Classification and Tumor Biology of NETs

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Classification of NETs

Type? Which organ? Hormones?


Stage? pTNM?
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

<table>
<thead>
<tr>
<th>Grade</th>
<th>mitosis (10 HPF)</th>
<th>Ki67-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>&lt;= 2%</td>
</tr>
<tr>
<td>Grade 2</td>
<td>2-20</td>
<td>2-20%</td>
</tr>
</tbody>
</table>

(Rindi et al., 2006)
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%
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
<table>
<thead>
<tr>
<th>Ki-67</th>
<th>PR/CR</th>
<th>SD</th>
<th>PD</th>
<th>PFS (95% CI)</th>
<th>OS (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;55%  (n=136)</td>
<td>15%</td>
<td>47%</td>
<td>38%</td>
<td>4 m (3.2-4.8)</td>
<td>14 m (10.7-17.3)</td>
</tr>
<tr>
<td>≥55%  (n=154)</td>
<td>42%</td>
<td>24%</td>
<td>34%</td>
<td>4 m (3.1-4.9)</td>
<td>10 m (8.4-11.6)</td>
</tr>
</tbody>
</table>

Nordic NEC
Patients with Ki-67 <55% had longer median survival (14 months) than patients with Ki-67 >55% (10 months) (p<0.001).
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
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

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

Bertino et al. Cancer October 1, 2009
## Lung Carcinoids

### New grading system

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic count (10 HPF)</th>
<th>Ki67 (%)</th>
<th>Necrosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>2</td>
<td>&lt;4</td>
<td>no</td>
</tr>
<tr>
<td>G2</td>
<td>&gt;2 – 47</td>
<td>4 – &lt;25</td>
<td>&lt;10</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;47</td>
<td>≥25</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

Rindi et al, Endocrine-Related Cancer 2014
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
  - Lower jejunum and ileum, appendix, and colon and rectum

### 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: T1s 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

<table>
<thead>
<tr>
<th>Grade</th>
<th>Ki67 index</th>
<th>Mitotic index (mitoses/10 HPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>≤2%</td>
<td>&lt;2</td>
</tr>
<tr>
<td>G2</td>
<td>3–20%</td>
<td>2–20</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20%</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>
Staging of Digestive NENs According to ENETS/WHO/AJCC

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

Cancer 2011;117:3332-41.
Tumor biology is related to the localization of the primary tumor.
Lessons from Hereditary Syndromes

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

MEN1, multiple endocrine neoplasia type 1 syndrome; HIF, hypoxia-induced factor.

Adapted from Yao et al. Best Pract Res Clin Endocrin Metab 2007
### Validation Set

**Somatic Mutations in 68 PETs**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency (%)</th>
<th>Type of mutations$^\S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN1</td>
<td>30/68 (44.1%)</td>
<td>18 indels; 5 ns; 2 sp; 5 mis</td>
</tr>
<tr>
<td>DAXX</td>
<td>17/68 (25%)</td>
<td>11 indels; 4 ns</td>
</tr>
<tr>
<td>ATRX</td>
<td>12/68 (17.6%)</td>
<td>6 indels; 3 ns</td>
</tr>
<tr>
<td>PTEN</td>
<td>5/68 (7.3%)</td>
<td>2 indels; 3 mis</td>
</tr>
<tr>
<td>TSC2</td>
<td>6/68 (8.8%)</td>
<td>1 indels; 1 ns; 3 mis</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>1/68 (1.4%)</td>
<td>1 mis</td>
</tr>
</tbody>
</table>

$mTOR$ pathway

$^\S$ 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

<table>
<thead>
<tr>
<th>Category</th>
<th>TC</th>
<th>AC</th>
<th>LCNEC</th>
<th>SCNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>≈ 47%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>≈ 70%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCNEC</td>
<td>≈ 52%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCNEC</td>
<td>≈ 41%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-53 mutations

<table>
<thead>
<tr>
<th>Category</th>
<th>TC</th>
<th>AC</th>
<th>LCNEC</th>
<th>SCNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>≈ 40%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>≈ 29%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCNEC</td>
<td>≈ 80%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCNEC</td>
<td>≈ 75%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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
Olfactory Receptor 51E1 (OR51E1)

Olfactory receptors (ORs) (Buck L and Axel R, Cell 1991)
Subgroups of SI-NET?
Genetically driven individual treatment?

- 5 Lymph node metastasis
- 7 Primary tumors
  - 12 Lymph node metastasis
- 7 Liver metastasis
- 10 Primary tumors
  - 8 No carcinoid syndrome
Future outlook

- Re-classification of NET G3
- Next Generation Sequencing (NGS)
- Epigenetics
- New biomarkers (miRNA, Multi-Transcript gen analysis)