Gastroenteropancreatic neuroendocrine tumors: classification, diagnostic pathology and molecular advances

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Tumeurs neuroendocrines du tractus gastro-intestinal et du pancréas
Classification et difficultés diagnostiques

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en collaboration avec Jean-Yves Scoazec
(Hôpital Edouard Herriot, Lyon)

What is a «neuroendocrine» tumor ?

- A tumor made of neoplastic cells of epithelial derivation, characterized by structural, phenotypic and/or functional properties recalling those of normal peptide- or amine-producing endocrine cells

Distribution of NETs

Digestive NETs	67,5 %
Bronchial NETs	25,3 %
Genito-urinary NETs	1 %
Other sites	6,2 %

Thymus, skin, breast, ear …

Modlin et al. Cancer 2003;97:934

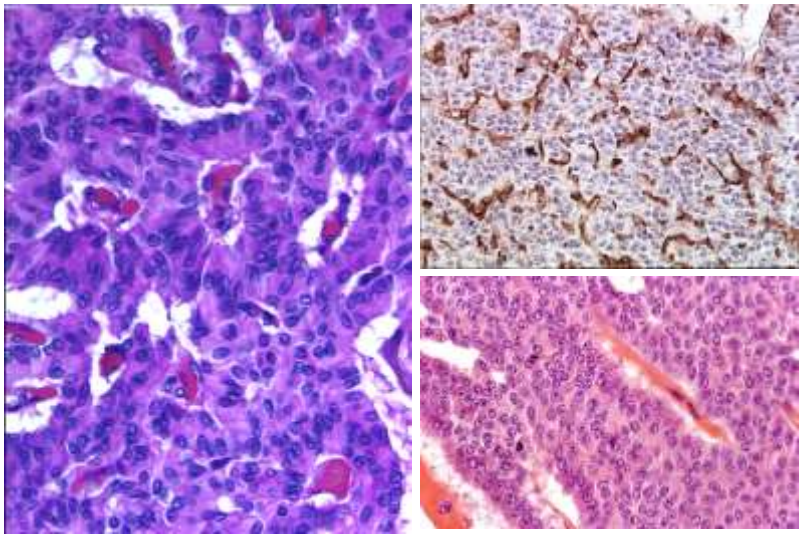
The contributions of the pathologist to NET diagnosis and … to patient care

- To identify the neuroendocrine nature of a tumor
- To evaluate its risk of malignancy and rate of progression
- To bring up additional informations useful for prognosis evaluation, treatment and follow-up

Diagnosis and classification

Diagnosis: how to identify a tumor as a «neuroendocrine» tumor ?

The diagnosis is easy when the tumor is well differentiated



The immunophenotype of peptide (or amine)-producing endocrine cells

Chromogranin A in the endocrine pancreas

Endocrine markers : chromogranins A et B, other granins

Large dense core granules containing mature hormones and their precursors

Neuro-endocrine markers : NCAM CD57

Small synaptic vesicles containing neuropeptides

Neuro-endocrine markers : synaptophysin, …

membrane

cytosol

Neuro-endocrine markers : NSE PGP9.5

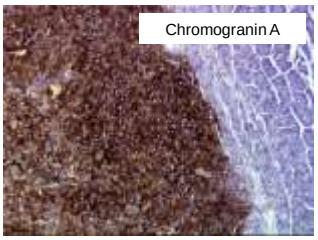
Synaptophysin expression by intestinal endocrine cells

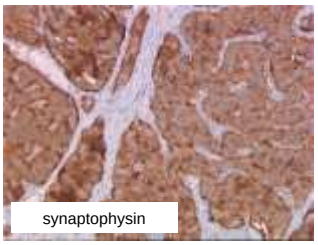
What is required for the pathological diagnosis ?

– One peptidergic endocrine marker
  • Chromogranin A

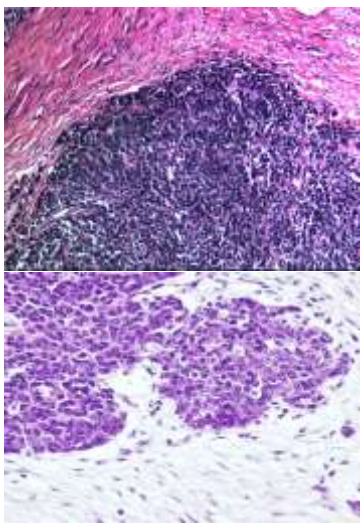
and

– One or several neuro-endocrine markers:
  • Synaptophysin
  • NCAM
  • Leu 7 (CD57)
  • PGP9.5





