported to be increased in other endocrine neoplasms such as medullary thyroid cancer, lung cancer, pheochromocytoma, and paraganglioma [48, 49].

5.2.7 Other Circulating Markers

Several peptide hormones have been reported to be secreted from NENs arising in different sites. Hereby we present the main circulating markers which have been recognized to cause a clinical syndrome. Besides, other peptides have been rarely reported to be secreted in NEN, even if often localized in extra GI and pancreatic site.

5.2.7.1 Adrenocorticotropic Hormone (ACTH)

Adrenocorticotropic hormone (ACTH) is a 39-amino acid hormone secreted from the

anterior pituitary gland. ACTH is part of the hypothalamic-pituitary-adrenal axis. It is synthesized in response to the hormone corticotropin-releasing hormone (CRH) released from the hypothalamus and acts on the adrenal gland increasing the production and release of cortisol.

For these reasons, an excess of ACTH leads to an increased secretion of cortisol outlining a Cushing syndrome. Typical features include muscle weakness, increased body weight, hypertension, hyperglycemia, hypokalemia, infections, bruising, osteoporosis, and psychiatric disorders [53].

ACTH ectopic secretion accounts for 10% to 20% of all cases of Cushing syndrome [53]. The source of ectopic ACTH syndrome is usually a small cell lung cancer, bronchial carcinoid, medullary thyroid cancer, and pheochromocytoma [54, 55].

5.2.7.2 Corticotropin-Releasing Hormone (CRH)

Corticotropin-releasing hormone (CRH) is a 41-amino acid peptide derived from a 191-amino acid precursor. CRH acts as hormone and neurotransmitter on the posterior pituitary stimulating ACTH synthesis in stress response. CRH is produced by parvocellular neuroendocrine cells (contained within the paraventricular nucleus of the hypothalamus). CRH secreting tumors are rare, and they may occur in patients with medullary thyroid cancer (about 33%) and pheochromocytoma (19%), small-cell lung carcinoma (about 10%), and small intestine NEN (5%) [56, 57].

CRH secretion from tumors results in increased ACTH levels. Thus, the main clinical features are those of Cushing's syndrome as reported above. Levels of cortisol are elevated (>900 nmol/l) as ACTH, dehydroepiandrosterone sulfate (DHEA-S). Overnight administration of dexamethasone does not suppress cortisol secretion [56].

5.2.7.3 Growth Hormone-Releasing Hormone (GHRH)

Growth hormone-releasing hormone (GHRH) is a 44-amino acid hormone released from neurosecretory nerve terminals of the arcuate neurons in the hypothalamus and acts on the anterior pituitary, where it stimulates the secretion of growth hormone (GH).

An increase in GHRH levels results in GH hypersecretion and acromegaly. Several hypothalamic tumors, such as hamartomas, gliomas, and gangliocytomas, may produce GHRH. Peripheral GHRH levels are usually not elevated in patients with hypothalamic GHRH-secreting tumors, as GHRH secretion into the hypophyseal portal system does not appreciably enter the systemic circulation. Excessive ectopic peripheral production of GHRH has been reported in several tumors, including pulmonary NENs and small-cell lung cancers (54%), pancreatic NENs (30%), small-intestine NENs (7%), adrenal adenomas, and pheochromocytomas. In these cases, peripheral GHRH levels are usually elevated. GHRH plasma levels evaluation provides a precise and cost-effective test for the diagnosis of ectopic acromegaly. Thus, elevated circulating GHRH levels in presence of a non-enlarged pituitary gland should drive the suspect of extra-pituitary production of GHRH [58].

5.2.7.4 Calcitonin

Calcitonin is a 32-amino acid peptide released from non-follicular C-cells of the thyroid. It is produced as a 136-amino acid precursor (procalcitonin) and processed in secretory granules to the active form. The synthesis and release of calcitonin are closely related to calcium serum levels.

Inappropriate secretion of calcitonin results in hypercalcemia. Calcitonin is raised in medullary thyroid cancer, where concentration may be thousand-fold the reference range. Medullary thyroid cancers frequently arise as part of MEN type 2 (MEN2) syndrome. Also, calcitonin has been reported to be raised in other solid neoplasms including pancreatic and pulmonary NENs, pheochromocytomas, neuromas, breast, prostate, and colorectal carcinomas [59, 60]. Usually, ectopic-produced calcitonin is a large molecule without biochemical activity [4].

5.3 Nonspecific Biomarkers

Several families of secretory proteins can be found in high concentrations in neuroendocrine cells and, in particular, in NENs, and these include the granins, neuron-specific enolase (NSE), and pancreatic polypeptide (PP). The chromogranin family consists of at least three different water-soluble acidic glycoproteins [chromogranin A (CgA), chromogranin B (CgB), and secretogranin II, sometimes called chromogranin C]. Both CgA and NSE show increased concentration levels in many NEN patients. CgA is the most commonly used biomarker for NEN disease, although its utility is controversial [61]. However, CgA is the only general biomarker that has been extensively investigated [61–63].

5.3.1 Chromogranin A (CgA)

Chromogranin A (CgA), which is an acidic glycoprotein of 439 amino acids and a molecular mass of 48 kDa, secreted by neurons and neuroendocrine cells, belongs to the granin family [64]. All granins—including CgB and C—are precursors of biologically active substances, involved in a series of biological pathways controlling protein (peptides, hormones, neurotransmitters, and growth factors) secretion upon secretagogue stimulation. CgA-derived peptides include vasostatins [65], pancreastatin [66], and catestatin [67]. Although all granins may be considered as biochemical markers of NENs, as recently reported for vasostatin [68], CgA is the only one routinely used in clinical practice. CgA is synthesized at the rough endoplasmic reticulum, then transported to the Golgi complex, packaged together with other secretory proteins (i.e., hormones and peptides) into immature granules, and then secreted by mature granules by exocytosis [61, 69]. The assessment of circulating CgA levels can be performed by several commercially available kits, which differ in methodology but all rely on antibody-dependent assays such as enzyme-linked immunosorbent assay (ELISA), immunoradiometric assay (IRMA), radioimmunoassay (RIA), and the more recent immunofluorescent assay based on time-resolved amplified cryptate emission (TRACE). Recently, a further method has been described [70] which employed a non-labeled monoclonal anti-CgA antibody and demonstrated highly sensitive CgA detection. CgA may be assessed in plasma or serum. A significant, positive relationship ($r = 0.9858$, $p < 0.0001$) has been reported between serum and plasma CgA, suggesting that either measurement provides an adequate estimate of circulating CgA [71].

Independently from the method used, CgA is found throughout the diffuse neuroendocrine system and has shown an overall sensitivity of 96% and 75% in functioning and nonfunctioning NENs, respectively, and a specificity ranging from 68% to 100% [72–77]. These diagnostic performances are only estimates of real operative characteristics of CgA, and these estimates often came from heterogeneous, undersized, case-control, uncontrolled studies. Nevertheless, CgA is generally considered a sensitive neuroendocrine marker, whereas its specificity might decrease (up to 68%), as it can be falsely positive in several conditions. CgA can raise in patients with other malignancies such as prostate cancer, small-cell lung cancer, breast cancer, colon-rectal cancer [78, 79], pancreatic adenocarcinoma, and hepatocellular cancer [2, 80] and different settings, including PPI therapy, steroids, and other drugs, chronic atrophic gastritis type A, renal insufficiency, untreated hypertension, liver disease, and inflammatory bowel disease [5, 81] (Table 5.3). Of note, treatment with PPIs induces hypergastrinemia, which in turn results in hyperplasia of enterochromaffin-like neuroendocrine cells; CgA levels can, therefore, increase (up to seven to ten-fold) in patients undergoing therapy with PPIs, and elevated concentrations can be observed up to 2 weeks following treatment discontinuation [82]. Moreover, CgA should always be measured in the fasting state, as food intake is likely to increase CgA levels, therefore, increasing the risk of false positives [83]. Furthermore, CgA levels are not always increased in all the patients with NENs, and normal levels can be found in almost all appendiceal NENs, most insulinomas, many

Table 5.3 Conditions associated with increased levels of Chromogranin A (CgA)

Conditions
Neoplasms Neuroendocrine neoplasms (NENs) Non-neuroendocrine neoplasms (prostate cancer, small-cell lung cancer, breast cancer, colon-rectal cancer, pancreatic adenocarcinoma, hepatocellular carcinoma, ovary cancer)
Diseases of the cardiovascular system Hypertension, heart failure, acute coronary syndrome, giant cells arteritis
Renal diseases Renal insufficiency
Gastrointestinal and liver diseases Chronic autoimmune atrophic gastritis, inflammatory bowel diseases, chronic hepatitis, liver cirrhosis, pancreatitis
Endocrine diseases Pheochromocytoma, hyperparathyroidism, pituitary tumors, medullary thyroid carcinoma, hyperthyroidism
Systemic inflammatory diseases Systemic rheumatoid arthritis, systemic lupus erythematosus, chronic bronchitis
Medications Proton pump inhibitors (PPIs), H-2 blockers intake, steroids

pulmonary NENs, tumors in the duodenum and rectum, some MEN-1 cases as well as poorly differentiated NEN [72, 84]. Caution is therefore suggested in its interpretation. In addition, one should keep in mind that CgA should not be considered a viable tool for screening [85].

While its role in tumor diagnosis is limited by several confounding factors, CgA is currently the most used liquid biomarker in the follow-up of NENs, as its concentration well correlates with disease progression and response to treatment [62, 86], and a correlation between tumor burden and serum CgA has been proven as well. In fact, both advanced tumor stages and the presence of metastases correlate with serum CgA levels [87, 88]; furthermore, a reduction in serum CgA concentrations in subjects undergoing treatment is a suggested surrogate marker of response to therapy. CgA levels decrease in cases of an adequate response, possibly even to the point of normalization, whereas persistently high concentrations are associated with poor clinical prognosis [62, 89, 90]. However, the measurement of CgA is less reliable than advanced imaging techniques, such as magnetic resonance imaging (MRI) or computed tomography (CT), which can also provide the morphological information needed for RECIST criteria (Response Evaluation Criteria In Solid Tumors) [91] and which can, therefore, provide additional information concerning the outcomes of treatment.

According to a recent meta-analysis, CgA seems to be an accurate marker to detect tumor recurrence/progression of GEP-NENs, and CgA levels should be always measured at first diagnosis and repeated during follow-up, particularly in those patients with baseline impaired levels [63].

In summary, CgA seems to be a reliable marker to monitor disease progression and response to treatment and for the early detection of recurrence after treatment, thus being more useful in the follow-up setting rather than in the diagnostic phase [63]. However, further studies are warranted to draw more robust conclusions, including specific cutoff levels to detect tumor recurrence.

5.3.2 Chromogranin B (CgB)

Chromogranin B (CgB) is the second most abundant member of the chromogranin family. Like CgA, it is a strongly acid protein containing approximately 25% acidic amino acid residues. It has 14 dibasic cleavage points but has been less well studied than CgA. Of note, CgB seems not to be affected by renal failure, atrophic gastritis, PPI therapy, and in tumors where CgA is not found (e.g., MEN1 patients and duodenal or rectal NENs), CgB may be increased, which explains the interest to measure CgB in addition to CgA in patients with GEP NENs [4, 23]. However, no robust evidence is available regarding the possible role of CgB as a neuroendocrine marker.

5.3.3 Neuron-Specific Enolase (NSE)

Neuron-specific enolase (NSE) is the neuron-specific isomer of the glycolytic enzyme 2-phospho-D-glycerate hydroxylase or enolase and is found in neurons and neuroendocrine cells.

NSE levels seem not to be related to any secretory activity of the tumor [10, 92]. NSE was introduced as a marker for neuroendocrine cells particularly to be used in the diagnosis of malignant tumors, and it was the first marker used to identify neuroendocrine cells [93]. However, assessment of NSE alone is rarely adequate for diagnostic purposes of NENs, given that only 30 to 50% of them secrete NSE [8, 77, 94]; additionally, NSE has low specificity and sensibility for differentiating NEN from non-endocrine tumors [7]. In fact, patients with other diseases, including thyroid cancer, prostate carcinoma, neuroblastoma, and small-cell lung carcinoma (SCLC), often show elevated levels of NSE [47], whereas patients suffering from neuronal damage exhibit decreased levels of NSE [95]. Of note, whenever a pulmonary mass is present, the detection of increased NSE levels is generally suggestive of an underlying SCLC with a negative prognostic significance. In details, overexpression of NSE by all tumors, including NENs, is usually suggestive of poorly differentiated tumors, and thus of poor prognosis for higher grade cancers [47]. Furthermore, the persistence of increased NSE levels after treatment is usually considered a negative prognostic marker for SCLC, even if the actual significance of post-treatment NSE levels for NENs is far from being clearly understood. In the recent study by Yao et al. [96], data on the impact of biomarkers on overall survival (OS) from the RADIANT-3 study were analyzed and NSE turned back to represent a poor prognostic factor for OS.

In summary, assessing NSE and CgA at the same time as part of the diagnostic process could increase the reliability of their measurement, providing further proof of the presence of an NEN; however, given the nonspecific nature of both markers, these tests provide little information concerning the site of the primary tumor.

5.3.4 Pancreatic Polypeptide (PP)

Pancreatic polypeptide (PP) is a single chain, 36-aminoacid peptide arising from the PP cells of the pancreas and is expressed in neuroendocrine cells of the gut and the pancreas. The function of PP is to self-regulate pancreatic secretion activities (endocrine and exocrine), and it also has effects on hepatic glycogen levels and GI secretions [8, 97]. Before methods for the measurements of CgA were available, PP was used as a general marker for NENs, although it is poorly specific. As a matter of fact, PP has been generally considered a marginal NEN marker with poor utility in everyday clinical practice, due to its low sensitivity and specificity (63% and 81%, respectively) [6]; in fact, less than half of pancreatic NEN patients show elevated serum PP [7]. Furthermore, serum concentrations of PP can be increased in several conditions, such as physical exercise, hypoglycemia, food intake, renal impairment, chronic inflammation, alcoholism, and elder age [8], as well as decreased by somatostatin and hyperglycemia. Moreover, PP has shown to be impaired in acute and chronic pancreatitis, even if determining PP in pancreatitis is quite controversial [98]. It has been hypothesized that the combination of PP with another marker, most commonly CgA, may increase diagnostic capability [99, 100], even if the diagnostic efficacy for the combination of CgA, PP, and gastrin analyzed in the setting of MEN-1 patients was still very low (AUC = 59.6%) [101, 102]. Given that 93% of its secretion can be traced back to the F cells in the pancreas [97], PP has always been considered most likely suggestive for pancreatic NENs; nevertheless the specificity of PP for the pancreatic origin of the primary tumor is not satisfactory as increased serum levels of PP have been reported in other GI NENs as well [7], thus caution is mandatory when interpreting PP level alterations. However, a decline in PP levels after any treatment can be considered as a good prognostic marker.

5.3.5 Human Chorionic Gonadotropin and Alpha-Fetoprotein

Human chorionic gonadotropin (hCG) is a heterodimeric glycoprotein that is physiologically synthesized during pregnancy by the placenta. As

a protein heterodimer, hCG is composed of two different subunits, named α and β with different characteristics. The α subunit is basically shared with the pituitary hormones such as LH (luteinizing hormone), FSH (follicle-stimulating hormone), and TSH (thyroid-stimulating hormone), whereas the β subunit (β-hCG) is unique. Various endocrine tumors, as well as non-endocrine, exhibit different patterns of expression for hCG [103], as tumors often lack the mechanisms to pair the two subunits. In detail, pituitary tumors and NENs are often characterized by increased expression of α subunit, while the β subunit is often secreted by pancreatic tumors. However, hCG is rarely used in everyday clinical practice for NENs [17].

Alpha-fetoprotein (AFP) is a peptide hormone produced by the yolk sac and the fetal liver during development. In adults, AFP has been historically considered as a biomarker for hepatocellular carcinoma [104] and testicular non-seminomatous germ cell cancer [105]. Increased serum AFP levels have been reported in NENs, suggesting its possible role as a marker for diagnosis [106, 107]; however, more recent evidence suggests that AFP might play a role as a marker of cellular dedifferentiation rather than representing a biomarker per se [108]. The decrease of AFP often highlights an adequate treatment, although the validity of this finding in the context of NENs is still far from being clearly understood.

In testicular tumors, combining hCG with other similar markers, such as AFP, could improve the efficacy of the measurement [108]. However, assessment of hCG and AFP is generally not recommended in NENs, since both lack the sensitivity or specificity of CgA.

5.4 Novel Circulating Markers

Since 1942, at least 40 circulating monoanalytes of different sensitivity and specificity have been developed [72]. The most recent developments have explored the use of new molecular marker technologies: in particular, a great interest has focused on the development of methods to detect circulating tumor cells (CTC), molecular multianalytes (miRNA), and circulating gene transcripts (known as NETest®). However, even if encouraging results have been found so far, these markers are costly and they have not yet been incorporated into routine clinical practice.

5.4.1 Circulating Tumor Cells (CTC)

Circulating tumor cells (CTCs) are released into the bloodstream from both primary tumor and secondary sites of disease and are considered metastatic precursors [109]. CTCs were first detected in patients with NEN in 2011 [110]. Khan and colleagues [111] demonstrated epithelial cell adhesion molecule (EpCAM) expression in NEN by immunohistochemistry. In details, in 79 patients with metastatic NENs, CTCs were detected in the midgut (43%), pancreatic (21%), and bronchopulmonary NENs (31%), and of note, the presence of CTCs had a prognostic significance as it was associated with disease progression, whereas their absence correlated with stable disease. Again, further evidence suggested that CTCs were associated with increased burden, increased tumor grade, elevated CgA, worse progression-free survival, and OS, being an independent prognostic factor for survival [112].

5.4.2 miRNA

The miRNAs are a family of 21- to 25-nucleotide small RNAs that regulate gene expression at the posttranscriptional level by binding to target RNAs, resulting in RNA degradation and inhibition of translation [113]. Several studies have reported the expression of miRNAs in pulmonary carcinoids [114–116], whereas data on GEP-NENs are scarce. However, both pancreatic and small bowel NEN progression appears to be characterized by a differential pattern of miRNA expression, even if with very little or no application of these findings in routine clinical practice so far.

5.4.3 Circulating Gene Transcripts

A multianalyte transcript assay with algorithmic analysis, namely NETest®, has been recently developed for NENs, and its efficacy has been compared with CgA. The NETest ® allows the objective measurement of multiple NEN-related genes in the blood [117]. The test is based on mRNA extraction from ethylenediaminetetraacetic acid (EDTA)-treated blood and subsequent cDNA production measured by polymerase chain reaction (PCR) [118]. Results are expressed as an activity index (NETest score) from 0 to 100 [119]. The normal score cutoff is less than 20%; NETest values between 21% and 40% represent stable disease, while values 41 and 100 reflect progressive disease [120]. The direct analysis of NEN-related genes limits the risk of test alterations due to food, medication, gender, ethnicity, or age [121]. According to available studies, NETtest appears to be more accurate than CgA for both NEN diagnosis [121] and in the follow-up phase [119]. As regard the diagnosis, NETest® accurately correlates with CT/MRI (92%) and functional imaging (94%) [120]. In the follow-up of GEP-NEN patients, the NETest® had demonstrated both prognostic and predictive utility. NETest® is effective in assessing the response of surgical treatment, SSA therapy, and peptide receptor radionuclide therapy. Moreover, the test has been shown to precede radiological progression by 6–24 months, allowing early implementation of effective treatment [119, 120, 122, 123].

However, to date, NETtest® is far more costly and less widely used than CgA in routine clinical practice.

5.5 Conclusion

Numerous biochemical markers have been identified which might be useful in the diagnosis and the follow-up of GEP-NENs; however, only a few are characterized by satisfactory both specificity and sensitivity. Circulating tumor biomarkers can be divided into general and specific biomarkers, the latter characterizing specific clinical syndromes (Table 5.1).

Among generic markers, CgA is the best known, available and used marker. However, it is not highly specific to GEP-NENs as it can be found in other malignancies and other non-tumor–related conditions. According to a recent meta-analysis [63], CgA seems to be more reliable when used to monitor disease progression and response to treatment and for the early detection of recurrence after treatment rather than in the diagnostic setting. It is not useful as a screening test.

Of note, new biomarkers have been developed with the use of new technological molecules: circulating tumor cells, molecular multianalytes (miRNAs), and circulating gene transcripts (NETest®) [72]. According to a recent study, the NETest® seems the most encouraging tool and probably it should be preferred over CgA in both the diagnostic and the follow-up setting due to its better accuracy [124]. However, these new markers are costly and not widely available in everyday clinical practice, and as a matter of fact, CgA is still considered as the most available general biomarker for NENs [6].

The dosage of specific markers is useful for marking the presence of clinical syndrome rather than a tumor. Specific markers include 5-HIAA, insulin, gastrin, VIP, glucagon, somatostatin, and GHRH. Among them, 5-HIAA is an accurate marker for carcinoid syndrome and its accuracy is particularly elevated when its levels are two-fold the upper normal limit. Furthermore, a strong correlation between 5-HIAA circulating levels and carcinoid heart disease onset and progression has been observed, which needs to be taken into account in the clinical evaluation of patients with carcinoid syndrome.

In summary, circulating biomarkers both general and specific offer a useful diagnostic tool in conjunction with radiology and tissue pathology for NENs. It is important to keep in mind that biomarkers both general and specific should be measured when there is a strong suspicion of NEN and never as a screening tool due to the high numbers of false-positive results; moreover, they are still widely used in the clinical practice, although caution is necessary when interpreting their results due to the high number of confounding factors that might affect their accu-

racy. They are more reliable when used in the follow-up of GEP-NEN patients rather than in the diagnostic setting. Biomarkers of diagnostic and prognostic value for NENs are urgently needed to improve patient management and tailor the therapeutic approach for each patient.

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Advances in Diagnostic Imaging

6

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6.1 Introduction on Imaging in NET

The definition "neuroendocrine tumours" (NETs) collects a variety of uncommon malignancies, broadly distributed in the body, but sharing the origin from the neural crest. The clinical onset of these tumours is unconventional with nonspecific symptoms, which reflects their possibility to arise in different anatomical regions and tissues. Moreover, NETs can keep the secretive activity of the cells they originate from in 60–70% of cases (functioning forms) or present as biologically inactive (non-functioning forms). This classification in non-functioning tumours impacts on the investigations needed for the diagnosis as per chosen modality and technical protocols. Despite their original functional attitude, notably, in the majority of cases, NETs are diagnosed when already advanced and metastatic, thus, symptomatic.

Accurate detection and characterisation of the primary tumour and the identification of the extent of disease are required to define an appropriate approach to treatment. Moreover, treatment monitoring and the detection of recurrent disease are crucial clinical objectives in the management of these tumours.

Clinical presentation, laboratory tests can guide the choice of the subsequent diagnostic imaging investigations: both Radiology and Nuclear Medicine can answer a wide array of questions on this challenging topic.

Either conventional imaging, namely ultrasound (US), computed tomography (CT) and magnetic resonance (MRI) or functional imaging through scintigraphy and PET-CT, contribute to the characterization of NETs. However, no single imaging technique represents the gold standard, and the sequence of exams needed for each tumour type may vary [1, 2].

It is then remarkable to underline that even though we live in an era of standardisation, personalisation of treatment (within a consensus guideline frame-shift) is often required to maximise the outcome, particularly in NETs, thus implying the need to build up a "multidisciplinary culture" approach.

From the morphological point of view, features like hyper-vascularisation, specific growth patterns and imaging appearance can help

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discriminate these tumours from other solid malignancies.

From the functional point of view, we have to consider that these diseases, albeit heterogeneous, hold some features derivative from their neural cell precursors, such as the amine pathway metabolism and the cellular overexpression of the somatostatin receptors (SSTR).

With these regard, nuclear medicine offers either scintigraphic techniques or PET-CT investigation to image both biological characteristics.

The two main categories of radiopharmaceuticals available aim to:

- enter the adrenergic pathway, namely the meta-iodo-benzyl-guanidine (MIBG), a norepinephrine analogue for scintigraphy and the F-DOPA for PET-CT imaging and,
- bind somatostatin receptors (both gamma-emitting and positron-emitting radiolabelled analogues are available).

Metabolic assessment of NETs, using FDG PET-CT, is also possible in selected cases, to better investigate the aggressive tumour attitude.

According to topography, we will distinguish NETs that origin from the gastrointestinal tract, usually called GEP (gastro-entero-pancreatic), and non-GEP which generally includes lung NETs (L-NETs), medullary thyroid carcinoma, Merkel, pheochromocytoma/paraganglioma and neuroblastoma.

Pulmonary NETs usually present as well-differentiated tumours, including low- and intermediate-grade malignant tumours, historically divided into typical and atypical carcinoids, sharing clinical and pathological traits, as opposed to the poorly differentiated high-grade large-cell neuroendocrine carcinoma and small-cell lung carcinoma.

Contrast-enhanced CT is the diagnostic gold standard for lung NETs, while, in the well-differentiated forms, somatostatin receptor imaging may visualise nearly 80% of the primary tumours and appears to be most sensitive for metastatic disease. The poorly differentiated lung NETs commonly benefit more from FDG PET-CT imaging [3].

All GEP NETs are potentially malignant: proliferation, differentiation and biological characteristics influence the metastatic widespread of disease, and understanding the natural history of these lesions has profoundly changed the approach from diagnosis to treatment in the last decades.

Morphological imaging is widely applied for the initial staging of the patients affected by GEP NETs. The evolution of the diagnostic tools with the introduction of improved multi-detector CT and MR, innovative contrast media, has profoundly influenced sensitivity and specificity. In contrast, functional imaging investigations contribute not only in detecting the lesions but lead towards a better understanding of the tumour behaviour, gaining a role in the prognostic evaluation and change of treatment and follow up management [4].

We aim at outlining the primary diagnostic imaging tools available for NETs—lung, GEP and other non-GEP—and discuss the possible future options of imaging.

6.2 Technical and Technological Aspects

6.2.1 Imaging

Current conventional diagnostic methods to evaluate NETs include morphologic modalities such as endoscopic US (EUS), abdominal ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI).

The endoscopic US finds its primary importance in the investigation and histological diagnosis of GEPs. Only afterwards, when grading and histological diagnosis are confirmed, complete tumour staging with whole-body CT or MRI should be performed. This sequence of events is in line with the current guidelines for the management of neuroendocrine tumours [5], and it is essential to assess the extent of disease and to plan the most appropriate treatment approach.

The abdominal US is an advantageous technique for the study of NETs mainly because of its immediateness and non-invasiveness,

especially in the case of pancreatic NETs and in the evaluation of liver metastasis. Also, it is suitable for the guidance of core needle biopsy and fine-needle aspiration cytology for histopathologic analysis. Nevertheless, it has low sensitivity, and it is operator-dependent, lacking reproducibility and it is then inadequate as a primary tool for diagnosis and follow-up of the disease [6].

For all of these reasons, a CT scan is often the initial imaging study in patients with signs and symptoms suggestive of NET. This technique, together with MRI, provides excellent anatomic detail of the tumours and of its relationship with nearby organs being essential for disease staging and surgical planning.

Nowadays, multi-detector CT scanners are used and characterised by high spatial resolution (even <1 mm). Moreover, CT allows for multiplanarity (axial, coronal, sagittal reconstructions) and volume rendering techniques which may help delineate the tumour itself, the organ involved and nearby structures. These details further improve accuracy and imaging interpretation [7] for a correct staging and therapeutic planning. Differently from the US, CT scan is reproducible, allowing to perform the exam with the same protocols and parameters. Moreover, it is a suitable and reliable imaging technique to compare baseline and follow-up images.

The characteristic behaviour of primary functioning NETs (mainly gastrointestinal and lung NETs) and of their metastases is the arterial phase hyper-enhancing after intravenous contrast media administration, describing highly vascularized lesions. On the contrary, non-functioning NETs appear as large masses with heterogeneous enhancement due to necrotic and haemorrhagic changes [8].

For example, in the case of gastrointestinal NETs, another option could be to perform contrast-enhanced CT scan with oral contrast material earlier than usual (i.e. before the portal venous phase). This technique may help detect small enhancing neuroendocrine tumours in the small intestine. Besides, CT protocols with similar modifications can help depict small enhancing neuroendocrine tumours in the stomach and rectum.

Significant limitations of CT are as follows:

- To date, no standardised parameters exist on the exact scanning delay of the contrast-enhancement phase and on the amount of contrast medium to administer, the latter is generally calculated based on the patients' weight.
- Small lesions and peritoneal lesions are challenging to identify; the identification of metastatic lymph nodes is especially challenging as size criteria, including RECIST criteria, still are of limited value.
- Iodinated contrast media makes this technique of limited use in patients at risk for allergic reactions and those with impaired renal function.

MRI is especially suitable for staging and restaging of liver metastases; it is not as useful as CT at detecting small intestine NETs, but it is very advantageous for the detection and localisation of primary pancreatic NETs, instead.

MRI has the advantage of a high spatial resolution (2–4 mm), which is amplified by examination at a higher field strength in a 3T scanner [9]. Currently, guidelines suggest the use of a magnetic field of at least 1.5T, which also allows the applicability of specific sequences. As for CT, the 3D acquisition allows for multiple anatomical planes viewing and reconstruction; thus, for a more accurate interpretation of the lesions.

Even MRI requires administration of intravenous contrast medium to increase tissue contrast and facilitate its characterisation, and the ability to contrast soft tissues is higher when compared to CT, which is one of the reasons why, as previously said, it is the most sensitive technique for the detection of liver metastases. The use of liver-specific contrast media can increase tissue contrast (Gadoxetate disodium—Primovist). Moreover, NETs are typically hyper-vascularised tumours and enhance after contrast injection in the late arterial phase. This characteristic also works for NET liver metastases even if, occasionally, some patients may show both

hyper-vascular and hypo-vascular liver secondary lesions.

Diffusion-weighted MRI (DWI) is an essential tool of this imaging technique, especially in the oncologic field. It is based on the restricted diffusion of water molecules in highly cellular tissue such as tumours Literature shows evidence that it has the potential for distinguishing high-grade from low-grade tumours by quantifying the tumour's apparent diffusion coefficient (ADC) in the images (ADC map). Also, evidence exists showing that DWI and ADC map analysis is even more sensitive than the commonly used T2-weighted fast spin-echo or dynamic gadolinium-enhanced sequences. Therefore, DWI is currently the most promising technique for investigating NETs [10, 11].

MR cholangiopancreatography is another important MRI tool and consists of specific cholangio-pancreatic sequences performed with the previous administration of oral negative contrast (e.g. pure blueberry juice). These specific sequences enable the radiologist to study the intra and extrahepatic biliary tree and pancreatic ducts. They, therefore, allow providing essential information to the surgeon for surgical planning. MRI with the administration of oral negative contrast should always be performed before surgical resection of a pancreatic NET [12].

Guidelines on MRI protocols exist for pancreatic NETs, but no validated protocols are available for the other GEPs and neuroendocrine tumours of different origin. As for pancreatic NETs, MRI should include T1- and T2-weighted MR sequences, dynamic three-dimensional (3D) sequence before and after intravenous administration of contrast medium (Gadolinium) with multiarterial, venous and delayed (>5 min) acquisition and diffusion-weighted (DWI) sequences. Fat suppression on T1- and T2-weighted images is useful to maximise the signal intensity differences between the pancreatic tumour and the adjacent normal pancreatic tissue.

To conclude, one of the most important advantages of MRI is the absence of radiation exposure, which confirms its vital role as a technique of choice, in young patients or in those with the long-standing disease who require repeated follow-up imaging studies. Nevertheless, the costs and the requirement for extensive patient compliance still make it, in general, an optional imaging modality to CT.

Regarding the evaluation of response to therapy, MRI shares with CT the same limitations. Additionally, MRI is unsuitable for the study of small thoracic lesions because of the motion artefacts due to cardiac and respiratory activity, the low signal-to-noise ratio in the lung and the lower spatial resolution as compared to CT [13].

To date, an emerging field of investigation is represented by Radiomics especially in the case of pancreatic neuroendocrine tumours; in many patients, they present as small volume tumours at diagnosis, thus volume definition is one of the most critical characterisations. Radiomics may support and aid at a more straightforward identification and volume definition. Nevertheless, the "gold standard" is still represented by manual delineation by an expert radiologist, notwithstanding inter-observer variability. Further studies are needed to confirm and implement Radiomics and, consequently, stable radiomic features in this field.

6.2.2 Molecular Imaging

As previously mentioned, molecular imaging investigates two main features of NETs: the amine precursors pathway and the expression of somatostatin receptors on the cell surface. From the technological point of view, scintigraphy, SPECT(CT) and PET-CT are available for both functional features.

Guidelines for nuclear medicine imaging of NETs, with (iodine-131 or iodine-123) MIBG [14], with 111In-pentetreotide (somatostatin receptor scintigraphy, SRS) or with 68Ga-DOTA-peptide and 18F-DOPA, have been published in the past years [15–18].

Further reading of these guidelines is recommended for more details. However, we will here give an outline of the leading nuclear medicine techniques available to study NETs.

Radiolabelled MIBG (the isotopes used are iodine-131 or iodine-123) can well be considered

a metabolic probe for the study of NET; it is an analogue of the norepinephrine that can be taken up via the vesicular monoamine transporters ($VMAT_1$ and $VMAT_2$) and then stored in the secretory granules of the neuroendocrine cells without being further metabolised in a significant way [19]. The result is a specific concentration in these cells, allowing their visualisation in contrast to non-adrenergic tissues. Clinical indications are the detection, staging and restaging of NETs, particularly in case of pheochromocytomas, ganglioblastomas or neuroblastomas, paragangliomas, MEN2 syndrome, with an overall sensitivity of 85% and specificity of 89%, as reported in the literature [20]. Other clinical applications are medullary thyroid carcinoma and Merkel cell carcinoma.

This imaging technique is also used to select patients for therapy with ^{131}I-MIBG, to evaluate treatment response and in follow-up. Being MIBG radiolabelled with radioactive, thyroid blockade, using Lugol solution of potassium iodide, is essential to avoid thyroid irradiation from iodine (a minimum amount of free iodine is often present in the solution of the radiopharmaceutical, consequently to prevent collateral thyroid irradiation, thyroid blockage ought to be ensured).

131I-MIBG should nowadays exclusively be used for therapy, but in some centres, it is still applied also for diagnosis. Planar and SPECT images are acquired with different timing: at 24 h for 123I-MIBG and at 24 h, 48 h and even later for 131I-MIBG. Dedicated spot images may be useful in order to investigate some areas of interest further.

18F-FDOPA could be considered the PET radiopharmaceutical "counterpart" of MIBG for the study of the NET, as the enhanced intracellular transport and decarboxylation of the amino acid DOPA is the diagnostic target of 18F-FDOPA PET imaging. It is mainly used in the diagnosis and staging of pheochromocytoma and paraganglioma and for staging and restaging of medullary thyroid cancer with elevated serum levels of calcitonin. Well-differentiated NETs of the digestive tract and another endocrine, digestive tumours can also be evaluated using 18F-FDOPA PET, especially when somatostatin receptor scintigraphy is negative [21].

On the other side of NET imaging, there is a significant chapter of somatostatin analogues and receptor imaging.

The first tracer being commercially available and registered in Europe for somatostatin-receptor (SR) imaging was 111In-DTPA-D-Phe1-octreotide also named 111In-pentetreotide (OctreoScan, Mallinckrodt Medical), showing a high affinity for the sstr2 and lower affinity for the sstr3, 5 and 4 respectively, with high accuracy in the diagnosis and localisation of primary NETs and secondary lesions. Sensitivity reported for somatostatin receptor scintigraphy ranges between 70% and 95% according to the type of NET, especially in GEP NETs, with a reduction to 20–60% in insulinomas [22].

Other impressive scintigraphic results have been reported for the 99mTc-EDDA/HYNIC-Tyr3-octreotide (99mTc-EDDA/HYNIC-TOC), available in some European Countries and registered in Poland (Tektrotyd—Polatom, Poland) and for the 99mTc-EDDA/HYNIC-Tyr3-octreotate (99mTc-EDDA/HYNIC-TATE) [23]. The clinical indication is for SR imaging in staging, restaging and follows up of GEP NET, pulmonary NETs, other forms arising from the skin as Merkel cell tumours. This radiopharmaceutical is also proposed for the study of tumours originating from the sympathoadrenal system. Moreover, this imaging is mandatory to select patients for peptide radio-receptor therapy (PRRT).

A gamma camera equipped with medium-energy parallel-hole collimator is needed; planar and SPECT images are acquired at 4 and 24 h, sometimes up to 48 h after injection (when at 24 h the activity in the bowel is still significant). CT hybrid imaging has shown increased sensitivity over gamma camera alone and planar imaging.

Different 68Ga-labelled peptides are available for SR PET-CT imaging, which differ in the affinity to the different SSTR subtypes. The most relevant radiopharmaceuticals in use are [68Ga-DOTA-Tyr3]-octreotide (68Ga-DOTA TOC), [68Ga- DOTA-Tyr3]-octreotate

(68Ga-DOTATATE) and [68Ga-DOTA-1-Nal3]-octreotide (68Ga-DOTANOC). We will generally speak of 68Ga-DOTA-peptide PET-CT [24].

Clinical applications of 68Ga-DOTA-peptide imaging are the detection and staging of the primary tumour, the restaging of recurrent or progressive disease and the assessment of somatostatin receptor expression to candidate patients for somatostatin analog and peptide radionuclide receptor therapy (PRRT) [25].

Breastfeeding should be interrupted and can be restarted when the radiation dose to the child would be lower than one mSv. Discontinuation of "cold" analogues is suggested by some authors in the weeks before the exam when "long-acting" analogues are used. Images are usually acquired between 45 and 60 min after intravenous injection of the tracer.

The heterogeneity of NETs and the different degree of differentiation may influence the affinity for 68Ga-DOTA-peptides and thereby the diagnostic performance. The reported pooled sensitivity and specificity of 68Ga-DOTA-peptide PET imaging is 96% and 100%, respectively [26].

High tracer uptake at this imaging reflects the increased density of somatostatin receptors, rather than malignant disease.

NETs usually do not show a high glucose turnover rate. Therefore 18F-FDG PET-CT is not routinely used to assess these tumours. However, FDG finds application in studying poorly differentiated forms and metastatic disease, then contributing to define the aggressiveness of the lesions, in a prognostic framing [27].

As for future perspectives, we would like to mention imaging based on glucagon-like peptide-1 receptor (GLP-1R), using 68Ga-DOTA-exedin-4 PET-CT. These receptors are overexpressed at a high incidence, and density in almost all benign insulinomas is, therefore, an ideal target for these tumours for which SR scintigraphy and PET can give suboptimal results. 68Ga-DOTA-exen-din-4, however, is not a state-of-the-art tracer, but an experimental and promising probe [28].

Other novel imaging radiopharmaceuticals, not in clinical use but showing impressive preliminary results, are the somatostatin antagonists. First-in-human studies showed the high potential of radiolabelled antagonists for imaging and also targeted radionuclide therapy. 111In- DOTA-BASS and 111In-DOTA-JR11 are such gamma-emitting tracers using somatostatin antagonists, whereas 68Ga-NODAGA-JR11 is one of the antagonists under evaluation for PET-CT imaging [29].

As seen for conventional imaging, there is a rising interest in the study of texture analysis and radiomics in PET-CT imaging as well, and to date, the impact of these researches is purely academic; still, they appear very intriguing.

6.3 NETs of the Lungs

Pulmonary NETs account for approximately 1–2% of all lung malignancies and approximately 20–30% of all NETs and display significant heterogeneity, ranging from well-differentiated to poorly differentiated neoplasms. In addition to the historical classification in typical carcinoid (TC) and atypical carcinoid (AC), the World Health Organization (WHO) classification of bronchial NETs distinguishes large-cell neuroendocrine lung carcinoma (LCNEC), small-cell lung carcinoma (SCLC) and mixed neuroendocrine/non-neuroendocrine forms (miNEN) [30].

Lung NETs (L-NETs) are also classified according to their origin in respect of the bronchial tree, into central and peripheral, but they can also occur throughout the lung parenchyma.

The central forms commonly present respiratory symptoms, such as recurrent chest infections, cough, haemoptysis, chest pain, dyspnoea and wheezing. The peripheral lesions more often are incidental findings at radiological procedures carried out for other reasons.

Rarely, lung NENs can be associated with carcinoid or Cushing's syndrome.

A full imaging work up with a combination of both morphological and functional imaging is necessary during the initial diagnosis, staging and therapeutic assessment.

Bronchoscopy, if necessary, with additional endoscopic ultrasonography and biopsies, is the

best procedure to study central bronchial NETs [31].

L-NETs can be detected already at standard chest x-ray in up to 40% of cases [32]. However, contrast-enhanced CT of the thorax is widely considered the gold standard, usually with a 20 s delay between contrast injection and image acquisition to allow better visualisation of the mediastinal structures. High-resolution CT must be considered in patients with clinical contraindication to contrast media (allergies or renal failure) [33]. The CT appearance of L-NETs is often similar to the that of adenocarcinoma, presenting as round-shaped peripheral lung nodules with smooth or lobular margins, usually with a slow growth pattern and high vascularity following intravenous contrast administration.

The level of contrast enhancement depicts the angiogenetic characteristics of the lesions.

Ground-glass appearance is also reported, usually as a sign of oedema around the lesions rather than intra-alveolar invasion.

At CT images, the intermediate forms are frequently associated with atelectasis and air trapping, indirect signs of obstruction; sometimes obstructive pneumonitis, bronchiectasis and lung abscess can be part of the imaging presentation. The typical CT presentation is with rounded or elongated nodules; the latter usually have their long axis parallel to the bronchi and vessels. Complete obstruction of the bronchus is rarely seen, as the extra-bronchial component is more often predominant to the endo-bronchial part [3].

Calcifications are detected in one-third of all cases, especially in the intermediate forms.

In the rare event of multiple synchronous carcinoids, high-resolution CT with an expiration study can help to show mosaic attenuation or air trapping in addition to multiple nodules [34].

Nodal involvement (particularly in the atypical carcinoids), as well as the presence of distant metastases, influences the prognosis and the treatment options, and imaging assessment of the spreading of disease is then crucial for the patient management.

Apart from mediastinal nodes, liver and bones are the most common sites of metastasis.

Multiphase CT, including arterial and portal phases, must be acquired to image the liver status accurately; CT with appropriate bone window setting may be useful to reveal bone metastases.

MRI should include dynamic acquisition and diffusion-weighted sequences for the study of the liver metastasis. In case spinal metastasis is suspected, MR is preferable to CT [35].

L-NETs, as well as NETs arising in other sites, are characterised by the ability to take up and concentrate amine precursors in order to produce amines and peptides and also express different membrane peptide hormone receptors (e.g. somatostatin receptors, SSTRs). These uptake mechanisms and the presence of membrane peptide receptors represent the basis for functional imaging of NETs [36].

Combined functional imaging using SSTR imaging and metabolic imaging allows in vivo demonstration of the overall biological behaviour of NETs [37].

Since 80% of typical bronchial carcinoids express SSTRs, somatostatin receptor scintigraphy (SRS) and 68Ga-DOTA-peptide PET-CT may be very informative.

Scintigraphy with 111I-labelled somatostatin analogue has been the most widely used method to assess somatostatin receptor expression in the last decades, but 68Ga-DOTA-somatostatin analogue PET-CT recently became the nuclear medicine test of choice for staging [38].

Well-differentiated NETs are typically not FDG avid and overexpress membrane receptors for somatostatin [39]. On the contrary, more aggressive bronchial NETs such as LCNEC and SCLC are characterised by higher FDG uptake and lower expression of somatostatin membrane receptors. Therefore, for poorly differentiated NETs, FDG PET-CT may result more sensitive and informative than somatostatin receptor imaging.

MIBG scintigraphy has no clinical role in the management of lung neuroendocrine cancer, while PET-CT with 18F-dihydroxy-L-phenylalanine and 11C-hydroxy-L-tryptophan might potentially be used in the future for therapy response evaluation (Figs. 6.1 and 6.2).

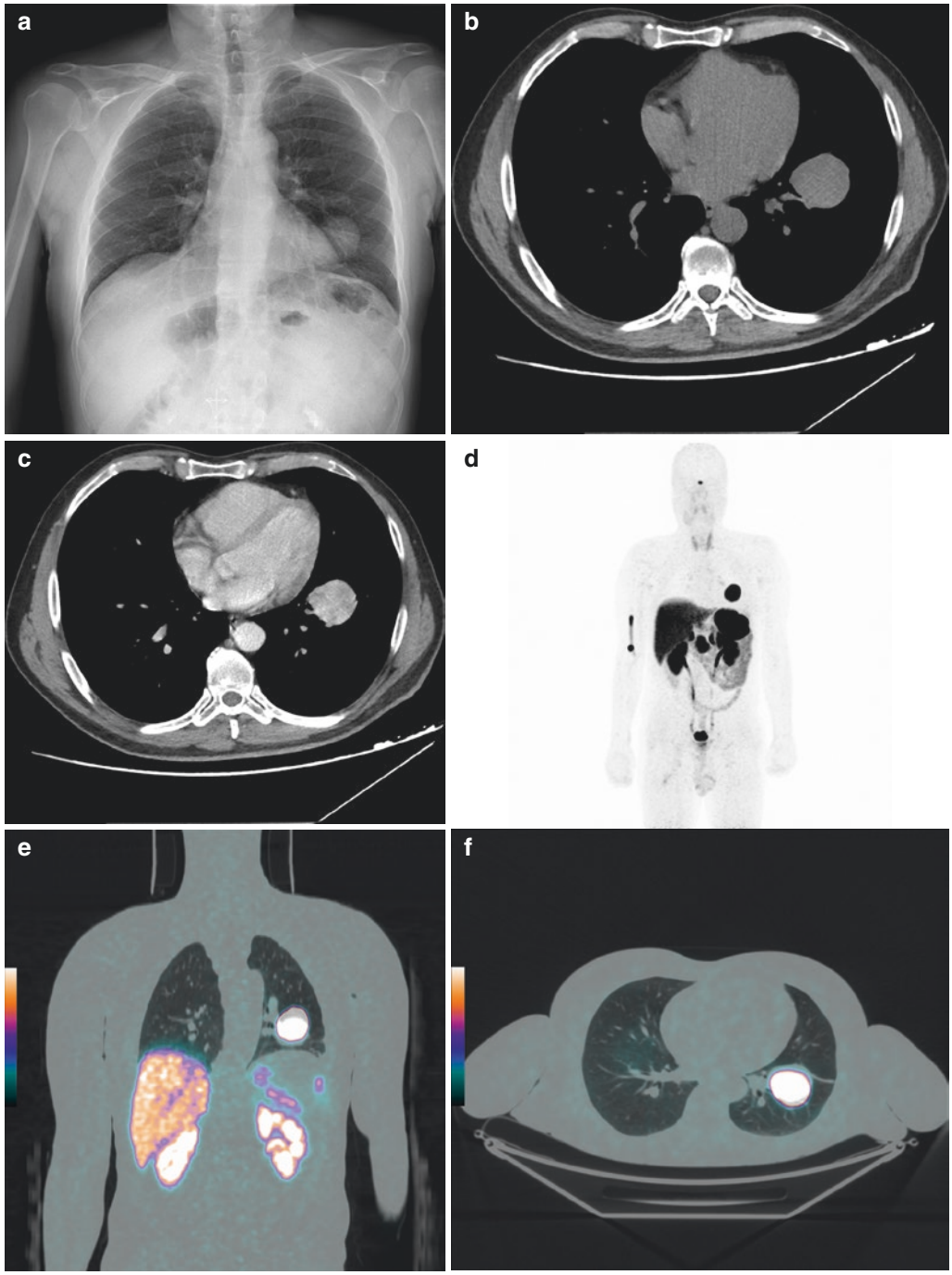


Fig. 6.1 A 50-year-old male patient referred to the hospital for pulmonary embolism. Chest x-ray (**a**) revealed an inferior left pulmonary mass. (**a**) Chest x-ray. The following CT of the thorax confirmed the lesion (**b**) that was characterised significant contrast enhancement (**c**). The intense contrast enhancement of the lesion documented at the CT scan coupled with the clinical data arose the suspect of a neuroendocrine tumour. (**b**) CT basal acquisition. (**c**) CT after contrast injection. A 68GaDOTATOC PET-CT (**d**, MIP, **e**, coronal, **f**, axial) was then performed showing intense tracer uptake of the lesion. Histology confirmed the diagnosis of typical carcinoid, ki67 = 8%. (**d**) 68Ga-DOTA-peptide PET-CT MIP. (**e**) 68GaDOTApeptide PET-CT coronal. (**f**) 68GaDOTApeptide PET-CT axial

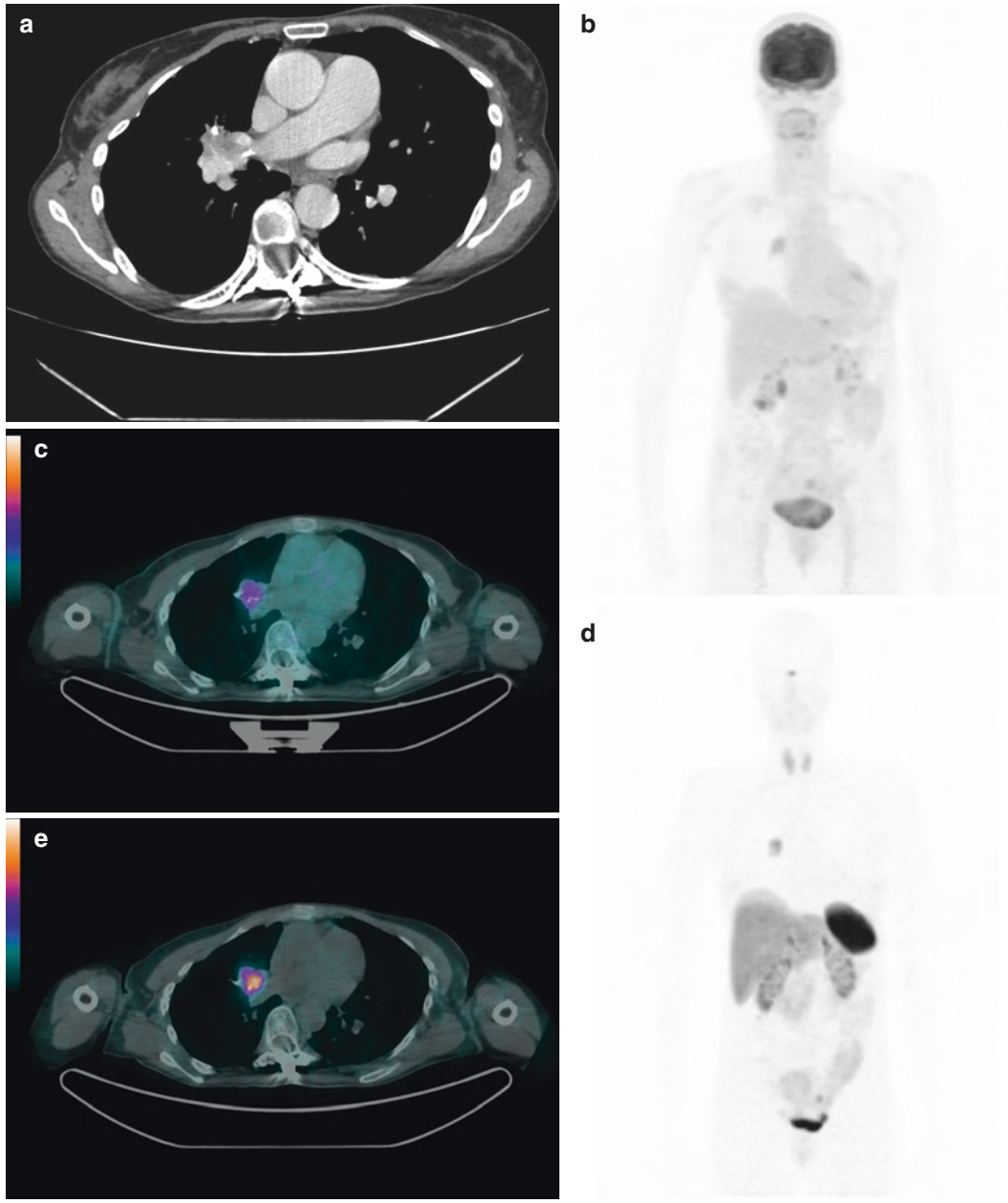


Fig. 6.2 A 50-year-old lady affected by atypical thoracic pain was scheduled to undergo a CT scan that showed a para-hilar right pulmonary node, with inhomogeneous contrast enhancement and some calcifications. (**a**) CT scan transaxial. At 18F-FDG PET-CT, the mass was confirmed, but the tracer uptake was mild (**b**, MIP and **c**, transaxial). Therefore, a fibro-bronchoscopy with biopsy was performed. Results were orientative towards a low-grade neuroendocrine tumour. (**b**) 18F-FDG PET-CT MIP. (**c**) 18F-FDG PET-CT transaxial. A 68 Ga-DOTATOC PET-CT was then requested. A focus of moderate tracer uptake was seen within the mass (**d**, MIP and **e**, transaxial). The multidisciplinary discussion proposed surgery upfront as no other lesions were detected. (**d**) 68Ga-DOTATOC PET-CT MIP. (**e**) 68Ga-DOTATOC PET-CT transaxial

6.4 GEP NETs

NETs arising in the gastrointestinal tract are the most represented forms (67%), with the most common origin in the distal tract of the ileum (up to 30% of GEP). These tumours are often quite small in size, making their identification challenging, especially for the ileal localisations [40].

The clinical presentation and tumour location, as already mentioned, profoundly influence the investigations required to achieve the final diagnosis.

The presence of hormonal hypersecretion must be assessed using laboratory analyses and endocrinological tests. Pathological analysis, whether possible, is required to confirm the diagnosis.

Functioning GEPs can either arise from the pancreas or the gastrointestinal tract, exhibiting specific hormonal syndromes according to the secreting abilities of the proliferating clone of cells. The clinical presentation can play a fundamental role in recalling the correct diagnosis, but from the imaging point of view it is not possible to discriminate functioning from non-functioning tumours; however, some general features are common findings in GEP NETs, such as the hyper-vascular attitude.

Usually, in case of non-functioning GEPs, the symptoms are mainly related to the compressive/obstructive effect of the mass on the surrounding structures and organs and include abdominal pain, obstructive jaundice, presence of abdominal mass, weight loss and intestinal obstruction. Therefore, the typical findings occurring in the clinical scenarios of abdominal discomfort are common to GEP NETs as well.

Well-differentiated, slow-growing GEP NETs are, nonetheless, quite often already metastatic at the moment of the diagnosis, hence the detection of primary together with the assessment of the disease extent is of paramount importance to guide staging and treatment.

Conventional imaging and functional imaging complement each other in the definition of these tumours, being nuclear medicine more effective in the biological characterisation of the lesions [41].

As a first step in the diagnostic workup, there is trans-abdominal ultrasonography (US), a non-invasive and widely available screening technique for the abdominal parenchyma. NETs typically appear on US images as a hypoechoic mass surrounded by a hyperechoic halo. US is mainly suitable for the investigation of solid organs but results inefficient at examining the gastrointestinal tract and mesentery.

The role of US seems to be limited, though, especially in the evaluation of the pancreas where it can turn out suboptimal due to partial obscuration by bowel gas, with an overall reported sensitivity of 13–27% [41].

Computed tomography shows high spatial and temporal resolution. Thanks to the multiplanar reconstructions and image display, and in consideration of the variety of protocols of contrast media injection and acquisition studies, it can survey different parts of the body in a more tailored fashion, providing detailed information on the tumour and its relationship with vascular structures and other close tissues and organs. These features gained its fortune, particularly in the pre-surgical evaluation.

Because of the known hyper-vascular aspect of the metastasis from GEP NETs, multiphase acquisition protocols are recommended for a more appropriate investigation of these lesions, usually more conspicuous in the early arterial phase of the acquisition [42].

It is also possible to perform basal scans with no contrast media injection to assess the presence of calcification and haemorrhage within the mass [43].

Multiphasic and multiplanar CT is usually performed at first. On average, arterial phase imaging is performed at 20–25 s following contrast injection. This timing takes into account the time for the contrast to reach the descending aorta at the level of the thoracoabdominal tract. Afterwards, a venous phase at approximately 50–60 s is scanned. All the phases must be performed for a complete examination and detection of eventual metastases, typically at the hepatic level.

Magnetic resonance (MR), even though more expensive, time-consuming and demanding either on patients cooperation or professional efforts to carry out a high-quality examination, together with the multiplanar acquisitions, offers superior intrinsic soft-tissue contrast and does not use ionising radiation. Multiphasic and multiplanar MRI is recommended for the study of

GEPs and considered superior to CT for lesion assessment in solid visceral organs.

At MRI, NET lesions are hypointense in T1-weighted sequences and hyperintense in T2-weighted sequences and, usually after contrast media injection, show a diffuse pattern of enhancement in the arterial phase. Typically, fat-suppressed contrast-enhanced T1-weighted sequences provide the best accuracy.

Molecular imaging techniques, especially with PET tracers, have a significant impact on patient management, including better localisation of occult tumours in the small intestine and pancreas as well as improved staging and restaging. Especially somatostatin receptor imaging continues to have a central role in the diagnostic workup of patients with well-differentiated GEP-NETs owing to its high accuracy and the theranostic potential [44].

111In-octreotide scintigraphy has a high sensitivity for detecting typical carcinoids and gastrointestinal pancreatic NETs, particularly, gastrinomas, non-functioning NETs, and functioning endocrine pancreatic tumours except insulinomas (because of the lack of expression of type 2 somatostatin receptor subtype) [45].

Somatostatin receptor scintigraphy (SRS) shows high accuracy in the diagnosis and localisation of primary NETs and secondary lesions. There is a consolidated experience on the use of SRS in GEP-NETs. It is well known for its usefulness in detecting small lesions of the small bowel that are difficult to identify on conventional imaging with a sensitivity of 80–100% [20].

Nonetheless, it is limited by low spatial resolution, low sensitivity in the detection of small tumours and high background activity in healthy organs, especially the liver, kidney and spleen.

The upgrade has introduced several improvements to tomographic and hybrid imaging by means of SPECT and SPECT/CT.

Finally, the development of PET tracers specifically designed for NETs originated a new paradigm in the staging and restaging of these tumours. Excellent signal-to-noise ratio, spatial resolution and high-quality imaging as early as 45 min after injection of the radiotracer are evident advantages.

Good sensitivity of 68Ga-DOTANOC was reported especially for cases with an unusual anatomic localisation and small lesions, particularly at the node and bone level. It also enables absolute quantification of tracer uptake (determination of the standard uptake value, SUV) and provides relevant information of SSTR expression, which has a direct therapeutic implication with PRRT [23, 46].

Regarding potential pitfalls in image interpretation, we would like to mention reactive nodes, benign meningiomas, accessory spleens, the physiological activity in the pancreatic uncinate process and physiologic activity at the adrenal level can cause false-positive results.

As already seen in general for NETs, GEP-NETs usually do not show a high glucose turnover rate. Therefore, the sensitivity of 18F-FDG PET/CT is low, especially in well-differentiated forms (G1 and G2).

FDG is useful in the poorly differentiated forms, which also seem to express lower levels of somatostatin receptors. Information deriving from the 18F-FDG PET seems to provide valuable prognostic elements that may contribute to select patients affected by a more aggressive disease.

MIBG is generally not used in the routine workup of GEP-NETs.

6.4.1 Gastric and Intestinal NETs

Gastric NETs (G-NETs) originate from enterochromaffin-like cells located in the gastric glands and are divided into three categories:

- Type 1 arises as neuroendocrine hyperplasia and ultimately neoplasms in the context of achlorhydric hypergastrinemia due to chronic atrophic (autoimmune) gastritis.
- Type 2 appears as a result of hypochlorhydria (hyper acidic) hypergastrinemia due to Zollinger-Ellison syndrome caused by one (or several) duodenal or pancreatic gastrinoma(s), usually in the context of MEN1 syndrome.
- Type 3 occurs sporadically and is not related to any gastric mucosal abnormality.

Type 1 and 2 mainly present as multiple small lesions due to the underlying diffuse (systemic)

growth stimulus; type 3 usually presents as a large-size solitary tumour with upper GI bleeding and is often characterised by more aggressive behaviour and worse prognosis with an increased risk of diffusion to regional lymph nodes and liver metastases [46].

Endoscopy, with biopsy, is the first imaging choice, but to assess the invasion of the surrounding structures and the spread of disease contrast-enhanced CT is required. Validated protocols exist: the patient should fast before the exam and have a couple of glasses of water right before the exam starts. This protocol enables the stomach to distend and its walls to be more visible. Also, water acts as a negative contrast allowing for better visualisation of the ampulla and thus identification of periampullary tumours. The thickness of the sections should be 1.25–2 mm as thin collimation of the scan is useful at identifying millimetric lesions. Iodinated contrast media is injected intravenously after an unenhanced scan is performed, particularly in case of lesions smaller than 2 cm.

Type 1 and type 2 tumours appear as numerous enhancing submucosal lesions similar to other small gastric tumours and polyps; type 3 lesions demonstrate an infiltrative morphology similar to that seen in adenocarcinomas and often show avid contrast enhancement [47].

Contrast-enhanced CT and MR are most crucial for staging distant metastases [48].

In G-NETs functional imaging, especially using PET-CT can contribute to staging and identification of disease spread. FDG PET-CT is most useful in the type 3 forms, to assess the diffusion of metastasis.

Moreover, radiologists should be aware of indirect signs and concomitant findings typical of this kind of tumours. In the case of gastrinomas, for example, a common accessory finding is represented by small-bowel mural thickening or oesophageal hyperenhancement (Figs. 6.3 and 6.4).

Duodenal NETs are rare tumours and comprise 1–3% of all duodenal neoplasms; they are generally small (<2 cm) and usually confined to mucosa or submucosa but in approximately 40–60% and 10% lymph node and liver metastases, respectively, have been reported.

The majority (90%) of duodenal NENs are non-functional, but an association with Zollinger–Ellison is reported as well as with carcinoid syndrome.

Upper gastrointestinal endoscopy is the most sensitive method of detection and diagnosis, while EUS can help determine the extension of the tumour invasion. CT, MRI and SSTRs functional imaging can be used in order to determine

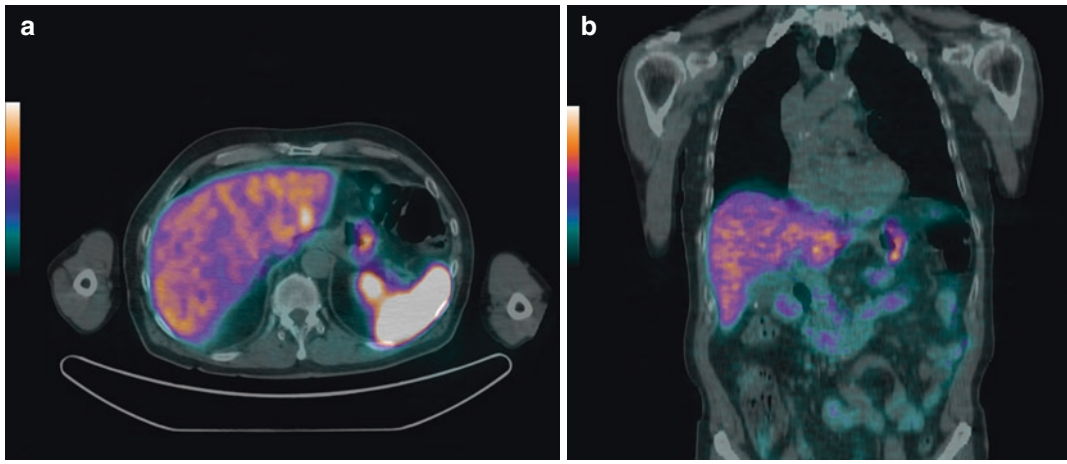


Fig. 6.3 A 72-year-old patient affected by anaemia, loss of B12 and gastritis. A biopsy of a polypoid mass seen at endoscopy demonstrated a well-differentiated NET G1 of the gastric body, ki67 = 1%. A 68Ga-DOTATOC PET-CT was performed for staging, confirming the gastric lesion and highlighting a hepatic metastasis in the third segment (**a**, axial, **b**, coronal). (**a**) 68Ga-DOTATOC PET-CT axial. (**b**) 68Ga-DOTATOC PET-CT coronal

the presence and the extent of metastatic disease [5] (Fig. 6.5).

Small intestinal neuroendocrine tumours (siNETs) derive from serotonin-producing enterochromaffin cells, and frequently they present with non-specific symptoms (abdominal pain, weight loss, bleeding or intermittent partial bowel obstruction). At the same time, 20–30% of patients develop carcinoid syndrome that is associated with liver metastases in more than 95% of cases.

SiNETs frequently present as multiple small lesions and have a high propensity to metastasise, as liver metastases are already seen at the moment of diagnosis in 80–90% of patients. However,

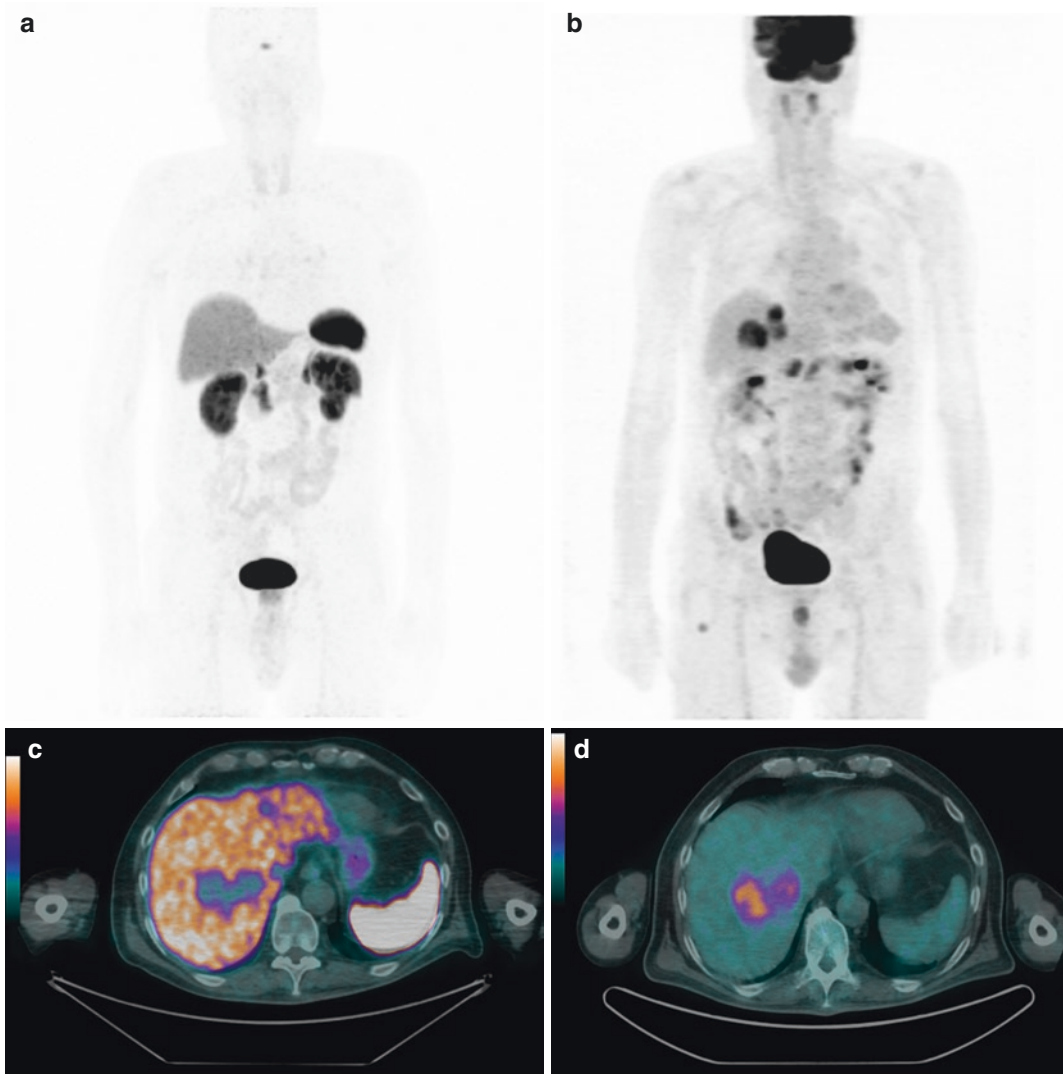


Fig. 6.4 A 76-year-old gentleman restaged for gastric NET (G2) relapsed after 3 years from surgery. 68Ga-DOTANOC PET-CT (**a**, MIP) and 18F-FDG PET-CT (**b**, MIP) were performed because of the clinical history and because of the moderate differentiation (G2). The residual gastric wall (after surgery) suspected for relapse of disease showed intense tracer uptake at the receptor PET-CT, whereas was not FDG avid. (**a**) 68Ga-DOTANOC PET-CT MIP. (**b**) 18F-FDG PET-CT MIP. Several liver lesions appeared as areas of no-uptake at 68Ga-DOTANOC PET-CT (**c**, axial) and as intense foci of uptake at the FDG images (**d**, axial). (**c**) 68Ga-DOTANOC PET-CT axial. (**d**) 18F-FDG PET-CT axial. Moreover, only FDG PET-CT revealed a bone lesion in the right femur neck (**e**, coronal and **f**, axial). (**e**) FDG PET-CT coronal. (**f**) FDG PET-CT axial

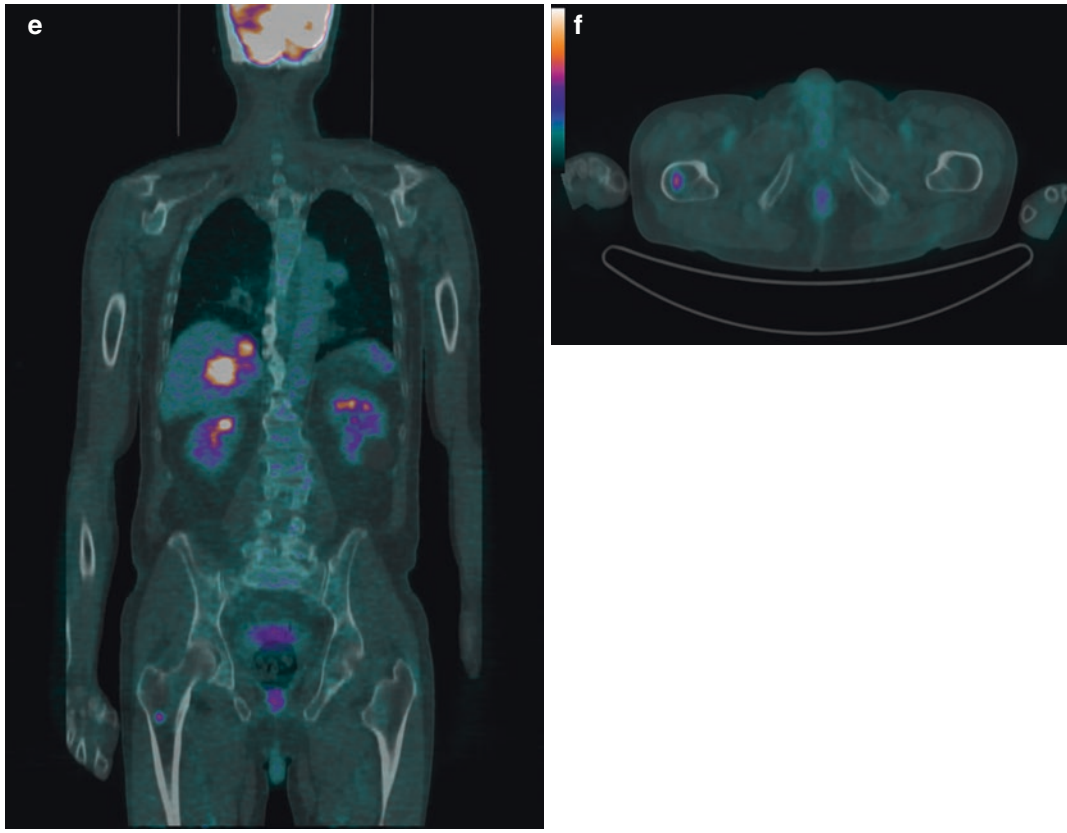


Fig. 6.4 (continued)

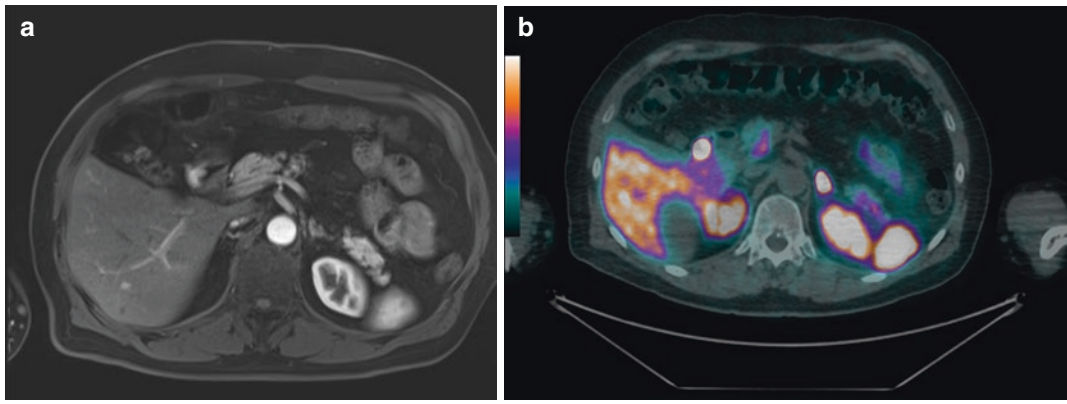


Fig. 6.5 A 67-year-old male patient was operated for gallbladder stones. Histology revealed the presence of a small amount of tissue with immune-reactivity orienting for the presence of a neuroendocrine tumour. Endoscopic ultrasound was performed, showing a lump in the distal part of the duodenum. The following MRI detected an area with early contrast enhancement in the duodenum, in keeping with a neuroendocrine lesion (**a**, axial, arterial phase); confirmed as an area of intense focal uptake at 68Ga-DOTATOC PET-CT (**b**, axial fused). (**a**) MRI axial, arterial phase. (**b**) 68Ga-DOTATOC PET-CT axial fused

despite their malignant behaviour, most of them belong to the G1 histopathological group.

CT or MRI, CT/MRI water enteroclysis or endoscopic techniques and SRS or 68Ga-DOTATOC PET can be helpful for the detection of the primary tumour and probable metastatic lesions while colonoscopy can detect tumours located in the terminal ileum.

Contrast-enhanced CT or MR imaging is often the preferred imaging techniques.

Small-bowel distention is often advisable to improve lesion detection using CT enterography and MR enteroclysis that have shown improved sensitivity (100% and 86%–94%, respectively) and specificity (96.2% and 95%–98%, respectively) for tumour detection [49].

These tumours usually appear as small, hypervascular, polypoid masses or as asymmetric or concentric bowel wall thickening. Another meaningful indirect sign is represented by mesenteric retraction (desmoplastic reaction), especially in the case of small-bowel lesions. This sign is crucial, and it may be more easily recognised than the primary lesion, at CT but also at MRI.

As with CT, multiphasic and multiplanar MRI is recommended for the study of GEPs and considered superior to CT for lesion assessment in solid visceral organs. Typically, fat-suppressed contrast-enhanced T1-weighted sequences provide the best accuracy.

A steep differential diagnostic consideration in these patients, concerning CT and MR, is chronic mesenteric panniculitis (also known as sclerosing mesenteritis) [50].

Given the technical difficulties to diagnose such small lesions at conventional imaging, functional imaging has gained over the past years an increasing role for siNETs, with an overall sensitivity of 80–90% for somatostatin receptor imaging [51] (Fig. 6.6).

