COLLEGE of AMERICAN PATHOLOGISTS

ARCHIVES
of Pathology & Laboratory Medicine

NEW ARTICLE

This article was posted on the *Archives* Web site as a New Article. New Articles have been peer reviewed, copyedited, and reviewed by the authors. Additional changes or corrections may appear in these articles when they appear in a future print issue of the *Archives*. New Articles are citable by using the Digital Object Identifier (DOI), a unique number given to every article. The DOI will typically appear at the end of the abstract.

The DOI for this manuscript is doi: 10.5858/arpa.2024-0410-RA

The print version of this manuscript will replace the New Article version at the above DOI once it is available.

Differentiation Versus Grade for Pancreatic Neuroendocrine Neoplasms

Guido Rindi, MD, PhD; Frediano Inzani, MD, PhD

• ***Context.***—**Pancreatic neuroendocrine neoplasia (panNEN) is a tumor disease with distinctive morphology and often poses diagnostic challenges.**

Objective.—**To present and discuss the current diagnostic criteria of PanNEN.**

Data Sources.—**PanNENs are classified by the World Health Organization (WHO) criteria into well-differentiated neuroendocrine tumor (panNET) and poorly differentiated neuroendocrine carcinoma (panNEC) of large or small cell type. panNETs are graded as G1-G3 on the basis of their proliferation capacity by mitotic count and/or Ki-67 proliferation index. Differentiation and grading are overlapping tools essentially directed to the definition of tumor cell resemblance to the normal cell counterpart (differentiation) and its proliferation capacity (grading). Both tools aim at defining the panNEN malignant potential, and ultimately the overall and event-free survival. The 2 panNEN families mirror different molecular backgrounds. The panNET genotype consistently displays mutation/copy number variation of *DAXX* (death domain associated protein), *ATRX* (ATRX chromatin remodeler), and *MEN1* (menin 1) genes, while in panNEC the usual cancer drivers *TP53* (tumor protein 53), *RB1* (RB transcriptional corepressor 1), and *KRAS* (KRAS proto-oncogene, GTPase) genes are mutated/abnormal. The more subtle differences measured by grade in panNET G1-G3 reflect a progressive gene disorder, with G3 often involving *TP53*. This rare genetic setting is usually associated with a difficult differential diagnosis between panNET G3 and panNEC. Immunohistochemistry for the informative genes *DAXX*, *ATRX*, *TP53*, *RB1, P16* (cyclin-dependent kinase inhibitor 2A), and SST_2 (somatostatin receptor 2) are the key to separating panNETG3 and panNEC; however, molecular investigation is often required and decisive. Nonetheless, ambiguous cases may remain unresolved.**

Conclusions.—**The WHO diagnostic criteria for panNEN are simple and effective tools for a clinically meaningful patient stratification. Areas of uncertainty remain and deserve further investigation.**

(*Arch Pathol Lab Med*. doi: 10.5858/arpa.2024-0410-RA)

The current World Health Organization (WHO) classification of neuroendocrine neoplasm of the pancreas (panNEN) entails the assessment of cell differentiation by morphology and of cell proliferation by grading.[1–4] In this article the definition and significance of both differentiation and grading in panNEN will be introduced while integrating such concepts with current molecular evidence. In addition, the pitfalls and problems inherent in these diagnostic tools will be discussed. Four points will be discussed: differentiation definition, grading definition, and molecular pathology features and problems.

DIFFERENTIATION

Two definitions of cell differentiation easily accessible on the Web are as follows: (1) "the process in which a stem cell changes from one type to a differentiated one"[5]; (2) "the evolutive mechanism(s) from visibly undifferentiated precursor cell to stable and persistent states of cell differentiation."[6] Although differently defined, both these definitions deliver the concept that differentiation is a plastic phenomenon of the cell life, describing its journey toward complete maturation and "adult" status. As such what we see on histology preparations is the photograph of a cell frozen by fixation at a specific evolutive step. In each cell population, it is expected that different evolutive steps are captured. This phenomenon takes place during development and is repeated continuously during adult life to maintain organ cell homeostasis, that is, the normal number of adult cells and ultimately organ trophism and function.

Adult cell status is variably defined for cells according to their different function, and in the pancreas, namely the ductal cell, the exocrine secretory cell, and the islet neuroendocrine (NE) cell. The mechanism through which a given cell acquires a specific cell type/adult status is defined as cell specification.