The diagnosis may be difficult when the tumor is poorly differentiated



- «Small cell» morphology (usually)
- « Large cell » morphology (rarely)
- High mitotic activity
- Frequent necrosis

Immunohistochemistry is mandatory for a correct diagnosis

- To demonstrate the neuroendocrine differentiation of tumor cells
- To rule out an undifferentiated tumor of other origin
- To confirm the poorly differentiated nature of the tumor in difficult cases

Endocrine markers	Chromogranin A	Frequently negative Sometimes positive
Neuroendocrine markers	Synaptophysin	Positive
	Leu7, PGP9.5, NCAM, NSE …	Frequently positive
Other markers	Keratins	Positive
	EMA Carcino-embryonic antigen	Frequently positive
	p53	Strongly positive

How to evaluate the risk of malignancy of a neuroendocrine tumor ?

Malignancy and site

- Incidence of malignancy according to the site of the primary:
  - Pituitary, parathyroids: exceptional
  - Bronchi: 20%
  - Digestive tract and pancreas: 60%

Three related questions

- How to identify an already malignant tumor ?
- How to identify a tumor which may eventually behave as a malignant tumor ?
- For malignant tumors, how to predict the rate of evolution ?

After the diagnosis ...

- Classification
- Grading
- Staging

Classifications

- Integration of morphological, biological and molecular informations
- in order to define categories of prognostic significance and clinical relevance (follow-up, therapeutic strategy)
- based on simple, robust, validated and reproducible criteria

After the diagnosis ...

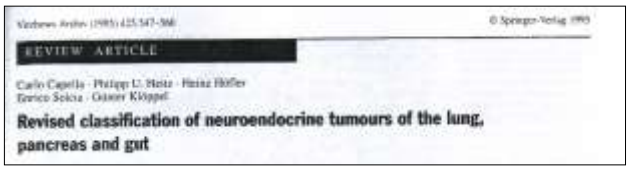
- Classification
- Grading
- Staging

- WHO
- ENETS/UICC
- TNM

All three informations are complementary and mandatory

Recent classifications

- The modern era starts with a common effort


REVIEW ARTICLE

Revised classification of neuroendocrine tumours of the lung, pancreas and gut

- Rapidly, classifications diverged


Thoracic NETs, 2004

GEP-NETs, 2000 -2004 -2010

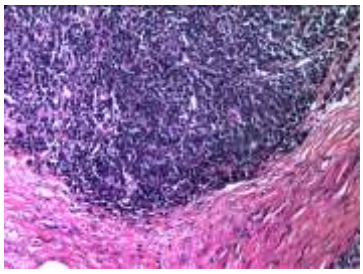
Digestive NETs: too much ?

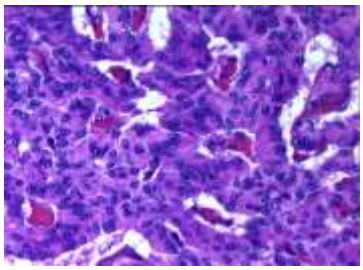
- Classification
  - WHO
    - 2000 ... 2004
    - 2010
- Grading
  - ENETS
    - ENETS 2006
    - ENETS/UICC 2010
- Staging
  - TNM
    - ENETS (2006-2007)
    - UICC (2010)

WHO classification, 2000

- Well differentiated endocrine tumors
  - Benign behavior
  - Uncertain behavior
- Well differentiated endocrine carcinomas
  - Low grade of malignancy
- Poorly differentiated endocrine carcinomas
  - High grade of malignancy

First criteria : morphological differentiation





Poorly differentiated :
Always malignant
High grade

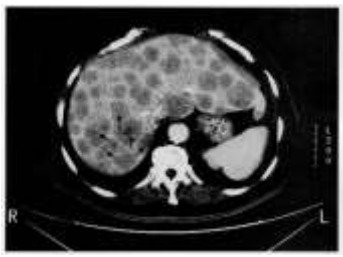
5-10%

Well differentiated :
Benign or malignant ?
Not predictive
of the behavior

90-95%

Second criteria : Evidence of objective signs of malignancy

- Metastatic dissemination
- Local invasion

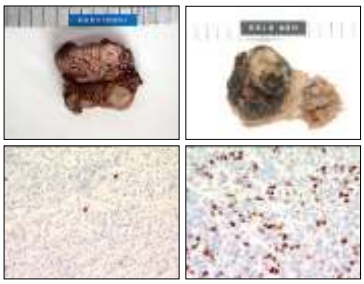


If yes, diagnosis :
Well differentiated
endocrine carcinoma
(low grade of malignancy)

