Classification and Tumor Biology of NETs

By
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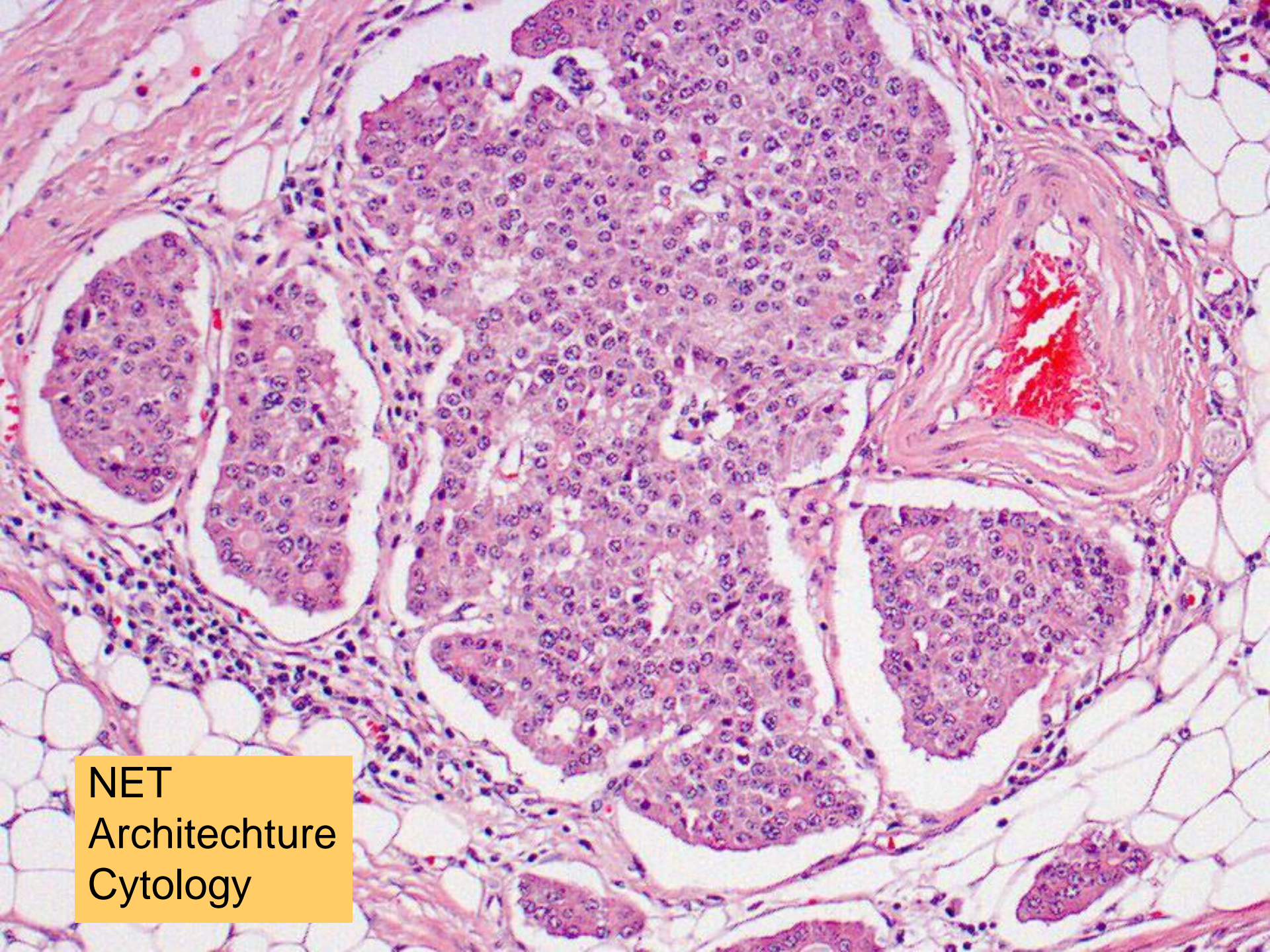
Classification of NETs
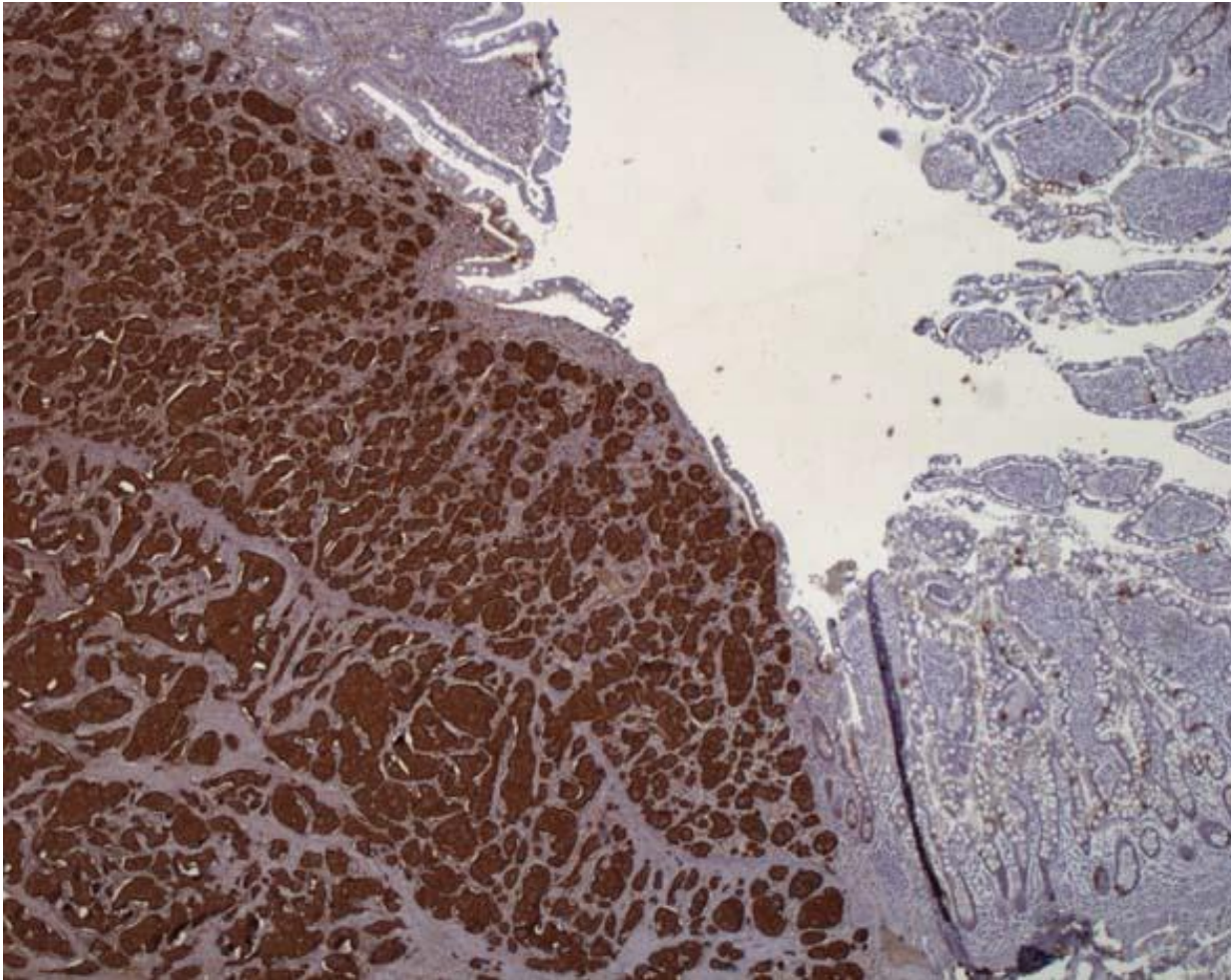
Type? Which organ? Hormones?

Grade? Benign, Well-diff., Poorly diff? G1/G2/G3

Stage? pTNM?