What is known of cell specification in the NE pancreas derives from embryogenesis studies. The cell-type specification

Accepted for publication January 14, 2025.

From The Section of Anatomic Pathology, Department of Life Sciences and Public Health and the ENETS Center of Excellence Fondazione Policlinico Universitario A. Gemelli IRCCS at Università Cattolica del Sacro Cuore, Rome, Italy (Rindi); Anatomic Pathology Unit, Department of Woman and Child Health Sciences and Public Health, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy (Rindi); the Anatomic Pathology Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy (Inzani); and Anatomic Pathology, Fondazione IRCCS San Matteo Hospital, Pavia, Italy (Inzani).

This work was in part supported by internal Catholic University Grant Line D1 R4124501397.

The authors have no relevant financial interest in the products or companies described in this article.

Presented in part at the Companion Meeting of the Pancreatobiliary Pathology Society at the 113th United States and Canadian Academy of Pathology Annual Meeting; March 24, 2024; Baltimore, Maryland.

Corresponding author: Guido Rindi, MD, PhD, Fondazione Policlinico Universitario A. Gemelli IRCCS, Anatomic Pathology Unit, Largo A. Gemelli, 8 00168, Roma, Italy (email: guido.rindi@unicatt.it).

takes place at certain embryonic days defined as primary (e8.5–12.5), secondary (e12.5–16.5), and tertiary (e16.5–postnatal); the latter indicates its continuous function after embryogenesis in adult life.[7–9] A cohort of transcription factor genes dictates the cell-type specification from the multipotent progenitor cell along different secretory lineage routes via complex interactions.[7–9] Key transcription factor genes include *NEUROG3* (neurogenin 3) for all 4 pancreas NE cell types, *ARX* (aristaless related homeobox) for alpha cell, and *PDX1* (pancreatic and duodenal homeobox 1) for beta cell, with *NOTCH* (notch receptor) and *HES1* (hes family bHLH transcription factor 1) playing a broader role at earlier development steps.[7–9] The interaction between transcription factor genes is extremely complex, only partially understood and dissected, and entails both the activation or blockage of several other transcription factor genes including *INSM1* (INSM transcriptional repressor 1) and *ISL1* (ISL LIM homeobox 1).[7–9]

The above-described complex phenomena normally take place in the pancreas for cell type homeostasis but also when regeneration/adaptation occurs following injury, in the physiological condition of pregnancy, or under the pathologic pressure induced by obesity and diabetes.[10] It is still not clear where in the NE pancreas compartment this powerful cell reservoir lies and whether the so-called NE stem cell exists, since no robust evidence supports the proposed theory of either ductal or islet cell origin of NE cells. For sure the phenomena of islet cell self-replication and islet cell conversion have been demonstrated and are currently accepted, indicating that a cell with an acquired adult phenotype may indeed rescue its replication capacity.[10] Similarly, transforming events may unlock uncontrolled proliferation capacity in mature NE cells. Indeed, experimental evidence in genetically engineered mice models indicate that directing powerful transforming reported genes in pancreatic NE cells results in transformation within islets and multiple instances of tumor development.[11,12]

The minimal requirements to meet the definition of an NE cell of epithelial lineage entail the demonstration of cytokeratin(s) (CK[s]) and the presence of the 2 regulated pathways of secretion associated with large dense core vesicles (LDCVs, hormone-storing) and small synaptic-like vesicles (SSVs, small mediator−storing). LDCVs and SSVs contain several antigens and respectively chromogranin-A (CgA) and synaptophysin (Syn), two antigens of use in daily pathology practice as general markers of NE differentiation.[13] In addition, the insulinoma-associated 1 (INSM1) transcriptional repressor gene recently proved to be expressed in normal and tumor NE cells as a novel powerful NE marker.[14] Overall, the minimal immunophenotype required for the definition of an NE cell of epithelial lineage is the positive demonstration of CK(s), chromogranin A and synaptophysin expression in the cytoplasm, and of INSM1 in the nucleus.[2–4]

This holds true also for transformed, neuroendocrine tumor (NET) cells of epithelial lineage. However, the phenotype of epithelial NET cells may vary significantly depending on their cytologic features, that is, whether they are like the normal islet counterpart or display severe and significant atypia. Of note, the absence of CK(s) expression in NE tumor cells points to a diagnosis of paraganglioma, a NEN of nerve lineage.[2,4]

PanNENs composed of NET cells like their normal counterpart by cytology and by cell-to-cell organization (islet-like, organoid structure) are considered to be well differentiated and defined NETs (Figure 1, A through E). PanNENs composed of cells with severe cell atypia and solid or loose structure are defined as neuroendocrine carcinoma (NEC) of small or large cell (SC, LC) types (Figure 2, A through E).