If not, diagnosis :
Well differentiated
endocrine tumor

Third criteria :
Evidence of predictive signs of malignancy

- Size (< or > threshold)
- Mitotic index or proliferation index (< or > threshold)
- Angioinvasion
- Perineural invasion
- Functioning syndrome (except insulinoma)



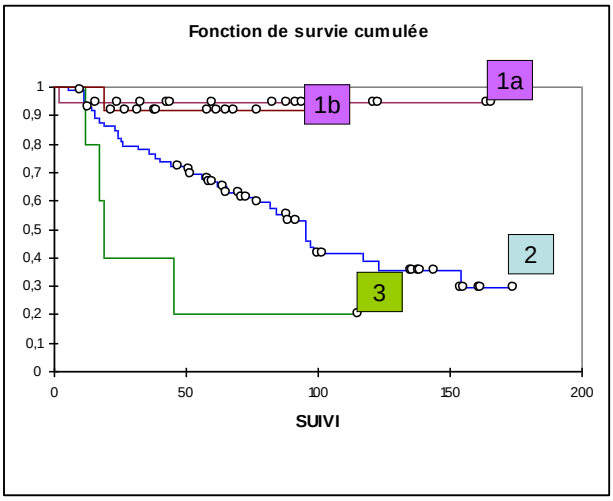
If no at all the criteria:
Well differentiated endocrine tumor, benign behavior
If yes at at least one criteria:
Well differentiated endocrine tumor, uncertain behavior

A complex assemblage of criteria, adapted to each anatomical site

WHO 2000	*Well differentiated endocrine tumor*		*Well differentiated endocrine carcinoma*	*Poorly differentiated endocrine carcinoma*
	Benign behavior	*Uncertain behavior*		
Histological differentiation	Well differentiated	Well differentiaéted	Well differentiated	Poorly differentiated
Local invasion	Gut: Mucosa and submucosa Pancreas: intra-pancreatic	Gut: Mucosa and submucosa Pancreas: intra-pancreatic	Gut: Muscularis propria and beyond Pancreas: Extra-pancreatic	
Metastasis	Absent	Absent	Possible	Possible
Size	Stomach, small bowel : ≤1 cm	Stomach, small bowel : >1 cm	Stomach, small bowel : usually >1 cm	
	Appendix, colon, rectum, pancreas : ≤2 cm	Appendix, colon, rectum, pancreas : >2 cm	Appendix, colon, rectum, pancreas : Usually >2 cm	
Angioinvasion **Perineural invasion**	Absent	Present *	Possible	Possible
Mitotic index	<2	>2	Usually >2	Usually >10
Ki 67 index	≤2%	>2%	Usually >2%	Usually >15%
Functional syndrome (clinical)	No (except insulinome)	Yes (except insulinome)		

*, except for appendiceal tumors

Clinical validation



Edouard Herriot Hospital

Problems

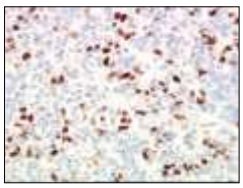
- Terminology
- Complexity
  - Combination of histological and clinical criteria
  - Combination of classification and staging
- Inadequacies
  - Not applicable to biopsies and cytological examinations
  - Lack of relevance of the category « well differentiated endocrine tumors of uncertain behavior »
  - Lack of accurate description and typing of poorly differentiated tumors
  - Lack of accurate prognostic evaluation of well differentiated carcinomas

Histological grading (ENETS, 2006)

Histological grading

Well differentiated carcinomas

Poorly differentiated carcinomas



Grading system (ENETS, 2006)

Table 4 Grading proposal for foregut (neuro)endocrine tumors

Grade	Mitotic count (10 HPF)[a]	Ki-67 index (%)[b]
G1	<2	≤2
G2	2–20	3–20
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[a]10 HPF: high power field=2 mm^2, at least 40 fields (at 40× magnification) evaluated in areas of highest mitotic density
[b]MIB1 antibody; % of 2,000 tumor cells in areas of highest nuclear labeling

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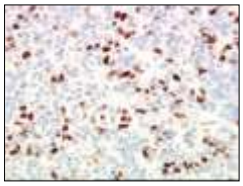
Virchows Arch (2006) 449:395–401

Histological grading

Well differentiated carcinomas



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[a]10 HPF: high power field=2 mm^2, at least 40 fields (at 40×

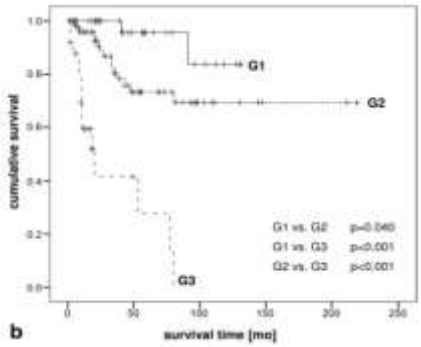
Treatment ? Follow up ?