NET
Architechture
Cytology

NET IHC

Epithelial markers (cytokeratins)
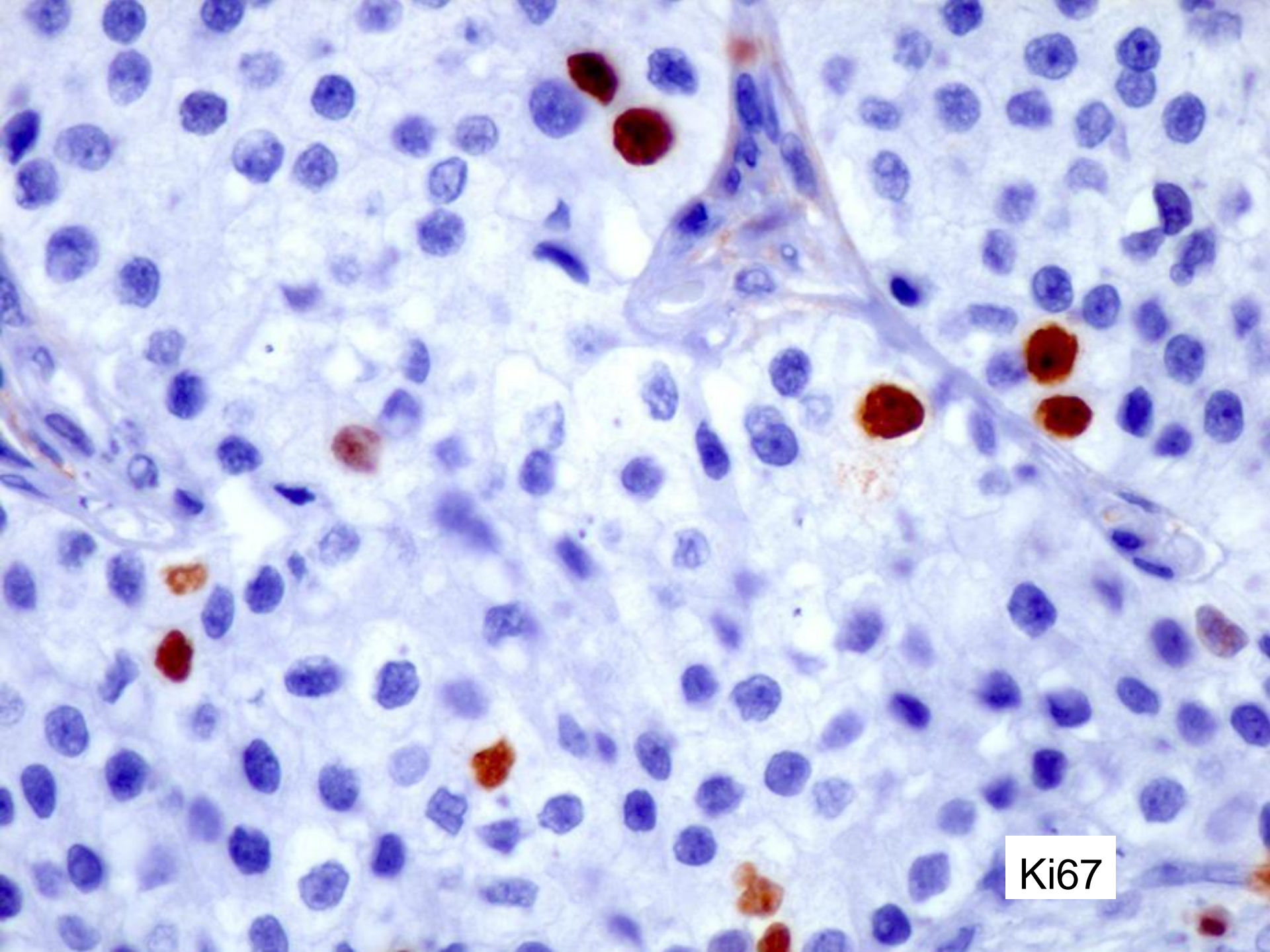Neurosecretory granules (CgA > Syn > SV2 > NSE > CD56)
Hormones

NET Grade, ENETS

Differentiated NETs

Well-demarcated, little atypia, few mitoses, often hormone production, low proliferation

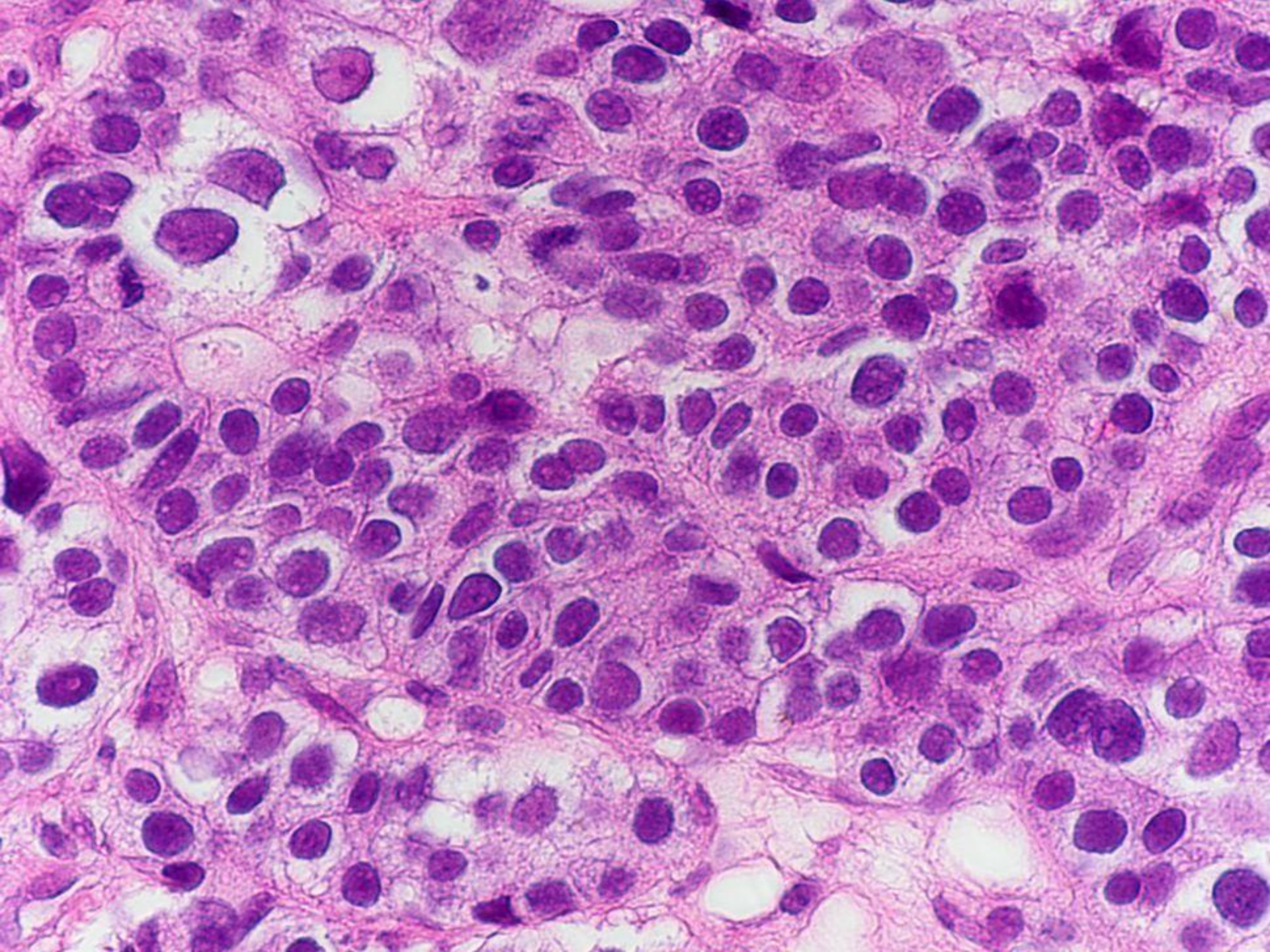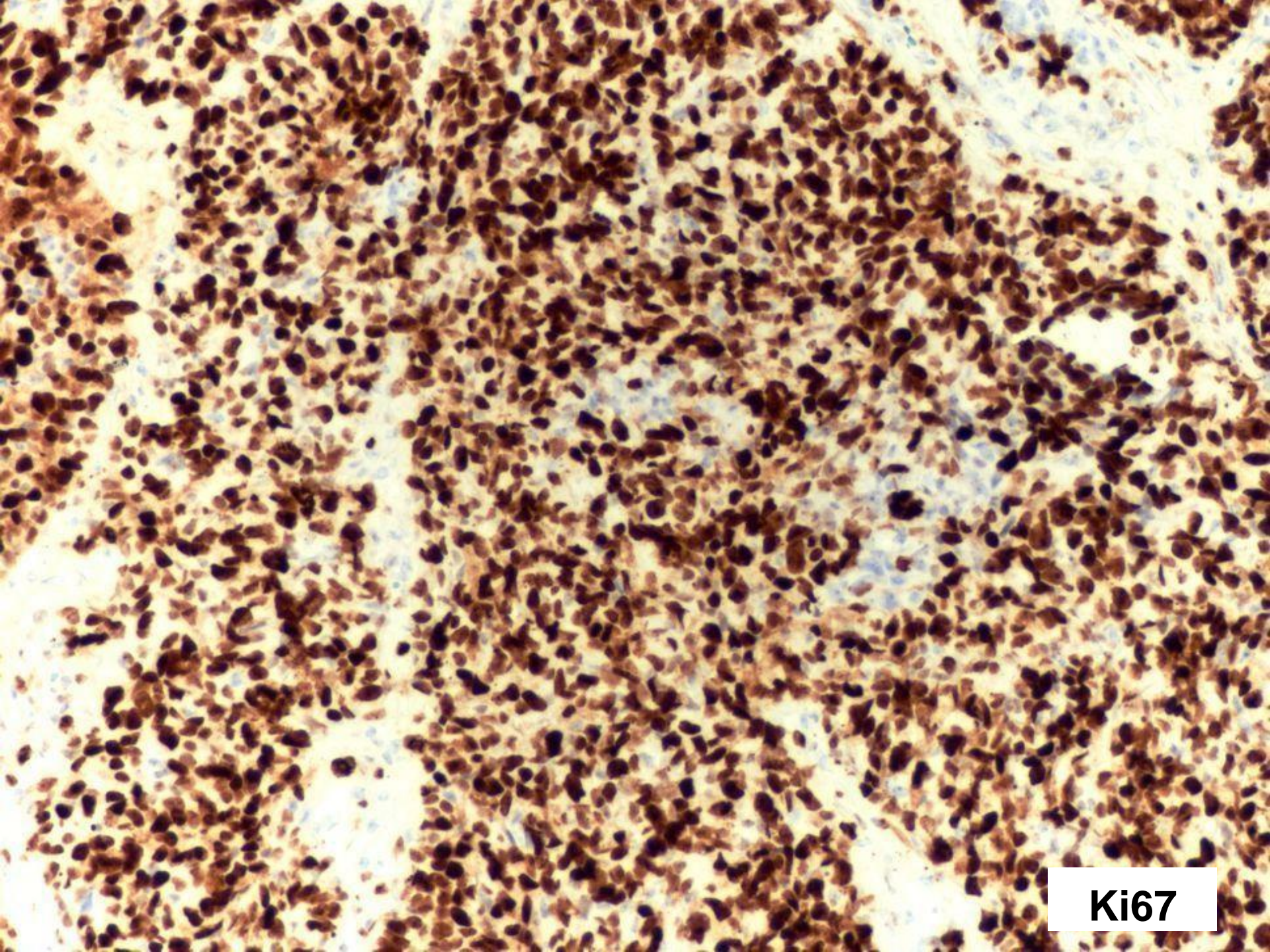|	mitosis (10 HPF)	Ki67-index
Grade 1	1	=< 2%
Grade 2	2-20	2-20%

