A well-differentiated neuroendocrine tumour of high grade

Seung Hyun Lee
Tu Vinh Luong

Abstract

Neuroendocrine neoplasms (NEN) are a group of epithelial neoplasms with neural and endocrine differentiation, one of the main sites of origin being the gastrointestinal tract and pancreas (GEP). One of the common groups of GEP-NEN, well-differentiated neuroendocrine tumour (WD-NET), is a biologically distinct group from poorly differentiated neuroendocrine carcinoma (PD-NEC). Accurate histopathological distinction of WD-NET from PD-NEC is critical for prognostication and deciding on optimal therapeutic approaches. However, high grade (grade 3) WD-NET can pose a diagnostic challenge; especially in the context of limited diagnostic material. Here, we present a difficult case of metastatic WD-NET of high grade (G3) in a liver biopsy. The case highlights some key pathological features of WD-NET and the importance of its accurate classification based on consideration of the up-to-date guidance, thorough examination and synthesis of clinical and radiological findings.

Keywords ENETS; European Neuroendocrine Tumor Society; gastrointestinal neuroendocrine tumour; GI-NET; NET; pancreatic neuroendocrine neoplasm; PanNEN; WD-NET

Case report

A 68-year-old gentleman with a complex surgical history was referred with a progressive metastatic neoplasm in the liver. He previously had a well-differentiated pancreatic neuroendocrine neoplasm (PanNEN), of intermediate grade (Well-differentiated Neuroendocrine Tumour, WD-NET, G2, WHO 2017 PanNEN classification, Ki-67: 10%). This was completely excised (R0 resection) with distal pancreatectomy, splenectomy, cholecystectomy and sleeve gastrectomy. 2 years later, a follow-up positron-emission tomography (PET) scan showed multiple liver, abdominal and pelvic lymph nodes, bone and small right middle lobe pulmonary metastases. They were Gallium-68 dotatate intensely avid, consistent with somatostatin receptor expression[1] that can be commonly seen in well-differentiated NEN.[1] Some liver metastases were also [18F] fluorodeoxyglucose (^{18}FDG) avid, which indicates aggressive clinical trajectory.[1] A partial response was obtained with internal radiotherapy in the form of peptide receptor radionuclide therapy (PRRT). However, a year later, the disease progressed with increased size and number of the FDG avid liver metastases. An 18-gauge liver biopsy of the growing liver metastases was undertaken to histologically examine the biology of this distant metastasis.

Histologically, this 20 mm needle core biopsy of the liver was densely infiltrated by confluent solid islands of a metastatic neoplasm (Figure 1a). The majority of cells possessed relatively uniform morphology with small to medium size and round to oval shape. Neuroendocrine differentiation was evident with ample eosinophilic and granular cytoplasm, with centrally located round nuclei and a stippled chromatin pattern i.e. well-differentiated. However, focal regions showed neoplastic cells with significant atypia including large, bizarre nuclei and prominent nucleoli. Nuclear moulding and tumour necrosis were not seen, while scattered apoptotic activity was appreciated (Figure 1b–e). A high number of mitotic figures was present (65 mitoses/2 mm^2).

Ancillary studies corroborated histomorphological findings. The epithelial marker (CAM5.2) and neuroendocrine markers (synaptophysin and chromogranin A) were diffusely positive, supportive of neuroendocrine differentiation (Figure 2). Serotonin was focally positive (not shown). Based on counting more than 500 neoplastic cells at hotspots, proliferation index on Ki-67 was approximately 60% (Figure 3). This far exceeded the proliferation index on his primary PanNEN (Ki-67: 10%). Of note, further immunohistochemical panels for broad differential diagnosis (e.g. p53 for PD-NEC) was not conducted in this case, given the limited amount of material, background of patient's previous medical history (WD-NET), radiological findings so far (Gallium-68 dotatate avidity and ^{18}FDG avidity) and current histological findings (WD-NET features detailed later in discussion). In particular, large cell pancreatic PD-NEC (PanNEC) was considered unlikely in this case. It is far more likely for the primary or secondary WD-NET to transform from low and intermediate grade (G1/G2) to high grade (G3) WD-NET; while it is considered a rare event for G1 and G2 WD-NET to progress to PD-NEC.[1,2] In the absence of the aforementioned features (e.g. no previous history of WD-NET and absence of morphological heterogeneity on the biopsy), a broader panel of immunohistochemistry would be necessary to rule in or rule out differential diagnoses. Depending on the differentials, a wide panel may be necessary as absence of a single marker expression may be limited in its sensitivity and specificity. E.g. Not all PanNECs are reported to bear aberrant expression (56%) for p53 immunohistochemistry.[2] Hence, absence of such aberrant expression would not exclude