In summary, differentiation in PanNEN defines a neoplasia of epithelial lineage displaying the standard epithelial NET immunoprofile ($CK^+/CgA^+/Syn^+/INSM1^+$), with NET or NEC of SC/LC-type morphology.

GRADING

Two definitions of grading accessible on the Web are as follows: (1) "a measure of the cell appearance in tumors and other neoplasms"[15]; (2) "the degree of tissue differentiation or to an ensemble of tissue features that have been found to be a good predictor of the aggressiveness of the tumor."[16] Both definitions indicate that grading is a tool to assess the aggressiveness of a given neoplasm. The current WHO grading of digestive NEN including panNEN is based on proliferation and entails the measurement of mitosis/2 mm^2 and Ki-67% (Table).[1,2] While NETs are graded, NECs are by definition high grade (G3).[4] Necrosis, considered for grading of NETs at other sites, is not part of the current grading system of panNETs, although its presence is recognized as a sign of histologic aggressiveness.[17] This system proved effective in defining patients' risk in panNEN, predicting both overall and event-free survival.[18]

In summary, grading is a tool to measure the proliferation capacity in panNEN. While the differentiation status at morphology may effectively separate NET and NEC (diagnostic/prognostic step 1) for NET only—proliferation measured by Ki-67/MIB-1 and/or mitotic count may define the NET-associated risk, ultimately predicting prognosis (diagnostic/prognostic step 2). The application of digital techniques for Ki-67 determination in panNET has been explored with promising results, especially in view of potential artificial intelligence (AI) applications in the near future.[19] Nonetheless, such a technique has not yet entered the methodology currently recommended by the WHO.

Molecular Pathology and Clinical Significance

Both differentiation and grading flag the molecular events taking place in the transformed NE cell. The recognized genetic landscape of panNET presents characteristic and recurrent gene features firstly defined and well delineated by Scarpa et al[20] in 2017. In general, the panNET displays low copy number variations and low mutational burden, with variable penetrance, including mutation of *MEN1* (menin 1), *DAXX* (death domain associated protein), and *ATRX* (ATRX chromatin remodeler) genes. Other genes that may be mutated in panNET include *MUTYH* (mutY DNA glycosylase), *PTEN* (phosphatase and tensin homolog), *TSC1-2* (TSC complex subunit 1/2). Of note, the abnormal gene function may result from other mechanisms including copy number variation rather than mutation. The application of genotyping in panNET consistently proved the above-defined genetic landscape, in addition to being tentatively used to associate genotype end clinical phenotype. The assessment of panNET genotype by tumor DNA sequencing proved useful in predicting the clinical behavior in different study cohorts. As an example, in a series of 87 panNETs less than 3 cm in size, copy number alterations