(Rindi et al., 2006)

Ki67

NET Grade, ENETS

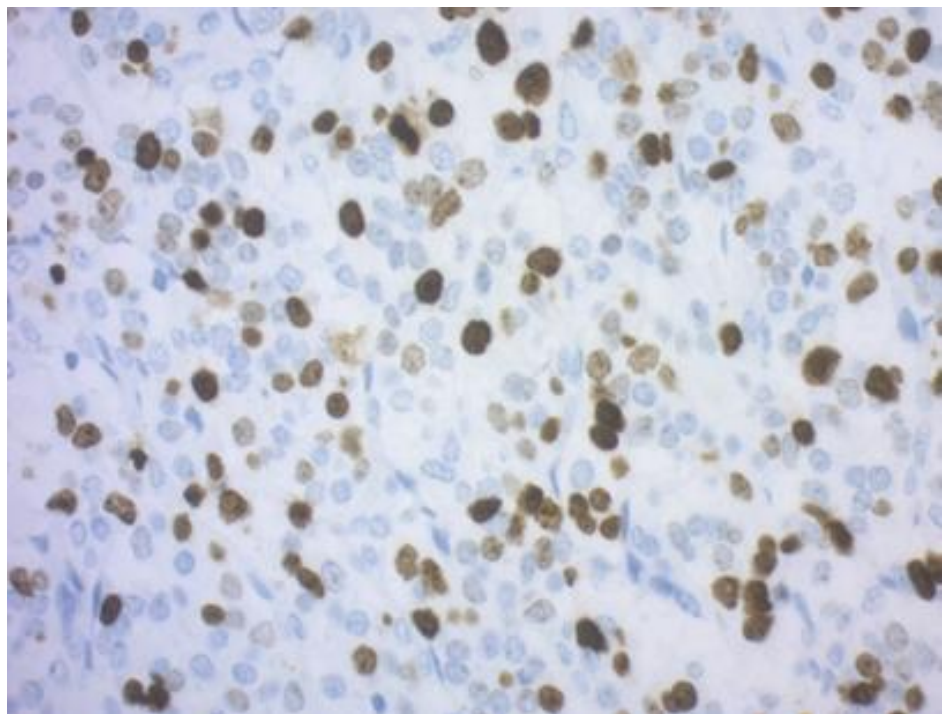## 2. Poorly differentiated neuroendocrine carcinoma (NEC)

Big, diffuse growth, infiltrative, atypia, plenty of mitosis, high proliferation, rarely hormone production, sometimes paramalignant phenomena

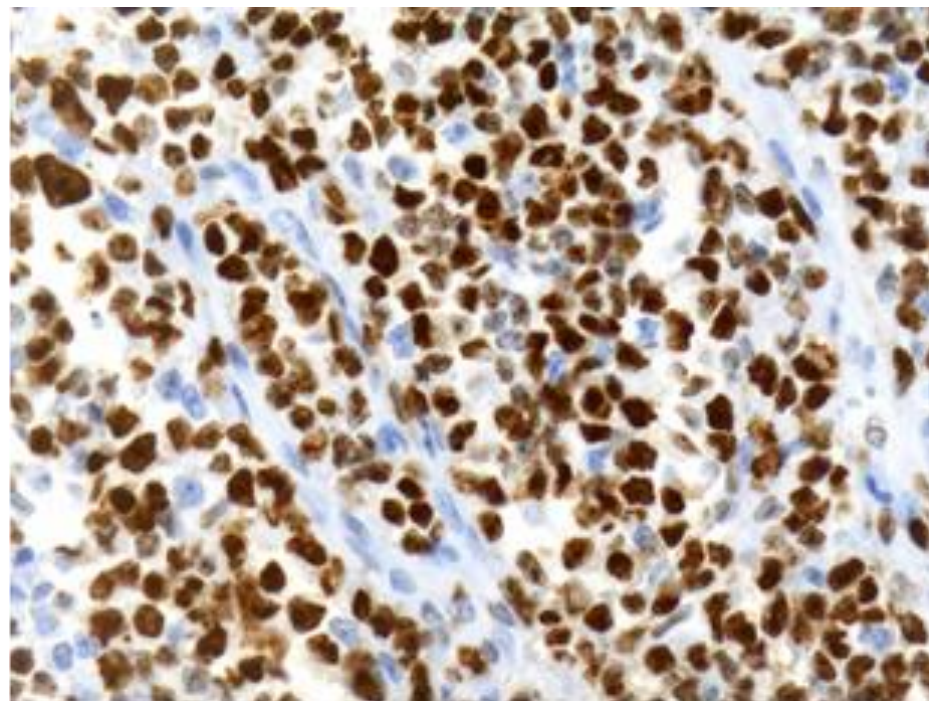|	mitosis (10 HPF)	Ki67-index
Grade 3	>20	>20%

Ki67

Ki-67 40%.   Ki-67 90%

Correlation between the proliferation rate (Ki-67) and response rate/ survival?

ROC curve analyse for cut-off for response rate: Ki-67; 55%

ROC = receiver operating characteristic

ROC of KI67 wrt. response rate (N=42)
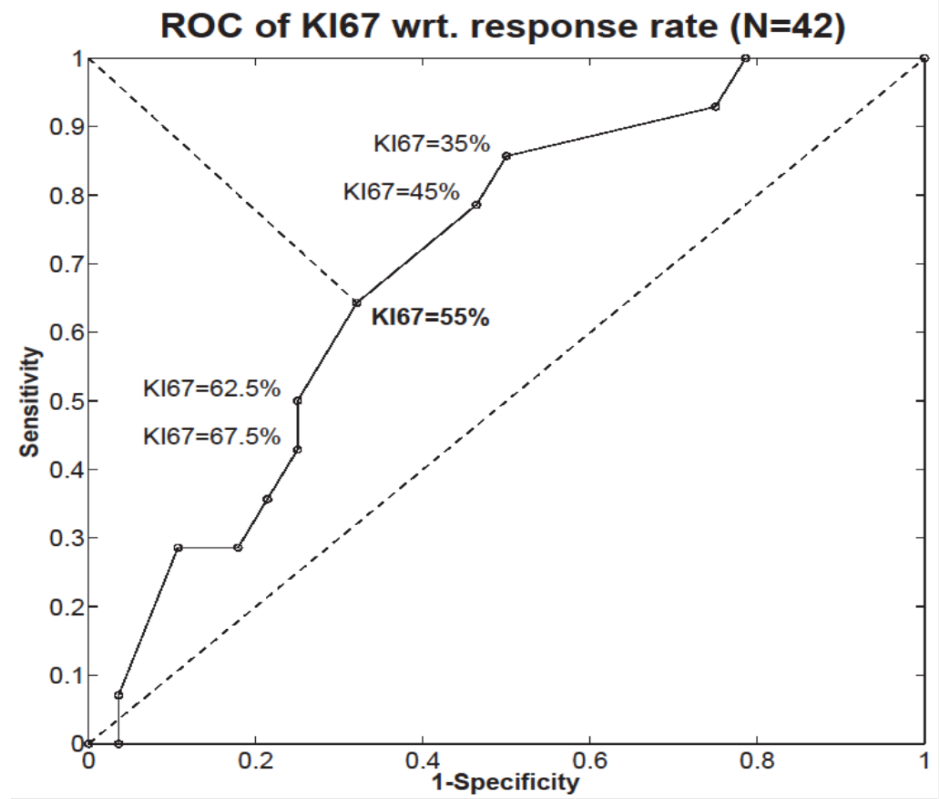
Ki-67	PR/CR	SD	PD	PFS (95% CI)	OS (95% CI)
<55% (n=136)	15%	47%	38%	4 m (3.2-4.8)	14 m (10.7-17.3)
≥55% (n=154)	42%	24%	34%	4 m (3.1-4.9)	10 m (8.4-11.6)