Seung Hyun Lee *BSc (Hons) MBChB Clinical PhD Fellow, Systems Oncology Group, Cancer Research UK Manchester Institute, Manchester, UK. Conflicts of interest: none declared.*

Tu Vinh Luong *MD FRCPATH Consultant Histopathologist, Royal Free London NHS Foundation Trust, UK. Conflicts of interest: none declared.*

This Short Case is brought to you in association with the Pathological Society of Great Britain and Ireland. Each month we feature a Short Case written by a member of the Trainees' Subcommittee of the Pathological Society of Great Britain and Ireland. This case, including its scanned slides, is published on the Pathological Society's website www.pathsoc.org/index.php/trainees/case-of-the-month (previous cases can be accessed by members via the education portal login). The Short Case includes a series of Test Yourself Questions at the end to check your understanding of the case. We hope you enjoy reading it.

Pathological Society
Understanding Disease — Guiding Therapy

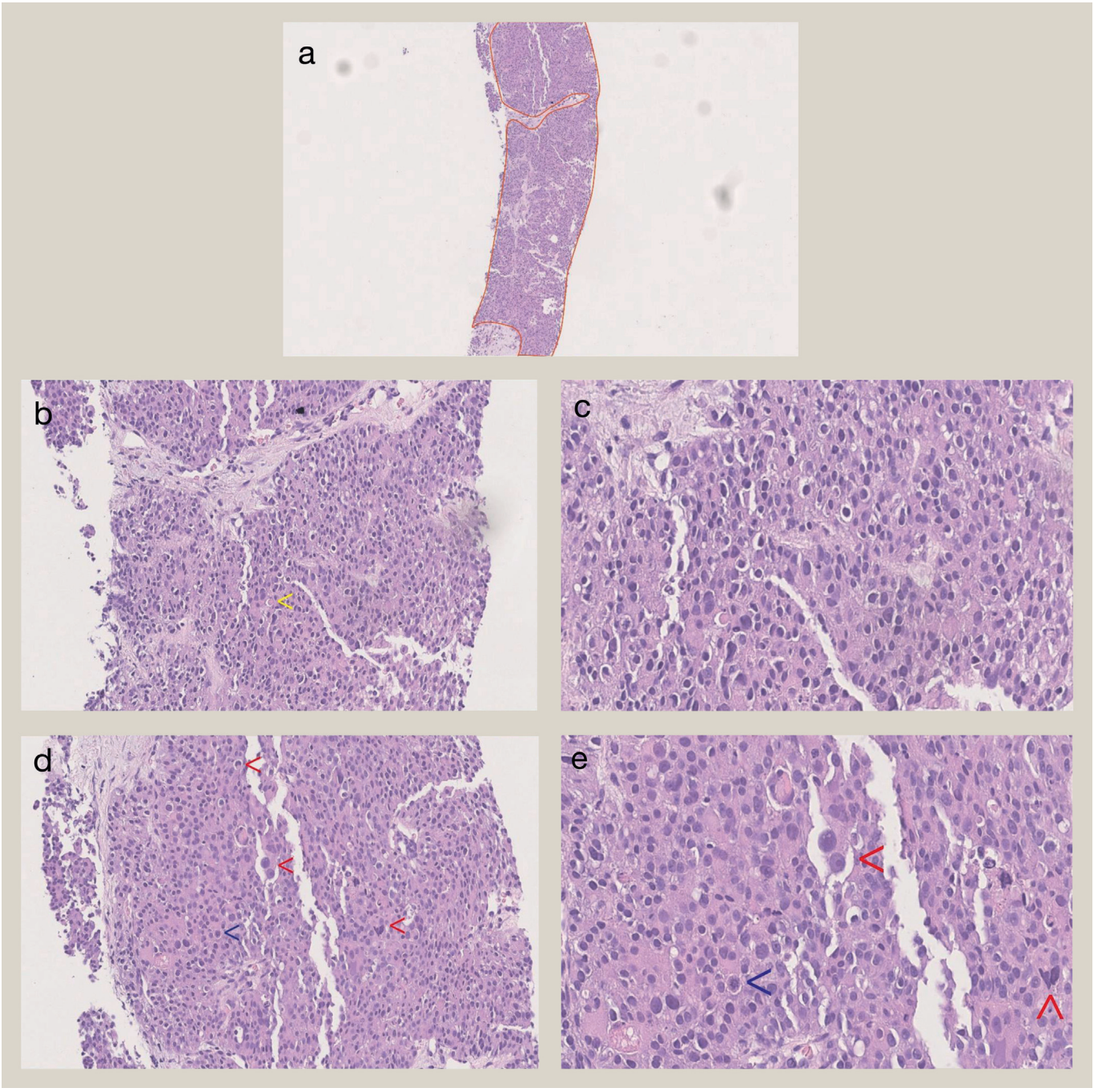

Figure 1 Morphological features of WD-NET with foci of intermediate (G2) and high grade (G3). (**a**) The needle core biopsy of liver parenchyma is densely infiltrated by confluent solid islands of WD-NET (marked with red outline) (Haematoxylin and Eosin, ×10). (**b** and **c**) On low (b, ×50) and high power (c, ×200) examination, neoplastic