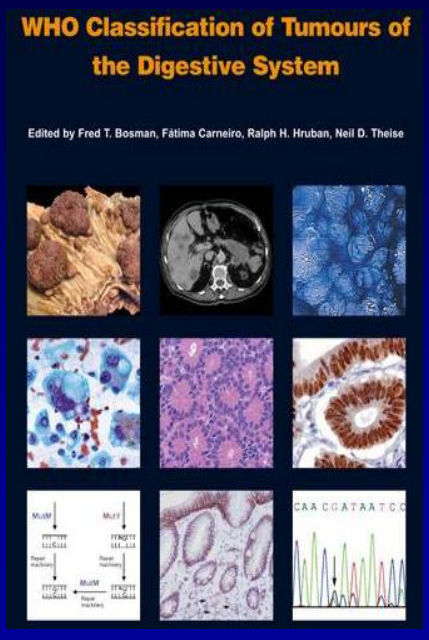
Gastro-entero-pancreatic neuro-endocrine tumors: frequently-asked questions.

Gianni Bussolati.
University of Turin, Italy and
Babes Institute, Bucarest, Romania.



The pathological diagnosis of neuroendocrine tumors: common questions and tentative answers

Volante M., Righi L., Berruti A., Rindi G. and Papotti M.

Virchows Arch. 458:393-402, 2011

FAQ 1: terminology of NETs (GEP system)

"can terms such as CARCINOID and NET be used interchangeably?"

FAQ 2: criteria to define malignant potential in NETs

can the malignant potential be defined in NETs (G1 and G 2)?

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are grading and staging necessary in all tumors?

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is the diagnosis of neuroendocrine tumors possible in small biopsy or cytology material?

FAQ 6: the role of Ki-67 in NETs

is Ki67 to be determined in all NETs?

FAQ 7: prognostic factors in NETs

are prognostic factors (markers) other than Ki67 to be assessed in all NETs?

FAQ 8: predictive factors in NETs

are factors (markers) predictive of response to therapy to be assessed in all NETs?

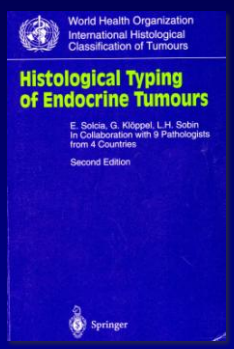
WHO 2000 classification of GEP endocrine tumors

Combined clinico-pathological parameters…

location, diameter, angioinvasion, presence of metastases

… and funtional data (clinico-pathological correlates)

type of hormonal secretion and clinical syndrome eventually present



WHO 2000 classification of GEP endocrine tumors

Well-differentiated endocrine tumor
- benign/uncertain behavior

Well-differentiated endocrine carcinoma
- Low grade malignant

Poorly differentiated endocrine carcinoma
- High grade malignant

Mixed Exocrine-Endocrine carcinoma / MEEC

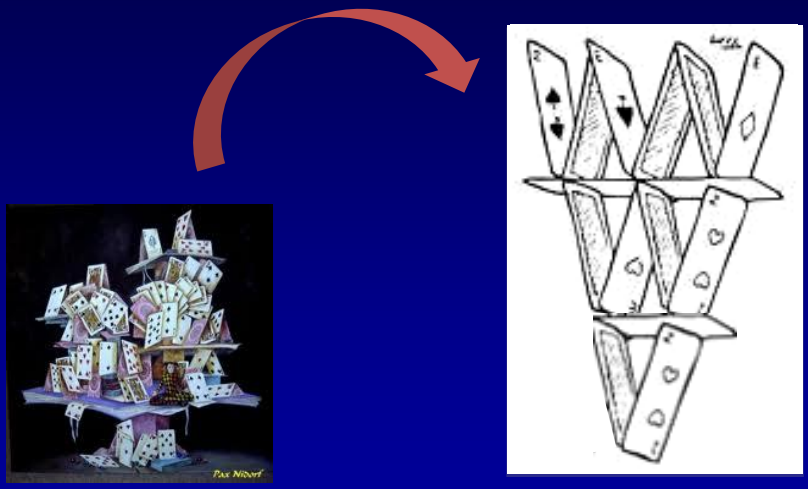
Neuroendocrine Neoplasms

WHO Classification 2010 of the Digestive System

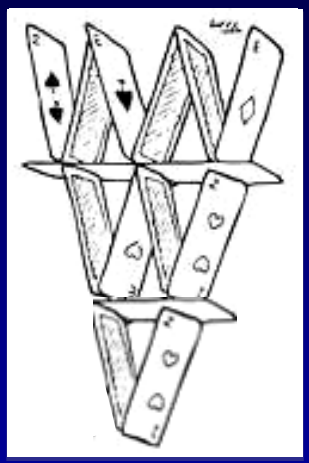
- **Working principles**
  - **"Neuroendocrine" defines the peptide hormone-producing tumours and share neural-endocrine markers**
  - **"Neuroendocrine neoplasm" includes well- and poorly differentiated tumours**
- **Premise: All neuroendocrine neoplasms (NENs) have a malignant potential**