Nordic NEC

OS by Ki-67

Survival Functions
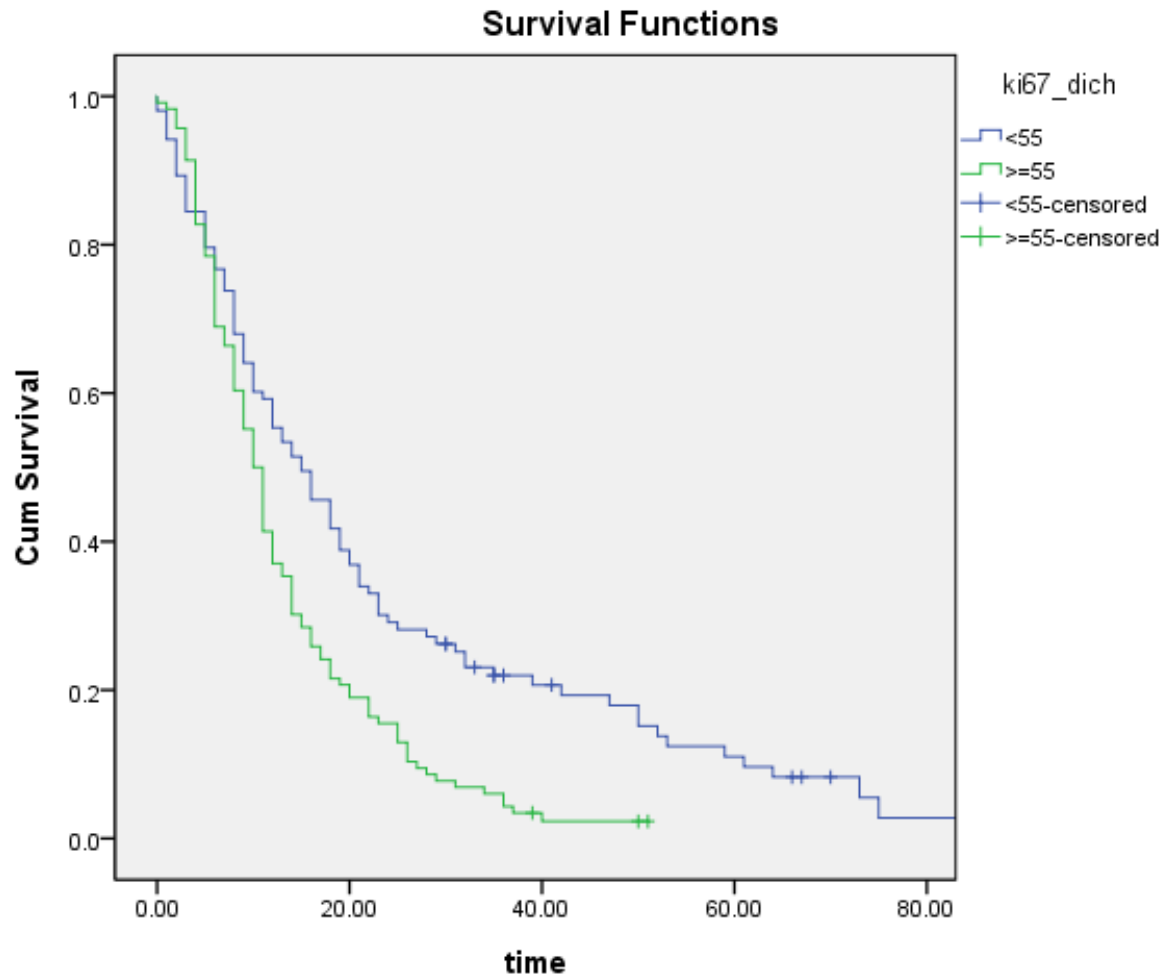
MANEC

Mixed adeno- neuroendocrine carcinoma

At least 30% of either component, adenocarcinoma and NEC,
By IHC and morphology..

DD: Adenocarcinoma with no signs of NE-differentiation
        NEC with scattered mucin droplets
        Adenocarcinoma with scattered CgA-positive NE-cells

Type?

fore-gut (-> duodenum)
        med.thyr.ca
        bronkial/lung carcinoid
        ECLoma
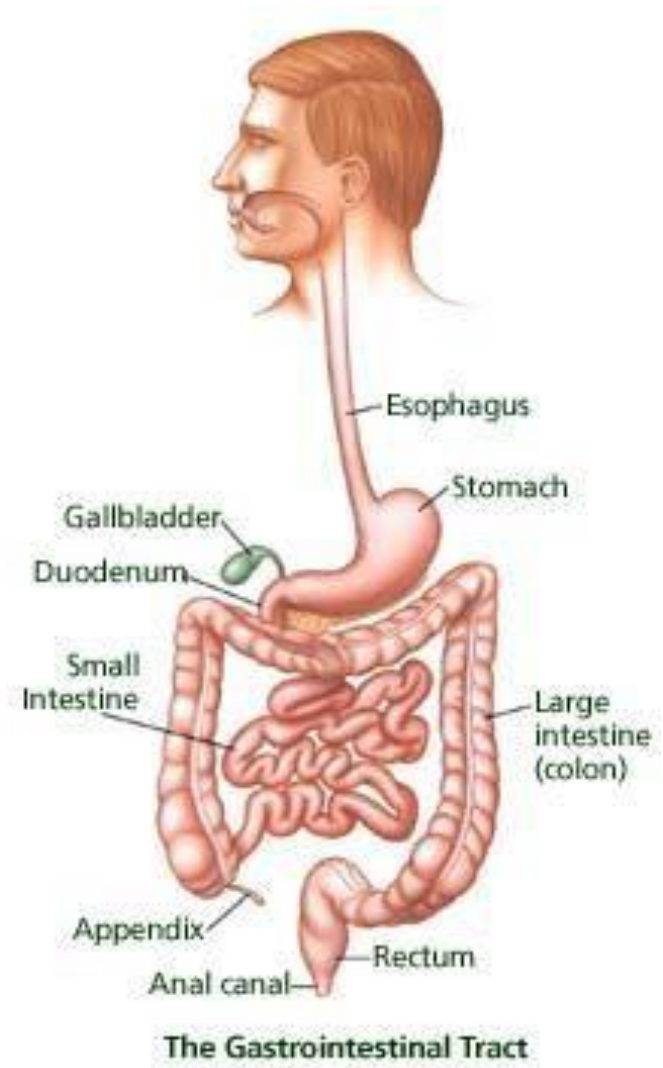   pancreatic NETs
        gastrinoma

mid-gut
        midgut carcinoid/
        small intestinal NET

hind-gut
        hind-gut carcinoid/
        rectal NET

The Gastrointestinal Tract
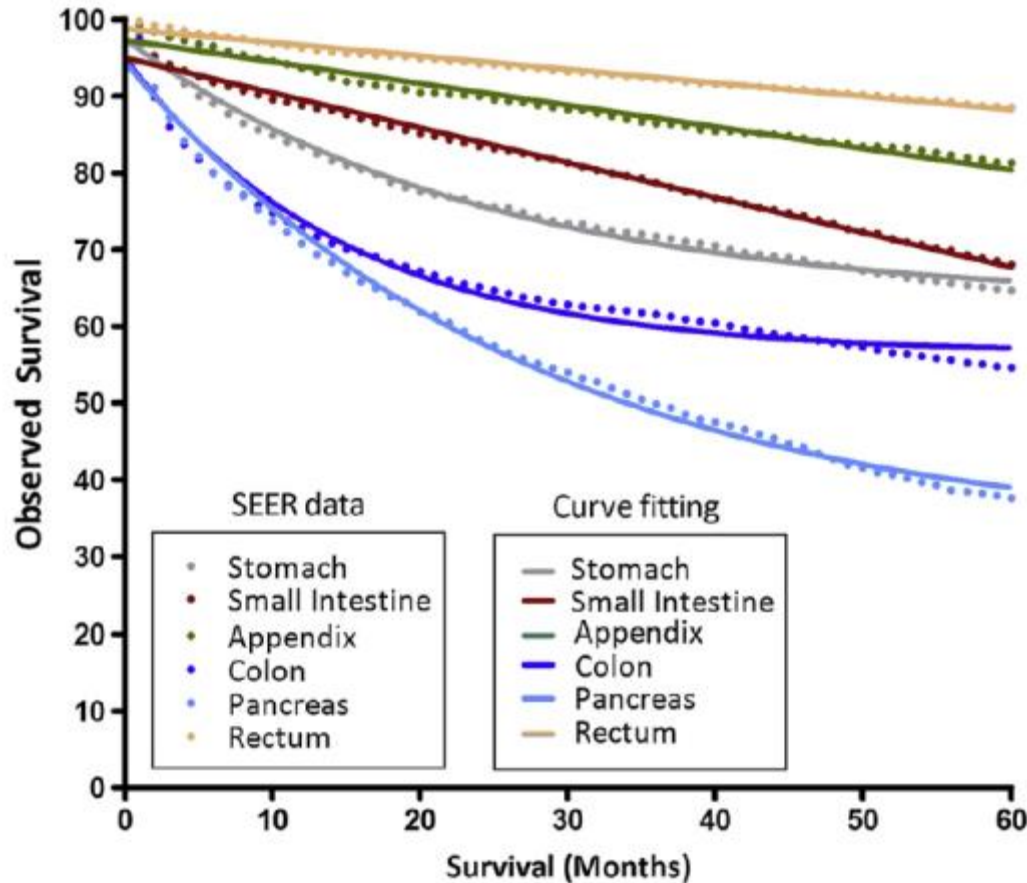
Observed 5-Year Survival for GEP-NET Primary Sites*