Virchows Arch (2006) 449:395–401

Clinical validation

Neuroendocrine Tumors of Midgut and Hindgut Origin: Tumor-Node-Metastasis Classification Determines Clinical Outcome

Cancer 2011;117:3332–41



Prognostic Validity of a Novel American Joint Committee on Cancer Staging Classification for Pancreatic Neuroendocrine Tumors

JOURNAL OF CLINICAL ONCOLOGY

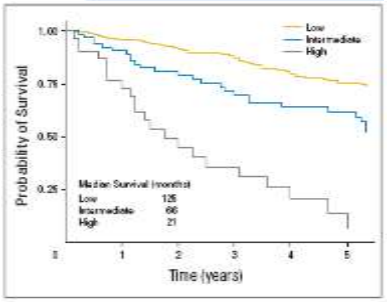
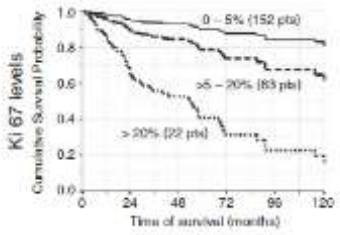


Fig 4. Kaplan-Meier estimate of overall survival, according to histopathologic grade.

Histological grading: issues and perspectives

- Is the determination of the proliferation index simple, robust and reproducible ?
- How accurate are the cut-offs currently in use ?

Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients



Staging: TNM classification

Integration between prognostic evaluation and clinical staging: ENETS proposals (2006-2007)

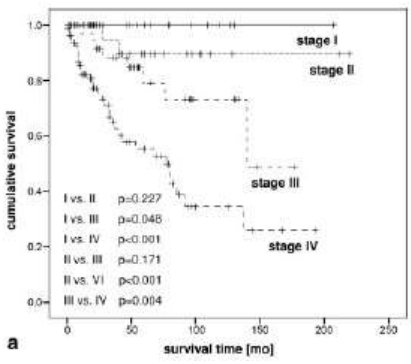
TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system

G. Rindi · G. Klöppel · H. Alhman · M. Caplin · A. Couvelard · W. W. de Herder · B. Eriksson · A. Falchetti · M. Falconi · P. Komminoth · M. Körner · J. M. Lopes · A-M. McNicol · O. Nilsson · A. Perren · A. Scarpa · J-Y. Scoazec · B. Wiedenmann · and all other Frascati Consensus Conference participants

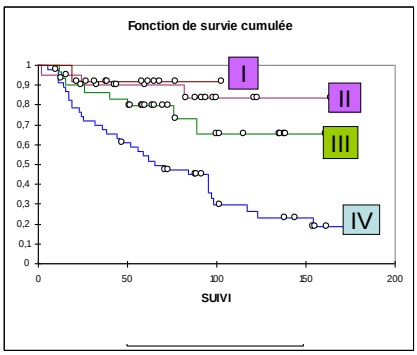
TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system

- for all digestive NETs:
  - «benign» and «malignant»
  - well and poorly differentiated
- based on criteria adapted to the anatomical location

Clinical validation



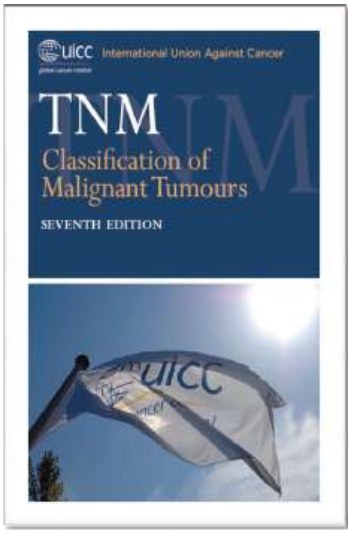
Cancer 2008;113:256



Hôpital Edouard Herriot

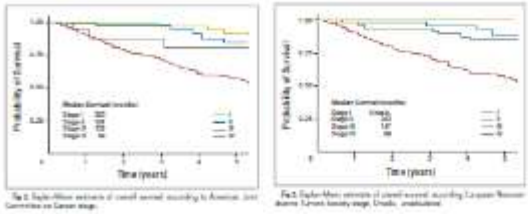
TNM classification: UICC (2010)

- Specific classification :
  - Well differentiated NETs of the digestive tract
- Classification identical to that of carcinomas of the same anatomical location:
  - Poorly differentiated NETs of the digestive tract
  - Pancreatic NETs (well and poorly differentiated)
  - Lung NETs (well and poorly differentiated)



Which are the differences between ENETS proposals and UICC/AJCC classification ?