Bosman FT, et al. *WHO Classification of Tumours of the Digestive System*. Lyon, France: IARC Press; 2010.

Neuroendocrine Neoplasms (NENs):
a stepwise diagnostic approach



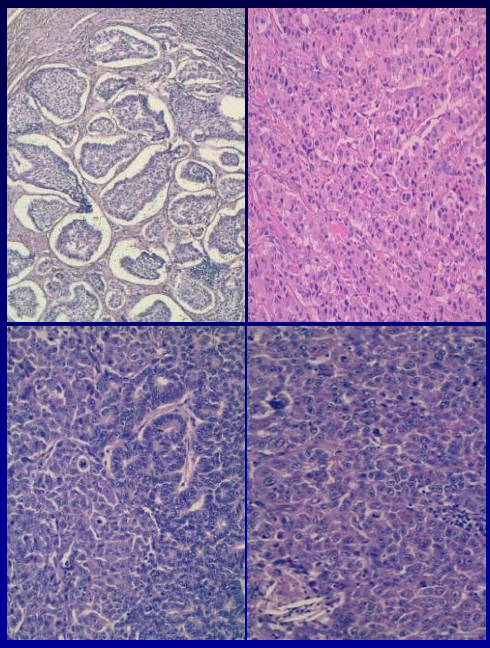
Neuroendocrine Neoplasms (NENs):
a stepwise diagnostic approach



0) NEN vs non NEN → morphology & NE markers

Morphological patterns in NENs

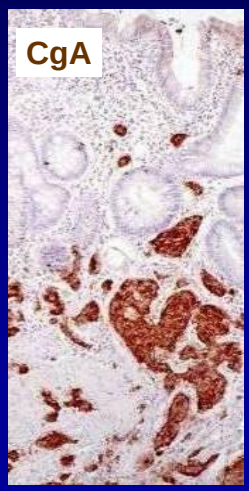
- ✓ Insular (nodular solid nests with peripheral invading cords)
- ✓ Trabecular (anastomosing trabeculae or ribbons)
- ✓ Glandular (tubules, acini or rosettes)
- ✓ Poorly differentiated with no well-organized growth pattern



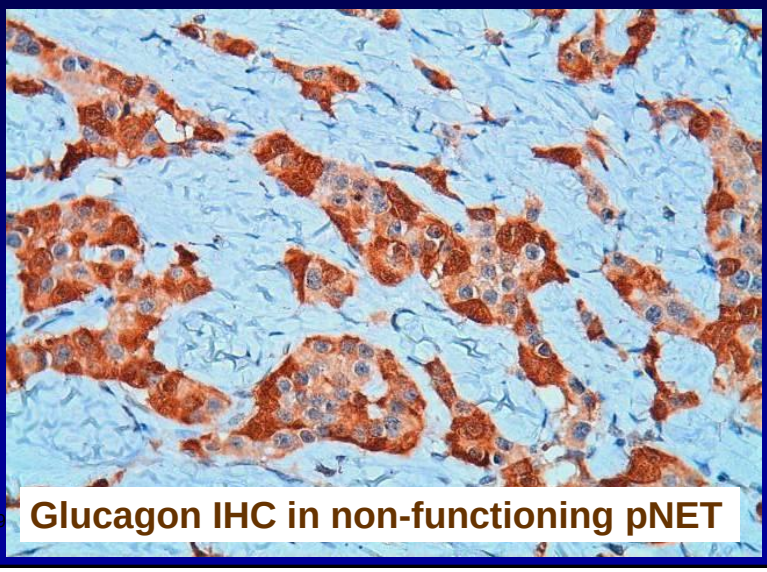
Immunohistochemical markers in NENs: *definition of NE phenotype*

Pan-endocrine markers

- ✓ Cytosolic (NSE, PGP 9.5)
- ✓ Related to secretory granules (chromogranin A)
- ✓ Related to synaptic vescicles (synaptophisin, VMAT)
- ✓ Intermediate filaments (NF, CK HMW)
- ✓ Adhesion molecules (N-CAM)