5-year survival rate for GEP-NET: 68.1%

- Pancreas: 37.6%
- Colon: 54.6%
- Stomach: 64.1%
- Small intestine: 68.1%
- Appendix: 81.3%
- Rectum: 88.5%

50% of patients have died at:

- 10.3 mo (colonic NETs)
- 16.7 mo (gastric NETs)
- 18.9 mo (pancreatic NETs)

*SEER 17 registry, 1973 - 2007

Classification of Lung NETs

1991 Travis et al; 2004 WHO

- Typical carcinoid
- Atypical carcinoid
- Large cell neuroendocrine carcinoma (LCNEC)
- Small cell neuroendocrine carcinoma (SCNEC)

Histologic Criteria for Pulmonary Neuroendocrine Tumors

	Typical Carcinoid	Atypical Carcinoid	LCNEC	SCNEC
Mitoses	<2/10HPF	2-10/10 HPF	≥11/10 HPF; Median, 70/10 HPF	≥11/10 HPF Median, 80/10 HPF
Necrosis	—	+ (punctate)	+ (large zones)	+ (large zones)
Nuclear pleomorphism, hyperchromatism	Uncommon	Sometimes	Frequent	Small cells (pleomorphic cells are rare unless mixed SCNEC/LCNEC)
N/C ratio	Moderate	Moderate	Low	High
Nucleoli	Occasional	Common	Very common	Absent or inconspicuous
Nuclear chromatin	Finely granular	Finely granular	Usually vesicular, may be finely granular	Finely granular
Shape	Round, oval, spindled	Round, oval, spindled	Round, oval, polygonal	Round, oval, spindled
Nuclear smear	No	No	Uncommon	Common
Azzopardi effect*	No	No	Uncommon	Occasional
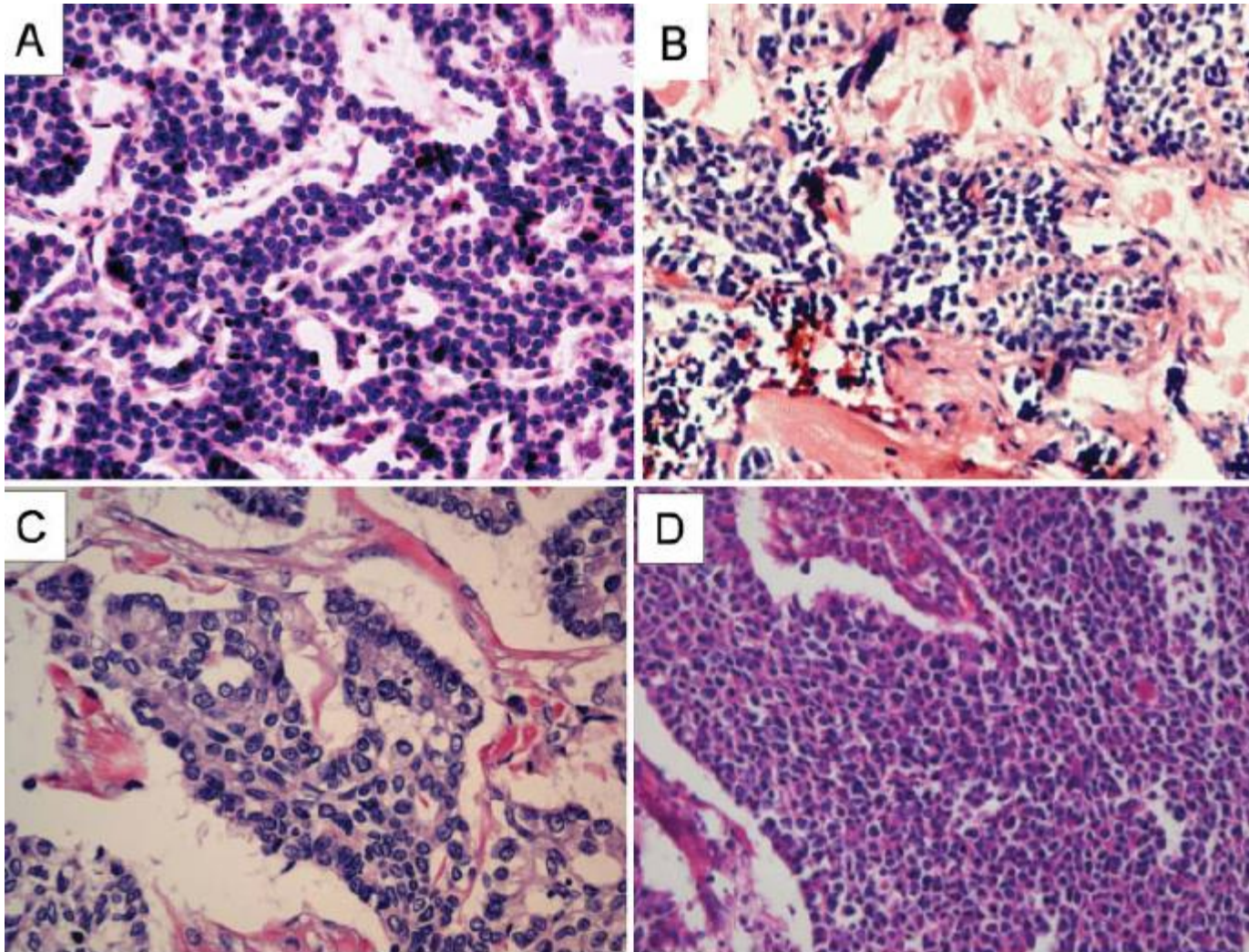
Lung Carcinoids
New grading system

Grade	Mitotic count (10 HPF)	Ki67 (%)	Necrosis (%)
G1	2	<4	no
G2	>2 – 47	4 – <25	<10
G3	>47	≥25	>10

Staging of NET According to Tumour-Node-Metastasis (TNM)

- The European Neuroendocrine Tumour Society (ENETS) and   American Joint Committee on Cancer (AJCC) have developed TNM staging systems

- Staging systems are developed for the following tumour locations:

- Gastric, duodenum/ampulla/proximal jejunum, pancreas[1]
- Lower jejunum and ileum, appendix, and colon and rectum[2]

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

T – primary tumour
x   primary tumour cannot be assessed
0   no evidence of primary tumour
1   tumour invades mucosa or submucosa and size ≤1 cm
2   tumour invades muscularis propria or size >1 cm
3   tumour invades subserosa
4   tumour invades peritoneum/other organs
     for any T add (m) for multiple tumours

N – regional lymph node metastasis
x   regional lymph nodes cannot be assessed
0   no regional lymph node metastasis
1   regional lymph node metastasis

M – distant metastasis
X   distant metastasis cannot be assessed
0   no distant metastases
1   distant metastasis

Stage:			
stage 0:	Tis	N0	M0 (stage 0: ENETS only)
stage I:	T1	N0	M0
stage IIa:	T2	N0	M0
stage IIb:	T3	N0	M0
stage IIIa:	T4	N0	M0
stage IIIb:	any T	N1	M0
stage IV:	any T	any N	M1

Grade	Ki67 index	Mitotic index (mitoses/10 HPF)
G1	≤2%	<2
G2	3–20%	2–20
G3	>20%	>20