cells with well-differentiated neuroendocrine morphology of neoplastic cells are demonstrated: uniform size, ample eosinophilic cytoplasm with granularity, centrally placed nuclei with stippled chromatin pattern. Apoptotic activity is marked with yellow <. (**d** and **e**) Small foci of high grade morphology are seen on low (d, ×50) and high (e, ×200) power examination. These cells have significantly larger size and bizarre nuclei (example marked with red <). Mitotic activity is marked with blue <.

PanNEC. More than 1 marker (e.g. p53, RB1, SMAD4) may be helpful if PanNEC is to be excluded on differential diagnosis.

Taken together morphological, immunohistochemical, previous history of lower grade NET and radiological findings, the diagnosis of a metastatic well-differentiated neuroendocrine tumour of high grade (NET G3, WHO 2019 GI-NET classification) was favoured. The patient commenced treatment with Carboplatin-based chemotherapy regimen.

Discussion

Neuroendocrine neoplasm (NEN) is a heterogeneous group of epithelial neoplasms with neural and endocrine differentiation. It can arise from the diffuse neuroendocrine system of any organ, predominantly from bronchopulmonary and gastro-enteropancreatic (GEP) sites.[1]

Understanding of the biology of GEP NENs, and subsequent improvement of clinical care for patients with NENs, have