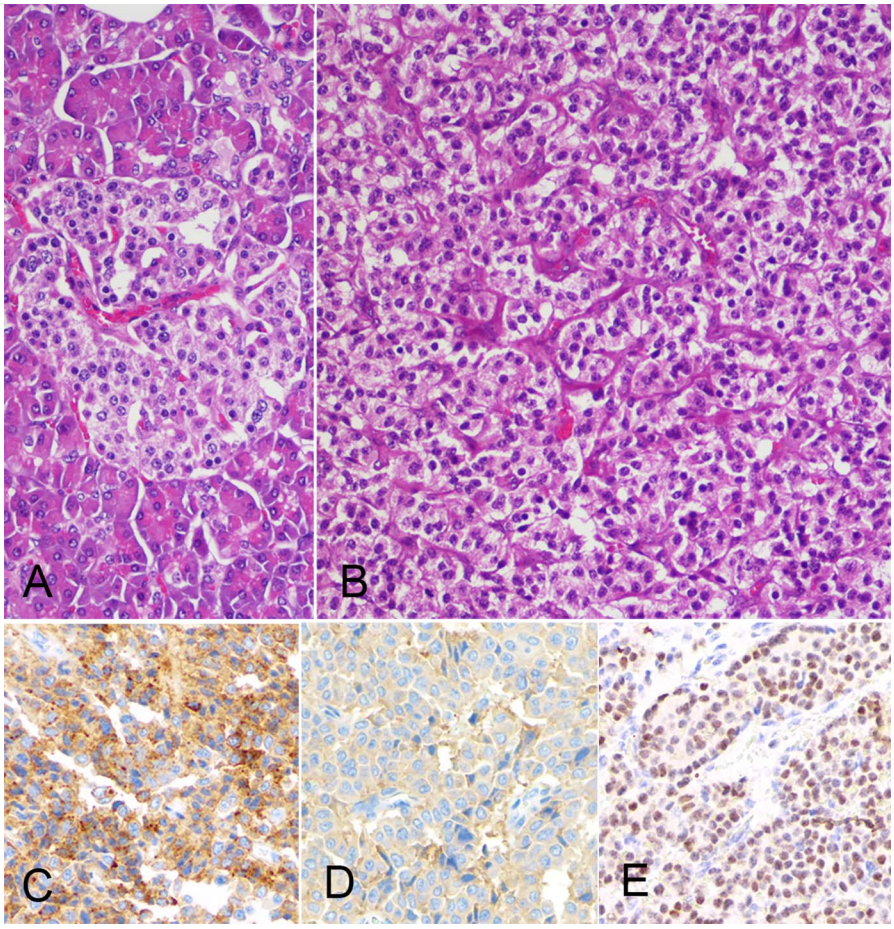

Figure 1. *Well-differentiated neuroendocrine neoplasia (NEN), neuroendocrine tumor (NET) of the pancreas (panNET). The features observed in the normal islet (A) are also observed in panNETs (B) with organoid, islet-like structure and usually mild atypia if any. PanNET cells consistently express the NEN family markers chromogranin A (C) and synaptophysin (D) in the cytoplasm and insulinoma-associated 1 (INSM1) in the nucleus (E) (hematoxylin-eosin, original magnification × 400 [A and B]; original magnification ×400 [C through E]).*

separated the panNET in 3 groups with different metastatic potential (group 1: metastasis 75%, with G2, *DAXX/ATRX*$^{-}$; group 2: metastasis 42%, low gene abnormalities; group 3: metastasis 35%, MEN1 loss).[21] In addition, the expected low copy number variation and "usual" mutant status involving *ATRX/DAXX/MEN1* (ADM) observed in a series of 80 patients with panNET evolved and acquired a hypermutation condition after alkylating agent treatment in 67% of cases, with