Staging of Digestive NENs
According to ENETS/WHO/AJCC
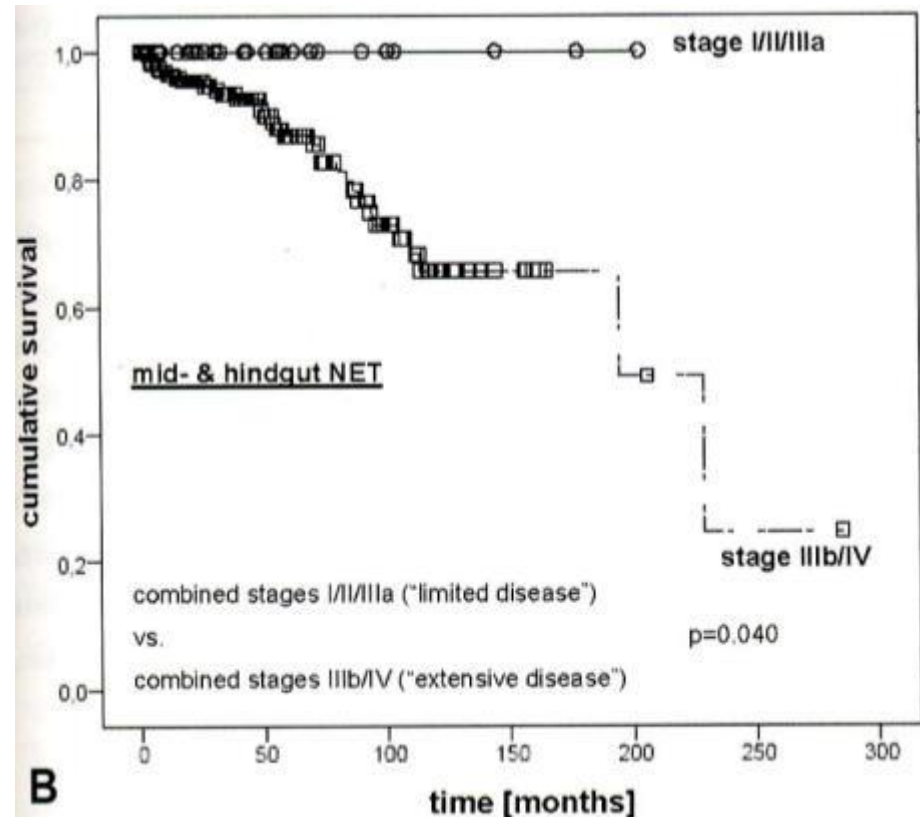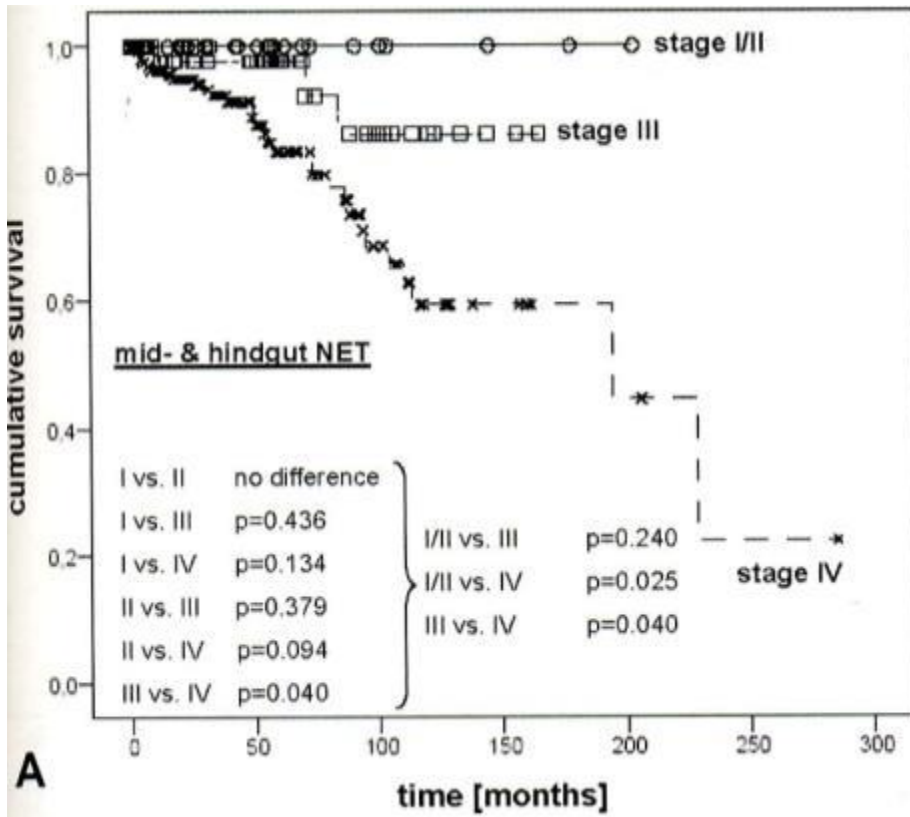
Neuroendocrine Tumors of Midgut and
Hindgut Origin: Tumor-Node-Metastasis
Classification Determines Clinical Outcome

Henning Jann, MD[1]; Stephanie Roll, PhD[2]; Anne Couvelard, MD[3]; Olivia Hentic, MD[4]; Marianne Pavel, MD[1];
Jacqueline Müller-Nordhorn, MD[2]; Martin Koch, MD[5]; Christoph Röcken, MD[5,6]; Guido Rindi, MD[7];
Philippe Ruszniewski, MD[4]; Bertram Wiedenmann, MD[1]; and Ulrich-Frank Pape, MD, MD[1]

Tumor biology is related to the localization of the primary tumor

Lessons from Hereditary Syndromes

Table 1. Genetic syndromes associated with the development of low-grade neuroendocrine tumors.

Syndrome	Gene (location)	Tumor location	
MEN1	MENIN	Pancreas, lung, thymus	Menin as part of a histone methyltransferase complex regulates gene transcription
Tuberous sclerosis 2	TSC2 (16p13.3)	Pancreas	Loss leads to constitutive mTOR activation
Neurofibromatosis	NF-1 (17q11.2)	Ampulla of Vater, duodenum, mediastinum	Loss leads to constitutive mTOR activation
von Hippel–Lindau	VHL (3p26–p25)	Pancreas	Loss lead to increase HIF activity

MEN1, multiple endocrine neoplasia type I syndrome; HIF, hypoxia-induced factor.

Validation Set

Somatic Mutations in 68 PETs

| Gene	Frequency (%)	Type of mutations[§]
MEN1	30/68 (44.1%)	18 indels; 5 ns; 2 sp; 5 mis
DAXX	17/68 (25%)	11 indels; 4 ns
ATRX	12/68 (17.6%)	6 indels; 3 ns
PTEN	5/68 (7.3%)	2 indels; 3 mis
TSC2	6/68 (8.8%)	1 indels; 1 ns; 3 mis
PIK3CA	1/68 (1.4%)	1 mis

mTOR pathway

[§] Indels, insertion or deletions; ns, nonsense; sp, splice-site mutations mis,missense.
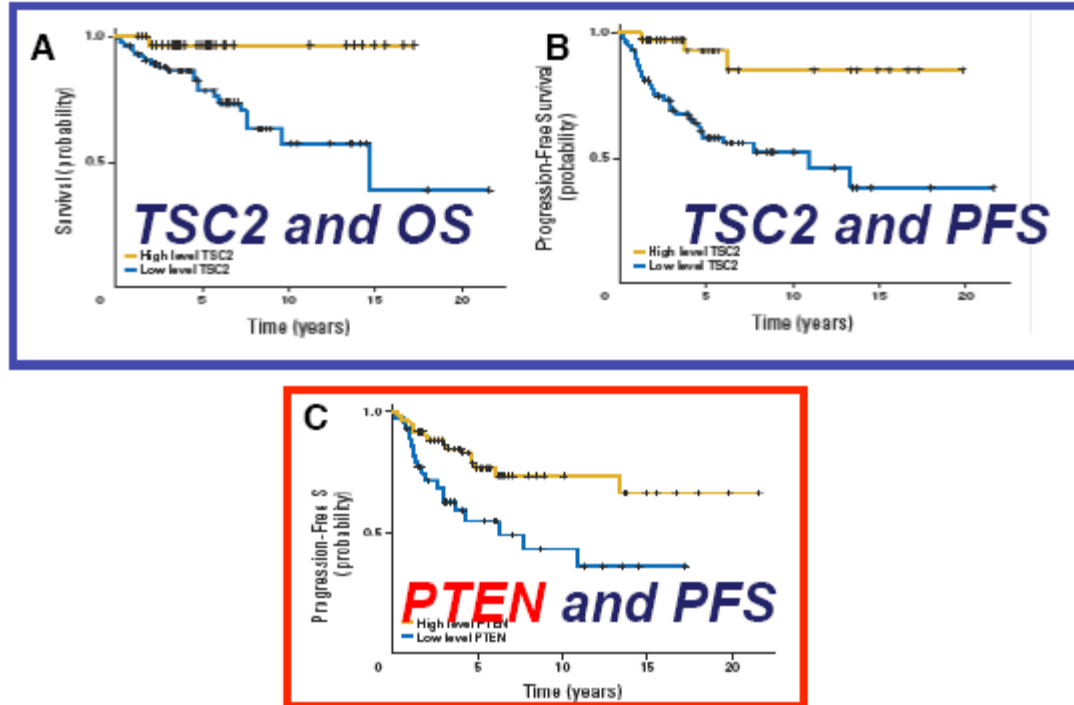
Somatostatin Receptors