- ENETS=UICC for :
  - Well differentiated neuroendocrine tumors of stomach, small bowel, colon and rectum
- ENETS≠UICC for :
  - Well differentiated neuroendocrine tumors of the appendix and pancreas
  - Poorly differentiated neuroendocrine tumors



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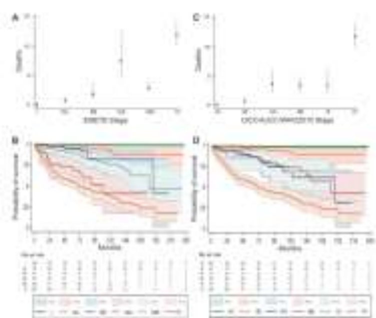
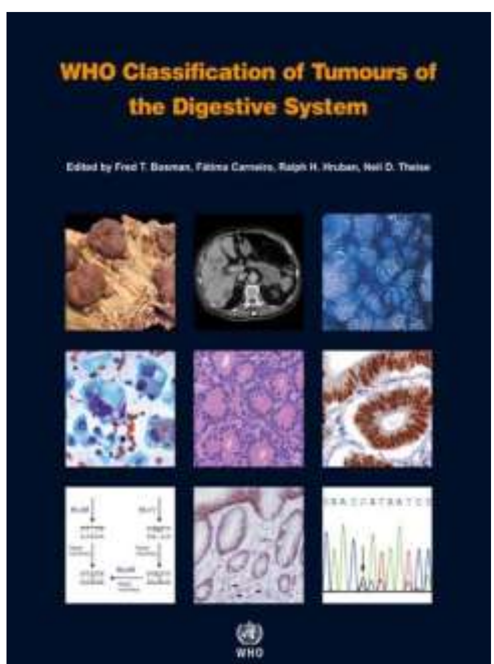
VOLUME 29 · NUMBER 22 · AUGUST 1 2011

JOURNAL OF CLINICAL ONCOLOGY

TNM Staging of Neoplasms of the Endocrine Pancreas: Results From a Large International Cohort Study

JNCI 2012 (May); 104:764-777





WHO classification, 2010

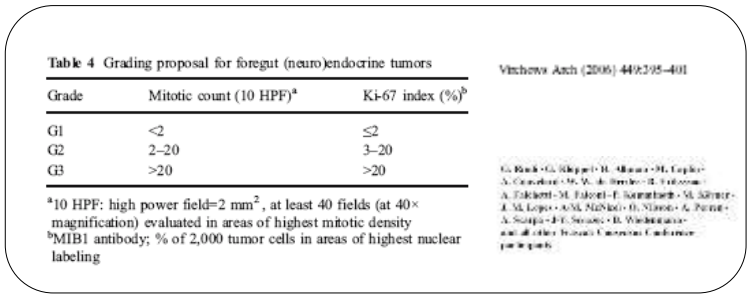
- Neuroendocrine tumor/neoplasm, G1
- Neuroendocrine tumor/neoplasm, G2
- Neuroendocrine carcinoma
  - Small cell type
  - Large cell type
- Mixed adeno-neuroendocrine carcinoma

WHO classification, 2010

- Neuroendocrine neoplasm, G1
  - Well differentiated morphology
  - MI <2 and Ki67 index ≤2%
- Neuroendocrine neoplasm, G2
  - Well differentiated morphology
  - MI: 2-20 and Ki67 index: 3-20%
- Neuroendocrine carcinoma
  - Small cell type
  - Large cell type
- Mixed adeno-neuroendocrine carcinoma

WHO classification, 2010


Nomenclature and classification of neuroendocrine neoplasms of the digestive system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Virchows Arch (2006) 449:395–401

Changes in terminology and classification mean changes in principles and concepts

- Changes in scope
  - Full separation between typing (WHO) and staging (TNM)
- Changes in basic concepts
  - All NET are potentially malignant
  - Proliferation fraction, and not only morphological differentiation, is a key parameter

Homologies ...