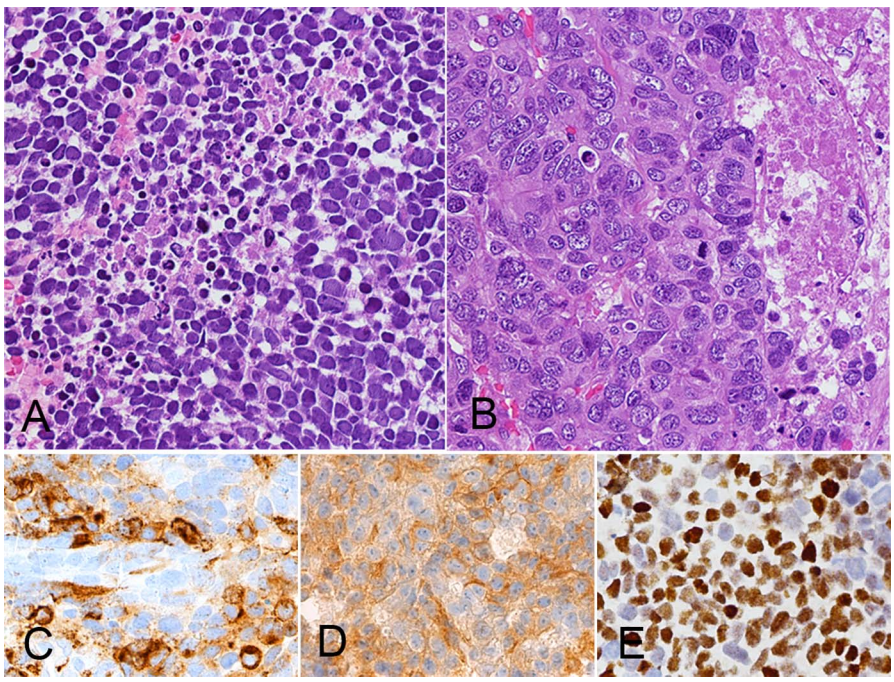

Figure 2. *Poorly differentiated neuroendocrine neoplasia (NEN), neuroendocrine carcinoma (NEC) of the pancreas (panNEC). PanNECs are poorly differentiated carcinomas, usually displaying solid structure with necrosis, either in single-cell or in large areas (geographical chart necrosis), are composed of severely atypical cells, either small with scarce cytoplasm and large nuclei ([A], small cell type, SC-NEC), or large with abundant eosinophilic cytoplasm ([B], large cell type, LC-NEC). PanNEC cells consistently express the NEN family markers chromogranin A (C) and synaptophysin (D) in the cytoplasm and Insulinoma-associated 1 (INSM1) in the nucleus (E) (hematoxylin-eosin, original magnification ×400 [A and B]; original magnification, ×400 [C through E]).*

Differentiation and Grading Features of Pure Neuroendocrine Neoplasia				
		Grading (Proliferation)		
Definition	Differentiation (Morphology)	Grade	Mitotic Count[a]	Ki-67 Index[b]
NET	Well differentiated	G1	<2	<3
	Well differentiated	G2	2–20	3–20
	Well differentiated	G3	>20	>20
NEC	Poorly differentiated	G3[c]	>20	>20

Abbreviations: G, grade; NEC, neuroendocrine carcinoma; NET: neuroendocrine tumor.

[a] In areas of 2 mm^2.

[b] Percentage of positive cells stained with Ki-67 clone MIB-1 in areas of highest nuclear labelling (hot spot).

[c] Grade not assessed in NEC.[1–4]

novel mutation in key cancer driver genes including *TP53* (tumor protein 53).[22] On the same scale, expanding the genetic analysis to RNA sequencing and DNA methylation in a cohort of 64 panNETs showed that the frequent ADM mutation status was associated with worse survival, distinct gene expression and methylation profile, and alpha-cell profile.[23] Gene expression profiling further provided prognostic stratification in a series of duodenopancreatic NENs, confirming a worse survival rate in association with a pancreatic alpha cell signature.[24] Finally, in a series of 29 panNETs, gene expression profiles and LINE-1 methylation status correlated with Ki-67 WHO grade, with gene expression profiles clustering by grade and not by case, with a profound transcriptome change characterizing the switch from G1/G2 to G3.[25] Overall, panNETs display a consistent gene "anatomy," with gene expression profiles aligning with grade and adverse prognosis as a promising area of further investigation.

Similar to NEC at other sites and well known for decades,[26] panNEC shows alterations in key cancer driver genes including *TP53*. Extensive, recent data obtained by whole genome sequencing/whole exome sequencing as well as gene expression profiling in a large cohort of digestive NECs, including 29 from pancreas, showed that panNECs (*TP53/RB[RB transcriptional coexpression 1]/KRAS* [KRAS protooncogene, GTPase] abnormal/mutant) are similar but different from NECs from other gastrointestinal sites (*TP53/RB* abnormal/mutant); as largely expected, panNECs are different from panNETs (ADM mutant), and panNECs may cluster into "ductal" and "acinar" types by expression profiling.[27]

Machine learning/AI application in a large cohort of panNENs compared to gastrointestinal-NENs (356 versus 156) for which clinicopathologic information and both gene "anatomy" and gene expression profiling were available proved that transcriptomic deconvolution algorithms could effectively predict NET, NEC, and define a grade distinguishing G3 NET and NEC, according to grading-independent molecular mechanisms.[28] Overall, panNECs display a consistent gene "anatomy," with distinctive gene expression profiles as a promising area of further investigation.

In summary, the molecular pathology of panNEN depicts a specific and consistent gene "anatomy" of panNET and panNEC, accurately separating the 2 entities with increasing and more profound genetic defects along their histologic and clinically more aggressive spectrum. Gene expression profiles for panNET and panNEC do reflect the increased genetic disruption associated with high grades with promising interpretative yield by machine learning/AI application.