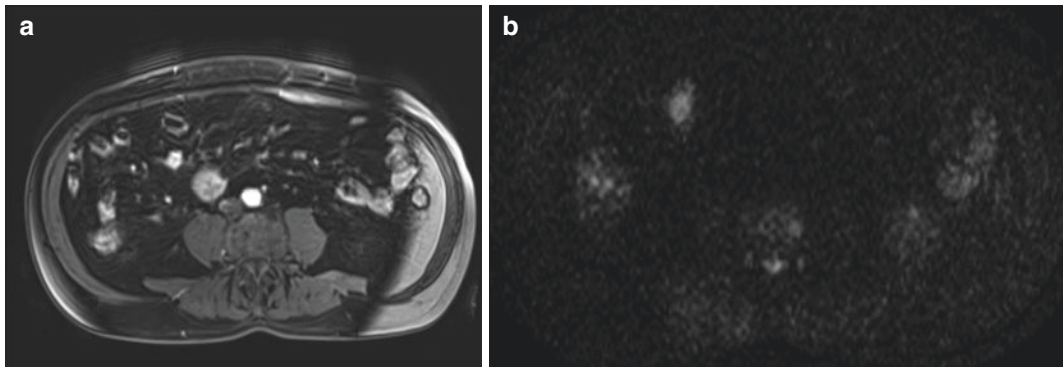


Fig. 6.6 A 79-year-old male patient with a 10-year history of a low-grade, multifocal, neuroendocrine tumour of the ileum. He was treated with surgery on the ileum and on the metastatic liver lesions that aroused in the second year after diagnosis. After 5 years of negative follow-up, an abdominal MRI for follow-up showed multiple solid nodules in the mesenteric adipose tissue with significant arterial contrast enhancement, thus suspicious for carcinoid tumour (**a**, MRI, dynamic sequence, arterial phase fat-saturated; **b**, MRI, DWI sequence b 800). (**a**) MRI, dynamic sequence, arterial phase fat-saturated. (**b**) MRI, DWI sequence b 800. After multidisciplinary consultation, a new surgery was performed on the mesenterial nodules and lymph nodes. No further treatment was suggested. Three years after surgery, during which the patient was negative for residual disease, a 68Ga-DOTATOC PET-CT scan was asked in the follow-up, detecting a pathological uptake in the mesenterial adipose tissue (**c**, axial fused). An area of uptake was also seen in the temporal region consistent with meningioma (**d**, axial fused). (**c**) 68Ga-DOTATOC PET-CT axial fused, mesenterial node. (**d**) 68Ga-DOTATOC PET-CT axial fused, meningioma. Also, MRI confirmed the presence of a mesenterial node (**e**, axial dynamic sequence "Lava MPh"). (**e**) MRI axial dynamic sequence "Lava MPh". Treatment with "cold" analogues of somatostatin was started, and MRI was the imaging chosen for follow-up, recording a progressive dimensional increase in the peritoneal lesion in the following 2 years, until the last MRI performed in November 2019 (**f**, MRI dynamic sequence, arterial phase fat-saturated; **g**, MRI axial dynamic sequence "Lava MPh"). (**f**) Dynamic sequence, arterial phase fat-saturated. (**g**) Axial dynamic sequence "Lava MPh". Subsequent multidisciplinary decision: "watch and wait" approach and continue with follow-up imaging studies

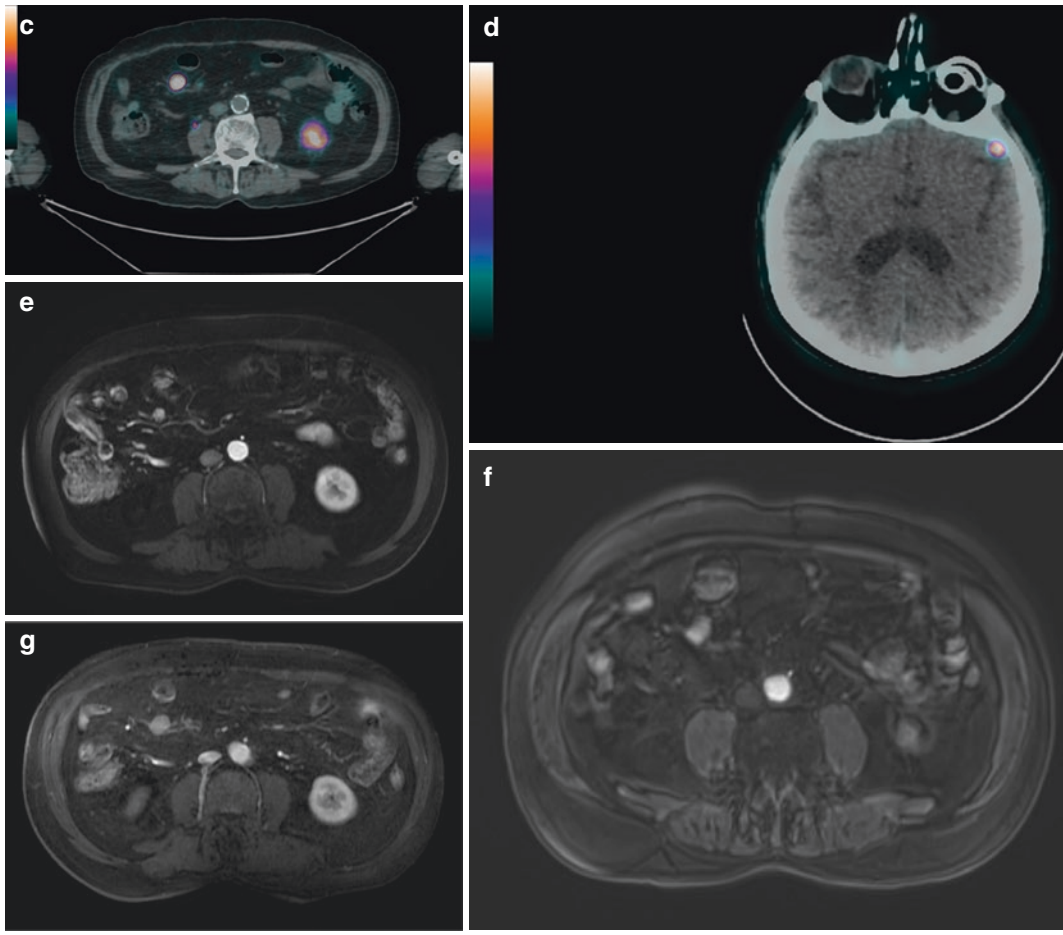


Fig. 6.6 (continued)

Appendix NETs are often incidentally discovered during appendicectomy and represent the most common neoplasm of the appendix. Despite they are generally considered indolent, approximately 49% display lymph node metastases while 9% present with distant metastases. The risk of distant metastases is associated with tumour size and is considered significantly increased for tumours >2 cm. For tumours >2 cm or with angioinvasion and infiltration of the mesoappendix, further imaging with abdominal CT/MRI and SRS or 68Ga-DOTATOC PET is recommended.

Colon NETs are often aggressive and metastatic at diagnosis while rectal neuroendocrine tumours are frequently low to intermediate grade and are associated with long-term survival.

EUS should be used to determine the depth of invasion while pelvic MRI is considered to be most accurate in determining local lymph node status [52] (Fig. 6.7).

6.4.2 Pancreatic NETs

Pancreatic neuroendocrine tumours are rare but represent the second most common pancreatic cancer. They can be functioning or non-functioning with a heterogeneous pattern of clinical presentation. They are often slow-growing lesions associated with prolonged survival, even in the presence of distant metastases.

Non-functioning pancreatic NETs show no symptoms as they are non-secreting lesions and are often detected when already of large size and usually in an advanced stage. Interestingly, the majority of non-functioning tumours are likely to

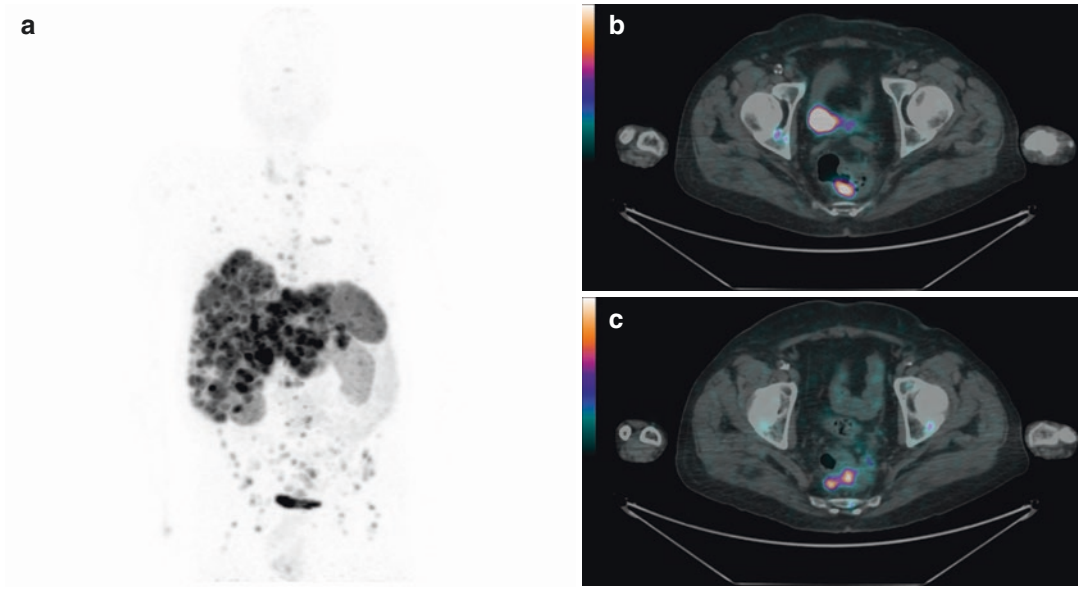


Fig. 6.7 A 68-year-old gentleman affected by a rectal mass revealed as moderately differentiated NET with an endoscopic biopsy. A 68Ga-DOTATOC PET-CT was performed for staging showing intense focal uptake of the primary lesion, regional lymphadenopathies, multiple liver and bone metastasis (**a**, MIP, **b**, axial fused, **c** axial fused). (**a**) 68Ga-DOTATOC PET-CT MIP. (**b**) 68Ga-DOTATOC PET-CT axial fused. (**c**) 68Ga-DOTATOC PET-CT axial fused

be malignant while functioning tumours, which have typical hormone-secreting clinical presentations, are benign [53].

Imaging techniques for the diagnosis of pancreatic NETs are the same already outlined for GEPs and especially in these tumours, the endoscopic US is of paramount importance due to its role for identification and histologic characterisation of lesions.

Multiphase multi-detector CT examination represents the first-line imaging test to evaluate pancreatic tissue with a detection rate rating between 69% and 94% in recent studies [4].

Functioning NETs are usually small in size (1–2 cm) and have a vibrant capillary network; therefore, present as homogeneously hypervascular lesions. When greater than 2 cm, they may show heterogeneity and degeneration patterns. Non-functioning tumours are instead well-defined, larger (> 4 cm) and show heterogeneous enhancement. This imaging characteristic is due to the possible cystic, necrotic or calcific components within the lesions.

Pancreatic NET secondary lesions are commonly seen in liver and locoregional lymph nodes, but retroperitoneal localisation can also occur. Moreover, as for pancreatic adenocarcinomas, also in the case of pancreatic NETs, the evaluation of locoregional vascular structures is mandatory. Pancreatic NETs tend to have a high rate of neoplastic vein thrombosis (splenic, portal and superior mesenteric veins) even though they show a lower rate of vascular encasement, more typical for pancreatic adenocarcinoma [54].

On MRI, most pancreatic NETs are hyperintense on T2-weighted images and hyper- or isointense during the arterial phase of the dynamic study. MR-DWI and ADC maps play an important complementary role to the other sequences, particularly at localising non–hyper-vascular tumours [55].

Despite the advances in the diagnostic approaches, in general, NETs are difficult to identify, and no single imaging test fulfils all the clinical expectations. For this reason, it is crucial to have a multimodal diagnostic approach that comprises invasive and non-invasive techniques.

Somatostatin receptor imaging, using scintigraphy or PET-CT is recommended because of the high expression of somatostatin receptors

generally occurring in these tumours, especially in the well-differentiated forms.

The reported sensitivity of somatostatin receptor scintigraphy, to detect islet cell tumours, is between 70% and 90%. However, it appears to be generally lower, ranging between 20% and 60% for insulinomas. Enthusiastic results with a detection rate of 100% for glucagonomas, 88% for VIPomas, 72% for gastrinomas, 82% for non-functioning islet cell tumours and 87% for other carcinoids is seen in some European experiences [20].

SRS has been widely adopted for diagnosis but also for the clinical management in the restaging after surgery to assess the therapy response and to plan further treatments.

The detection of unexpected sites of diseases, not found at other imaging modalities, is crucial to delineate the therapeutic strategy in the management of the patient. 68GaDOTA-peptide PET-CT has shown to be more sensitive than to SRS in the detection of primary pancreatic NETs, with sensitivity reported around 80–90%.

Versari et al. found similar figures for 68GaDOTATOC (sensitivity 92%) compared to multi-slice CT (sensitivity 91%) in detecting a duodenal-pancreatic tumour in a series of 19 patients [56].

Data on the comparison between 68Ga-DOTA-peptide PET-CT and MRI, particularly the diffusion-weighted MRI, are discordant on the superiority of one to the other, thus suggesting that the association of the two techniques is recommended to obtain the best performance [57].

68Ga-DOTA-peptide PET-CT also demonstrated to be superior to F-DOPA with a sensitivity of 96% and 56%, respectively [58].

FDG PET-CT is most indicated in patients affected by poorly differentiated or more aggressive forms and to complete staging when the disease is already metastatic, to assess the possible different behaviour of the different lesions.

MIBG scintigraphy has no clinical role in the study of pancreatic NETs (Figs. 6.8, 6.9, and 6.10).

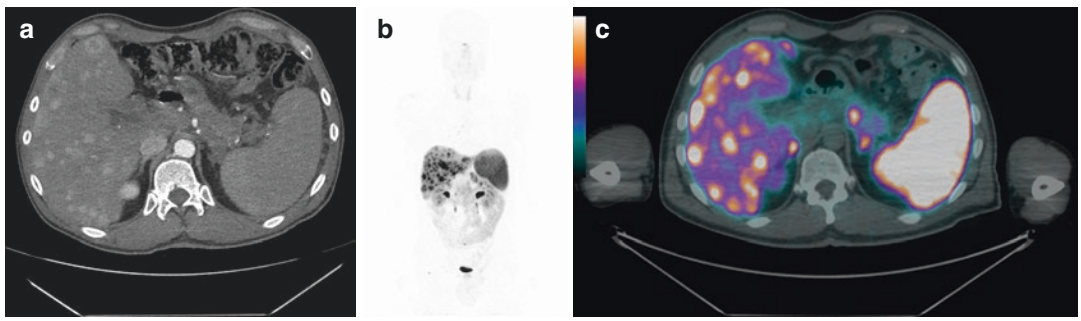


Fig. 6.8 A 44-year-old man suffered from weight loss in several months and a mild and unfocused abdominal pain. He underwent an abdominal ultrasound that showed multiple hepatic lesions, confirmed at a CT scan, particularly at the arterial phase for intense contrast enhancement. CT images also showed a 5 cm lesion in the pancreatic tail infiltrating the splenic hilum structures (**a**, CT axial, arterial phase). (**a**) CT axial, arterial phase. An ultrasound-guided biopsy was done on the liver. Histology revealed localisation of the well-differentiated NET tumour, with G1 aspects, ki67 = 2%, in keeping with the pancreatic origin. To complete staging a receptor PET-CT was performed and, because of the extension of disease, and FDG PET-CT was also scheduled. At 68Ga-DOTATOC PET-CT (**b**, MIP), the voluminous pancreatic mass in the organ tail was confirmed, showing inhomogeneous uptake of the radiopharmaceutical for a necrotic area in its context. Multiple foci of intense uptake were seen in the liver. The left adrenal appeared increased and almost fused with the pancreatic lesion; however, given the physiological adrenal uptake at receptor imaging, no conclusion was made on the effective adrenal pathological involvement (**c**, axial). Two bony lesions were seen, only at the receptor PET-CT, in the sacrum (**d**, CT of PET and fused) and the right femur (**e**, CT of PET and fused). (**b**) 68Ga-DOTATOC PET-CT MIP. (**c**) 68Ga-DOTATOC PET-CT axial pancreas and liver lesions. (**d**) 68Ga-DOTATOC PET-CT axial (CT of PET and fused) lesion in the sacrum. (**e**) 68Ga-DOTATOC PET-CT axial (CT of PET and fused) lesion in the right femur. At 18F-FDG PET-CT (**f**, MIP), the pancreatic mass appeared with moderate tracer uptake around a central area of necrosis. Only some of the multiple liver lesions were detectable. The left adrenal did not show pathological uptake (**g**, coronal). The patient was scheduled for systemic chemotherapy. (**f**) 18F-FDG PET-CT MIP. (**g**) 18F-FDG PET-CT axial

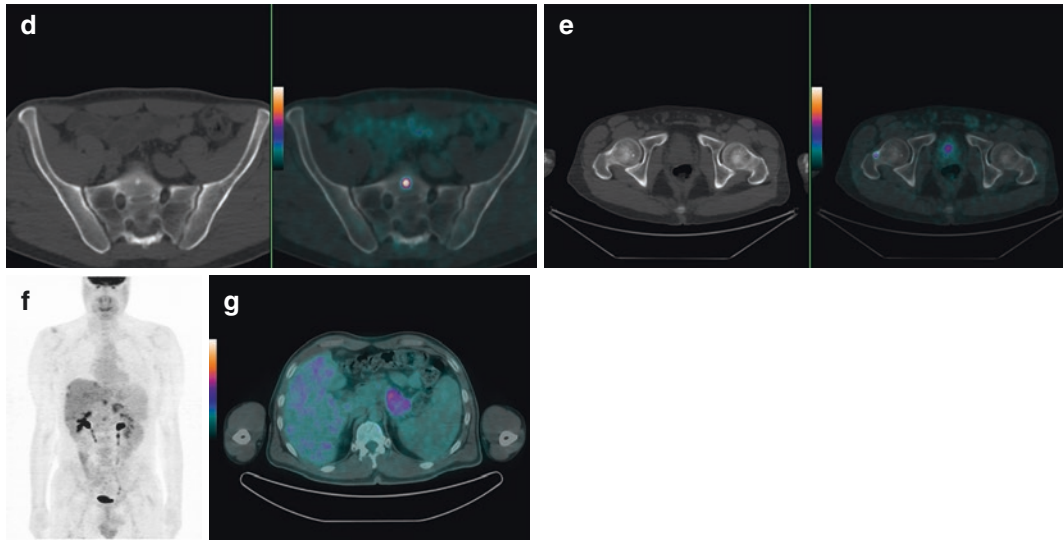


Fig. 6.8 (continued)

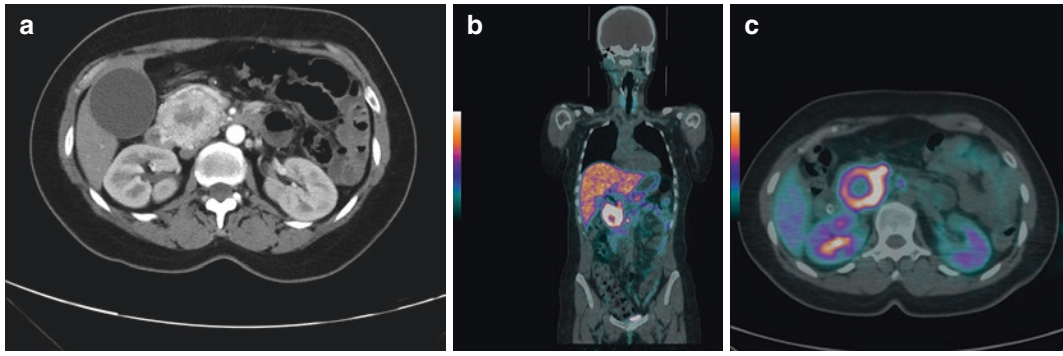


Fig. 6.9 A 47-year-old woman after an episode of jaundice was investigated using an abdominal ultrasound revealing a coarse mass in the pancreatic head. After an ultrasound-guided biopsy, the diagnosis of a well-differentiated pancreatic NET, G2, according to WHO 2000, Ki67 = 7%, was done. At contrast-enhanced abdominal CT, the lesion (4.2 × 4.8 cm and long 6.5 cm) early appeared in the arterial phase, with vivid contrast enhancement, an inhomogeneous aspect and a necrotic area within. Moreover, it appeared to have a compressive attitude towards the descending part of the duodenum (**a**, axial, arterial phase). (**a**) Contrast-enhanced abdominal CT, axial. At either 68Ga-DOTATOC (**b**, coronal, **c**, axial) or 18F-FDG PET-CT (**d**, coronal, **e**, axial), which followed the diagnostic CT, the lesion demonstrated to take up both tracers intensely and was characterised by a central area of no uptake due to necrotic changes. (**b**) 68Ga-DOTA-TOC PET-CT, coronal fused images. (**c**) 68Ga-DOTA-TOC PET-CT, axial fused images. (**d**) 18F-FDG PET-CT, coronal fused images. (**e**) 18F-FDG PET-CT, axial fused images

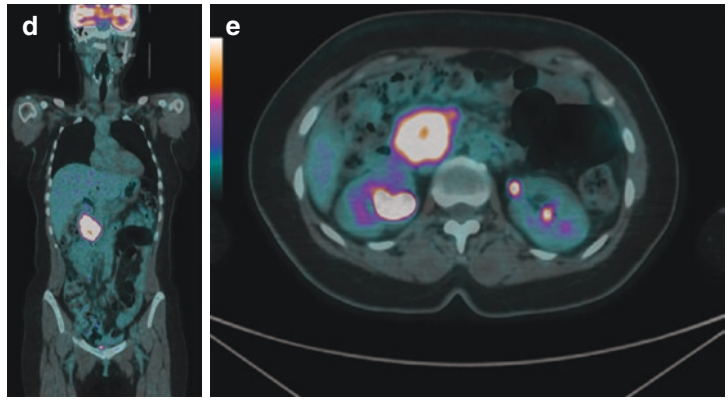
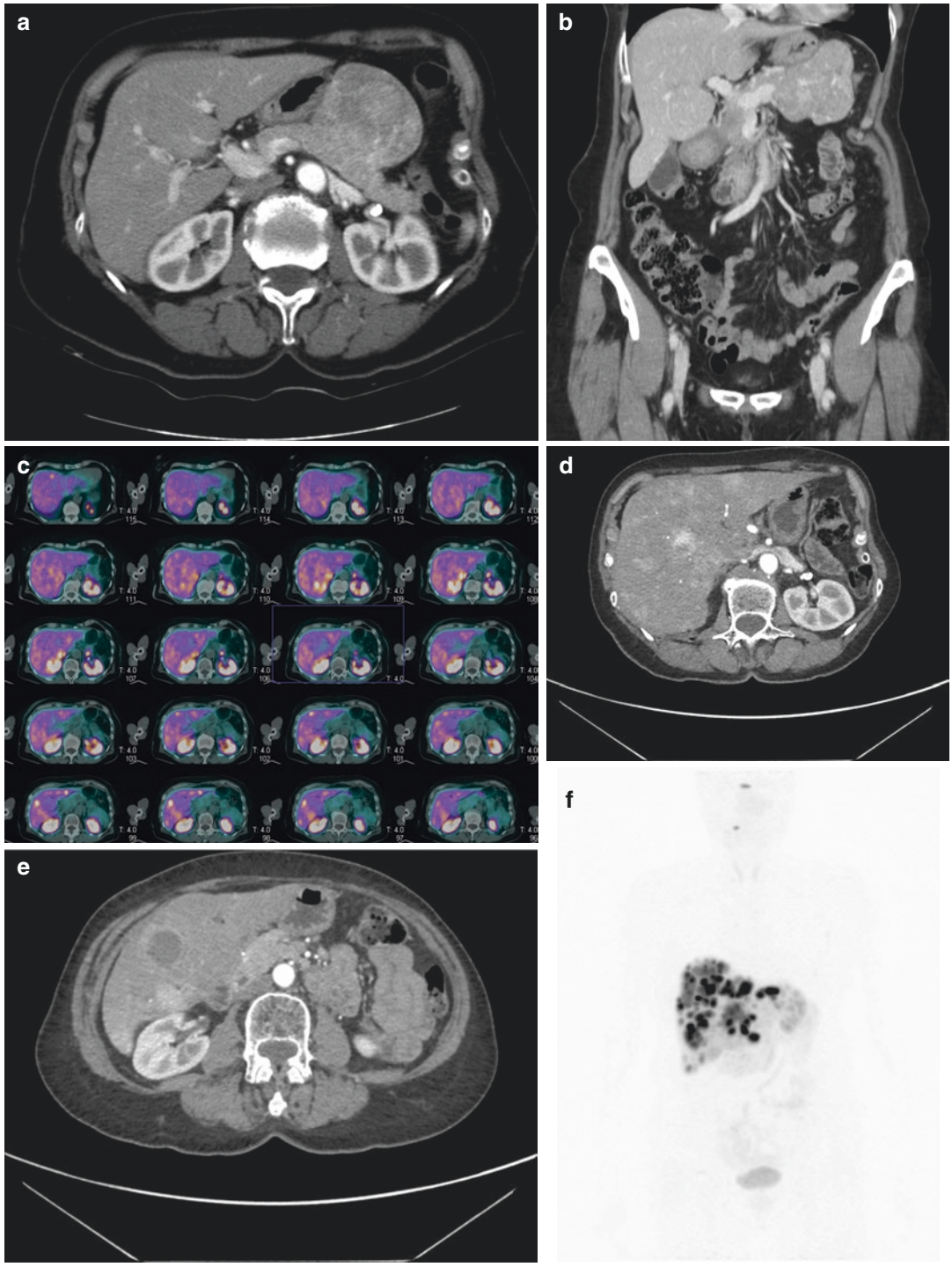


Fig. 6.9 (continued)

Fig. 6.10 In 2013 at the age of 71, this female patient had an incidental finding of a pancreatic lesion at CT scan done for abdominal pain (**a**, arterial phase axial and **b**, venous phase coronal). The following endoscopy and histological examination confirmed the presence of a pancreatic lesion, which resulted in a well-differentiated neuroendocrine tumour of the pancreatic gland, G2. After surgical evaluation, the patient was defined as suitable for resection and underwent distal splenic-pancreatectomy. (**a**) Abdominal CT scan, arterial phase axial. (**b**) Abdominal CT scan, arterial phase, venous phase, coronal view. Intraoperative US examination found bi-lobar liver lesions that were analysed histologically and resulted in metastatic localisations of the pancreatic NET (not seen at CT scan). A 68Ga-DOTA-NOC PET-CT was performed to complete staging after surgery with evidence of some foci of tracer uptake in the liver, in keeping with secondary localisation of the known neuroendocrine tumour of the pancreas (**c**, axials, fused). (**c**) 68Ga-DOTA-NOC PET-CT axial fused. After multidisciplinary discussion, treatment with somatostatin analogue was prescribed, with the progression of disease seen at 6 months of CT scan. Therefore, a treatment shift to chemotherapy was introduced. After the third cycle of chemotherapy, the follow-up CT scan showed a mixed response, but the treatment was stopped due to vascular complications, and 2 months later a substantial progression in number and size of the hepatic lesions was detected at CT (**d**, **e**), implying a new change of strategy with second-line chemotherapy. (**d**) Abdominal CT, arterial phase, axial view. (**e**) Abdominal CT, arterial phase, axial view. In the following 18 months, the disease remained stable. Then at the progression of liver disease seen at MRI, a palliative trans-arterial embolisation (TAE) of the accessible liver lesions was considered and performed. Successive follow-up CT scan: dimensional reduction of the liver lesions treated with TAE and stability of the other lesions; thus, a successive TAE was planned and performed. Follow-up abdominal MRI: progression in number and size of the untreated lesions, the stability of the treated lesions which appear inert. After further oncologic evaluation, taking into account the progression of the disease at a hepatic level even after TAE treatment, the latter was discontinued. In 2020, a new 68-GaDOTATOC was performed to evaluate the possibility to perform radio-receptor therapy (PRRT), which was started in April 2020. (**f**) 68-Ga-kDOTATOC MIP: multiple liver lesions and a bone lesion in C2


a
b
c
d
e
f

6.5 Conclusion

Imaging plays a fundamental role in the management of neuroendocrine tumours. Molecular and morphological information are available in a combined fashion and give a fundamental contribution to the diagnosis, staging, treatment election and treatment monitoring of these diseases.

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Part III

New Approaches for Treatment

7 Treatment of NET-Related Symptoms

Franco Grimaldi, Fabio Vescini, and Elda Kara

Abbreviations

ACTH	Adrenocorticotropic hormone
BM	Bowel movements
CC	Carcinoid crisis
CHD	Carcinoid heart disease
CS	Carcinoid syndrome
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
IFN-alpha	Interferon-alpha
LAR	Long-acting release
NETs	Neuroendocrine tumors
pNET	Pancreatic neuroendocrine tumors
PPI	Proton pump inhibitors
PRRT	Peptide receptor-targeted radionuclide therapy
PTH	Parathyroid hormone
PTHrP	PTH-related peptide
SA	Somatostatin analogs
u5-HIAA	24-hour urinary 5-hydroxyindoleacetic acid
ZES	Zollinger–Ellison syndrome

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7.1 Introduction

Generally, a delay of 53.8 months occurs in the diagnosis of neuroendocrine tumors (NETs) [1, 2], and patients with functioning NETs have a shorter overall survival than those with nonfunctioning NETs [3]. Beside overall survival and comorbidities, hormonal syndrome can also be related to quality of life, as for example, in the case of increased frequency of bowel movements (BM) in carcinoid syndrome CS [4]. For these reasons, timely diagnosis and proper treatment for syndrome control are crucial for patients with functioning NETs. This chapter will deal with the treatment of functioning NETs-related symptoms.

For the treatment of symptoms due to mass effects and for treatments aiming to reduce tumor burden in order to reduce hormonal secretion, see chapters on surgical procedures, locoregional treatments, and chemotherapy. Primary tumor resection in functioning NETs is controversial. There are data in the literature on improved survival after primary tumor resection of well-differentiated NETs metastatic to the liver [5]. Accordingly, some studies have demonstrated that this practice could help disease control [6–10] but data on survival improvement are scanty and hampered by many bias such as retrospective design of the studies [5]. Primary tumor resection should be carefully evaluated in a multidisciplinary team for patients with functioning NETs

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in order to reduce hormonal secretion (e.g., Zollinger–Ellison syndrome (ZES) patients, CS, insulinoma).

7.2 Carcinoid Syndrome

Carcinoid syndrome occurs in almost 20% of patients with well-differentiated NETs of the small bowel [11]. It usually presents with liver metastasis at diagnosis [11] and is rarely associated with NETs of other organs (pancreas, rectum, etc.) [12, 13]. CS can present as typical or atypical. In the first case (95% of cases), it is due to a huge production and release of serotonin and is characterized by diarrhea, abdominal pain, and flushing, [14] while atypical CS (5% of cases) is usually mediated by histamine and is characterized by prolonged flushing, ocular edema and hyperemia, bronchospasm, and hypotension [14]. Other substances such as tachykinins, prostaglandins, and callicrein can be secreted as biochemical mediators of CS.

Somatostatin analogs (SA) and especially long-acting SA remain the mainstay of CS treatment. Lanreotide and octreotide, the two agents commercially available, are equally effective for symptom control [15]. Literature provides us evidence of their antisecretory and antiproliferative effects [16, 17] along with a high tolerability [18]. Pasireotide, a multireceptor-targeted somatostatin analog that at the moment is not approved for NET treatment, has also been studied in patients with CS resistant or refractory to treatment with octreotide long-acting release (LAR) [19] showing symptom improvement. However, it was not superior to octreotide in a comparative trial [20]. In case of refractory CS, dose escalation above the upper labeled dosages should be considered [15, 21, 22], and successively, in case of persistence of symptoms, subcutaneous short-acting SA can be associated.

Interferon-alpha (IFN-alpha) is recommended as a second-line therapy in functionally active NETs [15]. It is recommended as an add-on therapy to SA. However, we should always keep in mind unfavorable side effects (especially flu-like symptoms, fatigue, thyroid dysfunctions) of INF-alpha while treating patients with it. A pegylated formulation with weekly administration can reduce side effects [23].

Telotristat is an oral inhibitor of peripheral serotonin synthesis, which acts by inhibiting tryptophan hydroxylase, the enzyme involved in the conversion of tryptophan to serotonin [24]. It may offer new possibilities for patients with refractory CS. In two prospective randomized clinical trials, telotristat demonstrated efficacy in reducing BM frequency and 24-hour urinary 5-hydroxyindoleacetic acid (u5-HIAA), and it also gave relief of symptoms during the assessment period [24, 25]. The percentage reduction of BM was greater in patients with greater percentage reduction of u5-HIAA. The clinical responses observed in these patients suggest that the assumption that diarrhea was mediated by serotonin and its reduction could improve symptoms was correct. Furthermore, telotristat etiprate was generally well tolerated, and there were no reports of depression and constipation [25] as reported previously for another tryptophan hydroxylase inhibitor [26]. Clinical trials that focused on telotristat safety showed a favorable safety profile and suggested that the depression observed [27, 28] could be related to the underlying disease or other causes recommending monitoring of patient's mood. Actually, it seems that telotristat does not cross the blood–brain barrier [29]. *Peptide receptor-targeted radionuclide therapy* (PRRT) with radiolabeled somatostatin analogs is an effective therapeutic option in patients with NETs [30]. PRRT usually involves administration of radiolabeled hormone analogs with high specificity to somatostatin receptors on tumor cells, leading to the internalization of the radioactivity into the tumor cells and consequent cell death [3]. The two most commonly used radiopeptides are ^{177}Lu-DOTATAE and ^{90}Y-DOTATOC [31, 32]. Netter-1 trial showed that PRRT is highly effective in controlling advanced progressive NETs along with a favorable safety and quality of life profile [33]. FDA

and EMA approval for the use of LutaThera™ in NETs will lead to increased use of PRRT in many countries. Patients with decompensated heart failure are not suitable candidates for PRRT, because it requires concomitant amino acid and fluid infusions before and along with peptide receptor radionuclide therapy [34].

Carcinoid heart disease (CHD) is a major cause of morbidity and mortality in patients with CS [34, 35]. Most frequently, it involves the pulmonary and tricuspid valves [35]. NT-proBNP for screening patients with carcinoid syndrome for evidence of clinically significant carcinoid heart disease and measurement of either 24-h urine 5-HIAA or plasma 5-HIAA are essential for diagnosis and follow-up of CS and CHD [34]. Furthermore, a 24-h u5-HIAA level >300 mmol/24 h seems to be a useful marker for identifying patients at risk for developing carcinoid heart disease [34]. Echocardiography and echocardiographic features seem to be the best modality in the evaluation of carcinoid heart disease and in the assessment of disease severity [34]. Cardiac magnetic resonance (CMR) and computed tomography (CT) scanning can be a valuable adjunct in the investigation of patients with CHD, especially where echocardiographic windows are poor or structures are difficult to visualize [36]. Patients with CHD and severe regurgitation should be referred for surgery, and the choice of valve prosthesis should be individually tailored [36]. An experienced medical (cardiologist, endocrinologist, oncologist), surgical and anesthetic team approach is mandatory for these patients in order to give them the best and complete management [34].

Carcinoid crisis (CC) is a life-threatening form of CS that occurs due to systemic release of a large surge of bioactive amines and peptides [3]. The classical (typical) CS is characterized by diarrhea, flushing, wheezing and shortness of breath, sudden changes in blood pressure, and hyperthermia [37]. CC can be precipitated by different conditions such as surgery, biopsies, PRRT, locoregional treatments, anesthesia, some kind of food, emotional stress, pain stimuli, certain medications, and alcohol intake. Some studies have identified patients with large tumor burden, already known CS, elevated chromogranin A and/or high 24-h u5-HIAA levels or preexisting CHD as high-risk patients for CC [3]. Other factors include increasing age, hepatic metastasis, previous exposure to octreotide, and increasing duration of anesthesia, but patients without these conditions can also develop intraoperative crises [38, 39]. PRRT is a procedure that increases the risk for hormonal crises [40], probably due to tumor lysis. According to the ENETS guidelines, long-acting SA should be discontinued 4–6 weeks before PRRT [41], while short-acting formulations can be given [41].

Electrolyte, vitamin, and protein abnormalities in CS patients should be corrected before surgery along with dehydration [42, 43]. Patients with severe diarrhea may require parenteral nutrition [44]. Patients with CHD who need to undergo surgery or other invasive procedures should also undergo preoperative evaluation by an expert cardiologist in CHD [44] to prevent low cardiac output syndrome due to right ventricular failure [44]. Various octreotide administration regimen and various schemes have been proposed [44]. Some authors suggest subcutaneous administration for low-risk patients and minor procedures [45], but intravenous octreotide infusions should be readily available since CC can be induced even by minor surgical procedures [44]. However, the intravenous administration is currently considered the most preferable one [46]. If patients already receive long-acting SA, they should be continued [44]. Some data in the literature suggest that patients pretreated with SA may require higher doses of octreotide infusion [46]. Most experts initiate prophylactic treatment with intravenous octreotide 12 h before surgery and escalate the dose as necessary to control symptoms at least 48 h after the operation [44]. It seems that intravenous octreotide at a starting dose of 50–100 microgr/h (mean dose 100–200 microgr/h) is currently used by most centers [42–44]. In addition, ondansetron may help for diarrhea control [47].

Patients with tumors originating from the foregut (especially lung, stomach, and duodenum) may present a less common atypical CS. Atypical SC is usually mediated by both histamine and serotonin and is characterized by patchy, intensely red flush, sweating, itching, cutaneous edema, bronchoconstriction, salivary gland swelling, lacrimation, and cardiovascular instability (mainly hypotension). In these patients, histamine urinary metabolite methylimidazole acetic acid must be controlled since 24-h u5-HIAA may be not elevated because of decarboxylation deficit [44]. In these patients, addition of H_1 receptor blockers and H_2 blockers is recommended, and sometimes also cortisone can be administered to block histamine peripheral actions [48, 49].

Specific recommendations about anesthesia and the drugs to prefer should also be considered in the management of CS patients. Appropriate pain relief and anxiety control can reduce catecholamines-mediated stress response, and this is very important since catecholamines are thought to contribute to the release of tumor products [42, 43, 50].

7.3 Hypoglycemia

Insulinomas are rare functional pancreatic neuroendocrine tumors (pNET) [51–53]. Symptoms of hypoglycemia (adrenergic and neuroglycopenic) with concomitant documented low blood glucose levels and the relief of symptoms by intake of carbohydrates (Whipple's triad) are strongly suggestive for the presence of an insulinoma [54]. However, documented levels of hypoglycemia with concomitant blood insulin, C-peptide, proinsulin, and β-hydroxybutyrate levels during a supervised 72-h fasting test, considered the gold standard, are needed to confirm the diagnosis of insulinoma [51, 54, 55] as well as the absence of sulfonylurea in plasma and/or urine [52, 53] and the absence of insulin antibodies [56].

Surgical exploration is recommended in all insulinoma patients with or without MEN1, if non-resectable metastatic disease is not present [57]. A laparoscopic approach is usually recommended in patients with sporadic disease and with imaged tumors [58]. In patients with a localized insulinoma, who are not candidates for surgery, ablative therapy either endoscopically or percutaneously with radiological guidance should be considered [57]. Prior to surgery or locoregional treatments, in order to control hypoglycemia and to reduce the risk of hypoglycemic crises during the procedure, medical therapy is very important. Besides treating patients with diazoxide (first-line treatment for hypoglycemia), 30–50% of them also respond to SA [57], but they need to be carefully monitored, because some of them may get worse [59–65] since SA also inhibit the secretion of counterregulatory hormones. Everolimus can be used in refractory hypoglycemia due to malignant insulinoma [2, 57], while treatment with glucocorticoids can be used because it induces hyperglycemia by inhibiting insulin secretion and increasing insulin resistance [2]. PRRT, even though experience in malignant insulinoma is very limited [2], may be an effective treatment option for hormonal syndrome control and tumor burden reduction or stabilization [33, 66, 67]. Sunitinib and pasireotide were also shown to be effective [57, 68], even though the experience is limited. However, it is not known which is the best and most effective therapeutic sequence in patients with malignant insulinomas.

7.4 Zollinger–Ellison Syndrome

Inappropriately elevated fasting serum gastrin in the presence of hypergastrinemia when gastric acid secretion (gastric pH < 2) is present suggests the diagnosis of Zollinger–Ellison syndrome (ZES) [57]. Routine surgical exploration is still not generally recommended in MEN1/ZES patients with pNETs < 2 cm [57]. Patients with sporadic gastrinomas and without contraindications should undergo surgical exploration by a gastrinomas dedicated surgeon [69]. Peritumoral lymph nodes should be removed in order to be assessed for prognostic

purposes and to increase the cure rate [57]. Enucleation is the generally recommended surgical procedure; pancreaticduodenotomy is reserved for selected cases [70–75]. pNETs with preoperative vascular involvement or invasion should be evaluated by a team well versed in this kind of surgery [57].

Proton pump inhibitors (PPI) remain the mainstay of medical therapy for gastric acid secretion control [57]. Hypomagnesemia and vitamin B_{12} deficiency can develop during long-term treatment [76–82]. Some epidemiological studies have also found an increased incidence of bone fractures even though this finding was not confirmed in other studies [57]. The high somatostatin receptor expression in gastrinomas makes them highly responsive to SA and supports the use of such drugs to control tumor growth in patients not amenable to surgical cure. However, only limited data exist to support the use of SSAs in advanced gastrinomas [83].

7.5 Glucagonoma

Glucagonoma is an uncommon neuroendocrine tumor arising from pancreatic islet alpha cells [84]. Its clinical manifestations include necrolytic migratory erythema, glucose intolerance or diabetes mellitus, and importantly weight loss [44, 84]. SSA, antibiotics, and amino acid infusion may improve syndrome control and may help heal skin lesions [44]. These patients are at high risk for deep venous thrombosis and pulmonary embolism. For this reason, they should also receive thromboprophylaxis especially before surgical procedures. Locoregional treatments and medical systemic therapy may also be considered based on disease extension and grading surgery.

7.6 VIPoma

VIPoma is generally a pNET secreting vasoactive intestinal peptide (VIP) that causes a clinical syndrome characterized by severe secretory diarrhea, which leads to severe hypokalemia, loss of bicarbonate, metabolic acidosis, and dehydration [44]. Patients must be treated for this life-threatening condition by correcting electrolyte abnormalities and dehydration. SA remain the treatment of choice for rare functioning NETs [44, 57] prior to surgery or if resection is not possible.

7.7 Somatostatinoma

Somatostatinomas are NETs of the pancreas, duodenum, or jejunum [57], and, at present, there are more than 100 cases described in the literature [57]. The clinical syndrome is characterized by diabetes mellitus, cholelithiasis, diarrhea, and steatorrhea [57, 85]. Primary tumor and metastasis surgery, when possible, can help treat symptoms due to tumor load, hormonal secretion, and obstructive symptoms [85]. With this aim, locoregional therapies may also be considered.

7.8 Ectopic Syndromes

Nonfunctioning NETs can also become suddenly functional NETs, and usually this is a poor prognostic factor [2]. Syndrome control is important in order to reduce comorbidities associated to the syndrome and in order to prepare the patient for surgical or other invasive procedures.

7.8.1 Syndrome of Inappropriate Antidiuresis (SIAD)

NETs syndrome of inappropriate antidiuresis (SIAD) is usually caused by small-cell lung carcinoma, but other neuroendocrine neoplasias can cause SIAD too [86]. SIAD is characterized by euvolemic hypotonic hyponatremia due to the antidiuretic effect of inappropriate levels of antidiuretic hormone (ADH). [87]. Hyponatremia correction is very important since it is a life-threatening situation. In severe symptomatic hyponatremia, infusion of 3% NaCl saline as

boluses or as a continuous infusion should be administered [88]. Vaptans, nonpeptide vasopressin receptor antagonists, cause serum sodium increase by inducing aquaresis [88]. Treatment is usually started with 7.5–15 mg per day. Cases of secondary resistance to tolvaptan in paraneoplastic SIAD have been reported despite increasing doses of tolvaptan [87]. The authors think that this can be due to extraordinarily high levels of ADH, rather than adaptive mechanisms at receptor level. Loss of aquaretic effect in these patients can represent disease progression [87].

7.8.2 Acromegaly

The incidence of acromegaly due to a pituitary adenoma is three cases per one million persons per year, and the prevalence is about 60 cases per million [89]. In less than 1% of cases, acromegaly may develop because of ectopic secretion of growth hormone (GH)-releasing hormone (GHRH) [90–95] or, more rarely, GH secretion from a nonpituitary origin, mostly from a neuroendocrine tumor (NET) [96–98]. Usually, it is secondary to pNETs or bronchial carcinoids, but NETs from other origin can also cause ectopic acromegaly [57]. There are reported cases of ectopic acromegaly in a patient with pheochromocytoma [99], lymphoma [98], and paraganglioma [89].

Surgical resection of the primary tumor [89] should be considered whenever possible in order to control syndrome, and, if it is not possible, curative SA should be used for their antiproliferative effect and hormonal excess control. After SA treatment, in case of persistence of high hormonal levels, pegvisomant, a GH receptor antagonist, should also be considered. In case of unresectable tumor or extensive metastasis, other systemic therapies, locoregional treatments, and radiotherapy should also be evaluated for syndrome control according to the extent, grading, and biological characteristics of the neuroendocrine disease [89].

7.8.3 Cushing Syndrome

Ectopic adrenocorticotropic hormone (ACTH) secretion has been reported primarily in patients with lung NETs [44], but can also be encountered in patients with gastrointestinal tumors [100, 101]. Syndrome control, especially before surgical procedures, is very important since ectopic hypercortisolism can cause severe hypokalemia, hyperglycemia, and high thromboembolic risk arising very quickly.

Drugs aiming at controlling hypercortisolism can act at the tumor level (SA, cabergoline), at the adrenals (metyrapone, ketoconazole), or at the glucocorticoid receptor. These drugs can also be used in association. Attention must be paid with ketoconazole that can interfere with the metabolism of other drugs such as anticoagulants, antibiotics, and chemotherapeutics. Hypokalemia must be corrected since these patients can present with rapid onset and severe hypokalemia that can cause cardiac rhythm alterations. Glucose levels control is also of crucial importance. Furthermore, these patients can present frailty fractures secondary to hypercortisolism, especially vertebral fractures, and for this reason adequate vitamin D supplementation and eventually antiosteoporotic treatment (such as zoledronate) should be considered. Finally, if medical therapy does not control hypercortisolism, bilateral adrenalectomy should be carefully considered by a multidisciplinary team and with a dedicated surgeon.

7.8.4 Hypercalcemia (PTHrP and MEN1)

Ectopic hypercalcemia may be secondary to ectopic production of parathyroid hormone-related peptide (PTHrP) [44] or, less commonly, to ectopic production of parathyroid hormone (PTH) [102]. In patients with uncontrolled hormonal syndrome, debulking surgery and hepatic locoregional treatment must be considered [44] despite systemic pharmacotherapy for the

neoplasia. The mainstays of medical treatment in case of severe ectopic hypercalcemia are patient rehydration and treatment with bisphosphonates (zoledronate, pamidronate) or denosumab with the aim to reduce bone resorption [103]. Hydrocortisone can inhibit calcium absorption and reduce extra renal calcitriol [103]. Treatment with furosemide is controversial. Cinacalcet mimics high levels of calcium to reduce PTH levels [104]. In case of patients unresponsive to pharmacotherapy or in case of severe hypercalcemia with important kidney failure, hemodialysis must be considered.

Hypercalcemic primary hyperparathyroidism can present in patients with pNETs in MEN1 syndrome. Although the optimum timing has not been defined, surgery for subtotal parathyroidectomy or total parathyroidectomy is recommended [105]. Total parathyroidectomy with autotransplantation may be considered. Patient management should be done by a NETs multidisciplinary experienced team that should include an experienced endocrine surgeon. Conventional open bilateral exploration is recommended, while minimally invasive parathyroidectomy is usually not recommended because multiple glands are typically affected [105]. Concurrent transcervical profilactic thymectomy is also suggested at the time of surgery [105]. While waiting for parathyroidectomy, hypercalcemia can be treated with cinacalcet and/or bisphosphonates.

7.9 Conclusions

Clinical syndrome control is paramount for comorbidities, mortality, and quality of life control in functioning NET patients. Whenever possible, surgery of the primary and/or metastasis should be considered in order to reduce tumor burden and consequently hormonal secretion. Because of their antiproliferative and antisecretive effects, SA are the mainstay for many hormonal syndromes in NETs. However, further investigation is needed for the use of multiple SA receptor pasireotide in the treatment of NET-related syndromes. According to the type of hormonal secretion, other medical treatments should be used alone or in combination therapy with SA in order to control symptoms, and to prepare the patient for procedures such as surgery, locoregional treatments, and PRRT. We still have to deal with syndromes that we cannot control at all, such as refractory CS, hypoglycemia in malignant insulinomas, or severe ectopic hypercortisolism. In the first case, telotristat has provided very promising outcomes, while for malignant insulinomas usually combination therapy with different sequences is recommended. For these reasons and with the aim to offer our patients the best treatment currently available, a multidisciplinary team approach is always crucial for treatment and follow-up.

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New Surgical Strategies

8

Valentina Andreasi, Stefano Partelli, Francesca Muffatti, and Massimo Falconi

8.1 Introduction

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are heterogeneous malignancies arising from neuroendocrine cells. These lesions represent the second most common digestive tumor in terms of prevalence [1], and their incidence has significantly increased during the last three decades [2], probably due to the extensive use of endoscopic procedures and high-quality diagnostic techniques [3]. These neoplasms are characterized by a broad spectrum of aggressiveness, as they comprise both slow-growing tumors with an indolent biological behavior and aggressive neoplasms presenting at diagnosis with invasion of nearby structures or distant metastases [4]. GEP-NENs can be defined as functioning or nonfunctioning, based on the presence or absence of a clinical syndrome related to hormone hypersecretion; pancreatic NENs (PanNENs) are nonfunctioning in the vast majority of cases (90%) [5], whereas small bowel NENs (SB-NENs), especially when metastatic, are frequently associated with a typical carcinoid syndrome [4, 6]. Furthermore, these neoplasms may display a variable aggressiveness depending on their site of origin. SB-NENs are characterized by a relatively high tendency to metastasize, but they are likely to have an indolent progression despite the unfavorable setting; conversely, gastric and rectal NENs have a low metastatic rate at initial diagnosis, but they rapidly progress once they have metastasized [4].

Therefore, the management of GEP-NENs should be tailored according to the characteristics of the tumor, including site of origin, stage, grade, functionality, and disease extent. Moreover, patient's features, such as age, comorbidities, and performance status, should be taken into account as well.

Surgical resection plays a pivotal role in the management of GEP-NENs, as it is the only chance of achieving a complete cure [4–8].

8.2 Management of Small and Incidentally Discovered Well-Differentiated Gastroenteropancreatic Neuroendocrine Tumors (GEP-NETs)

The incidental diagnosis of GEP-NETs is increasingly frequent, probably due to the widespread use of endoscopic and radiological examinations. Current guidelines [5, 7–10] report that nonfunctioning, well-differentiated,

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low-grade, incidentally discovered GEP-NETs might benefit from a conservative management consisting in endoscopic resection or active surveillance.

8.2.1 Pancreatic NETs

The incidence of small, asymptomatic, nonfunctioning pancreatic NETs (NF-PanNETs) has significantly increased during the last three decades [2, 11]. However, it is likely that the real prevalence of these lesions is much higher, as small PanNETs are frequently found incidentally at final pathological examination in patients submitted to pancreatic resection for diagnoses other than NETs [12]. This finding supports the fact that many individuals are probably affected by small PanNETs that will remain unchanged for their entire life.

Given the dramatic increase in the incidence of these small lesions, their indolent behavior, and the high morbidity associated to pancreatic surgery, a watchful strategy has been advocated. Several retrospective studies [13–15] demonstrated the safety of active surveillance for asymptomatic NF-PanNETs with a maximum diameter ≤2 cm. Based on these series, current guidelines [5, 7, 9] proposed a "wait and see" strategy. More recently, the long-term outcomes of active surveillance were investigated and no disease-specific survival advantage from surgery compared to active surveillance was found in patients with small NF-PanNETs [16]. Despite this evidence, the conservative management for small NF-PanNETs is still controversial in some challenging situations such as small lesions occurring in young patients or in the presence of radiological or pathological worrisome features, such as dilation of the main pancreatic duct [14, 17].

Nowadays, an *active surveillance strategy* with a 6-month radiological follow-up for the first 2 years from diagnosis and yearly thereafter is recommended for *NF-PanNETs ≤ 2 cm without features of aggressiveness* (nodal metastases, dilation of the main pancreatic duct or bile duct, vascular or nearby organs invasion) [18].

8.2.2 Gastric NETs

Gastric NETs (G-NETs) are increasingly recognized entities due to the expanding indications for upper gastrointestinal endoscopy [2]. *Type I G-NETs* (70–80%), which are characterized by the association with chronic atrophic gastritis, usually display small size, indolent biological behavior (mostly G1), and excellent overall survival rates [8]. Therefore, current European Neuroendocrine Tumor Society (ENETS) guidelines proposed that *conservative strategies* should be preferred over surgery, in order to reduce the risk of overtreatment [8]. In particular, *endoscopic surveillance* every 12 or 24 months is recommended for lesions smaller than 1 cm, whereas *endoscopic resection* (ER) by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) is suggested for lesions measuring >1 cm and limited to the submucosal layer [18, 19]. Some series reported a greater complete resection rate by using ESD compared to EMR [20, 21]. However, it should be pointed out that ER, especially ESD, is associated to a measurable risk of bleeding and perforation. The endoscopic (a) and eco-endoscopic (b) appearance of a 12-mm type 1 G-NET are shown in Fig. 8.1. On the other hand, patients with type I G-NETs invading the muscular layer should be excluded from ER and submitted to surgery [22]. Regarding *type II G-NETs* (5–6%), which are caused by hypergastrinemia in the setting of an underlying gastrinoma in patients with multiple endocrine neoplasia type 1 (MEN1), treatment is usually dependent on the simultaneous presence of duodenal or pancreatic NETs which require surgical treatment [8]. Nevertheless, a conservative management should be considered as well since these lesions may regress with a successful medical treatment of the underlying gastrinoma [18, 23]. Finally, *type III G-NENs* are usually large, unique, high-grade (G3), malignant lesions. Their incidental diagnosis is rare as they often present with symptoms at an advanced stage of disease [8, 19]. Therefore, current guidelines [8, 24] recommend surgical resection (partial or total gastrectomy with nodal dissection), whereas ER rarely represents an

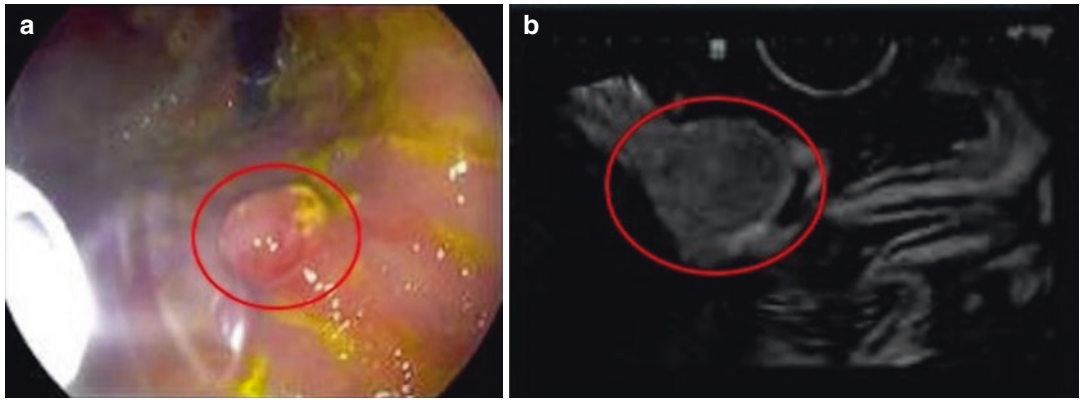

Fig. 8.1 Upper gastrointestinal endoscopy (**a**) and endoscopic ultrasound (EUS) (**b**) showing a 12-mm type I gastric neuroendocrine tumor (G-NET) G1 limited to the submucosal layer of the gastric body and subsequently treated by endoscopic submucosal dissection (ESD). The red circle indicates the tumor

acceptable treatment option [8]. In this regard, it has been reported that ER or wedge resection could be considered as initial treatment for type III G-NETs G1 <1.5–2 cm confined to the submucosal layer with no evidence of lymphovascular invasion [25, 26].

8.2.3 Duodenal NETs

Duodenal NETs (D-NETs) represent approximately 1–3% of all primary duodenal neoplasms and are often incidentally discovered during endoscopic or radiological examinations performed for other reasons [1]. Therefore, an increase in the detection of early-stage disease and a decrease in that of advanced disease have been observed [27]. Patients diagnosed with *G1 D-NETs ≤ 1 cm,* limited to the submucosal layer, can be submitted to *ER* (EMR or ESD) or to active surveillance [18] when the presence of nodal metastases has been ruled out [8]. The management of D-NETs with a diameter comprised between 1 and 2 cm is still not standardized, although an ER approach seems safe in the absence of aggressiveness features [8]. Currently, the endoscopic active surveillance approach is still debated as nodal metastases and microvascular invasion have been found even in few cases of small D-NETs G1 [28, 29].

8.2.4 Small Bowel NETs

Small bowel NETs (SB-NETs) represent approximately 30–50% of all neoplasms located in the small intestine and their incidence is increasing [1, 2]. An incidental diagnosis is rare and it may occur during colonoscopy performed for other reasons, if the lesion is located in the terminal ileum [30]. However, the vast majority of these tumors display features of aggressiveness, such as nodal metastases even when the lesion has a small size (50% of SB-NETs <1 cm) [31]. Therefore, all SB-NETs, even when small and incidentally detected, should be regarded as aggressive tumors and consequently treated with surgical resection associated to lymphadenectomy [7].

8.2.5 Colorectal NETs

Colorectal NETs are increasingly recognized entities, and they are rarely associated to the presence of a clinical syndrome, even when metastatic [32]. However, colonic and rectal NETs should be distinguished in terms of biology and treatment.

Colonic NETs (C-NETs) are often high-grade and poorly differentiated, therefore even an incidental diagnosis should be followed by surgical resection and careful postoperative follow up, based on the final pathological report [10].

On the other hand, rectal NETs (R-NETs), which represent approximately one-third of all digestive neuroendocrine tumors [2, 33], are usually small, low-grade (G1), and associated with a low risk of metastatic spread. According to the ENETS [10] and the North American Neuroendocrine Tumor Society (NANETS) [34] guidelines, their management depends on tumor size. Incidentally discovered *R-NETs* measuring less than 1.5 cm, limited to mucosa or submucosa and without features of aggressiveness (atypical endoscopic aspect, tumor grade G2-G3, and lymphovascular invasion) can be resected by *ER* [10, 33]. Several endoscopic resection techniques are available, but ligation-assisted endoscopic mucosal resection (ESMR-L) and endoscopic submucosal dissection (ESD) seem to be the most effective ones, since the high R0 rates and the low risk of recurrence [35, 36] especially for R-NETs measuring <10 mm. The endoscopic appearance of an 8 mm R-NET G1, limited to the submucosal layer of rectum and treated by ESD, is shown in Fig. 8.2. Another available option is *transanal endoscopic microsurgery* (TEM), which is a minimally invasive endosurgical technique allowing to combine a rigid rectoscope with magnified tridimensional vision and endosurgical instruments [33]. The advantage of TEM over endoscopic techniques is the possibility of performing a full-thickness resection of the lesion. TEM is nowadays the reference resection technique for T1 R-NETs measuring 10–15 mm and invading the submucosal layer, especially when located in the low or intermediate rectum, in order to avoid segmental resection surgery [37].

The follow-up of R-NETs after ER is not well-defined. No specific surveillance protocol is recommended for completely resected R-NETs <10 mm without high-risk features [10, 35]. As regards patients with completely resected (R0) R-NETs ≥10 mm, a surveillance rectoscopy at 1 year, 3 years, and then after 5 years should be proposed [33]. In case of ER in the presence of high-risk features or R1 resection without salvage therapy, one rectoscopy or endoscopic ultrasound (EUS) every 6–12 months for at least 5 years is indicated [38–40]. ENETS guidelines recommend also the execution of an abdominopelvic MRI on a yearly basis in order to detect perirectal and/or distant recurrence [33, 38].

8.3 Surgical Management of Localized Gastroenteropancreatic (GEP)-NETs

Surgery plays a pivotal role in the management of localized GEP-NETs. According to the site of the primary tumor, different surgical strategies are recommended.

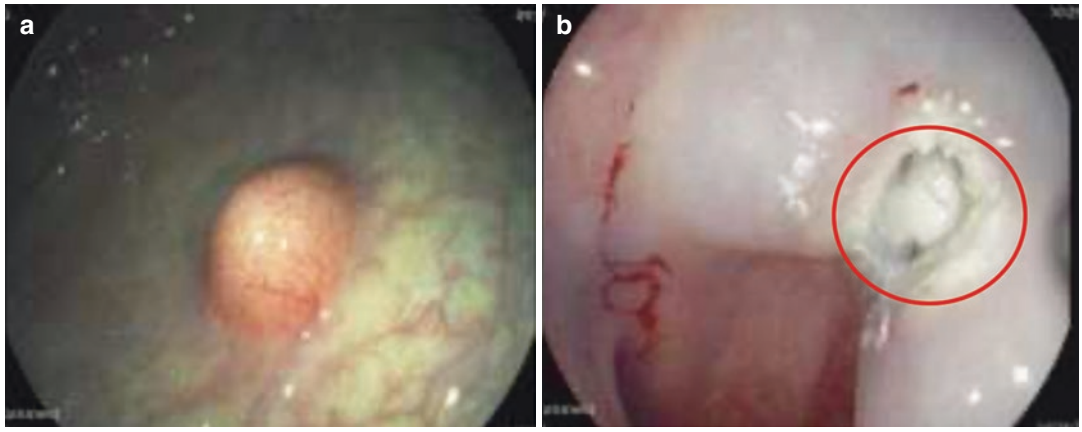

Fig. 8.2 Colonoscopy showing an 8-mm rectal neuroendocrine tumor (R-NET) G1 limited to the submucosal layer of the rectum, before (**a**) and after (**b**, red circle) endoscopic submucosal dissection (ESD)

8.3.1 Localized Pancreatic (Pan) NETs

Surgical resection represents the backbone for the management of localized PanNETs. The latest ENETS [5] and NANETS [9] guidelines recommend surgical resection for all patients diagnosed with any functioning PanNETs or with localized NF-PanNETs >2 cm in size as well as for those with symptoms or features of aggressiveness (evidence of nodal metastases, dilation of the main pancreatic duct or bile duct, invasion of nearby structures).

8.3.1.1 Formal Resections and Lymphadenectomy

A formal pancreatic resection (pancreaticoduodenectomy, distal pancreatectomy, total pancreatectomy) associated to a systematic lymphadenectomy is recommended for patients with NF-PanNETs >2 cm as well as for those with symptoms or radiological features of aggressiveness [5, 9, 41].

The presence of nodal metastases is one of the most powerful prognostic factors after radical surgery [42, 43], and the risk nodal involvement increases with increasing tumor grade and tumor size [44, 45]. More recently, it has been demonstrated that also the number of positive lymph nodes (LN) affects the risk of recurrence after surgery in these patients [46, 47]. Therefore, guidelines recommend that an adequate lymphadenectomy should be routinely performed [7]. Several cutoffs have been proposed as the minimum number of LN to be resected/examined in order to ensure a proper nodal staging according to the site of the tumor [46, 48]. There is still no consensus about this issue, but it seems that 12 LN and 7 LN represent an adequate number of nodes to be resected/examined after pancreaticoduodenectomy and distal pancreatectomy, respectively [46].

8.3.1.2 Parenchyma-Sparing Resections

Parenchyma-sparing resections, which comprise enucleation, middle pancreatectomy, and middle-preserving pancreatectomy, represent an alternative option for the surgical treatment of functioning PanNETs and small NF-PanNETs [7, 49]. The main advantage is related to the reduced risk of developing both pancreatic endocrine and exocrine insufficiency, as compared to formal pancreatic resections [50, 51]. On the other hand, these surgical procedures are associated to an increased risk of postoperative pancreatic fistula, although the postoperative mortality risk is lower [52]. The main concern regards the oncological appropriateness of parenchyma-sparing resections [53], due to the risk of inadequate clearance of surgical margins during enucleation and the absence of a standard lymphadenectomy [7]. This latter limitation may be partially overcome by performing a nodal sampling in the presence of suspicious LN. If nodal involvement is intraoperatively demonstrated, a formal resection instead of an atypical one should be then performed [7]. Therefore, current guidelines [7, 9, 41] suggest that *enucleation* should be proposed to patients undergoing surgery for NF-PanNETs ≤2 cm or to those affected by insulinomas, when located further than 2–3 mm from the main pancreatic duct [54]. An intraoperative image of enucleation is shown in Fig. 8.3. On the

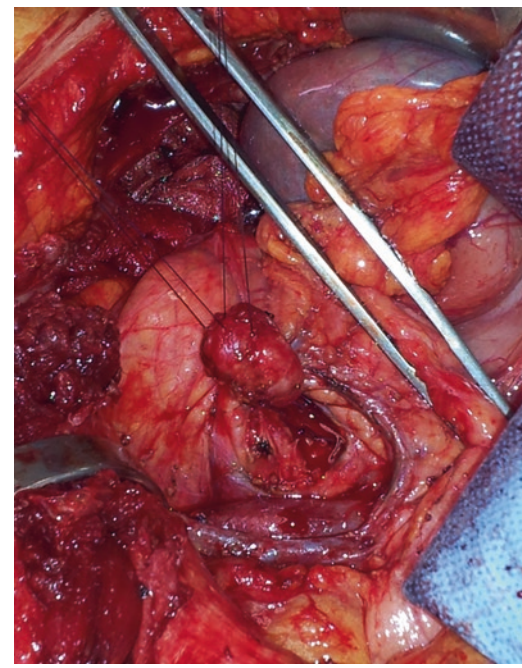

Fig. 8.3 Intraoperative image of an enucleation with open approach performed for an insulinoma

other hand, *middle pancreatectomy* should be offered to patients with small NF-PanNETs or insulinomas of the pancreatic neck/proximal body, when enucleation is not feasible, and the remaining parenchyma is enough to preserve a relevant pancreatic function [55]. Finally, *middle-preserving pancreatectomy* can be offered to patients with a multifocal neuroendocrine disease (i.e., MEN1 or Von Hippel Lindau) involving the pancreatic head and tail, but sparing the pancreatic body [49].

An additional option for reducing the risk of postoperative endocrine insufficiency is represented by *autologous islet transplantation* [56]. This procedure has a relatively low complication rate and can be proposed to patients, especially young, undergoing distal pancreatectomy for benign/borderline PanNETs located at the level of pancreatic body/neck [56–58].

8.3.2 Localized Gastric (G)-NETs

The management of localized G-NETs should be tailored according to the type. *Type I G-NETs* are usually managed conservatively. However, ENETS guidelines suggest to consider a local excision or partial gastrectomy when these lesions are T2 (or above) or with positive margins after endoscopic resection [8]. Concerning *type I* G-NETs which recur after endoscopic resection, antrectomy represents a possible option [19].

Patients with *type 2 G-NETs* should be discussed at multidisciplinary meetings, as their management depends on the concomitant presence of PanNETs or D-NETs that may require surgical resection. Regarding the extent of resection, local or limited excision may be appropriate for these lesions [8].

Finally, ENETS [8] and NCCN [59] guidelines recommend managing *type III G-NETs* in the same manner as gastric adenocarcinoma. Therefore, surgical resection with total or partial gastrectomy associated to lymph node dissection is indicated.

8.3.3 Localized Duodenal (D)-NETs

Radical surgery with nodal dissection is recommended when a D-NET measures ≥2 cm and/or extends beyond the submucosal layer and/or has an ampullary/periampullary location [8, 60]. Surgical resection is required whenever the risk of malignancy is high [61]. The endoscopic appearance and the preoperative computed tomography (CT) scan of patient with a NET of the major duodenal papilla are shown in Fig. 8.4.

Pancreaticoduodenectomy is the procedure of choice [62], especially when nodal metastases are

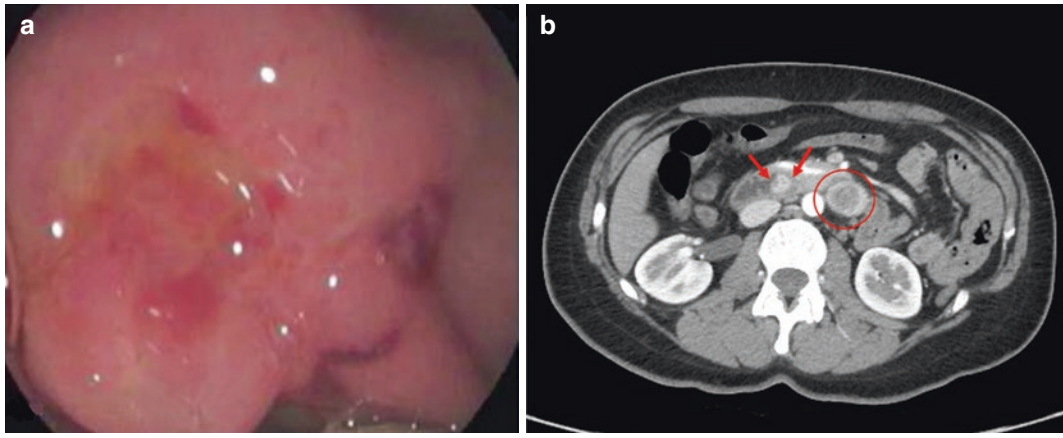

Fig. 8.4 Endoscopic image showing bulging of the major duodenal papilla (**a**) and preoperative CT scan with contrast medium (**b**) of a patient submitted to pancreaticoduodenectomy for a neuroendocrine tumor of the major duodenal papilla staged as pT2N1 G1 at the final pathological examination. The red arrows indicate the primary tumor, the red circle indicates a lymphadenopathy

preoperatively or intraoperatively detected, as it allows achieving a proper lymphadenectomy [61]. Nevertheless, the high rate of short-term and long-term postoperative complications associated with pancreaticoduodenectomy should be carefully evaluated for each patient [28, 63]. *Local excision* represents another possible option that can be considered when the risk of nodal involvement is low. This procedure can be used for patients with D-NETs located on the antipancreatic surface of the first part of duodenum, whereas *distal duodenectomy* can be performed for lesions arising in the fourth part of duodenum [8].

8.3.3.1 Localized Gastrinomas

Gastrinomas arise in the duodenum in approximately 70% of cases and represent the most frequent type of functioning D-NETs. These neoplasms frequently exhibit a malignant behavior, with *nodal involvement* being present in more than half of cases [5, 64]. Due to their small size (even below 5 mm), gastrinomas are frequently missed at preoperative imaging and the only radiological suspicious sign may be the presence of enlarged, hypervascularized lymph nodes [65]. Therefore, *surgical exploration* is indicated in all the patients with clinical and radiological signs of gastrinoma, even when the primary tumor is not clearly identified preoperatively [5]. Since the high rate of nodal metastases, *lymphadenectomy* should be systematically performed [5, 66] in any case, even if a pancreaticoduodenectomy is not the primary choice.

Gastrinomas affect patients with MEN1 syndrome in approximately one quarter of cases; in this setting, gastrinomas are frequently multiple and associated to nodal involvement. Despite these features, pancreaticoduodenectomy is curative in the majority of MEN1-associated gastrinomas [67].

8.3.4 Localized Small Bowel (SB)-NETs

Localized SB-NETs always require surgical resection, as their incidental diagnosis is rare and nodal metastases are frequently found even in the presence of small lesions [68]. The possibility to achieve a radical resection mainly depends on the presence of nodal metastases, which may be surrounded by a massive fibrotic reaction, neoplastic mesenteric deposits, and on their anatomical relation with the mesenteric vessel root and the retroperitoneum [69, 70]. A SB-NET is defined as "unresectable" when nodal metastases and/or mesenteric tumor deposits surround the mesenteric vessel root and/or extend to the retroperitoneum [7].

Current guidelines recommend a *radical open resection of the primary tumor(s)* associated to a *systematic nodal dissection* along the superior mesenteric root and around the mesentery in all the patients with localized SB-NETs, when technically feasible [7, 30, 71]. The surgical specimen of a patient submitted to ileal resection with lymphadenectomy for a SB-NET is shown in Fig. 8.5.

Nodal metastases are present in approximately 80–90% of patients with SB-NETs [72], and consequently, a standard lymphadenectomy must be always performed, independently from

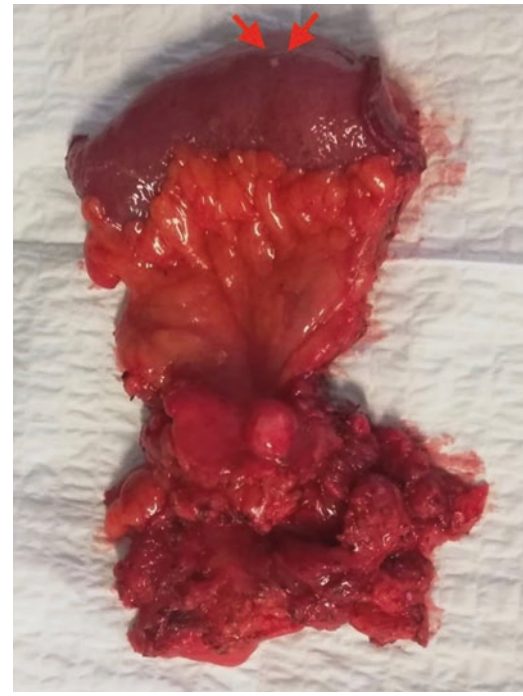

Fig. 8.5 Surgical specimen showing a 9-mm small bowel neuroendocrine tumor (SB-NET) staged as pT3N1 G1 at the final pathological examination. The red arrows indicate the tumor

the radiological evidence of suspicious LN [7]. A survival advantage has been demonstrated for patients undergoing a systematic lymphadenectomy as compared to those submitted to a selective nodal dissection [73]. The proper number of LN to be resected is still not well-defined, but it seems that at least 8 LN should be examined in order to obtain a proper nodal staging [74, 75]. As regards the extent of lymphadenectomy, it has been reported that there is no correlation between the number of resected LN and the length of small bowel resection [72]. Therefore, extended bowel resections ("pizza pie" rule) should be abandoned, as they are not necessary for achieving a proper nodal dissection [72]. On the other hand, "reverse" surgery, consisting in a wide lymphadenectomy followed by a limited small bowel resection, should be privileged [7].

Finally, an intraoperative bidigital palpation of the whole small intestine, from Treitz ligament to ileocecal valve, is always recommended in order to detect multifocal lesions, which are present in more than 80% of patients with SB-NETs [7, 71].

8.3.5 Localized Colonic (C)-NETs

C-NETs are usually diagnosed at an advanced stage of disease and they frequently appear as large tumors associated to metastatic spread. According to data from the Surveillance, Epidemiology and End Results (SEER) dataset, less than half of C-NETs (45%) are localized at the time of diagnosis [1]. In these cases, ENETS guidelines suggest that a *localized colectomy* associated to a *proper lymphadenectomy* is the treatment of choice [38].

8.3.6 Localized Rectal (R)-NETs

The management of R-NETs should be tailored according to the presence or absence of predictors of nodal metastases, including tumor size >15 mm, atypical endoscopic aspect (depression/ulceration of the lesion), invasion of the muscular layer (T stage ≥2), tumor grade (G3 or G2 versus G1), and lymphovascular invasion [10, 35, 76]. Nearly all the cases of R-NETs with nodal involvement include at least one risk factor [40]. In all these cases, a formal oncologic *low anterior resection* (LAR) with *total mesorectal excision* (TME) [10] should be proposed. Moreover, according to the location of the tumor, a very low anterior resection as well as an intersphincteric resection can be performed. Quality of life related to anal preservation is of paramount importance in these patients since the long-term postoperative outcome is generally good [33].

The optimal extent of lymphadenectomy for R-NETs is not yet well-defined. R-NETs usually develop LN metastases in the mesorectum, but sometimes pelvic LN as well as obturator canal LN are also involved [77]. Consequently, a lymphadenectomy extended to these areas should be performed whenever suspicious LN have been preoperatively identified [33, 77].

8.3.7 Role of Minimally Invasive Surgery

8.3.7.1 Minimally Invasive Surgery for PanNETs

Minimally invasive surgery (MIS) has gained wide acceptance over the last two decades [78], especially for the treatment of PanNETs. Current guidelines state that the minimally invasive approach (laparoscopic or robotic) is safe and feasible, especially for neoplasms located in the pancreatic body/tail [7, 9]. Various retrospective studies have demonstrated the *advantages of MIS* in terms of *short-term outcomes*, including reduced intraoperative blood loss, lower postoperative complication rate, shortened length of hospital stay, and better cosmetic results [79, 80]. As regards the long-term oncological outcomes of minimally invasive pancreatic resections, several retrospective series reported similar recurrence and survival rates between patients submitted to laparoscopic/robotic and open pancreatic resections [78, 80, 81]. However, prospective randomized trials on the oncological adequacy of MIS in PanNETs are still lacking.

Concerning the comparison between *laparoscopic* and *robotic* approach, some retrospective experiences compared laparoscopic and robotic distal pancreatectomy (DP) performed for PanNENs, reporting reduced blood loss [82] and lower conversion rates [83] favoring the robotic approach. On the other hand, a significant advantage was reported for the laparoscopic approach in terms of total costs, that represent the main limitation for the implementation of robotic procedures [82].

8.3.7.2 Minimally Invasive Surgery for G-NETs

Laparoscopic antrectomy may be an option for treating type I G-NETs, especially when recurrent. This procedure is associated to a lower risk of recurrence compared to endoscopic resection and reduces the discomfort related to the endoscopic follow up [19]. Furthermore, being less invasive compared to an open antrectomy, it has better short-term postoperative outcomes. In selected cases, a minimally invasive approach can be used for type II and type III G-NETs (total gastrectomy associated to nodal dissection).

8.3.7.3 Minimally Invasive Surgery for SB-NETs

The role of minimally invasive surgery has been poorly investigated in the setting of SB-NETs. *Open resection* is the *procedure of choice*, as it allows better vascular control in case of bleeding at the origin of superior mesenteric vessels and more sensitive bidigital palpation of the whole small intestine [84]. Therefore, current guidelines recommend that laparoscopic resection of SB-NETs should be limited to early lesions in the absence of gross nodal metastases [7].

8.3.7.4 Minimally Invasive Surgery for R-NETs

The outcomes of radical laparoscopic surgery performed for R-NETs have been scarcely investigated, due to the rarity of the disease. However, *laparoscopic resection* is nowadays the *standard surgical approach* for R-NETs, as it is associated with better outcomes as compared with transabdominal open surgery [33]. Radical laparoscopic surgery seems to be also an appropriate procedure in case of previous incomplete endoscopic resection [85].

8.4 Surgery for G3 GEP-NENs

High-grade (G3) GEP-NENs represent approximately 10–20% of all GEP-NENs, with a median overall survival ranging between 10 and 23 months [86]. According to the latest World Health Organization (WHO) classification, G3 GEP-NENs can be further classified as *well-differentiated*, defined as neuroendocrine tumors (G3 GEP-NETs), or *poorly differentiated*, defined as neuroendocrine carcinomas (G3 GEP-NECs) [87].