Thoracic NETs WHO 2004	Typical carcinoid	Atypical carcinoid 10	Large cell neuroendocrine carcinoma	Small cell neuroendocrine carcinoma
GEP-NETs WHO 2010	Neuroendocrine tumor G1 (or carcinoid)	Neuroendocrine tumor G2 20	Neuroendocrine carcinoma, large cell type	Neuroendocrine carcinoma, small cell type

mitoses

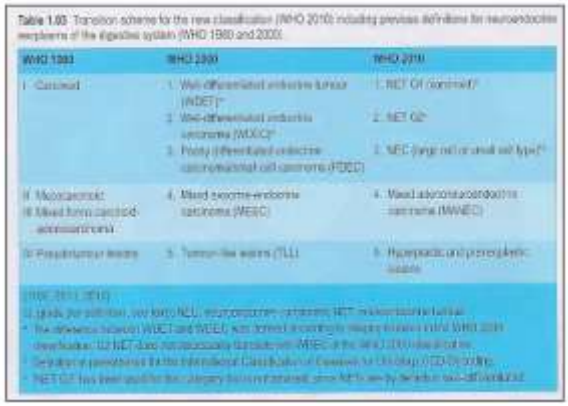
A progress ?

- *For diagnosis:*
  - + Applicable to small-sized tissue samples (biopsy, endoscopical resections …), even to cytological preparations
  - + A better definition of poorly differentiated gastroenteropancreatic tumors
- *For prognosis:*
  - + Over estimation of grading ?
  - + Under estimation of morphological differentiation ?

Some important issues

- A new terminology ...
  - Tumor/neoplasm
  - Neuroendocrine
  - Carcinoid: once more ...
  - Neuroendocrine carcinoma in 2010: always poorly differentiated !
- Caution: the reclassification of cases during their follow-up ...

Correspondence 2000/2010

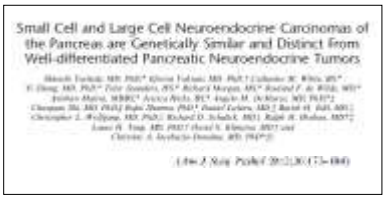


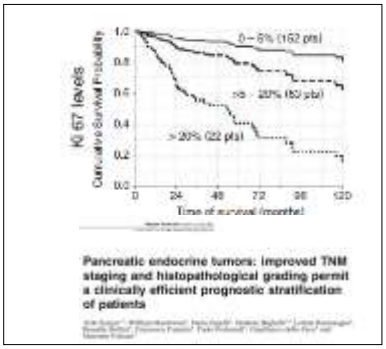
Correspondence 2000/2010

WHO 2010	WHO 2000
Neuroendocrine neoplasm G1	• Well differentiated endocrine tumor of benign behavior • Well differentiated endocrine tumor of uncertain behavior with mitotic index <2 and Ki67 index ≤2% • Well differentiated endocrine carcinoma with mitotic index <2 and Ki67 index ≤2%
Neuroendocrine neoplasm G2	• Well differentiated endocrine tumor of uncertain behavior with mitotic index 2-20 and/or Ki67 index 3-20% • Well differentiated endocrine carcinoma with mitotic index 2-20 and/or Ki67 index 3-20%
Neuroendocrine carcinoma, small cell type	Poorly differentiated endocrine carcinoma, small cell type
Neuroendocrine carcinoma, large cell type	*No corresponding category*
Adeno-neuroendocrine carcinoma	Mixed tumor

Future trends

- **Definition of NET spectrum**
  - Relation between well and poorly differentiated neoplasms: one or several diseases ?
- **Improvement of typing**
  - Neoplasms with well differentiated morphology, G3 ?
- **Refinement of grading**
  - Is Ki67 index a reproducible criteria ?
  - Are the current thresholds accurate and relevant ?
- **Validation of staging**
- **Integration of molecular informations**
  - Diagnostic and prognostic markers
  - Predictive markers in the era of targeted therapies


Small Cell and Large Cell Neuroendocrine Carcinomas of the Pancreas are Genetically Similar and Distinct from Well-differentiated Pancreatic Neuroendocrine Tumors



Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients

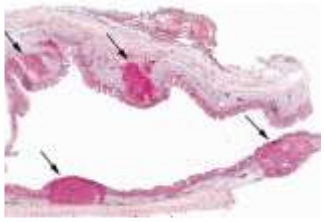
Minimal items for the pathological report