PROBLEMS/ISSUES

Currently, while the application of the WHO procedure for panNEN classification using the tools of differentiation and grading may well separate low-grade (G1, G2) panNET from NEC, problems exist in separating some high-grade NETs (NET G3) from NECs. Ideally, the tools provided and previously discussed should enable us to make this distinction; however, borderline/ambiguous cases of NET G3 exist

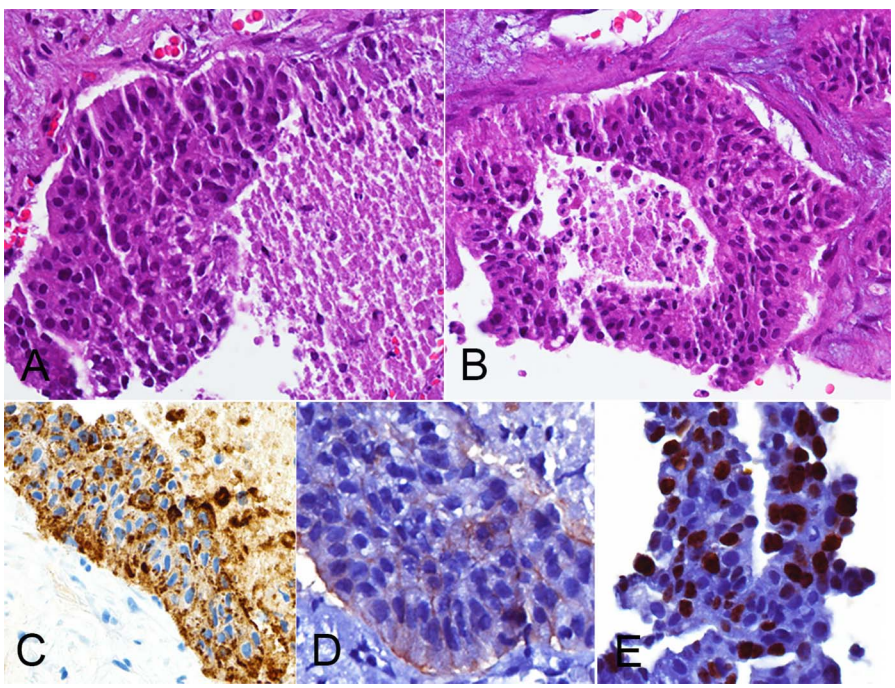

Figure 3. *A case of difficult pancreatic neuroendocrine tumor (panNET) G3 diagnosis. The diagnosis of this case proved difficult because the only available sample showed poorly preserved histology with diffuse artifacts. Although necrosis was evident, tumor cells showed relatively regular nuclei and ample cytoplasm (A and B), wide expression of chromogranin A (C), somatostatin receptor type 2 (SST_2) (D), with relatively high Ki-67 proliferation index (E). Based also on* ATRX *(ATRX chromatin remodeler) retention and partial* DAXX *(death domain associated protein) loss, the diagnosis of panNET G3 was favored and subsequently confirmed by the presence of* TSC1 *(TSC complex subunit 1): c.891T>A p.(Y297*) mutation and low tumor mutational burden (2.4 muts/mb) in the absence of* TP53 *(tumor protein p53),* RB1 *(RB transcriptional corepressor 1), and other classical cancer driver genes with next-generation sequencing (NGS) analysis of 500 genes (hematoxylin-eosin, original magnification ×400 [A and B]; original magnification ×400 [C through E]).*

that are classified with low level of efficacy even under expert eyes.[29] A simple diagnostic multimodal algorithm entailing immunohistochemistry for DAXX, ATRX, TP53, and RB, together with regular histology, clinicopathologic information, and molecular genetics, was proposed and proved effective for most cases.[29] The use of somatostatin receptor (SST_2) expression, in addition to the previously mentioned markers, was also deemed useful together with *P16* overexpression.[30] The issue becomes even more pressing in cases where small samples are the only available material for diagnosis and decision-making. These cases are made particularly complicated by the poor quality and amount of tissue available for "conventional" diagnosis (Figure 3, A through E). In such cases a definitive answer may be obtained by next-generation sequencing with an extensive multigene panel so that the classical NET or NEC genetic profile may emerge. Despite this effective multimodal effort, cases have been described that remain ambiguous even at the molecular level, indicating an unsolved gray area.[31]

In summary, the current problems/issues for panNEN diagnosis refer to the distinction between G3 NET versus NEC. Ancillary tests (SST_2/DAXX/ATRX/P53/Rb/P16) are needed and usually informative; however, deeper molecular investigation may be required. In this context, a small biopsy specimen may be very problematic, though molecular data may help finalize the diagnosis.