Current guidelines suggest that platinum-based chemotherapy represents the gold standard for the treatment of advanced grade 3 GEP-NENs [88, 89]. On the other hand, the role of radical surgery in this setting is more controversial. Although available data come from small retrospective series, it has been reported that *radical surgical resection of the primary tumor* is associated to a *survival benefit* compared to systemic therapies or palliative resection alone in patients with localized G3 GEP-NENs [90–93]. This advantage is particularly evident for *well-differentiated forms* (G3 GEP-NETs) [92, 93]. Furthermore, two recent studies reported a possible survival benefit also for highly selected patients with metastatic G3 PanNETs who underwent radical surgical resection [93, 94]. Therefore, the role of surgery should be evaluated separately for well- and poorly differentiated GEP-NENs. In conclusion, surgical resection represents a valuable option for patients with G3 GEP-NETs, in selected cases even when metastatic; on the other hand, surgery should be carefully considered for localized G3 GEP-NECs and completely avoided for metastatic G3 GEP-NECs.

8.5 Surgery for Metastatic GEP-NETs

Surgical resection plays an important role also in the setting of metastatic disease, since GEP-NETs usually display an indolent behavior and the liver is frequently the only metastatic site [7].

8.5.1 Surgery with Curative Intent

Current guidelines recommend that *surgery with curative intent* should be considered in the presence of *G1-G2 GEP-NETs with resectable or potentially resectable liver metastases*, when extra-abdominal disease has been ruled out [7, 9, 95]. Regarding liver involvement, three patterns of neuroendocrine liver metastases have been described: *type 1* when there is a single liver metastasis, *type 2* when an isolated metastatic bulk associated to smaller deposits is present (bilobar involvement), and *type 3* when there is a disseminated metastatic spread (bilobar involvement) [96]. Type 1 and 2 liver metastases are considered as resectable or potentially resectable. It has been reported that radical resection for GEP-NETs with type 1 liver metastases is associated to improved survival compared to medical therapies [97]. On the other hand, the survival benefit associated with surgery is less clear for patients with type 2 liver metastases. In the presence of this pattern of metastatization, a two-stage approach including resection of left metastases with right portal vein ligation followed by right hepatectomy could be an alternative, but the proper treatment sequence should be carefully discussed for each single patient in a dedicated multidisciplinary board [98, 99].

8.5.2 Palliative Resection of the Primary Tumor

Indications for palliative resection vary according to the site and the clinical presentation of the primary tumor. Generally, this surgical procedure is recommended to relieve symptoms related to tumor mass effect and hormonal hypersecretion (e.g., carcinoid syndrome).

Considering patients with functioning SB-NETs and unresectable liver metastases (type 3), debulking surgery should be considered with the aim of *controlling symptoms and/or complications* [7]. In patients with nonfunctioning SB-NETs, but symptomatic for small intestinal obstruction or ischemia, primary tumor resection is mandatory in order to prevent clinical deterioration and death [6]. Regarding PanNETs, a tumor located in the pancreatic body/tail is rarely symptomatic, and the management of complications related to tumors of the pancreatic head (jaundice or duodenal occlusion) usually does not require surgery.

Finally, several studies considering patients with GEP-NENs from different primary sites reported *improved survival* rates for patients undergoing *palliative resection of the primary tumor* in the presence of unresectable metastatic disease [100, 101]. This survival benefit was more evident for young patients with well-differentiated G1-G2 GEP-NENs [101, 102]. The reasons of this advantage in terms of survival are essentially unknown. It has been speculated that resection of the primary tumor may enhance the efficacy of other treatments, such as peptide receptor radionuclide therapy (PRRT), enabling to have only one target organ (i.e., liver) and a lower burden of disease [103]. Nevertheless, it should be pointed out that these results are from retrospective series and may have been affected by a possible selection bias.

8.5.3 Hepatic Cytoreduction

The management of *neuroendocrine liver metastasis* (NELM) is controversial, with some studies advocating an aggressive surgical strategy and others adopting a more conservative approach. Several retrospective series reported that cytoreduction of NELM may ameliorate both *symptoms* and *survival* [104]. Patients with functioning liver metastases are the one who benefit the most from surgical management [104]. A controversial issue regards the *threshold for hepatic*

cytoreduction: some studies recommend this surgical intervention when a debulking ≥90% can be reached [105, 106], whereas more recent series show that this procedure can give a benefit even when a 70% debulking is achieved [107, 108]. Hepatic cytoreduction may be helpful also in controlling an oligo-metastatic disease progression, allowing patients to continue their ongoing systemic therapy [109].

8.5.4 Liver Transplantation

Liver transplantation (LT) is an option for patients with unresectable NELM, as it may provide a substantial *survival benefit* [110]. However, due to high recurrence rates, patients have *to fulfil strict criteria* in order to have access to this treatment. Specific selection criteria for LT have been proposed by Mazzaferro et al. [111]. These criteria include histological confirmation of low-grade neuroendocrine tumor (Ki67 < 10%), primary tumor drained by the portal system previously removed with all the extrahepatic deposits, involvement of less than 50% of liver parenchyma, and stable disease/response to therapies for ≥6 months during the pretransplantation period and age <60 years old (relative criterion) [111, 112]. Patients with high-grade neuroendocrine tumors or carcinomas, other medical/operative conditions contraindicating LT (including previous neoplasms) as well as those with non-gastrointestinal NETs have to be considered not amenable of liver transplantation. Patients who meet the criteria and undergo LT have a significantly better survival when compared with patients undergoing alternative medical and surgical treatments [112, 113]. Transplantation-related survival increases over time and maximizes after 10 year [112].

8.6 Conclusions

Surgery plays a pivotal role in the management of GEP-NENs. Surgical resection represents the only chance of complete cure for patients with localized tumors. A conservative "watch and wait" strategy should be routinely considered for patients with incidentally discovered, asymptomatic, small lesions without radiological or endoscopic features of aggressiveness. Finally, even selected patients with metastatic or high-grade neoplasms may benefit from surgery in terms of both survival and quality of life. Therefore, surgery should be included in the context of a multimodal strategy for patients with advanced disease.

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9 New Approaches in Medical Therapies

Manila Rubino, Francesca Spada, Alice Laffi, and Nicola Fazio

9.1 Introduction

Advances in research of the molecular pathways associated with NETs have led to the discovery of multiple treatment options for patients with advanced NETs. Current available therapies include somatostatin analogs (SSA), peptide receptor radionuclide therapy (PRRT), the mammalian target of rapamycin (mTOR) inhibitor everolimus, and the tyrosine kinase inhibitor (TKi) sunitinib and interferon. Moreover, cytotoxic agents are indicated for the treatment of aggressive well-differentiated NETs and of poorly differentiated neuroendocrine carcinomas. Hepatic-directed treatments are recommended for patients with well-differentiated NETs and liver-predominant disease.

However, different drugs are currently under investigation in NET therapy, some molecules are similar to drugs already used in clinical practice, while others are approved in other tumors but not in NETs.

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9.2 Pasireotide

Pasireotide is a newer SSA with higher affinity for all subtypes of somatostatin receptors (SSTRs) 1, 2, 3, and 5, compared to octreotide and lanreotide, which mainly target SSTR2.

Pasireotide showed to be effective and well tolerated in controlling diarrhea and flushing related to carcinoid syndrome in patients with advanced NET refractory or resistant to octreotide LAR therapy [1]. However, pasireotide did not show a difference in symptoms control at 6 months in a randomized phase III trial compared to octreotide LAR, therefore, the study was interrupted early despite initial PFS improvements noted in the pasireotide treatment arm [2].

The phase II randomized trial, LUNA trial, assessed the efficacy of pasireotide alone or in combination with everolimus in lung and thymic carcinoids. The combination therapy with pasireotide and everolimus was not superior in mPFS compared to everolimus alone (12.5 months in the everolimus group and 11.8 months in the combination group), while a higher rate of adverse events was reported in combination group [3]. Several other monotherapy, combination, or increased dosage treatment strategies with pasireotide are currently being explored [4]. Pasireotide has not been approved for use in GEP-NETs but is certainly a focus of future research (Fig. 9.1).

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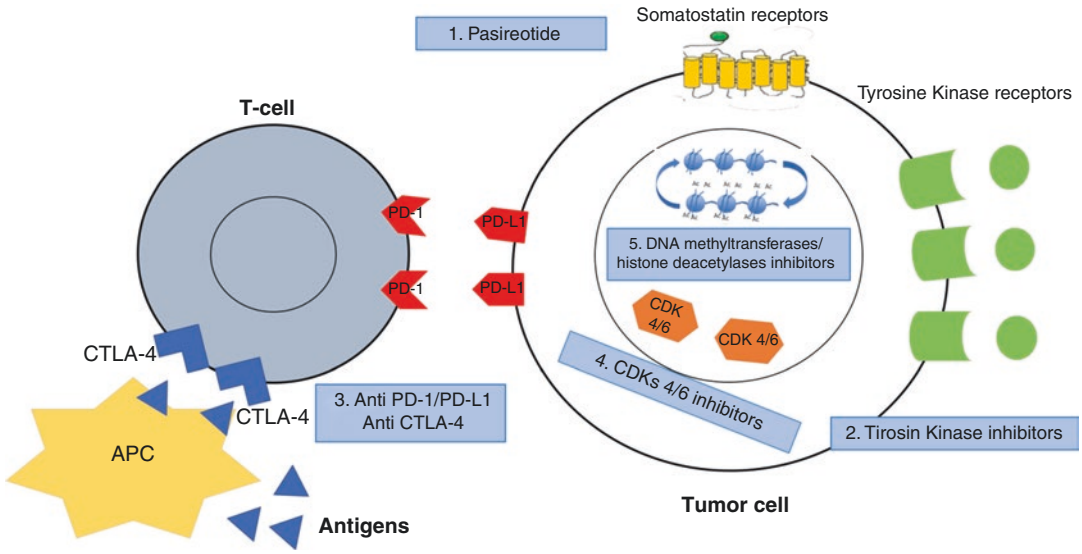

Fig. 9.1 Overview of new therapeutic strategies for NETs. *APC* antigen-presenting cell, *CTLA-4* cytotoxic T lymphocyte-associated protein-4, *PD-1* programmed cell death-1, *CDK 4/6* cyclin-dependent kinases

9.3 New Tyrosine Kinase Inhibitors

Neuroendocrine neoplasms are conventionally considered highly vascularized tumors, especially well-differentiated tumors that showed a high microvascular density [5, 6]. Tyrosine kinase pathways are involved in angiogenesis, tumor growth, and progression. For that reason, several molecules that act on specific tergets such as VEGFR, PDGFR, c-KIT, Flt-3, and RET [7] are under investigation.

Sunitinib is the only available TKi approved in PanNETs [8]. Clinical studies investigated other TKi with different activities on the multiple tyrosine kinase receptors (VEGFR, PDGFR, FGFR, KIT, RET, and MET).

Pazopanib is an oral multi-TKi that targets VRGFR1-2-3, PDGFR, c-Kit, and FGFR 1-2-3. Three phase II trials analyzed the activity and safety of pazopanib in advanced well-differentiated NETs from different primary tumors.

The study by Phan et al. explored the efficacy of pazopanib 800 mg/day and octreotide LAR (up to 40 mg every 3 weeks) in 44 patients with metastatic or locally advanced G1-G2 well-differentiated tumors from mixed primary sites. Thirty-two patients had pancreatic NETs. A partial response was observed in 22% of patients with a median PFS of 14.4 months and a median OS of 25 months [9]. Another study by Ahn et al. investigated the efficacy and safety of pazopanib 800/day in 37 patients with metastatic G1-G2 well-differentiated NETs and poorly differentiated G3 NECs from pancreatic and colorectal primaries. A PR rate of 19%, SD 57%, and mPFS of 9 months were observed [10]. In this study, patients had not been previously treated with other TKi or everolimus. On the contrary, PAZONET trial included patients after at least one prior systemic therapy, including other TKis. The study enrolled 44 patients with advanced well-differentiated G1-G2 NETs, and the median PFS was 9.5 months for the whole population, but it should be noticed that it was 12.4 months for patients pretreated with TKi and 6.8 for patients pretreated with mTOR inhibitors [11].

However, no phase III randomized clinical trials have been performed to date that define the role of pazopanib in the management of NENs, therefore, it is not currently approved for the treatment, although it could be useful in tumors resistant to standard therapy.

In general, pazopanib was well tolerated; the most common side effects were fatigue, nausea,

diarrhea, and hypertension and the most severe reactions (grade 4) being thromboembolism and hypertriglyceridemia that occurred in one patient each [10].

Cabozantinib is an orally available TKi that exerts a strong antagonist activity against MET and VEGFR2, but it also targets several kinases implicated in tumor pathology as KIT, RET, AXL, TIE2, and FLT3 [12].

A single-arm phase II trial in advanced well-differentiated pancreatic and small intestine NET showed that cabozantinib improved median PFS (21.8 and 31.4 months) for both PNET as well as small intestine NETs [13]. These results have led to a phase III trial (CABINET) that is now ongoing in USA, to assess the efficacy of cabozantinib in patients with advanced well-differentiated NETs who have progressed on everolimus (NCT03375320), but at the moment, this treatment is not currently approved for use in GEP-NETs. Main toxicities associated to cabozantinib therapy were hypertension, hypophosphatemia, and diarrhea.

Lenvatinib is an oral TKi that targets VEGFR 1–3, PDGFR, FGFR, RET, and SCFR. The effect of lenvatinib 24 mg/day has been investigated in a phase II trial, TALENT trial, in patients with G1-G2 advanced pancreatic (55 patients) and gastrointestinal (56 patients) NETs. Among patients with pancreatic primary tumors, 64% and 25% of patients were pretreated with everolimus or sunitinib, respectively. The study reported an overall response rate of 29%, in particular 42.3% for pancreatic primaries and 16.3% for gastrointestinal primaries. Median PFS and OS were 15.5 months and 29.2 months for pancreatic and gastrointestinal NETs, respectively. The most frequent grade 3/4 adverse events were hypertension, fatigue, and diarrhea, and in this study, almost 90% of patients experienced an adverse event.

Axitinib is an oral, second generation TKi that targets VEGFR 1-2-3, PDGFR, and c-KIT.

Axitinib has been studied at the dose of 10 mg/day in a phase II trial in 30 patients with well-differentiated advanced extrapancreatic (gastrointestinal, thoracic, and unknown primary) NETs. Interestingly, 53% of patients had history of carcinoid syndrome. Median PFS was 26.7 months and median OS was 45.3 months. Adverse events were mainly hypertension, thyroid dysfunction, and thromboembolism [14]. On the basis of this phase II trial, a phase II/III placebo-controlled trial is ongoing to evaluate the effectiveness of axitinib associated to octreotide LAR vs. placebo associated to octreotide LAR in G1-G2 NETs from extrapancreatic primary (NCT01744249).

Surufatinib is TKi that not only inhibits VEGFR1-2-3 but also targets FGFR1 and CSF pathways, which represent the supposed main acquired mechanism of resistance to anti-VEGF therapies. A phase I/II trial studied the efficacy of surufatinib in 81 patients, 42 with pancreatic, and 39 with extrapancreatic NETs. Overall response rate was 19% and 15%, while median PFS was 21.2 months and 13.4 months, in pancreatic and extrapancreatic NET, respectively. Grade 3/4 adverse events were mainly hypertension, proteinuria, and hyperuricemia. On this basis, the research with surufatinib moved to a next step and surufatinib demostrated efficacy vesrus placebo in two phase III trials in Asia in extra-pancreatic NETs (SANET-p) and pancreatic NETs (SANET-p) (Xu J et al. Lancet Oncol 2020) conducted in Asia [15]. Therefore it is not available yet in Western Countries for the treatment of pancreatic and extra-pancreatic NETs.

9.4 Immune Checkpoint Inhibitors

A new modality of immunotherapy has recently modified cancer treatment approach and has changed the treatment of some cancers, such as melanoma and lung cancer. However, the application of checkpoint inhibitors in the management of patients with NETs is still evolving. Many factors have been proposed as potential predictor of response to immune checkpoint inhibitors as programmed cell death-1 ligand (PD-L1) expression, lymphocyte infiltration, mismatch repair deficiency, and consequently tumor mutational and neoantigen load.

Levels of PD-L1 vary widely across published studies, suggesting that expression of this protein is heterogeneous in G1/G2 NETs. In particular,

PD-L1 expression has been associated with more advanced tumors as well as intermediate- to high-grade (G2-G3) GEP-NETs [16]. Lymphocyte infiltration is commonly observed in these tumors, but considering the low proportion of cases positive for PD-1/PD-L1, it is not clear if TILs are effectively activated by tumor neoantigens. Moreover, mechanisms of mismatch repairs appear to be efficient in most NETs, consequently, the mutational burden of these malignancies is relatively low, as only 3% of panNETs harbor >17 mutations/Mb, a cutoff usually used to predict response to immunotherapy [17].

Microsatellite instability (MSI) is considered a predictive biomarker for response to PD-1/PD-L1 inhibition. In well-differentiated tumors, high-level MSI has been demonstrated in sporadic insulinomas [18], but rarely in other GEP-NETs [19–21].

Taking into account this data, well-differentiated NETs do not seem good candidates for immunotherapy. In contrast, it seems to be more likely that NECs could be a target to checkpoint immunotherapy, given their mutational load and dense immune infiltration [22].

Checkpoint inhibitors utilize antibodies to target the programmed cell death receptor 1 (PD)-1/PD-L1 or cytotoxic T lymphocyte antigen (CTLA)-4 inhibitory axis found on immune cells to lower their threshold for activation and generate a more robust antitumor response. Several PD-1/PD-L1 antibodies are available for clinical use including pembrolizumab, nivolumab, and avelumab as well as ipilimumab for CTLA4 targets [23].

Pembrolizumab, a monoclonal antibody (mAb) targeting PD-1, has been investigated in the phase Ib study KEYNOTE-028. Two hundred-seventeen patients had been evaluated for PD-1 expression and 36% were positive. The trial enrolled 16 and 25 patients with pretreated PD-L1-positive pancreatic and extrapancreatic (nine lungs and seven guts) NETs. Objective responses were observed in 12% of carcinoid cohort and 6% of pancreatic cohorts; SD rates were 60% and 88% in carcinoid and pancreatic cohort, respectively. The 1-year PFS rate was 27% for either subgroups. This study showed higher response rates in tumors that had high mutational burdens as well as microenvironments that were T cell-enriched suggesting potential criteria that will be helpful in predicting eligibility for these treatments [24].

These results have been confirmed by another study that has investigated the efficacy of pembrolizumab (KEYNOTE-158) in a larger cohort of patients (107 patients) with well-differentiated NETs of the lung and gastroenteropancreatic. Sixteen percent of patients had PD-L1-positive tumors. ORR was 3.7% with 4 PR and no complete response. Median PFS was 4.1 months and median OS was 24.2 months [25].

Similar results have been recently reported in a study of 116 patients with well-differentiated G1-G2 gastroenteropancreatic and lung NETs as well as gastroenteropancreatic poorly differentiated NECs treated with spartalizumab (PDR-001), a mAb anti-PD-1. In this study, ORR was 7.4% in well-differentiated NETs and 4.8% in poorly differentiated NEC, to be noted, patients with lung carcinoids had higher ORR (20%). Main grade 3/4 adverse events were abdominal and back pain, anemia, dyspnea, and hypertension. PD-L1 expression was generally low, GEP NEC patients had a higher proportion of PD-L1 expression (43%) [26].

A phase Ib trial investigated the efficacy of toripalimab, an mAb anti PD-1 receptor, in 40 patients with NENs with Ki-67 > 10% progressing to first-line therapy. ORR was 20% (eight partial response and six stable disease) and median disease objective response was 15.2 months [27].

One strategy to increase the percentage of response to immunotherapy is to combine two treatments.

The phase II basket trial (DART trial) explored the combination of ipilimumab and nivolumab in rare tumors. In the NEN cohort, 32 patients had a non-pancreatic NEN, 56% had a NEC. Most common primary sites were gastrointestinal (47%) and lung (19%). The overall ORR was 25%, but in patients with NEC, ORR was 44%. Median OS was 11 months. The most common toxicities were hypothyroidism, fatigue, and nausea [28]. It is currently unknown whether prior

treatment with chemotherapy or peptide receptor radiotherapy or concomitant treatment with TKi may enhance the efficacy of immunotherapy in NETs. Strategies to enhance immune response and efficacy of immunotherapy in NENs are based on modulation of T cells and reverse immunosuppression, in particular the association of two immune checkpoint inhibitors, or the association of immunotherapy with chemotherapy, PRRT, and target therapy is under evaluation in several clinical trials [29]. Checkpoint inhibitors are an exciting option that deserve further investigation.

9.5 Cyclin-Dependent 4/6 Inhibitors

The cyclin-dependent kinases (CDKs) regulating cell cycle progression have been viewed as promising targets for cancer therapy. Palbociclib is an inhibitor of CDK4 and CDK6 approved together with other third-generation CDK4/6 inhibitors (ribociclib and abemaciclib) for the treatment of hormone receptor-positive and HER2-negative breast cancer in combination with either aromatase inhibitors or fulvestrant based on significant improvements in PFS [30]. It shows a potent antiproliferative activity in RB-positive tumor cells in vitro, inducing G1 arrest [31–33] in pNET cell lines overexpressing CDK4 [34].

The phase I trial by Fujivara et al. with abemaciclib in 11 patients with advanced tumors with different primaries found a reduction in tumor size >30% in two patients, one of them with a NET [35].

This encouraging result has not been confirmed by the phase II trial by Grande et al. assessed activity and safety of palbociclib 125 mg 21 of 28 days in 21 patients with advanced or metastatic G1-G2 pancreatic neuroendocrine tumors. All patients received at least one line of previous therapy, and 66% of patients received more than two lines of therapy. The median PFS was only 2.6 months and there were no objective response. Fifty-four percent of patients showed a disease stabilization for more than 6 months. Main toxicities were muscle weakness, neutrophil, and platelet count decrease. No correlation between the clinical outcome and the expression of RB1, Ki-67, and p16 on the tumor tissue was observed [36]. Translational studies correlating palbociclib activity with Ki-67 proliferation index are ongoing.

9.6 Epigenetic Drugs

The low mutation rate observed in NET compared to other tumors suggests that other mechanisms, such as epigenetic changes, could be involved in NET development and progression.

The term epigenetics is referred to external modifications to DNA, which do not alter the DNA sequence but change chromatin structure influencing gene expression and genomic stability. Epigenetic changes are transmitted in cells divisions and consist of DNA methylation and histone modification. Both mechanisms are deregulated in cancer, including NET, and contribute to tumor evolution.

Epigenetic drugs inhibit proteins implicated in the writing, the reading, or the erasing of epigenetic marks such as DNA methylation or post-translational modifications of histones. The main categories of this compounds are the inhibitors of DNA methyltransferases, such as azacitidine and decitabine, and the inhibitors of histone deacetylases [37].

Methylation profiles can be a predictive factor of response to chemotherapeutic agents and of survival, as for example, the methylation of MGMT promoter. MGMT (O6-methylguanine-methyltransferase) is an enzyme of DNA repair, and the methylation of the promoter seems to predict for better response to therapy with alkylating agent as temozolomide in panNET patients [38, 39]. Larger and randomized clinical trials should be conducted to confirm these findings.

Studies in vitro in panNET and small intestine cell lines used DNA methylases inhibitor and histone deacetylases inhibitor, showing results in terms of reducing cell viability and increasing gene expression [40–44]. Interestingly, decitabine increased the expression of SSTR2 and the Ga-DOTATOC uptake in BON1 tumour-bearing

mice, indicating a possible therapy implication in reexpression of somatostatin receptors for PRRT [45, 46].

These drugs are under investigation also in clinical trials, and the efficacy in NETs is under investigation.

A phase II trial with panobinostat, a histone deacetylases inhibitor, has been conducted in 15 patients with metastatic, low-grade NETs. The study was stopped at planned interim analysis based on a Simon two-stage design. There were no radiologic responses, but all patients have a disease stabilization. The median PFS was 9.9 months, and the median OS was 47.3 months. Fatigue (27%), thrombocytopenia (20%), diarrhea (13%), and nausea (13%) were the most common related grade 3 toxicities. The low response rate and the mPFS did not meet the prespecified criteria to open the study to full accrual [46].

A phase I trial with CC90011, a reversible oral inhibitor of the epigenetic target, lysine-specific demethylase 1A (LSD 1) showed in 50 patients (26 with neuroendocrine neoplasms) a complete response in 1 patient and a disease stabilization in 22 patients, with 7 patients with a duration of >4 months(five bronchial and two prostate NENs). Toxicity were thrombocytopenia and neutropenia. Retrospective studies on the epigenetic profile of neuroendocrine tumors would be necessary for future researches and specific treatments. Currently, multiple clinical trials are underway attempting to identify and use biomarkers for clinical use (NCT02630654, NCT02948946).

Given the clinical heterogeneity observed in NETs based on grade, anatomical location, etc., it is imperative that future efforts work toward an improved molecular understanding of NETs and their response to particular treatments.

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Locoregional Therapies of NEN

10

Franco Orsi and Guido Bonomo

10.1 Introduction

Interventional Radiology, also called Interventional Oncology (IO) when applied to the field of oncology, provides several treatment options alternatives, or sometime complementary, to the traditional ones. IO can also provide unique therapies for complex clinical situations, where no or not efficient standard options, are available. Some of the techniques developed within the IO field are nowadays upgraded to standard options and included into the clinical guidelines. It is especially true for hepatic tumors where, both in primary and metastatic disease, locoregional therapies can provide outstanding clinical results with minimal invasiveness.

Also in the field of Neuroendocrine Tumors, IO plays a very important role, in the management of metastatic stages, thanks to the several locoregional treatments, available from its wide armamentarium, ranged from the percutaneous techniques to the intra-arterial ones. Several indications for locoregional therapies of metastatic liver disease, from NET, are reported from the literature, whereas radical tumor ablation, tumor debulking, and hormone release control are the most common, also because of the increasing response to the medical therapy [1].

30–50% of patients with PNETs syndromes and 98–100% of patients with carcinoid syndrome due to a malignant GI-NET (carcinoids) have liver metastases at presentation [2–7]. These patients are rarely cured surgically and thus are candidates for various forms of liver-directed therapies, particularly when the primary tumor is resected [8–14]. In those clinical settings, liver-directed therapies allow for tumor debulking and/or hormone release control. However, treatment strategy is usually based on a multifactorial evaluation, mainly related to the general clinical conditions, the therapeutic options available, and, most importantly, on the histopathological tumor characteristics. According to its complexity, treatment strategy in NET setting requires a dedicated *multidisciplinary* team and very often an individualized approach [15]. The range of effective treatment options for NET liver metastases includes surgery (only limited to a small percentage of patients), medical therapies, interventional radiology, and nuclear medicine treatments [16, 17]; however, there is still a lack of evidence-based recommendations, regarding the ideal sequence of those treatments in these patients.

Liver surgery is reported as the treatment of choice for liver metastatic disease, with 5-year survival rates >70% in patients amenable to resection [18–20], but resection is often impossible due to the extent of the disease.

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Rationale for intra-arterial liver-directed therapies, such as arterial embolization (TAE), arterial chemoembolization (TACE), or arterial radioembolization (TARE), is based on the evidence that NET liver metastases are usually hypervascular and primarily supplied by branches of the hepatic artery, whereas normal liver parenchyma is fed by the portal vein. For that reason, the arterial route to the tumor is widely accepted for affecting liver metastatic deposits, by shutting down the blood flow to the tumor, alone (TAE) or with the coadministration of chemotherapeutic agents (TACE), or with radio-emitted beads (TARE) [9–12, 21, 22].

Mechanism of action for thermal ablation (TA) techniques is based on the sensitivity of any biological tissue to the high temperature. Cell death during exposure to heat is exponential and dependent on the temperature and length of exposure [23].

Different techniques are available for providing thermal damage to the cancer tissues, but Radiofrequency (RF) and Microwaves (MW) are the most common in clinical practice, for treating liver nodules, both primary and metastatic tumors.

Indications, results, and main technical aspects of IO liver-directed therapies, in liver metastatic NET, will be described within this chapter.

10.2 Intra-Arterial Therapies

More than 95% of liver metastases from NET are hyper-enhancing during the arterial phase on contrast-enhanced ultrasonography (CEUS), meanwhile on CT or MR, they could be hypointense or hypoattenuating during the same vascular phase, mostly because of a lower temporal resolution compared to CEUS [24]. However, the basic concept behind intra-arterial treatment strategy is that liver metastases from NET are mostly fed by the arterial system as any other liver tumor, whereas normal adjacent liver is mainly supplied by the portal venous system [25].

Embolization (TAE), chemoembolization (TACE), and radioembolization (TARE) have been shown to achieve objectives responses, tumor markers decrease, and control of tumor-related symptoms, in those patients with unresectable liver metastases and/or specific carcinoid symptoms, such as diarrhea, hypertension, abdominal pain, and flushing [26]. However, there is still no clear evidence, in terms of imaging response, symptomatic response, or impact on survival, about the superiority of one out of these three approaches [13, 17]. Liver-predominant disease and/or major uncontrolled symptoms in nonsurgical candidate patients are the two most common indications for hepatic intra-arterial therapies, meanwhile extrahepatic stable metastatic tumor and/or the presence of stable primary tumor are not considered absolute contraindications [4, 9].

10.2.1 Embolization/ Chemoembolization

Transarterial Embolization (TAE), also called "bland embolization," refers to the selective distal arterial embolization, with the aim of occluding small arteries, feeding the liver metastases and consequently tumor ischemia and necrosis [27]. During the last few years, more efficient embolic material has been developed, in order to achieve a better and more distal arterial embolization, with the specific goal to improve local results, meanwhile reducing the toxicity of surrounding healthy liver tissue. Small and round-shaped beads, with size ranged between 100 and 40 microns in diameter, could better reach a deeper level of tumor embolization, if compared with bigger and with irregular shaped embolics [28, 29]. The adoption of super-selective techniques, thanks to the more and more performing micro-catheters and the improved integrated imaging for guiding the intra-arterial procedures, may allow for really efficient selective embolization of liver tumors, mainly if hypervascular, such as HCC and metastatic NET (Fig. 10.1).

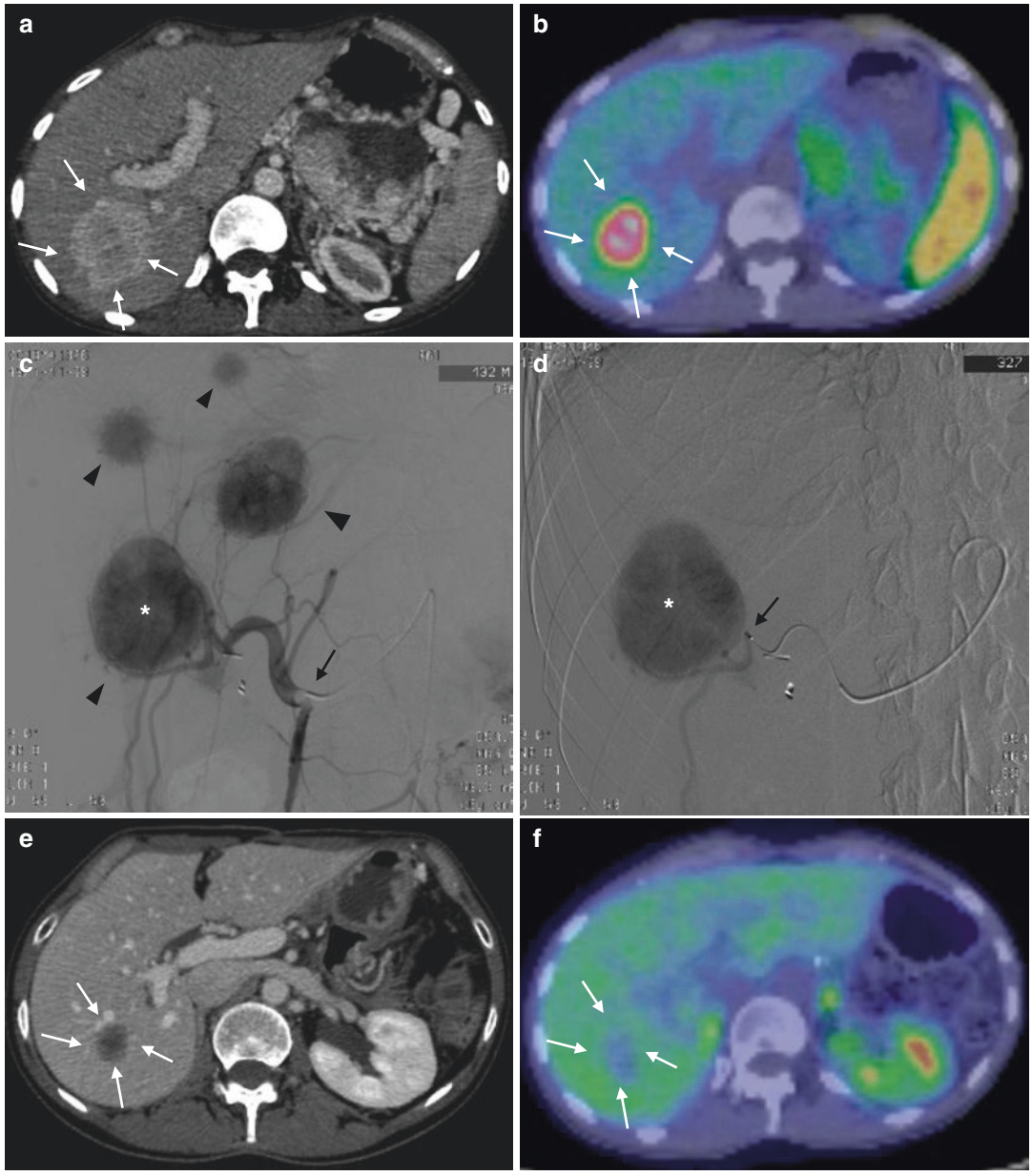

Fig. 10.1 NET G2 (Ki67 10%) of the pancreatic tail, with synchronous metastases on the right liver lobe; (**a**) CT shows the largest nodule, sited in S6 (arrows), and confirmed on Ga^{68}PET/CT (**b**). On NET-MDTB (IEO) was defined the indication to TAE, for control liver disease, followed by surgical resection of primary pancreatic tumor. Multiple sequential TAE sessions were super-selectively performed; (**c**) common hepatic artery (arrow) angiogram clearly shows how the liver lesions (arrowheads) are hyper-vascular; (**d**) super-selective angiography, performed with the micro-catheter tip (arrow) into the feeder of the lesion sited in S6 (*), confirms the right position for delivering the microbeads. After multiple sessions of TAE, patient underwent resection of the pancreatic tail. 24 months after last session of TAE, CT (**e**) shows sustained objective response at the level of S6, where the treated lesion is no more enhancing (arrows); (**f**) Ga^{68}PET/CT shows a photopenic area (arrows) at the same level

Transarterial chemoembolization (TACE) is based on the association of intra-arterial chemotherapy administration together with embolics. The rationale behind TACE is to increase the intra-tumor concentration of cytotoxic drugs, such as doxorubicin or epirubicin, with no or very few systemic side effects, if compared to the standard systemic chemotherapy, meanwhile concurrent or following embolization will reduce drug washout from the tumor, compared to drug infusion alone [30]. During conventional TACE (cTACE), the drug is emulsified with Lipiodol® (Guerbet) and selectively delivered into the tumor. In DEB-TACE, the drug is concentrated within small beads (DEB = Drug Eluting Beads), which will shut down the blood flow within the tumor and will elute the chemotherapeutic agent into the tumor microvasculature. The advantage of using eluting beads is mainly based on pharmacokinetic studies in HCC patients, which revealed that DEB-TACE resulted in a higher intra-tumor drug concentrations and a lower systemic exposure than TACE [31, 32].

Both TAE and TACE are usually performed for palliative treatment of liver-predominant disease, which is not surgically resectable, in order to reduce the hepatic tumor mass, and 25–85% of patients have an objective tumor response, with a mean response duration of 6–45 months. This approach has been particularly considered in patients with hepatic symptoms or refractory malignant F-NET syndromes [9–12, 21, 22]. In general, TACE/TAE result in a symptomatic response in 50–100% of patients, and numerous series as well as case reports have documented their control of symptoms in patients with both carcinoid syndrome and F-pNET syndromes [9–12, 21, 22, 33–39].

To date, no randomized study has sought to compare the efficacy of either embolization or chemoembolization in NET G3. CNCCN, NANETS, and ENETS guidelines include both TAE and TACE, within the list of local therapies for symptomatic and/or progressive NET liver metastases, on the basis of level IIB-3 evidence, but they offer no recommendation regarding the different techniques [13, 40–43]. No statistical difference in clinical efficacy of TAE versus TACE in the treatment of liver metastases from well-differentiated non-pNET in a prospective study has been reported [44]. According to this report, there are no data supporting the hypothesis of an additive clinical advantage of intra-arterial administration of chemotherapeutic agent compared to the arterial embolization alone. Meanwhile, some studies reported superior survival and/or outcomes of TAE compared to TACE [22, 45, 46]; two retrospective series have reported higher biliary complication rate after DEB-TACE [47, 48] compared to TAE, but there are still no definitive data regarding the superiority between cTACE and TAE.

10.2.2 Radioembolization

Radioembolization (TARE) delivers targeted radiation therapy to unresectable hepatic malignancies, by the injection of the β-emitting isotope Yttrium-90 (90Y) through micro-catheter, which is permanently bound to biocompatible, nonbiodegradable microspheres (glass or resin), into the arterial supply of the liver, in order to reach tumor microvasculature. It results in delivering doses of ionizing radiation, above 120 Gy, into the tumor compartment, with no intolerable toxicity to the healthy liver parenchyma [49, 50]. TARE demonstrated a close correlation between delivered dose to the tumor and local response [51]. The dose of the radioactive microspheres has also to be adapted to the lung shunting fraction, when present, and assessed before TARE by scintigraphy, obtained after intra-arterial infusion of ^{99}Tc-macroaggregated albumin (highest tolerable dose of the lung <30Gy).

Several authors reported data on the efficacy of TARE in the biological control of the disease and in the reduction of symptoms [52–55]. Interesting results about its feasibility and impact on survival compared to other locoregional therapies showed its possible application in particular series of patients [56–61]. One of the critical approaches in this kind of treatment is the calculation of the optimal dose that on the contrary is a

well known and investigated topic in HCC patients [62].

The most common side effects of TARE are abdominal pain, nausea, fever, and fatigue that last from 1 week to 1 month. Various complications have been described after or during the procedure of TARE. They are mainly caused by the delivery of radioactive beads to the normal liver parenchyma or to extrahepatic sites, such as the gastrointestinal system (e.g., gastroduodenal ulceration, radiation gastritis, cholecystitis, or pancreatitis), the abdominal wall (i.e., radiation dermatitis), and the lungs (i.e., radiation pneumonitis) [63]. Treatment toxicity is significantly related to the radiation activity delivered to the healthy liver, which is the main limiting factor making TARE less repeatable than TAE/TACE, because of the risk of irreversible damage of liver parenchyma. In 2008, Sangro et al. [64] described toxicity using the term "RadioEmbolization-Induced Liver Disease" (REILD), which is considered a form of sinusoidal obstructive syndrome (SOS) and includes ascites, weight gain, liver function impairment, and elevation of bilirubin levels. Furthermore, late changes in liver size and appearance, following TARE, have been described, with radiation injury potentially developing into sinusoidal congestion and portal hypertension [65, 66]. In a recent study, Yu-Kai et al. [67] evaluated the long-term (>2 years after treatment) hepatotoxicity of radioembolization in patients with mNETs, by reviewing imaging and laboratory findings and determining their correlation with clinical symptoms. They concluded that whole-liver 90Y TARE for patients with neuroendocrine tumors results in long-term imaging findings of cirrhosis-like morphology and portal hypertension in >50% of treated patients, with signs of hepatic decompensation that are more pronounced than those in patients treated with unilobar 90Y radioembolization. However, a majority of these patients will remain clinically asymptomatic. This evidence is crucial, as patients with mNETs have longer life expectancies than patients with other unresectable hepatic metastases [68]. Although this practice may result in an improved tumor response rate and overall survival, there may conceivably be manifestations of long-term hepatotoxicity from ^{90}Y. In the setting of a slowly progressive disease that remains localized into the liver for a long period of time, TARE should be considered in a very well selected patients and should be carried out with super-selective technique only (Fig. 10.2), in order to reduce the risks of early and late complications (i.e., REILD and late hepatotoxicity). TAE/TACE and TARE should not be considered competing therapies, but complementary tools. Many patients, according to their individual tumor and healthy liver characteristics, could be candidates for either TAE/TACE or TARE.

10.3 Percutaneous Liver Ablation

Radical resection is considered as the only curative treatment for liver metastases from NET, allowing for survival improvement [19, 69] and also recommended for tumor debulking in hormone-active metastases, for palliative purposes [70–72]. The overall survival after hepatic resection is 46–86% at 5 years and 35–79% at 10 years [19, 73, 74], but complete resection is achieved in only 20–57% with a local recurrence rate of 94% at 5 years [75]. Due to that high metastatic recurrence rate, repeated local treatments during patient's life might be required. Therefore, minimally invasive treatment, such as TA for liver metastatic disease, may play an important clinical role as an interesting tissue-sparing treatment, alternative to the conventional surgery, mainly for small tumor deposits. Repeatability and low invasiveness, together with a very high success rate, are the most relevant features of TA techniques for liver tumors.

TA refers to the application of high temperature to a tissue with the aim to effect tumor cell death. There are different methods and different energies for delivering the heat into the tumor, where percutaneous radiofrequency ablation (RFA) and microwaves ablation (MWA) are the most common and used in clinical practice.

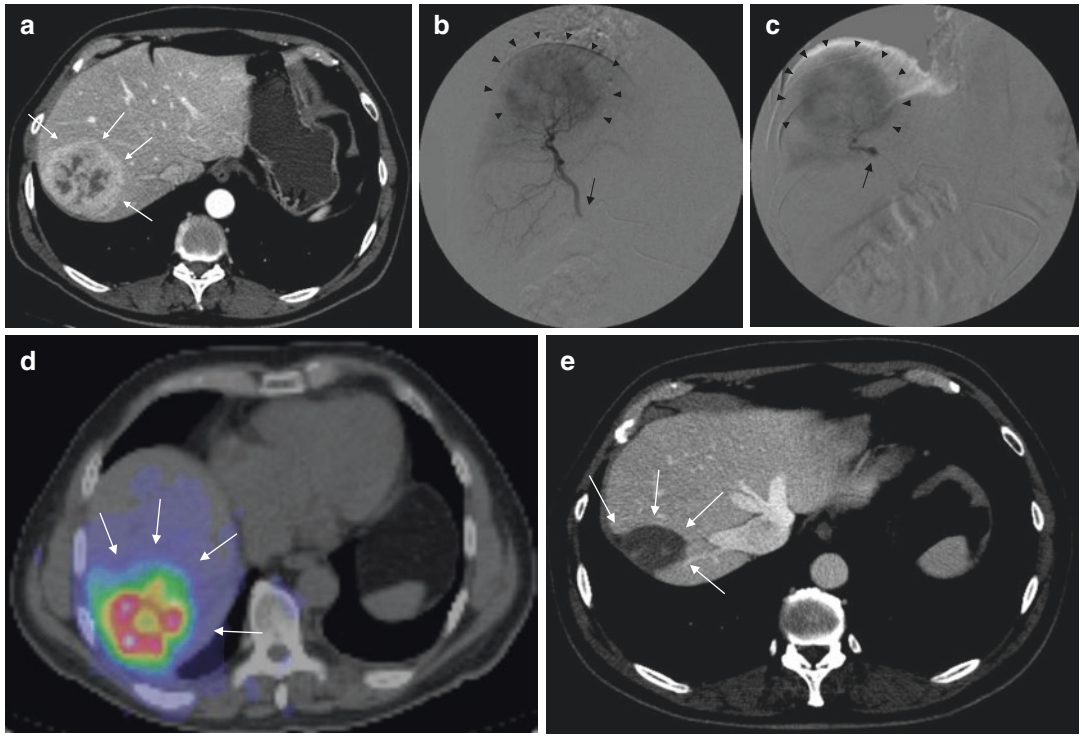

Fig. 10.2 Unknown primary site NET G1 (Ki67 1%), with single liver metastasis of right lobe. As the liver lesion was growing, the NET MDTB (IEO) put indication for local treatment. (**a**) CT scan in arterial phase shows the highly enhancing liver metastasis in S7 (arrows) (**a**), and according to its histology, super-selective TARE was indicated. (**b**) Angiography, obtained with the micro-catheter (arrow) in the right hepatic artery shows the hyper-enhancing tumor (arrowheads). (**c**) Super-selective angiogram from the tumor (arrowheads) feeder: the micro-catheter tip (arrow) is sited close to the tumor, distally to some lateral branches, in order reduce the healthy liver involvement during Y90-micro-particles injection. According to the low percentage of β + positron emission of the ^{90}Y, an abdominal PET/CT (**d**) was performed the day after TARE, in order to evaluate ^{90}Y-micro-particles distribution (arrows). The exam also shows no healthy liver was involved during the treatment. (**e**) CT scans, the last one (**d**) performed 4 years later showed a "scar" (arrows) in the site of the lesion, with no evidence of active pathologic tissue

The area affected by the heat is called "ablation zone," and its size and shape are dependent by many factors, some of them closely related to the tissue characteristics and some others to specific features of the different TA technologies. However, the size of the ablation zone should cover the whole tumor volume, including a peripheral safety margin (0.5–1 cm) of healthy liver [65]. For large lesions or for irregular shapes, multiple overlapping ablation zones might be necessary in order to achieve a complete tumor eradication [76–78].

TA can be performed percutaneously, laparoscopically, and during open surgery, where the imaging-guided percutaneous approach is the most common technique. Ultrasound and CT are the more common imaging modalities used, in clinical practice, for guiding percutaneous liver TA. The first modality has the unique feature to provide real-time imaging, which is essential for a safe needle penetration, from the skin surface to the target. The advantage of using CT is, first of all, the panoramic view and the higher spatial resolution. It is more and more emerging the need of both the two guidance modalities, during the same session, also integrated within the newer navigational tools [79], for a safer and more precise procedure. Contrast enhancement CT is also

essential for providing data regarding the outcome of the ablation, when it is performed at the end of the procedure. Clinical indications for liver ablation in liver metastases from NET are still not well defined, and patients have to be always discussed within a dedicated MDTB, meanwhile technical indications are well established and are mainly related to the tumor size, shape, and site. Generally speaking, acceptable size criteria for ablation may differ, according to the different techniques and devices used, ranging from 3 to 5 cm of largest diameter. However, it is well known that local recurrence and treatment failure are higher with larger lesions due to incomplete ablation at the periphery [80]. Ablation margin is actually reported as an independent factor affecting the local recurrence after laparoscopic RFA of liver NET metastases [81]. Hence, the precise placement of the RFA needle, which is deeply affected by the imaging modality used for guidance and ablation monitoring, has to be considered critical for achieving as large margins as possible, in order to obtain local tumor control.

10.3.1 Radiofrequency Ablation

In RFA, an alternating current is flowing between the uninsulated probe tip and a dispersive skin electrode-pad (unipolar) or between the different electrodes within one or multiple probes (multipolar). The current is converted into tissue heating by friction of the ions adjacent to the uninsulated tip of the RFA electrode [76, 82, 83]. RFA is the most frequently used ablation technique performed in this clinical setting, often in combination with surgery, especially to remove isolated metastases too deeply located for a safe resection. Several criteria regarding the possible indication for liver RFA in NET have been proposed and are mainly based on the number of lesions, size, and proximity to vital structures. Besides the use of RFA as an antitumor treatment, a number of studies have reported enhanced symptomatic control of functional NETs after its use [4, 9–11, 13, 21, 84–88]. In a series of 129 patients undergoing 177 sessions of laparoscopic RFA, for a total number of 770 liver metastases from NET, authors reported a 5- and 10-year overall survivals of 76% and 59%, respectively, and a median OS of 125 months, at a median follow-up of 73 months. Limitations of the technique include the poor efficacy of ablation in large tumors, where tumor size remains an independent predictor of poor overall survival [89].

10.3.2 Microwave Ablation

MWA is based on an oscillating electromagnetic field (0.9–2.450 GHz), generated by an antenna/needle, which induces water dipoles to continuously realign with the magnetic field. This kinetic energy induces heat in the tissue adjacent to the antenna, exposed at the magnetic field [90].

Compared to RFA, MW ablation is usually faster and less sensitive to the heat-sink effect, but its use is less common because it is more recently introduced in the clinical practice than RFA. Percutaneous application of MWA is more common in clinical practice, than during laparoscopy or open surgery, but due to the faster efficacy than RFA in destroying the tumor tissue, its use during open surgery is increasing, allowing liver tumors eradication, whereas resection only is not feasible or too invasive (Fig. 10.3).

A recent retrospective study comparing MWA, resection, and resection plus MWA, in patients affected by liver metastases from NET, reported a mean overall recurrence-free survival of 21.2 months (0 to 189 months), with no statistically significant difference when comparing patients treated with MWA only versus those who underwent surgical resection with or without MWA [91]. Moreover, patients treated with MWA only had a similar overall survival of 57 months, as patients undergoing resection with or without MWA. After MWA, the length of hospital stay is also reported to be significantly shorter than after surgery, with number of complications and their severity significantly reduced if compared with resection.

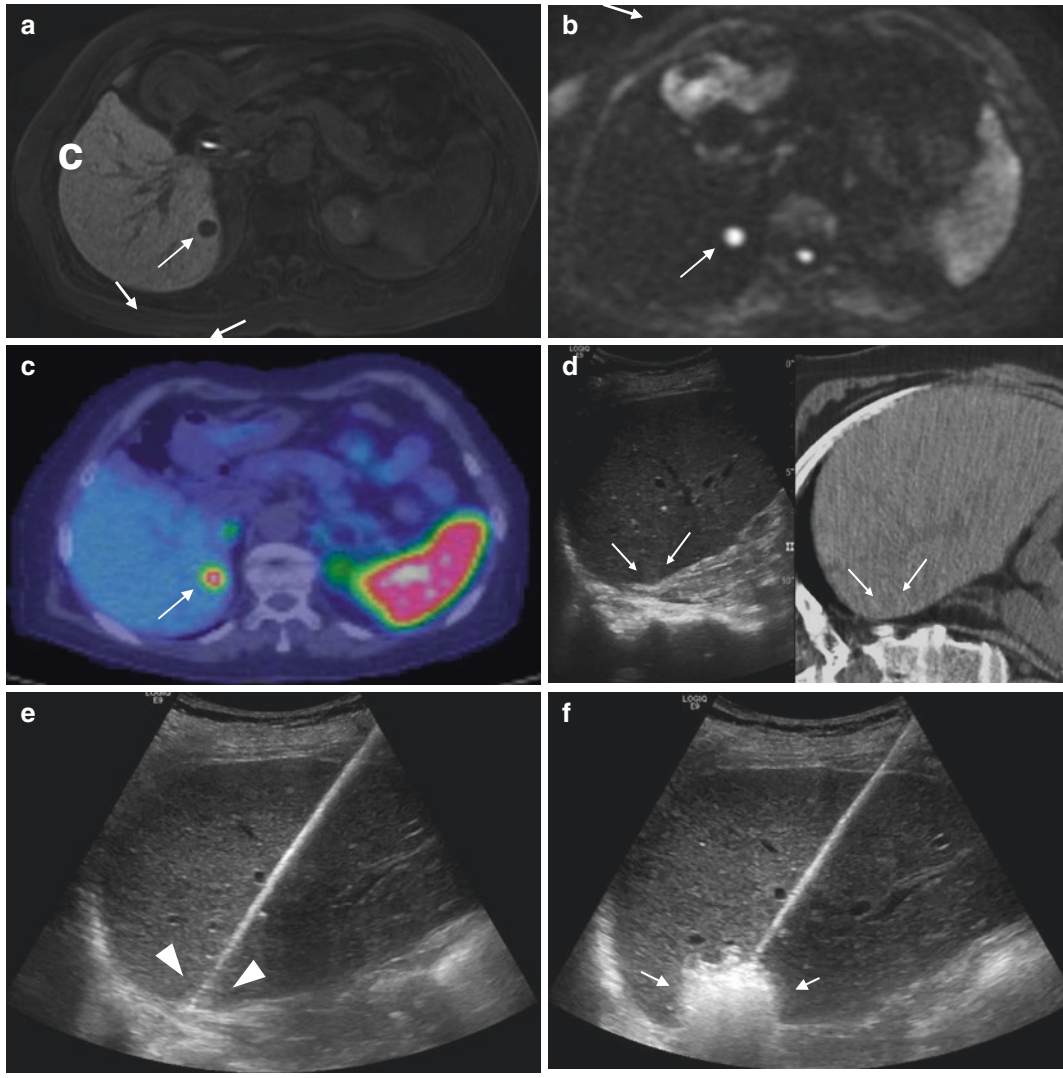

Fig. 10.3 Ileal NET G1 (Ki-67 1%) with two synchronous small liver metastases in S5 and S6. Patient previously received right emicolectomy, including the last ileal loop, and was subsequently treated with somatostatin analogues (SSAs). After 6 months, patient reported intolerance to SSAs. NET MDTB (IEO) puts indication for liver local treatment by percutaneous thermal ablation. Pretreatment-enhanced MRI clearly shows the lesion in S6 (arrow) both in T1w excretory phase (**a**) and in DWI (**b**); the lesion is also well defined on ^{68}GaPET/CT (**c**). Percutaneous MWA was performed to both the two lesions (S5 and S6) by using fusion-imaging technique (**d**) for a better precision; the lesion in S6 (arrows) is visible both on US and CT scan. (**e**) On US imaging, the tip of MWA-antenna located into the lesion (arrowheads); (**f**) as the result of the heat, tissue "vaporization" is visible at US as a white cloud (arrows). (**g**) CT performed 18 months after ablation clearly shows the hypodense scar of both the two treated liver metastases in S5 (arrowhead) and S6 (arrow)

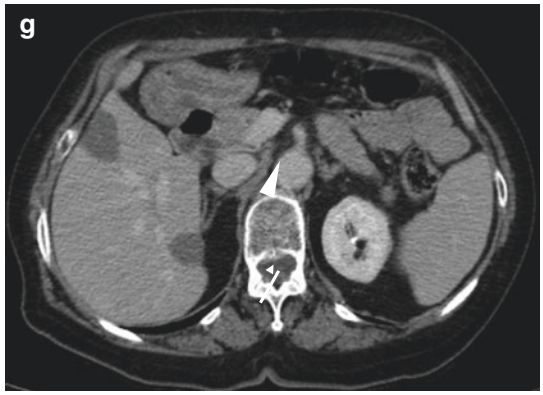

Fig. 10.3 (continued)

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Radioreceptor Therapy

11

Alice Lorenzoni, Marco Maccauro, and Ettore Seregni

11.1 Introduction

Peptide receptor radionuclide therapy (PRRT) is a highly effective anticancer treatment modality for patients with non-resectable, metastasized neuroendocrine tumors (NETs) that express highly and frequently somatostatin receptors (SSTRs), mainly subtypes 2 and 5. PRRT is an attractive therapy option for delivering cytotoxic radiation to tumor cells through specific binding of a radiolabeled peptide to a molecular target, representing the most successful paradigm of theranostics approach, in which the same (or very similar) agents are used for both diagnostic and therapeutic purposes. PRRT represents, in fact, the step following the initial development of the diagnostic technique for in vivo localization of NETs using the radiolabeled somatostatin analogue ^{111}In-pentetreotide. The logical sequel to somatostatin receptor imaging (SRI) for diagnostic purposes was to use the same receptor-binding concept for treatment. In 1992, using the specific physical characteristics of the Auger and conversion electrons of ^{111}In, the first NET patient with glucagonoma was successfully treated with high doses of ^{111}In-pentetreotide [1].

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After a few years of PRRT experience with ^{111}In-pentetreotide [2], it became clear that other radionuclides might be better suited for the regimen than ^{111}In because its short tissue range resulted in relatively modest tumor shrinkage. In addition, DOTA-chelated peptides, which could be more easily labeled with radioactive metals, also started becoming available. In the late 1990s and early 2000s, Yttrium-90 and Lutetium-17 coupled to various somatostatin analogues have been proposed for PRRT. From the early 90s, the clinical evidence of antineoplastic efficacy of PRRT was deriving only from limited, nonrandomized and I-II phase studies [3]. Recently, the randomized phase III NETTER-1 trial unequivocally demonstrated the efficacy of PRRT [4]. Consequently, ^{177}Luoxodotreotide (also known as ^{177}Lu-DOTA-TATE and Lutathera®) received marketing authorization in patients with metastatic and progressive midgut G1-G2 NET. Despite the great advances that PRRT represents in the management of NET, ^{177}Lu-DOTA-TATE is currently the only approved peptide-based radiotherapeutic agent. It is expected that approval of ^{177}Lu-DOTATOC (^{177}Lu-edotreotide) will follow the completion of the COMPETE-NCT03049189 phase III trial comparing ^{177}Lu-DOTA-TOC with the mTOR inhibitor everolimus in patients with inoperable, progressive NETs. These and other trials should further precise the position of PRRT in the

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current clinical algorithm with regard to other systemic therapies. PRRT are currently mainly performed with the medium-energy β-emitter ^{177}Lu, while α emitters (e.g., ^{225}Ac/^{213}Bi) are increasingly studied in various clinical applications. The use of alternative theranostic pairs of radionuclides, such as radioisotopes of scandium (43/44/47Sc) and terbium (149/152/155/161Tb), might open novel theranostic applications. An important evolution step is the use of radiolabeled SSTR antagonists instead of agonists. A number of clinical and preclinical studies suggest significant improvement in diagnostic sensitivity and therapeutic efficacy of the antagonists.

Furthermore, combining PRRT with other agents to achieve maximum benefits from the internal radiation therapy, while sparing nontarget organs from radiation toxicity, is an attractive perspective. Several mechanisms, to increase tumor perfusion, determine SSTR upregulation, and induce radiosensitization, may be used. Finally, more precise image-based dosimetry, to establish dose-effect relationship, and development of biomarkers, such as multitranscript gene blood assays, are expected to improve the prediction of outcome following PRRT.

11.2 Radiopharmaceuticals

Several radiolabeled SSAs have been proposed for PRRT, and they are different in terms of radionuclide, somatostatin analogue, and chelator.

Three types of radiation may be used in PRRT: β-particles (in particular ^{177}Lu and ^{90}Y), α-particles, and Auger electrons (Table 11.1).

Table 11.1 Physical characteristics of radionuclides for PRRT

Radioisotope	Type of emission	Max energy (MeV)	Half-life
^{111}In	γ, Auger electron	0.61 (Au)	2.8 days
^{177}Lu	β, y	0.49 (β)	6.68 days
^{90}Y	β	2.27	2.67 days
^{213}Bi	α	8.32	45.7 min
^{225}Ac	α	6.83	10 days

Beta particles have long range in tissues (0.05–12 mm) so that neighboring cells around the targeted cell are also irradiated (cross-fire effect). This is considered ideal for targeting large tumors with heterogeneous target distribution. In contrast, alpha particles (e.g., ^{213}Bi and ^{225}Ac) have a very short range in tissues (20–100 μm), irradiating volumes with cellular dimensions, therefore, sparing normal surrounding tissues from cytotoxic radiation. Their linear energy transfer (LET) is much higher compared to β-particles (50–230 vs. 0.2 keV/μm), which makes alpha radiation far more cytotoxic. Finally, Auger emitters (e.g., ^{111}In) have a very short range in tissue (<20 μm, subcellular dimensions) and intermediate LET (4–25 keV/μm).

Beta emitters, in particular Yttrium-90 and Lutetium-177, are currently used for PRRT. ^{90}Y electrons are highly energetic (Emax 2.27 MeV, penetration range max 11 mm, half-life 64 h) and penetrating, leading to better crossfire through the tumor, which is particularly valuable in larger tumors and when heterogeneous receptor and/or activity distribution exists. The shorter half-life of ^{90}Y allows a higher dose rate. ^{177}Lu, on the other hand, has lower energy and smaller particle range, allowing a better absorption probably in smaller tumors, also has less toxicity to bone marrow and kidney. In fact, ^{177}Lu is a medium-energy β-emitter (Emax 0.498 MeV, penetration range max 1.7 mm, half-life 162 h) and a low-energy γ-rays emitter (208 keV and 113 keV with 10% and 6% abundance, respectively), which allows SRI and subsequently to assess an internal dosimetry with the same therapeutic radiopharmaceutic agent [5]. Peptide-based radiotherapeutics require a chelator that stably chelates the radiometal in vivo, in order to exclude deposition of free radiometal in normal tissues. DOTA is the most used chelator agent due to its high affinity for radiometals such as Yttrium-90 and Lutetium-177. Both Tyr3-octreotide (TOC) and Tyr3-octreotate (TATE) are well-experimented somatostatin analogues in clinical trials. The somatostatin analogue TATE differs from TOC only in that the C-terminal threoninol is replaced with

threonine, resulting in a higher affinity for the somatostatin receptor subtype 2 [6].

11.3 Clinical Consideration

Candidates for therapy are selected based on somatostatin receptor scintigraphy or ^{68}Gallium-labeled synthetic SST analogues PET imaging. Such images should indicate an adequate uptake (at least equal to the uptake of normal liver) as evidence of adequate expression of targetable somatostatin receptors [7]. SRI is the most accurate noninvasive method to identify and confirm the overexpression of functioning SSTR. Other methods like immunohistochemistry, which provides similar information at the time of biopsy, are not practical from a clinical point of view. The in vivo use of functional SRI allows the simultaneous evaluation of the receptor density and the internalization capacity in all lesions with a single functional imaging approach. Certain tumors, such as the majority of highly malignant and high grade (Ki-67 > 55%) NETs, do not express adequate numbers of detectable somatostatin receptors. The success of PRRT is also influenced by the site of primary tumor and tumor burden. Patients with high tumor load and with massive liver involvement have lower chance to respond to PRRT [8].

Patients with poor performance status (e.g., Karnofsky score < 50) are not ideal candidates for PRRT even if unexpected and favorable responses are not rare in these clinically advanced patients. Exclusion criteria are also renal function, bone marrow, and impairment. Patient eligibility criteria and contraindications for PRRT are summarized in Table 11.2. The practical consideration of PRRT in patients with advanced, non-resectable NET should include the goal of therapy: carcinoid syndrome control resistant to somatostatin analogues, reduction of progressive tumor mass, or neoadjuvant treatment before surgery. Furthermore, the optimal sequence for using PRRT, chemotherapy, everolimus, and sunitinib will remain to be established.

Table 11.2 Patient eligibility criteria and contraindications for PRRT

Indication	
Tumor characteristics	NET G1-G2 NET G3 is considered (further data needed)
Disease stage	Inoperable/metastatic
SSTR expression	Tumor uptake on diagnostic SSTR imaging at least equal to the uptake of normal liver
Performance status	Karnofsky performance status >50%
Contraindication	
Child-bearing	Pregnancy or ongoing lactation
Kidney function	Severe renal impairment: Creatinine clearance <30 mL/min
Bone marrow	Impaired hematologic function: Hb < 5 mmol/L (8 g/dL); PLT < 75 × 10 9/L; WBC < 2 × 10 9/L Previous external beam radiation therapy involving more than 25% of bone marrow
Liver function	Severe hepatic impairment: Total bilirubin >3 × ULN; or both albumin <25 g/L and prothrombin time increased >1.5 × ULN
Heart	Severe cardiac impairment: New York Heart Association grade III or IV

11.4 PRRT Administration

^{90}Y/^{177}Lu-DOTATOC or ^{177}Lu-DOTATATE radiopharmaceuticals are systemically delivered in fractioned sequential cycles (generally four to six) every 6-9 weeks. The rhythm of administration is based on the time that has been determined as necessary to recover from potential hematological toxicity. The administered activities, in clinical trials, range from 2.8 to 3.7 GBq (80–100 mCi) for ^{90}Y-labeled SSAs and 5.5–7.4 GBq (150–200 mCi) for ^{177}Lu-labeled SSAs [9–11]. The recommended ^{177}Lu-DOTA-TATE (Lutathera®) acitivity is 7.4 GBq every 8 weeks for a total of four administrations [4].

The cumulative activity, fractionated in multiple cycles, is able to irradiate the tumor more efficiently, than single dose, without surpassing the conventional 25- to 27-Gy absorbed dose threshold to the kidneys, which are the dose-limiting organs. The biologic effective dose (BED) as opposed to the absorbed dose provides a dose threshold value that is slightly higher.

To reduce the renal dose of irradiation, patients are infused with a concomitant intravenous solution of positively charged amino acids (lysine and/or arginine), which are able to inhibit in a competitive manner the radiopeptide resorption in the nephron proximal tubuli.

The radiopeptide is intravenously administered slowly over 20–30 min. Mild adverse events may be experienced during the administration, in particular gastrointestinal symptoms, such as a slight nausea, and occasionally, vomiting. These symptoms may be related to the amino acids coadministration, but are controlled with appropriate medication [4].

11.4.1 Side Effects of PRRT

PRRT is generally well tolerated. Acute side effects include nausea and, more rarely, vomiting that are usually mild and self-limiting. In the majority of cases, these effects are attributable to amino acid infusion and can be effectively treated with antiemetics [12]. Other common adverse events include fatigue or asthenia, abdominal pain, and diarrhea, generally of mild entity. A severe but rare complication (incidence around 1% of cases) is a carcinoid crisis related to a massive release of biologically active amine or peptides [13]. This crisis develops shortly after infusion and, in any cases, within 24–48 h after the first radiopharmaceutical administration and requires proper clinical management. Another subacute adverse event is increased hair loss [10]. This transitory and mild side effect is observed in about one half of patients treated with ^{177}Lu-DOTATATE. Bone marrow suppression, occurring within 4–6 weeks after therapy, is usually mild and reversible. Severe hematological toxicities (WHO grade 3 or 4) have been reported in about 13% and 4% of patients treated with ^{90}Y-DOTATOC and ^{177}Lu-DOTATATE, respectively [10, 14–16].

Long-term serious side effects of PRRT are renal failure or myelodysplastic syndrome (MDS)/leukemia. With advances in expertise and knowledge about PRRT, cases of severe, end-stage renal damage are currently very rare. However, loss of kidney function can occur after PRRT, with a creatinine clearance loss of about 4% per year for ^{177}Lu-octreotate and 7% per year for ^{90Y}-DOTATOC [17]. Preexisting risk factors such as poorly controlled diabetes or hypertension seem to be correlated with more persistent renal damage. Serious side effects of PRRT on the bone marrow, such as MDS or leukemia, were reported by various groups. The frequency of MDS seems higher after ^{177}Lu-octreotate than after ^{90}Y-DOTATOC, but also in analyses with long patient follow-up, it does not exceed 2% of patients [3]. It's to be noted, however, that different factors related either to antineoplastic treatments (previous chemo or radiotherapy) or tumor evolution (bone marrow involvement) can affect the development of MDS and, in some cases, it is quite impossible to relate the MDS to PRRT.

11.4.2 Efficacy of PRRT in GEP-NETs

11.4.2.1 ^{90}Y-Labeled Somatostatin Analogues

^{90}Y-DOTATOC was evaluated in several phase I and phase II studies. Differences in cycle doses and administered cumulative dose, as well as differences in patient characteristics (included tumor types, patient performance status) make it virtually impossible to compare these studies. The reported objective responses range from 4% to 44%. Different studies report median progression-free survival (PFS) varying from 17 to 29 months and median overall survival (OS) from 22 to 37 months [18]. The evaluation of results of many trails using PRRT indices that treatment in a phase of early progression rather than a wait-and-watch approach was more efficacy. In fact, the assessment of the objective response according to the basal status indicated

that individuals stable at baseline demonstrated a better outcome than individuals with progressive disease. Overall, it was apparent that PRRT treatment in advanced stage disease was substantially less effective.

11.4.2.2 ^{177}Lu-Labeled Somatostatin Analogues

^{177}Lu-DOTATATE is currently the most widely used radiopeptide for PRRT. In a comparison study, ^{177}Lu-DOTATATE demonstrated a significantly longer tumor residence time than^{177}Lu-DOTATOC, leading to a higher absorbed tumor dose, with a comparable uptake in the kidneys, spleen, and liver. In several studies, ^{177}Lu-DOTATATE has demonstrated similar efficacy as compared with ^{90}Y-DOTATOC, while having a more favorable toxicity profile, particularly in terms of hematological and renal toxicity. The initial report of using ^{177}Lu DOTATATE was published by Kwekkeboom et al. in 2003 [19]. This study consists of 35 patients with gastroenteropancreatic (GEP)-NETs, all patients treated with 3.7, 5.6, or 7.4 GBq of ^{177}Lu-octreotate, up to a final cumulative dose of 22.2–29.6 GBq, with complete and partial responses in 38%. In the next study, the same group analyzed responses to ^{177}Lu-DOTATATE therapy according to tumor type at 3 months after the last therapy cycle in 310 patients [20]. The overall objective response rate (ORR) was 46%. Stable disease was noted in 16% of patients. Prognostic factors for predicting tumor remission were high uptake on diagnostic SRI and a Karnofsky performance score of over 70. The most important information was the impact of PRRT on survival, with a median OS over 48 months and a median PFS of 33 months. A direct comparison with data obtained from similar patients showed a substantial 40-month to 72-month survival benefit for PRRT-treated subjects. A categorization of overall response rate indicated that pancreatic NETs tended to respond better than other GEP- NETs, although functioning tumors (e.g., pancreatic gastrinomas) tended to relapse in a shorter interval (median time to progression 20 months vs. >36 in the remaining GEP-NETs). In a recent meta-analysis [21] which considered 473 NET patients submitted to ^{177}Lu-DOTATATE, ORR varied from 18% to 44%, with an average disease control rate of about 80%. Furthermore, PFS in patients with advanced and progressive NETs was about 36 months. Although these data are not derived from robust prospective-randomized phase III trials, this substantial survival difference probability reflects a real impact of PRRT as a very efficacy therapeutic approach in advanced non-resectable NETs.

Starting from these results, a randomized prospective phase 3 trial (NETTER-1) [4] was designed in order to demonstrate the efficacy of ^{177}Lu-DOTATATE therapy. NETTER-1 trial, evaluating 177Lu-DOTATATE vs. high-dose Octreotide LAR in 221 patients with non-resectable, progressive, midgut carcinoid tumors, showed that 177Lu-octreotate significantly improves PFS in patients with functional as well as nonfunctional tumors (PFS not reached vs. 8.4 months; hazard ratio 0.21, with a 79% reduction of the risk of progression). The response rates of 18% in the 177Lu-DOTATATE group and 3% in the control group were observed ($P < 0.001$).

Consequently, ^{177}Lu-DOTATATE (Lutathera®) has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in SSTRs-positive well-differentiated.

GEP-NETs, at a recommended fixed dosage of 7.4 GBq (200 mCi) every 8 weeks, for a total of four cycles. An example of objective response to PRRT is reported in Fig. 11.1.

11.4.2.3 Combination of ^{90}Y/^{177}Lu-Labeled Somatostatin Analogues

Protocols combining ^{177}Lu-peptides and ^{90}Y-peptides have been considered to take advantage of the different physical properties of both radionuclides. The combination of the two radioisotopes, in fact, would allow simultaneous treatment of both larger lesions (based on the higher energy and penetration range of the particles emitted by ^{90}Y) and small lesions (based on the lower energy and penetration range of ^{177}Lu). Initial data indicate that combination treatments

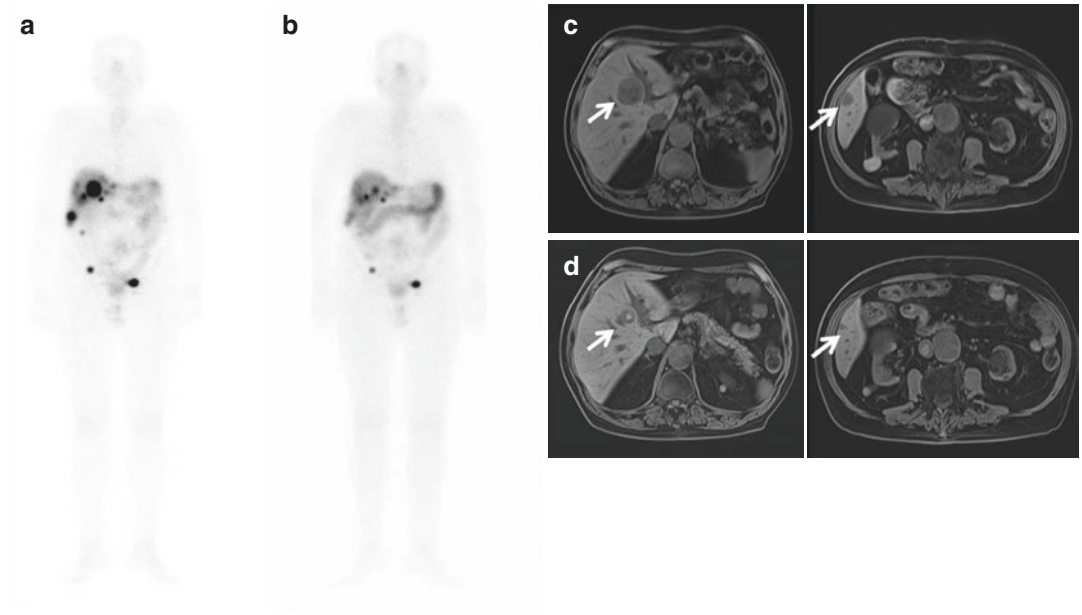

Fig. 11.1 Scintigraphic whole-body evaluation, performed after the first therapeutic administration of ^{177}Lu-DOTATATE, shows overexpressing somatostatin receptors liver lesions and pelvic nodes (**a**). Whole-body scintigraphy, performed after the four cycles of PRRT, shows partial response most clearly detectable in liver (**b**). Abdominal MRI confirms significant reduction of the hepatic secondary lesion (white arrows) after PRRT completion (**c**: baseline MRI; **d**: post-therapy MRI)

with the two isotopes of Y-90 and Lu-177 linked either to DOTA-TOC or to DOTA-TATE administered in sequential treatment cycles or as a cocktail infusion for several cycles improve survival.

In the study performed by Kunikowska and colleagues [22], therapy with tandem radioisotopes (^{90}Y/^{177}Lu-DOTATATE) provides longer overall survival than with a single radioisotope (^{90}Y-DOTATATE) in 50 patients with disseminated NETs. In a cohort study by Villard et al. [23], 486 patients with metastasized NETs were treated with repeated cycles of ^{90}Y-DOTA-TOC or with cycles alternating between ^{90}Y-DOT-TOC and ^{177}Lu-DOTA-TOC until tumor progression or permanent toxicity. The combination treatment was associated with improved overall survival compared with ^{90}Y-DOTA-TOC alone (5.51 vs. 3.96 years) in patients completing three or more cycles of treatment. Finally, the results of a pilot study performed by Seregni and colleagues [24] involving 26 patients treated with tandem regimen with ^{177}Lu/^{90}Y-DOTA-TATE showed objective response in 42.3% of the cases with a median PFS time of 25 months. This relatively new strategy, however, has been still be validated in clinical practice in a larger series of patients.

11.4.3 PRRT in Non-GEP-NETs

PRRT and functional imaging with labeled SSAs have been effectively applied for diagnosis and treatment of several SSTRs-expressing tumors. Beyond current applications, other PRRT potential targets in oncology include meningioma, bronchial NET (B-NET), malignant pheochromocytoma and paraganglioma, medullary thyroid cancer, and neuroblastoma. Applications of PRRT in paraganglioma treatment have been assessed in several small cohorts of patients with inoperable disease, with promising outcomes in tumor response and symptoms' control. It has been proven that PRRT is an effective treatment also in case of refractory or relapsed high-risk neuroblastoma, offering clinicians a valid

therapeutic option when chemotherapy and 131I-metaiodobenzylguanidine treatment are unfeasible.

A retrospective review of 114 advanced B-NETs treated with 90Yttrium-, a combination of 90Y and 177Lutetium-, or 177Lu-based PRRT, identified the median overall survival to be 58.8 months, with a median PFS of 28 months. Patients treated with 177Lu- DOTATATE, alone or in combination with 90Y-PRRT (n = 48 and 21, respectively), exhibited the longest 5-year overall survival (61.4% for both series, vs. 31.6% for 90Y-PRRT) [25]. In a recent, larger study (n = 34 bronchial NETs), the disease control rate was 80%, with 6% achieving a complete response, 27% a partial response, and 47% disease stabilization. The overall median PFS was 20.1 months [26]. The efficacy of PRRT in patients with bronchial NETs are encouraging, and they seem similar to those observed in GEP-NETs even if there are no perspective phase III trials necessary to establish the effective role of PRRT in the therapeutic algorithm of B-NETs.

11.5 Combined Strategies in PRRT

Combining PRRT with synergistic drugs that have ideally minimally overlapping toxicities could potentiate PRRT through several mechanisms. An option to enhance treatment efficacy of PRRT is the combination with radiosensitizing chemotherapy. Results of a nonrandomized phase II study treating patients with a combination of capecitabine and ^{177}Lu-octreotate showed a 24% partial response (PR), 70% stable disease (SD), and 6% progressive disease (PD) in 33 patients [27]. Median PFS and median overall survival had not been reached at a median follow-up of 16 months (range 5–33 months). Survival at 1 and 2 years was 91% (95% CI 75–98%) and 88% (95% CI 71–96%). Claringbold et al. treated patients with GEP-NETs with a combination of ^{177}Lu-octreotate, capecitabine, and temozolomide. Among 35 patients evaluated for tumor response, complete response (CR) was found in 15%, PR in 38%, SD in 38%, and PD in 9%. Median PFS was 31 months [28]. In a retrospective study, Kashyap et al. [29] showed favorable outcomes in patients with ^{18}F-FDG PET-positive GEP-NETs with advanced progressive ^{68}Ga-octreotate PET-avid disease with a combination of 5-FU and ^{177}Lu-octreotate. Among 52 patients, they report CR in 2%, PR 28%, SD 68%, and PD in only 2% of patients with median PFS for 48 months.