Somatostatin receptor 2 is predominantly expressed in NETs, with very strong staining in 30% of the patients
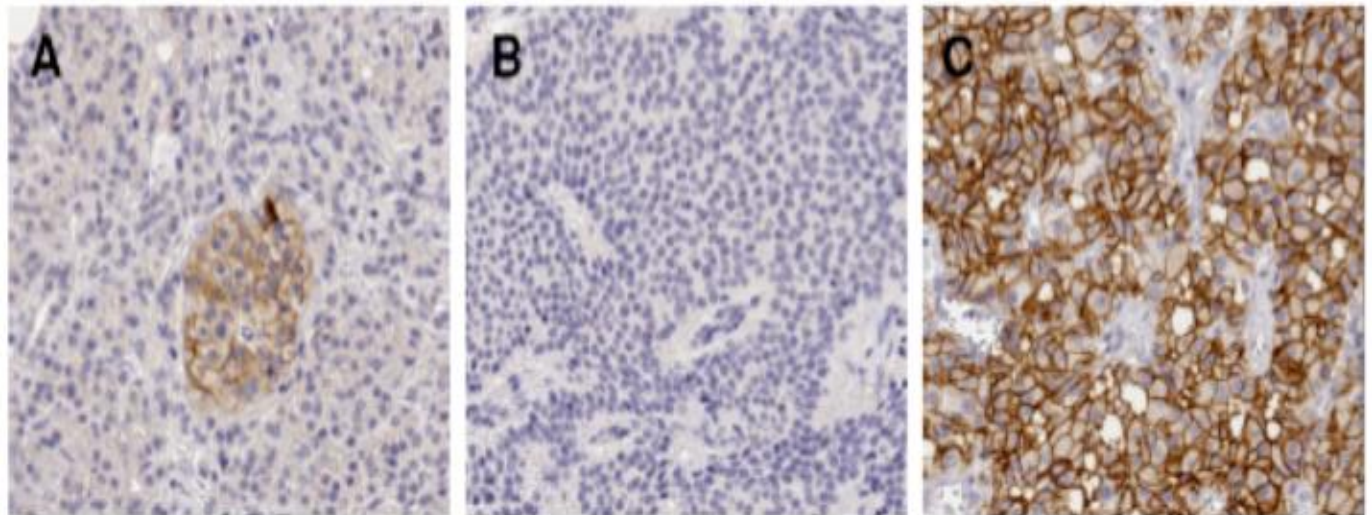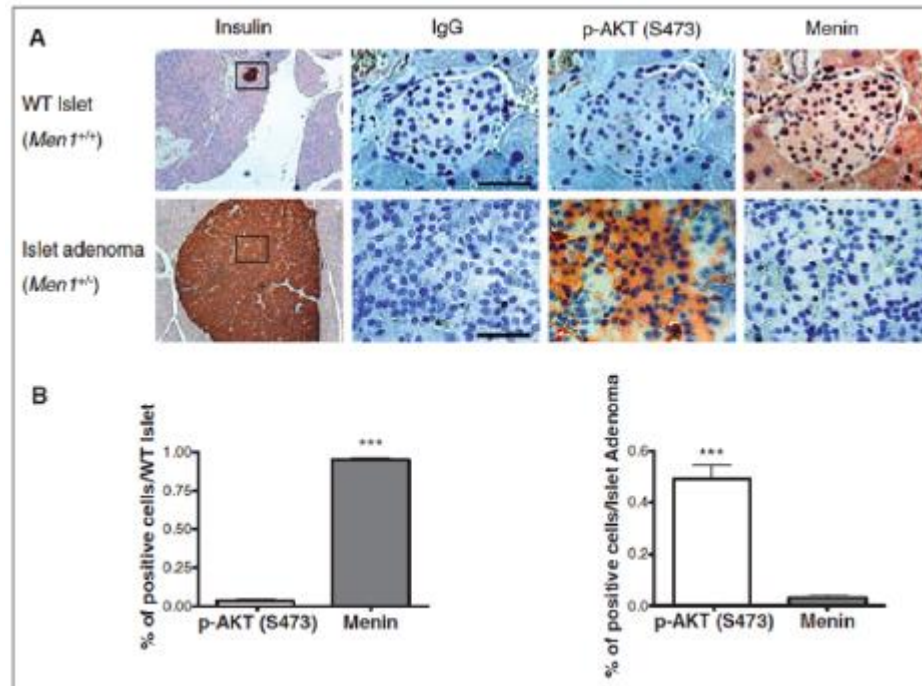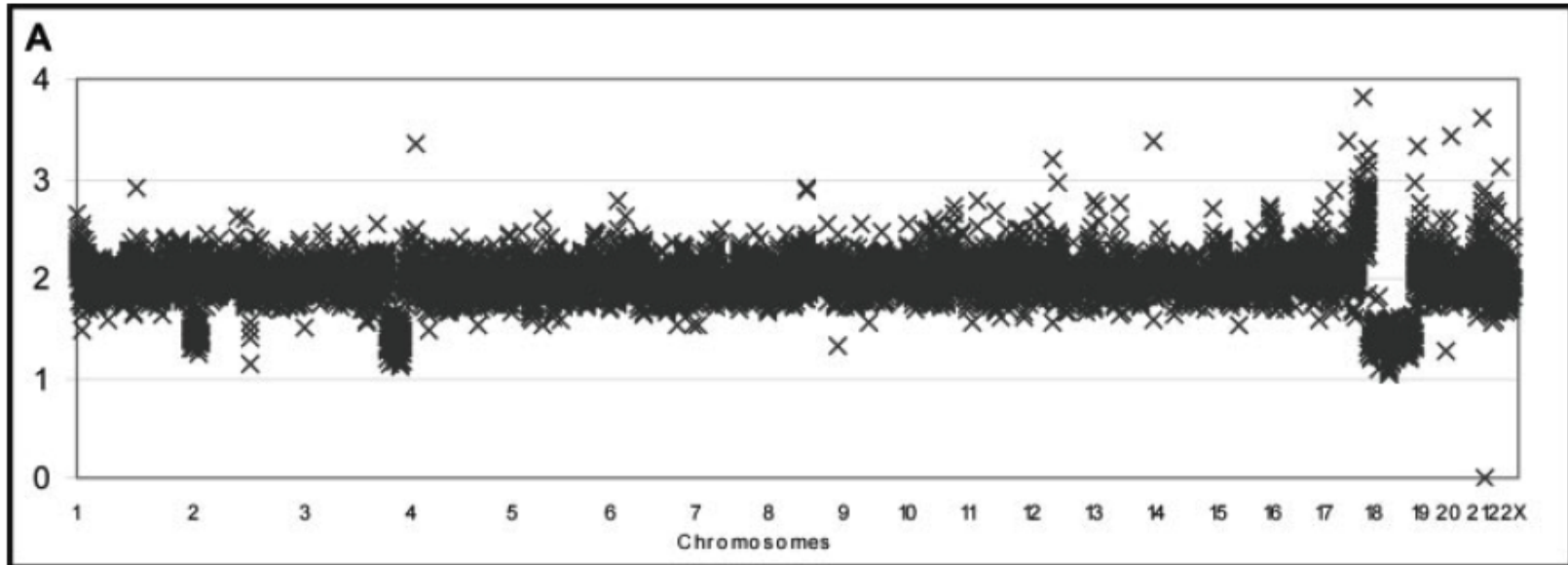
What we know: pNET Pathways
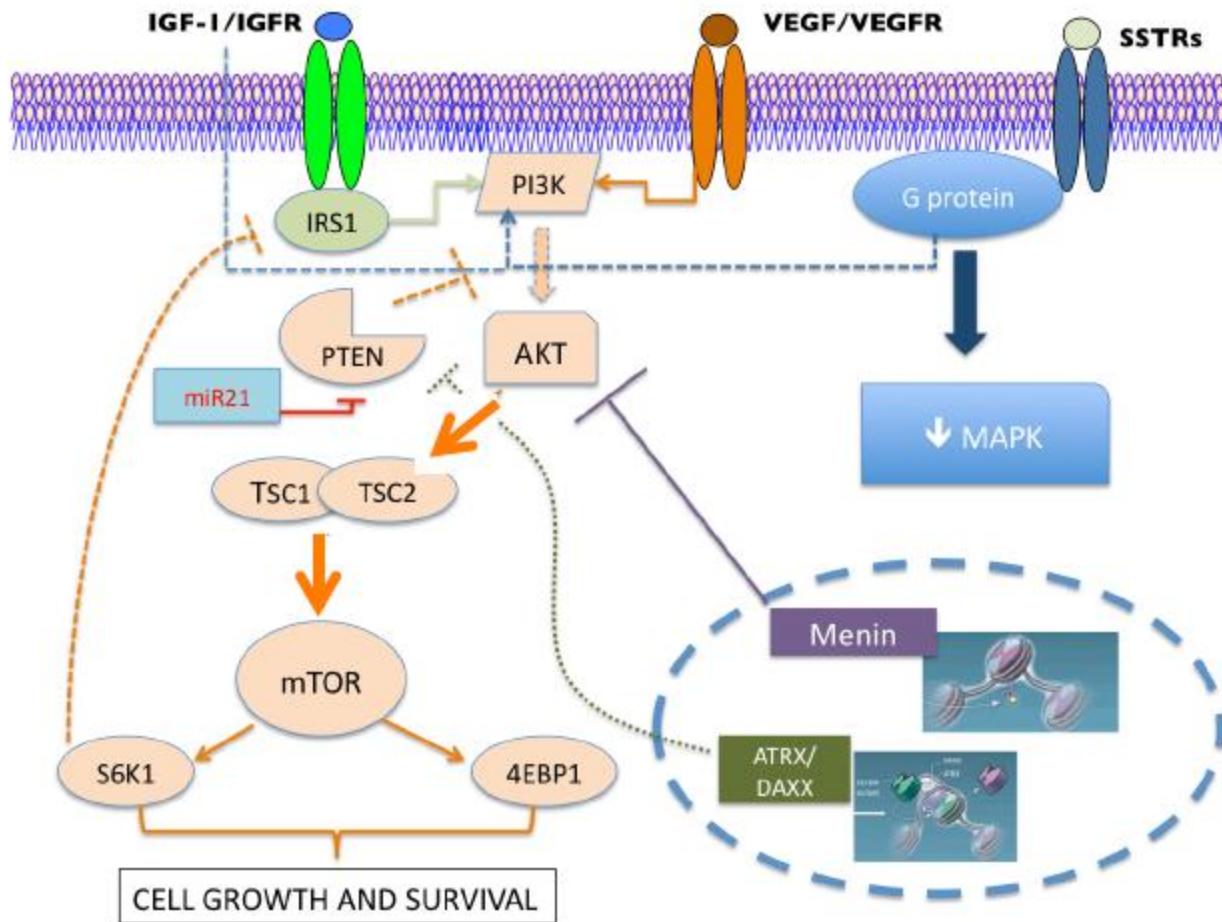
TSC2 and PTEN Down-Regulation Correlates with Poorer Prognosis