CONCLUSIONS

The current WHO classification provides effective tools to predict the clinical behavior of panNEN and to direct clinicians to the best possible patient management. Differentiation solidly defines NET and NEC by classic morphology and consistent immunoprofile (CK^+/CgA^+/Syn^+/$INSM1^+$/$SST_2^{+/-}$). Grading by Ki-67(MIB-1) and mitotic count complement this differentiation, defining prognostication for NET and consequent therapy approaches. Molecular pathology entailing genome/exome "anatomic" gene profiling consistently confirms the distinction between NET (*ATRX/DAXX/MEN1* mutant genotype) and NEC (*TP53/RB/KRAS* mutant genotype). Transcriptomic analysis and the application of machine learning/AI are promising tools, next in line on our bench. Still practical diagnostic problems may remain, usually regarding high-grade G3 cases and the differential diagnosis of panNET G3 versus panNEC. Most cases may be resolved by immunohistochemistry for key informative gene expression (*DAXX, ATRX, TP53, RB1,* and SST_2), although sometimes requiring deeper molecular investigation. Nonetheless, ambiguous cases may sometimes remain unresolved, in particular for small biopsy samples with poorly preserved tissue present in limited quantities. Indeed, difficult cases do exist and likely indicate a gray area of poor understanding.

We are indebted to Sammy Faris, BS, for thorough revision of the English grammar and usage.

References

1. Klimstra DS, Kloppel G, La Rosa S, Rindi G. Classification of neuroendocrine neoplasms of the digestive system. In: WHO Classification of Tumours Editorial Board, eds. *Digestive System Tumours*. 5th ed. Lyon: IARC Press; 2019: 16-19. *WHO Classification of Tumours*. vol 1.

2. Rindi G, Mete O, Uccella S et al. Overview of the 2022 WHO Classification of Neuroendocrine Neoplasms. *Endocr Pathol*. 2022;33(1):115–154.

3. Klimstra D, Asa S, Gill A, Klöppel G, Rindi G. Pancreatic neuroendocrine neoplasms: introduction. In: WHO Classification of Tumours Editorial Board, eds. *Endocrine and Neuroendocrine Tumours.* 5th ed. Lyon, France: International Agency for Research on Cancer; 2022. *WHO Classification of Tumours*. vol 10.

4. Rindi G, Asa S, Klimstra D, Papotti M. Neuroendocrine neoplasms in non-endocrine organs: introduction. In: WHO Classification of Tumours Editorial Board, eds. *Endocrine and Neuroendocrine Tumours*. 5th ed. Lyon, France: International Agency for Research on Cancer; 2022.*WHO Classification of Tumours*. vol 10.

5. Wikipedia contributors. Cellular differentiation. Wikipedia, The Free Encyclopedia. 2024. https://en.wikipedia.org/wiki/Cellular_differentiation. Accessed October 2, 2024.

6. Bernfield MR, Staehelin LA, Lodish HF, et al. Cell: Encyclopedia Britannica. 2024. https://www.britannica.com/science/cell-biology/Cell-differentiation). Accessed October 2, 2024.

7. Larsen HL, Grapin-Botton A. The molecular and morphogenetic basis of pancreas organogenesis. *Semin Cell Dev Biol*. 2017;66:51–68.

8. Arda HE, Benitez CM, Kim SK. Gene regulatory networks governing pancreas development. *Dev Cell*. 2013;25(1):5–13.

9. Dassaye R, Naidoo S, Cerf ME. Transcription factor regulation of pancreatic organogenesis, differentiation and maturation. *Islets*. 2016;8(1):13–34.

10. Zhou Q, Melton DA. Pancreas regeneration. *Nature*. 2018;557(7705): 351–358.

11. Rindi G, Bishop AE, Murphy D, Solcia E, Hogan B, Polak JM. A morphological analysis endocrine tumour genesis in pancreas and anterior pituitary of AVP/SV40 transgenic mice. *Virchows Arch A Pathol Anat Histopathol*. 1988; 412(3):255–266.

12. Rindi G, Efrat S, Ghatei MA, Bloom SR, Solcia E, Polak JM. Glucagonomas of transgenic mice express a wide range of general neuroendocrine markers and bioactive peptides. *Virchows Arch A Pathol Anat Histopathol*. 1991;419(2):115–129.