A recent randomized clinical trial by Ballal et al. [30] established clinical efficacy of ^{177}Lu-DOTATATE combination with capecitabine over PRRT alone. In the combination group, PR was achieved in 34%, SD in 50.2%, and PD in 6.8% of patients (compared to 6.3, 60.9, and 26.5% in the PRRT-only group, respectively), and the combination group was shown to have longer OS and PFS.

Other strategies to enhance PRRT efficacy include the coadministration of drugs that improve delivery of the radiopharmaceutical via increased tumor perfusion or through increased somatostatin receptor density at tumor surface.

It has been observed that the antiangiogenic drugs, which prevent neovascularization, could allow tumor vasculature to mature and become more efficient at the delivery of drugs, as well as radiopharmaceuticals. The antiangiogenic agent sunitinib has been shown to potentiate external beam radiotherapy (EBRT) in preclinical models and in a phase 2 clinical trial of patients with oligometastases from any primary sites, the head and neck being the most common. Its capacity to increase the delivery of ^{177}Lu-octreotate in patients needs to be examined. The mTOR inhibitor everolimus, whose efficacy is primarily attributed to its antiproliferative properties, also has an antiangiogenic effect. Recently, results from a study in 33 patients with pancreatic NET with liver metastases showed that everolimus-induced increased tumor blood volume, which was attributed to improved tumor perfusion [31]. In an Australian phase 1 study (NETTLE) in 16 patients, the combination of everolimus and PRRT appeared well tolerated. The maximum dose of everolimus was 7.5 mg/day and the median follow-up was 34 months [32].

Increasing the expression of SSTR-2 by the NET cells could result in a more effective at

equal or lower administered activities of ^{177}Luoctreotate, with limited additional risk of toxicity. A variety of drugs, as well as radiation, which have been shown to upregulate expression of SSTR in NETs, are potential candidates for ^{177}Lu-octreotate PRRT. Jin et al. [33] demonstrated that a combination of the radiosensitizer 5-FU with epigenetics drugs tacedinaline or decitabine can upregulate SSTR2 and radiosensitize the NET cells. Interestingly, this study also revealed that each of these agents alone acted as both a radiosensitizer and an SSTR-upregulating agent.

Furthermore, molecularly targeted therapeutics may be important to attain synergy with PRRT. For instance, concurrent inhibition of the DNA repair mechanisms, with the use of a poly-[ADP-ribose]-polymerase 1 (PARP-1) inhibitor results in increased DNA double-strand breaks. Recent preclinical studies show that PARPi sensitizes different NET cells and U2OS sarcoma cells expressing SSTR2 to ^{177}7Lu-octreotate [34, 35]. Both the studies showed that the inhibition of PARP leads to a greater accumulation of DNA damage after ^{177}Lu-octreotate treatment as seen with markers such as 53BP1 or γH2AX, which in turn increases apoptosis.

11.6 New Developments in PRRT

An important evolution step is the use of radiolabeled SSTR antagonists instead of agonists.

A number of clinical and preclinical studies suggest significant improvement in diagnostic sensitivity and therapeutic efficacy of the antagonists. ^{177}Lu-satoreotide tetraxetan (^{177}Lu-OPS201 or ^{177}Lu-DOTA-JR11) shows higher tumor accumulation and absorbed dose and higher numbers of.

DNA double-strand breaks than ^{177}Lu-DOTA-TATE [36]. This is partly due to the higher number of available binding sites and the longer tumor residence time for the antagonist. ^{177}Lu-OPS201 is currently under evaluation in a multicenter phase I/II study (NCT02592707) and in a single center study (NCT02609737).

Recently, a new long-circulating SSTR agonist has been developed. To improve the pharmacokinetics of SSTR2 analogs and reduce PRRT toxicity, Evans blue-based albumin was conjugated with octreotate (EB-TATE). In a SSTR2-positive AR42J xenograft model, ^{90}Y-DOTA-EB-TATE effectively accumulated in the tumor, resulting in complete regression of the tumors and full survival of the tumor-bearing mice with a single low dose of 3.7 MBq of ^{90}Y-DOTA-EB-TATE [37]. The first-in-humans study has recently explored the safety and dosimetry of ^{177}Lu-DOTA-EB-TATE in eight patients with advanced metastatic NETs [38]. ^{177}Lu-DOTA-EB-TATE showed remarkably higher uptake and retention in NETs (7.9-fold increase of tumor dose) compared to ^{177}Lu-DOTA-TATE, but at the cost of an even greater increase of renal and bone marrow absorbed doses, questioning its potential advantage over ^{177}Lu-DOTA-TATE.

Use of multi/heterovalent vectors to simultaneously target several receptors concomitantly expressed in the same cancer cell is an interesting approach to overcome tumor heterogeneity, resistance, and change of phenotype during disease progression. Recent in vitro studies have shown that, apart from somatostatin receptors, other peptide receptors are overexpressed in NETs, in particular the incretin receptor glucagon-like peptide 1 (GLP-1) receptor, the glucose-dependent insulinotropic polypeptide (GIP) receptor, and cholecystokinin (CCK) receptors (CCK1 and CCK2 subtypes). On the basis of the evidence that specific cancers express two or more peptide receptors, multireceptor targeting in vivo is an attractive perspective. The clinical development of ^{111}In-DOTA-exendin-4 and ^{68}Ga-exendin-4 targeting the GLP-1 receptor has been highly successful in insulinomas [39]. Preclinical evidence has shown that ^{68}Ga-DOTA-GIP can label GIP receptor-positive cancers in animals, although this method is not yet used in clinics.

Treatment of cancer with alpha-particle therapy (TAT) has been gaining popularity over the past few years. Alpha particles are positively charged and have a high particle energy ranging

from 5 to 9 MeV and a very short range of 40–100 μm. The range of the particle is thus equivalent to the thickness of 1–3 cell widths. Due to the short therapeutic range, intracellular accumulation of the alpha particle is preferred to ensure a higher chance of target damage to the cell's nucleus. Linear energy transfer (LET) is a term used in ionizing radiation to measure the ionizing density and hence molecular damage of a particle per unit length. LET is very high for alpha particles (80–100 keV/μm) throughout its range and three times greater at the end of the path range (the Bragg peak). The two principal therapeutic radionuclides used in preclinical and clinical TAT of the SSR are ^{213}Bi and ^{225}Ac. The first-in-human TAT study with ^{213}Bi–DOTATOC described the treatment In NETs patients with liver metastases refractory to treatment with ^{90}Y-DOTATOC or ^{177}Lu-DOTATOC [40]. Seven patients were treated with an intra-arterial infusion of ^{213}Bi-DOTATOC, and one patient with bone marrow carcinosis was treated with a systemic infusion of radiopharmaceutical. Enduring responses were observed in all treated patients. Kratochwil et al. [41] presented a dose escalation study data of single cycle and fractionation concepts for ^{225}Ac-DOTATOC in TAT on European Association of Nuclear Medicine (EANM) conference in 2015. Treatment was performed in 34 patients (46 treatment cycles) with progressive NET. They investigated tolerability of fractions—multiple fractions were tolerated with 25 MBq injected activity every 4 months or 18.5 MBq every 2 months up to cumulative activity of 75 MBq. It was no preference of a particular fractionation concept in the radiologic treatment response. At European Neuroendocrine Tumor Society (ENETS) conference 2018 was presented treatment results of ten patients with progressive metastatic NET, refractory to ^{177}Lu-DOTATATE therapy [42]. One to two cycles (average 1.2) of ^{213}Bi or ^{225}Ac-DOTATOC was done. Eight weeks post therapy PET/CT with ^{68}Ga-DOTANOC showed in 60% patients up to 40% reduction of target tumor volume. A phase I dose escalation study, examining the dose tolerance of ^{212}Pb-AR-RMX in PRRT-naive patients, is ongoing (NCT03466216). The first results were shown during 11th International Symposium on Targeted-Alpha-Therapy in May 2019 (TAT11) [43]. In nine enrolled patients, treatment was well tolerated with single ascending or the first multi-ascending doses of 212Pb-AR-RMX. Few mild adverse events were reported (nausea and mild hair loss in two of nine patients, abdominal pain and diarrhea in three of nine patients, fatigue in two of nine patients). There was no dose-limiting toxicity.

11.7 Conclusion

PRRT is an established therapy for patients with inoperable or metastasized NETs. In clinical practice, the indications are limited to G1-G2 well-differentiated NETs with high expression of SSTR, and its precise position in the treatment algorithm remains to be explored. PRRT is generally well tolerated by most of the patients. Chronic and permanent damage on the kidneys and bone marrow are generally mild. Combining PRRT with synergistic drugs might result in additive effects, through several mechanisms such as increased tumor perfusion, SSTR upregulation, and radiosensitization. The clinical experience with somatostatin-based targeted therapy in NET showed very promising results even in patients refractory to treatment with β-emitters.

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Part IV

Treatment NETs from Different Organs

NETs of the Lung

12

Natalie Prinzi, Roberta Elisa Rossi, Giovanni Leuzzi, and Sara Pusceddu

12.1 Introduction and Epidemiology

Neuroendocrine tumors (NETs) are malignant neoplasms arising from neuroendocrine system, mainly located in the gastrointestinal tract and lung [1]. An estimated 25–30% of all NETs have their origin in the bronchial tract and lungs. Lung NETs account for less than 1–2% of all pulmonary neoplasms [1–4]. Their incidence rate, which is 0.2–2/100000 population/year in Europe and United States, has dramatically risen over the past 30 years [1, 5, 6]. NETs of the lung comprise a heterogeneous population of tumors ranging from well-differentiated bronchial NETs to highly malignant and poorly differentiated small-cell lung cancer (SCLC) and large-cell neuroendocrine carcinoma (LCNEC) [7].

Well-differentiated NETs of the lung are also named lung carcinoids (LCs), including typical (TCs) and atypical carcinoids (ACs). TCs are more common than ACs, accounting for 90% of all LCs [5]. Both the subtypes arise mainly in female and in Caucasian, Hispanic, and Asian people [5, 8–10]. The median age at diagnosis is 45 years for TCs and 55 for ACs [5, 8–11].

Surgery is the gold standard in earlier stages. The 5-year overall survival (OS) for limited disease ranges from 87–90% for TCs to 44–78% for ACs, respectively [12–16]. Conversely, the patients with advanced stages show a poor prognosis with a median OS of 17 months and a 5-year survival rate of about 27% [17].

Given their rarity, only few available data from prospective studies are available, and no global consensus exists in regards to therapeutic management of LCs. Target agents such as everolimus, somatostatin analogues (SSAs), and chemotherapy treatments represent the options of choice in the advanced setting [5]. Peptide receptor radiotherapy (PRRT) and immunotherapy are emerging options. A multidisciplinary approach is strongly suggested in all clinical scenarios. Herein, we will provide a comprehensive literature review on diagnosis and management of advanced LCs.

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12.2 Etiology and Classification

The majority of LCs are sporadic neoplasms [18, 19]. Differently from high-grade lung NETs, no relationship between LCs and smoking habit has been proved so far [5]. In approximately 5% of the patients, the development of these neoplasms occurs in the context of multiple endocrine neoplasia type 1 (MEN1) syndrome [20–23], and the association to a rare pulmonary carcinoid tumor genetic syndrome is reported in sporadic cases as well [19]. Although rare, some patients may present with multiple lung nodules or tumorlets and widespread peripheral airway neuroendocrine cell hyperplasia. In this case, a diagnosis of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) can be made [24]. The mechanisms underlying progression and/or onset of LCs from DIPNECH are still unclear [7, 25].

12.3 Histopathological Features and WHO Classification

The availability of adequate tissue sample is required to distinguish lung NET subtypes.

The 2015 WHO classification has defined four lung NETs subtypes: TC, AC, LCNEC, and SCLC. SCLC and LCNEC are poorly differentiated, aggressive neoplasms, while LCs are well differentiated, indolent tumors (Table 12.1) [7].

The definition of LCs include lesions of more than 5 mm in diameter, while lung lesions of 5 mm or less are still classified as carcinoid tumorlets (DIPNECH) [7].

Two aspects are crucial to define lung NETs subtypes: the presence of necrosis and the number of mitoses. In particular, TCs have no evidence of necrosis and less than two mitoses per 2 mm^2 in the tumor area, while ACs are characterized by focal necrosis and 2–10 mitoses per 2 mm^2 [7]. However, the distinction between well- and poorly differentiated lung NETs based on the number of mitoses (less or more than 10/mm^2, respectively) is still debated [5, 7, 26]. Other biomarkers like synaptophysin, chromogranin A (CgA), and CD56 have to be combined to the WHO classification to confirm lung NET diagnosis [7].

The prognostic role of Ki-67 cell proliferation index by immunohistochemistry (IHC) has not been well defined in lung NETs, and its role is currently under debate. According to the WHO of 2015, a Ki-67 value lower than 20% characterizes LCs (≤5% in the TC and ≤20% in the AC), while a value >40% is typical of the high-grade pulmonary NETs [7].

Although the Ki-67 index is not currently accredited with lung NET subtyping due to some overlap of cut-off thresholds among biologically adjacent tumors (TC versus AC, AC versus LCNEC, LCNEC versus SCLC), its differential distribution between low- to intermediate-grade and high-grade tumors has made it an exceptional discriminator especially on biopsy/cytology samples, being its determination recommended on surgical specimens as well [7, 27, 28].

The reproducibility and clinical usefulness of Ki-67 in lung NETs is still under debate, and there is no current diagnostic role for Ki-67,

Table 12.1 WHO 2015 classification of lung/thymus neuroendocrine neoplasms

	TC	AT	LCNEC	SCLC
Tumor grade	Low	Intermediate	High	High
Histology	Well differentiated	Well differentiated	Poorly differentiated	Poorly differentiated
Mitosis/2 mm^2	<2	2–10	>10 (median 70)	>10 (median 80)
Ki-67	≤5%	≤20%	40–80%	50–100%
Necrosis	None	Focal, if any	Present	Present
Malignancy	Low-grade malignancy	Low-grade malignancy[a]	Highly malignant	Highly malignant

Legend: *TC* typical carcinoid, *AT* atypical carcinoid, *LCNEC* large cell neuroendocrine carcinoma, *SCLC* small cell lung cancer

[a]Considerable malignant potential

whereas the mitotic count has remained the only proliferation criterion in tumor classifications over time [7].

12.4 Diagnostic Workup

12.4.1 Clinical Presentation

TCs are usually located in the central parenchyma of the lung, while ACs often develop peripherally. TCs and ACs are usually diagnosed in earlier stages, but liver and bone metastases are common in advanced stages [5, 24]. Patients with LCs are often asymptomatic or present unspecific symptoms like hemoptysis, dyspnea, cough, or chest pain [24, 29]. Functional LCs are rare.

Although typical carcinoid syndrome occurs in only 10% of the cases [13, 30], NETs of the lung may secrete various hormones and vasoactive peptides.

In particular, LCs have been more often associated with adrenocorticotropic hormone (ACTH) production and can cause Cushing's syndrome [31, 32].

12.4.2 Imaging

Computed tomography (CT) is the gold standard to diagnose and stage LCs. A single round lesion is the most frequent radiological aspect [17, 24], but multiple calcified lesions may also be present [33]. Magnetic resonance imaging (MRI) may help in the detection of bone or liver metastases [5]. Approximately 80–90% of LCs express somatostatin receptors (SSTRs), thus functional imaging based on radiolabeled SSA plays a key role in both staging and defining treatment strategy [13, 29, 34, 35]. The octreoscan or somatostatin receptor scintigraphy (111In-pentetreotide/octreotide scan) demonstrates 93% sensitivity and 87% specificity, respectively, in LC diagnosis [34, 36]. However, more recently, the 68Ga-DOTATATE PET/CT has been introduced in lung NET management.

Considering the binding to SSTRs, 68Ga-DOTATATE showed a tenfold higher affinity than octreotide [37, 38]. A sensitivity of 81% and a specificity of 90% have been reported by Haug and collaborators, and in another study, the 68Ga-DOTATATE PET/CT detected a significantly larger number of NET lesions expressing type 2 SSTR than octreoscan ($p < 0.001$) [39–41].

Also taken into account the reduced scanning time and the better imaging resolution, PET/CT 68Ga-DOTATATE should be preferred in LC management [42, 43].

The role of fluoro-deoxy-glucose (FDG)-PET/CT in LCs is still debated. TCs are usually characterized by low or no uptake on FDG scan, whereas a higher uptake is possible in ACs with a high proliferative index [44]. In a small retrospective series of 20 LCs, Bongiovanni et al. showed a shorter progression-free survival (PFS) in LCs with positive 18-FDG PET/CT scan than in those with negative 18-FDG-PET/CT scan ($p = 0.015$) [45]. These data could support the possible prognostic role of 18-FDG-PET/CT and its potential ability in suggesting different therapeutic strategies for LCs according to the different FDG uptake.

12.5 Surgery

Surgical resection, with preservation of as much normal lung tissue as possible, is still the gold standard in case of limited and resectable disease [5, 24].

Regarding TCs, surgery is associated with excellent outcomes, with 5- and 10-year survival rates of ~90% and ~80%, respectively, and very low recurrence rates (3–5%) [46, 47]. In detail, recent analyses of the European Association of Thoracic Surgeons (ESTS) Neuroendocrine Tumours Working Group revealed that surgically resected TCs are associated with a 5-year survival rate of 94% [48]. On the other hand, 5- and 10-year survival rates following surgery in AC patients are lower (about 70% and 50%, respectively), given the higher rate of relapse (~25%) [46, 47, 49].

According to the Surveillance, Epidemiology, and End Results (SEER) database, lobectomy is the most common procedure (51.2%), compared to sublobar resection (wedge resection or segmentectomy, 24.1% of cases). Other less-used procedures are pneumonectomy, bronchoplasty, extended resection, and bronchoscopic ablation [50].

As a rule, the surgical technique of choice is lobectomy. On the other hand, given the indolent nature of TCs, sublobar resections may be taken into account in this setting as similar outcomes compared to lobectomy have been reported [51, 52]. Furthermore, segmentectomy and wedge resections should be considered in patients with compromised pulmonary function and/or severe comorbidities [53]. Further randomized studies are needed to better assess the differences in terms of perioperative outcome, long-term survival, and disease recurrence between the two approaches.

A minimally invasive approach, such as video-assisted thoracoscopic surgery (VATS), is recommended in experienced centers due to fewer complications and potentially increased survival rates [53, 54]. Some authors reported promising results also in the setting of minimally invasive bronchoplastic procedures [55].

The natural history of these tumors is strictly related to the lymph-node status. It has been reported that lymph-node metastases may be present in up to 25% of the cases in TCs and >50% in ACs, respectively [56]. Node-negative and N1 patients have similar outcomes. Conversely, N2 tumors have been reported especially in ACs and are associated with a dismal prognosis [16, 57]. Furthermore, in the majority of the patients, lymph-node involvement does not modify surgical indication, given that the neoadjuvant treatment does not improve the resectability rate or survival in LCs [58]. Thus, although the need for lymph-node dissection is still poorly defined in TCs, a complete radical lymphadenectomy should be performed in all cases.

From the surgical point of view, unknown lung lesions (that exhibit NET radiological features) undergoing upfront surgery should undergo wedge excisional biopsy. If intraoperative frozen section is consistent with NET and the margins are negative, systematic lymph-node dissection should be performed [59]. If the patient is node-negative, a completion lobectomy is not required. In node-positive patients with adequate pulmonary reserve, lobectomy should be performed regardless of histology [5, 59]. If atypical features are found during pathologic evaluation, an interval completion lobectomy may be considered in fit patients [5, 49]. When there is suspicion of N2 nodal involvement or after mediastinoscopy/endobronchial ultrasound (EBUS) showing N2 disease, multimodality treatment might be required [60].

Approximately 20% of all LCs present as pure endobronchial polyp-like lesions without gross radiologically detectable involvement of the bronchial wall and lung parenchyma [60]. Rarely, N1 lymph nodes may involve the bronchial takeoff. In both cases, bronchoplastic procedures (bronchial sleeve resection or wedge) with or without parenchymal resection are indicated [61] since they protect from the detrimental effects of pneumonectomy on respiratory functions as well as on quality of life. In the literature, the incidence of sleeve resections in the different series varies from 1.4% to 41% of cases [62]. Given the indolent behavior of these tumors, a complete resection with disease-free margins (~5 mm) is mandatory [62]. In rare cases, a pulmonary artery reconstruction can be associated as well [63]. Bronchial sleeve resections have been also described for more peripheral lesions involving the segmental bronchi [64], although this procedure is still debated. In order to assess the best surgical strategy, fiberoptic bronchoscopy should always be performed by the operating surgeon to have a precise idea of the anatomic details. The technical procedures are identical to those of bronchial sleeve resections performed for lung cancer. As a rule, the anastomosis can be encircled by intercostals pedicle flap, thymic, or mediastinal tissue in order to favor protection/revascularization of the bronchial anastomosis, to separate it from the arterial side when a combined bronchovascular reconstruction is performed (avoiding broncho-arterial fistulas)

and to contain a small dehiscence [62]. Concerning the perioperative outcome, one study only [65] compared bronchoplastic procedures between LCs and primary lung cancer, reporting less frequent anastomotic and nonsurgical complications in the LC group (probably because of the younger age of these patients).

Surgery is still considered a curative treatment for LCs, also in the metastatic setting [65]. Pulmonary resection is often recommended in patients with limited hepatic metastases, with ~20% achieving a cure [66]. If complete resection is not possible, palliative debulking surgery may be taken into account in particular cases to relieve symptoms or prevent complications (i.e., pneumonitis). However, resection of the primary tumor is not indicated in case of unresectable metastases when the primary site is relatively stable.

12.6 Endobronchial Resection

Due to their indolent clinical course, bronchoscopic excision can be taken into account in those cases presenting with centrally located intraluminal LCs. In the literature, a variety of endobronchial procedures have been reported as an effective alternative to surgical treatment such as YAG laser, diode laser, cryo or electrosurgery, argon-plasma coagulation, and mechanical debulking [67–71]. To date, these techniques are still considered suboptimal approaches and reserved for selected cases only. As a rule, extra-luminal tumor growth, larger tumor diameter, and suspected locoregional/distant metastases are generally considered contraindication for bronchoscopic excision [68]. Furthermore, bronchoscopic treatment is not always effective, especially when LCs extend to the segmental bronchi and when the tumor is in either the upper left or right lobes.

As reported by a recent study assessing prognostic factors for endobronchial ablation, only small intraluminal tumors smaller than 2 cm are suitable for this kind of procedure, whereas all other tumors should be treated with conventional surgery [68]. Another indication is the desobstruction and recanalization of the involved bronchus to obtain resolution of post-obstructive pneumonia [72] as well as to limit the extension of the subsequent surgical resection [73]. A recent systematic review reported excellent outcome results for TCs (5-year survival ranging from 89 to 94% in the literature) and a low rate of locoregional and distant recurrence in bronchoscopic-treated patients, ranging from 0–5% to 0–4% respectively [74]. Although these results seem promising, these studies are biased by the patients' selection (small tumors, young patients, usually indolent histology). Further prospective-randomized studies are, therefore, needed to better address this issue.

12.7 Somatostatin Analogues

For low-proliferating lung neuroendocrine tumors, treatment with SSAs is an option for functional tumors with clinical symptoms. SSAs constitute the gold standard for symptomatic control with >50% improvement in both flushing and diarrhea in gastroenteropancreatic (GEP) and LCs [75, 76]. Patients who have metastatic NETs and carcinoid syndrome should be treated with SSAs such as octreotide or lanreotide [77]. The long-acting release (LAR) formulation of octreotide is commonly used for the chronic management of symptoms in patients with carcinoid syndrome. Standard doses of octreotide LAR are 30 mg intramuscularly every 28 days. Dose and frequency may be further increased for symptom control as needed. Short-acting octreotide (usually 150–250 mcg subcutaneously three times daily) can be added to octreotide LAR for rapid relief of symptoms or for breakthrough symptoms [78, 79].

Lanreotide Autogel has a similar mechanism of action as octreotide, but is administered as a deep subcutaneous injection at the dose of 120 mg every 28 days [80, 81].

The more recent multicentric phase III ELECT trial randomized 115 patients with carcinoid syndrome (including LCs) who were either naïve or responsive to octreotide to receive 120 mg of

lanreotide or placebo and evaluated the number of days patients required use of rescue octreotide. Patients in the lanreotide arm required less-frequent rescue octreotide than those in the placebo arm (33.7% vs. 48.5%; $P = 0.017$), supporting the use of lanreotide for symptom control [82].

Moreover, interesting results have been reported by a specific study focused on advanced, functioning LCs, describing complete symptom control and normalization of urinary 5-hydroxyindoleacetic acid (5-HIAA) in 7 out of 126 ACs patients who received short-acting octreotide injections [83].

In nonfunctioning tumors, the use of SSAs is still controversial, but after the results reported by the PROMID and the CLARINET study indicating antitumor efficacy of octreotide LAR and Lanreotide Autogel drugs in GEP-NETs, it is now also widely accepted for nonfunctioning tumors of other origins [84, 85].

However, studies specifically focused on LCs are scanty. In a recent retrospective study, Bongiovanni et al. [45] investigated the efficacy of SSAs as first-line treatment of 30 metastatic nonfunctioning LCs and reported that, out the 30 patients, one patient (3.3%) achieved a partial response (PR) and 26 (86.6%) showed stable disease (SD), thus highlighting the antitumor activity of SSAs with a satisfiable safety profile.

A randomized double-blind, phase 3 study (SPINET trial, NCT02683941, clinicaltrial.gov) evaluating the efficacy and safety of lanreotide-autogel versus placebo is ongoing in patients with well-differentiated, metastatic, and/or unresectable TCs and ACs.

Due to the very limited level of evidence about the use of SSAs in nonfunctioning, advanced LCs, no clear consensus exists on the timing of octreotide or lanreotide initiation in asymptomatic patients with metastatic well-differentiated LCs. However, if patients with advanced low-grade LCs present with clinically significant tumor burden, initiation of octreotide and lanreotide may be considered [86].

12.8 Peptide Receptor Radiotherapy

PRRT with radiolabeled SSAs is an option in patients with NETs expressing high levels of SSTRs, namely well-differentiated forms [34, 87, 88].

Well-differentiated LCs frequently express subtype 2 of the SSTR family, and this can be identified by 68Ga-DOTATATE PET/CT scans, which constitute predictors of response [89].

PRRT with either 90Y- or 177Lu-peptides is generally well tolerated, and reported results are promising, even if mainly focused on gastrointestinal neoplasms.

So far, no prospective studies were performed with PRRT specifically in lung NETs, but only few retrospective study including LC patients are published [88–94].

A recent study examined the long-term efficacy, survival, and toxicity of 177Lu-dotatate in a group of 610 Dutch patients with metastatic GEP and lung NETs [95]. PFS and overall survival (OS) for all patients were 29 months and 63 months, respectively.

Other smaller studies also found improved OS (58.8 months) [90] and median PFS (20.1 months with TCs and 15.7 months with ACs) with PRRT treatment in patients with advanced LCs [93].

The phase III study NETTER-1 evaluated the efficacy and safety of 177Lu-dotatate in 229 patients with advanced, progressive, SSTR–positive midgut NETs who were randomly assigned to receive either 177-Lu-dotatate plus best supportive care (BSC) including octreotide LAR or octreotide LAR alone. Results of this study showed that treatment with 177Lu-dotatate was associated with a significant improvement in PFS (not reached vs. 8.4 months; $P < 0.0001$). Objective tumor responses were observed in 18% of patients who received 177Lu-dotatate versus 3% in the control group ($P < 0.001$). According to this trial, 177Lu-dotatate resulted in markedly longer PFS than high-dose octreotide LAR and was associated with limited acute toxic effects. Unfortunately, no LCs were included [96].

Given the results of this landmark trial, PRRT with 177Lu-dotatate was approved by the Food

and Drug Administration (FDA) in January 2018 for the treatment of patients with unresectable, low- or intermediate-grade, locally advanced, or metastatic GEP-NETs.

The National Comprehensive Cancer Network (NCCN) 2019 guidelines [86] recommend to consider PRRT with 177Lu-dotatate as a treatment option for some patients with advanced and/or metastatic gastrointestinal tract and lung NETs that are SSTR-positive on imaging and show disease progression while taking SSAs, if the tumor is either low-grade (typical) with clinically significant tumor burden or intermediate-grade (atypical).

In summary, only few, mainly retrospective, studies specifically dedicated on PRRT in LCs are available, but, based on data reported in literature, particularly in the setting of gastrointestinal NETs, PRRT might be considered as an effective option in progressive LCs with strong expression of SSTRs and high tumor burden.

12.9 Chemotherapy

No randomized trials on chemotherapy are available, and there is no currently an established standard regimen, being the role of chemotherapy in LCs under debate. Because of their low proliferative capacity, LCs are generally considered as chemoresistant neoplasms [97].

In general, available chemotherapy regimens for TCs and ACs include the use of streptozotocin plus 5-fluorouracil/doxorubicin, or capecitabine/oxaliplatin, temozolomide, dacarbazine, doxorubicin, etoposide, and cyclophosphamide [5, 26, 29, 98–101].

The objective response rates (ORR) with single-agent chemotherapy is generally not >20%, reserving this approach to pretreated patients or to patients with poor performance status [5, 26, 29, 101].

Poly-chemotherapy regimens (i.e., platinum-based chemotherapy, temozolomide combined with capecitabine or bevacizumab, capecitabine plus oxaliplatin) have demonstrated greater activity and are the best option in patients with good performance status and significant tumor burden. Patients are treated with poly-chemotherapy regimens SD in 30–50%, PR in 5–10%, and symptomatic response in 40–60% of the cases. However, these results derived from studies conducted on patients with NETs of any sites including only few patients with LCs [5, 26, 29, 98–103].

Among all the chemotherapy agents evaluated, temozolomide and oxaliplatin have shown the best clinical benefit in patients with LCs so far.

Temozolomide is a well-known alkylating agent, used in different types of cancer. The oral administration, the ability to cross the blood-brain barrier, and the possibility of being associated with other cytostatics represent the main strengths of this drug [104, 105]. Ekeblad and colleagues performed a retrospective analysis of 36 patients with histologically confirmed metastatic or inoperable malignant NETs treated with oral temozolomide (100–200 mg/m^2 for 5 days every 28 days). The study group included ten patients with TCs and three with ACs. After a median follow-up of 7 months (range 2–17 months), 31% of patients with LCs had SD and 31% showed a PR [106]. The most relevant toxicity was grade 3 and 4 thrombocytopenia in 14% of the cases.

Another retrospective study evaluated the activity of temozolomide in 31 patients affected by metastatic LCs, reporting PR in 14% of the cases and SD in 52% of the cases. The most common toxicity was, again, grade 3 and 4 thrombocytopenia [107].

Given these premises, a phase 2 study with the aim to evaluate the efficacy and safety of Lanreotide Autogel plus temozolomide in patients with advanced or unresectable LCs or thymus carcinoids is currently ongoing (ATLANT-NCT 02698410, clinicaltrial.gov).

Some data correlated the role of methylguanine DNA methyltransferase (MGMT) as predictor of response to temozolomide [108, 109]. MGMT is an enzyme promoting repair of DNA damage caused by alkylating agents. High levels of intracellular enzyme reduce the alkylating agents activity, whereas MGMT gene methylation reduces the levels of the intracellular enzyme.

Kulke et al. reported data about 95 patients with advanced NETs treated with temozolomide and showed that MGMT gene methylation is more common in pancreatic NETs compared with LCs.

This resulted in higher overall response rate with temozolomide in pancreatic NETs compared with LCs (34% versus 2% PR, respectively) [110]. Moreover, the recent study by Campana et al. evaluated the correlation between the outcome of 95 advanced NETs treated with temozolomide and the MGMT promoter methylation status. The authors showed an ORR of 51.8 and 17.7% in patients with or without MGMT promoter methylation, respectively, suggesting that the presence of MGMT promoter methylation represented a strong predictive factor for temozolomide response in NETs [109].

Finally, oxaliplatin has been reported as an active and potentially effective agent in retrospective analyses of patients with metastatic well-differentiated lung NETs alone or combined with other primary sites, treated with XELOX, GEMOX, CAPOX, or FOLFOX regimens [100, 110, 111].

Conversely, the use of cisplatin/carboplatin plus etoposide schedule, recommended by the international guidelines for the treatment of poorly differentiated lung NECs (SCLC and LCNEC), is not currently suggested as a treatment of choice in advanced LCs [34, 98, 101].

12.10 Targeted Therapy

12.10.1 mTOR (Mammalian Target of Rapamycin) Inhibitors

Although lung NETs are typically poorly represented in clinical trials of NET treatments, two phase III trials (RADIANT-2 and RADIANT-4), evaluating the efficacy of everolimus in advanced NETs, have recently reported specific results for LCs [112, 113].

In RADIANT-2, which evaluated the impact of combination therapy with the oral mammalian target of rapamycin (mTOR) inhibitor everolimus and the SSA octreotide LAR in patients with advanced NET and carcinoid symptoms, among them 6.9% of patients in the experimental group and 2.3% of patients in the control group were diagnosed with lung NETs. Overall, patients were randomly assigned to receive octreotide LAR 30 mg intramuscularly every 28 days combined with everolimus 10 mg per day (N = 216) or octreotide LAR plus a placebo (N = 213). Treatment with everolimus combined with octreotide was associated with longer PFS: 16.4 months in patients treated with everolimus and octreotide versus 11.3 months in control patients (P = 0.026); patients with lung NETs showed trend to improve PFS with everolimus plus octreotide (P = 0.228) [113, 114].

Based on these promising findings, a subsequent RADIANT-4 trial evaluated progressive, nonfunctioning, well-differentiated NETs, including LCs, where patients were treated with everolimus plus BSC versus placebo plus BSC [113]. Out of a total of 302 patients who were randomized to receive either everolimus or placebo (n = 97), the primary endpoint was PFS. In total, 175 patients had gastrointestinal NET and 90 had lung disease. Everolimus-treated patients showed a prolonged median PFS, as compared with those receiving placebo (11.0 vs. 3.9 months, HR 0.48; $p < 0.00001$). This benefit in PFS was consistent in all subgroup analyses: in particular, there was a 50% improvement in PFS for patients with lung tumors and a 44% benefit for those with gastrointestinal NETs [113, 115].

Finally, a trial specifically looking at lung NETs, the LUNA phase II trial, where patients were randomly assigned to everolimus, the SSA pasireotide (a novel multi-receptor ligand SSA with higher affinity for SSTR1, SSTR3, and SSTR5 than octreotide, but a lower affinity for SSTR2) [116], or the combination of both, was associated with antitumor activity and an acceptable safety profile. A total of 112 patients with LCs were included. The LUNA study achieved the preplanned statistical objective of a 9-month PFS rate >20% in all the three arms, supporting the efficacy of everolimus in lung NETs [117].

Since the results from these three phase II-III prospective trials have been published, everolimus, a selective mTOR inhibitor, has been

approved for the treatment of unresectable or metastatic, well-differentiated, nonfunctional NETs of lung origin in patients with progressive disease.

12.10.2 Antiangiogenic Agents

The potential role of antiangiogenic agents in LCs is still far from being clearly understood. Sunitinib is an orally administered kinase inhibitor small molecule with activity against a number of tyrosine kinase inhibitors including vascular endothelial growth factor receptor (VEGFR)-1, −2, −3, platelet-derived growth factor receptor (PDGFR)-a and -b [118]. A phase II study evaluated the activity of sunitinib in 109 NET patients including 41 with carcinoids of whom 14 were foregut including LCs and observed that sunitinib had antitumor activity in pancreatic forms, while its activity against carcinoid tumors could not be definitively determined [119].

The PAZONET trial [120] of pazopanib as a sequencing treatment in progressive metastatic NETs, including patients with LCs, observed a clinical benefit in 85% of patients treated with pazopanib.

Moreover, bevacizumab, an anti-VEGF monoclonal antibody, is also being evaluated for LCs. In the phase II study by Yao et al. [121] including 44 patients with advanced NETs of different origins (four were LCs), patients were randomized to either bevacizumab or pegylated interferon (IFN). The PFS rates after 18 weeks were 95% with bevacizumab versus 68% with pegylated IFN.

12.11 Immunotherapy

Immune checkpoint inhibitors have changed the clinical practice of different types of malignant neoplasms [122, 123]. The main evidence of the efficacy of immunotherapy in NETs is currently exclusively limited to poorly differentiated NETs of the skin (Merkel Cell Carcinomas), while results from clinical trials are disappointing for well-differentiated tumors [124]. In low-grade LCs, two immunotherapy agents have been evaluated in clinical studies so far, namely pembrolizumab and spartalizumab.

Pembrolizumab is a highly selective, humanized anti-programmed cell death protein 1 (PD-1) antibody. Antitumoral activity of pembrolizumab in carcinoids (lung and gut) and well-differentiated pancreatic NETs was initially evaluated in the phase 1b KEYNOTE-028 study at dose of 10 mg/kg, every 2 weeks. Out of 25 treated patients with LCs, three (12%) had objective response rate. Durations of response were 6.9, 9.2, and 11.1 months for the three LC responders, respectively. Stable disease rate was 60% (15 patients) [125]. The KEYNOTE-158 phase 2 basket study investigated the antitumor activity and safety of pembrolizumab (200 mg intravenously every 3 weeks) in different types of cancer, progressed after standard-of-care systemic therapy. One-hundred-seven patients with different types of NETs were enrolled and 14 of them were LCs. Median follow-up was 24.2 months (range: 0.6–33.4). ORR was 3.7%, with 0 complete responses and 4 PR (three pancreatic and one rectal). All the responses were in patients with PD-L1-negative tumors. Median PFS and median OS were 4.1 months and 24.2 months, respectively. Treatment-related grade 3–5 adverse events occurred in 21.5% of the patients. The authors concluded that pembrolizumab showed limited efficacy in pretreated advanced well-differentiated NETs [126].

Spartalizumab (PDR001), a high-affinity, humanized, anti-PD-1 antibody, was evaluated in a phase II, multicenter study in well- and poorly differentiated NETs. Primary endpoint was the ORR, and secondary endpoints included duration of response, biomarker analyses, and safety. In this study, PDR001 was administered at a flat dose of 400 mg, every 4 weeks. Of the 116 patients enrolled, 30 were thoracic NETs, 33 pancreatic NETs, 32 gastrointestinal NETs, and 21 GEP-NECs. After a median follow-up of 7.6 months in NET and 6 months in GEP-NEC, ORR was 7.4% in well-differentiated NETs and 4.8% in poorly differentiated NECs.

Patients with thoracic NETs had a higher ORR (20%) compared to GEP-NETs (1.5%) and GEP-

NECs (5%). Most common grade 3/4 adverse events (>2.5%) were hypertension, dyspnea, anemia, abdominal and back pain. Interestingly, PD-L1 expression was generally low, with a higher proportion of PD-L1 expression in immune cells >1% in GEP-NECs (43%) compared to thoracic NETs (19%) and gastrointestinal NETs (33%). These preliminary results might suggest clinical activity of PDR001 in thoracic NETs [127].

12.12 Summary and Conclusions

LCs are rare tumors with an incidence of 2% and 0.2% for TCs and ACs, respectively. However, due to the improvement in screening and imaging techniques and increased disease awareness, LC incidence has increased during the last three decades [1, 5, 6]. Given the disease rarity and the scarcity of prospective studies, some controversies still exist on diagnosis and management of LCs. A multidisciplinary approach is always recommended.

Surgery with preservation of as much normal lung tissue as possible remains the gold standard in case of limited and resectable disease. Bronchoscopic excision might be also taken into account in those cases presenting with centrally located intraluminal LCs, even though further prospective studies are warranted to validate this more conservative approach.

For advanced or progressive disease, no standard treatment or therapeutic algorithm is currently available. In advanced metastatic LCs, medical therapy represents the milestone and SSA, everolimus, chemotherapy, and PRRT treatments constitute the therapeutic armamentarium in this setting, being generally reserved to well-differentiated tumors with low proliferative index and SSTR positivity.

The majority of the studies exploring the efficacy of SSA therapy for both symptom and tumor growth control are retrieved from studies on GEP-NETs; however, the LUNA trial is the only prospective study dedicated exclusively to LCs [117], which reported that long-acting pasireotide, everolimus, or combination therapy with both agents was associated with antitumor activity and an acceptable safety profile. Furthermore, the SPINET trial is ongoing, evaluating the efficacy and safety of lanreotide versus placebo for the treatment of well-differentiated, metastatic and/or unresectable nonfunctioning LCs.

PRRT with radiolabeled SSAs is an option in patients with well-differentiated low-intermediate grade NETs expressing high levels of SSTRs; however, only few, mainly retrospective, studies specifically dedicated to PRRT in LCs are available. Systemic chemotherapy is reserved for those cases of locally advanced or metastatic disease; however, the standard chemotherapy regimen to be recommended in clinical practice is still unclear, and the choice of chemotherapy should be made by taking into account the characteristics of both patient and tumor.

The role of targeted therapies in LCs remains still limited with the exception of the only approved drug (everolimus) in clinical practice. Everolimus represents a therapeutic option in patients with progressive disease with advanced LCs.

The RADIANT 4 study will likely have a major impact on clinical practice, especially for lung NETs [115]. Indeed, it was the first randomized study to specifically show that everolimus is significantly effective in patients with LCs. We believe that this study represents a major breakthrough, as, to date, there has not been any properly established treatment algorithm for LCs. In particular, we think that everolimus may be particularly suitable for patients with more aggressive and rapidly progressing disease such as those with ACs, in whom upfront treatment can be suggested. Moreover, data on second-line therapy with everolimus are even more grounded, also compared with those available for chemotherapy and PRRT, which are mostly derived from retrospective series or nonrandomized studies in a mixed population of patients with TCs and subjects with ACs.

Conversely, there is a lack of studies focused on the potential role of antiangiogenic agents in LCs. Finally, immune checkpoint inhibitors have shown promising results in different tumors, and the main evidence of the efficacy of

immunotherapy in the neuroendocrine setting is only in Merkel Cell Carcinomas but not in well-differentiated forms, even though [124] in low-grade LCs, pembrolizumab and spartalizumab had shown preliminary promising results in terms of both efficacy and safety.

Further studies, specifically dedicated to LCs, are needed to draw more robust conclusions, particularly to better clarify the most adequate sequence and timing of systemic drugs in the management of this subgroup of rare neoplasms.

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Gastric Neuroendocrine Tumors

13

Davide Ravizza and Giancarla Fiori

13.1 Introduction

Gastric neuroendocrine neoplasms (g-NENs) should be defined according to the World Health Organization classification and staged according to the Tumor Node Metastasis system. The former is based on histological differentiation and grade, which relies on the proliferation index assessed by the Ki67 and mitotic index [1, 2]. Therefore, g-NENs are classified in well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs). NETs show a low to high proliferation grade, whereas NECs, by definition, are high-grade neoplasms [1].

Gastric neuroendocrine tumors (g-NETs), known as gastric carcinoid, were originally regarded as rare, but over the last few decades, their incidence has been growing (sevenfold to tenfold over the last 30 years) [3–5]. The increased incidence, frequently with lesions at early stage, may essentially be a consequence of the widespread use of endoscopy and imaging studies, improved immunohistochemical staining and increased awareness of the diagnosis [5, 6].

Recent epidemiological data show that g-NETs represent 6.9–8.7% of all gastrointestinal (GI) NETs and 0.3–1.8% of all gastric tumors [3, 6–11]. However, in a prospective Austrian study, g-NETs accounted for 23% of all NETs [10]. According to the last US epidemiological data (Surveillance, Epidemiology, and End Results - SEER), the age standardized incidence rate of g-NETs is approximately 0.4/100,000/year [12].

Most of the g-NETs develop from enterochromaffin-like (ECL) cells while a small proportion develop from non-ECL cells of gastric mucosa. Histologically, the diagnosis is confirmed by positive immunohistochemical staining of chromogranin A (CgA) and synaptophysin [13].

Gastric NETs are generally slow growing and often indolent neoplasms but can also be very aggressive and metastasize widely [11, 14–16]. They are divided into three types with different pathophysiology, clinical characteristics, aggressiveness, and prognosis (Tables 13.1 and 13.2) [17]. Type I and type II are associated with chronic hypergastrinemia causing ECL cells hypertrophy/hyperplasia and, ultimately, ECL cell NETs development [18]. In the former, the presence of a body chronic atrophic gastritis (CAG), mainly autoimmune, leads to achlorhydria which induces an appropriate hypergastrinemia [19, 20]. In the latter, the hypergastrinemia is inappropriate because it occurs in the presence of gastric acid hypersecretion, and it is due to an ectopic gastrin-producing G cell neoplasia

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Table 13.1 Clinical characteristics of gastric neuroendocrine tumors

	Type I	Type II	Type III
Prevalence (%)	70–80	5–6	15–20
Gender	Females	Females = males	Males
Age at diagnosis (years)	50–70	>50	>50
Associated conditions	CAG	Gastrinomas (ZES)	None
Other syndromes	Autoimmune polyglandular syndrome	MEN-1	None
Serum gastrin levels	Very high	Very high	Normal
Gastric pH	High	Low	Normal
Risk of metastases (%)	<10	10–30	50–100
Treatment	EMR, ESD or surgery	EMR, ESD or surgery	ESD or surgery
Tumor-related deaths (%)	None	<10	25–30

CAG chronic atrophic gastritis, *ZES* Zollinger–Ellison syndrome, *MEN-1* multiple endocrine neoplasia type 1, *EMR* endoscopic mucosal resection, *ESD* endoscopic submucosal dissection

Table 13.2 Endoscopic and pathological characteristics of gastric neuroendocrine tumors

	Type I	Type II	Type III
Cell of origin	ECL	ECL	ECL in most cases
Gastric mucosa	Atrophic ECL hyperplasia	Hypertrophic ECL hyperplasia	Normal
Endoscopic appearance	Polypoid/subepithelial	Polypoid/subepithelial	Polypoid/subepithelial
Location	Body and fundus	Body and fundus	Any region
Number	Multiple	Multiple	Single
Size (mm)	≤10	≤10	Often >20
Differentiation	Well differentiated	Well differentiated	Well differentiated
Grading	G1/G2	G1/G2	G1/G2/G3
Depth of invasion	Mucosa/submucosa	Mucosa/submucosa	Any depth
Angioinvasion (%)	Rare	<10	> 50

ECL enterochromaffin-like cells

(gastrinoma) in the context of a Zollinger–Ellison syndrome (ZES), almost exclusively associated with a multiple endocrine neoplasia type 1 (MEN-1) [21–24].

Although proton pump inhibitors (PPIs) can induce ECL cell hyperplasia, only rare cases of well-differentiated g-NETs developing after long-term PPI use are reported in the literature [25].

Type III g-NETs are not associated with any background gastric pathology, and serum fast gastrin levels are normal. These neoplasms have a more aggressive clinical behavior mimicking that of gastric adenocarcinoma [11, 26]. Occasionally, they are associated with an atypical carcinoid syndrome [4, 27, 28].

Gastric NECs are highly aggressive and, usually, at an advanced stage at the time of presentation. They are rare and solitary, mainly diagnosed in men over 60 years of age. NECs are high-grade and poorly differentiated epithelial neoplasms showing neuroendocrine differentiation by morphology and immunohistochemistry. Genomic evidence suggest that NETs and NECs are unrelated neoplasms. They have the worst prognosis among all g-NENs with 50% of the patients dying within 12 months [13, 29–32].

13.2 Clinical Presentation and Prognosis

13.2.1 Type I Gastric Neuroendocrine Tumors

Type I g-NETs are the most common, accounting for 75–80% of cases. They develop in response to hypergastrinemia because of achlorhydria

secondary to autoimmune CAG where gastric acid-producing parietal cells are destroyed by an autoimmune process [19]. Less frequently, they can also arise in the setting of *Helicobacter pylori*-induced CAG [33].

Type I g-NETs mostly occur in women in the fifth and seventh decades, although with the more extensive use of endoscopy, they are increasingly diagnosed at younger age, mainly in patients with multiple autoimmune disease (most frequently autoimmune thyroid disease and type I diabetes) [34, 35].

Most of the time, type I g-NETs are incidentally observed during endoscopic procedure in patients with macrocytic or iron deficiency anemia. In fact, gastric parietal cell loss in CAG impairs iron and vitamin B_{12} absorption through a reduced acid output and intrinsic factor availability. Moreover, patients may complain of dysmotility-like dyspepsia (due to slow gastric emptying associated with CAG) or other gastrointestinal symptoms [36–39].

Endoscopically, they generally present as smooth, rounded, subepithelial, or polypoid multiple lesions in the gastric fundus or gastric body with or without central depression and ulceration [40] (Figs. 13.1 and 13.2). Gastric folds are reduced, the mucosa is atrophic, and the NETs are usually less than 10 mm in size although they can be identified only in biopsies in 22.2% of patients [41]. At endoscopic ultrasonography (EUS) g-NETs appear as hypoechoic homogeneous lesions with clear and regular margins, usually placed in the first three echo layers of the gastric wall (the mucosa and the submucosa) (Fig. 13.3) [42].

Type I g-NETs are well-differentiated NENs, they have a low to moderate proliferation grade and show a very low malignant potential with an excellent prognosis and a 5-year survival rate of almost 100% [43]. However, rare cases of metastatic spread and

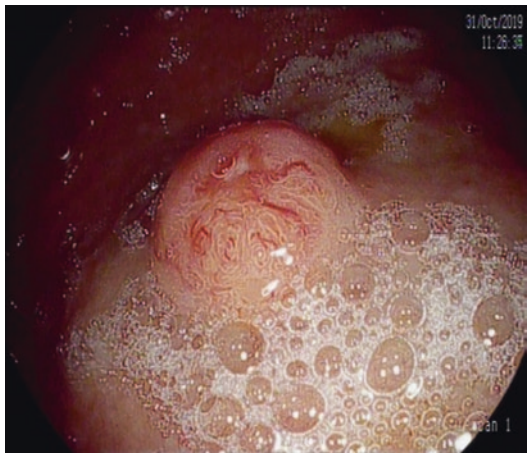

Fig. 13.1 A typical endoscopic appearance of type I g-NET with a rich superficial vascular supply

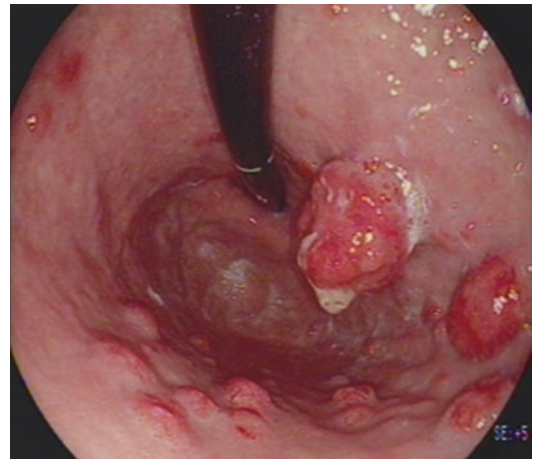

Fig. 13.2 Multiple type I g-NETs with marked atrophy of the surrounding mucosa

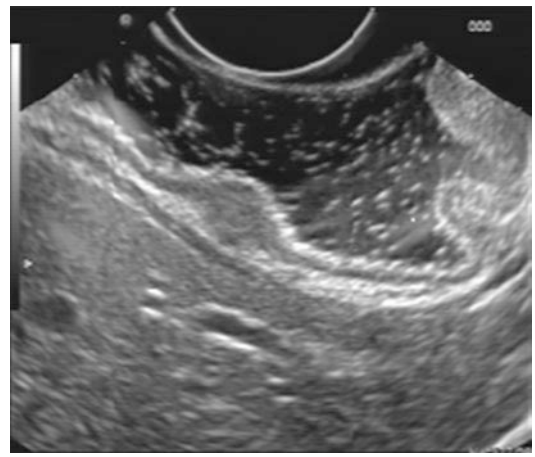

Fig. 13.3 Type I g-NET at endoscopic ultrasonography. A well-demarcated hypoechoic lesion with regular borders, placed in the first three echo layers of the gastric wall (the mucosa and the submucosa)

extraordinary tumor-related death at follow-up have been described [11, 14–16].

13.2.2 Type II Gastric Neuroendocrine Tumors

Type II g-NETs are the least common, accounting for 5–6% of cases. They develop in response to hypergastrinemia in the setting of hyperchlorhydria due to neoplastic secretion from gastrinomas, mostly in ZES-MEN1 patients, rarely in sporadic ZES [21–24]. For this reason, in type II g-NET patients, a screening for other associated tumors in the pituitary and parathyroid is required. Germline testing for MEN-1 should be considered. Type II g-NETs are equally frequent in men and women, with a clinical presentation characterized by severe peptic disease and diarrhea, both caused by an excessive gastric acid production [4, 44]. Endoscopically, they have the same presentation of type I g-NETs but with a hypertrophic background gastric mucosa (Fig. 13.4).

Type II g-NETs are well-differentiated NENs with a low to moderate proliferation grade, but unlike type I, they show a more aggressive behavior, with an increased metastatic potential (10–30% of cases) [4]. The 5-year survival rate of these patients is good (70–90%) although their prognosis is dominated by the behavior of the concomitant gastrinoma [45].

13.2.3 Type III Gastric Neuroendocrine Tumors

Type III g-NETs account for 15–20% of cases. They are generally observed in male patients over the fifth decade and are not associated with hypergastrinemia or any background gastric mucosa pathology. These NETs develop from ECL cells in most cases, in the absence of gastric mucosa ECL cells hyperplasia.

It is not uncommon that type III g-NETs diagnosis is made in asymptomatic patients when searching for a primary tumor in the setting of liver metastases of unknown origin. However, patients usually complain of pain, weight loss, and iron deficiency anemia as seen in adenocarcinoma of the stomach [29].

Mostly non-functioning, type III g-NETs are infrequently associated with an atypical carcinoid syndrome due to histamine production [4, 27, 28].

Endoscopically, they are generally larger than 2 cm and solitary with an infiltrative growth pattern, arising everywhere in the stomach on a normal-looking gastric mucosa (Fig. 13.5).

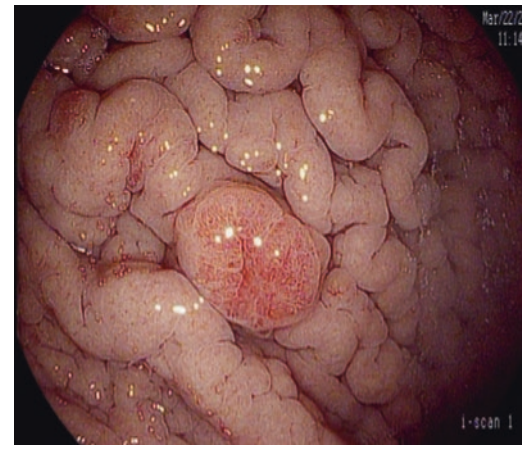

Fig. 13.4 A type II g-NET with significantly hypertrophic adjacent gastric folds in a patient with Zollinger–Ellison syndrome and multiple endocrine neoplasia type 1

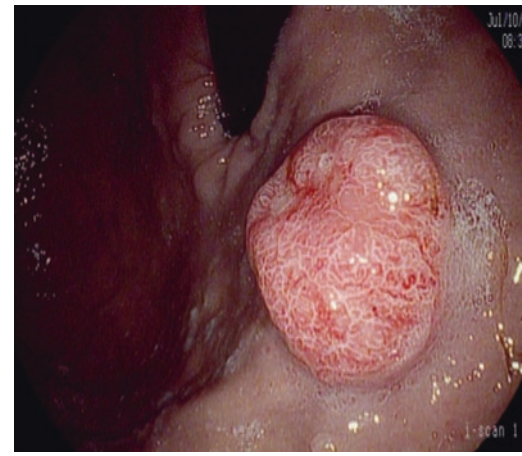

Fig. 13.5 A type III g-NET of the proximal gastric body. The lesion is larger than 25 mm, sessile, with a broad base and central depressed region

Type III g-NETs are well-differentiated NENs with a low to high proliferation grade. Frequently, at diagnosis local and distant metastases are observed (>50%). Type III g-NETs show the worst prognosis among all g-NETs with a 5-year survival rate of less than 35% [11, 17, 26, 30, 34, 46].

13.3 Diagnosis and Tumor Staging

Upper GI endoscopy with careful evaluation of the tumors is the gold standard in diagnosing g-NETs. In addition to assess site, number, and size of the lesions, it allows their adequate pathological diagnosis and characterization by the biotic sampling. Multiple random antrum, corpus, and fundus biopsies should also be taken to search for etiologic orientation, such as the presence of CAG (whose diagnosis is essential to define type I g-NETs), and to assess the presence of ECL cell hyperplasia and *Helicobacter pylori* infection. In type II g-NETs, upper GI endoscopy is also necessary to search for duodenal gastrinomas and to verify adequate control of gastric hypersecretion (healing of peptic disease) [47–49].

Endoscopic ultrasonography is recommended in g-NETs that appear resectable, except for lesions <10 mm in size, to define parietal invasion and regional lymph nodes status. Furthermore, it allows lymph node cytological assessment by fine needle aspiration. Moreover, in ZES-MEN1 patients with normal conventional imaging studies, EUS has a pivotal role to search for small pancreatic gastrinomas [47–50].

Contrast-enhanced abdominopelvic computerized tomography (CT) scan and magnetic resonance imaging (MRI) with gadolinium-enhanced and diffusion-weighted sequences are of very limited value for small type I and II g-NETs. However, they are mandatory in all patients with an increased risk of regional/distant tumor spreading such as type I and II g-NET patients with a tumor size ≥10 mm and/or muscularis propria invasion and type III g-NET patients [15, 35, 49]. Transabdominal ultrasonography can be used in situations with a very low risk of local or distant metastases. Somatostatin-receptor imaging [Somatostatin-receptor scintigraphy and 68Ga-DOTA positron-emitting tomography (PET)] should be performed in all g-NETs associated with liver metastases or if there is concern for metastatic disease or lymph node involvement [35, 49]. 18Fluorodeoxyglucose-PET is helpful in higher grade g-NETs, and its positivity is an independent poor prognostic factor [49, 51].

Laboratory tests should be performed for diagnosis and during follow-up. The measurement of gastrin values is crucial for diagnostic purposes. In patients with type I and II g-NETs, serum gastrin levels are always elevated differently from patients with type III who have normal serum gastrin levels. Hypergastrinemia is also observed in approximately one third of patients with NECs. Gastrin measurement during follow-up is not necessary. It is worth to keep in mind that PPIs alter serum gastrin levels whose dosage should be preferably performed 14 days after the interruption of these drugs (except in ZES patients, in whom PPIs must not be stopped to prevent rebound acid secretion, possibly leading to peptic ulceration and GI bleeding) [35, 52, 53].

Serum CgA levels are always elevated in type I and II g-NETs because of the hypergastrinemia-induced ECL cells hypertrophy/hyperplasia. For this reason, the measurement of this biochemical marker is not necessary neither for the diagnosis nor during the follow-up of these patients. However, in patients with type III g-NETs in which serum gastrin levels are normal and liver metastases are frequently observed, plasma CgA may be useful [35]. In fact, it is well known that CgA has a higher sensitivity for metastatic NETs in comparison with localized NETs [54]. CgA false-positive results may be observed during treatment with PPIs or in patients with heart disease and severe kidney failure [55]. As well as serum gastrin levels, serum CgA evaluation should be preferably performed 14 days after PPI interruption (see the above comment about PPI withdrawal) [54, 56].

Urinary 5-hydroxy-indolacetic acid dosage should also be considered in type III g-NET

patients in the rare cases with associated symptoms suggestive of the carcinoid syndrome.

In patients with type I g-NETs anti-parietal cell and anti-intrinsic factor antibodies should be evaluated in the context of autoimmune CAG. *Helicobacter pylori* should be searched because its eradication may modify the natural history of gastric atrophy [17, 57].

It must be highlighted that is of paramount importance that patients with type I g-NETs, particularly if elderly, are screened for iron and vitamin B_{12} deficiency at diagnosis and mainly during follow-up. In fact, iron deficiency anemia has been found to be the presenting feature in more the 50% of CAG patients, whereas vitamin B_{12} deficiency is frequently observed in these patients and can be responsible of significant health consequences (neurological, cognitive, psychotic, and mood impairment) [57].

Thyroid function, thyroid peroxidase antibodies, and thyroglobulin antibodies should be assessed in type I g-NETs because of the possible association of autoimmune CAG with autoimmune thyroiditis [41, 57].

13.4 Treatment and Follow-Up

An expert NEN-dedicated multidisciplinary team should be involved to individualize treatment.

13.4.1 Localized Disease

13.4.1.1 Type I Gastric Neuroendocrine Tumors

Due to the indolent course of type I g-NETs, a conservative management is to be preferred over surgery [48]. In these patients, tumor size ≥1 cm is a potential predictor of lymph nodal metastases and should be the lesion characteristic considered first when their management is planned [14, 15].

Lesions <1 cm should be removed without any additional evaluation, although nothing suggests a less favorable evolution if they are left in place and followed up [58]. Endoscopic resection is the treatment of choice for these tumors, ranging from polypectomy and endoscopic mucosal resection (EMR) to endoscopic submucosal dissection (ESD) [48].

Complete resection of g-NETs is difficult with conventional polypectomy because most of them are not confined to the mucosa but, rather, they invade the submucosa, resulting in frequent involvement of the resection margins. This might account for the high recurrence rates observed in some series [41]. EMR and ESD can satisfactorily achieve the en bloc resection of these lesions without any difference in complication (bleeding and perforation) incidence, although ESD is more time-consuming than EMR [59–62]. However, the rate of vertical resection margin involvement has been observed to be significantly lower in the ESD-treated lesions than in those treated with EMR [61, 62]. Moreover, EMR and ESD might be used to resect remnant tumor after an initial incomplete endoscopic resection as observed in incompletely resected rectal NETs [63, 64].

Recently, a novel endoscopic therapeutic technique, the endoscopic full-thickness resection (EFTR), has been used for the treatment of gastric subepithelial tumors. ETFR allows a full-thickness resection of the gastric wall showing interesting results for the treatment g-NETs [65].

In the case of type I g-NETs ≥1 cm, CT scan or MRI is necessary to rule out lymph nodal and/or distant metastases. EUS evaluation is mandatory to exclude invasion beyond the submucosal layer or regional lymph nodal invasion. If the lesions do not reach the muscularis propria layer, then endoscopic resection, preferably using the ESD technique, should be performed [48].

After endoscopic resection, an endoscopic surveillance is required. First, because type I g-NETs are recurring disease. Second, because of the underlying CAG, to monitor the risk of development of intestinal metaplasia, dysplasia, and adenocarcinoma [66, 67]. Endoscopic surveillance is suggested every 12 months for patients with recurring neoplasms and every 24 months for those with non-recurring lesions [35, 41].

Surgery (wedge resection or total gastrectomy with lymphadenectomy) should be considered for lesions not amenable to endoscopic resection

(lymph nodal and/or distant spread, extensive multifocal diffusion), in case of involvement beyond the submucosa (at EUS or at pathological examination of an endoscopically resected tumor), in the presence of positive margins after endoscopic resection and if vascular and/or lymphatic invasion are observed [48]. Any surgical treatment should be planned considering patient-related parameters (age, comorbidity) and the well-known usually indolent course of type I g-NETs also in the presence of recurrence and local or distant spread [14, 41, 59].

Antrectomy is a further surgical option for the treatment of type I g-NETs. It can be considered for extensive recurrent or multifocal lesions not amenable of less invasive treatment [48]. Antrectomy removes the source of the hypergastrinemia which is the cause of ECL cell hypertrophy/hyperplasia and, ultimately, ECL cell NET development [28]. Patients treated with antrectomy have a lower risk of recurrence and need fewer follow-up endoscopies than those treated with endoscopic resection [68]. However, given the evidence that some lesions recur after hypergastrinemia interruption, the improvement in endoscopic techniques, the complications and side effects of surgery, and the possibility of medical treatment, its use is debated and rarely practiced [48, 69].

Long acting somatostatin analogs (SSAs), because of their antiproliferative, antiangiogenetic, and antisecretive effects, are widely used as a medical treatment of both functioning and non-functioning NENs [70]. They inhibit gastrin release from antral G cells suppressing hypergastrinemia, the leading cause of ECL cell NET development, and directly inhibit endocrine cells proliferation. When administered continuously, SSAs have been demonstrated to reduce the number and size of type I g-NETs. However, after their withdrawal, lesions recur early and increase in size [71–76]. SSAs must be given by injection and are generally well tolerated, although some adverse drug reaction (ADR) such as diarrhea, headache, gallstones development, and hyperglycemia are non-infrequently observed [70]. Because of the high costs of SSAs, their ADR profile and the usually excellent prognosis of most type I g-NET patients, these drugs might be proposed in selected cases, as for recurrent or multifocal lesions and when endoscopic resection is not feasible or radical. Randomized controlled trials comparing SSA treatment efficacy to endoscopic management are needed. ENET guidelines suggest their use only according to expert opinion [35, 48].

Another potential medical option in type I g-NET treatment is Netazepide, an orally active, highly selective, competitive gastrin/cholecystokinin 2 receptor antagonist. In 16 patients treated once daily for 12 weeks, it significantly reduced the number of tumors, the size of the largest tumors, and the circulating CgA within the normal range. Serum gastrin values were unaffected. Netazepide is safe and well tolerated; however, the tumors regrow quickly after the drug is discontinued [77, 78]. The same results in terms of efficacy, safety, and tolerability were observed in 13 patients treated with netazepide daily for 52 weeks. It is interesting to note that also circulating CgA increased again after netazepide was stopped. ECL cells, both in g-NETs and in CAG, are the source of CgA, and its normalization is consistent with netazepide inhibiting ECL cell growth. Thus, CgA might be used to monitor treatment [79].

Despite these initial favorable experiences, placebo-controlled studies in a larger number of patients and for a longer time are needed to confirm the use of netazepide for the treatment of type I g-NETs.

13.4.1.2 Type II Gastric Neuroendocrine Tumors

Even more than in type I g-NETs, treatment strategy of type II g-NET patients should be planned in a NEN-dedicated multidisciplinary team. Their management needs to be individualized and to be approached in the context of MEN-1 syndrome whose treatment is first influenced by the presence of duodenal or pancreatic gastrinomas for whom surgical resection is recommended whenever it is possible.

Because of the more aggressive clinical behavior than type I g-NETs, type II should always be treated, and local or limited excision

are recommended. Endoscopic resection is reserved for lesions limited to the gastric wall and without invasion beyond the submucosa otherwise surgery is recommended. As in type I g-NETs, further treatments will be evaluated in relation to the pathological examination of the resected lesions. For the endoscopically successfully managed patients, endoscopic surveillance is suggested yearly [18, 35, 48].

Some case series have shown that SSA treatment resulted in reduction in size and number of type II g-NETs [80].

In type II g-NET patients, high-dose PPI therapy is mandatory to control acid hypersecretion and to prevent life-threatening complications from peptic ulceration [81].

13.4.1.3 Type III Gastric Neuroendocrine Tumors

At diagnosis, most of type III g-NETs show invasion beyond the submucosa, lymphoinvasion, angioinvasion, and local or distant spread. They should be managed aggressively following the same guidelines for gastric adenocarcinomas. Resectable disease often undergoes partial or total gastrectomy with lymphadenectomy [48].

Endoscopic management by means of EMR or better with ESD for small (generally ≤2 cm) type III g-NETs might be considered as initial treatment if an appropriate and careful preoperative staging is unremarkable. The pathological examination of the resected lesion will dictate the need for further treatments [60, 82, 83]. A close endoscopic and radiological (CT scan or MRI) follow-up is then mandatory for these patients.

13.4.2 Advanced Disease

Treatment options for advanced g-NETs, include SSAs, systemic chemotherapy and molecular targeted agents. Liver metastases can be treated also with locoregional therapies (transarterial chemoembolization and radiofrequency ablation), peptide-receptor radionuclide therapy, and surgery [84].

The treatment strategy should be planned on a case-by-case basis and discussed by an expert NEN-dedicated multidisciplinary team. Previous treatment, cumulative toxicity, the impact of treatment on patient's quality of life and the long survival of g-NETs must properly weighted.

13.5 Conclusions

Gastric neuroendocrine tumor diagnosis is on the rise, and they are more frequently diagnosed at an early stage, allowing a conservative approach for most of them. Based on pathophysiology, three types of g-NETs are recognized. Type I are the most frequent and associated with CAG. They are slow-growing neoplasms with an excellent prognosis also in the presence of local or distant spread which is infrequently observed. Endoscopy is a powerful and suitable technique to manage most of them in terms of both diagnosis/staging and treatment. Because of CAG, it is of paramount clinical relevance to screen type I g-NET patients for micronutrients deficiency and gastric adenocarcinoma development.

Types II and III g-NETS are less frequently observed, but they behave more aggressively. The former, usually managed as type I, should be approached in the context of MEN-1 syndrome. The latter have the worst prognosis among all g-NETs. They are surgically managed although the endoscopic resection may be adequate in selected cases.

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14 Treatment of Pancreatic Neuroendocrine Tumors

Carlo Carnaghi and Elettra Merola

14.1 Introduction

This chapter focuses on the medical treatment of metastatic pancreatic neuroendocrine tumors (PanNETs) not suitable for curative surgical treatment or loco-regional therapies. In this setting, medical treatment is the cornerstone for improving survival and preserving the quality of life.

Over the last 10 years, the landscape of medical treatment of PanNETs changed completely not only because of the availability of several new effective drugs but also for the better comprehension of tumor biology which emphasized that PanNETs are heterogeneous tumors requiring individualized approaches.

Presently, the clinical management of metastatic PanNETs is a challenge. The low number of randomized and sequence trials implies a low level of evidence for therapeutic options as the lack of predictive biomarkers of response and survival does not allow the patient's selection.

This algorithm and treatment combinations can be argued, and it is recommended to discuss PanNET patients in a dedicated and specialized multidisciplinary tumor board.

The different therapeutic medical options will be detailed below according to recent data.

14.2 Somatostatin Analogs

Somatostatin analogs (SSAs) are usually adopted as the first line in advanced gastroenteropancreatic NETs (GEP-NETs) expressing somatostatin receptors. Standard doses are octreotide LAR 30 mg every 4 weeks and lanreotide extended-release (autogel) 120 mg every 4 weeks.

Their antiproliferative effect in PanNETs G1-G2 is based on the results of the CLARINET trial [1], which included a subgroup of 91 patients with the pancreatic primary site and Ki67 up to 10% and suggested a benefit in the lanreotide arm when compared to placebo (median PFS, "not reached" vs. 12.1; HR, 0.58), with a good safety profile. Most of the adverse events were mild, mainly represented by diarrhea, hyperglycemia, and cholelithiasis. An ongoing trial enrolling only PanNETs with Ki67 < 10% treated with lanreotide will provide more details about tumor control by SSAs in this subset of patients (NCT03947762).

Although SSAs are currently adopted also for PanNETs with Ki67 > 10%, data about the efficacy of this therapy in this subgroup of patients are still scanty. From the real-world setting, Jann

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H [2] showed, in a retrospective analysis of seven patients treated with octreotide LAR, median time to progression is 4 months (95% CI, 1.434–6.566), but the metastatic pattern or further patients' features were not detailed. Preliminary results from a retrospective, cooperative study [3] presented at the 17th ENETS Conference have shown a benefit from SSAs in 59 metastatic PanNETs with Ki67 10%–35%, with a median PFS of 11.9 months (95% CI, 6.7–14.1 months), and 5-year OS rate of 64.2% (SE:8.9%). In this series, the hepatic tumor burden has been confirmed as a prognostic factor for disease control with SSAs.

An increase in SSA doses at disease progression is also adopted in clinical practice for PanNETs, although data in literature supporting this therapeutic strategy derives from retrospective cohorts. A very recent publication by Diamantopoulos [4] retrospectively analyzed the clinical outcomes of GEP-NETs treated with SSA administration every 3 weeks, observing in 105 included patients a median PFS of 25 months (95% CI: 16.9–33.1) and disease control rate in half of the population. Results from a prospective study will provide soon further data about this indication (NCT02651987).

14.3 Peptide Receptor Radionuclide Therapy

Peptide receptor radionuclide therapy (PRRT) exploits the expression of somatostatin receptors, binding radiolabeled somatostatin analogs. Toxicity usually includes transient nausea and vomiting, hematologic events (e.g., lymphopenia in 9%, thrombocytopenia in 2%, leukopenia in 1%, and neutropenia in 1%) [5].

The NETTER-1 trial [5] is the largest prospective study regarding this treatment. It randomized 229 patients with well-differentiated, metastatic midgut NETs to receive 177Lu-Dotatate (116 patients) plus best supportive care including octreotide long-acting vs. octreotide alone (113 patients). Progression-free survival at month 20 was 65.2% (95% CI, 50.0–76.8) in the 177Lu-Dotatate group and 10.8% (95% CI, 3.5–23.0) in the control group. The response rate was 18% in the 177Lu-Dotatate group vs. 3% in the control group ($P < 0.001$). Although PRRT has been approved for GEP-NETs thanks to the NETTER-1 study, pancreatic cases were not enrolled in this trial. Data investigating the efficacy of this treatment in PanNETs derive from retrospective cohorts [6], and just a few of them focused on pancreatic cases only. The reported median disease control rate was 83% (range, 50%–94%), median PFS ranged from 25 to 34 months, and median OS ranged from 42 to 71 months [7].

Sharma et al. [8] described a median time to progression from the first PRRT cycle of 37.0 months (6.5–48.0) for PanNETs, with a median OS of 37.3 months (18.1–48.0). Zandee et al. [9] described, in 34 metastatic, functioning PanNETs, median progression-free survival was 18.1 months (interquartile range: 3.3 to 35.7); disease control rate was reached in 78% of cases with baseline progression, and in 71% of patients with uncontrolled symptoms, a clinical improvement. Regarding toxicity, subacute hematological toxicity, grade 3 or 4 occurred in four patients (12%) and a hormonal crisis in three patients (9%).

No phase III trials on PRRT in PanNETs are currently available, while some trials enrolling also pancreatic cases are ongoing, investigating the association of PRRT with capecitabine (NCT02736448, NCT02736500, NCT02358356, NCT04194125) or the comparison with everolimus (NCT03049189).

The role of PRRT as a neoadjuvant treatment for PanNENs has been explored in a few retrospective series, suggesting an increase in the stroma and the preservation of somatostatin receptors expression after treatment [10]. An ongoing trial will provide prospective results about this PRRT application (NCT04385992).

14.3.1 Everolimus

Everolimus is the most extensively investigated target of rapamycin (mTOR) inhibitor in NETs. The standard dosage is 10 mg/day as continuous

oral intake, with a positive impact on response rates and OS for cumulative dose >3000 mg [11]. In the case of toxicity, a dose adjustment to 5 mg/day is possible. This targeted drug is approved for progressive GEP-NETs and lung carcinoids as a result of several clinical studies, also supported by "real-life" experiences.

A phase II trial (RADIANT-1) [12] focused on metastatic pancreatic NETs (PanNETs) after chemotherapy failure and assessed the efficacy in tumor control of both everolimus alone (10 mg/die) and in combination with LAR octreotide, observing a median PFS of 9.7 months and 16.7 months, respectively.

The phase III RADIANT-3 study [13] enrolled 140 PanNETs with disease progression in the last year and investigated the real benefit from everolimus in comparison to placebo. A significantly different median PFS was described between the two subgroups: 11.0 vs. 4.6 months, respectively ($P < 0.01$).

The efficacy of everolimus in advanced progressive NETs and the good safety profile were confirmed also in a "real-life" setting [14]. In 169 patients treated in "compassionate use," median PFS was similar to the RADIANT-3 study (12 months). Median OS was 32 months, and similar disease control rates were observed for PanNETs and non-PanNETs. Toxicity was acceptable but higher for patients previously treated with PRRT and chemotherapy, suggesting to adopt everolimus before these other options.

Bajetta et al. [15] adopted the combined regimen of everolimus and LAR octreotide in naïve advanced NETs, with positive conclusions. In detail, 18% and 74% of the 50 cases showed objective response and disease stabilization for at least 6 months, respectively.

The efficacy of everolimus associated with SSA was also verified in a "real-world" setting by a Spanish study [16], retrospectively analyzing the outcome of 57 NETs, describing a median time to progression of 25.8 months.

The COOPERATE-2 study [17] compared the antiproliferative effect of everolimus alone vs. everolimus + pasireotide in advanced progressive PanNETs. Grade 3/4 fasting hyperglycemia was respectively reported in 11% and 37% of patients. Furthermore, no significant difference was observed between the two groups in terms of PFS, OS, or disease control rates. Considering these results and toxicity profile, this drug combination has shown less encouraging results than the other SSA formulations.

Focusing on chemotherapy, the combination of everolimus and temozolomide offered interesting results in advanced PanNETs. In 40 patients [18] treated with these two therapies for 6 months, no synergistic toxicities were observed, and 40% of patients experienced a partial response. The median PFS rate was 15.4 months, while the median OS was not reached.

An ongoing trial is currently recruiting advanced progressive NETs to receive everolimus in comparison to PRRT (NCT03049189).