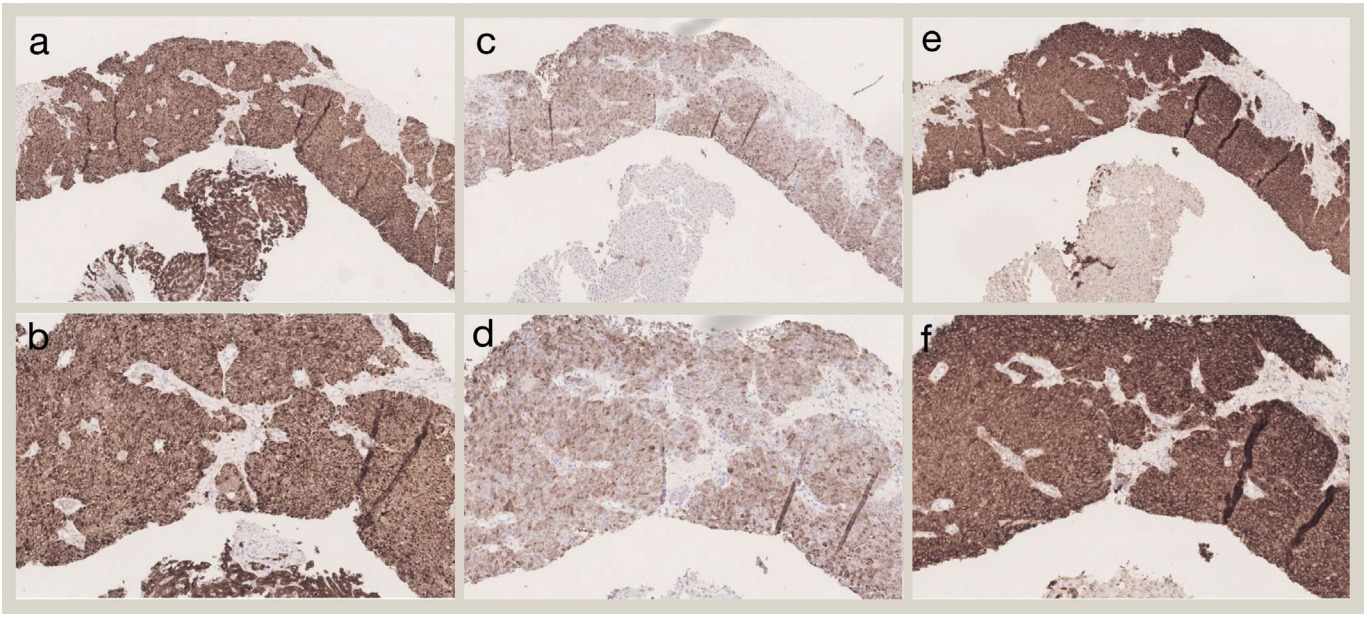

Figure 2 Pertinent immunohistochemical expression of WD-NET. The neoplasm shows diffuse positive staining for epithelial and neuroendocrine markers. (**a** and **b**) CAM 5.2 staining demonstrates diffuse strong positivity (immunohistochemical staining for CAM 5.2, ×50 and ×200). (**c** and **d**) Chromogranin A shows diffuse medium to strong positivity (immunohistochemical staining for chromogranin A, ×50 and ×200). (**e** and **f**) Synaptophysin shows diffuse strong positivity (immunohistochemical staining for synaptophysin, ×50 and ×200).