13. Rindi G, Wiedenmann B. Neuroendocrine neoplasia of the gastrointestinal tract revisited: towards precision medicine. *Nat Rev Endocrinol*. 2020;16(10): 590–607.

14. Rosenbaum JN, Guo Z, Baus RM, Werner H, Rehrauer WM, Lloyd RV. INSM1: a novel immunohistochemical and molecular marker for neuroendocrine and neuroepithelial neoplasms. *Am J Clin Pathol*. 2015;144(4):579–591.

15. Wikipedia contributors. Grading (tumors). Wikipedia, The Free Encyclopedia. 2024. https://en.wikipedia.org/wiki/Grading_(tumors). Accessed October 2, 2024.

16. Costa J. Cancer: Encyclopedia Britannica. 2024. https://www.britannica.com/science/cancer-disease/Evaluation-of-tumours. Accessed October 2, 2024.

17. Fuchs TL, Chou A, Ahadi M, et al. Not all tumour necrosis is granular necrosis: authors' response. *Pathology*. 2023;55(6):904–906.

18. Rindi G, Klersy C, Albarello L, et al. Competitive testing of the WHO 2010 versus the WHO 2017 Grading of Pancreatic Neuroendocrine Neoplasms: data from a large international cohort study. *Neuroendocrinology*. 2018;107(4):375–386.

19. Luchini C, Pantanowitz L, Adsay V, et al. Ki-67 assessment of pancreatic neuroendocrine neoplasms: systematic review and meta-analysis of manual vs. digital pathology scoring. *Mod Pathol*. 2022;35(6):712–720.

20. Scarpa A, Chang DK, Nones K, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. *Nature*. 2017;543(7643):65–71.

21. Pea A, Yu J, Marchionni L, et al. Genetic analysis of small well-differentiated pancreatic neuroendocrine tumors identifies subgroups with differing risks of liver metastases. *Ann Surg*. 2020;271(3):566–573.

22. Raj N, Shah R, Stadler Z, et al. Real-time genomic characterization of metastatic pancreatic neuroendocrine tumors has prognostic implications and identifies potential germline actionability. *JCO Precis Oncol*. 2018;2018:PO. 17.00267.

23. Chan CS, Laddha SV, Lewis PW, et al. ATRX, DAXX or MEN1 mutant pancreatic neuroendocrine tumors are a distinct alpha-cell signature subgroup. *Nat Commun*. 2018;9(1):4158.

24. Diedisheim M, Dermine S, Jouinot A, et al. Prognostic transcriptome classes of duodenopancreatic neuroendocrine tumors. *Endocr Relat Cancer*. 2021; 28(8):563–571.

25. Simbolo M, Bilotta M, Mafficini A, et al. Gene expression profiling of pancreas neuroendocrine tumors with different Ki67-based grades. *Cancers (Basel)*. 2021;13(9):2054.

26. Ubiali A, Benetti A, Papotti M, Villanacci V, Rindi G. Genetic alterations in poorly differentiated endocrine colon carcinomas developing in tubulo-villous adenomas: a report of two cases. *Virchows Arch*. 2001;439(6):776–781.

27. Yachida S, Totoki Y, Noe M, et al. Comprehensive genomic profiling of neuroendocrine carcinomas of the gastrointestinal system. *Cancer Discov*. 2022;12(3):692–711.

28. Otto R, Detjen KM, Riemer P, et al. Transcriptomic deconvolution of neuroendocrine neoplasms predicts clinically relevant characteristics. *Cancers (Basel)*. 2023;15(3):936.

29. Tang LH, Basturk O, Sue JJ, Klimstra DS. A practical approach to the classification of WHO grade 3 (G3) well-differentiated neuroendocrine tumor (WD-NET) and poorly differentiated neuroendocrine carcinoma (PD-NEC) of the pancreas. *Am J Surg Pathol*. 2016;40(9):1192–1202.

30. Konukiewitz B, Schlitter AM, Jesinghaus M, et al. Somatostatin receptor expression related to TP53 and RB1 alterations in pancreatic and extrapancreatic neuroendocrine neoplasms with a Ki67-index above 20. *Mod Pathol*. 2017; 30(4):587–598.

31. Umetsu SE, Kakar S, Basturk O, et al. Integrated genomic and clinicopathologic approach distinguishes pancreatic grade 3 neuroendocrine tumor from neuroendocrine carcinoma and identifies a subset with molecular overlap. *Mod Pathol*. 2023;36(3):100065.