Immunohistochemical markers in NENs: *definition of hormone production*

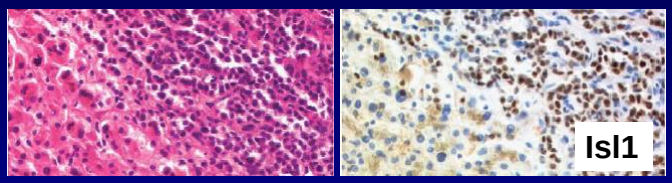


19 Glucagon IHC in non-functioning pNET

Immunohistochemical markers in NENs: *definition of origin*

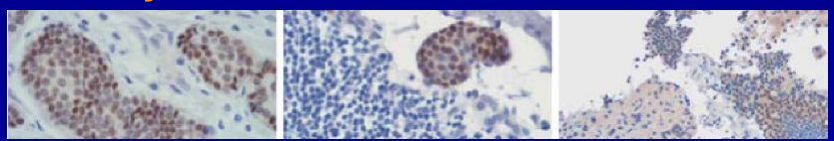
Islet 1 (Isl1) expression is a reliable marker for pancreatic neuroendocrine tumors and their metastases[1]

Liver mts



CDX2 as a marker of intestinal EC-cells and related well-differentiated neuroendocrine tumors[2]

Primary ileal **LN mts** **Liver mts**

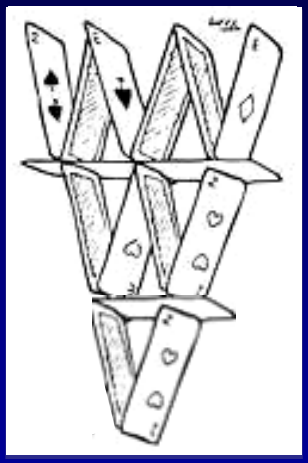


1. Schmitt AM et al Am J Surg Pathol. 2008 Mar;32(3):420-5, 2. La Rosa S et al Virchows Arch. 2004 Sep;445(3):248-54

Definition of NE phenotype

- ✓ Compromise between sensitivity and specificity
- ✓ Do not rely on a single marker to establish or disprove the diagnosis of NEN
- ✓ Immunohistochemical findings must be interpreted in the context of the microscopy (and, if necessary, the clinical and biochemical picture)

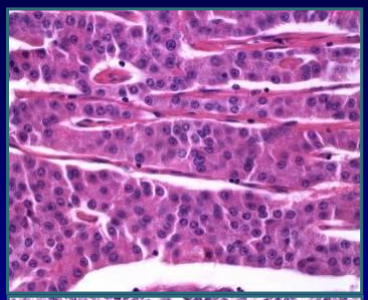
Neuroendocrine Neoplasms (NENs): a stepwise diagnostic approach

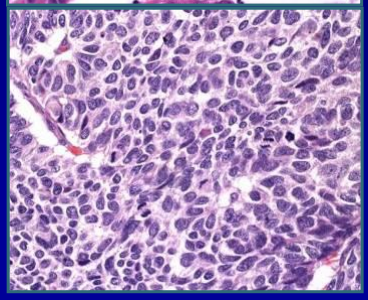


1) NET vs NEC → structure + grade

0) NEN vs non NEN → morphology & NE markers

Neuroendocrine Neoplasms
WHO Classification 2010 of the Digestive System





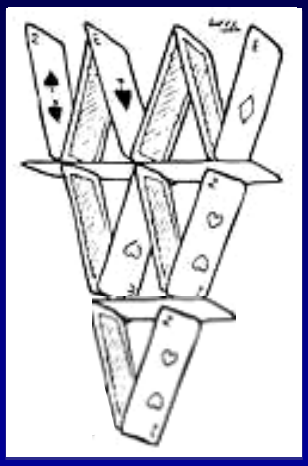
Neuroendocrine tumor/NET (Carcinoid)

Neuroendocrine carcinoma / NEC

Neuroendocrine Neoplasms
WHO Classification 2010 of the Digestive System

WHO 2000	WHO 2010
Well-differentiated endocrine tumour (WDET)	Neuroendocrine tumour
Well-differentiated endocrine carcinoma (WDEC)	
Poorly differentiated endocrine carinoma/small-cell carcinoma (PDEC)	Neuroendocrine carcinoma