Preclinical studies had suggested a potential antiproliferative effect of everolimus also in G3 cases [19]. Experiences were so far mainly based on well-differentiated cases, e.g., the NET G3 patients [20]. A 15 cases NET-G3 series was reported [20] (including 4 naive cases) with ki67 ranging from 20% to 55%. Median PFS was 6 months, and median OS was 28 months. Disease stabilization was maintained in 40% of cases for at least 12 months. These encouraging results have been so far reached only in a retrospective setting. However, two trials are investigating the efficacy of everolimus in G3 patients: the EVINEC study (NCT02113800), using everolimus for G3 neuroendocrine patients after platinum-based chemotherapy failure; results from another trial (NCT02248012) will show the potential synergy of everolimus and temozolomide in G3 NET patients (ki67: 20%–55%).

As far as toxicity of everolimus is concerned, most common adverse events are represented by aphthous ulceration, abdominal pain, perimalleolar edema, and fatigue. A meta-analysis of RCTs [21] described stomatitis in 67% of patients with solid tumors treated with everolimus. A longer PFS was observed in patients experiencing stomatitis at first after 8 weeks from treatment start when compared to cases not presenting stomatitis. Other possible symptoms can be diarrhea, rash, bone marrow toxicity, and metabolic impairment (increase in cholesterol, risk of

diabetes especially if associated with SSAs), risk of infections (sometimes leading to sepsis), and frequent respiratory tract. A typical everolimus complication is the occurrence of interstitial pneumonitis in 10.4% of patients, as reported by a meta-analysis of 2223 patients extracted from RCTs [22].

14.4 Sunitinib

Sunitinib is an oral multikinase inhibitor competing with ATP for binding within the intracellular domain of various wild-type and/or mutated receptor tyrosine kinases. It is currently approved for advanced progressive PanNETs at a standard oral daily dosage of 37.5 mg.

Following the first phase II study adopting this drug in NETs [23] with a schedule of a 6-week cycle (50 mg/d for 4 weeks, then 2 weeks off treatment), Raymond et al. [24] performed a double-blind phase III RCT enrolling 171 advanced progressive PanNETs, treated with sunitinib or placebo. The trial was discontinued early due to significantly different outcomes and toxicity between the two arms. Median PFS was 11.4 months with sunitinib group vs. 5.5 months with placebo, while rates at 6 months were 71.3% vs. 43.2% ($P < 0.01$). Objective response rate respectively was 9.3% vs. 0%, with disease control rate in 72% and 60%. Rate of OS at 6 months respectively was 92.6% vs. 85.2% ($P = 0.02$).

Some data about sunitinib comes from "real-world settings." In a study [25] adopting sunitinib in 21 advanced progressive PanNETs, the disease control rate was reached in 57% with a median PFS of 7.0 months. In a Chinese monocenter series [26], 18 progressive PanNETs treated with sunitinib had a median PFS of 12 months, with disease control rate in 77.8%–88.9%.

The combination of sunitinib with SSA [16] adopted in 50 NET patients lead to a "not reached" median PFS, with disease control in 86% of cases. Conclusions reporting a better outcome than RCT [24] might suggest a synergistic effect of the combination therapy, but they come from "real-world" studies and may be limited by retrospective design including heterogeneous population.

Sunitinib was also investigated to potentiate tumor control after transarterial hepatic embolization (TAE) in NETs [27]. In 23 enrolled patients, a 34% increase of circulating VEGF was observed after TAE. Sunitinib was administered for 1 year after the procedure, with a median PFS of 15.2 months and an overall response rate of 72%. The authors concluded that sunitinib can be safely administered after the embolization procedure.

The most frequently reported adverse events during sunitinib treatment are gastrointestinal symptoms (diarrhea, nausea, vomiting) in 33%–59% of PanNETs and fatigue (41% of patients) [28, 29]. Other possible side effects can be the "hand-foot syndrome," hypertension, headache, and neutropenia.

A re-analysis [30] of the phase III RCT by Raymond et al. [24] has shown no significant difference in the quality of life between treatment and placebo arm during the study. Only a worsening of diarrhea affected patients receiving sunitinib ($P < 0.05$ vs. placebo) that besides this side effect significantly provided a benefit in PFS without adversely worsening patient life.

Regarding G3 cases, literature reports only a small series of poorly differentiated pancreatic G3 carcinoma [31]. These five naïve NEC refused chemotherapy and received standard-dose sunitinib (37.5 mg/day, 5 patients) associated with SSA (30 mg Sandostatin LAR monthly). Toxicities (acute and late) were manageable, and no toxicities were needed to stop the treatment. All patients progressed within 15 months and presented a minimum OS of 24 months. Further cohorts are surely needed to validate this experience.

14.5 Locoregional Treatments for Liver Metastases

Locoregional treatments for hepatic metastases include several approaches, such as surgery, radiofrequency ablation (RFA), hepatic arterial embolization (HAE), radioembolization, or

radiation. In a recent series of 69 GEP-NETs treated with local treatment for focal progression, the median time to new systemic treatment was 32 months (95% CI, 16.5–47.5 months). The median time to any additional intervention was 19 months (95% CI, 8.7–25.3 months) [32].

In a retrospective series including 71 metastatic PanNETs receiving embolotherapy, median PFS was 9.3–18.5 months, and hepatic tumor burden was shown as a prognostic face for tumor response.

As far as radioembolization is concerned, Su et al. [33] described a cirrhosis-like morphology in 26.7% of patients, ascites in 13.3%, and varies in 6.7%.

An ongoing trial is investigating tumor control by locoregional treatments in metastatic GEP-NETs (NCT02724540).

14.6 Chemotherapy

Chemotherapy represents the standard for PanNET situations in which the objective is to downsize the disease that may be necessary to plan surgery or to alleviate the symptoms related to tumor burden. The most studied category of drugs are alkylants including streptozotocin, dacarbazine, and temozolomide [34–36]. Prior to the development of PRRT, chemotherapy represented conventional second-line therapy after somatostatin analog failure. The first evidence of the efficacy of streptozotocin dates back to 1968 when its efficacy was documented on the control of syndrome and on the control of tumor growth in a malignant insulinoma. Its usefulness in combination with 5-fluorouracil and doxorubicin has therefore been studied in a randomized trial conducted by 105 patients from Moertel [34]. Despite the methodological limitations of this study, since the 1990s the combination regiments based on streptozotocin have remained the standard of care for metastatic PanNETs. However, it should be stressed that the high response rates observed by Mortel with the combination regimens (overall response rate 69% and 45%) have not been confirmed in subsequent studies and that to date they are not the only predictive response criterion identified is a proliferative activity >5% [37]. Furthermore, the combination regimens, especially with anthracyclines, are characterized by rather high toxicity that does not allow their prolonged use over time [38]. With the aim of improving the tolerability profile, various authors have tested combination regiments where streptozotocin was replaced by dacarbazine, another alkylating agent [35, 39]. The results obtained were interesting and apparently similar to those observed with streptozotocin, but, in the absence of large randomized studies, the chemotherapy of choice for PanNETs are always the combination regimens with streptozotocin. In the last 10 years, attention has therefore been shifted to temozolomide, an oral prodrug of dacarbazine, already widely used in brain tumors and melanoma. The drug, whose main toxicity is represented by myelotoxicity, was developed first in monotherapy and subsequently in combination therapies mainly with capecitabine, an oral prodrug of 5-fluorouracil [36, 40, 41]. The data initially observed by Fine on 18 patients and confirmed in a subsequent phase II study on 30 patients showed that in addition to being well-tolerated, the combination is particularly active (ORR 61%, 2-year survival 92%) [42]. A phase II randomized study not yet published but of which the final data presented at ASCO 2018 is available, compared to temozolomide monotherapy with the combination of temozolomide and capecitabine. The progression-free survival (PFS) figure was favorable for the combination regiment (22 vs. 18 months) despite the presence of similar response rates between the two regiments [43]. It is difficult to define the impact on the survival of these regimens as PanNETs can not only behave very differently from each other but at the same time, the evolution of knowledge makes available to the clinician a series of treatment options to be used ideally sequentially in order to maximize the ultimate survival benefit.

Unfortunately, also for temozolomide as for the other alkylating agents, there are no certain predictive response criteria. MGMT, an enzyme involved in repairing DNA damage, has been studied extensively. The hypothesis was that his

deficit could be associated with a good response to temozolomide. Kulke's retrospective data supported this hypothesis, however, confirmed by subsequent studies [44–46]. In the absence of large prospective randomized MGMT studies, it cannot yet be considered a validated predictive response criterion.

With regard to platinum agents, their effectiveness should be restricted to the NEC/NET G3 group only. In fact, the few experiences conducted with the traditional combination of cisplatin and etoposide in well-differentiated forms have proved very unsatisfactory due to the poor efficacy shown by this regiment [47].

14.7 Conclusions

Medical treatments are still the cornerstone for patients with metastatic or advanced PanNETs unsuitable for surgical or locoregional treatments. Choosing the optimal treatment for each individual patient is difficult not only due to the multiplicity of treatment options (somatostatin analogs, chemotherapy, target therapies, PRRT) but also due to the lack of randomized studies.

In order to define the optimal treatment, clinicians are asked to carefully analyze the data studied by integrating these data with the patient and tumor characteristics and updated scientific guidelines. This process should be conducted as part of a multidisciplinary tumor board including a panel of expert clinicians with specific expertise in NETs.

The decision-making process must be based on some key elements: the Grade (G 1,2,3), the proliferation index (Ki67), expression of somatostatin receptors, the rate of growth of the disease over time, and the presence of tumor-related symptoms.

Watch and wait strategy is not commonly used in metastatic pancreas neuroendocrine tumors. The only exception is for asymptomatic patients with a low tumor burden where they have not progressed over the last 12 months. For well-differentiated (G1–2), SSTR-positive, low-growth (ki67% <10%) PanNETs, the treatment of choice is somatostatin analogs (lanreotide or octreotide) that can be useful also as a second-line treatment at increased doses [1, 48].

The most difficult therapeutic choice is how to treat a metastatic SSTR-positive G2 PanNET with KI67 > 10%. In this setting, the use of everolimus, sunitinib, chemotherapy, and PRRT is adequately supported by scientific data, and there are no clear criteria that favor the choice of one treatment over the other [7, 9, 13, 24].

In the presence of patients where a rapid tumor shrinkage is needed (i.e., presence of symptoms, imminent risks of visceral failure, bringing inoperable patients to a surgical program), chemotherapy with CAP-TEM regimen seems like the right choice due to the high rate of objective response [42, 43]. In this setting also PRRT seems to be effective, but it takes more time to achieve significant tumor shrinkage.

The use of everolimus and sunitinib is supported by strong scientific evidence from large randomized studies that achieved very similar results in terms of progression-free survival, overall survival, and low response rate. Due to the lack of predictive factors, the choice between these two options must be made on the basis of the different toxicity profiles of the two drugs [13, 24].

Regarding G3 PanNETs, it should clearly be distinguished between well-differentiated tumors (NET) and poorly differentiated carcinoma (NEC). Very limited prospective data are available for the treatment of G3 PanNETs, and guidelines suggest a therapeutic approach similar to G2 PanNETs with ki67 > 10% [49].

Finally, in G3 PanNEC, platinum-based chemotherapy is the only available treatment option although the results in terms of overall survival are disappointing [50].

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Treatment of Intestinal NETs (Including Appendix)

15

Francesco Panzuto and Maria Rinzivillo

15.1 Introduction

The treatment of intestinal neuroendocrine tumors (NETs) remains a challenge for physicians, requiring a multidisciplinary approach and a tailored patient's evaluation [1]. Prognosis of this disease depends on a number of factors, including specific primary tumor site, tumor grade (expressed as Ki67 value), staging, and expression of somatostatin receptors (sstr). Among these factors, grading is widely considered the most powerful, with significant role in terms of predicting tumor behavior and patients' prognosis. The recent WHO 2019 classification identifies four different categories, based on Ki67 values and tumor differentiation [2]: NET G1, well-differentiated morphology and Ki67 < 3%; NET G2, well-differentiated morphology and Ki67 3–20%; NET G3, well-differentiated morphology and Ki67 > 20%; NEC G3, poorly differentiated morphology and Ki67 > 20%. The majority of intestinal NETs are included in NET G1 to NET G2 groups, whereas NET G3 and NEC are considered rare entities. Tumor spontaneous behavior, response to treatments, and thus patient's clinical outcome strictly depend on grading. In fact, in some cases, intestinal NETs may present as indolent, slow-growing diseases, whereas in other cases, tumor may be more aggressive resulting in a worse clinical outcome. From a clinical point of view, intestinal NETs may be divided into two major categories: "functioning tumors" when a specific clinical syndrome (usually a carcinoid syndrome, mainly characterized by diarrhea and flushing, with cardiovascular disease and difficult to breath in advanced stage) related to secretion of active substances by the tumor exists; otherwise, the tumor is defined as "non-functioning" when only generic mass-related symptoms are present.

Irrespective of the tumor functionality, intestinal NETs are commonly diagnosed at advanced stage, with distant metastases which are most frequently found in up to 75% of patients [3, 4]. Nevertheless, long-term survival rates are fairly good, ranging from 45% to 75% depending on the above-mentioned prognostic factors [4–6] and the efficacy of therapeutic management.

Current scientific evidences demonstrate a range of efficient therapies to treat advanced intestinal NETs, including somatostatin analogs (SSAs), targeted therapy, peptide-receptor radionuclide therapy (PRRT) (Table 15.1, Fig. 15.1) which, in addition to surgery and liver-directed ablative treatments, need to be carefully considered when approaching the therapeutic sequence of these patients.

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Table 15.1 Randomized-controlled trials performed in advanced NENs over time

Reference	Number of patients evaluated	Drug used	Main finding
Arnold et al. CGH 2005 [7]	105 gastrointestinal and pancreatic	Octreotide vs. octreotide + interferon	Combination treatment was not superior to monotherapy concerning progression-free and long-term survival
Yao et al. JCO 2017 [8] (SWOG study)	427 gastrointestinal	Octreotide + interferon vs. octreotide + bevacizumab	No significant differences in PFS were observed between the bevacizumab and IFN arms
Faiss et al. JCO 2003 [9]	80 gastrointestinal and pancreatic	Lanreotide vs. interferon vs. lanreotide + interferon	Comparable antiproliferative effects among the three arms
Kolby Br J Surg 2003 [10]	68 midgut carcinoids	Octreotide alone vs. octreotide plus interferon	Addition of IFN-α to octreotide may retard tumor growth in patients with midgut carcinoid tumors
Rinke et al. JCO 2009 [11] (Promid study)	85 midgut carcinoids	Octreotide vs. placebo	Octreotide LAR significantly lengthens time to tumor progression compared with placebo
Caplin et al. NEJM 2014 [12] (Clarinet study)	73 midgut (total)	Lanreotide 120 mg vs. placebo	Lanreotide was associated with significantly prolonged progression-free survival among patients with metastatic gastroenteropancreatic neuroendocrine tumors of grade 1 or 2
Strosberg et al. NEJM 2017 [17] (Netter-1 study)	229 midgut carcinoids	[177Lu]Lu-DOTA-TATE vs. high dose octreotide	^{177}Lu-Dotatate resulted in markedly longer progression-free survival
Pavel et al. Lancet 2011 [24] (Radiant-2 study)	224 small intestine	Octreotide + everolimus vs. octreotide + placebo	Everolimus plus octreotide LAR, compared with placebo plus octreotide LAR, improved progression-free survival in patients with advanced neuroendocrine tumours associated with carcinoid syndrome
Yao et al. Lancet 2016 [25] (Radiant-4 study)	302 gastrointestinal or lung	Everolimus vs. placebo	Everolimus was associated with significant improvement in progression-free survival

For each study, the number of enrolled patients, the therapeutic schedule, and the main finding are reported. Further data may be found in the text

15.2 Medical Treatment for Advanced Disease

15.2.1 Somatostatin Analogs

Synthetic somatostatin analogs octreotide and lanreotide are widely considered the first-line therapy for patients with well-differentiated G1 and G2 intestinal NETs. Up to 90% of NETs carry sstr on tumor cell membrane and are therefore optimal candidate to receive SSAs-based therapy. Since their introduction in the early 1980s, they showed a clear activity to improve diarrhea and flushing in patients with carcinoid syndrome, as well as to decrease tumor markers chromogranin A and urinary 5-HIAA, by inhibiting the release of neuropeptide. In the following years, several retrospective and phase 2 trials proposed their ability to reduce tumor growth in patients with well-differentiated NETs. The antiproliferative activity of octreotide was definitively demonstrated in 2009, when the phase 3 PROMID trial was published [11]. In that trial, a clear benefit in terms of progression-free survival (PFS) was observed in patients with advanced "midgut carcinoid" (mainly small intestine NETs) receiving octreotide LAR 30 mg every 4 weeks, compared with placebo, with a 66% reduction in risk of disease progression. This figure was confirmed and further corroborated in the CLARINET study [12], the largest phase 3 trial ever published on SSAs and NETs, including 204

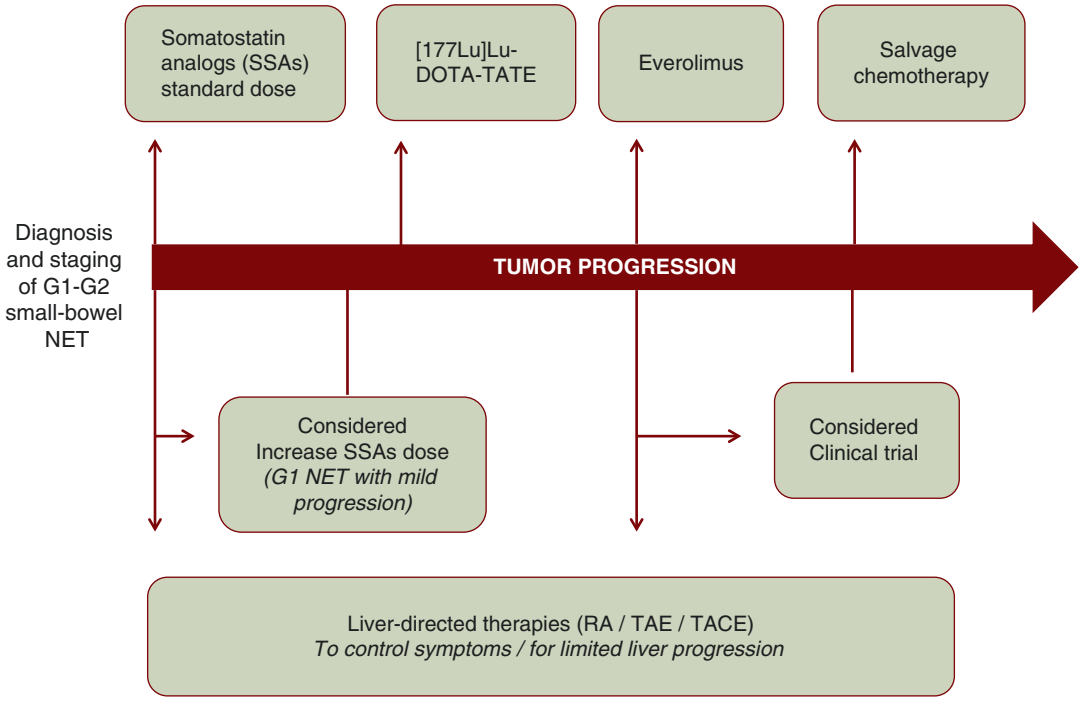

Fig. 15.1 Proposed therapeutic algorithm for the treatment of unresectable well-differentiated G1–G2 small-bowel NET

patients with advanced well-differentiated NETs rising from gastrointestinal tract or pancreas. Lanreotide extended-release (autogel) at a dose of 120 mg every 4 weeks significantly decrease the risk of tumor progression compared with placebo (−53%), even in those tumors with relatively high proliferation (NET G2 with Ki67 < 10%) or presenting metastatic liver involvement >25%. Both trials showed an excellent safety profile in patients receiving SSAs, most frequent serious adverse events (AEs) being diarrhea, abdominal pain, cholelithiasis, and flatulence.

Given the findings reported by those phase 3 trials, octreotide LAR 30 mg/4 weeks and lanreotide extended release (autogel) 120 mg/4 weeks are recommended by international guidelines as first-line therapy for well-differentiated, slow-growing NET G1 and G2 gastro-entero-pancreatic NETs expressing sstr [13, 14].

Meanwhile, several studies have suggested that an escalation of SSAs dosage might provide additional antiproliferative activity compared with the above-mentioned standard doses. Higher doses of SSAs, also referred to as non-conventional SSA doses, are achieved by either increasing administered dose (increased dose intensity; e.g., octreotide LAR 60 mg) or reducing interval between administrations (increased dose density; e.g., lanreotide autogel 120 mg every 21 or 14 days). Above-label doses of SSAs are being used frequently for the management of NETs in clinical practice in patients with disease progression or uncontrolled symptoms while on standard dose therapy without excessing toxicity [15, 16]. However, solid data regarding the role of non-conventional SSA doses are lacking, given the absence of large, prospective trials focusing on this topic. To date, increasing SSA dose above the standard may be proposed in selected NET patients progressing with the standard SSA dose after a multidisciplinary discussion has been made, carefully considering the kind of tumor progression (increase in number of lesions and/or increase in tumor size), presence of uncontrolled NET-related syndrome, patient's

age and comorbidities, and potential therapeutic alternatives including PRRT and everolimus-based target therapy.

Clinical trial with lanreotide high doses has recently been completed, and data will be published shortly (https://clinicaltrials.gov/ct2/show/NCT02651987).

15.2.2 Peptide Receptor Radionuclide Therapy

Peptide receptor radionuclide therapy (PRRT) is the result of a combination of a radionuclide and a peptide conjugate with an appropriate chelator that specifically binds to sstr delivering a cytotoxic radiation to the tumor. Upon binding of the radiolabeled peptide to the receptor, after an internalization of the compound has took place, the emission of ionizing radiation from the bound radionuclide occurs, inducing selective tumor cell destroy. In clinical practice, this model of radio-labeled targeted therapy consists of [177Lu]Lu-DOTA-TATE, a complex compound in which 177Lutethium is conjugated with a chelator (DOTA) and a targeting peptide (octreotate).

After almost 20 years during which several non-randomized studies, often retrospective, proposed this treatment to be effective in different kinds of NETs, the first phase 3 randomized trial NETTER-1 definitively confirmed the clear benefits of [177Lu]Lu-DOTA-TATE in advanced, progressive intestinal NETs [17]. In that trial, 229 patients were randomized to receive [177Lu]Lu-DOTA-TATE or high-dose octreotide (60 mg/4 weeks); the risk of tumor progression or death was reduced in the active arm by 79%, and an objective tumor response in terms of significant reduction in tumor size was observed in 18% of patients. The treatment was well-tolerated, with most important AEs being lymphopenia, vomiting, diarrhea, nausea, and abdominal pain. Given the impressive results obtained by the NETTER-1 trial, [177Lu]Lu-DOTA-TATE has been approved by international regulatory agencies FDA and EMA for treating patients with advanced, progressive gastrointestinal or pancreatic, well-differentiated G1 and G2 NETs expressing somatostatin receptors. A number of publications outside the regulatory trial further corroborates data from NETTER-1 study. In fact, similar findings were reported by a very large retrospective study including a mixed population of 1214 patients with NETs from different sites treated with [177Lu]Lu-DOTA-TATE over 15 years period of time [18]. The reported median overall survival rate was 58 months in the subgroup of patients with intestinal NETs and documented progressive disease, a promising figure if compared with data deriving from other therapeutic strategies. Again, a significant ability to induce objective tumor response was reported in 30% of patients. Safety data analyses were in agreement with those reported by the phase 3 trial. While [177Lu]Lu-DOTA-TATE PRRT is considered a well-tolerated therapy with few, usually transient, AEs, some concerns have been raised concerning long-term toxicity. The risk of persistent renal toxicity has significantly decreased over time after specific administration protocols including kidney protection with amino acids infusion has been applied [18, 19]. As far as hematological toxicity is concerned, myelodysplastic syndrome and acute leukemia have been reported to rarely occur in the late follow-up of patients treated with PRRT. Although severe, these conditions represent very rare event, being reported in nearly 1% of patients, particularly in those previously treated with alkylating agents [19, 20]. This observation further highlights the need to properly plan the optimal therapeutic sequence in NET patients, to provide optimal anti-tumor activity and to avoid unnecessary toxicity.

Tumor size is considered a prognostic factor for patients treated with [177Lu]Lu-DOTA-TATE, and an inverse correlation between tumor burden and treatment effectiveness has been proposed in the past. However, a recent sub-analysis from the NETTER-1 trial showed clear [177Lu]Lu-DOTA-TATE activity regardless of baseline liver tumor burden and presence of large target lesions [21].

Beyond efficacy, [177Lu]Lu-DOTA-TATE PRRT has showed to have a positive impact on

patients' quality of life (QoL), by prolonging global health time to QoL deterioration, as well as by improving both physical and role functioning in treated patients [22]. Maintaining QoL is particularly important in patients with NETs, given the relatively indolent course of these diseases which gives patients the possibility to receive several therapies during the long clinical course.

Furthermore, there is a promising emerging evidence supporting the effectiveness of PRRT in somatostatin-receptor imaging (SRI)-positive G3 disease. In fact, favorable clinical outcome, in terms of both disease control rate (69–78%) and median PFS (11–16 months) have been also observed in patients with highly proliferating G3 tumors with Ki67 ranging between 20% and 55% [23], thus suggesting that PRRT might play a significant role in the therapeutic sequence also in this more aggressive setting of disease.

Although placing [177Lu]Lu-DOTA-TATE PRRT in the therapeutic sequence of intestinal NETs still remains an interesting open question which need to be definitively answered, it is reasonable to consider this treatment as second-line therapy after failure of SSAs.

15.2.3 Everolimus

Everolimus is an inhibitor of the mammalian target of rapamycin (mTOR) used as a systemic therapy in lung and gastroenteropancreatic neuroendocrine tumors at a dose of 10 mg/day. In the last decade, its activity in different settings of NETs has been extensively investigated. Concerning intestinal NETs, two phase 3 RCTs have focused on the activity of this compound in this subgroup of NET. The Radiant-2 trial [24] enrolled 429 patients with advanced progressive gastrointestinal NET with previous history of carcinoid syndrome; although it failed to reach the pre-specified statistical significance threshold, it showed an advantage in terms of PFS for patients receiving everolims compared with the control group who received octreotide. This initial promising finding was confirmed in the subsequent Radiant-4 trial [25], which clearly demonstrated a benefit in PFS for patients treated with everolimus vs. those receiving placebo. The median PFS was 11 months, and the risk for tumor progression was reduced by 52%. Both studies reported a proportion of objective response rates ranging <10%. Most frequent side effects of everolimus include hyperglycemia, cytopenias, oral ulcers, rash, diarrhea, and atypical infections. Basing on the findings from the above-mentioned trials, everolimus was approved in advanced, progressive, well-differentiated lung and gastrointestinal NETs, thus including intestinal primaries.

15.2.4 Chemotherapy

Cytotoxic agents are the cornerstone of therapy for patients with poorly differentiated NEC, irrespective of the primary tumor site. Given the extreme rarity of NEC rising from the small intestine, their use in this setting of patients is a rare event. As far as well-differentiated intestinal NETs are concerned, disappointing data have been reported by using different chemotherapeutic agents. Well-designed studies in this peculiar clinical scenario are particularly scant, in fact most of the available literature is based on retrospective studies including heterogeneous small series of NET patients.

Even a phase 3 study including 64 patients randomized to receive streptozotocin/5-fluoruracil or interferon failed to demonstrate any difference in terms of PFS and OS between the two groups, and only one patient achieved partial response in the chemotherapy group [26]. Similarly, negligible activity with single-agent or temozolomide-based regimens has been reported by other studies, again confirming that chemotherapy plays a minor role in treatment of intestinal well-differentiate intestinal NETs [27, 28]. To date, there is no evidence of clinical outcome benefit by using systemic chemotherapy in well-differentiated intestinal NETs.

Conversely, chemotherapy might be considered in the setting of highly proliferating tumors, however without good quality scientific data supporting it.

15.3 Liver-Directed Treatments for Hepatic Disease

Several liver-directed approaches have been proposed for treating NETs hepatic metastases, including radiofrequency ablation (RA), trans-arterial embolization (TAE) and trans-arterial chemo-embolization (TACE). The main goal of these treatment is to control symptoms in patients with functioning tumors and related carcinoid syndrome. Potential benefits on patients' survival have been proposed, however with no solid evidence-based data supporting it.

Radiofrequency ablation is a thermal ablative technique based on the cytotoxic effects of high temperature locally administrated in the liver through electrode needles, inducing coagulation necrosis, which can be performed percutaneously under ultrasonography guidance or intraoperatively [29]. This technique has showed to improve symptoms in approximatively 90% of syndromic NET patients, with a relief duration ranging from 14 to 27 months [30]. The treatment-related mortality is below 1%, usually related to uncontrolled carcinoid syndrome exacerbated by ablation, whereas morbidity is around 10%, consisting of hemorrhage, abscess, perforation, bile leakage, and transient live insufficiency [25]. An early computed-tomography is usually performed within the first week after ablation, to identify incomplete ablation and to establish subsequent follow-up. The risk of local disease recurrence is quite high, recurrence rate being reported to range between 5% and 25% [30]. Unfortunately, there is not sufficient amount of prospective trials nor comparative studies able to give reliable information concerning the impact of RA on long-term patient survival.

Other ablative techniques are mainly represented by microwave ablation, cryotherapy, and percutaneous ethanol injection, which however are less frequently performed compared with RA for safety reasons, and due to their lower efficacy.

Trans-arterial embolization (TAE) and chemo-embolization (TACE) consist of the intravascular delivery of therapeutic agents via selective catheter placement under radiological guidance. The rational basis consists of the highly arterial vascularization of NET liver metastases. Trans-arterial embolization (TAE) involves the infusion of embolic agents like lipiodol, absorbable gelfoam particles, or non-absorbable bland microspheres into the artery, which will stop the blood flow. The principle of TACE is to perform intra-arterial injection of cytotoxic agents, usually doxorubicin, or streptozotocin, or a combination of chemotherapy agents before embolization. The treatment consists of multiple embolizations performed every 4–8 weeks, until symptomatic control and/or objective tumor response is achieved. The choice to prefer TAE or TACE in liver metastases from intestinal NETs still remains an unanswered question. Comparing TAE and TACE is difficult because the majority of studies are retrospective, with few patients, including heterogeneous NET populations. A better tolerance has been reported by some authors by using TAE. Concerning efficacy, no significant difference was observed in terms of both objective response and patient's progression-free survival [29–31]. Symptomatic relief is achieved in 60–85% of syndromic NET patients after embolization is performed, whereas objective response is observed in approximatively 50% of patients, with a median PFS of 18–24 months [32]. The most common complication is the so-called post-embolization syndrome, consisting of fever, leukocytosis, abdominal pain, nausea, and transient impairment of liver function tests. Morbidity may be reduced by fractioning treatment in different procedures targeted to embolize each liver segment separately.

Selective internal radiation therapy (SIRT), also known as radioembolization, is a recently developed technique in which 90Y-labeled microspheres are deposited in the hepatic artery. Objective tumor response is reported between 40% and 65% [32]. Again, there is limited data regarding the real impact of this procedure on patients' survival.

15.4 Appendiceal Neuroendocrine Tumors

The appendix is one of the most common sites for NENs. Appendiceal NENs are found in approximatively 0.3%–0.9% of patients undergone appendectomy for acute appendicitis. In a large retrospective analysis performed on 1237 appendectomies, a total of five appendiceal NENs were found, accounting for 0.4% [33]. There is not a specific clinical syndrome related to appendiceal NENs, since the vast majority of them are incidental findings in post-appendectomy specimens. They are slightly more frequent in females, occurring at an average age of 40–50 years; however, they have also been reported in a series of pediatric patients. The prognosis of this kind of NEN is usually excellent, with several series reporting 5-year survival rate of 100% [34]. However, in some cases, they present a more aggressive behavior determining a less favorable patient's clinical outcome. Metastatic disease is a rare event; however, it may occur in those patients with large tumor.

The most powerful prognostic factor of these NENs is tumor size. A diameter above 2 cm is well-recognized as a major negative feature, being associated with presence of metastases in up to 40% of cases [35]. Conversely, tumors sized <1 cm are usually considered with negligible risk of metastases, although some studies have reported few patients with lymph node involvement even in case of such small primary tumors.

International guidelines propose to assess risk profile of appendiceal NENs based on the following criteria: tumor size, specific localization within the appendix, extent of invasion (if any) into the meso-appendix and vascular invasion, proliferative index Ki67 determining tumor grading, lymphatic invasion. Assessing the potential risk of malignancy of appendiceal NENs is pivotal when approaching patients with incidental NEN diagnosed after appendectomy, to understand whether this minimally invasive surgical treatment may be considered curative or not. In the presence of risk factors, right emicolectomy with standard lymphoadenectomy should be performed to prevent the risk of late metastatic occurrence (Fig. 15.2).

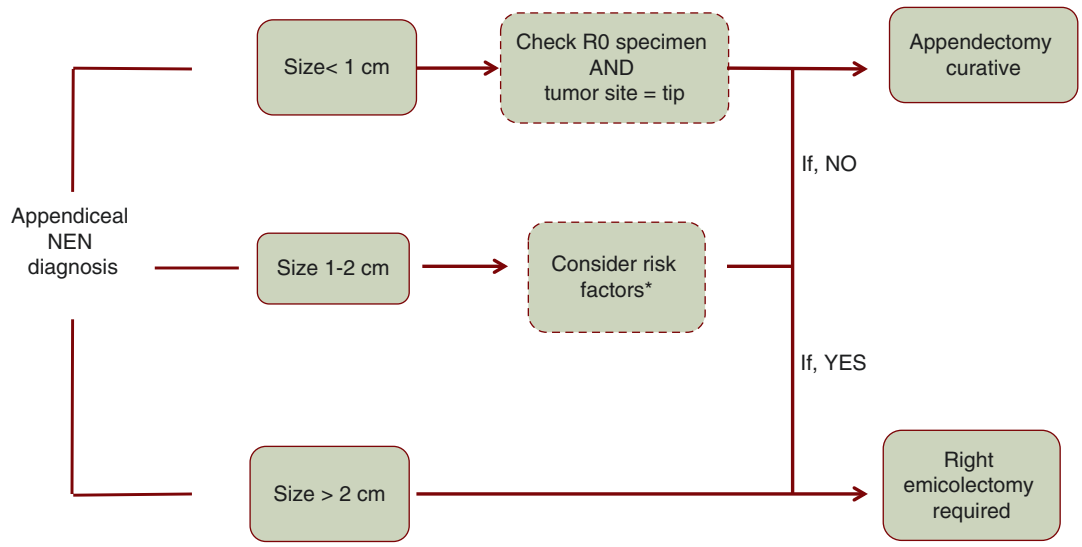

Fig. 15.2 Prognostic stratification and proposed therapeutic approach to appendiceal NENs. Risk Factor: infiltration of mesoappendix (cut-off 3 mm); grading (other than G1); lympho-invasion; angio-invasion

To date, right-emicolectomy with lymph node resection is recommended in those patients with tumor sized >2 cm, if tumors are located at the base of appendix, when the surgical margin is involved after appendectomy (R1 tumors), in selected cases with tumor sized <2 cm if risk factors are present (>3 mm infiltration of meso-appendix, presence of lympho-angioinvasion, grading G2) (Fig. 15.2) [34]. However, planning the optimal treatment for appendiceal NENs with small tumors and presence of risk factors remains a clinical challenge. In a recent multicenter large retrospective analysis, tumor size >1.5 cm, grading G2 (Ki67 3–20%) and lympho-vascular infiltration were independent risk factors related to nodal metastases, suggesting that in the presence of at least one of these factors, right emicolectomy should be suggested [35].

Although the majority of tumors <2 cm do not harbor any risk to develop metastases and may be considered cured after appendectomy, several controversies remain for some of these patients, in whom several risk factors have been identified. Those patients may have to undergo an additional operation and a proportion of them will need long-term follow-up [36]. Well-designed clinical trials, with long-term patients' follow-up, are definitively understand the prognostic impact of those risk factors which could be associated with regional or distant metastases and potentially adverse outcomes [36].

15.5 Conclusions

Clinical management of intestinal NETs still remains a challenge for physicians dealing with this rare kind of cancer. In the last decades, therapeutic landscape of these tumors has dramatically changed, given the introduction novel therapies (Fig. 15.1), including targeted agents and radiolabeled compounds, which may be used when the first-line therapy based on somatostating analogs fails to control tumor growth. Peptide receptor radionuclide therapy is an established treatment for progressive intestinal G1 and G2 NETs, with solid scientific data confirming its ability to induce tumor regression and prolong both progression-free survival and overall survival. Ablative liver-directed treatments may be helpful to reduce hepatic tumor load and to control symptoms in functioning tumors.

Appendiceal NETs need to be separately considered, given their peculiar biology and frequent indolent behavior. In these tumors, an accurate prognostic stratification is mandatory to reduce the risk of tumor recurrence and to avoid unnecessary surgical procedures.

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Treatment of NETs from Rare Origin

16

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16.1 Introduction

Rare NETs closely resemble their lung and gastroenteropancreatic (GEP) counterparts, although it is still unknown whether they also share similar genetic alterations. Therefore, it is unknown whether they may benefit from treatment reserved for lung and GEP NENs or, on the contrary, should be treated according to protocols used for epithelial cancers of the specific primary site. The impossibility to conduct large-scale studies on rare NETs leads to a lack of definitive biologic, epidemiologic and prognostic information, as well as standardized treatment guidelines. However, a comprehensive genomic characterization of NENs arising in uncommon sites represents an essential starting point for advancing our understanding of their biological behaviour and leading to the identification of reproducible diagnostic markers and of genetic alterations that could be exploited therapeutically in some subgroups of patients. The demonstration of a common molecular background shared by similar histotypes could be of great clinical relevance for treatment planning, broadening the spectrum of therapeutic options, regardless of the primary site of diseases. In this chapter, we will discuss clinical presentation, diagnosis and treatment of oesophageal, thymic, renal/genitourinary and breast neuroendocrine tumours.

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16.2 Oesophageal Neuroendocrine Tumours

Oesophageal neuroendocrine tumours (OeNETs) occur in the middle-low third of the oesophagus as a reflection of the distribution of neuroendocrine cells and account for 0.4–2% of oesophageal malignancies [1–3]. The estimated prevalence of oesophageal primary site among GEP NETs is approximately of 0.04–4.6% [2, 6]. Prevalence is highest in Japan, Korea and China, showing a predominance in men in their seventh to eighth decades of life [1, 4–6]. The major risk factors include smoking, alcohol abuse, a prior

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history of achalasia, gastroesophageal reflux disease and Barrett metaplasia. Lastly, OeNETs can arise as part of MEN1 and MEN2 familial cancer syndrome, von Hippel–Lindau disease, type 1 neurofibromatosis and tuberous sclerosis [6–8].

The 2010 WHO classification for digestive system NETs has divided OeNETs into low-grade (G1) and intermediate-grade (G2) NETs, and high-grade (G3) NECs, which include small-cell and large-cell oesophageal carcinoma (SCEC and LCEC) [9, 10]. Virtually all oesophageal NETs are high-grade poorly differentiated NECs, with the "small cell" histotype being the most common finding, especially in the context of mixed neuroendocrine/non-neuroendocrine neoplasms (MiNENs) [4].

OeNETs are characterized by rapid progression (metastases are found in 31–90% of cases at presentation, mainly in the lymph nodes, liver, lungs and bone, while brain metastases are relatively rare), resulting in a poor prognosis, even in the setting of clinically localized disease [1–3, 6, 11]. Given their aggressiveness, the time to diagnosis is lower than that for other histological types [11]. Prognostic factors include age, stage (median survival time can reach 20 months for limited disease vs. 6–12 months of the extended disease), biochemical parameters and type of treatment [2, 12, 13]. Among the biochemical markers, circulating NSE levels ≤17 ng/mL seem to be associated with a better prognosis [14], and patients with LGR5 (leucine-rich repeating-containing G-protein-coupled receptor 5) overexpression seem to present more frequently an advanced stage, lymph node metastasis, poor response to chemotherapy and a worse prognosis [14, 15]. Regarding the histotype, although few cases of LCEC have been reported so far, no apparent differences have emerged in survival rates in comparison to the small cell subtype if they are equally treated [16].

16.2.1 Clinical Manifestation and Diagnosis

The most common clinical onset of OeNETs consists of progressive dysphagia, hoarse voice, anorexia, fatigue and weight loss. Less frequently, the initial presentation may include retrosternal/epigastric pain or painful swallowing, dysphonia, dyspnoea, emesis and digestive bleeding. Rarely the recurrent left laryngeal nerve can be injured, resulting in a vocal cord paralysis. Often the diagnosis occurs randomly on endoscopic examination, and sometimes, it is related to metastatic sites. While carcinoid syndrome is rarely described, paraneoplastic syndromes are more frequently reported (i.e. inappropriate anti-diuretic hormone syndrome and watery diarrhoea-hypokalaemia-achlorhydria syndrome) [4, 5].

The assessment of circulating markers, including CgA, NSE, carcinoembryonic antigen (CEA) and pro-gastrin-releasing peptide (pro-GRP) may contribute to the diagnosis and management [3, 4]. Preliminary investigations may include a barium oesophagography and/or an esophagogastroduodenoscopy (EGD) that usually depict a single elevated polypoid or nodular lesion expanding the oesophageal lumen. Subsequently, the diagnostic workup is typically accomplished by conventional cross-sectional contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI) scans, generally including the chest, abdomen and pelvis [4, 9]. Somatostatin receptor-based imaging (i.e. 68Ga-DOTATATE or DOTATOC-PET) is instrumental for NET G1-G3; whereas 18-F fluorodeoxyglucose PET-CT scanning is recommended for NECs [17]. Given their poor differentiation and aggressive behaviour with a propensity to metastasize extensively, the FDG-PET/CT has been proposed also for the detection of any recurrence during the follow-up. Finally, an endoscopic ultrasound with biopsy remains the most contributory technique for delineating the anatomic extent of oesophageal wall invasion and lymph node malignancy and establish a preoperative histological diagnosis [4]. Positive immunostaining for common neuroendocrine markers (chromogranin A, CgA, synaptophysin, Syn, and neuron-specific enolase, NSE) as well as for CK (cytokeratin) and CD56 (cluster differentiation 56) is mandatory for the

diagnosis of oesophageal NETs, with synaptophysin proving the most sensitive marker, whereas SCECs are often CgA- and Syn-negative and NSE-positive [1, 4].

16.2.2 Treatment

The treatment of OeNETs strictly depends on grade and stage [1, 5]. While there is general consensus that well-differentiated NETs (G1 and G2) should be treated surgically or, in case of inoperability or diffuse disease, with somatostatin analogues, different treatments have been evaluated for poorly differentiated NECs in an attempt to improve survival rates [2, 9, 12, 18].

16.2.2.1 Surgery

Surgery is indicated for localized well-differentiated OeNETs. It generally consists of *minimally invasive transhiatal esophagectomy* that, unlike *classic transhiatal esophagectomy*, does not involve radical lymph node dissection, thus reducing the risk of pulmonary complications. The minimally invasive procedure is recommended for early cancers in the middle (below the level of carina) and lower (type I and II esophagogastric junction tumours) third of the oesophagus. It is also performed for advanced oesophageal cancers in patients who are not fit to undergo a thoracotomy [19]. *Transthoracic esophagectomy* has the advantage of a more extensive resection and may be associated with a longer disease-free survival (DFS) [20]. However, it deeply affects short- and long-term health-related quality of life (QoL), with the postoperative recovery period potentially taking more than half or even all the patient's remaining life expectancy, generally not showing any improvement to the preoperative level until 9 months post-surgery [21].

16.2.2.2 Chemotherapy (CT)

Since surgery alone is rarely curative, a multimodality approach including systemic CT is recommended even for patients with limited-stage disease [22, 23]. Taken singularly, CT seems superior to surgery or radiotherapy alone for NEC treatment, with a further survival improvement by combining it with one of these two treatments [4, 6, 24–28]. Standard first-line CT schedule for metastatic NEC consists of cisplatin/carboplatin combined with etoposide or irinotecan (reported median survival time of 12.8 and 9.4 months, respectively). Even though the optimal duration of treatment is not established, performing four to six cycles of therapy seem reasonable, but if a patient is still responding and tolerating well the treatment, continuation of CT to at least maximal response is appropriate. Based upon data on small-cell lung cancer (SCLC), patients with platinum-sensitive disease may benefit from retreatment with a platinum and etoposide combination if relapse occurs at least 6 months after discontinuation of first-line treatment [24–28]. Second-line regimens, although not yet evaluated rigorously, include temozolomide-, fluoropyrimidine-, irinotecan-, and oxaliplatin-based regimens [7, 29, 30].

16.2.2.3 Radiotherapy (RT)

RT alone has shown disappointing results in terms of survival, notably when compared to CT alone (5 vs. 24 months) or surgery alone (7 vs. 17 months) [31]. Moreover, the optimal radiation dose for localized SCEC has not been established: locoregional recurrence is observed in approximately half of the patients receiving <50 Gy, and in 14–22% of those receiving >60 Gy [11, 32].

16.2.2.4 Peptide Receptor Radionuclide Therapy (PRRT)

PRRT with 177Lu-DOTATATE was found to be effective in a single patient with OeNET [33], but further studies are required to better evaluate its efficacy.

16.2.2.5 Multidisciplinary Modalities

Combination of local and systemic treatments has been largely reported to improve median survival time in comparison with local treatments alone in patients with SCEC [6, 8, 12, 22]. Interestingly, the concurrent administration of CT plus RT (chemoradiation, **CRT**) seems more effective than sequential scheme. Definitive **CRT** seems even superior to surgery plus CT for

locoregional oesophageal NECs (3-year OS of 50% vs. 24%) [23]. Long-term relapse-free survival (RFS) is possible among patients with localized disease who are treated with multimodality therapy, as demonstrated by a single-institution retrospective review of 25 patients with oesophageal SCEC (14 with limited-stage disease, LD, and 9 with extensive disease, ED), in which six (24%) remained alive at a median follow-up of 38 months, one with ED and five with LD [22].

Therefore, a possible treatment algorithm for oesophageal NECs depending on the stage was proposed.

- Stage I/IIA. Surgery is suggested for its effectiveness in comparison to RT (29 vs. 17 months), without a further median survival improvement given by adjuvant CT.
- Stage IIB/III. Administration of postoperative CT increases median survival time compared to patients who do not received it (13 vs. 6 months).
- Stage IV. CRT is more effective in extending median survival time than CT alone (13.2 vs. 8.9 months) [15].

16.3 Thymic Neuroendocrine Tumours

Thymic neuroendocrine tumours (T-NETs) represent the least common primary thymic neoplasms, accounting for 2–5% of the total [34]. Specifically, a primary thymic site constitutes about 0.4% of all NETs, corresponding to an estimated annual incidence of approximately 0.2 per million [35, 36]. Almost all cases have been reported in adults (median age of 54 years), with a male preponderance (male to female ratio of 3:1) [34, 35]. Up to 25% of T-NETs occur in patients with multiple endocrine neoplasia type 1 (MEN1) [37], and T-NETs are broadly categorized as low-grade (typical carcinoid), intermediate-grade (atypical carcinoid) or high-grade (large-cell and small-cell neuroendocrine carcinomas, LCNECs and SCNECs) [38]. Most T-NETs are classified as atypical carcinoids; however, even if well differentiated, these tumours are characterized by relatively aggressive behaviour and high propensity for locoregional invasion, local recurrence and distant dissemination [34, 39, 40].

16.3.1 Clinical Manifestation and Diagnosis

T-NET typically presents as a large locally advanced mass in the anterior mediastinum, inducing symptoms from local mass effects (e.g. cough, dyspnoea and chest pain to superior vena cava syndrome, and hoarseness due to recurrent laryngeal nerve invasion) [39]. About one third of them are asymptomatic findings on radiographic study done for an unrelated cause or for MEN1 surveillance [34]. Among paraneoplastic syndromes, Cushing's is the most common [41]. Finally, 20–40% of patients can manifest symptoms and signs related to distant metastases (lung, pleura, chest wall, bone, liver and pancreas) [34, 39, 40]. Mediastinal lymph node localizations are present in approximately 50% of patients at presentation [39, 40].

Most T-NETs consist of high-grade tumours, generally characterized by aggressive behaviour, resistance to standard therapy, tendency to recur locally and to metastasize over up to 20 years, resulting in an average poorer prognosis compared to NETs of similar stage and grade arising elsewhere [42–44]. The main prognostic factors are: surgical resectability and completeness of resection (patients who were able to undergo surgical therapy had a significantly longer median survival than those who did not, 109 vs. 46 months, respectively), completeness of resection being a strong prognostic factor for overall survival [35, 40]; disease stage (in the SEER database, the median survival for patients with localized, regional and distant metastases were 110, 59 and 35 months, respectively) [35]. Interestingly, tumour grade/differentiation did

not seem to affect survival, but span to recurrence/progression [39]. Tumour size also impacts outcomes, 10-year rate of survival being significantly higher for tumours <7 cm compared with those 7 cm or larger (91 vs. 29%) [44].

Most T-NETs are nonfunctioning. Given the high incidence of Cushing's syndrome in patients with non-MEN1-associated thymic NETs, some experts recommend measurement of serum cortisol levels and a 24-h urine collection [45]. Contrast-enhanced chest CT scan is the imaging procedure of choice for anterior mediastinal masses. However, a cardiac MRI can be helpful in assessing invasion of the adjacent cardiovascular structures [45]. A T-NET usually manifests as a large, lobulated, invasive mass with indistinct margins and heterogeneous enhancement that may exhibit areas of haemorrhage and necrosis, as well as punctate and dystrophic calcifications. It may be difficult to distinguish a thymic NET from other thymic or non-thymic malignancies based on radiographic imaging [45, 46]. A preoperative histological diagnosis obtained by means of a CT-guided core needle biopsy should be performed to guide decision-making about neoadjuvant therapy [47]. If a diagnosis of a T-NET is established, it is recommended to complete the evaluation with cross-sectional imaging of the abdomen and somatostatin-receptor-based scans (68Ga DOTATATE PET/CT) in order to exclude a primary NET in another site, identify metastatic disease and evaluate the possible benefit from somatostatin analogues (SSA) or peptide receptor radioligand therapy (PRRT) for advanced disease [48]. However, specificity is somewhat limited because somatostatin receptors can be expressed also in malignant thymic epithelial tumours, granulomas and autoimmune diseases. Moreover, sensitivity may be limited because many thymic NETs do not express high levels of somatostatin receptors [37, 40]. This, in addition to the aggressiveness of most thymic NENs, brings out the utility of 18-F fluorodeoxyglucose/PET scans in the further characterization of negative or equivocal radiolabelled somatostatin analogues diagnostic imaging [49].

16.3.2 Treatment

Treatment of T-NETs depends on their size, extension and the presence of metastases.

16.3.2.1 Locally Resectable (or Borderline Resectable) Tumours

Surgery is the mainstay of therapy for resectable cases. A total extended thymectomy accompanied by hilar and mediastinal lymph node sampling implies removal of all mediastinal tissue anterior to the pericardium from the innominate vessels to the diaphragm and laterally to each phrenic nerve. Maximal resection of anterior mediastinal masses can be achieved via different approaches. Since most of the thymic NETs are diagnosed at a size that is not amenable to minimally invasive techniques (transthoracic, transcervical, video-assisted thoracoscopic surgery or robotic thymectomy), standard *transthoracic thymectomy* via *a median sternotomy* is usually the approach to choose to achieve the highest oncologic efficacy, avoiding tumour spillage and incomplete resection. Unfortunately, microscopically radicality is uncommonly attained for tumours that invade contiguous structures such as major blood vessels, pericardium or phrenic nerve [47].

RT plays a role in subtotally resected or locally advanced unresectable nonmetastatic disease as adjuvant and neoadjuvant treatment, respectively, while evidence supporting benefit for definitive RT is limited. Although few series and case reports have proven a better local control thanks to adjuvant RT after complete resection, there is no evidence that this confers any survival advantage [35, 40].

Adjuvant systemic therapy is based on chemoradiotherapy (CRT) and long-acting somatostatin analogues (LA-SSA). If the tumour is resectable upfront, a postoperative consolidation approach based on the concurrent use of radiosensitizing doses of fluorouracil or capecitabine in conjunction with platinum/etoposide-based CT, as typically used for small-cell lung

carcinoma (SCLC), is considered more appropriate than RT alone for patients with moderately to poorly differentiated tumours (atypical carcinoids and NECs), even when a complete resection has been obtained, in order to reduce the risk of recurrence and to achieve prolonged disease control [50, 51]**.** The role of consolidation therapy with SSA after surgical resection, possibly followed by CRT, is not established in the absence of endocrine secretion syndrome [52, 53]**.**

Neoadjuvant systemic therapy may be delivered in locally advanced disease at the time of diagnosis with invasion of intra-thoracic neighbouring structures to reduce the tumour burden, possibly allowing subsequent local treatment with a curative intent. In such cases, the feasibility of neoadjuvant CT or CRT to increase resectability has been demonstrated, but whether this approach improves outcomes over maximum resection followed by adjuvant RT is unknown [52–54]. Guidelines from the National Comprehensive Cancer Network (NCCN) on the treatment of thymic NETs does not address the utility of neoadjuvant therapy [50].

16.3.2.2 Unresectable, Recurrent and Metastatic Disease

Options for recurrent and/or metastatic disease include resection (when possible), RT and systemic therapy. Survivals as long as 12–15 years have been reported with aggressive surgical resection of both local and distant metastases [1]. If not surgically manageable, metastatic and/or unresectable disease should be properly treated by systemic therapy, which relies on cytotoxic chemotherapy (temozolomide- and platinum-based regimen for well- and poorly-differentiated NETs, respectively), somatostatin receptor therapy (LA-SSA and PRRT) and everolimus [50, 55–58]. LA-SSAs should probably be the first-line treatment for patients with relatively low-volume and asymptomatic somatostatin-receptor-positive disease. However, the stabilizing effect on tumour growth exerted by these agents on some GEP-NETs is unclear and supported by few data for thymic carcinoids [50, 59, 60]. Moreover, there are no evidence for selecting or sequencing these treatments except that PRRT should be limited to patients with somatostatin-receptor-expressing tumours. Even in these cases, there is no real basis for choosing PRRT over everolimus, or vice versa, as the second-line treatment [50, 55–58]. Given the difficulty to perform randomized trials focusing only on thymic NETs because of their rarity and considering their biological and clinical similarity to pulmonary NETs, the potential efficacy of everolimus has been extended to progressive thymic NETs on the basis of the results obtained from the RADIANT 4 study, which demonstrated a significant improvement in progression-free survival (PFS) in patients with progressive lung NETs treated with everolimus compared with those receiving placebo [55].

Among patients with advanced midgut NETs, the benefits of PRRT have been shown in the NETTER-1 trial, which demonstrated significant improvement in objective response rate, PFS and overall survival (OS) with PRRT compared with high-dose LA-octreotide in patients whose disease had progressed on standard-dose SSA therapy (median PFS not reached versus 18 months). However, data on thymic NETs are limited to two reported patients treated with 177Lu-dotatate, one of whom had stable disease as the best response. Since the 2018 FDA approval did not cover thymic NETs, off-label use could be considered in appropriate patients [57, 58].

Poorly differentiated high-grade NECs should be treated, similarly to SCLC, with combined CT including platinum plus etoposide [61].

Whether **surgical debulking** of large tumours without curative intent may confer any survival benefit is unclear; however, surgical resection of a large tumour causing compression disturbance as well as palliative radiation on a symptomatic site may provide benefits in terms of symptom control and therefore is suggested [34, 50]. Palliative resection could also be considered in case of debilitating uncontrolled hormonal secretory condition such as Cushing's syndrome, hypercalcemia or carcinoid syndrome. The use of LA-SSA and/or adrenal steroidogenesis inhibitors (i.e. ketoconazole, metyrapone, etomidate, aminoglutethimide and others) has proven to attain a satisfactory hypercortisolism control

prior to surgery in thymic ACTH-producing NETs. Particularly, LA-SSAs has been reported to rapidly lower ectopic ACTH secretion, although not having any effect on the mass reduction [41, 62]. However, it is much important to reach a mitigation of Cushing's syndrome for the purposes of a safer perioperative course that some authors have suggested recurring to mitotane or bilateral surgical adrenalectomy in the event of SSA and adrenal enzyme inhibitors failure [62, 63].

There are no evidence-based guidelines for posttreatment surveillance but, given the potentially long delay between primary treatment and the development of metastases, long-term surveillance is recommended. A shared strategy is to perform first imaging within 6 months, then every 4–12 months depending on tumour grade. After 5 years, if there is no evidence of recurrence, imaging frequency can be decreased but continued at least annually and possibly in association with tumour markers for at least 10 years [50–54].

16.4 Renal Neuroendocrine Tumours

Primary renal neuroendocrine tumours (R-NETs), including well-differentiated (WD) carcinoid tumour and poorly differentiated large- and small-cell carcinoma (PD-LCNEC and PD-SCNEC), have an estimated annual incidence of 0.13 per one million persons [64–67]. Only 2.5% of PD-NECs occur in extrapulmonary sites, including the genitourinary tract, where they have been more commonly reported in the urinary bladder and prostate, with only ~50 cases described in the kidney so far [65, 68]. Carcinoid tumours arising from the genitourinary tract constitute less than 1% of all NETs and less than 1% of all genitourinary neoplasms, with kidney being the second most frequent site of WD-NETs in each sex, following testis in men and ovaries in women [67, 68]. Overall ~150 cases have been reported in literature to date, mostly deriving from small series focusing on WD-NETs, in which different grading methods, often not including the Ki-67 proliferative index, and no official American Joint Committee on Cancer (AJCC) staging system have been applied [64–68]. However, some consistent findings have emerged across the series [64–68]. R-NETs affect equally men and women in their 50s (median age at diagnosis of 57 years). The pathogenesis is unclear, since neuroendocrine cells are not normally found in adult renal parenchyma. This would suggest an origin either from neural crest cells entrapped during embryogenesis or from activation of gene sequences common to neuroendocrine programmed cells in multipotent stem cells within foci of metaplastic or teratomatous epithelium. This hypothesis is further supported by the strong association seen between renal NENs and congenital or acquired anomalies of the kidneys, such as metaplasia of the urothelium induced by chronic inflammation, mature cystic teratoma and horseshoe kidney (20–30% of renal NENs arise within a horseshoe kidney, with a calculated relative risk ranging from 62 to 120, markedly greater than that for Wilms tumour or transitional cell carcinoma), albeit their clinical course appears to be more benign than that of the non-horseshoe variant [67, 69]. Regional lymph nodes involvement and distant metastasis to liver and bone are common at diagnosis (reported in up to 92% and 46% of cases, respectively), with most of patients (75%) experiencing secondary localizations at any point of the disease course [64–67].

16.4.1 Clinical Manifestation and Diagnosis

Abdominal, back or flank pain, accompanied by haematuria or fever, is the most common presenting symptom; an incidental diagnosis is made in 25–30% of cases and carcinoid syndrome occurs in less than 15% of patients. Even rarer are symptoms related to other neuroendocrine syndromes, such as glucagon-induced constipation, Zollinger–Ellison, Verner–Morrison and Cushing's syndromes [70–73]. Usually diagnosed at a large size (>4 cm in 75% of cases) with invasion of the perirenal or sinus/hilar fat or of the renal vein in

almost half of the cases, R-NETs show no distinctive pattern on CT or MRI. They can present as well-circumscribed, lobulated, bulging and heterogeneous (in the 60% of cases) solid masses, occasionally associated with cystic components and calcifications (up to 30% of cases), characterized by non- or slight enhancement). The presence of necrosis and haemorrhage indicates a more aggressive behaviour while calcifications are often associated with long-standing tumour growth or presence of teratomatous elements [3, 4]. Along with conventional radiographic techniques, 68Ga-DOTATATE PET is instrumental for the diagnosis, staging and follow-up of carcinoid tumours [66, 67, 74]. A preoperative histopathological diagnosis through a renal trucut biopsy is recommended.

16.4.2 Treatment

Treatment of R-NETs depends on their size, extension and the presence of metastases.

16.4.2.1 Localized Disease

If it is commonly accepted that a **radical/partial nephrectomy**, eventually accompanied by a **lymph node dissection**, is the gold standard treatment for early stage disease, no trial so far has shown its direct impact on survival, as well as the utility of any neoadjuvant/adjuvant treatment. Close follow-up after surgery is strongly recommended [67].

16.4.2.2 Advanced Disease

Given the high rate of successful **total resection** and the evidence of improved outcomes in patients with other metastatic NENs undergoing hepatic debulking or transarterial embolization [75], an aggressive surgical approach (i.e. radical nephrectomy, lymphadenectomy and eventual hepatic metastasectomy) has been proposed as the cornerstone of treatment for primary R-NETs [64–67]. However, given their indolent clinical course, the possible development of metastatic recurrence in most patients even after radical resection and the lack of data comparing the survival of patients who have not undergone surgery or complete resection, the superiority of such an aggressive strategy in terms of survival advantage has to be demonstrated [64–67]. Moreover, the role of debulking/cytoreductive surgery, CT, RT, LA-SSAs, and targeted therapy in the management of advanced disease remains an open question [67].

Systemic therapy plays a role in case of inoperability, residual disease or surgically not-accessible recurrence [64–67]. The survival benefit of chemotherapeutic regimens, including cisplatin/carboplatin, etoposide and 5-FU, in the management of renal carcinoid tumours has not been proven [67]. If consistent responses to CT have not been observed, the use of LA-SSAs as a first-line therapy has shown disease stabilization in almost half of patients, with another half of them rescued by the second-line agent everolimus. The only patient who received simultaneous everolimus and SSA in the first-line setting showed documented radiographic disease regression at 3 months, but progression at 7 months [64]. In the absence of a solid clinical experience with LA-SSAs in renal NENs treatment, it seems reasonable to consider them as a first-line therapy for patients with advanced WD-NETs, relying on the abovementioned few publications and, mostly, on the results of well-designed trials on GEP NETs that demonstrated not only a good control of hormonal excess by means of these agents, but also an antineoplastic activity in both functionally active and inactive NETs, resulting in a significantly prolonged time to disease progression (14.3 vs. 6 months in the "placebo" group) [59]. Similarly, based on the evidence of an extension of the progression-free survival (PFS) time using everolimus in patients with metastatic non-functional lung and GEP NENs, it seems appropriate to consider the mTOR inhibitor as a first-line therapy for patients with renal NENs that do not express somatostatin receptors, other than as a second-line choice in alternative to the tyrosine kinase inhibitor **sunitinib** and PRRT for patients with progression during SSA therapy [55, 59, 76]. The radiologic response in three of six patients

who underwent **RT** (five with palliative purpose on metastatic disease and one as adjuvant chemoradiotherapy, CRT, to the tumour resection bed), with the other three showing disease stability, gives reason to palliative RT of symptomatic metastases [64, 67]. Based on the results of a phase III randomized clinical trial, **PRRT** with 177Lu-dotatate has been recently approved for the treatment of GEP NETs after progression during LA-SSA therapy, and this therapeutic technique seems also effective, often resulting in durable disease stability, for pancreatic and thoracic NENs [57, 77–79]. Consequently, the indication for PRRT as a second-line therapy option can be extended to WD R-NETs expressing somatostatin receptors [64].

Combined approaches have been used for PD renal NECs, but with suboptimal results, as demonstrated by the median OS of 8–10 months reported by two reviews on patients with renal SCNECs [80]. These neoplasms are indeed overly aggressive, often presenting with extrarenal extension not amenable to complete resection, bringing about a rapidly fatal outcome regardless of the therapeutic strategies adopted. However, both adjuvant RT on the residual disease and upfront systemic platinum-based CT seem to improve the prognosis [65]. In fact, although palliative in nature, the initial relative chemosensitivity of SCNECs makes cytotoxic chemotherapy to become the mainstay of treatment of these high-grade malignancies, with best results demonstrated if it is given preoperatively [64, 65, 67]. Moreover, platinum-based CT alone has yielded a better, although not statistically different, survival compared to surgery alone in genitourinary SCNECs [81]. As a result, it could be reasonable to offer CT as first-line therapy to patients with recognized PD renal NECs, reserving nephrectomy only for those selected cases with high risk of local complications [68].

Metastasis of renal NENs are possible even years after treatment, indicating the need for long-term follow-up, with images taken within 6 months after primary treatment and then every 6–12 months on an individual basis [64–67].

16.5 Gynaecologic Neuroendocrine Tumours

NETs of the female genital tract, often occurring in association with other epithelial and germ cell neoplasms, account for less than 2% of all gynaecologic cancers [82–85]. The most common site is the cervix (cNETs), where the high-grade forms are much more prevalent, followed by the ovaries (oNETs), where most are clinically benign carcinoid tumours arising within mature cystic teratomas, and the uterus (uNETs) [82–84]. Primary NETs of the vagina and vulva (vNETs) are sporadically reported as high-grade neuroendocrine carcinoma (NEC) at both sites and as Merkel cell carcinoma in the vulva, often presenting metastatic disease and showing extremely aggressive behaviour [86, 87].

While the classification of primary NTEs occurring at endometrium, cervix, vagina, and vulva has been updated in the WHO 2014 to match the GEP terminology that grouped typical and atypical carcinoids into low-grade NETs and neuroendocrine carcinoma of small-cell (SCNEC) and large-cell (LCNEC) type into high-grade NETs, the current ovarian classification does not adopt a separate category for ovarian NENs, including low-grade NETs among the tumours arising from a dermoid cyst and the high-grade SCNEC of pulmonary type in the miscellaneous category [86–89]. The largest retrospective review, including more than 500 patients with gynaecologic NET (43% cNET, 30% oNET, 20% uNET, 7% vNET), demonstrates an incidence on the rise over 25 years (from 0.3 per million in 1987 to 1.3 per million in 2012), without any signs of improvement in OS which usually results in less than 2 years [90].

16.5.1 Cervical Neuroendocrine Tumours

Cervical neuroendocrine tumours (cNETs), representing 0.9–1.5% of the tumours of the uterine cervix with an annual incidence of 0.06/100000 women, are mostly poorly

differentiated and aggressive HPV-associated neoplasms (high-risk HPV DNA and overexpression of p16 are detected in over 95% of high-grade cNETs and in no one of low-grade cNETs), diagnosed at a relatively young age (median age: 37–46 years) and at an advanced stage [91–93]. Even neoplasms with a minor component of high-grade NEC may behave aggressively, commonly presenting lymph-vascular space invasion (LVSI), regional and distant lymph node involvement, and local or distant relapses (mainly in the lung, liver, bone and brain), and therefore carrying an ominous prognosis (a median DFS of 16 months and a median OS of 24–54 months have emerged from different reports) [85–87, 93–95]. Since their exceptional rarity in this site, the prognosis of typical carcinoids is difficult to be evaluated, whereas atypical carcinoids and NECs, frequently associated with subclinical lymphatic and hematogenous spreading even in apparently early disease, have overall low survival rates [86, 87, 90–95]. Other than histotype, the tumour stage is the strongest prognostic factor, with age [91], smoking, tumour size [96], depth of invasion, LVSI, lymph node involvement [97] and margin status [96] being other relevant prognostic variables. The stated 5-year OS for SCNEC, the most common oNEC subtype, was 30–60% for early stages and 0–17% for advanced stages [93, 96]. The combination of negative HER-2/neu and positive EGFR expression had the worst impact on LCNEC patients' survival, where a median OS of 16.5 months has been reported [98].

No prospective, well-designed clinical trials are currently available, making cNETs a therapeutic challenge for clinicians. Different multimodality approaches, mainly adapted from those of lung NETs [24, 82, 99, 100], have been reported even in early stage patients and, specifically, radical hysterectomy followed by adjuvant chemotherapy (ACT) or concurrent chemoradiation (CCRT) for early stage disease; definitive CCRT sometimes preceded by neoadjuvant chemotherapy (NACT) and followed by ACT for locally advanced disease; palliative CT for metastatic disease.

In this respect, locoregional treatment alone has proved inadequate both to control local disease and to prevent distant metastases; as recently demonstrated by a SEER database analysis reporting equally poor outcomes in cNET patients undergoing surgery alone and those undergoing primary RT, in which loco-regional recurrences outside of the irradiated fields (specifically in para-aortic lymph node or vagina) frequently occurred [91, 93]. CT both in NACT and ACT setting and in concomitance with CCRT demonstrated to improve the OS in patients with all stages when compared with other treatment modalities [99]. The most effective and frequently used regimen involve the combination of cisplatin and etoposide [94]. On multivariable analysis, besides early stage disease, the use of radical hysterectomy and any CT were independent prognostic variables for improved OS [91, 93, 97]. However, recurrence and progression frequently occur, leading to a lower OS at all stages compared to that for squamous cell carcinoma of the cervix [91–97, 99]. In a series of approximately 60 patients with LCNEC, perioperative CT was an independent prognostic variable for longer OS. Unfortunately, most of these patients undergoing radical surgery and adjuvant CT or CCRT developed a recurrence and died within 6–24 months after the operation [101].

Conversely, no information on the use of somatostatin analogues (SSAs), mTOR inhibitors and antiangiogenic agents are currently available for cNETs [93].

In Table 16.1, there is a proposal of stage-based therapeutic strategies drawn from a "systematic review" that has conducted an in-depth analysis of the different treatment modalities available so far in the literature [93].

The high recurrence rate of patients with early clinical stage and the poor prognosis of those with advanced disease make the detection of novel therapeutic options strongly warranted for the management of cNETs. The statistically significant PFS benefit observed in patients with recurrent SCNECs who received a combination of chemotherapeutic agents and *bevacizumab* compared to those who received regimens not containing bevacizumab [102] and the apparent

Table 16.1 Therapeutic algorithm proposed by Gaducci et al. [93]

Stage disease (FIGO)	Suggested treatment strategies
IA-IIA1	– Radical hysterectomy + pelvic and Para-aortic lymphadenectomy followed by ACT (PE regimen) – Adjuvant cisplatin-based CCRT could be added in case of lymph nodes positivity or surgical margins involved (extra-cervical disease ± positive lymph nodes)
IB–IIA2	– NACT (PE regimen for 3 cycles), followed by radical hysterectomy with pelvic and Para-aortic lymphadenectomy – ACT (3 additional cycles of PE) in case of complete or optimal partial response (persistent residual disease with <3 mm stromal invasion and negative lymph nodes) – Adjuvant cisplatin-based CCRT followed by 3 additional cycles of CT (PE regimen) in case of persistent intra-cervical residual disease (>3 mm stromal invasion and negative lymph nodes) or residual extra-cervical disease ± positive lymph nodes
IIB–IVA	– NACT (PE regimen for 3 cycles), followed by cisplatin-based CCRT (on both pelvis and Para-aortic area) and then by 3 additional cycles of CT (PE regimen)
IVB	– NACT (PE regimen for 3 cycles), followed by palliative tailored RT on the pelvis ± on single distant metastases

negative prognostic implication of *survivin* expression [97] suggest a promising future for the incorporation of targeted therapies into the treatment of these tumours [93]. Conversely, hormonal therapy has no clinical usefulness, considering the extremely low rate of positive oestrogen receptor and progesterone receptor staining [98].

16.5.2 Ovarian Neuroendocrine Tumours

Ovarian neuroendocrine tumours (oNETs) arise from the neural crest tissue present in the stroma and surface epithelium or within teratoma [88]. Indeed, although histologically like other typical/atypical carcinoid tumours, low-grade NETs in the ovary are often present as a component of a "specialized" teratoma. Well-differentiated oNETs can manifest four histological patterns: insular, trabecular, stromal and mucinous, with the first being the most common and the latter being the most unfavourable, potentially associated with advance stage, pelvic spread and metastasis, whereas poorly differentiated oNECs are morphologically identical to LCNECs in the lung [87, 88]. To date, only 58 cases of ovarian LCNECs (of which only 15 were pure LCNECs) have been reported, all characterized by extreme aggressiveness and lethal outcome even when diagnosed at an early stage [103]. Data from major series including patients affected by oNEC of small or large or mixed cell type pointed out a mean age at diagnosis of 55–60 years (with 20–40% of the patients younger than 50 years), a unilateral involvement in 60% of cases, a high prevalence of metastatic disease on initial presentation (70%), and a median OS of 16–30 months [90, 94, 103, 104]. In the SEER registry, with a mean survival of 27 months, close to that of ovarian carcinosarcoma, the oNEC histotype, luckily representing only the ~0.4% of all ovarian malignancies, resulted the most aggressive epithelial ovarian cancer (including serous, endometrioid, mucinous and clear cell) [87, 104]. The 1-, 3- and 5-year survival was of 58%, 33% and 27%, respectively [104]. Demographic characteristics including race, age and the year of diagnosis did not influence the prognosis; while early clinical stage (FIGO stage I/II), low tumour grade and surgical treatment independently predicted a better survival [104].

Due to a lack of clinical research into ovarian NETs, individualized treatment guidelines have not been established, and whether these tumours should be treated according to guidelines for GEP NETs or those for ovarian cancer remains unclear [104]. At present, most ovarian NENs are treated according to ovarian cancer protocols, whose unquestionable cornerstone is primary surgical debulking, which results in undoubted benefit for the patient [89, 105]. In line, data from

the SEER database showed that an important strategy for improving survival rates was the achievement of a complete surgical resection that, therefore, should be undertaken as a primary treatment modality for ovarian NECs [90, 104]. Specifically, the standard procedure includes a debulking surgery (bilateral salpingo-oophorectomy, extra-fascial hysterectomy, prophylactic omentectomy and, when necessary, pelvic and para-aortic lymph node dissection, appendectomy and bowel resection) with the goal of a complete macroscopic tumour resection, followed by ACT based on carboplatin and paclitaxel in line with the treatment standards for epithelial ovarian cancer [88, 89, 103, 105]. Although routinely performed as an adaption from ovarian cancer surgery, whether such a radical procedure provides an added benefit is unclear at this point [89]. Accordingly to what stated by the most recent European Society for Medical Oncology (ESMO) guidelines with regard to the epithelial ovarian cancer treatment, conservative surgery alone (unilateral salpingo-oophorectomy with a fertility-sparing approach) can be applied to those patients with Ki-67 $\leq$ 5% and FIGO Ia, especially in younger patients with unfulfilled wish for a child [103]. In any case, to preserve the fertility of patients undergoing ACT and/or pelvic irradiation, oocyte and embryo cryopreservation and ovarian transposition (oophoropexy) should be offered in routine reproductive clinical practice [103].

For poorly differentiated NECs, different ACT regimens (including platinum, paclitaxel, etoposide and bleomycin) and, less frequently, postoperative RT have been used [88, 89, 103, 105]. The paucity of data still suggests that platinum-based CT, mainly associated with etoposide, may be of benefit to patients, inducing some authors to propose it as the adjuvant treatment-of-choice in high-grade ovarian NECs, mandatory for stages III–IV, in line with the recommendations for GEP and pulmonary NECs [89, 94, 103]. General opinion is to prefer a neuroendocrine-aimed platinum-etoposide regimen in the case of pure NEC or if the neuroendocrine component is much more prevalent and to decide on a first-line paclitaxel plus carboplatin treatment in the presence of a prevailing proportion of epithelial elements in mixed high-grade neoplasms [103]. However, considering the substantial side effects (i.e. nausea, hair-loss and hemotoxicity) and the scantiness of data not confirming a definitive benefit of adjuvant platinum-based CT, it is unclear if it should be always administered in early stages [89, 103].

In general, there is limited evidence to use systemic CT in slowly growing low-proliferative NETs (suggested Ki-67 cut-off of <30%), where rather alternative approaches such as somatostatin receptor-targeted therapies (SSAs or PRRT) should be considered [89]. The efficacy of SSAs in slowing tumour growth and improving carcinoid symptoms is reported in few reports on oNETs limited to well-differentiated neoplasms and should only be discussed in poorly differentiated tumours when the Ki-67 index remains below 30% [106]. Finally, novel targeted therapies, such as CDK4/6 inhibitors, are being studied in pre-clinical and clinical research [107].

16.5.3 Endometrial Neuroendocrine Tumours (eNETs)

High-grade eNETs account for 0.8% of all endometrial carcinomas, with nearly 130 cases reported in the literature, mainly as SCNEC and mixed with other more typical histotypes [86, 87]. Usually described as bulky, ill-defined, large endometrial-based masses with deep myometrial invasion, eNETs manifest clinically with vaginal bleeding, similarly to other uterine malignancies, and rarely with symptoms associated to a paraneoplastic syndrome [94, 108]. According to the largest published series, the average age at diagnosis is approximately 60 years without clear risk factors, an advanced stage (FIGO stage $\geq$IIIA) is reported in over 60% of cases with distant metastasis present in 30–50% of patients; median OS is about 50 months with a quarter of patients surviving more than 5 years [90, 94, 108]. The recurrence rate and mean OS in stage I–II vs. stage III–IV disease have been reported to be 50% vs. 88%

and 22 vs. 12 months, respectively [94]. Typically, eNETs are positive for at least one neuroendocrine marker in at least 10% of the tumour cells and present frequent lymph-vascular space invasion, geographic necrosis and a high mitotic index [87, 94, 108].

Analogously to the therapeutic strategy adopted for cervical SCNECs, multimodal approaches have been employed for endometrial equivalents. In all cases, surgery (hysterectomy and bilateral salpingo-oophorectomy with variable lymphadenectomies) was performed as a first-line therapy, followed by different adjuvant treatments (platinum-based CT or CCRT or pelvic RT). Regardless of the therapeutic strategy, all patients with stage IVB disease died with a median OS of 9 months [89, 108].

16.5.4 Vaginal Neuroendocrine Tumours

Approximately 28 cases of SCNEC arising in the vagina have been described in literature, two of them presenting with Cushing's syndrome. In these extraordinary cases, a diagnosis of exclusion must be performed, ruling out metastasis from elsewhere. The median age of diagnosis is 55 years, with 65% having stage I–II and 35% stage III–IV disease [109]. Surgery or radiotherapy alone have been adopted to treat early stage vaginal NECs, while CCRT using a PE regimen has been the most common treatment modality for advanced stage disease. Despite the combined approaches, this aggressive malignancy maintains a high mortality rate with a mean OS of 10 months and only two reported cases with a relative long survival (2 and 3.5 years) [109].