The Tumor Suppressor Protein Menin Inhibits AKT Activation by Regulating its Cellular Localization

Wang et al. Cancer Res 2010

Loss on chromosome 18

Kim et al, Genes, Chromosomes and cancer, 2008

Molecular Genetics of Lung-NET

MEN-1 gene mutations   TC   ≈ 47%

                                      AC   ≈ 70%

                                      LCNEC   ≈   52%

                                      SCNEC   ≈   41%
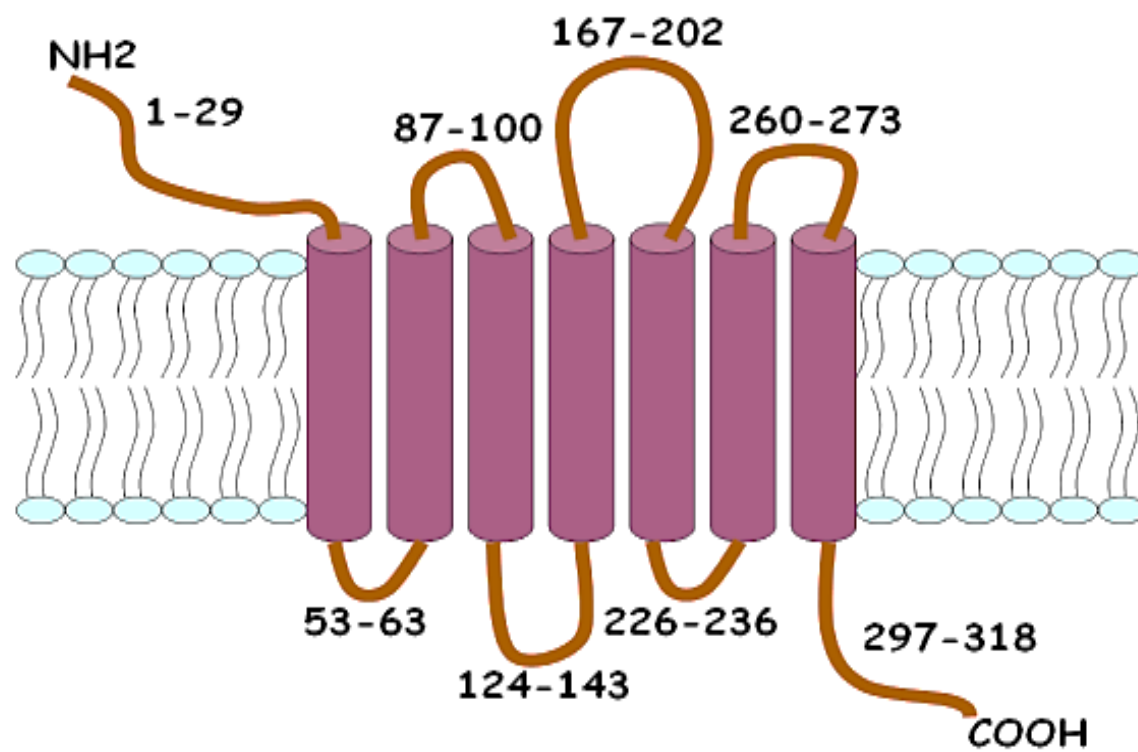
p-53 mutations   TC   ≈ 40%

                                      AC   ≈ 29%

                                      LCNEC   ≈   80%

                                      SCNEC   ≈   75%

E-caderin and beta-catenins are expressed in ≈ 80% of LCNEC/SCNEC compared with ≈ 40% in TC/AC
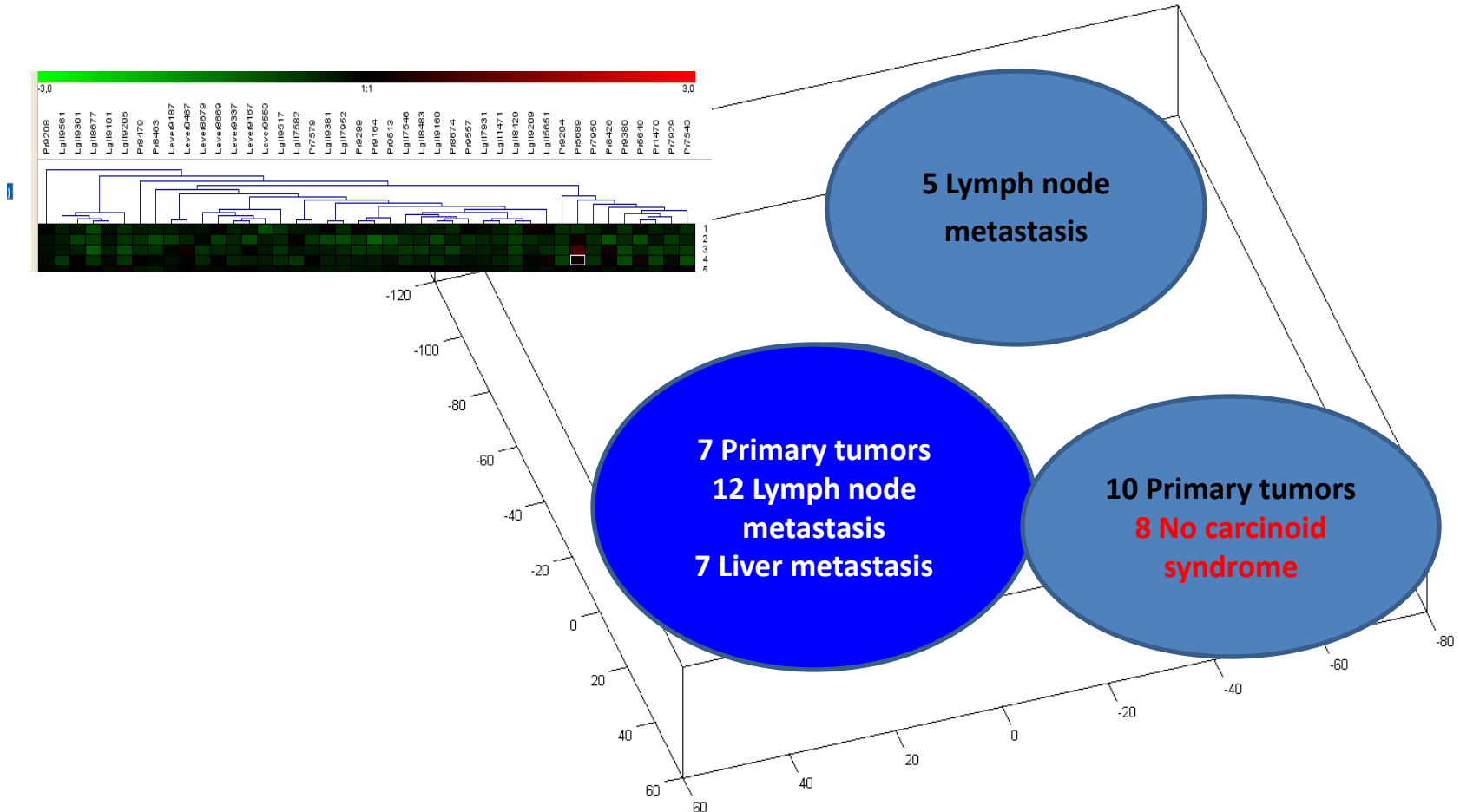
Targets

- Somatostatin receptors

- Interferon receptors

- Growth factor receptors
  - EGFR/HER-2
  - IGFR1

- Receptor tyrosine kinases

- Intracellular kinases

- Enzymes

- Circulating ligands

- Others

Subgroups of SI-NET?
Genetically driven individual treatment?

Future outlook

❑ Re-classification of NET G3

❑ Next Generation Sequencing (NGS)

❑ Epigenetics

❑ New biomarkers (miRNA, Multi-Transcript gen analysis)