Neuroendocrine Neoplasms (NENs): a stepwise diagnostic approach



size & invasion

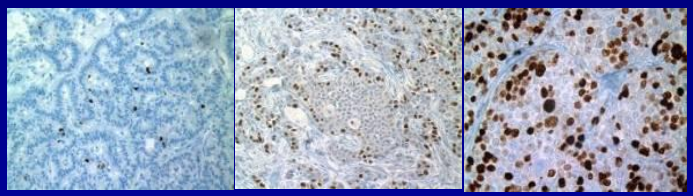
2) Grade 1-2-3 → mitoses & Ki67

1) NET vs NEC → structure + grade

0) NEN vs non NEN → morphology & NE markers

Grading of GEP-NENs According to ENETS/WHO/AJCC

Grade	G1	G2	G3
Ki67 index	≤2	3–20	>20
MI	<2	2-20	>20



1. Rindi G, et al. *Virchows Archiv.* 2006;449:395-401. 2. Rindi G, et al. *Virchows Archiv.* 2007;451:757-762.

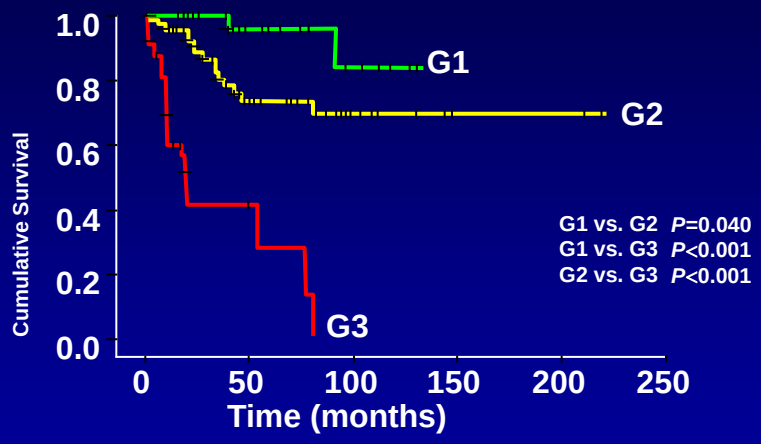
Ki67 Counting

- **Options to quantify Ki67**
  - **Systematically counting a defined number of tumors cells (500-2000) and calculating the positive percentage**
  - **Using a computerized digital image analysis system to measure the positive percentage**
  - **A general "eyeballed" estimate of the percentage of positive cells**
- **The result should be reported as a single percentage reflecting the average of the regions counted, rather than a range of value**

Mitotic index evaluation

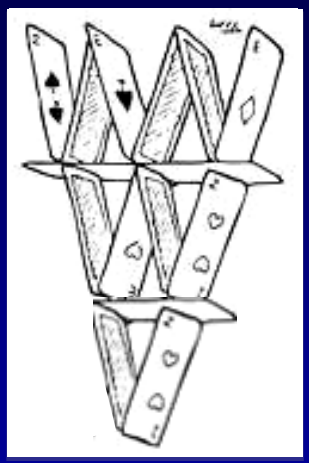
- ✓ **A total of 50 fields should be counted**
- ✓ **The mitotic rate should be expressed based on the number in 10 high power fields (2 mm^2)**

Grading of GEP-NENs according to ENETS/WHO/AJCC



Pape UF et al. *Cancer.* 2008;113:256-265.

Neuroendocrine Neoplasms (NENs): a stepwise diagnostic approach



3) TNM Stage I-II-III-IV → size & invasion

2) Grade 1-2-3 → mitoses & Ki67

1) NET vs NEC → structure + grade

0) NEN vs non NEN → morphology & NE markers

TNM Classification of GEP-NENs

- ✓ SITE-specific
- ✓ Based on depth of invasion and size

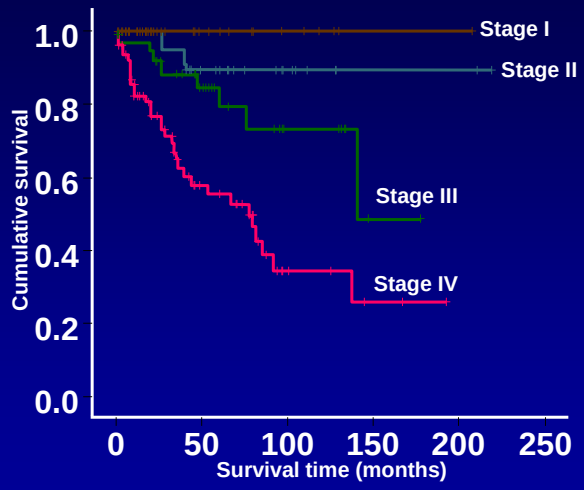
ENETS: 2006/2007
Rindi, Klöppel, Ahlman,Wiedenmann.TNM staging of foregut, midgut and hindgut (neuro) endocrine tumours: A consensus proposal including a grading system. *Virchows Archiv.* 2006;449:395-401, and 2007;451:757-762.