16.6 Breast Neuroendocrine Tumours

Breast neuroendocrine tumours (bNETs) are a rare condition that constitutes approximately 0.5–1% among all breast cancers, and it mostly affects postmenopausal women, of Caucasian ethnicity, between sixth and seventh decades of life; but it is also described as rare cases in male patients [110–112]. Unlike other types of breast invasive ductal carcinomas (BIDC), the bNET is frequently characterized by a greater propensity to metastasize to the loco-regional lymph nodes and often it is diagnosed at stage II of the disease. The real prevalence of bNET is likely underestimated because of little use of immunohistochemical techniques in routine pathological anatomy [111]. Indeed, despite some morphological features may suggest the possible neuroendocrine origin of a neoplasm, the certain diagnosis of a NET is based on the positivity of specific neuroendocrine markers. Among these, synaptophysin and chromogranin are the most specific and sensitive, and their positivity respectively characterize about 50% and 100% of poorly differentiated carcinomas. Furthermore, in the majority of well-differentiated neuroendocrine carcinomas and in more than half of those poorly, it is possible to identified the presence of specific sexual hormonal markers. Commonly, the bNETs are featured by immunophenotypic pattern luminal B (ER and/or PR positive, Her2 negative, high proliferation rate); this aspect is more representative bNET than the others BIDC. However, in some studies, an equivalent prevalence of luminal A (ER and/or PR positive, Her2 negative, low proliferation rate) and luminal B pattern has been reported, while other studies showed the prevalence of luminal A pattern [110, 113]. Like other small-cell carcinomas, the poorly differentiated bNETs often express the thyroid transcription factor 1 (TTF1), and, in about 45% of cases, they present androgen receptor in co-expression with gross disease fluid protein 15 (GCDFP15) [112]. Nowadays, the classification of these tumours is still impaired because of the rarity of this specific histological type. Based on the current classification of neuroendocrine tumours and carcinomas, the bNET could be classified into well-differentiated NET (breast carcinoid tumours NET G1, G2 or G3); Ductal carcinoma in situ (NEC-DCIS), with neuroendocrine in situ differentiation; invasive carcinomas BIDC, solid invasive papillary carcinomas with neuroendocrine differentiation and mucinous carcinomas, currently considered as breast non-neuroendocrine

carcinomas; poorly differentiated neuroendocrine carcinomas, that is considered a sub-type of invasive cancer. In some rare cases, characterized by remarkable aggressiveness, bNETs show Merkel's cellular features.

16.6.1 Clinical Manifestation and Diagnosis

The clinical presentation of bNET is like other histologic type of BIDC; however, according to their neuroendocrine origin, it is possible to observe hormonal hypersecretion syndrome [112]. The bNETs are still a heterogeneous entity with nosographic and histological difficulties. Indeed, the diagnostic path and the knowledge about their biological behaviour are not yet certain due to the limited number of cases. The characterization of neuroendocrine markers is not routinely performed except in cases where the pathologist has a suspicion for the presence of a neuroendocrine component, so the incidence of bNETs may be underestimated. Despite the limitations related with the poor representation of different study population, now neuroendocrine tumours of the breast are treated similarly to other invasive breast carcinomas. Surgery is the first and best choice of the treatment for early bNETs [114], while no specific studies are present for adjuvant radiation treatment. Radiotherapy should be considered following the already known recommendations just given for the other kinds of invasive breast cancer. Tumour size and nodal metastases represent the prognostic factors for evaluating the possible relapse for bNETs, as for other types of breast cancer [115, 116].

16.6.2 Treatment

CT can be used as AT in patients with a high risk of relapse or as neoadjuvant therapy in cases of locally advanced or not operable bNETs. Patients with hormone receptor-positive bNET are normally candidates to undergo adjuvant endocrine therapy [117]. The negative prognostic value of proliferation index, patients with hormone receptor-positive and high Ki67 may also benefit from adjuvant chemotherapy in addition to endocrine therapy [118]. Different chemotherapy schedules have been suggested such as anthracyclines and/or taxanes, also combinations of platinum agents and etoposide, fluorouracil/epirubicin/cyclophosphamide followed by docetaxel, docetaxel/epirubicin/cyclophosphamide, cyclophosphamide and doxorubicin, cyclophosphamide/methotrexate/fluorouracil, paclitaxel alone, carboplatin/paclitaxel, carboplatin or cisplatin and etoposide, and cisplatin and irinotecan [117–119]. HER2 is rarely expressed by bNET [120–122], and its prognostic role in bNET is not so clear. bNETs are able to metastasize even many years therefore a long-term follow up is suggested. For bNETs, the expression of somatostatin receptors should theoretically support the PPRT and LA-SSAs treatment in patients positive for SSTR at 68Gallium PET-CT [123]. Future perspectives underline the possible use of different molecules targeting for specific pathways such as PIK3CA, fibroblast growth factor receptor (FGFR) family members [124, 125]. The mutation of vascular endothelial growth factor receptor 2 (VEGFR2) (activating mutation) [126] seems to be strongly expressed in bNET [127]. This observation supports a rationale for the possible use of antiangiogenic molecules. It is well known that the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway is involved in the pathogenesis and progression of pancreatic neuroendocrine tumours (pNETs) [128]. Everolimus (mTOR inhibitor) is efficacy in well- and moderately differentiated pNET [76]. Taking into account that PI3K/AKT/mTOR pathway seems to be implicated also in the mechanism of the resistance to hormone therapy in oestrogen receptor (ER)-positive breast cancer [129], the addition of everolimus to the aromatase inhibitor exemestane seems to be able to significantly prolong progression-free survival of these patients [130, 131].

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Part V

NENs with Peculiar Biology and Features

17 Neuroendocrine Neoplasms with Peculiar Biology and Features: MEN1, MEN2A, MEN2B, MEN4, VHL, NF1

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Abbreviations

AKT	Protein kinase B
ATA	American Thyroid Association
CDK	Cyclin-dependent kinase
CEA	Carcinoembryonic antigen
CLA	Cutaneous lichen amyloidosis
CT	Computed tomography
DOPA	3,4-Dihydroxyphenylalanine
d-pNET	Duodeno-pancreatic NET
EUS	Endoscopic ultrasound
FDG	Fluorodeoxyglucose
FNA	Fine-needle aspiration
GEP	Gastroenteropancreatic
GLP-1R	Glucagon-like peptide-1 receptor
gNET	Gastric NET
HIFs	Hypoxia-inducible factors
MEN1	Multiple endocrine neoplasia type 1
MEN2	Multiple endocrine neoplasia type 2
MEN4	Multiple endocrine neoplasia type 4
MIBG	Metaiodobenzylguanidine
MiNEN	Mixed neuroendocrine non-neuroendocrine neoplasm
MRI	Magnetic resonance imaging
MTC	Medullary thyroid cancer
mTOR	Mammalian target of rapamycin
NEC	Neuroendocrine carcinoma
NEN	Neuroendocrine neoplasm
NET	Neuroendocrine tumor
NF1	Neurofibromatosis type 1
PD1	Programmed cell death protein 1
PDGF	Platelet-derived growth factor polypeptide
PET	Positron emission tomography
PFS	Progression-free survival
PGL	Paraganglioma
PHEO	Pheochromocytoma
PHPT	Primary hyperparathyroidism
PI3K	Phosphoinositide 3-kinase
pNEN	Pancreatic NEN
PNMT	Phenylethanolamine N methyltransferase
PPIs	Proton pump inhibitors
PRRT	Peptide receptor radionuclide therapy
RET	Rearranged during Transfection
SPECT	Single photon emission computed tomography

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SSAs	Somatostatin analogs
SST	Somatostatin
SUV	Standardized uptake value
TGFα	Transforming growth factor α
TKI	Tyrosine kinases inhibitor
US	Ultrasound
VEGF	Vascular endothelial growth factor
VHL	Von Hippel–Lindau disease
VIPoma	Vasoactive intestinal polypeptidoma
WDHA	Watery diarrhea, hypokalemia, and achlorhydria
ZES	Zollinger–Ellison syndrome

17.1 Introduction

A subgroup of neuroendocrine neoplasms (NENs) show a hereditary background and occur in the context of genetic endocrine neoplastic syndromes, such as multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 2 (MEN2), variants MEN2A and MEN2B, multiple endocrine neoplasia type 4 (MEN4), Von Hippel–Lindau disease (VHL), and neurofibromatosis type 1 (NF1) [1–5]. It has been estimated a rate around 10% of patients with gastroenteropancreatic (GEP) NENs associated with a hereditary endocrine neoplastic syndrome [1, 2]; this rate is higher in case of pancreatic NENs (pNEN), while thyroid NENs are associated with MEN2 in 20–30% of cases [6].

The genetic origin of the neoplasm greatly influences its natural history, since the diagnosis of NEN is generally made toward the sixth decade of life in the case of sporadic forms, while the forms associated with hereditary syndromes are diagnosed approximately two to three decades in advance, sometimes in adolescence [1, 7]. NENs associated with hereditary syndromes are generally well differentiated, the so-called neuroendocrine tumors (NET), low proliferating, multiple, and multifocal [1, 2]. MEN1-related duodeno-pancreatic NETs (d-pNETs) are in most cases grade 1 or 2, while no case of neuroendocrine carcinoma (NEC) is generally found [2].

D-pNETs are found in 70–80% of patients with MEN1, while VHL is associated with pNET in up to 30% and NF1 with dNET in 1% of cases [8–12]. These tumors are frequently associated with functioning endocrine syndromes and highly express somatostatin (SST) receptors. MEN2A and B are mainly characterized by the development of thyroid NET, the so-called medullary thyroid cancer (MTC), in about 100% of cases [6]. Lung and thymic carcinoids as well as gastric NET (gNET) arise in less than 10% of MEN1 patients [8]. Together with malignant tumors, neuroendocrine adenomas could arise in these genetic syndromes. Pituitary and parathyroid adenomas are common in MEN1, while they represent the main lesions of MEN4 [8, 13]. Parathyroid adenomas also develop in MEN2A [8]. Pheochromocytoma (PHEO) is common in MEN2 (~50%), VHL (10–20%), and less common in NF1 (~5%) [14]. Extra-adrenal PHEO, the so-called paragangliomas (PGLs), can occur in VHL as well as NF1. These tumors frequently result in hormone hypersecretion syndromes, such as hyperprolactinemia, hyperparathyroidism, and hypersecretion of catecholamines. Rarely adrenomedullary tumors and very rarely pituitary as well as parathyroid tumors present malignant behavior in patients with hereditary endocrine neoplastic syndromes. Other tumors of non-neuroendocrine origin are described in all the hereditary syndromes associated with NEN. They are less frequent in MEN1, MEN2, and MEN4, while VHL and NF1 represent the main manifestations and have negative prognostic impact [1–5].

An update of diagnosis and treatment of NENs in patients with MEN1, MEN2A, MEN2B, MEN4, VHL, NF1 is here described.

17.2 MEN1

17.2.1 Overview

MEN1 is an autosomal dominant genetic syndrome characterized by the occurrence of NENs arising mainly in parathyroid glands, pancreatic islet cells, and anterior pituitary gland [8]. The syndrome is caused by mutations in the tumor suppressor *MEN1* gene, located on chromosome

11 (11q13), consisting of 10 exons, encoding a 610 amino acid nuclear protein, named menin [15]. Menin, in association with 50 different proteins, contributes to DNA repair, cell signaling, cytoskeletal structure, cell division, adhesion, and motility. The main mechanism underlying tumorigenesis related to menin loss in MEN1 syndrome needs to be fully elucidated [16]. Phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway, which appears to be inhibited by menin [17, 18], is shown in Fig. 17.1.

In 90% of patients, the mutation is inherited from an affected parent, and only in 10% there is a de novo *MEN1* germline mutation [19]. Currently, contrary to what occurs in MEN2, a clear correlation between phenotype and genotype has not been found [20].

The prevalence of MEN1 is 1–10/100,000 [21]. It has been estimated to be 1–18% in patients with primary hyperparathyroidism

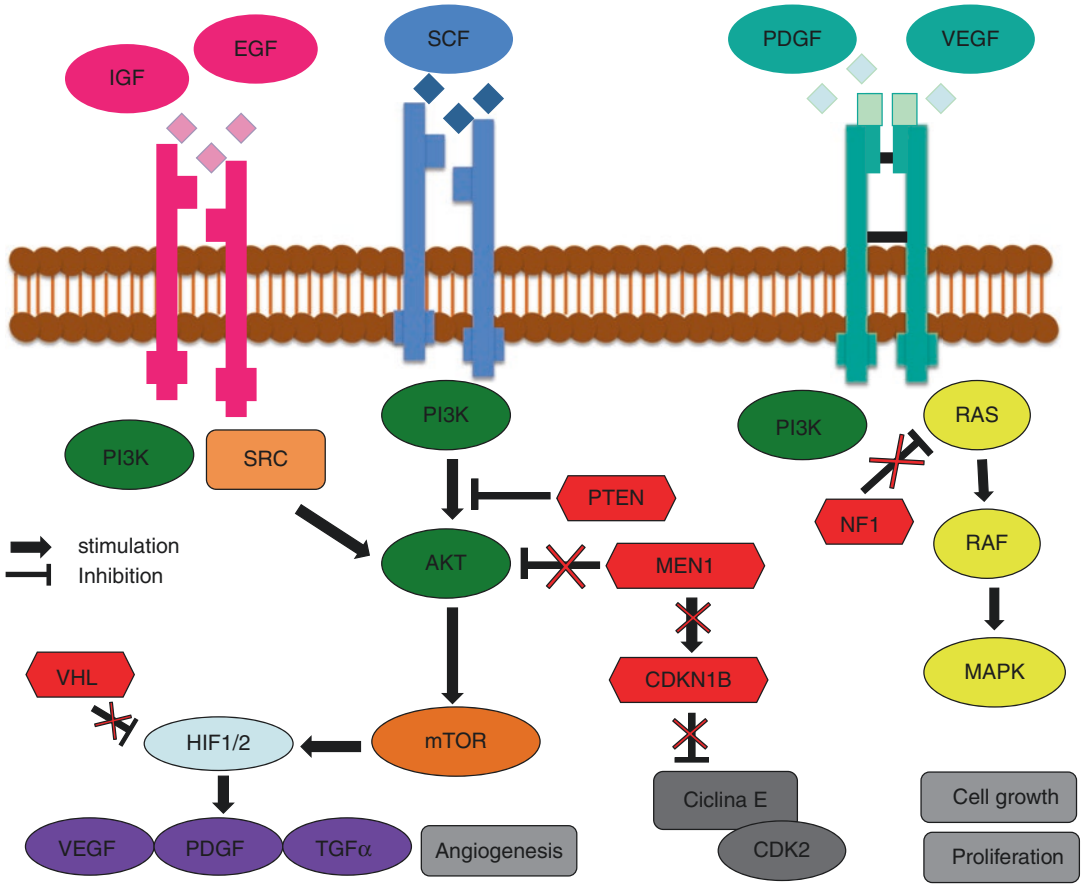

Fig. 17.1 Molecular pathogenesis of NENs in hereditary endocrine neoplastic syndromes (MEN1, MEN4, VHL, NF1). The red boxes indicate onco-soppressor genes acting by negative regulation of various pathways involved in cell growth and proliferation (PI3K/AKT/mTOR; RAS/RAF/MAPK; p27/Ciclina E/CDK2) and angiogenesis (HIF1/2). The inactivating mutations of these genes are responsible of oncogenic events in the corresponding syndrome (MEN1, MEN4, VHL and NF1). *IGF* insulin growth factor, *EGF* epidermal growth factor, *SCF* stem cell factor, *PDGF* platelet derived growth factor, *VEGF* vascular endothelial growth factor, *PI3K* phosphoinositide 3-kinase, *SRC* sarcome tyrosine kinase, *AKT* protein kinase B, *mTOR* mammalian target of rapamycin, *PTEN* phosphatase and tensin homolog, *VHL* Von Hipple Lindau, *HIF* hypoxia inducible factor, *TGFα* transforming growth factor, *MAPK* mitogen activated protein kinase, *MEN1* multiple endocrine neoplasia type 1, *CDKN1B* cyclin-dependent kinase inhibitor 1 B, *CDK2* cyclin-dependent kinase 2, *NF1* neurofibromatosis type 1

(PHPT), 16–38% in patients with gastrinomas, and <3% in patients with pituitary adenomas [8]. The syndrome affects patients with an age ranging from 5 to 81 years [8].

The syndrome can be diagnosed using the 2012 Endocrine Society Clinical Practice Guidelines criteria as follows: (1) clinical diagnosis, occurrence of at least two endocrine tumors typically associated with MEN1, (2) familial diagnosis, presence of one MEN1-related tumor in a first-degree relative of a patient with a clinical diagnosis of MEN1, (3) genetic diagnosis, detection of a germline *MEN1* mutation in an asymptomatic subject with no evidence of tumor by biochemical or imaging examination [8].

The typical MEN1 manifestations are PHPT, occurring in >90% of patients and due to adenoma/hyperplasia generally involving all parathyroid glands, pituitary adenomas, occurring in 30–40% of cases and characterized by prolactin hypersecretion in about half of cases, and NENs, which are observed in 70–80% of patients, mainly located within pancreas and duodenum but also found in other sites within digestive and respiratory system [8, 10] (Table 17.1).

17.2.2 MEN1-Related NEN: Diagnostic and Therapeutic Update

Among NENs, those arising in duodenum and pancreas are the most frequent in MEN1 (up to 80% of cases) [8, 10]. MEN1-related NENs are divided into functioning and non-functioning tumors. A variety of hormones are secreted excessively by functioning tumors such as gastrinomas, insulinomas, glucagonomas, vasoactive intestinal polypeptidomas (VIPomas), and several of them are associated with specific clinical syndromes [8] (Table 17.2).

Non-functioning tumors could be either non-secreting or secrete inactive polypeptides such as pancreatic polypeptide, chromogranin A, neurotensin, neuron-specific enolase, or ghrelin. In most cases, such tumors are detected incidentally or, rarely, patients could exhibit symptoms related to tumor mass. In case of functioning tumors, the clinical features are dependent on the secreted hormone. Gastrinoma's clinical presentation often includes abdominal pain, heartburn, nausea, gastrointestinal bleeding, and diarrhea (steatorrhea) [22, 23]. The presence of hypergastrinemia and recurrent peptic ulcerations, caused by the secretion of gastrin, allow the diagnosis of Zollinger–Ellison syndrome (ZES) which occurs in 21–70% of patients with MEN1 [8, 21, 24]. Insulinomas cause fasting hyperinsulinemic hypoglycemia accompanied by autonomic and neuroglycopenic symptoms [22, 23]. The pathognomonic combination of necrolytic migratory erythema, weight loss, anemia, and stomatitis may be absent in MEN1-related glucagonomas, so they can be detected just by glucose intolerance and hyperglucagonemia [8, 22]. Watery diarrhea, hypokalemia, and achlorhydria (WDHA) are characteristics of the Verner–Morrison syndrome (WDHA syndrome), caused by VIPomas [8].

Contrary to the sporadic counterpart, MEN1-related d-pNENs occur at a younger age and are multifocal and generally well-differentiated, low-grade tumors (G1-G2 NET) [2, 25, 26]. Moreover, the presence of d-pNENs in patients with MEN1 is correlated with an increased mortality [10, 27, 28], and tumor size has been proven to be directly related to a higher risk of metastatization and death regardless of hormone secretion [29, 30]. Another factor which is independently associated with an increased risk of distant metastases is the presence of ZES [29, 31]. Nevertheless, ZES, which used to be the major cause of death in patients with MEN1 [21], nowadays, seems not associated with an increased mortality; however, this evidence needs further confirmations [27–29].

Bronchopulmonary and thymic NENs occur in about 2% of MEN1 patients, gNENs (the type II gastric carcinoid of the clinical classification) in <10%, and PHEOs in <1% [8] (Tables 17.2 and 17.3). Thymic NENs in MEN1 are particularly aggressive and are associated with a significantly increased risk of death, even in absence of distant metastases [29].

Table 17.1 Phenotypic spectrum across the hereditary endocrine neoplastic syndromes

	PHPT	Pituitary adenoma				MTC	PTC	ACA	PHEO	PGL	Gastroenteropancreatic NET					Lung carcinoid	Thymic carcinoid	NEC
											Duodenum–Pancreas				Stomach			
		PRL	GH	ACTH	NF						ZES	INS	Other	NF				
MEN1	X	X	X	X	X		X	X	X		X	X	X[a]	X	X	X	X	
MEN2A	X					X			X	X								
MEN2B						X			X									
MEN4	X	X	X	X	X		X	X			X			X	X	X		X[b]
VHL	X								X	X				X				
NF1									X	X		X	X[c]	X				

PHPT primary hyperparathyroidism, *PRL* prolactin, *GH* growth hormone, *ACTH* adrenocorticotrophic hormone, *NF* non-functioning, *MTC* medullary thyroid cancer, *PTC* papillary thyroid cancer, *ACA* adrenal cortex adenoma, *PHEO* pheochromocytoma, *PGL* paraganglioma, *NET* neuroendocrine tumor, *ZES* Zollinger–Ellison syndrome, *INS* insulinoma, *NEC* neuroendocrine carcinoma

[a]Glucagonoma, vipoma

[b]Small cell cervical NEC reported in one case

[c]Somatostatinoma

Table 17.2 Main features of neuroendocrine neoplasms associated with hereditary endocrine neoplastic syndromes: comparison with the sporadic counterpart

Neuroendocrine neoplasms					
	MEN1	MEN4	VHL	NF1	Sporadic tumors[a]
Rate of NENs	Up to 80%	17%	20%	1%	–
Age at tumor diagnosis	<50 years	50–60 years	35 years (mean age)	<50 years	56–66 years (mean age)[b]
M:F ratio	1:1.4	Only F	1:1.1 to 1:1.6	M=F	M>F (si-, d-, p-, rNET) F>M (g-, a-, ceNET)
Site (from high to low frequency)	Pancreas, duodenum, stomach, lung, thymus	Pancreas, duodenum, stomach, lung, cervix	Pancreas	Duodenum and peri-ampullary region, pancreas, rectum	All sites
Histology	Well differentiated low tumor grade (G1–G2 NET) No G3 reported	Well differentiated low tumor grade (G1 NET/ typical carcinoid) NEC in cervix	Well differentiated low tumor grade (G1–G2 NET) G3 NET extremely rare	Well differentiated low tumor grade (G1–G2 NET) Psammoma bodies in SSoma	Well differentiated (G1–G3 NET): 90% Poorly differentiated (NEC): 10%
Metastases at diagnosis	13.7%	Metastases in 4/8 cases (lymph node and distant)	Rare distant metastasis at diagnosis	Common lymph nodes, rare distant metastases	G1 NET: 21% G2 NET: 30% G3 NET or NEC: 50%
Clinical manifestation (from high to low frequency)	– Non-functioning: incidental diagnosis, symptoms in case of complications – ZES – Insulinoma – Extremely rare: VIPoma; glucagonoma; SSoma	– Non-functioning: incidental diagnosis, local symptoms – ZES	– Non-functioning: incidental diagnosis, symptoms in case of complications	– Non-functioning: incidental diagnosis, local symptoms, pain, and jaundice – SSoma (often asymptomatic) – Insulinoma	– Non-functioning: incidental diagnosis, symptoms in case of complications – Carcinoid syndrome – ZES – Insulinoma – Rare: VIPoma; glucagonoma, SSoma
Neuroendocrine markers	CgA, specific markers according to clinics	CgA, gastrin	CgA NSE	CgA, specific markers according to clinics	CgA, NSE, specific markers according to clinics
Conventional imaging	Gastrointestinal endoscopy, endoscopic US, CT/MRI	Gastrointestinal endoscopy, endoscopic US, CT/MRI	Endoscopic US, CT/ MRI	Gastrointestinal endoscopy, endoscopic US, CT/MRI	Gastrointestinal endoscopy, endoscopic US, CT/MRI
Functional imaging	^{68}Ga-DOTA PET-CT (^{18}F-FDG PET-CT)	^{68}Ga-DOTA PET-CT (^{18}F-FDG PET-CT)	^{68}Ga-DOTA PET-CT (^{18}F-FDG PET-CT)	^{68}Ga-DOTA PET-CT (^{18}F-FDG PET-CT)	^{68}Ga-DOTA PET-CT (^{18}F-FDG PET-CT)

Treatment	– Radical surgery in d-pNET ≥2 cm or tumor growth rate >0.5 cm/year or functioning tumor (if possible, enucleation) – Radical surgery in lung/thymic NEN – Endoscopic resection in gNET >1 and <2 cm – Surveillance in non-functioning nonprogressive d-pNET <2 cm and gNET <1 cm (SSA alternative approach) – SSA in unresectable/ metastatic disease and, in case of progression, PRRT or targeted therapy – CHT in metastatic tumors non-responsive to previous therapies	– Radical surgery in d-pNET ≥2 cm or tumor growth rate >0.5 cm/year or functioning tumor (if possible, enucleation) – Radical surgery in lung NEN – Endoscopic resection in gNET >1 and <2 cm – Surveillance in non-functioning nonprogressive d-pNET <2 cm and gNET <1 cm – SSA in unresectable/ metastatic disease and, in case of progression, PRRT or targeted therapy – CHT in metastatic tumors non-responsive to previous therapies or NEC	– Radical surgery in pNET >2 cm (if possible, enucleation) – Surveillance in pNET <2 cm – SSA in unresectable/ metastatic disease and, in case of progression, PRRT or targeted therapy – CHT in metastatic tumors non-responsive to previous therapies or rare G3 NET	– Radical surgery (pancreaticoduodenectomy) in d-pNET >2 cm (local resection if possible) – Endoscopic resection or transduodenal surgical ampullectomy in small tumor (<1–2 cm) – SSA in unresectable/ metastatic disease and, in case of progression, PRRT or targeted therapy – CHT in metastatic tumors non-responsive to previous therapies or MiNEN	– Radical surgery in local and locoregional NET if resectable – Enucleation or endoscopic resection, if possible, in stage I tumors, according to tumor site, size, grade, and presence of functioning syndrome – Surveillance in non-functioning nonprogressive pNET <2 cm and d-gNET <1 cm – SSA in unresectable/ metastatic disease and, in case of progression, PRRT or targeted therapy – CHT in metastatic tumors non-responsive to previous therapies or G3 NEN

yrs years, *M* male, *F* female, *NEN* neuroendocrine neoplasm, *NET* neuroendocrine tumor, *siNET* small intestine NET, *dNET* duodenal NET, pancreatic NET, *rNET* rectal NET, *gNET* gastric NET, *aNET* appendiceal NET, *ceNET* cecum NET, *NEC* neuroendocrine carcinoma, *VIP* vasoactive intestinal polypeptide, *ZES* Zollinger–Ellison syndrome, *SS* somatostatin, *CgA* chromogranin A, *NSE* neuron-specific enolase, *US* ultrasound, *CT* computed tomography, *MRI* magnetic resonance imaging, *PET* positron emission tomography, *FDG* fluorodeoxyglucose, *MIBG* metaiodobenzylguanidine, *SSA* somatostatin analog, *PRRT* peptide receptor-targeted radiotherapy, *CHT* chemotherapy, *MiNEN* mixed neuroendocrine non-neuroendocrine neoplasms

[a]References: Garcia-Carbonero et al. ENETS Consensus Guidelines for High-Grade Gastroenteropancreatic Neuroendocrine Tumors and Neuroendocrine Carcinomas, Neuroendocrinology. 2016;103(2):186–94; Cives M, Strosberg JR. Gastroenteropancreatic Neuroendocrine Tumors.CA Cancer J Clin. 2018 Nov;68(6):471–87

[b]Mean age at diagnosis was younger in appendiceal NEN

Table 17.3 Main features of pheochromocytoma and paraganglioma associated with hereditary endocrine neoplastic syndromes: comparison with the sporadic counterpart

Pheochromocytoma and paraganglioma						
	MEN1	MEN2A	MEN2B	VHL	NF1	Sporadic tumors[a]
Rate of PHEO/PGL	<1%	70–80% of MEN2	5% of MEN2	10–20%	7.7–14.6%	–
Age at tumor diagnosis	<50 years	<35 years	<35 years	<30 years	>40 years	30–50 years
M:F ratio	M = F	M = F	M = F		F>M (++ malignant tumors)	M=F
Site (from high to low frequency)	Monolateral PHEO, rarely bilateral Anecdotical PGL	Multicentric and bilateral PHEO 65% of cases Metachronous in up to 25% of cases	Multicentric and bilateral PHEO 65% of cases Metachronous in up to 25% of cases	Mostly PHEO, usually multiple and bilateral Less frequent PGL (more functioning)	85% monolateral adrenal PHEO 9.6% bilateral PHEO, 6% PGL	Mostly monolateral PHEO
Malignancy rate	15%	1–4%	1–4%	5%	11.5%	10–17%
Clinical manifestation (from high to low frequency)	Symptomatic: hypertension, headache, sweating, palpitations, flushing	– Symptomatic: hypertension, headaches, sweating – Asymptomatic in 1/3 at the time of diagnosis	– Symptomatic: hypertension, headaches, sweating – Asymptomatic in 1/3 at the time of diagnosis	– Symptomatic: mostly related to norepinephrine secretion – Rarely dopamine secretion or asymptomatic	– Asymptomatic: incidental diagnosis – Symptomatic related to epinephrine and norepinephrine secretion	– Symptomatic: hypertension, headache, sweating, palpitations, flushing – Asymptomatic: incidental diagnosis or mass effect
Neuroendocrine markers	Plasma free or urinary fractionated metanephrines	Plasma free or urinary fractionated metanephrines	Plasma free or urinary fractionated metanephrines	Plasma free or urinary fractionated metanephrines, (methoxytyramine)	Plasma free or urinary fractionated metanephrines, (methoxytyramine)	Plasma free or urinary fractionated metanephrines
Conventional imaging	CT or MRI	CT or MRI	CT or MRI	CT or MRI	CT or MRI	CT or MRI for adrenal mass; MRI for extra-adrenal mass
Functional imaging	^{123}I-MIBG (^{18}F-DOPA PET-CT/ ^{18} FDG PET-CT)	^{123}I-MIBG (^{18}F-DOPA PET-CT/ ^{18} FDG PET-CT)	^{123}I-MIBG (^{18}F-DOPA PET-CT/ ^{18} FDG PET-CT)	^{123}I-MIBG (^{18}F-DOPA PET-CT/ ^{18} FDG PET-CT)	^{123}I-MIBG ^{18}F-DOPA PET-CT	^{123}I-MIBG In metastatic disease: ^{18} FDG PET-CT

Treatment	– Laparoscopic adrenalectomy for primary tumors – ^{131}I-MIBG-PRRT, CHT, sunitinib, everolimus for metastatic tumors	Laparoscopic cortical sparing adrenalectomy Total adrenalectomy for recurrence	Laparoscopic cortical sparing adrenalectomy Total adrenalectomy for recurrence	– Laparoscopic cortical sparing adrenalectomy for primary tumors – ^{131}I-MIBG/PRRT, CHT, sunitinib, everolimus for metastatic tumors	Laparoscopic adrenalectomy for small and unilateral tumors – Posterior retroperitoneoscopic adrenalectomy for large or bilateral tumors – Laparoscopic cortical sparing adrenal surgery for bilateral tumors – ^{131}I-MIBG, CHT, sunitinib for metastatic tumors	– Laparoscopic adrenalectomy for primary tumors – ^{131}I-MIBG/PRRT, CHT, sunitinib, everolimus for metastatic tumors

yrs years, *M* male, *F* female, *PHEO* pheochromocytoma, *PGL* paraganglioma, *CT* computed tomography, *MRI* magnetic resonance imaging, *MIBG* metaiodobenzylguanidine, *PET* positron emission tomography, *DOPA* dihydroxyphenylalanine, *FDG* fluorodeoxyglucose, *PRRT* peptide receptor-targeted radiotherapy, *CHT* chemotherapy

[a]Reference: Lenders JWM et al. Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline- J Clin Endocrinol Metab. 2014 Jun;99(6):1915–42. doi: 10.1210/jc.2014-1498

The importance of an early diagnosis is highlighted by the high prevalence and unfavorable prognostic significance of d-pNENs in MEN1.

As reported in the current guidelines, besides a clinical diagnosis associated with plasma biochemical evaluation of hyperexcreted hormones, there is not a well-established consensus for the best radiological screening of MEN1-related NENs [8]. The minimum suggested imaging protocol includes annual abdominal magnetic resonance imaging (MRI), contrast-enhanced triphasic computed tomography (CT), or endoscopic ultrasound (EUS) [8]. Chest CT or MRI performed every 1–2 years is recommended for the detection of thymic and bronchopulmonary NENs. In patients with hypergastrinemia, a gastroscopy with eventual biopsy every 3 years is performed to detect peptic ulcer and type II gastric carcinoids [8].

^{68}Gallium positron emission tomography (PET) is widely used in sporadic NEN diagnosis, staging, and restaging [32]. Moreover, it can also provide prognostic information [33] and lead to therapeutic decisions, e.g., cold or radiolabeled somatostatin analogs (SSAs) [34]. The high sensibility and specificity of ^{68}Gallium PET-CT has been demonstrated in detecting also MEN1-related NENs [35–37]. Its diagnostic accuracy is high in both primary and metastatic tumors [38]. Given its higher diagnostic performance, ^{68}Gallium PET-CT should replace ^{111}In-pentetreotide single-photon emission computed tomography (SPECT) in the diagnostic work-up of MEN1-related NENs [39] and should be included in the radiologic screening and follow-up of these patients due to its capability to significantly adjust patient's therapeutic management [35, 36]. ^{68}Gallium PET-CT should be considered in the diagnostic work-up also when an insulinoma is suspected. Contrary to preliminary studies using ^{68}Ga-DOTANOC PET-CT which showed a low detection rate of insulinomas, with a sensitivity of 25% [40], ^{68}Ga-DOTATATE/DOTATOC PET-CT can identify up to 90% of sporadic insulinomas, and in case of MEN1 syndrome could be able to exclude the presence of additional pancreatic lesions not detected by anatomic imaging [41, 42].

Recently, due to the overexpression of glucagon-like peptide-1 receptor (GLP-1R) in benign insulinomas [43], PET-CT with ^{68}Ga-NOTA–exendin-4 has been studied in these patients. This new functional imaging has shown to be highly sensitive in the localization of sporadic benign insulinomas [44] and seems promising also in MEN1-related insulinomas, with a potential role in leading selective and pancreas-sparing surgery [45].

^{18}F-Fluorodeoxyglucose (FDG) PET avidity in sporadic metastatic NENs is strongly related to tumor differentiation and WHO tumor grade [46]. Moreover, it has also a prognostic role, and, regardless of Ki-67 index and histologic classification, the overall survival of patients with a positive ^{18}F-FDG PET scan is significantly lower than negative ones [47]. Given its prognostic role, ^{18}F-FDG PET-CT is suggested in MEN1 patients to identify lesions with a higher malignant potential, above all for pancreatic [48], pulmonary, and thymic lesions [49].

Recently, EUS has emerged as the most sensitive technique to detect small and intrapancreatic tumors [50]. Among its advantages, EUS allows a precise evaluation of pNEN size and can be utilized to assess serial changes in pNEN dimensions. Finally, fine-needle aspiration (FNA) can be associated with EUS to obtain a histological diagnosis guiding the clinician in therapeutic decisions [9, 51].

Medical therapy to control gastric hypersecretion includes proton pump inhibitors (PPIs) and H_2 receptor antagonists [8]. Surgical management of gastrinomas is controversial; however, surgical excision is the suggested treatment for ZES-related gastrinomas >2 cm. Surgical technique should be tailored to the patients considering preoperative findings, patient history, and preference [8, 52]. A more extensive surgery, such as pancreaticoduodenectomy with lymphadenectomy, is not performed routinely because of its higher operative mortality and long-term complications [8, 52].

In MEN1 patients with insulinomas, surgery ranges from tumor enucleation to distal pancreatectomy or partial pancreatectomy. It is the gold standard treatment in case of non-metastatic disease [8, 52]. EUS-guided ethanol ablation and CT-guided radiofrequency ablation can be performed in selected cases [52]. Before surgery, and in case of recurrent and metastatic insulinoma, patients need medical treatment. Besides frequent carbohydrate meals, also diazoxide, SSAs, the mTOR inhibitor everolimus, peptide receptor radionuclide therapy (PRRT), or hepatic artery embolization is effective in controlling hypoglycemia [52].

Regarding the other rarer functioning NETs, a curative resection is recommended in patients with pNENs >2 cm, and SSAs is the treatment of choice to control the hormone-excess prior to surgery or for unresectable lesions [8, 52].

Surgical resection is indicated for non-functioning pNEN more than 1–2 cm in size or a doubling of tumor size, over a 3- to 6-month interval and exceed 1 cm in size. Enucleation or local resection is preferred over pancreaticoduodenectomy [8]. Conservative management is safe for patients with lesions of ≤2 cm and is associated with a low risk of disease-specific mortality [53, 54]. However, recent evidence suggested that treatment with lanreotide autogel can improve progression-free survival in MEN1-related pNENs <2 cm, so avoiding or delaying surgery in a significant rate of patients [55].

Surgical treatment with curative intent is the treatment of choice for resectable thymic and bronchial NENs and PHEOs [8].

Small type II gastric carcinoids (<1 cm) may be endoscopically surveilled. Endoscopic resection or local resection with partial or total gastrectomy is reserved for larger tumors.

Similarly to sporadic NENs, in case of non-resectable or metastatic disease, SSAs (octreotide or lanreotide) are considered the first-line treatment, while PRRT is now available for NENs progressing under SSAs. Targeted therapy (everolimus or sunitinib) and chemotherapy (streptozotocin, 5-fluorouracil, doxorubicine, capecitabine/temozolomide) are effective therapies that could be employed for progressive disease [8, 56].

17.3 MEN2

17.3.1 Overview

MEN2 is an autosomal dominant genetic syndrome characterized by the occurrence of NENs arising most commonly in thyroid and adrenal glands [57] (Table 17.1). MEN2 is further classified into two subcategories: MEN2A that also presents primary PHPT (20%–30%) and MEN2B. MEN2A is further categorized into the following four subtypes: (1) classical MEN2, (2) MEN2A with cutaneous lichen amyloidosis (CLA), (3) MEN2A with Hirschsprung disease (HD), (4) familial medullary thyroid cancer.

In both MEN2A and MEN2B, there is an occurrence of multicentric NEN formation in all organs where *REarranged during Transfection (RET)* proto-oncogene is expressed.

The syndrome is caused by mutations in the *RET* proto-oncogene, localized on chromosome 10q11.2, which encodes a receptor tyrosine kinase. It appears to transduce growth and differentiate signals in several tissues, particularly those arising from neural crest cells. Some cytogenetic mutations have been reported; these may involve intracellular and extracellular domains of the RET protein signaling pathway. The germline *RET* mutations in MEN2 result in a gain of function of this tyrosine kinase receptor. This is different from many other inherited predispositions to neoplasia that are due to heritable "loss-of-function" mutations that inactivate tumor suppressor proteins [57] (Fig. 17.2).

The majority of the mutations in MEN2A variants occur in the cysteine-rich region of RET protein's extracellular domain (coded by the genes in exon 10 and 11). Mutations in the intracellular tyrosine kinase 2 domain cause MEN2B-associated tumors. A single 918 Met to Thr mutation (M918T) in exon 16 is responsible for

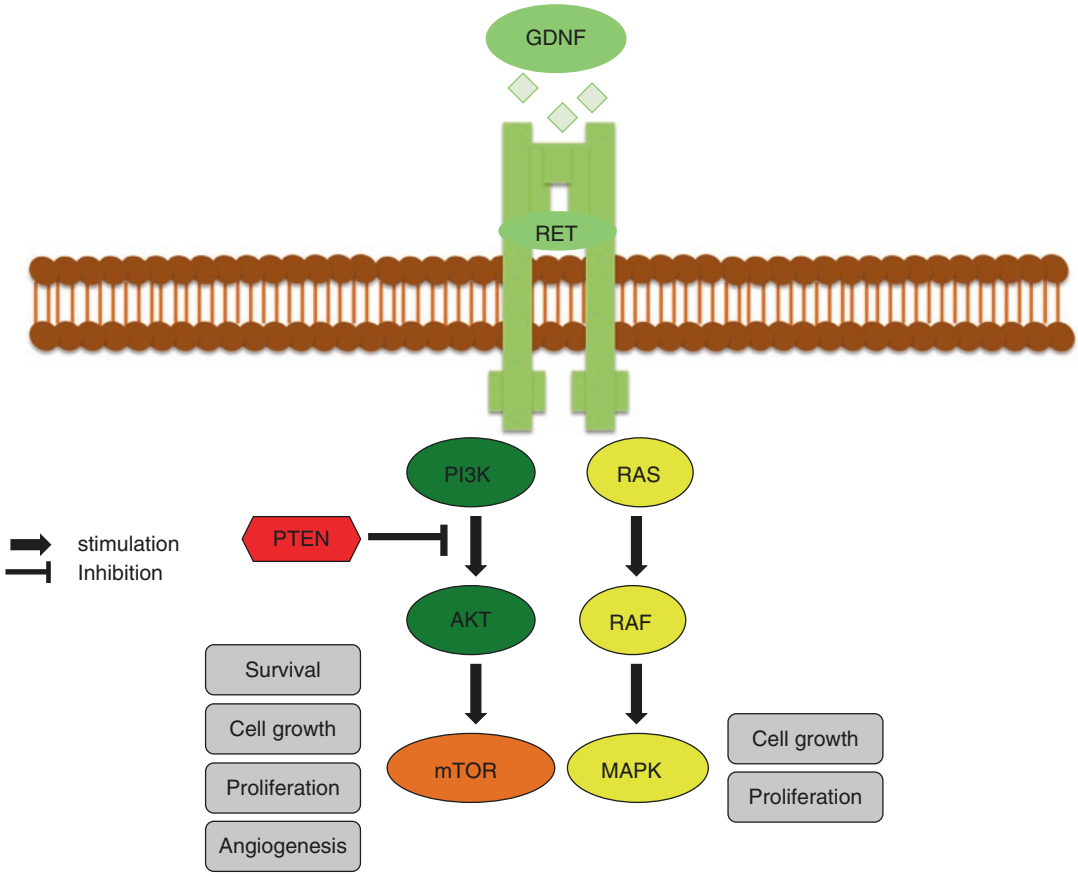

Fig. 17.2 Molecular pathogenesis of NENs in MEN2 syndrome. The proto-oncogene RET encodes for a Receptor Tyrosin Kinases that, activated by the GDNF-family ligands, regulates intracellular pathway involved in cell survival, growth, proliferation and angiogenesis. Constitutively activating mutations of RET are responsible of oncogenic event in MEN2 syndrome. *GDNF* glial cell-line-derived neutrophic factor, *RET* RErranged during Transfection receptor protein, *PI3K* phosphoinositide 3-kinase, *AKT* protein kinase B, *mTOR* mammalian target of rapamycin, *PTEN* phosphatase and tensin homolog, *MAPK* mitogen activated protein kinase

over 95% of cases of MEN2B. Other less common mutations are associated with both MEN2A and MEN2B divided into high-risk, moderate-risk, and low-risk categories [57–59].

The total prevalence of all MEN2 worldwide variants is approximately 1/35000. MEN2A accounts for about 95% of cases, MEN2B for 5%. In approximately 50% of MEN2B cases, a de novo germline *RET* mutation gives rise to the disease.

MEN2 should be suspected in any patient diagnosed with MTC or PHEO, particularly when the age of presentation is very young (<35 years). Any patient with diagnosed MTC or family history of MTC should be tested for *RET* proto-oncogene mutations for both MEN2A and MEN2B. The patients who are diagnosed with PHEO at an earlier age than sporadic forms should be tested for MEN2. The classic symptoms of PHEO are the paroxysms of a headache, anxiety, diaphoresis, palpitations, and high blood pressure. The presence of these symptoms in the third decade, particularly in between 25 and 32 years, should prompt to screen for MEN2 [57].

Other possible physical examination findings include marfanoid habitus (decreased upper to lower body ratio), mucosal neuromas (red papules) over lips and tongues, and joint hyperlaxity associated with MEN2B. MEN2A is also

suspected in patients with clinical features like purity, scaly, pigmented papules in the interscapular region, typical features of CLA [60]. The presence of PHPT alone does not indicate for further testing as it is less than 20% associated with MEN2A and no associated with MEN2B.

17.3.2 MEN2-Related NEN: Diagnostic and Therapeutic Update

17.3.2.1 MTC

Virtually all patients with MEN2A develop MTC. MTC is multicentric and occupies preferentially the upper and middle portions of each thyroid lobe. The tumor remains confined to the thyroid gland for a variable period of time before spreading to the regional lymph nodes and subsequently to the liver, lung, bone, and brain. Histologically, 20% of the tumors have a predominantly cellular growth pattern, 40% have a fibrous pattern with more than half of the cellular component replaced by a calcified acellular stroma, and the remaining 40% display an intermediate pattern with neoplastic nests of cells separated by bands of fibrous tissue. The stroma is composed primarily of full-length calcitonin, which has staining properties similar to amyloid [61].

The tumors should be appropriately staged using the synoptic cancer worksheets proposed by the College of American Pathologists [62]. Multifocality or C-cell hyperplasia in the contralateral lobe should be assessed, because those features indicate a strong likelihood of germline *RET* mutation and inherited disease [63].

It is important that clinicians who first see children with MEN2B recognize the characteristic signs and symptoms associated with the syndrome, because the MTC is highly aggressive in this setting, and there is a narrow window during which thyroidectomy may be curative [64–67].

Measurement of serum calcitonin levels, especially after the administration of the provocative secretagogues calcium, served as the primary method for screening family members at risk for hereditary MTC [68].

In line with the American Thyroid Association (ATA) management guideline for adult patients with thyroid nodules [63], the thyroid ultrasound (US) examination represents the first diagnostic choice. The US features suggestive of MTC could be hypoechoic, solid with smooth borders, round or oval shape nodule, and particularly the presence of micro- or macrocalcifications [69, 70].

The cytologic appearance of MTC on FNA can be variable, causing misdiagnosis with follicular neoplasm or sarcoma. A more accurate method of diagnosing MTC is to measure calcitonin in FNA washout fluid. FNA calcitonin is more sensitive than cytology for diagnosing MTC, reaching a 100% accuracy using a threshold value of 39.6 pg/mL (range reported in literature 7.4–67 pg/mL) [71], or a FNA calcitonin/serum calcitonin ratio >1.39 [72].

Immunocytochemistry staining of FNA specimens for calcitonin, carcinoembryonic antigen (CEA), and chromogranin can also be performed, increasing the sensitivity of cytology to 89.2% (95% CI: 74.6%–96.9%) [71].

The revised ATA guideline for MTC now recommends measurement of calcitonin in FNA washout fluid and immunocytochemistry for calcitonin, CEA, and chromogranin when cytology is inconclusive or suggestive of MTC (grade B recommendation based on fair evidence); however, the guideline does not recommend a threshold value for calcitonin [73]. CEA is not a specific MTC biomarker, but it is useful for monitoring disease progression. In addition, baseline levels of calcitonin can indicate distant metastases when they are higher than 500 pg/mL, recommending systemic imaging [74].

Approximately 50% of patients with MTC have metastatic disease on initial presentation [75]. Palpable thyroid nodules are associated with a 70% rate of lymph node metastasis and a 10% rate of distant metastasis [76].

Recommended imaging studies include neck US, CT of lungs and mediastinum, three-phase contrast-enhanced multi-detector liver CT or contrast-enhanced MRI of liver, and bone MRI or scintigraphy [77]. ^{18}F-FDG PET-CT and ^{18}F-dihydroxyphenylalanine [DOPA] PET-CT are less sensitive in detecting metastases and therefore are not recommended [78, 79].

Specific *RET* mutations are associated with disease aggressiveness and dictate early timing of thyroidectomy [80]. Before MTC is treated, diagnosis of a PHEO is essential to avoid a hypertensive crisis during surgery [80]. The preferred therapeutic option is total thyroidectomy with dissection of lymph nodes in the central neck. Additional lymph node compartments are dissected if there is evidence of metastases on preoperative imaging studies, or at the time of thyroidectomy. Currently, the generally accepted practice is to use a combination of genetic testing and the basal or stimulated serum calcitonin level to decide the timing of thyroidectomy. In families with hereditary MEN2B, the disease may be apparent at or soon after birth, when thyroidectomy may be curative; however, the MTC is aggressive in this setting, and rarely, infants have regional lymph node metastases at the time of thyroidectomy [81].

Lifelong follow-up is indicated, beginning every 3 months postoperatively, and at longer intervals if there is no evidence of persistent or recurrent disease in the first year after thyroidectomy. Serial measurements of serum calcitonin and CEA levels are useful in documenting disease progression, and especially their the doubling time.

For the patients with persistent or recurrent MTC, the treatment option is systemic therapy with orally available tyrosine kinases inhibitors (TKI), such as vandetanib, a selective inhibitor of RET, vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR) signaling, and cabozantinib, targeting MET, VEGFR2 and RET [82]. Recently LIBRETTO 001 (NCT03157128), a phase I-II trial on the efficacy of selpercatinib, a selective RET inhibitor, has been published. In RET-mutated thyroid cancer, including also a group of 55 patients affected by MTC, objective response was 69% [83]. Phase III trial comparing selpercatinib with cabozantinib or vandetanib in tirosin kinase naive patients is currently ongoing (NCT 04211337). Another selective *RET* inhibitors (BLU-667, Blueprint Medicines, Inc., Cambridge, MA, USA) is currently being evaluated in a phase II clinical trial (NCT03037385).

17.3.2.2 Pheochromocytomas and Paragangliomas

PHEOs develop in approximately 50% of patients with MEN2A and MEN2B, the clinical presentation and behavior are similar in the two syndromes. The mean age of presentation is 36 years, and the diagnosis is made after MTC in 50% of cases, concurrently with MTC in 40% of cases, and before MTC in 10% of cases. In patients with PHEO, the adrenal tumors are almost always benign and confined to the gland. In 65% of cases, they are multicentric and bilateral. Patients with unilateral PHEO usually develop a contralateral PHEO within 10 years [84].

There is significant morbidity and mortality associated with an undiagnosed PHEO; thus, in patients with known MEN2A or MEN2B, it is critical to rule out this tumor before interventional procedures. In MEN2B, over 90% of patients with PHEO have gastrointestinal symptoms characterized by abdominal pain, constipation, and alternatively diarrhea, bloating, and megacolon. The gastrointestinal symptoms are particularly evident in children and young adults and may require a surgical procedure to relieve symptoms [85]. Of note, about one-third of the patients were not symptomatic (hypertension, headaches, sweating) at the time of diagnosis [86]. Then systematic screening should thus be performed regularly even in the absence of clinical signs suggestive of PHEO.

The development of PHEO in MEN2 is usually progressive, and bilateral PHEOs are not always synchronous: metachronous PHEOs have been reported in up to 25% of cases after a mean period of 5–10 years [86, 87], requiring a prolonged follow-up after the first surgery. PHEO represents the most prevalent disease of MEN2 given the fact that young familial cases are treated by prophylactic thyroidectomy.

Positive diagnosis is based on increased plasma metanephrines and normetanephrines (drawn from a supine patient after an overnight fast), or 24-h urinary fractionated metanephrines and normetanephrines or plasma or urinary fractionated metanephrine and normetanephrine [88]. MEN2-associated PHEOs express phenylethanolamine N methyltransferase (PNMT), the

enzyme that converts norepinephrine to epinephrine, hence the association with predominant epinephrine secretion and elevated metanephrines [88]. Serum chromogranin A is elevated in 48% of patients with PHEO [88]. Diagnostic utility of chromogranin A is, however, constrained by poor specificity due to its elevation in several conditions [88].

Imaging should be performed only when biochemistry becomes positive [89]. US can detect PHEO in 80–90% of cases [90] where it may be visible as a well-defined mass, which may be solid (75% in one case series) or cystic or mixed [91]. CT scanning and MRI are used to localize PHEO. The sensitivity (90%–100%) and specificity (70%–80%) are similar for the two procedures [92, 93].

Several specific radiopharmaceuticals (^{123}I-metaiodobenzylguanidine [MIBG], ^{18}F-DOPA PET, and ^{111}In-pentetreotide (Octreoscan, Covidien) and ^{68}Gallium PET) have been used for functional imaging [92, 94, 95]. The main advantage of ^{18}F-DOPA compared to other radiopharmaceuticals is the absence or faintly uptake by normal adrenal glands. ^{18}F-DOPA PET-CT can also detect residual MTC in patients with persistent hypercalcitoninemia [96–100]. MIBG is the most common and available functional imaging used in the assessment of PHEO. The uptake of radiotracer is proportional to the number of neurosecretory granules within the tumor [92, 94, 95]; therefore, the characteristic appearance of a PHEO is unilateral focal uptake within the tumor [101]. Octreoscan and ^{68}Gallium PET can detect PHEO, because they express SST receptors [95].

Excepting very unusual circumstances, a PHEO should be resected before the MTC if both are present. Preoperative preparation is with alpha-adrenergic blockade and if necessary beta-adrenergic blockade. Subtotal sparing adrenalectomy is indicated to preserve adrenocortical function [102, 103]. The idea of adrenal sparing surgery is to take off the PHEO while maintaining one third to one fourth of the gland to allow maintenance of a normal cortisol and aldosterone function. As there is only a very low 1–4% risk of malignancy for MEN2 PHEO [104], this procedure should be systematically considered in all patients with MEN2 PHEO. The standard procedure is laparoscopic adrenalectomy [105, 106]. Recurrence after adrenal sparing surgery will be mainly treated by total adrenalectomy, or in some very experienced centers, by another partial adrenalectomy [107].

17.4 MEN4

17.4.1 Overview

MEN4 is a recently characterized autosomal dominant genetic syndrome characterized by the occurrence of NETs arising mainly in parathyroid glands and anterior pituitary gland [13]. The syndrome is caused by mutations in the tumor suppressor *CDKN1B* gene, located on chromosome 12 (12p13), consisting of three exons, encoding a kinase inhibitor protein named p27, primarily inhibiting the complex cyclin E/cyclin-dependent kinase (CDK)2 [108] (Fig. 17.1). MEN4 is generally observed in patients with MEN1 phenotype but no *MEN1* gene mutations, the so-called MEN1 phenocopies, or patients with an intermediate phenotype between MEN1 and MEN2 without *MEN1* and *RET* mutations. The incidence of *CDKN1B* mutations in MEN1 phenocopies has been estimated in the range of 1.5–3.7% [109, 110]. To date, 48 subjects have been reported as *CDKN1B* mutated, including 23 MEN4 patients and 25 carriers. Nineteen different heterozygous loss-of-function *CDNK1B* mutations have been identified in patients with MEN4, including nine missense, six nonsense or frameshift, and four mutation/deletion within the 5'-UTR region [109–123]. As a whole, *CDKN1B* mutations causing MEN4 affect p27 cellular localization, stability, or biding with Cdk2 or Grb2 [108].

All typical MEN1 endocrine tumors are observed also in MEN4 (Table 17.1). As in MEN1, PHPT is the most frequent endocrine disorder in MEN4 (83%), while pituitary adenoma occurs in 39% and is mostly ACTH- and GH-secreting, conversely to MEN1 where the prolactin-secreting adenoma is the main type.

NENs presented a lower penetrance in MEN4 than MEN1, being reported in 17% of the *CDKN1B* positive subjects reported in the literature. However, the rate of NENs is double (35%) if we consider the 23 cases with MEN4 reported.

17.4.2 MEN4-Related NEN: Diagnostic and Therapeutic Update

Due to the very small number of cases, it is not possible to achieve specific conclusions for MEN4-related NENs. These included GEP NEN in six cases, lung and cervix in one case each [109, 110, 112, 114, 116, 117, 120, 123] (Table 17.2). All GEP NENs were well-differentiated tumors (NET) as well as the lung one, which was a typical carcinoid. A small cell poorly differentiated cervical NEC occurred in one patient. Among the GEP NETs, four were pNET, in combination with dNETs in three cases, and two gNET. A ZES was the only functioning endocrine syndrome, reported in two patients with d-pNETs. No other functioning syndrome such as insulinoma, glucagonoma, VIPoma, and ectopic hormone syndrome has been reported. When reported, Ki-67 index was 1% (G1). Three pNET, two of whom associated with dNETs, were metastatic, as well as the lung carcinoid, while the gNETs were localized.

In all cases, NENs were diagnosed in females at age ranging 42–79 years (median, 57 years). In all cases but one, a PHPT was also detected, while pituitary adenomas were in three out of the eight patients with NEN (acromegaly, Cushing's disease, and nonfunctioning pituitary adenoma respectively).

As a whole, a diagnosis of MEN4 has to be considered in all patients with MEN1-related tumors and no *MEN1* mutation. MEN4-related NENs are usually NETs located within the duodenum–pancreas tract. They are well differentiated and low proliferating, resulting in tumor diagnosis in the sixth decade as average.

In the lack of specific studies for MEN4-related NENs, the diagnostic work-up of these tumors should be made in the same way as for MEN1 NENs, where contrast-enhanced triphasic CT scan or MRI, in combination with EUS, is the best diagnostic procedure to detect the small NETs which are located in duodenum and pancreas. Endoscopy and EUS are the optimal tool to characterize gNETs. As for either MEN1 or sporadic NETs, ^{68}Gallium PET is the best functioning imaging technique in MEN4-related NETs, to perform tumor staging in combination with CT, as well as to candidate these tumors to therapy with cold or radiolabeled SSAs.

As for MEN1, ZES can be associated with MEN4 d-pNETs and should be therefore investigated by measuring serum gastrin levels, after exclusion of all other conditions of hypergastrinemia, first of all the use of PPIs. Chromogranin A is the general neuroendocrine marker to be assessed after the histological diagnosis of NET, as potentially useful biochemical marker for follow-up.

An optimal strategy to perform an early diagnosis of NEN in MEN4, is to perform the mutational analysis of *CDKN1B* in all patients with MEN1 phenotype and negative *MEN1* analysis. Care should be in particular for females affected with PHPT.

When a MEN4 patient is identified, a familiar genetic screening has to be performed in order to recognize asymptomatic patients and gene carriers. All *CDKN1B*-positive subjects should undergo a clinical, biochemical, and radiological work-up, which has to be addressed not only to parathyroid glands and pituitary but also to NENs, in particular d-pNETs.

Therapy of MEN4-related GEP NENs could be the same as in MEN1. Surgery has to be considered for tumors >1.5–2.0 cm within pancreas or duodenum or tumor progressing during active surveillance or those associated with ZES. As in MEN1, radical surgery has not to be taken in account because of high morbidity and mortality rate, while tumor enucleation, distal pancreatectomy, and duodenectomy are reasonable procedures for this kind of patients.

SSAs are the first therapeutic option in MEN4-related d-pNETs with uncontrolled ZES or tumor progression. MEN4-related GEP NENs are expected to be clinically controlled and

radiologically stabilized for long time with SSA therapy. In case of SSA failure, PRRT is a new option for all SST-positive tumors. MEN4 NETs likely express SST at high grade and therefore are potential candidates for PRRT. Alternatively, a targeted-therapy with everolimus could be performed, especially in consideration of the peculiar molecular pathway underling these tumors, where the AKT-mTOR complex results to be hyperactivated. Finally, chemotherapy is another option that could be considered in metastatic NETs with high tumor burden, not responding to previous therapies.

17.5 Von Hippel–Lindau

17.5.1 Overview

VHL disease is an autosomal dominant genetic syndrome caused by a germline mutation in the *VHL* gene. *VHL* is a suppressor gene located on chromosome 3p25 [124]. This gene has three exons which encode for two different mRNA and, consequently, two isoforms of VHL protein [125]. Both the isoforms are required for VHL protein actions. *VHL* is a tumor suppressor gene, so tumors arise in patients after the inactivation of the wild-type allele. VHL protein, localized in the nucleus or cytoplasm, binds elongin B, elongin C, and Cullin 2 [126]. The multi-protein complex is responsible of the inhibition of transcription elongation and ubiquitin-mediated degradation of various proteins, including the α subunits of hypoxia-inducible factors (HIFs) 1 and 2 [127] (Fig. 17.1). Consequently, abnormal or absent VHL protein is implicated in tumorigenesis by enhancing HIFs and, consequently, stimulating glucose uptake and expression of angiogenic and mitogenic factors as VEGF, platelet-derived growth factor (PDGF), and transforming growth factor α (TGFα) [4, 128, 129].

Prevalence of VHL disease is 1/36,000 live births [4]; the majority of VHL cases are familial but up to 20% are caused by de novo mutations [130]. Penetrance is almost complete by the age of 75 years [131]. VHL patients show inherited susceptibility to many kinds of benign and malignant tumors including renal clear cell carcinoma, hemangioblastomas of the retina and of the central nervous system, endolymphatic sac tumors, simple cysts, pancreatic serous cystadenomas, and NENs [132]. Clinically, VHL syndrome is classified into two types according to the absence (type 1) or presence (type 2) of PHEO. Type 1 can be subclassified in accordance with high (1A) or low (1B) risk of renal cell carcinoma. Type 2 VHL is further categorized into type 2A (associated with other tumors different from renal cell carcinoma), type 2B (associated with renal cell carcinoma), and type 2C (only PHEO, also called autosomal dominant familial non-syndromic PHEO). Interestingly, different family members can have different disease manifestations as well as different VHL subtypes [133, 134].

Clinical diagnosis is based on the discovery of a classical VHL-associated tumor (central nervous system hemangioblastoma, retinal hemangioblastoma, renal cell carcinoma, PHEO) in a patient with positive familial history or, for sporadic cases, diagnosis is based on the presence of at least two classical VHL-associated tumors (in particular, two hemangioblastomas or one hemangioblastoma associated with one visceral tumor) [133]. Genetic testing is always recommended for confirmatory diagnosis, familial screening, genetic counseling, and genotype-phenotype predictions [132, 135]. The identification of asymptomatic carriers of *VHL* mutation is essential for early detection of VHL-related tumors in order to limit morbidity and mortality [135, 136].

NENs associated with VHL include pNENs and PHEO/PGLs, while PHPT is anecdotally reported (Table 17.1).

17.5.2 VHL-Related NEN: Diagnostic and Therapeutic Update

17.5.2.1 Pancreatic NEN

Patients with VHL syndrome have a lifetime risk of developing one or more pNENs of 20%. Table 17.2 reports the main features of VHL-associated pNETs. Histologically, VHL-related

pNETs are similar to the sporadic counterpart, even if they can present clear cell features [137]. Classical neuroendocrine cells are medium size, uniform cells with eosinophilic cytoplasm, round to oval nuclei, and "salt-and-pepper" granular chromatin, usually organized in trabecular structures [138]. Similarly to MEN1-, MEN4-, and NF1-related NENs, VHL-related NENs are usually grade 1 or 2 NETs. Women have a slightly higher risk of pNET development, with male to female ratio ranging from 1:1.1 to 1:1.6 [139, 140]. Mean age of presentation is 35 years, about 20 years before sporadic pNET [141]. The youngest patient affected by pNET was 11 years old [141]. Half of these tumors are localized in the head of the pancreas [142]. VHL-related pNETs differ from sporadic ones because of their tendency to be multiple [143] but with overall indolent behavior, even if a variable proportion of patients ranging, in larger studies or metanalysis, from 12.8 to 20% have metastatic disease [141, 144].

The great part of VHL-associated pNETs are non-functioning [139], and only sporadic reports demonstrated that they can secrete ACTH, causing ectopic Cushing's syndrome [145]. Patients are therefore usually asymptomatic, and symptoms arise in case of compression of nearby structures [146].

Diagnosis is based on radiological findings. Morphological imaging commonly used for the detection of pancreatic lesions are contrast-enhanced CT and MRI and EUS [147]. CT shows a well-defined solid mass, usually with rounded or lobulated borders, characterized by early enhancement [148]. Pancreatic MRI usually shows hypointense T1-weighted sequences and hyperintense T2-weighted sequences lesions, which can contain hemorrhagic, necrotic, and calcified portions [149]. EUS is the most sensitive method for the diagnosis of small solid pancreatic tumors [147]. Functional imaging is recommended in case of locally advanced or metastatic disease; moreover, it could play a role for helping differential diagnosis and for identifying tumor recurrence [147]. In sporadic pancreatic tumors, ^{68}Gallium-DOTA PET showed a better sensibility compared to SST receptor scintigraphy [39, 150]. This data has been confirmed also in VHL-associated pNET [141, 151]. In a study on 197 patients affected by VHL-related pancreatic lesions, Sadowski et al. demonstrated that ^{18}F-FDG PET-CT was able to correctly characterize pNETs using a standardized uptake value (SUV) cut-off of 4 (sensibility 92%, specificity 75%) and in three patients also gave the possibility to recognize metastatic sites not previously detected by total-body CT scan [152]. Routine use of biopsy in these patients is not recommended, because tumors are nearly often correctly identified by morphological and functional imaging, and pNET in VHL disease are known to be well differentiated, although a biopsy would be useful to characterize tumor biology in selected patients [147]. Chromogranin A can be useful for follow-up in some patients with high basal levels [153].

Natural history of pNET is variable among VHL patients, so it is of a great importance to consider prognostic factors in order to identify the best treatment strategy.

Blansfield et al., in a study on 108 VHL-related pNETs, described that more aggressive tumors, with higher metastatic potential, had three characteristics: size >3.0 cm, presence of a mutation in exon three and tumor doubling time less than 550 days [139]. Similarly, Krauss et al. underwent to comparable conclusions: size >2.8 cm and mutation in codon 161/167 of exon three were the main prognostic factors [141].

According to these findings, surgical resection is the therapy of choice for larger masses. Guidelines recommended surgery in case of diameter >3.0 cm in pancreatic tail and body, considering the higher risk of metastases, and >2.0 cm in pancreatic head and uncinate process, in order to prevent main pancreatic duct involvement, which implicates a more radical resection [147]. After guidelines publication, an original article on 2330 VHL patients, 273 of which had pNETs, demonstrated a longer 10 years disease-free survival also in small pNET surgically treated compared to surveillance [154]. On the other hand, surgically treated patients had a high rate of postoperative complications. In particular, early postoperative complications were fistula,

abscess, or cholangitis (23%), while long-term postoperative complications were diabetes mellitus and exocrine insufficiency (41%) [141]. Taking into account ENETS consensus guidelines for the management of pNET [52], it is reasonable to suggest a surgical resection of VHL pNETs with one of the following characteristics: diameter >2.0 cm, growth rate >0.5 cm per year or in case of functioning tumors.

In case of surgical resection, given the possibility of multiple lesions, intraoperative US should be suggested [155]. When it is possible, enucleation of pNETs is recommended for sparing pancreatic tissue [143], even if no comparison study between enucleation and classical resection is currently available. Lymphadenectomy is recommended for correct staging [147].

After surgical excision, annual imaging with CT or MRI is recommended [155]. In case of locally advanced or metastatic disease, no specific data are available in VHL patients, so patients are treated according to the beforementioned ENETS guidelines [52]. Briefly, surgical intervention can be considered for reducing tumor burden or in case of complications, as obstruction, compression, or hemorrhage [147]. Systemic first-line therapy is based on SSAs, which has demonstrated in CLARINET study to increase progression-free survival (PFS) in entero-pancreatic NETs [156]. In case of disease progression, it is possible to consider PRRT [52] or targeted therapy [157]. Specific targeted therapies could play an important role in VHL-related tumors, even if no dedicated clinical trials are currently available in VHL-mutated patients.

17.5.2.2 Pheochromocytomas and Paragangliomas

PHEO/PGLs arise respectively from chromaffin cells localized in the adrenal medulla and in extra-adrenal paraganglia. The percentage of VHL patients developing PHEO/PGLs is estimated from 10 to 20% [4, 132]. Table 17.3 reports the main features of VHL-associated PHEO/PGLs. For definition, PHEO occurs only in type 2 VHL. Mean age of presentation is <30 years, and the risk of malignancy is lower than 5% [158]. More than 900 *VHL* mutations have been described, including deletions, missense substitutions, and mutations causing the synthesis of a truncated protein. PHEO often occurs in association with specific alleles, usually due to missense mutations rather than deletions or premature termination [125]; particularly, the mutation at nucleotide 238 in exon 3 is associated with a 62% risk for PHEO [159]. The reason could be that PHEO development requires partial but not complete loss of function in VHL protein [134]. Interestingly, HIF-2α is highly expressed in the adrenal medulla and in the organ of Zuckerkandl, and the gene encoding for tyrosine hydroxylase, implicated in adrenal catecholamine production, is a HIF target gene [160].

PHEO in VHL disease can be bilateral and multiple [4] and most commonly secrete norepinephrine, although a small percentage can produce dopamine [161]. Clinical presentation includes intermittent or sustained hypertension, palpitations, tachycardia, headaches, anxiety, sweating, pallor, and flashes up to hypertensive crisis [130].

PHEO/PGLs are histologically characterized by neoplastic cells, with oval nuclei, granular cytoplasm, and evident nucleolus, gathered in nest or "zellballen" pattern, surrounded by S-100-positive sustentacular cells [132, 138].

Diagnosis is based on the dosage of plasmatic or urinary fractionated metanephrines. Plasmatic normetanephrines seem to have the greatest sensitivity and specificity compared to other plasma catecholamines and urinary catecholamines, and vanillylmandelic acid [162]. Urinary fractionated metanephrines can be used alternatively [163]. Endocrine Society Clinical Practice guidelines on PHEO/PGL recommend drawing blood sample for plasma testing in supine position, using liquid chromatography with mass spectrometric or electrochemical detection methods and checking possible pharmacological interferences [163].

Confirmatory clonidine suppression test demonstrated 97% sensibility and 100% specificity in a retrospective study [164], but no data are available in VHL syndrome, and this test should be performed in centers with an adequate experience and only in selected patients.

In case of biochemical alterations, morphological imaging is recommended. CT is usually preferred for the detection of adrenal masses, considering the great sensibility and special resolution, while MRI is more accurate for PGL identification [163]. PHEOs/PGLs appear as homogeneous or heterogeneous mass, usually necrotic, with some calcifications [165]. MRI usually shows hyperintense mass in T2-weighted image [166].

Functional imaging is particularly relevant in case of extra-adrenal PGLs or for metastatic disease. Guidelines recommend ^{123}I- MIBG scintigraphy for the high accuracy in PHEO diagnosis (sensibility 92%, specificity 94%) [167] and for predicting response to radiotherapy using ^{131}I-MIBG. In metastatic cases, diagnostic accuracy of ^{18}F-FDG PET seems better than ^{123}I-MIBG [168]. Few studies analyzed diagnostic accuracy of ^{18}F-DOPA PET-CT in VHL-related PHEOs/PGLs. Weisbrod et al., in a study on 52 VHL-mutated patients, demonstrated that ^{18}F-DOPA PET-CT was able to identify lesions not detected by conventional imaging in 9.6% of patients, even if CT and MRI generally identified a larger amount of masses. The authors concluded that ^{18}F-DOPA PET-CT should be used as complementary diagnostic technique [169]. Another study on 101 patients, including 19 VHL mutated patients, with known or suspected PHEOs/PGLs, demonstrated a high sensibility and specificity of ^{18}F-DOPA PET-CT, respectively 93% and 88% [170].

Surgery is the treatment of choice and should be performed even in asymptomatic patients. Best surgical management for VHL-associated PHEO is laparoscopic cortical sparing mass excision, in order to maintain corticosteroid independence [171, 172]. In case of functioning PHEO/PGL, patients require previous preparation therapy with α-adrenergic receptor blockers. Objectives of pre-surgical treatment are reduction of diastolic blood pressure and heart rate, and minimization of the risk of postoperative hypotension [173].

β-Adrenergic receptor blockers are indicated for controlling tachycardia but can be used only after starting α-adrenergic receptor blockers, and calcium antagonist can be added for controlling blood hypertension [174].

In metastatic PHEOs/PGLs, debulking surgery can improve overall survival [175]. In case of stable disease or slow progression, a follow-up strategy or radionuclide therapy using ^{131}I-MIBG or ^{177}Lu-DOTATATE is recommended [176].

Systemic treatment includes chemotherapy with cyclophosphamide, vincristine, and dacarbazine. This protocol is burdened by serious adverse events and determines a partial response in about 37% of patients [177].

Recently, new targeted therapies are under evaluation for the treatment of metastatic PHEOs/PGLs. Antiangiogenic therapy with TKI has been studied as potential treatment in malignant lesions [178], and an international randomized study on sunitinib is now ongoing (FIRSTMAPPP, NCT01371201). Sunitinib seems particularly promising in VHL syndrome, considering the role of sustained angiogenesis in VHL mutated tumors [179].

Finally, immunotherapy has been proposed in patients with alterations in proteins associated with the regulation of hypoxia-inducible factor-α, as in VHL disease [176, 180]. The programmed cell death protein 1 (PD1) is one of the checkpoints that impedes the efficacy of cytotoxic T lymphocyte response, and pembrolizumab is a humanized monoclonal antibody directed against PD1 [181]. Only one phase II trial on pembrolizumab is now recruiting (NCT02721732), and the results could be very interesting for VHL-associated PHEO/PGL.

VHL guidelines [131, 182, 183] recommend that screening for PHEO/PGL should begin in early childhood (5 years) and should be repeated every 12 months, using blood pressure monitoring and evaluation of fractionated metanephrines (paying special attention to normetanephrine) in plasma or 24-h urine collection. Imaging protocol includes annually abdominal US examination from 8 to 15 years, reserving MRI or functional imaging in case of biochemical alterations. After the age of 16 years, abdominal imaging is performed annually, alternating abdominal US and

MRI, which is preferred to CT for reducing exposure to ionizing radiation. Clearly, abdomen examination is performed also for the early diagnosis of renal cell cancer and pNET.

17.6 Neurofibromatosis Type 1

17.6.1 Overview

NF1, also known as von Recklinghausen disease, is an autosomal dominant disorder with a complete penetrance and variable expression, caused by germline mutations in the *NF1* tumor suppressor gene. *NF1* gene, located on chromosome 17q11.2, encodes for neurofibromin, a protein acting as negative regulator of the RAS-RAF-MAPK pathway, involved in cell growth and proliferation (Fig. 17.1). So the loss of neurofibromin expression, as seen in NF1, leads to increased cell growth and survival through hyper-activation of RAS. NF1 belongs to a group of inherited disorders referred to as phakomatoses or neurocutaneous syndromes.

Its prevalence is estimated in 1/3000 live births, with half of cases showing a family history and half arising with a de novo mutation. NF1 can affect multiple organ systems and has a wide range of variable clinical manifestations.

Approximately all individuals with NF1 develop pigmentary lesions (café-au-lait macules, skinfold freckling, and Lisch nodules) and dermal neurofibromas. Some individuals show skeletal abnormalities (scoliosis, tibial pseudarthrosis, and orbital dysplasia), brain tumors (optic pathway gliomas and glioblastoma), peripheral nerve tumors (spinal neurofibromas, plexiform neurofibromas, and malignant peripheral nerve sheath tumors), learning disabilities, attention deficits, and social and behavioral problems, which can negatively affect the quality of life. Life expectancy in people with NF1 is reduced by 10–15 years mainly due to a high risk of malignant tumors [184]. NENs can occur in the context of NF1 including either GEP NENs or PHEOs/PGLs (Table 17.1).

17.6.2 NF1-Related NEN: Diagnostic and Therapeutic Update

17.6.2.1 Gastroenteropancreatic NEN

NF1-related GEP NENs (Table 17.2) are reported in about 1% of individuals with NF1 with special affinity for the duodenal and periampullary region [185]. In the most recent review of gastrointestinal tumors associated with NF1, tumor sites were duodenum (60%), ampulla (31%), pancreas (5%), or bile duct/gallbladder (4%), with SST-positive NET, the so-called somatostatinoma, as the most common histology (40%) [5]. The peri-ampullary somatostatinoma is almost patognomonic of NF1, because a rate of 26–41% of these tumors has been reported in association with NF-1 [5]. A recent study of whole-exome sequencing of six NF1-related dNETs confirmed the importance of somatic inactivation of the wild-type *NF1* and suggested that loss of chromosome 22 is another genetic determinant in at least a subset of cases [186].

The NF1 somatostatinomas, compared to sporadic ones, occur at younger age (<50 years) and are smaller in size, probably because of the earlier diagnosis due to local symptoms (i.e., pain and jaundice) related to peri-ampullary localization and the clinical screening of this kind of NET in the context of NF1 [185]. They are well differentiated (NET), with low tumor grade (G1–G2) and high incidence of psammoma bodies (psammomatous calcifications), which are helpful in guiding the diagnosis. Very rarely mixed neuroendocrine non-neuroendocrine neoplasms (MiNENs) of periampullary region, expressing SST, have been reported [187]. The majority of peri-ampullary and dNENs in NF1, although express SST, are non-functioning somatostatinomas, so they occur in the absence of the characteristic syndrome, including diabetes mellitus, steatorrhea, cholelithiasis, and weight loss. Most patients with gastrointestinal tumors associated with NF1 are symptomatic (92%), but clinical features are variable depending on tumor localization, size, and spread [5].

The most frequent symptoms are attributed to the mass effect: jaundice and non-specific abdominal pain are the most common, occurring in approximately two thirds of patients, followed by weight loss, gastrointestinal bleeding, and anemia. Due to the high risk of NF1-related malignancies, patients with abdominal symptoms may show one or more intra-abdominal, synchronous, or metachronous tumors, especially gastrointestinal stromal tumors, associated with dNETs [188].

The imaging features of a peri-ampullary mass in a patient with NF1 are clinically relevant in making the differential diagnosis, since somatostatinoma usually presents as a focal intraluminal mass [185].