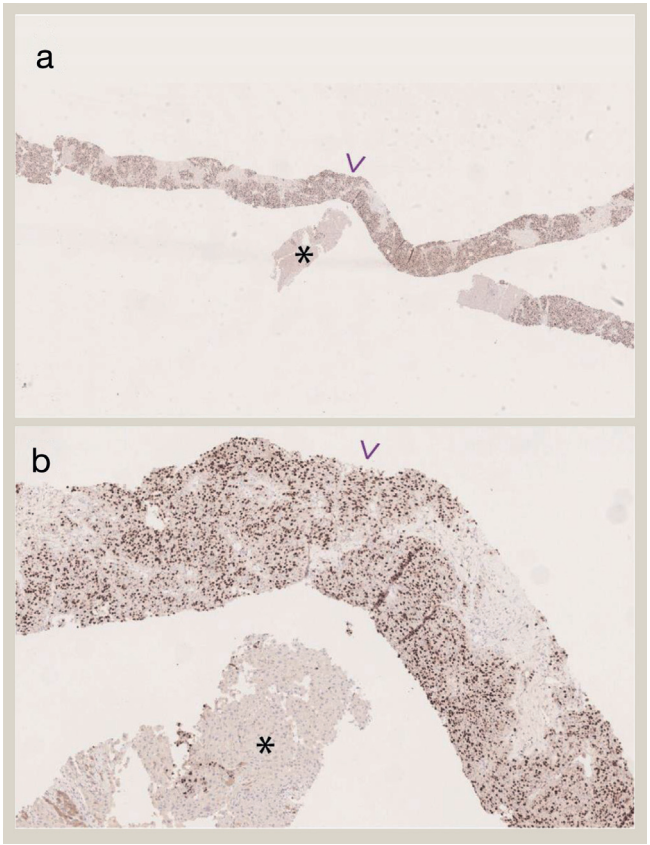

Figure 3 Proliferative activity of WD-NET. The neoplasm shows striking positivity for Ki-67. (**a**) Ultra-low power examination of the neoplasm evidences high proliferative activity (marked with purple <). This contrasts with minimal proliferative activity of the background liver parenchyma (marked with *). (immunohistochemical staining for Ki-67, ×10). (**b**) Counting of more than 500 cells at spots show proliferation activity of approximately 60% (immunohistochemical staining for Ki-67, ×50).

accelerated over the past decade.[1,3] This is reflected in its evolving nomenclature and classification, led and harmonised by the European Neuroendocrine Tumor Society (ENETS) and World Health Organisation (WHO).[4] While it can be confusing to navigate these changes,[4,5] application of the up to date classification system for each organ system (e.g. the WHO 2017 classification for pancreatic neuroendocrine neoplasms) is critical to guide patient management and prognostication.[4]

Specifically, well-differentiated neuroendocrine tumour (WD-NET) is a distinct pathological category from poorly differentiated neuroendocrine carcinoma (PD-NEC). It possesses divergent pathological features, molecular underpinnings and has a more indolent clinical trajectory.[1,4–6] Biological behaviour of WD-NET is further stratified by grading (low/intermediate/high), which is assessed on quantification of proliferation index and mitotic activity.[4,5] Specific guidance on diagnostic criteria, grading and staging can be found in the Royal College of Pathologists dataset for histopathological reporting of neuroendocrine neoplasms of the gastroenteropancreatic tract.[4]

While differentiating WD-NET from PD-NEC is of clinical importance, this can be complicated by the heterogeneous phenotype of WD-NET. Intratumoral and intertumoral heterogeneity is a prominent feature of WD-NETs. For example, WD-NET can demonstrate different tumour grades in different areas of the same tumour, as well as between primary and metastatic sites.[2] This case exemplifies the latter scenario. Specifically, the metastatic tumour generally retained uniform morphology, centrally located round nuclei, stippled chromatin pattern and absence of large areas of tumour necrosis (i.e. well-differentiated). However, concerning features associated with poor differentiation were also present in focal areas: solid confluent architecture, scattered atypical cells, remarkably increased proliferation activity on mitotic count and Ki-67 immunohistochemistry.[4] This case proves that a diverse and heterogeneous phenotype can occur within WD-

NET. Conversely, PD-NECs maintain a more homogenous morphology and generally maintain the high-grade features within the same tumour and between primary tumour and metastases.[2]

A useful tool to easily and confidently delineate WD-NET of high grade from PD-NEC is increasingly needed, given the significance of this pathological distinction for personalised care. Firstly, exercising one's clinical acumen is of the utmost importance. In this case, for example, pathological features of this biopsy alone could potentially overlap with one of large cell PD-NEC. It was imperative to be aware of the previous pathology (WD-NET G2) and to consider radiological features (e.g. expression of somatostatin receptor and FDG-avidity on functional imaging), which helped to arrive at the diagnosis of WD-NET G3. It is important to note that WD-NET bears distinct molecular alterations (e.g. mutations in *DAXX, ATRX* and *MEN1*) from PD-NEC (e.g. mutations in *TP53 and RB1*).[1,2] Further, even in the presence of high grade component, WD-NET has significantly better prognosis (55 months median disease-specific survival) than PD-NEC (11 months).[2] In terms of antiproliferative treatment, a range of new options is available for WD-NET including somatostatin analogues (e.g. octreotide), peptide receptor radionuclide therapy (PRRT) and targeted treatment (e.g. mTOR inhibitor).[1,5] The first-line antiproliferative treatment for PD-NEC usually includes platinum-based chemotherapy.[1,2] More details on treatment options for WD-NET can be found on the review by Hofland, Kaltsas and de Herder (2020).[1] Overall, precise classification of GEP-NEN and accurate diagnosis of WD-NET of high grade is critical for patient management.