UICC/AJCC: 2009
Sobin, Gospdarowicz, Wittekind. *TNM Classification of Malignant Tumours*. Wiley-Blackwell. 7th Edition; 2009.

Comparison of ENETS 2006/2007 and UICC/AJCC 2009 TNM Classifications

- ✓ Similar TNM classifications:
  - Stomach
  - Duodenum
  - Jejunum/ileum
  - Colon/rectum

Staging of upper digestive NENs according to ENETS/WHO/AJCC



I vs II	*P* = .227
I vs III	*P* = .048
I vs IV	*P*<.001
II vs III	*P* = .171
II vs IV	*P*<.001
III vs IV	*P* = .004

Pape UF et al. *Cancer.* 2008;113:256-265.

Comparison of ENETS 2006/2007 and UICC/AJCC 2009 TNM Classifications

- ✓ **Similar TNM classifications:**
  - Stomach
  - Duodenum
  - Jejunum/ileum
  - Colon/rectum
- ✓ **Different TNM classification**
  - Appendix
  - Pancreas

T Category Criteria for Appendiceal NENs is Different Between ENETS and UICC/AJCC

	ENETS TNM	UICC/AJCC TNM
T1	≤1 cm; invasion of muscularis propria	T1a: ≤1cm T1b: >1– 2 cm
T2	≤2 cm; and <3 mm invasion of subserosa/ mesoappendix	>2–4 cm; or invasion of cecum
T3	>2 cm; or >3 mm invasion of subserosa/ mesoappendix	>4 cm; or invasion of ileum
T4	invasion of peritoneum/ other organs	invasion of peritoneum/ other organs

Pathology report of NENs

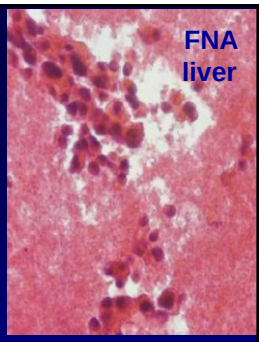
- Define location and tumor type based on WHO classification
- Define tumor grade (including Ki-67 proliferative index)
- Describe the presence of additional histologic features (multicentric disease, non-ischemic tumour necrosis, vascular or perineural invasion)
- Assess the TNM stage
- Define the resection margins
- Define the hormonal production, if any

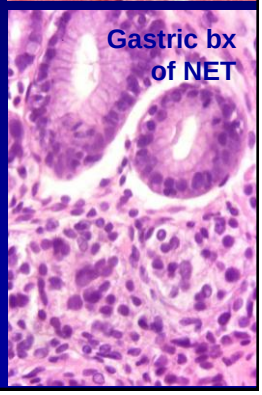
Upon request, assess prognostic or predictive factors useful for target therapy (e.g. somatostatin receptors, mTor pathway molecules, other target enzymes, …)

See also: Klimstra D, et al. *Am J Surg Pathol.* 2010;34:300-313.

WHO 2010 applicability in the "preoperative" setting?

- ✓ Definition of a NE neoplasm possible (suspect + markers)
- ✓ Definition of NET vs NEC usually possible (cell size, atypia, necrosis, growth pattern)
- ✓ Grading applicable, although with some limitations





WHO 2004 Criteria and CK19 are Reliable Prognostic Markers in Pancreatic Endocrine Tumors

Anja M. Schmitt, MD, Martin Anlauf, MD,† Valentin Rousson, PhD,‡ Sonja Schmid,* Andreas Kofler, MD,* Florian Riniker,* Juliane Bauersfeld,† Andre Barghorn, MD,* Nicole M. Probst-Hensch, MD,* Holger Moch, MD,* Philipp U. Heitz, MD,* Guenter Kloeppel, MD,† Paul Komminoth, MD,§ and Aurel Perren, MD*‖*