- Diagnostic arguments
  - Morphological arguments
  - Immunohistochemical arguments
- Classification
  - WHO 2010 (WHO 2000 in brackets)
- Histological grading
  - Absolute values
- pTNM
  - UICC 2010 (along with ENETS, especially for appendix and pancreas)

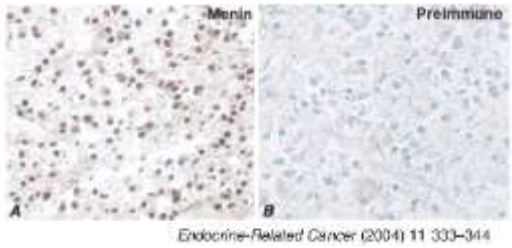
The pathologist may provide useful additional informations

Identification of a syndrome of genetic predisposition: MEN-1

- Histological arguments
  - Endocrine hyperplasia in target tissues
- Molecular arguments
  - Loss of expression of target proteins



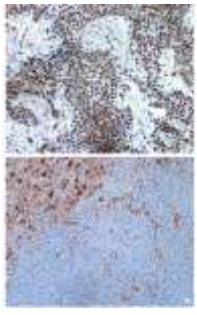
Menin



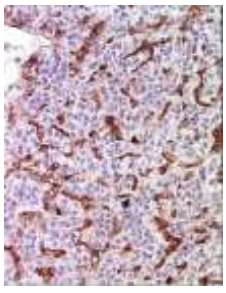
Endocrine-Related Cancer (2004) 11 333–344

Identification of factors predictive of the response to treatment

sst2



MGMT



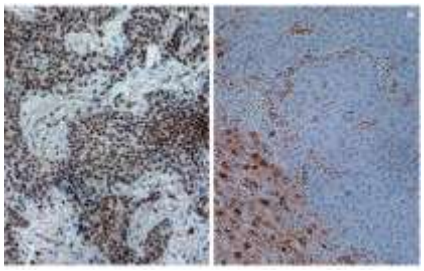
CD34

EGFR

pmTOR

Predictive markers: MGMT and temozolomide

- MGMT
  - Interest in NETs and pheochromocytomas
  - Loss of protein expression and/or gene silencing associated with increased response
  - Evaluation:
    - Immunohistochemistry
    - Promoter methylation



Kulke et al. Clin Cancer Res 2009;15:338

MGMT deficiency:
51% pancreatic NET
0% gut NET

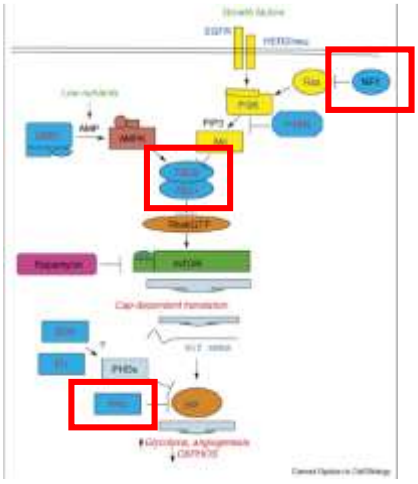
Molecular advances

Molecular mechanisms involved in early tumorigenesis and tumor progression are poorly known

- Tumorigenesis
  - No role for the «conventional» oncogenes and tumor suppressor genes known to be involved in epithelial carcinogenesis
  - Several genes involved in syndromes of familial predisposition to endocrine tumors, behaving as tumor suppressor genes with a role in early tumorigenesis
    - *MEN-1, but also NF1, TSC1/2, VHL …*
  - Some candidate genes emerging for sporadic pancreatic NETs
    - *MEN-1, DAXX/ATRX,* genes of the mTOR pathway
- Tumor progression
  - Limited knowledge about the molecular mechanisms involved in local invasion and metastatic dissemination

Molecular mechanisms

- Several genes involved in predisposition syndromes encode proteins of the mTOR pathway or of the hypoxia response pathway
- Some of these genes are also involved in sporadic tumors



Shaw RJ. Curr Opin Cell Biol. 2006;18:598-608.