In conclusion, accurate pathological classification of WD-NET from PD-NEC leads to differential patient management but diagnosis requires a circumspect and wholistic assessment, particularly in the presence of a high-grade component. Diagnosticians should be familiar with the histopathological and clinical features of WD-NET of high grade and perform a thorough examination of the often limited diagnostic material available. ◆

REFERENCES

1 Hofland J, Kaltsas G, de Herder WW. Advances in the diagnosis and management of well-differentiated neuroendocrine neoplasms. *Endocr Rev* 2020; **41:** 371–403.

2 Tang LH, Untch BR, Reidy DL, et al. Well-differentiated neuroendocrine tumors with a morphologically apparent high-grade component: a pathway distinct from poorly differentiated neuroendocrine carcinomas. *Clin Cancer Res* 2016; **22:** 1011–7.

3 Alexandraki KI, Spyroglou A, Kykalos S, et al. Changing biological behaviour of NETs during the evolution of the disease: progress on progression. *Endocr Relat Cancer* 2021; **28:** R121–40.

4 Luong TV, Watkins J, Chakrabarty B, et al. Dataset for histopathological reporting of neuroendocrine neoplasms of the gastroenteropancreatic tract 4ed. London: The Royal College of Pathologists, 2019.

5 Rindi G, Klimstra DS, Abedi-Ardekani B, et al. A common classification framework for neuroendocrine neoplasms: an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert consensus proposal. *Mod Pathol* 2018; **31:** 1770–86.

6 Scarpa A. The landscape of molecular alterations in pancreatic and small intestinal neuroendocrine tumours. *Ann Endocrinol* 2019; **80:** 153–8.

Acknowledgement

Seung Hyun Lee holds a Clinical Research Training Fellowship from the International Alliance for Cancer Early Detection, an alliance between Cancer Research UK [C19941/A27859], Canary Center at Stanford University, the University of Cambridge, OHSU Knight Cancer Institute, University College London and the University of Manchester.

Practice points

- NEN from the gastrointestinal tract and pancreas is a heterogeneous group of epithelial neoplasm with neuroendocrine differentiation. Two predominant classifications include well-differentiated neuroendocrine tumour (WD-NET) and poorly differentiated neuroendocrine carcinoma (PD-NEC)
- WD-NET has a distinct molecular alterations and biological behaviour to PD-NEC and implicates different patient management strategies and prognostication. WD-NET is subdivided into well, intermediate and high-grade tumour, graded by mitotic activity and proliferation index on Ki-67 IHC
- WD-NET can present with features of mixed grades within the primary or secondary tumour. Thorough examination based on up to date classification system and clinical, radiological and pathological correlation aid in accurate diagnosis of WD-NET of high grade

Self-assessment MCQs

1. Which one of below is/are GEP-NEN?

a. Poorly differentiated neuroendocrine carcinomas (NECs)
b. Well differentiated neuroendocrine tumours (NETs)
c. Colorectal adenocarcinoma with scattered neuroendocrine cells
d. None of above
e. a and b

Correct answer: e

2. Which is true from statements below?

a. GEP-NET is graded into either low grade or high grade according to ENETS grading system.
b. GEP-NEC is graded into either intermediate or high grade.
c. GEP-NEC is derived from neural crest origin.
d. Grading by GEP-NET is done by assessing mitotic activity and examining at least 500 cells on Ki-67.
e. GEP-NET does not express somatostatin.

Correct answer: d

3. Which mutations can be associated with NET G3?

a. p53
b. MEN1
c. ATRX
d. b and c
e. None of above

Correct answer: d