Am J Surg Pathol 2007;31:1677–1682

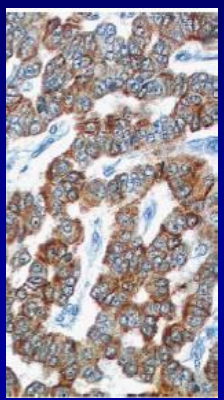


TABLE 1. Multivariate Analysis Including WHO 2004 Classification

	Multivariate *P* Value DFS	Multivariate *P* Value TTD
WHO classification	0.000	0.001
CK19 (positive)	0.019	0.001
Necrosis	0.057	0.130
Nodular fibrosis	0.052	0.518

10 HPF indicates 10 high-power fields, corresponding to an area of 2 mm^2.

PREDICTIVE MARKER

LINKED TO CURRENT THERAPY

+

METHODOLOGY OF ANALYSIS AVAILABLE

=

CLINICAL RELEVANCE

Expression of Somatostatin receptor types 1-5 in 81 cases of gastrointestinal and pancreatic endocrine tumors. A correlative immunohistochemical and reverse-transcriptase polymerase chain reaction analysis.

Papotti M, Bongiovanni M, Volante M, Allìa E, Landolfi S, Helboe L, Schindler M, Cole SL, Bussolati G.

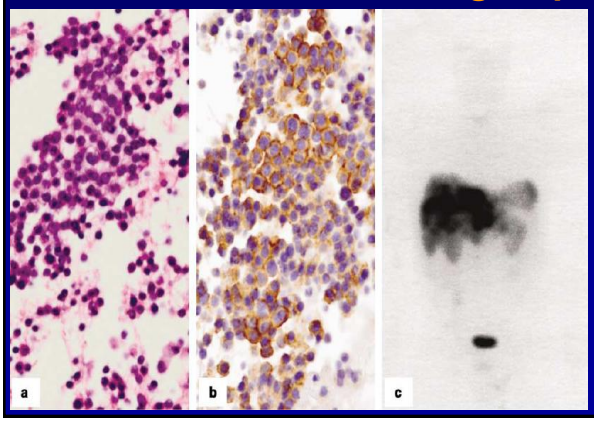
Virchows Arch. 2002 May;440(5):461-75

Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy

Modern Pathology (2007) 20, 1172–1182

Marco Volante[1], Maria Pia Brizzi[1], Antongiulio Faggiano[2], Stefano La Rosa[3], Ida Rapa[1], Anna Ferrero[1], Gelsomina Mansueto[4], Luisella Righi[1], Silvana Garancini[5], Carlo Capella[3], Gaetano De Rosa[4], Luigi Dogliotti[1], Annamaria Colao[2] and Mauro Papotti[1]

107 cases… including 41 pre-operative samples



Correlation with

Scintigraphy: 77%
(107 cases)

Tx response: 75%
(28 patients)

Integration of SSTR IHC and Somatostatin Analog-based Imaging (SRS):

SSTR IHC	SRS
✓ Cost effective	✓ Identification of "functional" receptors
✓ Detection of protein	✓ Detection of SSTR expression in the whole tumor mass
✓ Identification of SSTR subtype	
✓ Identification of cell type expressing SSTR	
✓ Applicable retrospectively	

Current therapy modalities in NETs

- ✓ **Biotherapy (somatostatin analogs and interferon)**
- ✓ **Peptide receptor radionuclide therapy**
- ✓ **Cytotoxic treatment**
- ✓ **Targeted therapies**

FAQ 1: terminology of NETs (GEP system)

"can terms such as CARCINOID and NET be used interchangeably?"

Context:

Carcinoid vs. (neuro) endocrine tumor/ carcinoma terms

Relevance:

Terminology reflects classification and prognosis

Criticism:

Not accepted worldwide

FAQ 1: terminology of NETs (GEP system)

"can terms such as CARCINOID and NET be used interchangeably?"

Answer:

NO

(the term "carcinoid" fails to convay informations related to clinical behaviour)

FAQ 2: criteria to define malignant potential in NETs

can the malignant potential be defined in NETs (G1 and G 2)?

Context:

Combination of clinical (presence of metastases) and morphological parameters

Relevance:

Determine the classification (and prognosis) of a NET

Criticism:

Change according to the tumor site; some equivocal/difficult to assess

FAQ 2: criteria to define malignant potential in NETs

can the malignant potential be defined in NETs (G1 and G 2)?

Answer:

YES,
applying a site-specific TNM and Grading

FAQ 3: nomenclature for functioning versus non functioning tumors

is any immunohistochemically detected hormone production sufficient to label a GEP NET with a suffix "oma" folloving the hormone?