Molecular mechanisms

DAXX/ATRX, MEN1, and mTOR Pathway Genes Are Frequently Altered in Pancreatic Neuroendocrine Tumors

Yuchen Jiao,[1*] Chanjuan Shi,[2*] Barish H. Edil,[2] Roeland F. de Wilde,[2] David S. Klimstra,[4] Anirban Maitra,[5] Richard D. Schulick,[3] Laura H. Tang,[4] Christopher L. Wolfgang,[3] Michael A. Choti,[3] Victor E. Velculescu,[1] Luis A. Diaz Jr.,[1,6] Bert Vogelstein,[1] Kenneth W. Kinzler,[1†] Ralph H. Hruban,[5†] Nickolas Papadopoulos[1†]

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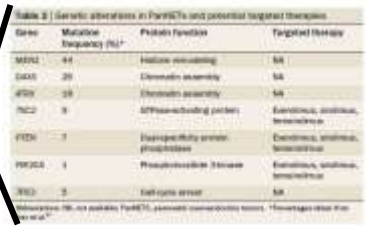
Table 1. Comparison of commonly mutated genes in PanNETs and PDAC based on 68 PanNETs and 114 PDACs.

Genes*	PanNET	PDAC†
MEN1	44%	0%
DAXX, ATRX	43%	0%
Genes in mTOR pathway	15%	0.80%
TP53	3%	85%
KRAS	0%	100%
CDKN2A	0%	25%
TGFBR1, SMAD3, SMAD4	0%	38%



Prognostic relevance

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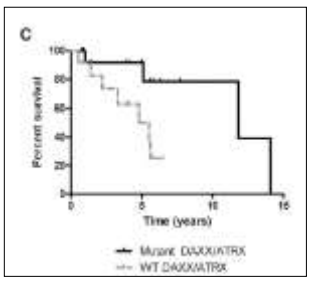
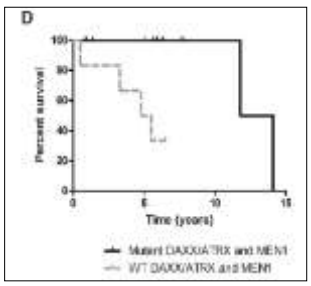


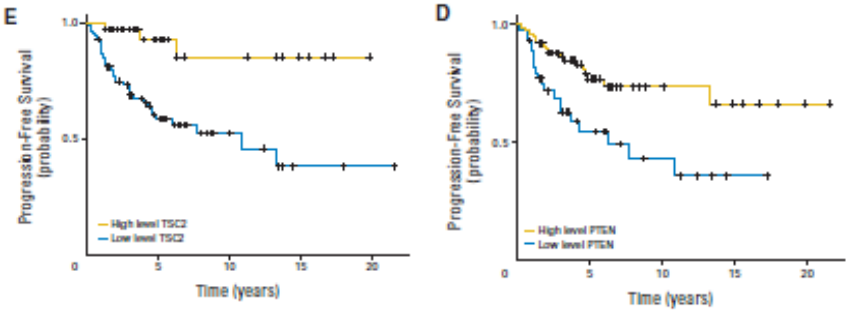


Prognostic relevance

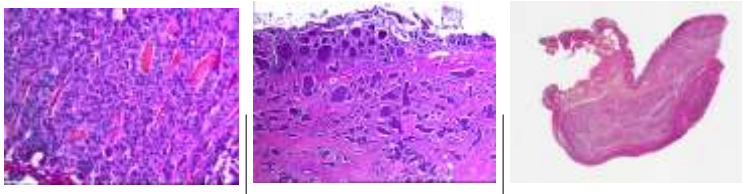
Pancreatic Endocrine Tumors: Expression Profiling Evidences a Role for AKT-mTOR Pathway

J Clin Oncol 28:245-255. © 2009

Edoardo Missiaglia, Irene Dalai, Stefano Barbi, Stefania Beghelli, Massimo Falconi, Marco della Peruta, Lorenzo Piemonti, Gabriele Capurso, Alessia Di Florio, Gianfranco delle Fave, Paolo Pederzoli, Carlo M. Croce, and Aldo Scarpa



Pancreatic NETs



foregut

Stomach
Duodenum
Pancreas
Bronchi

MEN1, DAXX/ATRX
TSC1/2, NF1, PTEN
VHL

Right colon

Midgut NETs

LOH (X, 3, 17, 18)
PDCD4
Methylation (CIMP-like)

Stomach
Duodenum
Pancreas
Bronchi

Jejunum
Ileum
Right colon

Left colon
Rectum

Messages

- The diagnosis of endocrine tumors is usually easy: *however, be aware of the possible pitfalls !*
- Their classification is in a state of flux: *validation studies are needed !*
- New predictive factors would be hepful for a better evaluation of the prognosis and for the design of new therapeutic strategies: *more basic, translational and clinical research is required !*

INSTITUT NATIONAL DU CANCER

TENpath

Importance of networks
and collaborative studies