Since these tumors express SST receptor subtypes 2 and 5, SST receptor-based imaging techniques are useful to localize them, but also to predict the response to therapy with cold or radiolabeled SSAs. Therefore, the ^{68}Gallium-DOTATATE PET-CT, in combination with upper gastrointestinal endoscopy, EUS, CT, and MRI should be considered for diagnosis, staging, and preoperative assessment of these tumors [189].

In NF1 individuals, NETs very rarely metastasize. Local and node invasions are more frequent, but the preoperative imaging study and endoscopic biopsy are often inaccurate regarding lymph node involvement and depth of invasion [190].

For tumors smaller than 1–2 cm, there is no consensus regarding management. Endoscopic excision or transduodenal surgical ampullectomy have been suggested [191]. Endoscopic ampullectomy could be an option when the tumor is limited to the mucosal layer without lymphovascular involvement [190]. Otherwise, transduodenal surgical ampullectomy could be suggested for relatively small tumors with suspected submucosal invasion [190]. However, ENETS guidelines for gastroduodenal sporadic NETs smaller than 1 cm suggest a more aggressive approach for peri-ampullary lesions with surgical resection, whereas an endoscopic management for not peri-ampullary localizations. On the contrary, for NF1-related NETs ≥2 cm, surgical resection is recommended, and local lymphadenectomy should also be considered due to the risk of submucosal invasion and lymph node involvement. Pancreaticoduodenectomy with regional lymphadenectomy should be limited to larger tumors with more aggressive behavior. Postoperative treatments in cases with node metastases have not been established. Response to chemotherapy with etoposide and cisplatin has been reported in a metastatic NET [190], while to 5-fluoroacil and oxaliplatin in a patient with MiNEN [192].

pNETs in NF1 patients are rare with only seven cases reported in the literature, five of them showed an aggressive behavior, suggesting that might be some biological differences between peri-ampullary and pancreatic NF1-related NETs. Histology was insulinoma in three cases, somatostatinoma in two cases, and non-functioning NET in two other cases [193].

In only two NF1 patients, rectal NETs have been described. They were multiple, with different and nonspecific clinical symptoms, that include changes in bowel habits, hematochezia, and abdominal pain. This clinical picture is similar to those of the most frequent rectal diseases such as hemorrhoids, rectal polyps, and colorectal adenocarcinoma, thus making difficult to achieve an early diagnosis [194].

17.6.2.2 Pheochromocytomas and Paragangliomas

PHEOs/PGLs in patients with NF1 (Table 17.3) show a debatable prevalence. While the mostly cited prevalence in the literature is 0.1–5.7% based on a retrospective review [195], subsequent studies showed that the prevalence might be higher if patients were screened prospectively (7.7–14.6%) [196, 197]. In the last few years, an increased number of incidental diagnosis was evident in normotensive and asymptomatic patients due to the large use of advanced imaging and a better knowledge of the disease genetic basis. Individuals with PHEOs/PGLs associated with NF1 were predominantly women in the fourth decade of life with no family history of PHEOs/PGLs.

The mean age was younger in NF1 than in patients with sporadic PHEOs/PGLs, while older as compared to patients with other genetic

syndromes, probably due to lack of routine screening for adrenal medulla in NF1 and consequent delayed identification [198, 199].

Approximately 84% of individuals with PHEO/PGL have solitary adrenal tumors, 10% bilateral adrenal tumors, and 6% have extra-adrenal tumors in the abdominal sympathetic chain, the organ of Zuckerkandl or the bladder [200]. Individuals with NF1 are at higher risk of malignant PHEO/PGL than sporadic ones (11.5% vs. 4%) and can present with distant metastases [200]. A recent study found that all cases of bilateral, metastatic, and recurrent PHEOs/PGLs occurred in women [201].

NF1-related PHEOs/PGLs, whether or not secreting, are mostly asymptomatic. When symptomatic, patients can show typical symptoms of catecholamines hypersecretion: hypertension, sweating, palpitations, headache, or flushing.

NF1 PHEOs produce both epinephrine and norepinephrine attributable to the activity of the PNMT enzyme, the terminal enzyme in catecholamine synthesis, which converts norepinephrine to epinephrine [202, 203]. In these patients, the increased plasma and urinary levels of metanephrine (indicating epinephrine overproduction) and normetanephrine (a norepinephrine metabolite) help to discriminate NF1 from VHL tumors that express only a noradrenergic phenotype [202, 203]. All patients with NF1, such as patients with MEN2, presented with tumors characterized by increased plasma concentrations of metanephrines, in contrast plasma-free methoxytyramine was elevated only in 39% of patients with NF1 [203]. Therefore, when suspected, PHEOs are diagnosed by assessing the levels of plasma-free metanephrines and performing abdominal imaging (CT or MRI), combined with functional imaging using ^{123}I-MIBG or ^{18}F-DOPA PET [196].

Clonidine suppression testing can also be used in case of an indeterminate adrenal nodule associated with elevated urinary metanephrine levels [204].

Over the last few years, the potential significance of systematic screening for PHEO/PGL in patients with NF1 has been questioned in the literature. If individuals with other hereditary endocrine neoplasia syndromes are routinely screened for PHEO/PGL, contrarily, both adult and pediatric NF1 guidelines not recommend routine biochemical screening for these types of tumors. They recommend that patients with NF1 should have a specialist clinic visit once a year with blood pressure measurement, given the association with renal artery stenosis and PHEO. According to the American College of Medical Genetics and Genomics, only patients with NF1 and hypertension, aged ≥30 years, who are pregnant, and/or symptomatic should be considered for biochemical or imaging screening. Some recent studies suggest that systematic biochemical screening should be a part of the routine evaluation in patients with NF1, by regular measurements of plasma-free or urinary fractionated metanephrines, starting from early adolescence and repeated every 3 years [198, 205, 206]. Patients with undiagnosed PHEO/PGL are at risk of developing life-threatening cardiovascular complications due to catecholamine crises triggered by tumor manipulation, anesthesia, drugs, pregnancy, or rarely metastatic disease [206], so biochemical testing should also be carried out prior to elective surgical procedures and conception [201, 206].

Surgical resection with laparoscopic adrenalectomy is the standard treatment for these tumors. Posterior retroperitoneoscopic adrenalectomy is a reasonable approach with a more direct access to the adrenal gland in cases with significant history of abdominal surgeries and bilateral adrenal tumors [207]. However, in last years, management of hereditary PHEOs has drastically evolved and cortical sparing adrenal surgery may be proposed to avoid definitive adrenal insufficiency especially in case of bilateral PHEOs with low risk of malignancy, the most great experience was in patients with MEN2 and VHL [208].

Patients with NF1 had the most volatile intraoperative hemodynamic course and more severe postoperative complications that may be related to large tumors associated with abundant catecholamine secretion [202].

The treatment of malignant PHEO should be focused on symptomatic control of the hypersecretion using alpha and beta adrenergic blockade.

If possible, a surgical excision or a debulking procedure should be performed. No effective treatment currently exists for PHEO with distant metastases. Internal radiotherapy with ^{131}I-MIBG and chemotherapy, using cyclophosphamide, vincristine, and dacarbazine, have been widely used with poor responses. Sunitinib, an oral receptor TKI, inhibits catecholamine synthesis and secretion in PHEO tumor cells and may prove to be useful in the treatment of malignant PHEOs in the future even in the context of genetic syndromes [209].

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18 Mixed Neuroendocrine and Non-neuroendocrine Neoplasms (Mi NEN)

Alice Laffi, Nicola Fazio, Manila Rubino, and Francesca Spada

18.1 Introduction and Terminology

Among epithelial neoplasms, the coexistence of neuroendocrine and non-neuroendocrine components is a rare but possible phenomenon. These entities encompass a heterogeneous category of neoplasms whose two histological components could be represented in different proportions in the whole tumoral mass. Furthermore, they can be characterized by a different morphology, proliferation index, and, thus, degree of differentiation in both neuroendocrine and non-neuroendocrine components. Despite their rarity, with the improving use of immunohistochemistry, the incidence of new cases increased compared with the past [1].

Indeed, during the years, the variability in proportions of the two different patterns gave birth to a large number of definitions, creating some clinical and pathologic controversies. In 2006, Volante et al. tried to explain the wide spectrum of histologic entities compositions, ranging between the two extremes: on the one hand, pure neuroendocrine and, on the other hand, pure non-neuroendocrine neoplasms [2].

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In the 2010 classification system of digestive tract neoplasms, the World Health Organization (WHO) introduced the definition of "mixed adeno-neuroendocrine carcinomas" (MANECs) for those entities composed of a neuroendocrine and a non-neuroendocrine component and in which each histologic entity represented at least 30% of the whole tumor mass [3].

This threshold was adopted also by the 2017 WHO classification of pancreatic mixed neoplasms [4]. The cut-off of 30% mostly derived from the statement of the study by Lewin et al. that restricted the definition of "adeno-endocrine cell carcinomas" only to those neoplasms with a neuroendocrine component proportion of at least 30–50%. This assumption was not based on demonstrated, statistical differences in clinical or prognostic terms, but rather on the feeling that a minor component of one of the two histologies did not influence the biologic behavior of the whole tumor [5].

Anyway, the term "MANECs," in addition to not being officially accepted for mixed neoplasms arising of extra gastro-entero-pancreatic (GEP) tract, did not include all mixed neoplasms without a poorly differentiated neuroendocrine component and an adenocarcinoma as exocrine histology.

Indeed, both the patterns can show different features in terms of morphology for the neuroendocrine component (well and poorly differentiated) and type of non-neuroendocrine component

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Table 18.1 Evolution in terminology for mixed forms of neuroendocrine neoplasms

WHO 1980	WHO 2000 (GEP)	WHO 2010 (GEP)	WHO 2017 (pancreas)	WHO 2019 (GEP)
Mixed forms of carcinoid adeno-carcinomas	Mixed endocrine exocrine cell neoplasms (MEEC) [7]	Mixed adeno-endocrine carcinoma (MANEC) [3]	Mixed neuroendocrine non-neuroendocrine neoplasms (pancreatic MiNENs) [4]	Mixed neuroendocrine non-neuroendocrine neoplasms (GEP MiNENs) [6]

GEP gastro-entero-pancreatic

depending on the arising site for exocrine component.

Therefore, to overcome this issue in 2017 for pancreatic neoplasms and then for whole digestive tract in 2019, WHO classification introduced the definition of "mixed neuroendocrine non-neuroendocrine neoplasms" (MiNENs). The new term was proposed, on the one hand, in order to include also other exocrine variants as squamous cell type and, on the other hand, the well-differentiated neuroendocrine tumors (NETs) [6]. The evolution of the WHO classifications is reported in Table 18.1.

18.2 Epidemiology

Despite the progressively increasing detection of the disease, MiNENs represent a rare category of neoplasms, and their epidemiology is challenging due to missing and debatable data. Indeed, during the years, changes in terminology and in the International Classification of Diseases (ICD) made difficult to find a real epidemiologic data.

According to the Surveillance of Rare Cancers in Europe Registry (RARECAREnet), in 2008 the crude incidence was 0.01 cases per 100,000/year, with 147 cases collected between 2000 and 2007 in RARECAREnet database [8]. The Surveillance, Epidemiology, and End Results (SEER)-18 database reported an incidence of gastrointestinal MANECs between 0.23 and 1.16 cases per 1,000,000 in 2000–2016 [9]. The Associazione Italiana Registri Tumori (AIRTUM) reported 17 cases in 2015 with a crude incidence <0.01 per 100,000/year, without an estimation of the 1- or 5-year survival [10].

The Italian Association of Medical Oncology (AIOM) [11], the European Society for Medical Oncology (ESMO) [12], the European Neuroendocrine Tumor Society (ENETS) [13], and the National Comprehensive Cancer Network (NCCN) [14] guidelines do not report the epidemiology of MiNENs as separate subgroup of neuroendocrine neoplasms. Due to the rarity of the disease, the difficult of detection, and the different classifications during the years, epidemiologic data regarding MiNENs patients (pts) mostly derived from case series or retrospective analyses.

18.3 Pathogenesis

The pathogenesis of MiNENs is a debated argument, and the lack of prospective data limits the resolution of this controversial issue among pathologists. The two more corroborated hypothesis are the following:

- The first is that two distinct neoplastic clones (neuroendocrine and non-neuroendocrine) grow up in a synchronous or in a metachronous fashion, giving rise to two different histologic patterns.
- The second concern is the possibility of a pluripotent stem cell that, under the same stimuli, undergoes to a divergent differentiation (*common precursor theory*).

In some cases, the detection of amphicrine cells, defined as cells in which neuroendocrine and non-neuroendocrine constituents can be found together, strongly supports the hypothesis of a common precursor [15].

In case of the presence of a poorly differentiated neuroendocrine component, the mutational pattern, common to the two components, mostly

suggests a monoclonal origin of the two histologies, different in terms of morphology and immunophenotype [16].

A high concordance has been found between the mutational pattern of the poorly differentiated neuroendocrine and the non-neuroendocrine components in MiNENs; on the other hand, the same gene abnormalities are not found in well-differentiated NETs.

This evidence suggests a multistep progression from a common precursor that gives rise to neuroendocrine cells from non-neuroendocrine ones, disproving an evolution from two different clones [17, 18].

Recently, a retrospective, small case series by La Rosa et al. reporting about mixed adenoma well-differentiated neuroendocrine (MANET) of the digestive system seemed to confirm the hypothesis of a multipotent stem cell with a divergent differentiation. In this study, all MANETs presented a transitional area between the two components intimately admixed, and also the molecular analysis demonstrated the same mutational pattern (the lack of mutations of Kirsten rat sarcoma viral oncogene homolog—KRAS; v-raf murine sarcoma viral oncogene homolog B—BRAF; phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha—PIK3CA aberrations) in both components. Furthermore, in this analysis, neuroendocrine and non-neuroendocrine components showed a lack in KRAS aberration, usually mutated in pure adenoma, and a high immunoreactivity of beta-catenin in neuroendocrine component, commonly lower in pure NETs. Again, this evidence supported the monoclonal origin of the two components in MiNENs, differently from the pathways of development of pure adenomas and pure NETs [19].

18.4 Diagnosis

Pure neuroendocrine neoplasms represent a very heterogeneous entity. While the morphology remains the same within each lesions of the same neoplasm, different proliferation indexes can be commonly found between the primitive lesion and metastases (or even between distinct metastases). Indeed, several studies have reported cases of changes in Ki-67 between the different sites of biopsy, leading to an upgrading of the whole well-differentiated neoplasm [20–22].

Considering this, the diagnosis of MiNENs is even more challenging for pathologists.

Over to the possibility of a misgrading of the well-differentiated neuroendocrine component, biopsy could not be representative of the effective proportions of both the histologies. Indeed, small bioptic specimens could even virtually not include one of the two components, and not distinguish MiNENs from their pure counterparts.

The diagnosis of MiNENs is then likely underestimated, often until the surgical specimen becomes available [23].

This happens particularly because the definition of MiNENs is essentially based on the threshold of 30% [24]. As aforesaid, the quantitative cut-off is not supported by prospective studies, and its clinical role is actually unknown. Some studies proposed other thresholds (10 and 20%) and reported a statistically better prognosis for those pts. with a neuroendocrine component lower than these cut-offs [25, 26].

Anyway, in these studies, also carcinomas with neuroendocrine morphology and differentiation were included (as has been described in neoplasms of other sites, as lung) [27], but, according the WHO 2019 classification, the mere immunohistochemical expression of neuroendocrine markers by non-neuroendocrine cells should not be considered for the diagnosis of MiNENs.

As general rule, La Rosa et al. proposed the use of an appropriate panel of stains (hematoxylin and eosin) as mandatory to recognize both neuroendocrine and non-neuroendocrine components and to formulate the suspect on MiNENs, also in case of small specimens [1].

Synaptophysin represents one of the most reliable markers in diagnosis of neuroendocrine neoplasms. On the other hand, chromogranin A, mostly used in several studies of the past as unique marker of neuroendocrine differentiation, has an intensive and diffuse expression in well-differentiated NETs, but is usually lacking

in the poorly differentiated neuroendocrine component [28].

According to a recent systematic review by Frizziero et al., the neuroendocrine component mostly presented a poorly differentiated morphology (39.5%), even if the feeling is that this percentage may be smaller than reality. Indeed, the grade of differentiation was available only for 124 of 2427 included pts. (5.1%). The non-neuroendocrine component was mostly represented by adenocarcinoma (92.2% of cases) and only in 2.5% by squamous cell carcinoma.

The immunophenotype of well-differentiated neuroendocrine component changes based on the site of the origin: CDX2 suggests an intestinal origin [29, 30], while PDX1 and ISL1 point toward a pancreatic origin [31, 32] and TTF1 to a pulmonary one [33, 34].

On the other hand, when a poorly differentiated neuroendocrine component is present, the immunophenotype does not show a correlation with the site of origin.

The morphology of well-differentiated neuroendocrine components is characterized by monomorphic cells with large nuclei and focal necrosis. Poorly differentiated neuroendocrine components are composed of poorly formed and solid nests of cells with large areas of necrosis [1].

The 2019 WHO classification added morphology to proliferation index as main diagnostic criteria only in digestive NENs, dividing the category into five subgroups. Grade 1 neuroendocrine tumors (NETs), grade 2 NETs, and grade 3 NETs are characterized by a well-differentiated morphology with ≤2%, 3–20%, and >20% Ki67 index, respectively. On the other hands, grade 3 NECs present ki67 index >20% and a poorly differentiated morphology. Therefore, the introduction of the MiNENs category only for GEP-NENs leads to the virtual possibility that, in these new entities, one of the subgroups above could variably combine with its non-neuroendocrine counterpart.

Even if the 2019 WHO classification has officially accepted the term of MiNENs only for those GEP-neoplasms that are composed of a neuroendocrine and a non-neuroendocrine component, in which each represents at least 30% of the whole tumor mass; mixed neoplasms could virtually arise from every site and the features of both components should depend on the tissue of origin.

Krugmann et al. reported two cases of ovarian mixed neoplasms. In both of them, neuroendocrine component was associated with a 10% serous papillary component. The authors emphasized that neuroendocrine neoplasms in the genital tract mostly be associated with smaller other non-neuroendocrine components rather than in pure form [35].

In another paper by Ramalingam et al., two cases of cervix MiNENs were reported. In both the cases, the neuroendocrine component was represented by NEC and the non-neuroendocrine component by a moderately differentiated endocervical adenocarcinomas (EAC). Immunohistochemistry revealed a positivity for p16 and for high-risk human papillomavirus (HPV) in both NEC and EAC, while neuroendocrine component was positive to synaptophysin [36].

As in GEP MiNENs, these evidences support a common origin by a stem cell undergoing a divergent differentiation and, even more in these cases, suggest the hypothesis of a common pathogenetic pathway of tumorigenesis. While, on the one hand, the result of the non-neuroendocrine differentiation seems to depend on the site of origin (embryogenic tissue and its stimuli), and on the other hand, the neuroendocrine component seems to derive, first of all, from non-neuroendocrine differentiation and then on the gain of further, distinct genetic traits.

Again, as in GEP MiNENs, also the threshold of 30% seems to be mostly an assumption rather than a cut-off defined by clinical and prognostic criteria.

In uterine cervix mixed carcinomas, the presence of 17.5–55% of poorly differentiated neuroendocrine component was associated with a worse prognosis compared to pure non-neuroendocrine carcinomas [37]. Furthermore, Bressenot et al. presented a case of a mixed neo-

plasm of the kidney, composed of 90% of the tumor mass by clear cells and 10% of a well-differentiated neuroendocrine component, with metastases only from the NET [38].

All these evidences, as well as the need of the threshold of 30% to be revised, suggest that extra-GEP MiNENs should be considered as a distinct clinicopathologic entity, even in the absence of a specific classification of the category [39, 40].

18.4.1 The Exception that Proves the Rule

Since the challenging diagnosis of MiNENs, several authors tried to clarify the criteria to standardize the pathologic approach. Lewin et al. defined all those lesions with a variable proportion (30–50%) of endocrine and non-endocrine epithelial cells as mixed tumors. The authors distinguished mixed tumors from other two categories that could likely be mistaken with MiNENs: collision and amphicrine tumors.

Collision tumors are neoplasms composed of a neuroendocrine and a non-neuroendocrine component juxtaposed to one another, without any intermixing between the two patterns. As general rule, the key to identify a collision tumor (and to distinguish it from MiNENs) is represented by the coexistence of two distinct, juxtaposed histologies and well distinguishable from each other, the absence of histologic admixture between the two patterns, and the growth in the same site [41]. Thus, a collision tumor is the result of a two neighboring independent neoplasms that grow in the same neoplastic mass [42].

On the other hand, amphicrine tumors differ from mixed neoplasms since they are not composed of two different histologic patterns but of cells that express both endocrine and non-neuroendocrine constituents at the same time [43]. Furthermore, the presence of amphicrine cells in MiNENs is not unusual, and it has been proposed by several authors in order to support the hypothesis of a common precursor stem cell capable of divergent differentiation [15].

A separate comment should be reserved to goblet cell tumors, which represent the first example of mixed endocrine and non-endocrine neoplasms of the appendix [2]. During the years, the plethora of definitions proposed for goblet cell carcinoid (mucinous carcinoid, adenocarcinoid, and crypt cell carcinoma) has contributed to the uncertainty surrounding this entity. Goblet cell tumors are composed of an intimate mixture of both epithelial (glandular) and neuroendocrine elements containing goblet cells. Diagnosis is mostly incidental, e.g., after an appendectomy due to an appendicitis. Furthermore, immunohistochemical staining for synaptophysin or chromogranin could not be so indicative: the positivity could highlight only the periphery of the goblet cell tumor in which are scattered the neuroendocrine cells [44].

Considering all pitfalls above, Volante et al. proposed a practical algorithm in order to identify non-neuroendocrine neoplasms with a neuroendocrine differentiation and distinguish those entities from MiNENs. The clinical and prognostic significance of neuroendocrine differentiation in non-neuroendocrine carcinoma remains controversial. Since no differences in clinical outcome has ever been proven by prospective studies for breast, lung, and gastrointestinal tract [45–50], a clinical issue has been raised about prostatic adenocarcinoma. Indeed, in this kind of neoplasms, the presence of chromogranin A-positive neuroendocrine cells is linked to unfavorable prognostic factors [51]. Therefore, the algorithm by Volante et al. defined chromogranin A as the first immunostaining choice, followed by synaptophysin, CD56, Ki67, and somatostatin receptors (proposed more to a systemic treatment rather than a diagnostic role).

Furthermore, it has to be mentioned that, not so commonly, the suitability of the sampling has been took in consideration by the studies as a potential source of discrepancy, reducing the quality of the evidence in literature.

In conclusion, as general rule, an adequate sampling and a panel of pan-neuroendocrine markers (not a single antibody) are needed for

MiNENs diagnosis in order to identify, quantify, and characterize the proportion of the two components.

18.4.2 Mutational Landscape

During the years, the improvement in even more specific targeted therapies was the result of a progressively increased knowledge in matter of mutation patterns driving disease pathogenesis. The rarity and the diagnostic issues mentioned above represent the most important obstacle to the collection of high-quality data; anyway, some reports about MiNEN genetic aberrations are arising in literature. A case report by La Rosa et al. recently described clinicopathologic data, methylation profile, chromosomal gains and losses, and mutation analysis of a case of sinonasal mixed exocrine-neuroendocrine carcinoma. The proportions were verified in order to confirm the diagnosis of extra-GEP MiNEN: the two components were represented by a non-neuroendocrine one resembling colorectal carcinoma and by a poorly differentiated NEC. Immunohistochemistry revealed that both components expressed Cytokeratin (CK) 8, CK20, CDX2 while were negative CK7 and Transcription Termination Factor 1 (TTF1). The Methylation-Specific Multiplex Ligation Probe Amplification (MS-MLPA) assay used to investigate the genetic and epigenetic profiles showed concurrent copy number changes in both the components. Aberration in chromosome regions was found in both non-neuroendocrine and neuroendocrine components: 17p13 (tumor protein p53—TP53), 14q24 (MutL-homolog3—MLH3), and 19q13 (Kallikrein-Related Peptidase 3—KLK3), 5q21 (adenomatous polyposis coli—APC), 7q21 (cell division protein kinase 6—CDK6), 9q34 (death-associated protein kinase 1—DAPK1), 12p13 (tumor necrosis factor receptor superfamily member 1A—TNFRSF 1A, CDKN1B), 13q12 (breast cancer type 2—BRCA2), 17p13.3 (hypermethylated in cancer 1—HIC1), 18q21 (B-cell lymphoma 2—BCL2), and 22q12 (tissue-inhibitor of metalloproteinase 3—TIMP3). Furthermore, the mutational analysis did not detect KRAS (exons 2–4), BRAF (exon 15), and p53 (exons 4–10) in both the components. Interestingly, only the neuroendocrine component was found to be characterized by a deletion and aberrant methylation of APC gene (resulting in its inactivation) and an aberrant methylation of *DAPK1* genes. Immunohistochemistry showed a difference between the two components too: synaptophysin, chromogranin A, serotonin and glicentin were found positive only in NEC.

These evidences supported the pathogenetic theory of the common precursor, suggesting the monoclonal origin from a stem cell undergoing a dual differentiation. In this setting, NEC seems to be the result of a further differentiation of the non-neuroendocrine component through a specific endocrine pathway due to the acquisition of additional mutations [52].

A systematic review about MiNENs by Frizziero et al. reported genetic and molecular information for pts. with colorectal disease, which is available for 49.1% of the 381 included pts. The most frequent mutations involved the following genes: *TP53*, *RB1* (retinoblastoma tumor corepressor 1), *PTEN* (phosphatase and tensin homolog), *APC*, *PI3KCA*, *KRAS*, *BRAF*, and *MYC* (v-myc avian myelocytomatosis viral oncogene homolog). The cluster of these aberrations above has been defined as a trunk of driver genes involved in tumorigenic process [24].

In another retrospective study, 19 samples of colorectal MiNENs and 8 NECs from the same site were analyzed with next-generation sequencing. MiNENs were also examined for microsatellite instability analysis and MLH-1 promoter methylation status; in three of them, the two components were separately analyzed.

The authors found that the two components of MiNENs, when compared, shared the same mutational pattern, expressing the driver genes implicated in colorectal carcinogenesis. Furthermore, compared with pure colorectal NECs, MiNENs showed a higher rate in BRAF

aberration (37%; $p = 0.006$); instead, APC (16%; $p = 0.001$) and KRAS resulted less frequently mutated (21%; $p = 0.043$) [53]. Thus, compared to the non-neuroendocrine component, the neuroendocrine counterpart seems to present a higher rate of acquired genetic aberrations, and this phenomenon could correlate with their more aggressive behavior.

Also Capdevila et al. have reported that colorectal carcinoma and NECs arising from the same site shared a similar mutational pattern with frequent mutations in *TP53*, *APC*, *KRAS*, or *BRAF* genes; furthermore, the authors demonstrated a relatively higher rate of BRAF mutations in colorectal NECs compared to colorectal adenocarcinomas (28% vs 15.86%, respectively) [54].

These evidences not only suggested that a BRAF mutation could play a fundamental role in differentiation of neuroendocrine neoplasms (pure or mixed), but also that this mutation is likely to have a predictive role in the innovative targeted therapies.

La Rosa et al. reported the results of a clinical-pathological analysis of 14 digestive MANETs. The immunostaining for p53 was negative in all 10 analyzed neuroendocrine components and its positivity did not exceed 10% in 9/10 non-neuroendocrine components. A perfect overlap was reported for beta-catenin expression between NETs and adenomas components. None of the investigated case (4/14) showed mutations in KRAS, BRAF, PIK3CA, and MSI between the two components.

On the one hand, the differences in mutational pattern and immunohistochemistry between MANECs and MANETs suggest that the term MiNENs could be inclusive of probably distinct subcategories that need further specifications. Furthermore, the international classification systems may extend this term to the other extra-GEP neoplasms.

On the other hand, the mutational pattern and immunohistochemistry shared between neuroendocrine and non-neuroendocrine components of each entity above seems to support the monoclonal pathogenesis.

18.4.3 Morphologic and Functional Imaging

The diagnosis of MiNENs is basically histopathologic, but imaging represents a fundamental tool for staging and follow-up. The choice between computed tomography (CT) scan or magnetic resonance imaging (MRI) is mostly driven by the site of origin and, in the absence of specific guidelines, follows the diagnostic and stadiative recommendations of their pure counterparts [13]. The available data in literature are mainly represented by case reports or small case series, and it is likely that no useful conclusion can be drawn on the basis of the current evidences. A recent paper by Semrau et al. reported the case of a woman with a metastatic rectal MiNEN: initial diagnosis was performed with a chest and abdomen CT scan, followed by an MRI for a better stadiation of the rectal primitivity and the liver metastases. Follow-up was carried out with both imaging techniques [55]. The use of both CT scan and MRI was justified by the evidence that MRI alone seems to be less sensitive in the detection of pathological lymph nodes in MANECs than in pure adenocarcinomas [56]. In a recent review of the literature about extrahepatic biliary tract MANECs, the authors underlined the sensibility of MRI not only in detection of carcinomas of this site, but also in macroscopic description of the growing pattern [57, 58].

Concerning the use of functional imaging, assuming that poorly differentiated neuroendocrine components show the same high glucose metabolism of NECs, positron emission tomography with fluorodeoxyglucose (FDG PET) may be suggested to complete the staging of MANECs. On the other hand, 68-Gallium PET (68 Ga-PET) seems not to be appropriate due to the loss of somatostatin receptor expression [59].

According to the same principle, 68 Ga-PET may be indicated for MANETs. A recent case report by Both et al. dealt with a young woman with a mixed neoplasm composed of an acinar cell carcinoma as non-neuroendocrine component and a well-differentiated Grade 3 NET as neuroendocrine component. The neoplasms not

only showed a high uptake on 68 Ga-PET but also demonstrated a response to peptide receptor radionuclide therapy (PRRT) in numerous metastatic lesions [23].

Considering the high rate of false positives, functional imaging techniques alone do not substitute histologic diagnosis [60]. Thus, histologic diagnosis should not be delayed in order to perform various kinds of imaging once the lesions are detected.

18.4.4 Therapeutic Management

According to a recent systematic review, more than 81% of MiNENs pts. presented with a localized disease at diagnosis, with or without locoregional nodal involvement but in the absence of distant metastases. Advanced or metastatic disease accounted for almost 18% of cases [24]. In the absence of specific guidelines on MiNENs, the cumulative analysis conducted by Frizziero et al. on retrospective studies reported that 98.3% of pts. with localized disease underwent surgery with curative intent. Perioperative chemotherapy, when mentioned, mostly followed the guidelines for adenocarcinomas from the same sites of origin. In a few cases, (neo)adjuvant chemotherapy was based on NEN regimens, even if these therapeutic protocols are mostly part of a clinical practice rather than being supported by prospective studies, which are lacking. As general opinion, radical surgery represents the only therapeutic option to cure MiNENs, even if no clear surgical rules have been defined in prospective trials and the possible impact of perioperative chemotherapy on prognosis has not been defined yet [61].

Pokrzywa et al. recently released the retrospective data of 57,804 pts. who underwent surgery for stage I–III pancreatic neoplasms; 515 of them (0.9%) had pancreatic MiNENs. Median 5-year overall survival (OS) on MiNENs pts. was 37%, placed between acinar cell carcinomas (51%) and pure NECs (20%). Data concerning other systemic treatments were available for 49% of the sample: 41% and 8% received adjuvant and neoadjuvant chemotherapy, respectively, and regimens were not mentioned [62].

Milione et al. reported data about 160 MANECs pts. who underwent surgery. A better OS was reported for pts. with early stage (22.1 months [mo]), compared to stage IIIb pts. (12.7 mo) and stage IV pts. (15.7 mo), and for pts. with Ki67 index <55% in the NEC component (40.5 mo vs 12.2 mo, $p < 0.0001$) [63].

In the largest retrospective studies, a significant variability in terms of OS for pts. with localized disease has been described: from 14 and 75 mo.

This fact can be explained by the lack of information about staging, site of origin and morphologic feature of both MiNENs components: no distinction has ever been done between MANETs and MANECs.

Indeed, in case of MANETs, surgery (endoscopic resection) seems to be the best approach as it is for their non-neuroendocrine counterparts (polypoid adenomas).

A recent metanalysis by La Rosa et al. reported that the neuroendocrine component of MANETs is generally in the deeper portion of the polyp and surgery represents together a diagnostic and a therapeutic approach. Except for a single patient who died due to a likely occult NEC that became metastatic, all pts. were alive (with a median of follow-up of 6 years), 80% free from disease and 20% with a residual local disease, irrespective of the site of origin, stage and neuroendocrine component Ki67. This evidence supported that MANETs, with their indolent biologic behavior, should be considered and managed as a distinct entity from MiNENs with poorly differentiated neuroendocrine component [19].

On the other hand, in case of advanced or metastatic disease, surgery and radiotherapy seem to play a palliative role, while chemotherapy has the predominant one.

No specific regimens have been proposed, and retrospective analyses did not report a preferred scheme of systemic therapy. Indeed, where data were available, the percentage of pts. treated with adenocarcinomas or neuroendocrine regimens was similar (22.1% and 27.4%, respectively). ENET guidelines listed some regimens used for MANECs (platinum/etoposide, irinotecan-based regimens, S-1) that follow the indications for

pure NECs, suggesting that prognosis of MiNENs likely depends mostly on the neuroendocrine component. This statement is supported by the evidence that the 60.8% of pts. with MiNENs presented synchronous or metachronous metastases with predominant poorly differentiated neuroendocrine component.

In the analysis by Frizziero et al., median OS for pts. with metastatic disease ranged between 10 and 18 mo, considering the results by Milione et al. on MANECs and what aforesaid about MANETs, this evidence suggests that the higher percentage of metastatic MiNENs pts. had a poorly differentiated neuroendocrine component.

Since the diagnosis is often performed after a radical surgery, we can find several studies suggesting for MANECs the same chemotherapeutic regimes as for their non-neuroendocrine counterparts from the same origin. Actually, in these studies, the treatment was administrate relying on a bioptic diagnosis that demonstrated only the non-neuroendocrine component; thus, the misunderstood MiNEN diagnosis was revealed only in retrospect [64].

Data about MANET treatments are even more scarce. In a recent paper by De Both et al., a case of a misunderstood metastatic MANET of the pancreas received the same treatment accepted for pancreatic NETs (sunitinib, temozolomide, and capecitabine and PRRT). The authors reported a certain response to all the systemic treatments [23].

In this scenario, a multimodality approach, shared in a multidisciplinary team, seems to be the best choice for MiNEN pts. management.

Kanazawa et al. reported the case of a man with MiNEN of the colon with synchronous thyroid and liver metastases. The combination of surgery of the three sites of cancer and chemotherapy based on small-cell lung cancer (SCLC) regimens (cisplatin and etoposide, carboplatin and irinotecan, amrubicin) lead to 7 years of no evidence disease follow-up [65].

In a recent retrospective study investigating the correlation between the 2019 WHO classification and the prognosis of ovarian NENs, pure NECs and mixed neoplasms of the ovary were treated as the same entity and, also in this case, almost the 60% of pts. received platinum-based combination chemotherapy [66].

Even if in largest retrospective analyses, no statistical differences were reported between therapeutic schemes, several authors reported the efficacy of non-neuroendocrine regimens in treatment of MiNENs.

Tagai et al. reported a case of a young woman affected by an ascending colonic MANECs who experienced a partial response to streptozotocin monotherapy and a stable disease to capecitabine + oxaliplatin and 5-fluorouracil/oxaliplatin/bevacizumab Scheme [67].

In other cases, MiNEN pts. received therapeutic regimens indicated for non-neuroendocrine neoplasms which arise from the same site. Toptas et al. described a successful treatment with carboplatin and taxol for pts. with an endometrial MANEC [68].

In addition to the lack of prospective trials, few retrospective studies and metanalyses about MiNEN management are burdened by a delayed or, worse, autoptic diagnosis.

Indeed, because the diagnosis is often performed after surgery, we can find several studies in literature that reported data about underestimated MiNENs, treated as their non-neuroendocrine counterparts, presenting all the results together after the diagnosis has been revealed [64].

In the era of molecular patterns and target therapies, customized treatments based on specific gene alterations are becoming a stronger idea. Indeed, in the literature, the first studies about the efficacy of the target therapy in rare neoplastic entities are arising [54].

With these premises, Quaas et al. reported the cases of a man with a metastatic MiNEN from ileum [69]. After the failure of two therapeutic lines, a tumor-DNA analysis was performed. A BRCA-1 mutation was revealed. Therefore, the patient was successfully treated with a combination of carboplatin, paclitaxel, and the poly (ADP-ribose) polymerase (PARP) inhibitor Olaparib analogs (according the protocol described by Oza et al. [70]). The patient experienced a reduction of all liver metastases, allowing

the surgical removal of them. The authors reported that the patient was alive after 25 months from the diagnosis, without evidence of further metastases [69].

These evidences suggest the need for prospective studies whose aim would be to better understand the biologic behavior and personalize the treatments for each entity of this heterogeneous category.

18.4.5 Prognosis

Prognosis of pts. affected by MiNENs is another issue still to clear due to the lack of high-quality data in literature.

First, the most part of studies did not distinguish between MANECs and MANETs, and some of them, due the rarity of these entities, considered MANECs together with NECs [36].

According to the analysis by Frizziero et al., MiNENs, that are mostly composed of a poorly differentiated neuroendocrine component [71], presented a worse outcome than well-differentiated NETs. On the other hand, prognosis of MiNENs compared to pure NECs remains debated.

According to some studies, in poorly differentiated GEP MiNENs, prognosis does not differ from pure NECs, except for gastric MiNENs pts. for whom a better survival has been observed compared to pure NECs [72]. Controversially, in the retrospective analysis by Milione et al., pts. with colorectal MANECs had a significantly poorer OS compared with pts. with esophageal or pancreato-biliary MANECs (12.2 months vs 17.3 months, $p = 0.001$) [63].

A recent analysis by Zeng et al. reported no statistical differences in terms of disease-free survival (DFS) and OS between NECs and MANECs of biliary tract ($p = 0.152$ for DFS, $p = 0.150$ for OS) [73]. On the other hand, a study by Pokrzywa et al. recently reported a better prognosis for pts. with MiNENs compared to pts. with pure NECs (5-year OS 37% vs 20%, respectively) [62].

This topic remains debated in the field of extra-GEP MiNENs too; the issue concerns not only the prognostic difference between MiNENs, pure non-neuroendocrine counterparts, and pure NECs, but also between MiNENs from different site of origin.

In urinary bladder, MiNENs composed of small-cell neuroendocrine component and high-grade urothelial carcinomas present a worse prognosis than pure non-neuroendocrine carcinomas [74]. On the other hand, mixed bladder neoplasms with a small-cell morphology for neuroendocrine component seem not to have significantly differences in terms of outcome compared to pure NECs ($p = 0.8734$) [75].

Despite the limits of the following comparison, the survival between extra-MiNENs from the various sites seems to be very different. In case of advanced disease, a mean OS of 16.5 months for a case series of sinonasal MiNENs pts. was reported [52], while, in another report, median OS was only 3 months for cervix MiNENs pts. [36].

Morphologic feature and proliferation index of neuroendocrine component could determine the outcome of MiNENs pts. too. It is not a case that in the retrospective analysis on MANET by La Rosa et al. all pts. were alive at median follow-up of 9 years (range 1–27 years) [19].

Furthermore, the prognosis seems to depend on stage (until 75 months for localized disease and 18 mo for metastatic disease of every sites) [24], morphologic features, and proliferation index of neuroendocrine components [63] and, likely, on treatments. In the absence of mindshare guidelines, the multimodality approach may be the best choice.

18.4.6 Conclusion

MiNENs represent a category of neoplasms extremely heterogeneous in terms of site of origin, morphologic features, proliferation index of neuroendocrine component, and stage of the disease at presentation. The relative novelty of this definition, the sequence of ambiguous terminologies used during the years, and the rarity of the disease made it hard to collect high-quality evidence to understand the state of the art about MiNENs.

The 2019 WHO classification seems to have finally specified what the term "MiNENs" is referring to: a category of neoplasms composed of a neuroendocrine and a non-neuroendocrine component representing at least 30% of the whole tumor mass.

Anyway, the 2019 WHO classification adopted this term only for digestive NENs, leaving uncovered mixed neoplasms of all other sites.

The strict conditions for setting out what the "MiNENs" term means (the presence of two different histologies, the threshold of 30%, the need of an adequate sample which allows to determine morphology and Ki67 index of the neuroendocrine component) lead to the underestimation of the disease. The first hot topic that the international associations should address is to define the good clinical practice for the diagnosis of MiNENs, in order to avoid misunderstandings of pathologists and clinicians. The controversy around the cut-off of 30% should be resolved in prospective studies, in which the role of exocrine neoplasms with neuroendocrine components <30% (and vice versa) should be defined. These limits, together with the rarity of the disease itself, justify the lack of prospective trials enrolling pts. with this diagnosis.

Second, another unmet need is represented by the definition of a standardized management for each subcategory of MiNENs, divided according to the site of origin and the characteristics (morphology and Ki67) of neuroendocrine component. Indeed, it is likely that biologic behavior and prognosis of MiNENs are driven by this component, considering the evidences above.

Surgery seems to be the only curative approach, mostly in localized disease, while its role needs to be better explored in the palliative setting of a metastatic disease, especially distinguishing according to the morphology of the neuroendocrine component.

Perioperative therapies and systemic treatments are an even more unexplored field.

Assuming as true the pathogenetic hypothesis of the common precursor, neuroendocrine component should represent the result of a progressive differentiation of a non-neuroendocrine element toward a neuroendocrine cell, due to the sum of chromosomal and gene aberrations.

Furthermore, to our knowledge, no MiNENs with a high-grade non-neuroendocrine component and a low-grade neuroendocrine component has been described before. Therefore, if the statement is that the prognosis of NECs is poorer than their high-grade non-neuroendocrine counterparts and the prognosis of NETs is poorer than their low-grade non-neuroendocrine components, it is likely that systemic treatment depends on the features (morphology and proliferation index) of the neuroendocrine component.

There is no doubt that prospective studies are needed, but until the results of them are not available, each new case of MiNENs should be discussed within multidisciplinary team. With the improvement of targeted therapies and immunotherapies, the better knowledge of the mutational pattern and the pathogenetic pathways of the disease could help to identify new therapeutic strategies by the participation of MiNENs pts. in basket and/or umbrella trials. At present, the vast majority of clinical trials do not allow the enrollment of pts. with mixed neoplasms. Considering the rarity of the disease, the choice of the investigators could be to alter the purity of a case series or to design specific multicenter trials, aware of the slow accrual. We hold the idea that, in order to improve our knowledge about MiNENs, the best way to study these diseases is through prospective, observational studies with a centralized review of clinical, histopathological, and radiological data.

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Merkel Cell Carcinoma

19

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19.1 Introduction

Merkel cell carcinoma (MCC) is a rare and highly aggressive primary cutaneous carcinoma of the skin with epithelial and endocrine features. Its origin—neuroendocrine—is still uncertain. The first description of MCC was performed by Toker who defined it as a "trabecular cancer of the skin" [1]. This tumor is rare, but its incidence is dramatically increasing. This chapter examines the incidence, pathogenesis, and molecular abnormalities of this malignancy. It discusses the most frequent clinical presentation, the diagnostic approach, modern treatments, and their results. It also reviews the patient prognosis and follow-up procedures. Because of the complexity, aggressive nature, and individuality of each case, MCC is best treated by a multidisciplinary team.

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19.2 Epidemiology

The incidence of Merkel cell carcinoma (MCC) has increased worldwide [2].

Australia and New Zealand register both the higher incidence of MCC cases than anywhere else in the world [3–5]. This geographic predominance seems strictly related to the combination of exposure to UV (ultraviolet) radiation and fair-skinned population. In Queensland Australia, the average annual incidence reached 1.6/100,000 between 2006 and 2010, peaking at 20.7 per 100,000 for persons 80 years or older [5]; men accounted for around two thirds of all cases (68%), with a median age at diagnosis of 75.5 years compared with 78.0 years for women [5].

Differently, in Western Australia, the incidence was estimated at 0.82/100,000 between 1993 and 2007 with a higher value in older age (15.5/100,000 in the ≥85 year age group) [4]. In New Zealand, the age-adjusted incidence was 0.96/100,000 between 2000 and 2015: MCC affected mainly sun-exposed areas and the rate increased with age [3]. The age-adjusted incidence for MCC of males was 1.45 times that of females [3].

Data from Surveillance Epidemiology and End Results (SEER-18) database, derived from United States (US) registries, confirmed a rising

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incidence rate of MCC from 0.5 cases per 100,000 in 2000 (95% CI 0.4–0.5) to 0.7 per 100,000 in 2013 (95% CI 0.7–0.8), corresponding to 2488 cases in 2013 [2]. Moreover, the incidence of MCC increased with age, varying from 0.1 cases/100,000 for ages 40–44 to 9.8/100,000 for ages more than 85, and it was higher in men than women, across all age groups. The total annual incidence of MCC has been predicted to increase to 2835 cases in 2020 and 3284 cases in 2025: this growth has been mainly related to the aging of the population and to an increased diagnostic accuracy [2, 6].

In European population, a growing incidence of MCC was documented in the different registries, as well: in Nederland, from 0.17 in 1993 to 0.35 per 100,000 in 2007 [7]; in Sweden from 0.23 in 1993 to 0.49 per 100,000 in 2012 [8] and in France from 0.57 in 2006 to 0.74 per 100,000 in 2010 [9].

Five-year survival rate for MCC differs between different states and was estimated approximately at 60% in US (1986–2004) [10], 64% in Western Australia (1993–2007) [4], and 40% in Queensland, Australia (2006–2010) [5].

19.3 Pathogenesis

The cell of origin of MCC is still uncertain and under debate. Tumor cells share morphologic and histologic features with the normal Merkel cell; however, there is no evidence of direct evolution from normal Merkel cells into tumor cells, and no precursor lesions have been identified [11].

The etiology divides MCCs into two types: Merkel cell polyomavirus (MCPyV) positive and MCPyV negative. The second one being associated to accumulation of UV-induced mutations. Virus-positive tumors are found in 80% of cases in the northern hemisphere, whereas virus-negative tumors are found in a majority of southern hemisphere cases. Although it is clear that these two categories have different mechanisms of promoting cell growth and replication, the pathogenesis of MCC is not yet completely understood [12].

19.3.1 Virus-Positive MCC

Merkel cell polyomavirus (MCPyV) belongs to Polyomaviridae family, a typical mammalian polyomavirus with a double-stranded DNA genome [13]. The potential oncogenic role of polyomaviruses was firstly demonstrated by Gross et al. who discovered the murine polyoma virus in 1953 [14]. In mice, the transforming mechanism of mouse polyoma was related to the integration of virus DNA into the host genome [15].

MCPyV has been mainly isolated from the skin, even though it is not known which cells support its replication [16]; recent evidences suggest monocyte as the possible reservoir for the virus [17].

Primary infection of MCPyV does not cause any signs or symptoms in healthy individuals, and the prevalence of subclinical infection increases with age. The viral genome possesses an early and a late regions, which contain genes encoding proteins for viral replication and viral capsid [18]. The early region encodes for four proteins: large T antigen (LT), small T antigen (sT), ALTO (alternate frame of the large T open reading frame), and 57 kt antigen transcript. The late region encodes for viral coat proteins: VP1 and VP2 and microRNA that regulates the T-antigen transcripts [18]. Productive viral infection is associated with cell death, rather than oncogenic transformation [19]. After entry into a host cells, the inhibition of viral replication commonly occurs and typically the virus defaults to a latent, non-replicative state after infection [18, 20].

Although MCPyV mostly causes a persistent innocuous infection, it may rarely generate an aggressive skin cancer known as Merkel cell carcinoma (MCC) [18]. An immunosuppressed state likely contributes to viral integration, mutagenesis, and carcinogenesis [21].

In 2008, Feng et al. discovered that MCPyV infection is related to MCC [22]. They documented that the viral genome was integrated into tumor genome in the virus-positive (VP) MCC samples analyzed [22]. This was the first association of a polyomavirus with human tumorigenesis.

Due to its oncogenic properties, the World Health Organization-International Agency for Research on Cancer has attributed to MCPyV the classification of probably carcinogens for human (group 2A) [23].

The incidence of infection among MCC patients has been estimated 80–90% [22, 24]. In the Northern hemisphere, the majority of MCC cases are of viral etiology [25].

The precise mechanism involved between MCPyV infection and MCC development remains unknown. Mongha et al. demonstrated the biological impact of UV radiation on MCPyV replication and biology, providing the potential explanation for the role of repetitive solar exposure in the pathogenesis of MCPyV positive MCC [26]. UV lights may also promote cancer through their immune-suppressive effect on tumor microenvironment [27].

Oncogenic transformation by MCPyV is hypothesized to require two events: integration of the viral genome into the host genome and truncation of LT to render the viral genome replication deficient [22]. The resulting truncated LT lacks the viral replication capacity, while it retains the ability to bind and inactivate the tumor suppressor retinoblastoma (RB1) protein, which leads to a dysregulation of cell cycle progression [28]. LT-Ag also downregulates expression of TLR (toll-like receptor) 9, a key receptor in the host innate immune response, liberating infected cells from host immune surveillance [29]. An additional LT-Ag property is to activate survivin, an inhibitor of apoptosis [30]. Unlike other polyomaviruses, MCPyV LT-Ag lacks TP53-binding capacity [31], so p53 disruption in MCC is independent from LT-Ag [32]. Therefore, virus-positive MCC displays a pattern of wild-type TP53, differently from the virus-negative counterpart [32].

The viral oncoprotein sT enhances MCC oncogenesis independently from LT properties. LT-stabilizing domain (LSD) is an sT domain, essential for its oncogenic activity [19]. LSD allows sT to inhibit the cellular SCF (complex of Skp1, Cul1, and F-Box protein) ubiquitin ligase protein complex, SCF^{Fbw7}. F-box/WD repeat-containing protein 7 (Fbw7) ubiquitin ligase protein complex normally promotes the degradation of proto-oncogene products such as cyclin E, c-Myc, c-Jun, mTOR, Notch, NFKB2 [33]. Recent findings support the concept that the sT-Ag inhibits the degradation of LT-Ag, c-myc, and cyclin E, thus enhancing the oncoprotein stability [19, 28, 34].

During transformation, another sT property, independent from the Fbw7, is the modulation of the mammalian target of rapamycin (mTOR) pathway: sT preserves the hyperphosphorylation of the eukaryotic translation initiator factor 4E-binding protein 1 (4E-BP1). This event results in a dysregulation of cap-dependent translation and favors carcinogenesis [35].

Moreover, sT is a suppressor of nuclear factor KB (NFKB) activation, which likely impairs the local innate immune response to MCPyV infection [36].

The VP tumors typically express sT and the truncated LT both considered the main oncogenic triggers, while the expression of the capsid proteins, VP1 and VP2, is frequently lost [37].

In addition to the expression of sT and LT viral protein, VP tumors contain mutations that activate PI3K pathway (HRAS, KRAS, PI3KA, PTEN) [18].

19.3.2 Virus-Negative MCC

The pathogenesis and the consequent molecular profile distinct virus-negative (VN) MCCs from virus-positive (VP) variants [38]. The higher prevalence of this subtype in Australia, in comparison to Europe or North America [39], and the predilection of sun-exposed fair-skinned population suggest that UV radiation is central in the pathogenesis of VN-MCC [39, 40]. UV radiation

is a known risk factor for many skin cancers [41]. It can cause mutagenic, carcinogenic, and immunosuppressive effects [42, 43]. UVB, in particular, promotes DNA damage, generates reactive oxygen species, and induces the immunosuppressive effect both locally and systemically. The decrease in DNA repair and subsequent immunosuppression contribute to carcinogenesis [42, 44]. Immunosuppressive mechanisms include depletion and downregulation of Langerhans cells presenting antigens, UV-induced regulatory T cells (CD4+ CD25+), secretion of cytokines such as interleukin-1, interleukin-10, and tumor necrosis factor [27]. UV-B rays are less prevalent than UV-A rays, but they are much more intense and destructive. UV-B induces mutations in the tumor suppressor p53 and Ha-RAS genes, which increase the risk of cancer [44]. UV-A has also been reported to induce MCC. This long-wavelength UV corresponds to deeper penetrance beyond the epidermis into the dermis and is a significant contributor to UV-induced immunosuppression [44, 45].

19.3.3 Mutational Landscape of Virus-Positive (VP) and Virus-Negative (VN) MCCs

The genome landscape differs significantly between virus-positive (VP) and virus-negative (VN)-MCC. VP-MCCs typically harbor few somatic mutations or copy number alterations and no definitive mutational signature [18, 46]. As detailed above, genome sequencing revealed the mutations in tumor suppressors and oncogenic mutation and the relative lack of UV-damaged DNA in VP tumors [46]. In contrast, VN-MCCs harbor a specific pattern of genomic alteration, suggesting a different etiology in comparison to VP tumors [46]. VN-MCCs showed a high mutational burden associated with UV damage, typically seen in other skin cancers associated to sun exposure such as melanoma, basal cell carcinoma, and squamous cell carcinoma [46, 47]. An enrichment in C > T transition, a typical somatic signature UV-related, had been demonstrated in this variant [46, 47].

Virus-negative MCCs harbor highly recurrent inactivating mutations in tumor-suppressor genes such as *RB1*, *TP53*, and genes encoding member of Notch family [20, 38, 46, 48, 49]. The mutations of *RB1* and *TP53* are clonal, occurring early during the tumor evolution, and they are shared by the primary tumor and by the metastases [20]. Hot-spot mutations of different oncogenes such as HRAS, KRAS, PIK3CA have been described both in VP and VN-MCCs [20]. Other oncogenes specifically associated with VN-MCCs have been characterized, including mutations in AKT1, EZH2 [20, 48, 50]. The mutational burden of VN-MCC is fivefold higher than non-small-cell lung cancer and melanoma [46]. The high incidence of point mutations generates non-self-peptides that may be involved in the immunogenicity of virus-negative tumors [46].

Epigenetic changes may also play a role in the pathogenesis of MCC and jeopardize its prognosis. Epigenetic tumor suppressors silencing may play a role in MCC oncogenesis [28]. Main alterations are represented by DNA modifications, with promoter hypermethylation or by histone modifications [28].

19.4 Risk Factors

Advanced age is associated with an increased incidence of MCC [2]. Immuno-senescence, defined an imbalance between inflammatory and anti-inflammatory mechanisms, may explain the prevalence of MCC in older patients (aged 70 years or older): a remodeling of the immune system leads to weakened immune functions in the elderly [51]. This "inflammaging" state contributes to an impairment of both adaptive and innate immunity and, consequentially, to age-related disorders and neoplastic disease [51].

Other risk factors for MCC are represented by white skin pigmentation, sustained exposure to sun or UV light, or history of other skin cancers [52].

Iatrogenic or disease-related immunosuppression may also account for an increased risk of

developing MCC. A compromised immunosurveillance may favor proliferation of atypical cells. The mechanism by which immunosuppression interacts with MCPyV and UV radiation exposure in the pathogenesis of MCC is unknown [53]. Immunosuppressed patients account for 10% of MCC population [54], and the age of onset of MCC is lower than in immune-competent patients [55]. Moreover, immunosuppression was demonstrated to be a stage-independent predictor of worse MCC-specific survival [54–56].

Immunosuppression-related risk factors include HIV/AIDS, organ transplantation, lymphoproliferative disorders, and autoimmune diseases [53, 57].

HIV infection has been associated with a tenfold increased MCC risk in comparison to general population [57]. The pathogenesis of HIV-related MCC is not well elucidated. It is plausible that HIV-1/AIDS predisposes to virus-positive MCC, but it should be noted that HIV-1/AIDS increases the risk for developing UV-induced skin cancers (such as squamous cell carcinomas and basal cell carcinomas) and therefore may also increase the risk for virus-negative MCC [55].

Solid organ transplant increases the risk of malignancies, including MCC [53, 58]. After solid organ transplantation, the overall risk of MCC was increased 23.8-fold compared to general population [53]. The combination regimen of azathioprine and cyclosporine was associated with the highest MCC risk. MCC rose with advancing time since transplant, suggesting an etiologic role of long-term, chronic immunosuppression [53].

Lymphoproliferative disorders such as non-Hodgkin lymphoma, chronic lymphocytic leukemia, and multiple myeloma may also be associated to an increased risk of MCC [59]. Patients with lymphoproliferative diseases have weakened humoral and cell-mediated immune-response that could be advocated in MCC increased risk [21].

Chronic inflammatory disorders, such as rheumatoid arthritis, are also associated with higher incidence of MCC. Autoimmune disorders impair the natural immune response, creating an environment that makes an affected individual particularly vulnerable to secondary malignancies [60].

19.5 Histology and Immunohistochemistry

Merkel cell carcinoma is a primary cutaneous neuroendocrine cancer, characterized by high grade and poor differentiation [61]. Neoplastic proliferating cells are usually located in the dermis and frequently invade deeply into the hypodermis. The tumor growth pattern is nodular or infiltrative: the first appears as single- or multiple-well circumscribed nodules, the second is composed of single cells, nests, trabeculae, or rows, infiltrating the surrounding tissue (derma and soft tissue). The papillary dermis and adnexa are usually spared [62]. Ulceration may occur.

In about 10% of cases, cancer cells may display epidermotropism, with nested or pagetoid pattern [63–65]. In rare cases, only intraepidermal component has been described [66].

Tumor cells are characteristically small, uniform, with round to oval nuclei, finely dispersed chromatin, not prominent nucleoli and scant cytoplasm [67].

Mitoses are numerous. Apoptosis and necrosis are frequent [68] (Fig. 19.1). Even though necrosis can be prominent, it is not needed for the defi-

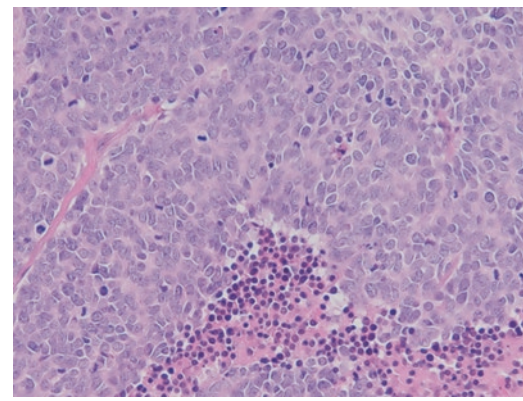

Fig. 19.1 MCC intermediate variant with necrotic foci and high mitotic activity (H&E, 20×)

nition of high-grade tumor. Lymphatic and vascular invasions are common.

Three main histologic variants are described: trabecular, intermediate, or small-cell.

The trabecular subtype was first reported by Toker [1]. This is the least frequent histological pattern. Cells have more abundant cytoplasm and are arranged in distinctly organoid clusters, with trabeculae and occasional ribbons. This type of tumor usually occurs adjacent to adnexal structures, particularly hair follicles [1].

The intermediate subtype is the most frequent histological subtype, observed in more than 50% of cases [68]. It exhibits large and solid nodules with basophilic sheets of irregular and hyperchromatic cells [69]; cytoplasm is less abundant than in the trabecular type, and nuclei may be more vesicular with small nucleoli. Mitoses and focal areas of necrosis are frequent. These tumors usually arise adjacent to adnexa and may invade the epidermis [62].

The small-cell type mimics small-cell tumors of other sites, e.g., small-cell lung cancers. The tumors are composed of solid sheets or cluster of cells, with frequent crush artifact and nuclear molding. The clinical behavior of this subtype appears to be as similar as the small-cell tumors of other origins [63].

Rarely, tumors might display a large cell and sometimes spindle cell morphology. Nevertheless, no clinical implication has been correlated to the morphological variants or cytologic characterization, because most MCCs display overlapping features and transitional forms [70].

Besides pure endocrine forms, some cases of MCC are combined with different skin tumors (more frequently SCC, infiltrating, or in situ) or show divergent differentiation (squamous or adnexal morphology) [71, 72].

The MCC has a characteristic immunohistological profile, with expression of both epithelial and neuroendocrine markers. Tumor cells stain positively for several type I and II cytokeratins such as CK8, CK18, CK19, and CK20. CK20 is considered a very sensitive and specific marker for MCC, since it is positive in 75–100% of cases. A wide range of neuroendocrine markers are immunoreactive with neoplastic cells, such as neuron-specific enolase (NSE), chromogranin A, synaptophysin, and CD56 [62, 63, 67].

Thyroid transcription factor 1 (TTF1) and CK7 are essentially negative [62, 67, 73].

The CK20 positivity, combined with thyroid transcription factor-1 (TTF-1) and CK7 negativity, is commonly used to distinguish MCC from other primary cutaneous tumors and metastasis of extracutaneous neuroendocrine carcinomas [73], in particular small-cell lung cancer (SCLC).

However, a subgroup of MCC may lack CK20 expression [74, 75] and rare case may be positive for CK7 or TTF1 [67, 70, 76, 77].

This unusual or aberrant immunohistochemical staining pattern makes the differential diagnosis more challenging.

In the past few years, additional markers have been studied and suggested as specific for Merkel cell carcinoma. Tumor cells are found to express Merkel cell markers: neurofilament (NF), frequently positive in MCC with dot-like pattern [74, 78], and special AT-rich sequence-binding protein 2 (SATB2) [74]. Atonal homolog 1 (ATOH1) is an additional Merkel cell marker, involved as a transcription factor driving cell differentiation; unfortunately, his expression has been found in other neuroendocrine tumors, and it is not selective [74, 79].

Insulinoma-associated protein 1(INSM1) is a marker of neuroendocrine differentiation. It may be useful in confirming the neuroendocrine nature of MCC, with respect to poorly differentiated skin tumors, but it is not consistent in the differential diagnosis with extracutaneous neuroendocrine tumors [74, 80, 81].

Few cases of MCC can express paired box protein 5 (PAX5), terminal desoxynucleotidyl transferase (TdT), CD99, and cell surface-associated mucin 1 (MUC1). Nevertheless, all of these markers are not relevant in the diagnosis, because they are shared with other kind of tumors and other neuroendocrine carcinomas [74, 82–84].

Positivity for the oncoprotein huntingtin-interacting protein 1 (HIP1) has been observed in

the majority of cases (almost 90%), and it contributes to the mechanism of MCC development, maintenance, or progression [85].

Staining for tumor protein 63 (p63) and survivin has been observed and has been linked to a worse prognosis, with more aggressive clinical course [86].

The detection of Merkel cell polyomavirus has also been suggested for MCC diagnosis. Different experiences have assessed morphological and immunohistochemical differences between MCPyV positive and negative tumors [74, 87, 88]. Based on morphological criteria, MCPyV negative tumors may display marked heterogeneous cytologic features, intraepithelial component and combination with other skin tumors, more frequently with squamous cell carcinoma [87–89]. Even though no marker has been reliably associated selectively with either virus-positive or -negative MCC, differences in immunohistochemical profiles are recognized. The classical panel (CK7−, CK20+, chromogranin+, synaptofisin+, NF+, and TTF1−), usually found in MCPyV-positive MCC (Fig. 19.2), is not commonly encountered in MCPyV-negative tumors. MCPyV-negative MCC, both in pure (neuroendocrine) and in combined (with SCC) form, may present reduced expression of CK20, chromogranin, and NF, with more frequent positivity for CK7 and TTF1 [74, 76, 88]. CD99 with dot-like expression pattern has appeared as a suitable biomarker of MCPyV positivity [74].

Recent evidences suggest a significant correlation of lymphocytes diffusely infiltrating tumor (brisk TILs) and high global PDL1 signal with MCPyV-positive tumors contrasted with virus-negative cases. MCPyV seems to convey greater immunogenicity to MCCs than the high mutational burden/greater neoantigen load of MCPyV-negative cases [72].

Methods for the detection of MCPyV in tumors include immunohistochemistry (IHC), PCR, RNA, or DNA in situ hybridization, and next-generation sequencing [20]. These assays vary significantly in sensitivity and specificity for detection of tumor-associated MCPyV [20].

The common approach used for MCPyV detection is represented by immunohistochemistry for the expression of T-antigen proteins.

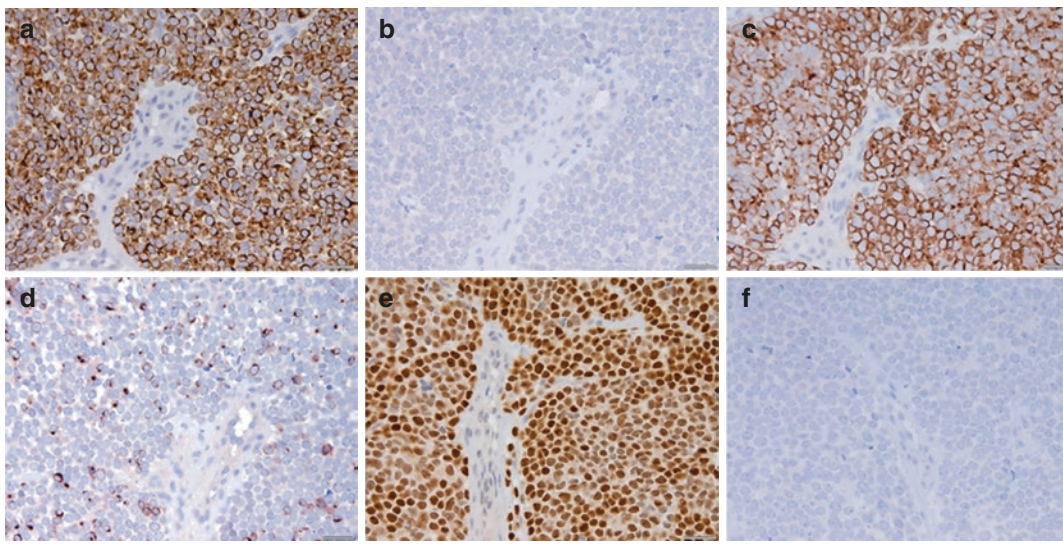

Fig. 19.2 Classical immunohistochemical panel in MCPyV-positive MCC: (**a**) diffuse CK20 expression with cytoplasmic and dot-like pattern; (**b**) negativity for CK7; (**c**) diffuse chromogranin expression with pattern similar to CK20; (**d**) NF expression with dot-like pattern; (**e**) high and diffuse nuclear expression of MCPyV; (**f**) negativity for TTF1

CM2B4 is a commercially available antibody to detect LT and has approximately 88% sensitivity and 94% specificity (compared to multimodal approaches combining PCR and IHC) [90].

Another method commonly used for the assessment of MCPyV is PCR. Quantitative PCR allows for the detection of number of copies of viral DNA integrated in a tumor. Copy number estimation by this method may range from extremely low (<1 MCPyV copy per 100 cells) to thousands of copies per cell [91].

A multimodal approach incorporating PCR and IHC results may be the most sensitive and specific method for confirming MCPyV status by commonly used assays [90].

19.6 Pathologic Report

The College of American Pathologists (CAP) provided a protocol for the examination of specimens from MCC patients. AJCC T-stage requirements include the description of maximum tumor diameter and tumor extension (invasion of fascia, muscle, cartilage, and bone) (as detailed below) [92]. Analysis from National Cancer Database (NCDB) supported the prognostic role of tumor size [93]. Furthermore, tumor diameter was significantly associated with nodal involvement and outcome [94]. A retrospective study showed that the tumor size and the deepest anatomic compartment involved by tumor inversely correlated with prognosis, at univariate analysis [95]. In accordance with these results, the NCBD confirmed the prognostic role of T4-stage category [93].

The CAP protocol completes the description of primary tumors with some other parameters such as:

- Tumor site.
- Margin status.
- Lymphovascular invasion.

In addition, other optional primary histopathologic features have been considered by CAP protocol, whose prognostic role is still debated:

- Tumor thickness.
- Mitotic rate.
- Tumor-infiltrating lymphocytes (TILs).
- Tumor growth pattern.
- Presence of second malignancies.
- Specimen laterality.

19.6.1 Tumor Location

Tumor location has a prognostic role mostly in head and neck MCCs. Scalp tumors present more likely with distant metastasis, lip tumors have the highest rate of invasion into bone, cartilage, and muscle, and ear tumors have the highest rate of nodal metastasis [96].

19.6.2 Margins

The CAP protocol suggests recording the status of margins: for margins uninvolved, the distance (in millimeters, mm) of the carcinoma to the margins should be reported. When margins are involved, their location should be described [92]. A large experience on 6901 patients from National Cancer Database (NCDB) confirmed a relation between positive margins and poor survival across all stages (from I to III MCCs), at multivariate analysis [97]. Close or positive margins were correlated to local recurrence after surgery alone [98].

19.6.3 Lymphovascular Invasion

The identification of lymphovascular invasion was found to be strongly associated with sentinel node biopsy positivity [99] and had been related to worse prognosis [95].

19.6.4 Tumor Thickness

Tumor thickness should be recorded as for Breslow depth in melanomas, and it is defined as the distance in millimeters between the top of the

granular layer of the epidermis and the deepest point of tumor invasion [92]. In a recent retrospective experience, tumor thickness and diameter were both moderately correlated, and each of them was independently associated with increased likelihood of positive sentinel node and worse overall survival [100].

19.6.5 Mitotic Index

In the CAP protocol, mitotic index, defined as the number of mitotic figures per square mm, is preferred than reporting the number per high-powered field (HPF) because the definition of HPF varies and depends on the technology available in each institution. No uniformly accepted threshold for low or high mitotic rate has been uniformly established [92]. The prognostic role of mitotic index varies across different studies with conflicting results [92, 101–103]. Nevertheless, some evidences suggest the correlation between mitotic rate, infiltrative growth pattern, and lymphovascular invasion [104] and between mitotic rate and sentinel node positivity [101].

19.6.6 Tumor-Infiltrating Lymphocytes

Tumor-infiltrating lymphocytes (TILs) are defined as lymphocytes present at the interface of the tumor and the stroma. In the absence of specific accepted guidelines for the assessment of TILs, the CAP recommended to report TILs as is done in cutaneous melanomas [92]:

- TILs not identified: No lymphocytes present, or lymphocytes present but do not infiltrate tumor at all.
- TILs non-brisk: Lymphocytes infiltrate tumor only focally or not along the entire base of the vertical growth phase.
- TILs brisk: Lymphocytes diffusely infiltrate the entire base of the dermal tumor or the entire invasive component of the tumor.

Even though the literature produced conflicting data about the prognostic role of TILs [103], in more recent experience, the presence of TILs seemed to correlate with a better outcome [95, 105].

19.6.7 Tumor Growth Pattern

Two tumor growth patterns have been characterized by the CAP protocol: nodular and infiltrative [92]. Nodular pattern is defined as tumors with a relatively well-circumscribed interface, and it has been associated with a better prognosis [92, 95, 100]. Infiltrative pattern does not exploit a well-circumscribed interface with the surrounding tissue, showing single cells, rows, trabeculae, or strands of cells infiltrating through dermal collagen or deeper soft tissue [92]. Retrospective experiences have documented the correlation between growth pattern and sentinel lymph node positivity with a higher risk for infiltrative variant [99, 101].

19.6.8 Second Malignancies

MCC has been observed contiguous to or intermingled with other skin malignancies, particularly cutaneous squamous cell carcinoma, including Bowen disease [106]. A significant overexpression of p53 has been recently described in combined tumors [107]. Interestingly, MCPyV is not found in cases of MCC associated with cutaneous squamous cell carcinoma, indicating that it does not play a part in these combined tumors [107].

19.6.9 Nodal Evaluation

The CAP protocol recommends to complete hematoxylin and eosin staining with immunohistochemistry to allow the identification of cancer cells in clinically occult lymph nodes [92]. Stains may include AE1/AE3, CK116, Cam 5.2, CD56, CK20, synaptophysin, and/or chromogranin [92].

19.7 Staging System

The AJCC eighth edition was based on an analysis of 9387 patients from the National Cancer Database (NCDB) diagnosed with MCC between 1998 and 2012. This classification provides important information for the management of prognosis of patients with MCC [93, 108].

19.7.1 T Category

T1 is defined as a tumor with a maximum clinical diameter not greater than 2 cm, T2 as tumor with a maximum clinical diameter greater than 2 cm but not greater than 5 cm, T3 as tumor with a maximum clinical diameter greater than 5 cm, and T4 as tumor that invade muscle, fascia, cartilage, or bone [108].

19.7.2 N Category

N stage is categorized as N1, defined as regional lymph node metastasis without in transit lesions; N2, as in transit lesions without node metastasis; N3, as combination of node metastasis and in transit lesions. N1 is further categorized as N1a for clinically occult metastasis or N1b for clinically or radiologically assessed nodal metastasis. In particular, N1a(sn) is defined as clinically occult lymph node metastasis detected only at sentinel node biopsy, while N1a is defined as clinically occult lymph node metastasis detected after lymph node dissection. For N1b, a histopathological assessment is necessary to confirm the lymph node involvement [108].

19.7.3 M Category

M0 is defined as the absence of metastatic spread, while M1 is tumor with distant metastasis. M1 is subcategorized as M1a for cutaneous/subcutaneous metastasis, M1b for lung metastasis, M1c for all the other metastatic sites other than M1a or M1b [108].

19.7.4 Stage

In eighth edition, both clinical and pathological staging groups are provided. Stage I is defined as T1 tumor without nodal or distant involvement. Stage II is subdivided into stage IIA (defined as T2 or T3 tumors) or stage IIB (defined as T4), both without nodal and distant disease.

Stage III recognizes two subgroups without distant metastasis: stage IIIA is defined as T1–4 with clinically occult nodal spread (N1a (sn) or N1a) or as unknown primary tumor (T0) with clinically/radiologically detected lymph node metastases (N1b); stage IIIB includes all the other categories (T1–4 N1b–3).

Stage IV is defined as any T and any N with M1 spread [108].

19.7.5 Prognosis

The AJCC eighth edition provides also a correlation between stages and clinical outcomes: survival data were based on the findings of an analysis of 9387 patients from the NCDB diagnosed with MCC between 1998 and 2012. Five-year overall survival (OS) estimates for local disease (n = 6138), regional metastatic disease (n = 2465), and distant metastatic disease (n = 784) were 50.6, 35.4, and 13.5%, respectively [93].

In patients with localized disease, without regional or distant metastatic spread, survival rate at 5 years was 55.8% in T1, 41.1% in T2–3, and 31.8% in T4.

Increasing tumor diameter was demonstrated to be predictive of poor survival (overall survival and disease-specific survival) and sentinel node involvement [100].

In patients with regional nodal involvement, the 5-year survival rate was found to be 39.7% for patients with clinically occult node metastasis (but pathologically positive lymph node), 26.8% for clinically detected nodal metastasis and 41.4% for patients in transit involvement. In the subgroup with occult

primary lesions and clinically evident lymph node metastases, the proportion of 5-year surviving was 42.2% [93].

Clinical nodal staging that could hide a high rate of occult nodal disease is less reliable in the prediction of survival than pathological nodal staging. For the same specific T stage (from T1 to T4), a significant difference in OS was observed comparing clinical node negative patients (cN0) with pathological node negative patients (pN0). These observations led to widespread use of the sentinel node biopsy [93].

19.8 Clinical Features

MCC classically presents in older patients with asymptomatic, rapidly growing, red or violet cutaneous nodule, typically on sun-exposed skin of the head and neck or upper extremities [54]. Geographic areas at higher incidence of MCCs, such as Queensland Australia or New Zealand, are characterized by a similar pattern of clinical presentation: MCCs occur more commonly on the face and ears (35% of patients) in both sexes, with a predominance in male; women are more likely than men to have diagnosed MCCs on lower limbs [3, 5].

Current United States MCC incidence shows a major susceptibility of non-Hispanic white individuals in comparison to Hispanic, Blacks, or Asians [2, 109]. A dataset of 3431 MCCs from SEER registry diagnosed during 1973–2014 showed that age < 65 years, male sex, and tumor sites (trunk versus head-neck or limbs versus head-neck) were predictors of late-stage at diagnosis at multivariate analysis [110].

Heath et al. conducted a study on 195 patients with MCC to identify key features associated to MCC. They created the AEIOU acronym to aid clinicians in MCC diagnosis: *a*symptomatic/lack of tenderness, *e*xpanding rapidly (less than 3 months), *i*mmune suppression, *o*lder than age 50 years, and location on *U*V-exposed areas on a person with fair skin (Fig. 19.3). In this study, 89% of patients presented with three or more of the AEIOU criteria [54].

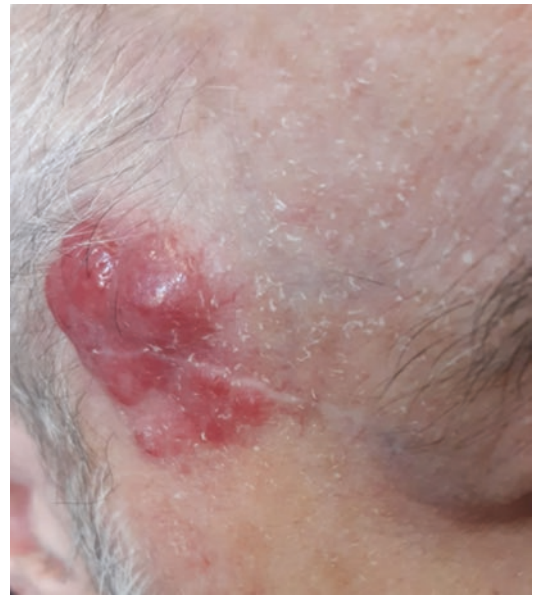

Fig. 19.3 A clinical example of Merkel cell carcinoma: a rapidly expanding red cutaneous lesion arose on sun-exposed area of the head, in an old man

About 15% of patients presents with MCC of unknown primary origin (MCC-UP) with a clinically positive nodal disease without an identifiable cutaneous primary lesion [111]. The diagnosis confirmation of MCC-UP requires immunohistochemical staining to exclude metastatic neuroendocrine carcinoma from other sites of primary origin such as the lung [73].