Context:

Terms such as insulinoma, gastrinoma, etc. and the concept of functioning tumors

Relevance:

Functioning tumors are defined by the presence of clinical symptoms related to hormone production

Criticism:

Terminology reflects clinical picture rather than pathological features; no link with malignancy

FAQ 3: nomenclature for functioning versus non functioning tumors

is any immunohistochemically detected hormone production sufficient to label a GEP NET with a suffix "oma" folloving the hormone?

Answer:

NO

(unless specific information was provided)

FAQ 4: grading and staging of NETs

are grading and staging necessary in all tumors?

Context:

Necessity to apply novel grading and staging systems

Relevance:

NET stage (ENETS and AJCC) and grade (ENETS) are of prognostic value

Criticism:

Recent, to be validated prospectively, sometimes confusing and discordant

FAQ 4: grading and staging of NETs

are grading and staging necessary in all tumors?

Answer:

YES

(applying the criteria of the 2010 WHO classification of digestive system tumors)

FAQ 5: pathological diagnosis of NETs in biopsy/cytology material

is the diagnosis of neuroendocrine tumors possible in small biopsy or cytology material?

Context:

The type of information that may be determined in preoperative material from NETs

Relevance:

In inoperable cases, biopsy/cytology material may be the only tissue sample available

Criticism:

Classification criteria not applicable; immunoprofile (included Ki-67) affected by sampling error

FAQ 5: pathological diagnosis of NETs in biopsy/cytology material

is the diagnosis of neuroendocrine tumors possible in small biopsy or cytology material?

Anwer:

YES

(although classification limited to some cases)

FAQ 6: the role of Ki-67 in NETs

is Ki67 to be determined in all NETs?

Context:

Diagnostic vs. prognostic vs. predictive role of Ki-67 determination in NETs

Relevance:

Most relevant marker in NETs (upon NE differentiation is established)

Criticism:

Still poorly reproducible; diagnostic vs. prognostic vs. predictive uses often erroneously interpreted

FAQ 6: the role of Ki-67 in NETs

is Ki67 to be determined in all NETs?

Answer:

YES

(it is presently the best prognostic/predictive parameter for NETs we have. An accurate and reproducible procedure to assess Ki67 index is mandatory)

FAQ 7: prognostic factors in NETs

are prognostic factors (markers) other than Ki67 to be assessed in all NETs?

Context:

Molecular and immunohistochemical markers of prognostic value

Relevance:

Correct classification and proliferative index are is the only prognostic features of relevance, to date

Criticism:

Several molecules proposed, none turned to be useful in clinical application

FAQ 7: prognostic factors in NETs

are prognostic factors (markers) other than Ki67 to be assessed in all NETs?

Answer:

NO
(not yet. Work in progress.)

FAQ 8: predictive factors in NETs

are factors (markers) predictive of response to therapy to be assessed in all NETs?

Context:

Molecular and immunohistochemical markers of predictive value

Relevance:

Predictive interest of detection of Somatostatin Receptors (5 types)

Criticism:

Several molecules proposed, none turned to be useful in clinical application

FAQ 8: predictive factors in NETs

are factors (markers) predictive of response to therapy to be assessed in all NETs?

Answer

NO

(only upon request by the clinician. Most requested: Somatostatin Receptors, but data are questionable)

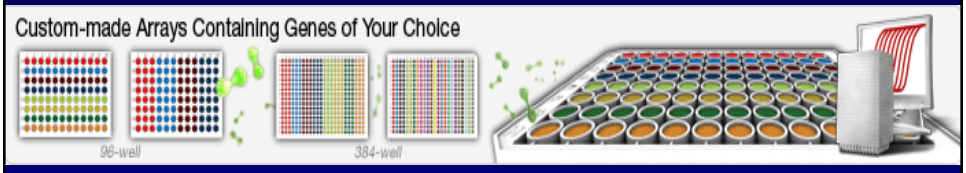
FAQ 9: what next?

open issues…

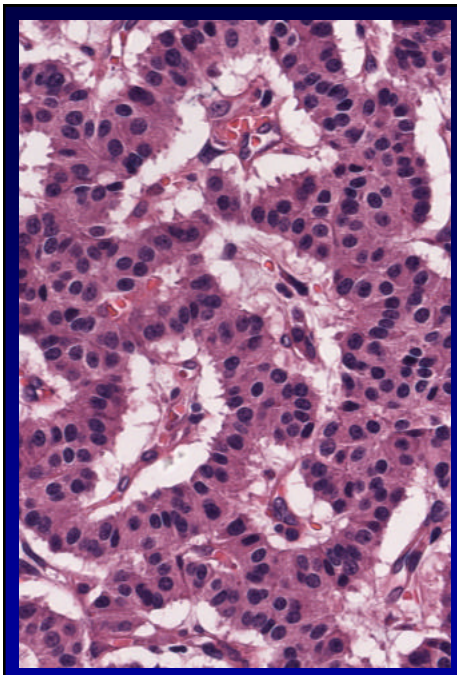
- ✓ Applicability of new WHO classification to be tested
- ✓ Grading to be implemented (G2, location)
- ✓Optimal staging system to be validated

- ✓ Prognostic/predictive markers to be validated
- ✓Quantitative and reproducible evaluation of immunohistochemical markers

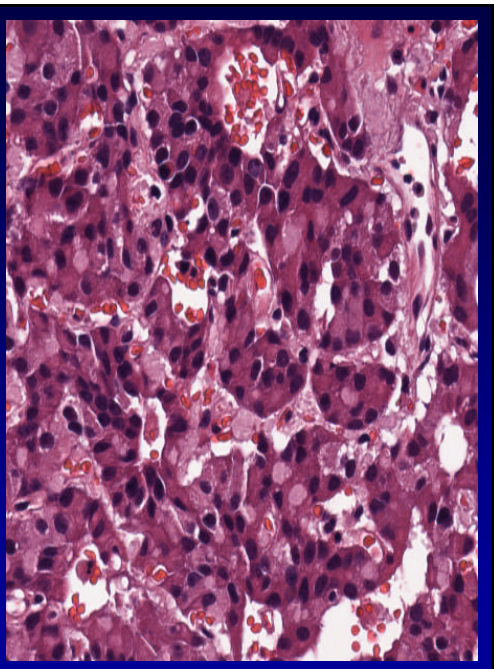
- Gene expression profiling



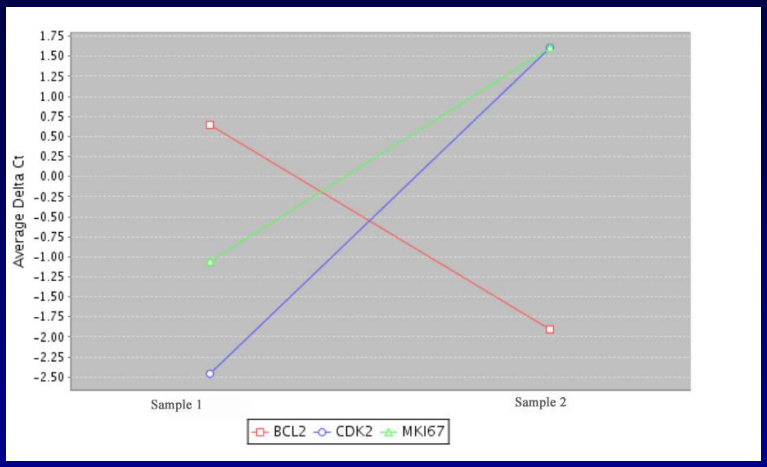
→ Gene expression profiling by RT-qPCR on Formalin-fixed Paraffin embedded tissues.



977/94 NET Pancreas

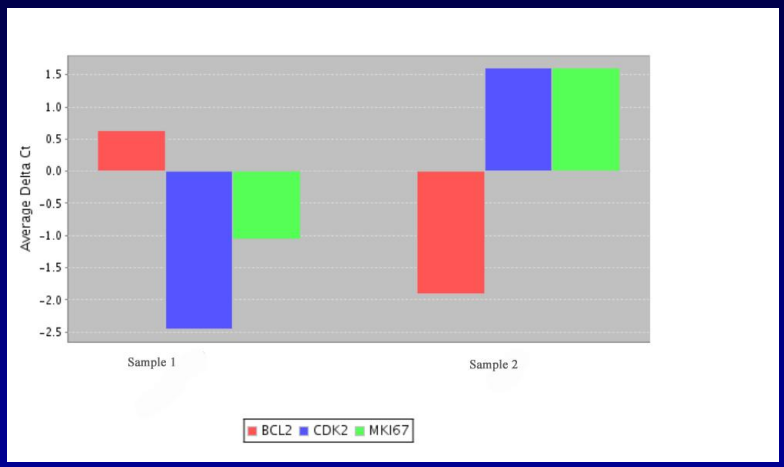


8943/96 malignant Insulinoma



Sample 1: 977/94 NET

Sample 2: 8943/96 Malignant Insulinoma



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