Several experiences postulated that MCC-UP could be characterized by a regression of the primary skin tumor, due to an immune-mediated mechanism. Recent evidences suggested that patients with nodal or metastatic MCC-UP had a better MCC-specific survival as compared to patients with known primary tumor (MCC-KP) [112]. Vandeven et al. recently explored the immune-mediated mechanisms underlined to MCC-UP: this cohort of patients had a higher MCPyV oncoprotein antibody titer and a higher mutational load in comparison to MCC-KP patients. These findings collectively suggest that enhanced immune function may underlie the development of MCC-UP through elimination of the primary skin lesion [112].

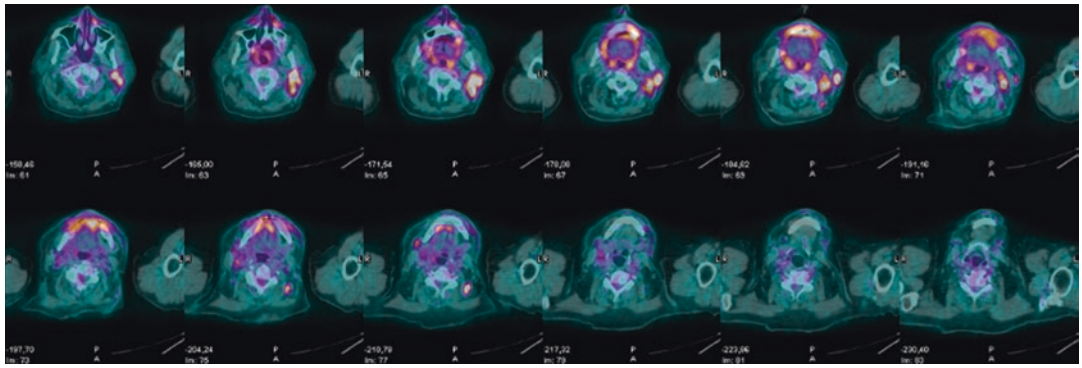

Fig. 19.4 PET-CT scan with fluorodeoxyglucose: these images refer to a cervical lymph node progression of Merkel cell carcinoma

19.9 Staging Assessment

Imaging studies are necessary to assess tumor extension, once a biopsy has confirmed the diagnosis of MCC. Computer tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) with fluorodeoxyglucose (FDG) has been validated to detect primary tumors, lymph node metastases, or distant metastases [63, 113] (Fig. 19.4).

These modalities may influence the subsequent management in stage II–III patients [114]. However, generalizable data are lacking about the sensitivity and specificity of imaging, as well as the utility of imaging in clinical management decisions and disease outcomes. Imaging is secondary to sentinel lymph node biopsy to stage patients, demonstrating a low sensitivity to detect nodal involvement [115].

19.10 Management of Local/Locoregional Disease

Once a tumor biopsy has confirmed the diagnosis of MCC, a multidisciplinary approach is recommended to ensure an appropriate patient management.

Surgery is the primary treatment option for patients with localized MCC, also allowing an appropriate staging of both the primary tumor and the locoregional nodes. Besides surgery, the lack of prospective clinical trials prevented the achievement of high-level clinical evidence. Therefore, the different strategies adopted, and the results obtained, as detailed in the subsequent paragraphs, are based upon the retrospective experiences available so far [63, 116].

19.10.1 Management of Primary Tumor

As mentioned previously, surgery of the primary MCC tumor is the treatment of choice and should be performed whenever possible. In a retrospective study on 2454 patients with local or locoregional MCCs from the NCDB, surgery as a part of the initial treatment improved overall survival as compared to definitive radiotherapy [117].

Several retrospective studies explored the correlation between surgical margin status (positive or negative) and local control and patient outcomes (progression free survival, recurrence-free survival, and overall survival), but conflicting results emerged [97, 118–123]. The variability of these results is due to confounding variables and in homogenous patient selection. As an example, in some studies, postoperative radiotherapy (RT)

to the primary site could have led to a reduction of the risk of relapse, associated to residual disease [97, 118–123].

These limitations notwithstanding, negative surgical margins seemed to correlate with better local control and survival, in patients with stage I–II disease treated with surgery alone [124]. Furthermore, in one retrospective study, positive histologic (<1 mm) or close margins (1–9.9 mm) were directly related to local recurrence in patients who underwent narrow excision alone, without receiving adjuvant radiotherapy to primary site [98]. Accordingly, wide local excision (WLE) with 1–2 cm margin to the investing fascia of muscle or pericranium is recommended by the European and American guidelines [63, 125]. Safety margin is more intended to remove microsatellites than ensure clear margin of the primary tumor [125]. WLE is not always clinically feasible, due to unacceptable functional and cosmetic implications, especially when MCC involves the head and neck regions. Mohs micrographic surgery may represent a valid surgical approach, alternative to WLE and resulting in similar results in terms of survival and recurrence, even if limited by time and experience constraints [118–120, 125].

After surgery, patients may undergo observation or adjuvant RT of the primary site. Nowadays, no clear data exist about the role of adjuvant RT due to several limitations of the retrospective case series published: nonetheless, the American and European guidelines currently recommend considering adjuvant radiotherapy [63, 125]. NCCN guideline suggests some risk factors indicating the need for adjuvant RT to the tumor bed such as the presence of a primary tumor ≥1 cm, positive or limited surgical resection margins, lymphovascular invasion, a head and neck primary, and an immunocompromised host [63, 116].

Finally, radiation therapy may represent a definitive approach to treat primary lesions, for patients who are deemed inoperable or for patients who refuse surgery [126, 127].

19.10.2 Management of Regional Lymph Nodes

Before 1996, patients with a diagnosis of MCC underwent complete lymph node dissection. From 1996 onwards, sentinel lymph node biopsy (SLNB) has been routinely adopted in clinically node-negative patients, in place of extensive nodal approach [121, 128].

Lymphatic mapping is usually recommended to detect sentinel node [128]. Furthermore, a pooled patient-level metanalysis confirmed that SLNB had a higher sensitivity in detecting regional nodal disease, compared to computer tomography (CT) scan [115]. SLNB should be performed prior to or at the time of definitive surgery of the primary tumor, and SLN positivity has been reported for 30–38% of patients with clinically node-negative MCC [63].

SLNB is a staging tool that provides a better prognostic characterization, according to pathological stage groups [93].

Results from retrospective studies exploring the prognostic value of SLN status are still conflicting [129]. Some findings showed an association between SLN negativity and lower risk of recurrence [115, 130] and better survival [131]; conversely, some others provided opposite results [122, 129]. Nonetheless, information derived from SLNB guides the subsequent clinical decision [115].

A large retrospective cohort of 8044 MCC patients from NCDB assessed the relationship between primary tumor size and nodal positivity: the risk of nodal involvement was 14% for 0.5 cm tumors, 25% for 1.7 cm tumor (median sized) and >36% for ≥6 cm tumors [94]. Moreover, some pathologic features of primary MCC have been demonstrated to be predictive of SLNB positivity such as tumor diameter, thickness, mitotic rate, and infiltrative tumor growth [101].

Routine use of both hematoxylin-eosin staining and immunohistochemistry allows for the identification of micrometastases in the sentinel node [101]. CK20 immunostaining should be included in the pathologic assessment [101].

In an analysis on 721 MCCs, the overall false-negative rate of SLNB was estimated to be 17%; nodal recurrence after a negative SLNB may be related to technical errors in localization and removal of sentinel nodes [129]. Contributing factors to technical failure include: the complex pattern of lymphatic drainage, the presence of multiple ipsilateral and contralateral sentinel node, the rapid tracer time transit, the close proximity of primary to sentinel nodes and primary site of head and neck [130]. Nodal recurrence after negative SLNB may also be classified as biological or pathological. A biological nodal failure is defined as a nodal relapse subsequent to a local or in transit relapse; pathological failure is derived from an incorrect classification of SLN as negative when metastasis is present [129].

The role of SLNB is less clear for primary lesions arising from head and neck region, since the lymphatic drainage is variable [129]. Nevertheless, SLNB should be considered in patients fits for surgery [63]. In a systematic review, patients with head and neck MCCs had a similar rate of SLNB positivity (about 30%) as those with non-head and neck location and a similar risk of false-negative findings [115, 130].

Lymph node dissection (LND) and/or radiotherapy (RT) to the nodal basin should be discussed in the presence of SLNB positive [63, 116]. It is currently unclear if LND could prolong survival in micro-metastatic sentinel node patients [129]. For patients undergoing complete lymph node dissection, the positivity of non-SLNs is predictive of poor overall survival and disease-free survival [49].

Retrospective experiences comparing node dissection to definitive radiotherapy in nodal MCCs showed conflicting results, in terms of survival advantage [97, 117]. Nowadays, it is unclear which treatment (nodal surgery or radiotherapy) is more effective for stage III MCCs, due to the absence of prospective studies. Accordingly, enrollment in clinical trials is the preferred choice in SLNB-positive patients [63, 116].

Finally, the American guideline suggests the role of adjuvant radiation therapy following LND in the presence of extra-capsular extension or multiple nodes involvement, even for patients with clinically node-negative disease [63, 116]. Adjuvant RT is not indicated after LND for patients with low tumor burden on SLNB [63, 116].

For patients with clinically node negative, not candidate for SLNB, adjuvant radiotherapy to regional nodes should be considered. As supported by a French prospective trial, the addition of adjuvant nodal irradiation to wide local excision in stage I MCC resulted in a reduction of regional recurrence: a survival benefit was not demonstrated as a consequence of a premature interruption due to poor accrual and to the routinely introduction of SNLB [132].

In patients with clinically node positive, a fine-needle aspiration, or a core biopsy is recommended to confirm the diagnosis [63, 116]. Retrospective analyses confirmed a poorer outcome for patients with clinically positive nodes than those with clinically negative, but pathologically positive nodes [93, 122]. Surgical approach to nodal basin and/or radiotherapy (RT) should be considered in this setting, and the aggressiveness of nodal treatment should be commensurate to the extent of nodal disease [63, 116].

Even in the absence of prospective studies, NCCN panel members commonly recommend LND as the treatment of choice, while adjuvant RT is recommended after LND in the presence of extra-capsular extension or multiple nodes involvement [63, 116].

19.10.3 Radiotherapy

MCC is a radiosensitive tumor. The role of post-operative radiotherapy (RT) has been largely explored. Several retrospective studies have attempted to determine the benefit of adjuvant RT in terms of survival and reduction of local relapse, with conflicting results. The range of MCC stages, the variability of surgical approaches (SLNB, LND or none, differences in surgical margins), the differences in radiation's fields and dosing could have contribute to the opposite

results, with some studies confirming a correlation between adjuvant RT and favorable outcome and others failing to show a significant correlation [97, 118, 121, 123, 131, 133–135].

In node-negative patients, no clear recommendation about the role of adjuvant RT emerged from the literature. It should be noted that the frequency of locoregional relapse in pathologic node-negative patients is estimated to be about 10% [135]. A prospective trial tried to demonstrate the role of adjuvant RT on draining basin, in stage I patients with clinically node negative: this trial was prematurely interrupted due to a drop in the recruitment attributed to the adoption of sentinel node dissection. The preliminary results revealed a significant reduction of locoregional relapse in the radiation therapy arm without a survival benefit, in patients not addressed to sentinel node biopsy [132].

Several retrospective studies tried to explore the association between postoperative RT and relapse/survival in pathologic node-negative patients (stage I–II). Unfortunately, these results were inconsistent: some studies confirmed that adjuvant radiotherapy could lead to an improvement in survival in comparison to surgery alone in stage I–II patients [97, 123, 136], while others did not confirm these results [133, 135]. Noteworthy, radiation therapy was not standardized across the studies, and it could involve the primary site, the nodal basin, or both.

As suggested by NCCN guideline, if SLNB is negative, observation of the nodal basin is appropriate [116]. Patients who are at high risk of disease progression may be considered for RT to the nodal basin [116]. These include patients with profound immunosuppression and those with factors associated with increased risk of false-negative SLNB: technical failure (e.g., removal of non-sentinel nodes secondary to a rapid radiotracer transit), anatomic features (e.g., close proximity of primary MCCs to the SLN or previous history of surgery including WLE), and location (head and neck region could be associated to aberrant lymph node drainage and frequent presence of multiple SLN basins) [130].

In stage III patients, retrospective studies about the role of postoperative RT showed conflicting results. The largest of these experiences used data from MCC cases in the NCDB, exploring the role of adjuvant radiotherapy in stage III patients. This analysis showed no survival benefit of adjuvant RT in nodal MCCs [97]. The authors hypothesized that, in this cohort of patients, survival was mostly driven by the presence of subclinical distant metastases [97].

In contrast to these results, in a retrospective study from the Moffitt Cancer Center adjuvant radiotherapy was associated with an improved local control and disease specific survival, in clinically or pathologic node-positive patients but not in node-negative patients [133]. In particular, CLND was performed in 30 patients with SLN positivity (57.7%) and in 17 patients with clinically nodal involvement (100%) [133].

In conclusion, as detailed by the American and European guidelines, adjuvant RT to the lymphatic drainage area cannot be recommended in general after therapeutic node dissection but could be discussed in a multidisciplinary approach to improve local disease control mainly in the case of extracapsular nodal involvement or multiple nodes involvement [63, 125].

19.10.4 Chemotherapy

The role of adjuvant chemotherapy (CT) is uncertain and nowadays not recommended for patients with localized disease.

No randomized trials explored the role of postoperative chemotherapy; most of the data came from retrospective experiences where chemotherapy was not associated to a survival benefit, even in the presence of nodal disease [97, 134, 137]. In a large trial including 6908 stage I–II–III MCC patients, neither chemotherapy alone nor chemoradiotherapy was associated to a survival benefit [97].

The prospective TROG 96:07 study evaluated the role of chemoradiotherapy in a selected group of 53 patients, classified at higher risk of recurrence (based upon a recurrence after

initial treatment, involved nodes, primary tumor size greater than 1 cm, gross residual disease after surgery, or occult primary with nodes). Treatments consisted of RT and synchronous carboplatin and etoposide. Wide surgical clearance of the primary site was not required or recommended, and having nodal disease resected or positive margins re-excised were not prerequisites. The 3-year overall survival, locoregional control, and distant control rates were 76, 75, and 76%, respectively [138]. However, a comparison of these data with those of a historical control group treated without CT suggested that the adjuvant chemotherapy did not affect the overall survival compared with standard local regional approaches consisting of surgery plus RT [139].

The most relevant study supporting the role of adjuvant chemoradiotherapy was a retrospective evaluation on 4815 patients with head and neck MCCs from NCDB [134]. Chemoradiotherapy was associated with improved overall survival over adjuvant RT alone, in patients with positive margins, male sex, and tumor size more than 3 cm. Both chemoradiotherapy and radiotherapy alone provided a survival benefit over surgery, as well. Furthermore, postoperative chemotherapy alone was associated with decreased OS in comparison to surgery alone. These results suggested the potential role of adjuvant chemoradiotherapy, but not chemotherapy alone, in patients at higher risk of relapse [134].

Noteworthy, the immunosuppressive effects of chemotherapy may interfere with the relevant role of the immune system against MCC. In Javelin Merkel 200 trial, patients exposed to adjuvant chemotherapy had a lower response to avelumab in comparison to those who received avelumab as first-line treatment [140, 141].

Recent evidences about the role of immunomodulating agents in metastatic MCCs have led to explore the same strategy in the postoperative setting: several trials are evaluating the role of adjuvant immunotherapy, using nivolumab (NCT02196961; NCT03798639), pembrolizumab (NCT03712605) or avelumab (NCT03271372; NCT04291885).

19.11 Recurrent and Metastatic Disease

The management of patients with recurrent or metastatic disease should require a multidisciplinary evaluation to better select treatments. Instrumental assessment should be ruled out to stage the extension of the disease.

Systemic therapy is often the treatment of choice. Radiotherapy or surgery may be considered in selected cases for primary and recurrent disease (oligometastatic disease or symptomatic lesions) [63, 116].

19.11.1 Systemic Treatment: From Chemotherapy to Immunotherapy

Historical systemic approach in metastatic MCC consisted of chemotherapy, even if no randomized trial confirmed its role in this setting. Cisplatin/carboplatin plus etoposide was the initial treatment option; alternative regimens included cyclophosphamide plus doxorubicin and vincristine [142]. As documented in retrospective experiences, despite high response rates of chemotherapy as first-line treatment (ranging between about 50 and 60%), median duration of complete and partial responses ranged from 6 to 3 months, respectively; overall survival benefit was not shown [143, 144]. These results suggested a rapid emergence of chemoresistance. Moreover, treatment toxicities such as febrile neutropenia, sepsis, fatigue, nausea, vomiting, especially in older patients affected treatment tolerability and compliance [144].

Several experiences evidenced the relation between immunosuppression and MCC oncogenesis supporting the role of immunotherapy as a promising approach. Merkel cell polyomavirus is present in 80% of tumors, and the integration of the virus into the host cell DNA favors tumor proliferation. In virus-negative tumors, the ultraviolet radiation exposure is associated to high mutation burden, necessary to the oncogenesis.

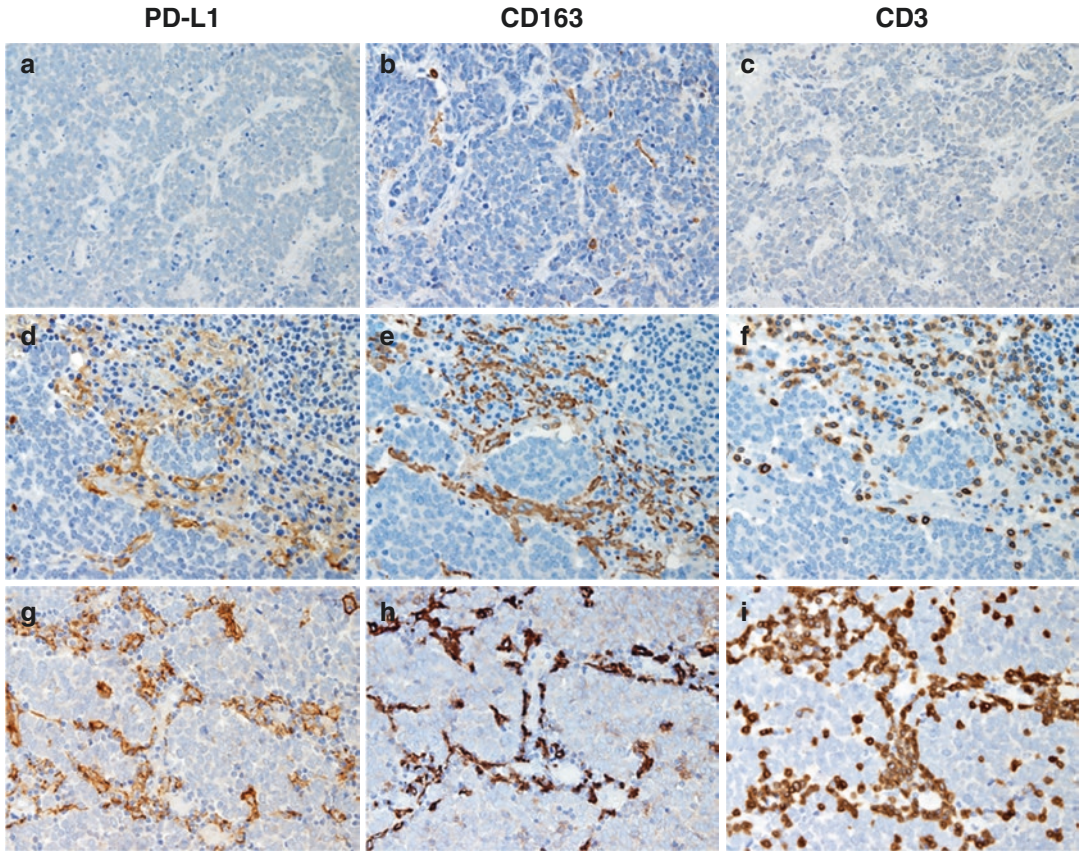

Fig. 19.5 Macrophage expression of PDL1 in Merkel cell carcinoma (MCC). Serial sections are from there cases of Merkel cell carcinoma stained for PDL1 (clone E1L3N, 1:200, Cell Signaling Technology), CD163 (clone10D6, 1:50, Thermo Scientific) and CD3 (clone LN10, 1:70, Leica Biosystem) and revealed with Novolink Polymer (Dako) followed by DAB. Case #1 (**a**–**c**) represent a cold MCC lacking PDL1 expression, whereas case #2 (**d**–**f**) and case #3 (**g**–**i**) contain PDL1

These mechanisms heighten the immune response, due to the presence of viral antigens in VP-MCCs or to the increase of neoantigens in VN-MCCs.

Programmed cell death ligand 1 (PDL1) was discovered to be expressed by MCC cells and by the adjacent immune system cells (Fig. 19.5). A significant association was observed between polyomavirus infection, inflammatory response and tumor cell PDL1 expression [145]. Moreover, specimens with PDL1-positive tumor cells were associated with immune infiltrate. These findings suggested that a local tumor-specific and a virus-specific immune response drove tumor PDL1 expression [145].

Nowadays, immunotherapy represents the preferred first-line treatment for selected patients with advanced MCC, while chemotherapy retains a role in patients who progressed to immunotherapy.

Avelumab is a monoclonal antibody against programmed cell death ligand 1 (anti-PDL1). The phase II Javelin Merkel 200 trial aimed to demonstrate the efficacy of avelumab in MCC patients progressing after at least to one previous line of chemotherapy [141]. This prospective and multicenter study enrolled stage IV patients with metastatic MCC, refractory to chemotherapy. Systemic treatment with corticosteroids or immunosuppressive agents was not permitted.

Patients with HIV, immunosuppression, hematological malignances, or solid organ transplant were excluded. Moreover, patient selection was not based on PDL1 expression or MCPyV status. The study enrolled 88 metastatic patients: 41% of them had received at least two or more lines of chemotherapy and visceral disease was assessed in 53% of patients. Samples were assessed for PDL1 and MCPyV: 79% [58] were PDL1-positive while 60% [46] were MCPyV positive. The primary endpoint was treatment response [141]. With a minimum follow-up of 2 years (median follow-up of 29.2 months), there were 29 objective responses (33%) with 10 complete response (11%) [146]. Clinical activity was demonstrated regardless of the tumor expression of PD-L1 and MCPyV status. Two-year progression-free survival (PFS) was 26% while 2-year OS was 36% [146, 147]. Treatment-related adverse events occurred in 76% [67] patients, with grade 3 toxicity reported in 11.4% [10] of patients. No grade 4–5 toxicity was registered and 17% of patients had G1–2 infusion reaction; 2% of patient permanently discontinued treatment due to adverse events [146, 147].

Javelin Merkel 200 part B was a prospective trial of first-line treatment [140, 148]. The study included untreated stage IV MCC patients. In a pre-planned analysis on 29 patients with at least 3 months of follow-up, objective response rate (ORR) was 62.1%, with 14 of 18 responses ongoing at the time of analysis (77.8%). Among 39 patients assessed for safety, G3 treatment-related adverse events (TRAE) toxicity was detected in eight patients (20.5%), and no G4–5 TRAE were documented [140]. The primary analysis for part B of this trial after ≥15 months of follow-up in the full patient population showed that the median OS was 20.3 months (95% CI: 12.4 months to not estimable) and the 12-month OS rate was 60% (95% CI: 50–68%) [148].

Important data are also available from expanded access program. Between December 2015 and March 2019, 494 patients received avelumab: ORR was 46.7% and DOR was 71.2% [149].

These results have led to the approval of avelumab for the treatment of metastatic MCC.

Pembrolizumab is an anti-PD1 receptor agent demonstrated to be also effective in the treatment of MCCs. A phase II trial of 50 treatment-naïve patients with stage IIIB unresectable or stage IV explored the role of pembrolizumab (2 mg/kg every 3 weeks) for up to 2 years [150, 151]. After a median follow-up of 14.9 months, ORR was 56% (12 complete responses and 16 partial responses). The Kaplan–Meir estimation of duration of response was 79% at 24 months. No statistical difference was observed in response rates based upon polyomavirus status (53% in VN-MCCs and 59% in VP-MCCs) and on PDL1 expression. Median PFS was 16.8 months, and 2-year PFS was 48.3%; median OS had not been reached, and 2-year OS was 68.7%. TRAEs of any grade were detected in 48 patients (96%), while G3 or greater in 14 patients (28%). Seven patients (14%) discontinued treatment due to adverse events and one death due to treatment toxicity [151].

Recently, the effects of nivolumab, an anti-PD1 agent, have been explored in a phase I–II study (Checkmate 358). Twenty-five patients who had received ≤2 treatment lines were enrolled and candidate to nivolumab (240 mg every 2 weeks). ORR was 68% (in 22 evaluable responses): in treatment-naïve patients, responses were 71%, while in patients with 1–2 previous treatment responses were 63% (without differences in both VP and VN-MCC). Any grade TRAEs were registered in 68% of patients and G3–4 in 20% of patients [152].

19.11.2 Target Agents

Different target agents have been explored in the metastatic setting, without a clear evidence of efficacy. Imatinib, pazopanib, and cabozantinib have been documented in case reports, with a limited experience [153–156].

MCC expresses somatostatin receptors, and the efficacy of somatostatin analogs (octreotide and lanreotide) has been explored, as well. Responses have been documented in case reports; however, a formal prospective clinical trial has never been conducted [156–158].

Peptide receptor radionuclide therapy could also have a role in this setting of disease, even if large experiences have not been reported, till now [156, 159].

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20 Therapy in Poorly Differentiated Neuroendocrine Neoplasms (NEN G3)

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20.1 Introduction and Histopathological Classification and Characteristics

According to the WHO classification, NeuroEndocrine Carcinomas (NEC) are defined as poorly differentiated NeuroEndocrineNeoplasms (NEN) with Ki-67 > 20% and hence G3. Although lately, increasing evidence suggests that G3 NEN are not a homogenous entity and can be further subclassified into biologically different subgroups, according to both morphological and pathological characteristics other than Ki-67 alone. In fact, not all the neoplasms with high Ki-67 levels have histological characteristics of poor differentiation [1].

A separation based on the proliferative index (Ki-67 > 55%) showed to have clinical prognostic and predictive implication: NEC with Ki-67 > 55% has high sensitivity and good response to platinum-based chemotherapy but a poorer prognosis than G3 NEN in the lower proliferative range (20–55%) [2].

Recent data show that morphological differentiation associated with Ki-67 is essential in defining prognostic and pathological subgroups among G3 NEN, and therefore, a separation of well-differentiated G3 NeuroEndocrine Tumors (NET) from poorly differentiated G3 NEC is emerging [3].

The WHO 2017 classification for pancreatic NEN refers to these tumors as NET G3, whose median Ki-67 rate is 30% compared with 70–80% for GastroEnteroPancreatic (GEP) NEC. These neoplasms are different morphologic, molecular, clinical, and prognostic entities if compared to NEC. However, differentiation between the two and the pathological criteria for subdivision in G3 NEN and NEC are not entirely straightforward and are evolving to more precise criteria. Clinically, NET G3 and NEC differ substantially from NET G1–G2. The prognosis is worse: metastatic disease is usually present at diagnosis, and the treatment of metastatic disease is different. NET G3 can have high proliferative index but rarely exceed 50–60%, different response to chemotherapy (low benefit from platinum–etoposide-based chemotherapy, better with oxaliplatin and temozolomide), high expression of SRI, and Chromogranin A (100% vs. 70%) [3].

Based on an analysis by Milione et al., new insight in the GEP G3 NEN have been identified, with a median follow-up of 81 months, the median OS was 12.9 months. At multivariate analysis, morphological differentiation, Ki-67 index, MMRd, stage, and CD117 expression were independent prognostic markers in NECs. Three different prognostic categories of NECs

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Table 20.1 Histological and molecular features of G3 NEN

	Histomorphology	Molecular features	Ki-67, %
NET G3	Regular cells presenting round or oval nuclei with "salt and pepper" chromatin Minimal to moderate atypia, with organoid growth pattern with apposition of capillary vessels to tumor cells lacking geographic necrosis	Abnormalities of *MEN1*, *DAXX*, and *ATRX* genes Chromogranina A staining in 91–100% SSTR2A staining in more than 90%	20–60%
NEC	Highly proliferative atypical cells, solid growth pattern lacking organoid features, rosette formation and palisading, and apoptotic bodies and necrosis	Abnormal immunolabeling for p53, Rb1 loss, and KRAS mutation Chromogranina A staining in 60–80% SSTR2A staining in 20%	>50%

were identified according to the degree of morphologic differentiation (well vs. poorly differentiated) and Ki-67 index (<55% vs. ≥55%). On this basis, median OS was 43.6 months in well-differentiated neoplasms with a Ki-67 index 20–55% (named type A), 24.5 months in poorly differentiated neoplasms with a Ki-67 index 20–55% (type B), and 5.3 months ($p < 0.0001$) in poorly differentiated neoplasms with a Ki-67 index ≥55% (type C) [3].

NET G3 is more frequent in younger patients, primary tumors mostly located in the pancreas (65%), and the disease appears metastatic since the diagnosis in 62–70%, sometimes appearing with functional syndrome (14%) compared to NEC (2%). For pancreatic primaries, the median Ki-67 has been reported in pancreatic NET G3 to be 29–47% (range, 21–80%), compared with pancreatic NEC with a median Ki-67 of 70–80% (range, 21–100%). Also the NET G3 prognosis is better than NEC. The median survival for metastatic patients was 41 months for GEP NET G3 versus 17 months for non–small cell GEP NEC. Several retrospective studies support the prognostic value of histological differentiation.

Till now, classification and diagnosis based on morphologic differentiation alone are challenging. The pathological and biological criteria for subdivision in G3 NEN have not been entirely established yet and are evolving to achieve a standardization.

The ENET Society recommends that a pathology report on GEP NEN G3 should include morphology concerning both differentiation (well-differentiated or poorly differentiated) and small cell versus large cell, as well as proliferation rate as an absolute Ki-67 value [4]. Moreover, it is important to establish histopathological criteria, marking the difference between NET G3 and NEC, because the Ki-67 value alone cannot distinguish between the subgroups. In fact, there is an overlapping of Ki-67 value among NET G3 and NEC, especially in the area of 30–50%, although a Ki-67 of greater than 60% is rare in NET G3 (Table 20.1).

20.2 Pulmonary Neuroendocrine Poorly Differentiated Neoplasms (LUNG NEN)

20.2.1 Histopathological Classification and Characteristics

The 2015 WHO classification has grouped the four histologic variants of lung NETs, namely typical carcinoid (TC), atypical carcinoid (AC), large-cell neuroendocrine carcinoma (LCNEC), and small-cell lung carcinoma (SCLC), into a unique box of neuroendocrine (NE) cell proliferations to facilitate their taxonomy and improve diagnostic recognition. Behaviorally, TCs are low-grade tumors with good prognosis. ACs are intermediate-grade tumors with a more aggressive clinical course benefitting from multimodality therapy.

LCNEC and SCLC are high-grade carcinomas with dismal prognosis usually treated by chemoradiotherapy. A grading system independent of histology could prove useful in the setting of a metastatic disease, where morphology alone could not match adequately with the pathologic

and clinical grade to support the best therapy choices.

The classification of lung NETs is a process based on cytological and histological features other than the evaluation of mitotic count and necrosis extent. Defining criteria of carcinoids include organoid growth patterns (rosettes, trabeculae, ribbons, festoons, lobular nests, palisading), absent to focal punctate necrosis (not just apoptotic bodies), up to 10 mitoses per mm^2.

On the contrary, SCLC and LCNEC are clustered into poorly differentiated tumors, showing trabecular to solid to diffuse growth patterns, extensive necrosis, mitotic count higher than 10 mitoses per mm^2 with no upper limits and uneven cell expression for pan-NE markers, especially. LCNEC is a tumor category defined upon pan-NE IHC markers to exclude histological mimics such as LCC-NEM and basaloid carcinoma, or identify non-NE components in combined variants. The diagnosis of SCLC relies primarily upon morphology in both the lung and elsewhere. Ki-67 antigen has been extensively evaluated in lung NET with several diagnostic, prognostic, and grading implications. Although Ki-67 level is not currently accredited in lung NET subtyping due to some overlap of cut-off thresholds among biologically adjacent tumors, its distribution between low- to intermediate-grade and high-grade tumors has made it a very important prognostic and predictive factor. A Ki-67 level up to 20–25% has the highest specificity and sensitivity for low- to intermediate-grade versus high-grade tumors, in the setting of metastatic disease. It is important to note that Ki-67 reflects tumor biology, such an advantage holds particularly true for AC and LCNEC. Not unexpectedly, Ki-67 is typically 5% or less in TC and usually 80% or more in SCLC [5–7].

On the basis of actual knowledge, we can identify four different subgroups of lung NETS:

1. First two groups comprehend low and low-to intermediate tumors, with Ki-67 lower than 20–25% (TC with Ki-67 < 5%) and AC and LCNEC with Ki-67 up to 20–25%, with mainly indolent clinical behavior. The second group includes low-to-moderate malignant tumors showing Ki-67 level up to 20–25%, which correspond mostly to AC and even some LCNEC with a molecular profile similar to carcinoids.
2. The third group consists of moderate to higher malignant tumors with Ki-67 level ranging from 25% to 50–60%, biologically corresponding to more uncommon aggressive AC or LCNEC with a molecular profile similar to NSCLC. They can be treated with alkylating drugs or others chemotherapy (such as gemcitabine, paclitaxel, or vinorelbine), but they do not have good response to platinum/etoposide-based chemotherapy.
3. The last group is composed of highly malignant tumors with Ki-67 ranging from 60% to 100%, biologically corresponding to aggressive SCLC and SCLC-like LCNEC on molecular grounds, which should be treated with platinum/etoposide-based chemotherapy and have a very poor prognosis.

20.2.2 Poorly Differentiated Lung Neuroendocrine Carcinomas NEC (SCLC and LCNEC) Treatment

Even though TNM staging classification has been approved for lung NEC, the old classification, dividing this category into limited stage (LS) and extended stage (ES) disease, remains a gold standard to define treatment strategy.

Lung NEC are characterized by a very aggressive behavior with fast clinical progression and metastatic spread and extremely low survival time in the absence of treatment, with most of the patients diagnosed with advanced disease at diagnosis.

20.2.2.1 Limited Stage Disease Treatment

Radiochemotherapy

Small- and large-cell neuroendocrine tumors of the lung which involve only thoracic organs

(lung, nodes, and pleura) are considered limited stage (LS) disease and should undergo multimodal therapy, comprehending chemotherapy and radiotherapy, both sequentially or concomitant. In fact, chemotherapy alone results in poor intrathoracic disease control, with early failures occurring in 75–90% of patients. The addition of thoracic radiotherapy (TRT) to chemotherapy leads to a significantly lower rate of intrathoracic failure, to 30–60%.

In order to address this issue, two meta-analyses were performed [8, 9]. The results from both analyses confirmed that multimodal treatment can reduce risk of death and prolong progression free and overall survival (PFS and OS) over chemotherapy alone.

Platinum and etoposide doublets are the landmark chemotherapy for lung NEC, achieving high response rate (up to 70–80%) even though a rapid progressive disease often occurs after treatment discontinuation or during therapy. Adding radiotherapy (both sequentially and concomitantly) can improve and prolong response rate in limited disease with a reduction in death risk of 14% and prolonging OS and PFS and is considered the gold standard in LS disease.

Prophylactic Cranial Irradiation (PCI)

The incidence of central nervous system (CNS) metastases in lung NEC is very high, up to 50% even in limited disease with good response after radiochemotherapy, and is the main cause of disease progression and death. PCI demonstrated to reduce the risk of metastatic spread to the CNS and therefore to increase disease control rate and prolong survival time, with a reduction of relative risk for death of 16% [10].

PCI should be proposed in all patients achieving complete or major response after radiochemotherapy. Recent data support the use of prophylactic brain irradiation even in patients with extended disease, achieving major response after first-line chemotherapy. The factors associated with the recommendation for the use of PCI included the fitness of the patient, young age, and good response to chemotherapy. PCI was recommended by the majority of experts for non-elderly fit patients who had at least a partial response (PR) to chemotherapy [11].

20.2.2.2 Advanced Stage Disease Treatment

First-Line Chemotherapy

Small-cell lung cancer (SCLC) is highly sensitive to first-line chemotherapy, leading to rapid clinical and radiological improvement; unfortunately, this benefit is transient, and relapse is expected either during or shortly after completing chemotherapy. Upon relapse, SCLC is relatively refractory to second-line treatment, and survival with first-line platinum-based chemotherapy rarely exceeds 10 months. Despite this poor outcome, standard first-line therapy has been unchanged in the last three decades with platinum–etoposide combination being the most active treatment and should be considered even in elderly and patients in poor clinical conditions.

Platinum (cisplatin and/or carboplatin) and etoposide combination demonstrated to be very active and has been the standard of care for SCLC since 1990s. A randomized trial published in 1992 [12] confirmed the cisplatin and etoposide combination as the standard of care as first line in advanced SCLC, demonstrating better outcomes than CAV (cyclophosphamide, doxorubicin, and vincristine) with a 8.6 median OS and a 61% partial response (PR) and 10% complete response (CR) rate. Subsequent meta-analyses suggested improved survival with the use of first-line platinum-based regimens compared with other alkylating agents [13].

The next major advance to first-line therapy was the substitution of cisplatin with carboplatin, always in association with etoposide (both iv and oral). This regimen offered a different toxicity profile (higher hematological but lower gastrointestinal, clinical, and neurological toxicity rate) but was not associated with any difference in efficacy. The COCIS meta-analysis compared outcomes with these two platinum agents confirming substantial equivalence between cisplatin and carboplatin in combination with etoposide [14].

With the aim of improving the outcome of first-line therapy, other combinations chemotherapy have been investigated during last decades, with inconclusive and controversial results. The most promising one was cisplatin and irinotecan, although initial promising results in a Japanese phase III trial in comparison with platinum and etoposide, demonstrated higher survival (median OS 12.8 months vs. 9.4 months, $p = 0.002$) and 1-year survival rate (58.4% vs. 37.3%) [15], further studies failed to confirm this benefit, showing no substantial differences among irinotecan and etoposide in combination with platinum [16]. Although dismal, cisplatin and irinotecan could be considered as an alternative (even if not a new standard of care) to platinum-etoposide combination in first-line treatment of SCLC. Finally, maintenance chemotherapy after completion of first-line treatment did not demonstrate to improve patients' outcome and should not be considered in advanced lung NEC.

Second-Line Chemotherapy

Even if a high response rate is expected from first-line treatment, this result is of short duration, and a rapid disease progression is observed both during and within few months from the end of treatment. Second-line treatment has a very small probability to be active in SCLC, and topotecan is the only approved drug, with CAV (cyclophospamide, doxorubicin, vincristine) being potentially considered as an alternative in case of patients in good clinical conditions. The phase III trial comparing topotecan and best supportive care demonstrated an advantage in term of PFS (25.9 vs. 13 weeks) with topotecan over BSC (best supportive care), with a 7% PR (partial response) and 44% SD (stable disease), and a higher probability of symptoms control [17]. In order to improve this dismal results and define potential alternative, other drugs have been investigating in (irinotecan, paclitaxel, docetaxel), but any of them demonstrated an advantage among topotecan. Other new drugs have been compared with topotecan, but with disappointing results, for example, amrubicin and cabazitaxel did not show any advantage over topotecan in phase III trials. Prognostic and predictive factors for second-line topotecan activity are clinical conditions, LDH levels, and time to progression after first-line chemotherapy (when >90 days it was associated with better outcome). Finally in some cases, platinum/etoposide rechallenge could be considered in patients achieving good response to first-line chemotherapy and with a long time to relapse (3–6 months).

Immunotherapy

After a decade of failure in improving the results of first-line chemotherapy in SCLC, lately immunotherapy appeared to have partially changed the landscape of lung NEC. Given the founding of high rate of somatic mutations in SCLC immunotherapy was expected to be an effective treatment for SCLC. Phase I and II trials demonstrated promising results with anti-PD1 and PDL1 drugs in terms of survival rate in advanced stage disease in second- and third-line treatments (nivolumab and ipilimumab combination and pembrolizumab).

According to these promising results, concomitant immunotherapy and chemotherapy in first-line setting has been investigated. Despite the combination of cisplatin and etoposide with ipilimumab did not showed any improvement in patients outcome but increased treatment-related toxicity, other immunotherapies (atezolizumab and durvalumab) demonstrated to increase survival when administered with first-line chemotherapy.

The addiction of atezolizumab (anti-PDL1 antibody) to first-line chemotherapy (carboplatin and etoposide) was evaluated in the IMpower 133 [18], a phase I/III trial which included patients with advanced SCLC, who were randomized to receive chemotherapy with atezolizumab or placebo, followed by atezolizumab/placebo maintenance. Atezolizumab improved both PFS (HR 0.77; HR 0.77; 95% CI, 0.63–0.96) and OS, with an improvement from 10.3 months to 12.3 months and a HR of 0.70 (95% CI, 0.54–0.91). Atezolizumab improved the 1-year survival rate from 38.2 to 51.7%. There was no difference in

response rate, and no new safety signals were identified.

Other than atezolizumab, durvalumab (an anti-PDL1 antibody) demonstrated to improve results of first-line platinum and etoposide in a phase III trial (CASPIAN) [19] in which we randomly assigned 805 patients to receive chemotherapy alone or in combination with durvalumab or durvalumab and tremelimumab, followed by durvalumab maintenance. The addition of the anti-PDL1 antibody again improved survival, with a median OS of 13 months with durvalumab compared with 10.3 months with chemotherapy. The 1-year survival rate was higher with durvalumab (54% vs. 40%), even in this case, no significant difference in term of PFS and response rate was observed.

These results, even if practice changing, need to be confirmed and further investigated, above all in terms of definition of predictive biomarker of response to define the subgroup of patients who can really benefit from immunotherapy; in fact, until now, PDL1 expression did not serve a predictive role in both study.

20.3 Extrapulmonary G3 Neuroendocrine Neoplasms

Extrapulmonary G3 NEN are very rare, they mostly arise in gastrointestinal tract (35–55%) with pancreas and colon representing the most frequently primary site followed by stomach and esophagus. They can be both small-cell and large-cell neuroendocrine carcinoma, mainly with high Ki-67% (above 60%) with the exception for primary pancreatic G3 NEN, which have a high Ki-67 level (>55%) in about 30% of cases. Other primary tumor sites are even more infrequent such as prostate, gynecological, and urothelial tract.

Unfortunately, due to the rarity of these tumors, prospective data regarding treatment strategy are lacking, and the consensus on treatment is mainly based upon retrospective data and mutuated from analogous lung NEC (despite potental differences both in terms of biology and treatment response).

On the basis of a retrospective analysis on 305 patients diagnosed with gastroenteropancreatic NEC (NORDIC NEC) [3], most extrapulmonary NEC are diagnosed in advanced and metastatic stage (60–65%) and have a poor prognosis. Ki-67% level, which is considered the main prognostic and predictive factor, appears higher than 55% mainly in esophageal and rectal tumor, while it was mainly lower in pancreatic neoplasia (only 33% with Ki-67 higher than 55%). Median OS is about 1–2 months in patients who do not receive oncological treatment and 11 months in patients who undergo chemotherapy.

20.3.1 Treatment of Extrapulmonary Neuroendocrine Carcinomas

20.3.1.1 First-Line Chemotherapy

Chemotherapy plays a prominent role in advanced extrapulmonary NEC, compared with their G1–2 counterpart. Median OS is about 11 months, and 1 month for patients receiving chemotherapy or not, respectively. Unfortunately, due to the small number of cases of extrapulmonary NEC, no prospective or randomized data are available in support of chemotherapy, so far the first-line treatment is mutuated from data regarding SCLC. Platinum and etoposide doublets remain the standard of care for extrapulmonary NEC; this is usually given for 4–6 cycles, with a RR (response rate) of 30%, with PFS of 4–5 months and OS of 11 months. As previously mentioned, there is no randomized trial investigating this regimen, but several retrospective studies confirm the efficacy and safety of this approach [20].

According to the NORDIC NEC study [3] response rate after first-line chemotherapy with platinum/etoposide combination was 31%, but it was different according to Ki-67 (higher in Ki-67 > 55% than 21–54%: 42% vs. 15% respectively) suggesting that high Ki-67 index may predict response rate. Patients with lower Ki-67

(<55%) had longer survival than those with higher Ki-67 levels (14 vs. 10 months). On the other side, ORR appears to be indipendent from tumor morphology or chromogranin A staining. Finally, colonic primary tumor had a worst prognosis (8 vs. 15 months) than other tumors.

More recently, retrospective data have been published, and platinum (both cisplatin and carboplatin) and etoposide combination chemotherapy has been confirmed as the standard of treatment, achieving a good response rate (from 40% to 65%), with 11.5 months OS and 6 months PFS. Tumor response was mainly unrelated to primary site, endocrine hyperfunction, or prior therapy experience (Table 20.2).

Another regimen which has been investigated is cisplatin/irinotecan, even in this case mutuated on results observed in phase III study in SCLC, a retrospective study for patients with advanced NECs included patients treated with both cisplatin/etoposide and cisplatin/irinotecan (Table 20.2). The response rate and OS (13 vs. 7 months) was higher in cisplatin/irinotecan arm; however, the difference was not statistically significant due to the imbalance with respect of primary site. Similar results were observed in another study, enrolling both extrapulmonary NEC and NET G3. Cisplatin and irinotecan combination was effective in NEC with a RR of 51% and 8 months median OS, but did not show any activity in NET G3 (no partial response observed, with a median OS of 5.4 months) meaning that while the combination of cisplatin and irinotecan may have activity in patients with poorly differentiated neuroendocrine tumors, it has little or no activity in patients with well-differentiated histologies.

According to clinical results of NORDIC NEC study, patients with Ki-67 lower than 55% did not have good response to platinum/etoposide (EP) combination (even with a better prognosis), suggesting the use of alternative

Table 20.2 First-line chemotherapy in NEC G3

	Histology (number of patients)	Regimen	Response rate	Overall survival (months)	Progression-free survival (months)
Moertel et al. [21]	Anaplastic neuroendocrine tumor (18)	Cisplatin/ etoposide	ORR 67%	19	8
Mitry et al. [22]	PDNEC (41)	Cisplatin/ etoposide	CR 9.8% PR 31.7% SD 34%	15	9.2
Frizziero et al. [23]	Extrapulmonary PD NEC (113)	Carboplatin/ etoposide (iv or oral)	CR 7% PR 40% SD 26%	11.5	6
Iwasa et al. [24]	Gastroenteropancreatic NEC (21)	Cisplatin/ etoposide	CR 0 PR 14% SD 48%	5.8	1.8
Sorbye et al. [2]	GEP NEN (252)	Cisplatin or carboplatin/ etoposide	PR 31%	11	6
Du et al. [25]	GEP NEC (11)	FOLFIRI	PR 63%	13	6.5
Li et al. [26]	Gastroenteropancreatic NEN G3 (40)	Cisplatin/ irinotecan	PR 51% (NEC) PR 0% (NETG3)		5.7 (NEC) 8.9 (NET G3)
Rogowsky et al. [27]	G3 NEN (32)	Capecitabine/ temozolomide	PR 70% (NETG3) PR 30% (NEC)	22 (NET G3) 4.6 (NEC)	15.3 (NETG3) 3.3 (NEC)
Bajetta et al. [28]	PD G3 NEN (40)	XELOX	PR 23% SD 7%	11	5

chemotherapeutic regimen in this subgroup. On the basis of this further subclassification, retrospective data are available, and oxaliplatin-(FOLFOX or CAPOX) or temozolomide-based treatment can be considered as an alternative to EP in gastroenteropancreatic NEC with Ki-67 < 55% (Table 20.2).

20.3.1.2 Second-Line Chemotherapy

Evidence for second-line chemotherapy in patients with progressing disease after platinum-etoposide is very limited, and no prospective data are available; therefore, thee is not consensus regarding optimal second-line chemotherapy [20]. Overall response rate (observed in NORDIC NEC study) is quite low (about 18%) even if small retrospective series have documented higher response rate (30–40%, in selected patients), with short benefit and an estimated PFS of 3–4 months and OS lower than 6 months [3].

Actual data regarding second-line chemotherapy mainly derive from retrospective analysis on small number, in fact a low percentage of patients is able to receive further treatment after failure of first-line chemotherapy due to rapid clinical worsening related to tumor aggressiveness.

The most active regimens investigated are FOLFIRI, oxaliplatin-based chemotherapy (FOLFOX and CAPOX) and temozolomide (both alone or in combination with capecitabine). No prospective or randomized data have been available until now, and clinical results are mainly difficult to compare due to the heterogenous population included in these analyses (mainly both G3 NET and NEC).

FOLFIRI showed quite interesting results in retrospective analysis (comprehending both G3 NET and NEC), with 31% response, 31% stable disease with a median PFS of 4 months and 8 months OS (Table 20.3).

Another potential alternative is oxaliplatin-based chemotherapy (FOLFOX or CAPOX) (Table 20.3) which is demonstrated to be effective as second-line chemotherapy with a documented response rate of 20–40% and a median OS up to 6 months.

Finally, temozolomide can have a role in the treatment of progressive disease, both alone and in combination with capecitabine, it has demonstrated encouraging results especially in tumors with Ki-67 lower than 55% (Table 20.3).

Finally, some data are available for biological treatment, with some activities observed for both everolimus and sunitinib in GEP G3 NEN (both NETG3 and NEC), supporting further analysis in order both to confirm these data and to better define the role of these drugs other than identify predictive factors of response (Table 20.3).

Second-line chemotherapy should be considered according to clinical characteristics of patients, performance status, and tumor features. In some cases, platinum/etoposide re-treatment can be considered, in case of prolonged response

Table 20.3 Second-line chemotherapy in NEC G3

	Histology (number of patients)	Regimen	Response rate	Overall survival (months)	Progression-free survival (months)
Hentic et al. [29]	NEC (19)	FOLFIRI	PR 31%	18	4
Hadoux et al. [30]	NEC (20)	FOLFOX	PR 29%	9.9	
Welin et al. [31]	PD NEC (25)	Temozolomide/ CAPTEM	CR 4% PR 33% SD 38%	22	6
Pellat et al. [32]	PD G3 NEN (31)	Sunitinib	PR 66% (NETG3) PR 55% (NEC)	6	1.5
Okuyama [33]	Pancreatic NEC (25)	Everolimus	PR 0% SD 39%	7.5	1.2
Panzuto et al. [34]	Pancreatic NEC (15)	Everolimus	SD 73%	28	6

to first-line chemotherapy (at least 3–6 months relapse free survival).

20.4 Treatment of Extrapulmonary G3 Neuroendocrine Tumor (NET)

The optimal sequence of treatments for NET G3 remains unclear, as this category has been recently identified. NET G3 is considered a molecularly, radiologically, and prognostically distinct entity compared to NEC and NET G1/G2. Although NET G3 have been treated mainly with platinum-based chemotherapy, retrospective data showed that this treatment has limited effectiveness in this group of neoplasms.

Predictive factors for treatment benefit in NET G3 are scarce, and few prospective studies are available. Much more research is, therefore, needed to aid clinicians selecting the best personalized therapy. Until further data are available, NET G3 treatment choice has to consider several factors: tumor differentiation, tumor stage, primary tumor location, Ki-67 index, and clinical course as well as each patient's specific features. Again, most chemotherapy studies are a mixture of NET G3 and NEC and specific data on the NET G3 subgroup are few and based on a very small number of patients.

20.4.1 Chemotherapy

Chemotherapy has a central role in the treatment of advanced NET G3 and should be considered the standard of treatment in first-line setting. Several retrospective data and few prospective trial have been evaluating and investigating the role of chemotherapy and the potentially active drugs [35].

20.4.1.1 Temozolomide

The role of temozolomide, an alkylating agent, has been defined for advanced well-differentiated G1 and 2 pancreatic NET in multiple studies. It demonstrated to be effective, both as a single agent and in combination with capecitabine in NET G3 (other than G2), the main part of the results being about pancreatic NEN, and some reports about lung NET. The activity of CAPTEM (Capecitabine and Temozolomide) was recently evaluated in NEN G3: NET G3 had a better response compared to NEC group in terms of DCR (70% vs. 30%), PFS (15.3 months vs. 3.3 months), and median OS (22 months vs 4.6 months).

A retrospective Australian study reported the activity of CAPTEM in patients with metastatic NET G2 (66%) and G3 (34%). ORR was 46.9% in the overall population with 15.6% of patients having stable disease. A retrospective multicenter study evaluated the activity of temozolomide-based therapy in patients with G3 NENs, showing a time to treatment failure (TTF) in patients with well-differentiated G3 NETs was 5.8 months, OS and ORR for the same group were 30.1 months and 52%, respectively. The phase II clinical trial (ECOG-ACRIN EA2142) will better help to assess the activity of CAPTEM compared to platinum and etoposide combination in patients with advanced GEP-NEN G3 excluding small-cell histology (Table 20.4).

A number of studies have shown that O6-methylguanine-DNA methyltransferase (MGMT) can be a predictor of temozolomide efficacy in patients with advanced NENs. However, the mechanism behind the association between MGMT and temozolomide is unclear. A lack of MGMT deficiency in patients with NENs, as shown by immunohistochemistry, has been demonstrated in 24–51% of cases, whereas MGMT deficiency in cases of gastrointestinal NENs has not yet been reported. Further studies and clinical trials are required to demonstrate the relationship between MGMT and temozolomide.

Similar reports have been observed even in lung NEN, CAPTEM regimen is associated with a high response rate and a tolerable toxicity profile in lung NENs with 30% patients exhibited a partial response, 55% stable disease, and 10%

Table 20.4 Treatment of G3 NET

	Histology (number of patients)	Regimen	Response rate	Overall survival (months)	Progression-free survival (months)
Chan et al. [36]	Gastroenteropancreatic G3 NEN (118)	Temozolomide/CAPTEM	CR 1% PR 39% SD 22%	18	5
Sahu et al. [37]	G3 NET (32)	CAPTEM	ORR (26.9%) SD 15%	24	15
Thomas et al. [38]	G3 NEN (116)	CAPTEM (second line)	DCR 73%	38	13
Spada et al. [39]	G3 NET	Oxaliplatin-based chemotherapy (GEMOX; CAPOX; FOLFOX)	PR 26% SD 54%	32	8
Pellat et al. [32]	PD G3 NEN (31)	Sunitinib	PR 66% (NETG3) PR 55% (NEC)	6	1.5
Okuyama [33]	Pancreatic NEC (25)	Everolimus	PR 0% SD 39%	7.5	1.2
Panzuto et al. [34]	Pancreatic NEC (15)	Everolimus	SD 73%	28	6

progressive disease, and promising results in term of PFS and OS (Table 20.4).

20.4.1.2 Platinum-Based Chemotherapy

In general, first-line treatment for G3 NEC is platinum-based chemotherapy, and multiple retrospective cohorts suggest a low response to platinum-based therapy in NET G3 patients ranging from 0% to 17%. The NORDIC NEC study included patients with GEP NEN G3 treated with platinum-based regimens, demonstrated that G3 NET (Ki-67 <55%) had lower probability of response (even if better survival) compared to NEC with Ki-67 >55%, ORR was 15% vs. 42%, respectively, when treated with platinum/etoposide. According to these data, an alternative treatment should be considered in G3 NET and extrapulmonary NEC with Ki-67 lower than 55%. A retrospective analyses by Fazio et al. suggest that oxaliplatin-based chemotherapy can be active with a manageable safety profile in advanced NETs irrespectively of the primary sites and tumor grade [capecitabine/oxaliplatin (CAPOX), 6% gemcitabine/oxaliplatin (GEMOX), and 29% leucovorin/fluorouracil/oxaliplatin (FOLFOX-6)] [39]. Similar results have been observed in a study by Bajetta et al., which demonstrated activity of oxaliplatin and capecitabine combination in G3 NET with a 30% DCR (23% PR and 7% SD) in second-line treatment, this study demonstrated a lower activity profile in G1–2 NET and NEC (Table 20.4).

20.4.2 Biological Treatment

Both everolimus and sunitinib (both alone and in association with somatostatin analog (SSA) are the standard of care in progressive low to intermediate NET. While sunitinib demonstrated survival advantage only in pancreatic NET, everolimus is the standard of care in low to intermediate NET of pancreatic, lung, and non-functioning gastrointestinal origin. Some activity evidences have been shown for both drugs in G3 NET and NEC.

Everolimus: Actually, we only have some case reports, and a retrospective study about everolimus activity in NET G3. An Italian study included patients with advanced pancreatic NET G3 with a Ki-67 of 55% or less (median, 30%); everolimus was given mainly after first-line treatment. Median PFS was 6 months and OS was

28 months; 40% had disease stabilization for at least 12 months [33, 34].

Sunitinib seems to show activity also in NET G3. Mainly two studies have evaluated the activity of sunitinib in NEN G3 after progression to chemotherapy. In an open-label phase II, non-randomized prospective trial, 31 patients with GEP-NEN G3 (six patients with NET G3 were included) mainly pretreated with chemotherapy, received sunitinib. Among 31 patients, DCR was 58% while ORR was detected in 12.9%. There was no correlation between tumor differentiation and response to therapy.

In a larger retrospective study, 60 patients with pancreatic NEN treated with sunitinib ORR (in the overall population) was 33.3% with 48.3% stable disease; while G3 NET patient ORR was 60% and 30% SD; PFS in NET G3 was similar to well-differentiated NET (but PFS data was not statistically significant). Otherwise, NEC G3 had a worse prognosis and no response to sunitinib [32] (Table 20.4).

20.5 Immunotherapy in GEP G3 NEN

Immunotherapy has demonstrated to be active and improved patients' survival in combination with first-line chemotherapy in SCLC. Evidence points to an important role of immune phenomena in the pathogenesis and treatment of NENS, and the presence of inflammatory infiltrated can be considered a poor prognostic factor. Even if still lacking, some evidences showed PDL1 expression in metastatic gastroenteropancreatic (GEP)-NENs, in particular in high-grade tumors, poorly differentiated NENs, and GEP-NECs. Furthermore, PD-1 and its ligands appear to be also expressed in well-differentiated intestinal and pancreatic NETs. This molecular and genetic profile could explain potential activity of immune check-point inhibitors in GEP G3 NEN [40].

Currently, there are only preliminary data on the effects of immune checkpoint inhibition from controlled trials in GEP NEN patients. Actual data regards mainly pembrolizumab which did not demonstrate high response rate, but a good percentage of disease control in G1–2 NET. Even the association of pembrolizumab with platinum containing chemotherapy in NEN G3 resulted in low response rate. Interesting and promising data derive from the DART trial [43]: the combination of immunotherapy with ipilimumab and nivolumab in the treatment of NEN G3 resulted in a 44% of ORR in NEN G3.

Although NEN G3 has high mutational burden, making them potential target for immune checkpoint inhibitors, the role of immunotherapy still remains unclear ant its role is a is currently evaluated in several phase II studies (Table 20.5).

20.6 Locoregional Therapy

Very little is known about the role of regional therapy such as surgery, radiation, ablative therapy, and embolotherapy in patients with G3 NEN. The treatment recommendation for patients with apparently localized disease is not based on prospective data, and supporting evidence from heterogenous studies is limited [3, 20]. Curative surgery is usually attempted in localized disease, although retrospective

Table 20.5 Immunotherapy in G3 NEN

	Histology (number of patients)	Regimen	Response rate	Overall survival (months)	Progression-free survival (months)
Mehnert et al. [41]	G1–2 NET (carcinoid 170; pNET 106)	Pembrolizumab	12% (carcinoid) 6.3% (pNET)	–	Not reached in pNET 9.2 in carcinoid
Vijayvergia et al. [42]	G3 NEN (29)	Pembrolizumab	PR 3.4% SD 20.7%		
Patel et al. [43]	G3 NEN (32)	Nivolumab/ ipilimumab	ORR 44% (0% in low-grade tumor)	11	31%, 6 months PFS

series indicate that it is rarely curative as a sole therapeutic modality. There is expert consensus that surgery alone is rarely curative and that patients with limited disease should probably receive multimodality-based treatment. Surgery, as a part of the treatment, can be curative in patients with localized disease even with regional nodal metastasis; however, retrospective data often do not distinguish between NET G3 and NEC G3. The 5-year survival for localized disease depends of the primary tumor site: 40–50% for colorectal, gastric, and pancreatic neoplasms and 25% for anal and esophageal primaries. Surgery as a part of the treatment should be considered for all localized GEP NEC with the exception for esophageal cancer [20].

Until more data become available, the locoregional approach for G3 NET should follow the treatment paradigms for NET G2 and intended curative resection/ablation suggest a survival benefit, especially in tumors with a Ki-67 less than 55%. Retrospective analysis showed a trend toward a better survival in patients with colorectal and pancreatic NEC who underwent primary tumor resection, in a multimodal approach (comprehending chemotherapy and radiotherapy) [44, 45].

In pancreatic NET G3, resection of primary tumor seems to be associated with better survival than chemotherapy alone, although debulking surgery cannot be recommended in NEC because of the tumor aggressiveness and the absence of clear benefit from retrospective data. An Italian retrospective analysis suggests that surgery with radical intent could be discussed in pancreatic G3 NEN, even though a better survival was observed in patients with Ki-67 lower than 55% and G3 NET. Given the high relapse rate observed after radical surgery, most clinicians would advocate platinum-based adjuvant therapy in this setting, while some authors propose neoadjuvant chemotherapy followed by definitive surgery, although data to support this approach are scarce [20].

In patients with important comorbidities or where the tumor's anatomical site makes surgical resection not advisable due to high morbidity (i.e., esophagus), a definitive course of radiotherapy and chemotherapy is a reasonable treatment strategy. Debulking and locoregional treatment for liver metastasis are not recommended and also discouraged in poorly differentiated NEC.

20.7 Peptide Receptor Radionuclide Therapy (PRRT)

Peptide receptor radionuclide therapy (PRRT) delivers highly localized radiation by targeting specific somatostatin receptors on tumor cells. PRRT is comprised of three main components: a high activity radionuclide (177Lutetium or 90Yttrium), linked via a chelator (DTPA or DOTA) to a somatostatin receptor (SSR)-binding ligand which is typically a somatostatin analog (octreotide or octreotate). PRRT has been approved for somatostatin-positive GEP-NETs after failure of previous therapy, according to the results of NETTER 1 phase III trial, which did not include G3 NET [46].

Peptide receptor radionuclide therapy (PRRT) has previously not been recommended for GEP NEN G3 due to the assumption that these tumors lacked SSR expression, and the growth rate was too rapid to expect any benefit from PRRT. However, several retrospective studies have shown that high-grade tumors can display a high tumor SSR expression, and these patients seem to benefit from PRRT [29]. Last evidences demonstrate that NET G3 can have somatostatin receptor expression (87–92% positive on SRI), so PRRT could be a potential therapeutic option in these patients. SRI positivity has been reported for both NET G3 and NEC, and expression of somatostatin receptor 2A has been shown with immunohistochemistry.

According to these evidences, some studies evaluated PRRT in progressive G3 NEN [47, 48], demonstrating interesting activity profile, which need to be further confirmed. The larger one included 149 patients with progressive disease NEN G3. Results from these studies are mainly based on patients with pancreatic primary NET G3 or low NEC (Ki-67 <55%). However, the PFS and OS seem impressive as second- and third-line therapies, especially for the NET G3 and low

NEC group (40% PR and 38% SD, with median OS of 44 and 19 months in NEN G3 and low NEC, respectively). For NEN G3 with a Ki-67 <55% and specifically the low NEC subgroup, available data suggest a possible substantial benefit of PRRT. The outcomes of PRRT in GEP NEN G3 with a Ki-67 >55% are based on limited number of cases, and a possible benefit is therefore difficult to assess, additionally response rate was similar between NEC and G3 NET (40–50%), the duration of response was limited in NEC with frequent immediate progression. Another potential option is the combination of PRRT and chemotherapy in NEN G3, several studies have used concomitant chemotherapy including infusional 5FU, oral capecitabine or capecitabine and temozolomide with favorable responses and acceptable toxicity for patients with metastatic NEN.

Pending further research such as NETTER 2 (https://clinicaltrials.gov/ct2/show/NCT03972488) investigating the role of PRRT in high grade G2 and G3 NEN with Ki-67 <55% is warranted to define the effective role of PRRT in G3 NEN. Up to now PRRT for high-grade GEP NEN with a high uptake on SRI showed promising response rates, disease control rates, progression-free survival, and overall survival. Until further data are available, PRRT could be considered for all NET G3 cases and NEC cases with a Ki-67 21–55% with high uptake on SRI even if it cannot be considered a standard of care yet. The crucial clinical questions are to decide which NEN G3 patients should be offered PRRT and when it should be used. ENETS guidelines recommend that PRRT can be considered in SRI-positive NET G3 [20], with Ki-67 <55% and could be discussed in NEC with SRI, whereas the National Comprehensive Cancer Network (NCCN) recommend only the use of PRRT in GEP NEN when Ki-67 is ≤20%.

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Part VI

Conclusions

Conclusions: NEN Management Today and Looking at the Future

21

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Neuroendocrine neoplasms (NENs) are a very heterogeneous group of neoplasms with peculiar clinical and biological characteristics. NENs are largely heterogeneous, featuring different biological behavior, and malignant potential. NENs originate from the diffuse neuroendocrine system and, therefore, can arise in any part of the body. They are usually considered rare neoplasms when compared, in terms of incidence, with non-neuroendocrine neoplasms. The annual incidence rates varies from 1 to 5 per 100,000 worldwide and is increasing more across population and sites, stages, and grades although with different magnitude of change. Their relative rarity, combined with their variability and body wide presentation, frequently determines a delay in diagnosis that has been reported in patients between the first symptoms and the correct diagnosis. However, the continuous improvement in the diagnostic tools, from laboratory to the instrumental procedures, leads to facilitate timely diagnosis and therefore disease management. This type of tumors is a fertile field of new scientific knowledge, and this brings many challenges in identifying new ways of classification, diagnosis, and treatment. Due to these peculiarities, NENs constitute today a good subject for an exciting scientific debate and, from the clinical point of view, a matter for a multidisciplinary approach.

Pathology provides relevant indicators of tumor aggressiveness. NENs are classified into different subgroups according to their morphological and biological features, tumor cell differentiation and proliferation index: G1 NeuroEndocrine Tumor (NET) (well-differentiated morphology, <2 mitosis/10 HPF, and/or Ki-67 <3%); G2-NET (well-differentiated morphology, 2–20 mitosis/10 HPF, and/or Ki-67 3–20%); G3-NET (well-differentiated morphology, >20 mitosis/10 HPF and/or Ki-67 >20%—considerate; NEC (poorly differentiated morphology, >20 mitosis/10 HPF, and/or Ki-67 >20%). Besides, the main prognostic factors are: the site of the primary tumor (for example, pancreatic NENs show a worse prognosis than NENs in the rectum), the stage according to TNM and

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the World Health Organization (WHO) histopathological classification, which expresses both the morphological aspect of the tumor cells and their proliferative activity in terms of the number of mitoses or proliferation index (Ki-67).

The advent of large-scale genomics and transcriptomics in the last 10 years has allowed to accumulate an impressive amount of new information on NENs, confirming both the common alterations and a large molecular heterogeneity even within morphologically compact subgroups. Recent studies confirmed the involvement of SWI/SNF chromatin remodeling genes in pancreatic (*ATRX*, *DAXX*) and pulmonary (*PSIP1*, *ARID1A*) NETs. These alterations seem to have prognostic value in pancreatic NETs, being associated to a poorer prognosis in tumors expressing an alpha cell-like phenotype. On the contrary, small intestinal NETs did not show a definite mutational pattern and seem to be rather driven by chromosomal alterations. Also several epigenetic changes have been investigated in these last years. Global methylation analysis of both small intestinal and pancreatic NETs showed different patterns in each tumor type. Small intestinal NETs evidenced several variation subtypes. Pancreatic NETs revealed some focal differences in a reduced number of genes. Differences and changes across NETs of different sites were related to a reduced number of core pathways, including cell cycle regulation DNA damage repair, phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling, chromatin remodeling/histone methylation, and telomere alteration. The impressive contribution of these biological informations should be implemented and integrated with further research in order to provide a better stratification of patients and allow a better knowledge of the great heterogeneity of behaviors displayed by these tumors.

In the diagnostic process, the role of imaging has gained great importance during the last decades, not only to depict the presence of tumors, but also to select patients for the most appropriate therapies and to monitor the diseases during their course. No doubts that imaging is today essential for planning a correct patient management. Surgical and medical therapy, radiopharmaceutical therapy, interventional radiology, and radiation treatments cannot be carried out without the support of diagnostic imaging. The visualization of NETs can be obtained through the conventional morphologic imaging including, ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and the functional imaging through scintigraphy and PET-CT. Some typical features such as hyper-vascularization, specific growth patterns, and imaging appearance help physicians to discriminate NETs from other solid malignancies. These diseases hold several metabolic aspects based on the products of their neural cell precursors, such as the amine synthesis and secretion and the cellular expression of somatostatin receptors (SSTR). On these basis nuclear medicine offers either scintigraphic techniques or PET-CT studies to integrate morphology with biology. Two categories of radiopharmaceuticals are available: the first includes the meta-iodo-benzylguanidine (MIBG), a norepinephrine analog used for scintigraphy and the 18F-DOPA used for PET-CT imaging; the second one gamma and positron-emitting radiolabeled somatostatin analogs that bind somatostatin receptors used for both scintigraphy and PET-CT imaging. The study of glucose metabolism of NETs, using FDG PET-CT, can be usefull, in selected cases, to better investigate tumor aggressiveness. Due to the continuous technological advancement of the instrumentations and the related softwares, no single imaging technique represents the gold standard, and the sequence of exams needed for each tumor type may vary. It is important to remember that, even though we live in an era of standardization, personalization of treatment (within a consensus guideline frame-shift) is often required to maximize the outcome, particularly in NETs, thus implying the need to build up a “multidisciplinary culture” approach.

Considering the physiopathology of NENs and its impact on the daily clinic, we must not neglect the traditional old classification of NETs based on the capacity to secrete peptides and neuroamines. So well- and moderately differentiated NETs can be grouped into functioning and nonfunctioning forms. The functioning tumors

synthesize, store, and secrete in the bloodstream peptides and neuroamines that cause distinct clinical syndromes (carcinoid syndrome being the most common), while the non-functioning forms are clinically silent, being diagnosed in advanced stage because of their mass effects. Functioning NETs require symptoms control, which often assumes primary clinical relevance since it can affect survival, quality of life, surgical procedures, and peptide receptor-targeted radionuclide therapy outcome. Somatostatin analogs with their antiproliferative and antisecretive effects are the cornerstone for the treatment of many hormonal syndromes in functioning NETs. The use of multiple receptor somatostatin analog pasireotide is under investigation, and according to the various types of hormone secretion, other medical treatments are available alone or in combination with SA in patients with NET-related syndromes, and also to prepare patients eligible for procedures such as surgery, locoregional treatments, and PRRT.

Speaking about the current NENs therapy, we cannot avoid underlining some concepts that are emerging from recent acquisitions. First, this is an extremely dynamic field of research and debate which is ready to design new horizons for facing this disease. Alongside the traditional prognostic parameters, other novel biologic indicators have been investigated in light of genomic and proteomic studies, as we reported above. It is likely that the NENs classification should be revised, as new prognostic molecular indicators will be validated, potentially affecting both the development of new treatments and the strategies for their management. Furthermore, it appears increasingly clear how NENs that arise from different organs, even if belonging to the same histological type, do not show the same response to therapies. Clinical evidences demonstrated that gastrointestinal NENs should be managed in a different way than lung NENs. Pancreatic NENs display different clinical outcome and intrinsic characteristics than other neuroendocrine tumors, and they should be considered as a separate group. Besides, other groups of NENs have been defined on the basis of histologic characteristics, such as MiNEN, undifferentiated NECs, and Merkel cell carcinoma, and they require particular protocols. We are aware that we are living at the border of a revolution, and we are experiencing this progressive and continuous change.

Surgery represents today the main curative option of NEN management. Due to the high variability of presentation and variable disease aggressiveness of NEN, surgical treatment should be tailored according to the tumor characteristics and patient's features. A surveillance strategy has been even proposed for the management of incidentally discovered, asymptomatic, small lesions without radiological or endoscopic signs of malignancy. It goes without saying that formal resections associated or not with lymphadenectomy remain the gold standard for patients with localized tumors for whom a conservative strategy is not acceptable. In addition, surgery may play a role for selected patients with metastatic or high-grade well-differentiated neoplasms, who could benefit from this strategy. The application of robotic surgery can find place, in selected patients, for the treatment of both primary and metastatic tumors, in peculiar anatomic conditions and in centers with adequate expertise. Of course, surgery has to be always considered together with the options of medical, radiation, and radiopharmaceutical treatments, alone or in combination, adopting a multidisciplinary approach. In particular conditions, when the tumor is limited to a single organ or a defined anatomic district, some loco-regional surgical techniques combined with endoscopy, laser-ablation, or thermo-ablation can be proposed. These are the cases that could benefit also from alternative strategies including interventional radiology and radiation treatments. In patients with functioning tumors, whenever possible, surgery of the primary and/or metastasis should be considered in order to reduce tumor burden and consequently hormonal secretion.

The loco-regional therapies are another interesting option for the treatment of patients with NETs with liver metastases. The intra-arterial therapies, included in the area of interventional oncology, comprising intravascular (such as embolization, chemoembolization, and radioembolization) and ablative (percutaneous

radiofrequency ablation and microwave ablation) procedures, are the most common choices. All intra-arterial therapies, that in principle were introduced for treating HCC in clinical practice, demonstrated to be successful in liver metastatic NENs, because of the outstanding results in symptoms control and prolonging survival, with no or very limited side effects. On the other hand, liver ablation, first introduced for percutaneous imaging-guided approach and more recently also applied in open and laparoscopic setting, can provide eradication of liver metastases with less invasiveness than traditional surgery. Moreover, due to the high rate of relapse after radical treatments, new liver metastases can be further eradicated with percutaneous ablation, with no cumulative toxicity such as with repeated resections.

Peptide receptor radionuclide therapy (PRRT), recently proposed to be named as radioligand therapy (RLT), is another possible therapy for patients with unresectable or metastatic well- and moderately differentiated NENs. Three types of radiation may be used in PRRT: β-particles (in particular ^{177}Lu and ^{90}Y), α-particles, and Auger electrons. ^{90}Y/^{177}Lu-DOTATOC and ^{177}Lu-DOTATATE are the radiopharmaceuticals currently used, and they are systemically delivered in fractioned sequential cycles. In clinical practice, the indications should be limited to G1–G2 well-differentiated NETs with high expression of SSTR. The precise position in the treatment algorithm remains to be explored. PRRT is generally well tolerated by most of the patients. However, chronic and permanent damage on the kidneys and bone marrow are described. Combining PRRT with synergistic drugs may result in additive effects, through several mechanisms such as increased tumor perfusion, SSTR upregulation, and radiosensitization. In recent years, great interest has been shown in PRRT with alpha particle-emitting radionuclides (bismuth-213 or actinium-225) and in PRRT agents based on SSTR antagonists. The clinical experience with somatostatin-based targeted therapy in NET showed promising results even in refractory disease to β-emitters treatment.

Evidence-based medical treatment options available in clinical practice include: somatostatin analogs (SSAs), everolimus (m-TOR inhibitor), sunitinib (thyrosine kinase inhibitor), chemotherapy, PRRT, and locoregional therapies. New treatment options under investigation are new somatostatin analog (pasireotide) or new thyrosine kinase inhibitors (pazopanib, cabozantinib, lenvatinib, axitinib, surufatinib). Moreover, immune checkpoint inhibitors (ICIs) have also been investigated in NETs. Several studies with ICIs as both monotherapy and combination have been published. No clear role of ICIs emerged; however, some encouraging results in lung NETs and NECs deserve further investigations. Epigenetic drugs were also investigated in NETs and NECs; some promising results derived from clinical trials investigating histone deacetylase (HDAC) inhibitors, like panobinostat, and a lysine-specific demethylase (LSD)-1 inhibitor. Furthermore, CDK 4/6 inhibitors, such as ribociclib and palbociclib, were investigated in clinical trials of NETs.

In conclusion, the goal of this book was to provide a general update in prognosis, diagnosis, and therapy, covering the current knowledge on the whole family of neuroendocrine neoplasms. We adopted the definition of neuroendocrine neoplasia (NEN) with the aim to include all grades of malignancies. We included also MEN-related NEN, MiNEN, NEC, and Merkel cell carcinoma. We collected from various distinguished experts the most recent update on the management of neuroendocrine neoplasia and discussed what is going to change in this area on the basis of the results of the recent researches. The structure and the content of the text wanted to follow the philosophy that at present is becoming more and more evident of the concept that among the big family of tumors taking origin from neuroendocrine cells, the traditional paradigm that classifies neuroendocrine tumors as a single entity is not sufficient to explain the great differences often observed in the prognosis and tumor responsivity of the various groups of patients with different “neuroendocrine neoplasia.”

Finally, we hope this approach will capture the reader’s interest and generate a critical dis-

cussion. This book wants to help healthcare workers in their choices, in moving in this labyrinth, looking for the best strategy through a multidisciplinary approach. In case of a positive result, we have been successful in providing a valuable insight for all colleagues interested in the management of this particular neoplasms.

21.1 Dedication

The idea of proposing and writing this book was born several months ago.

In this period, the outbreak of COVID pandemic has changed many scenarios in the world view, but it must not change the continuous search for better and better treatments for our patients.

Unfortunately, in Bergamo, COVID took from us our colleague *Italo Nosari*, distinguished endocrinologist, an exquisite person, and a very valid professional who participated and gave his scientific support in all our multidisciplinary groups before his illness.

Our work is dedicated to him and to all those who have suffered in this period.