Gastrointestinal Neuroendocrine Tumors: Current Concepts

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**Developmental Origin of (Neuro)Endocrine Cells and Organs**

- **Ectoderm**
  - Anterior pituitary
  - Merkel cells (?)
- **Neural Crest**
  - Adrenal medulla
  - Paraganglia
  - C cells of the thyroid
- **Mesoderm**
  - Adrenal cortex

- **Endoderm**
  - Thyroid
  - Parathyroid
  - Pancreatic islets
  - Gastrointestinal neuroendocrine cells
  - Pulmonary neuroendocrine cells

Neural Crest Migration

“Neuroendocrine” vs. “Endocrine”
Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors, 2007

- Public and physician education
- Identification of molecular markers for early diagnosis and therapeutic monitoring
- Improved imaging modalities and molecular prognostication
- Development of a standardized pathological classification system
- Creation of regional centers of expertise

Modlin I et al., JNCI 2008; 100: 1282-9

Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors, 2007

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- Creation of regional centers of expertise

Modlin I et al., JNCI 2008; 100: 1282-9

Neuroendocrine Tumors

- Diverse but related group of tumors
  - Lung, thymus, pancreas, GI tract, other sites
- Microscopic “organoid” patterns
- Characteristic nuclear morphology
- Immunohistochemical evidence of neuroendocrine differentiation (chromogranin / synaptophysin)
- Can be either well differentiated or poorly differentiated
Differentiation:

“Extent of resemblance of the cells of a neoplasm to their normal cellular counterparts”

- Usually closely linked to grade (for NETs)

Differentiation vs. Grade in NETs

- **Differentiation = resemblance to normal**
  - Well differentiated  Poorly differentiated
- **Grade = inherent biological aggressiveness**
  - e.g., benign, low grade malignant, high grade malignant
  - Low grade  Intermediate grade  High grade
- Correlated but not synonymous

- Poorly differentiated → High grade
- Low grade → Well differentiated

Grading of NETs

- Based primarily on proliferative rate (mitoses / Ki67)
  - G1 vs. G2 vs. G3
  - Low grade vs. Intermediate grade vs. High grade
  - “Typical carcinoid” vs. “Atypical carcinoid” vs. “High grade neuroendocrine carcinoma”

- Generally correlates well with prognosis
- ? Dynamic feature of NETs

- Criteria vary by site of origin
Grade vs. Stage in NETs

- **Grade** = inherent biological aggressiveness
  - e.g., benign, low grade malignant, high grade malignant
- **Stage** = extent of disease
  - e.g., organ confined, locally invasive, metastatic, etc.
- Both are prognostically relevant
- Can be independent

“Classification Systems” for NETs

- Combination of nomenclature, grade, and stage
- 2000/2004 WHO classifications
- Provide “overall” prognostic stratification

Well Differentiated vs. Poorly Differentiated NETs

- Two different families of neoplasms
- Both share neuroendocrine differentiation
- Can be difficult to distinguish
- Fundamentally different
  - Cell of origin
  - Relationship to non-NE neoplasia
  - Genetic background
  - Clinical aggressiveness
  - Treatment

<table>
<thead>
<tr>
<th></th>
<th>Well Diff.</th>
<th>Poorly Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association with MEN1?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Association with neuroendocrine bodies and tumorlets?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Association with smoking?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Association with NSCLC component?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Combined with other grade?</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Well Differentiated vs. Poorly Differentiated Neuroendocrine Tumors of the Lung: Molecular Differences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Well Diff.</th>
<th>Poorly Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoblastoma Gene</td>
<td>Normal</td>
<td>Abnormal (95%)</td>
</tr>
<tr>
<td>TP53 Gene</td>
<td>Normal</td>
<td>Abnormal (50%)</td>
</tr>
<tr>
<td>N-myc Oncogene</td>
<td>Normal</td>
<td>Amplified (30%)</td>
</tr>
<tr>
<td>MEN1 Gene</td>
<td>Abnormal (50%)</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Well Differentiated NETs
- Well differentiated NET (pancreas, GI tract, etc.)
- Carcinoid tumor (lung, thymus)
- (Islet cell tumor)

Poorly Differentiated NETs
- Small cell carcinoma
- Large cell neuroendocrine carcinoma
- High grade neuroendocrine carcinoma
- Mixed neuroendocrine carcinoma (with component of adenocarcinoma, squamous cell carcinoma, etc.)

Problems with the Pathology of NETs: Starting Point, 2007

- Nomenclature
  - Varied by classification system
  - Varied by primary site
  - Varied by stage of disease
- Grading systems
  - Multiple proposals; not standardized
- Staging systems
  - Largely did not exist
- What pathology data should be reported?

Diversity of NETs

- Cells of origin / differentiation
- Underlying mechanisms
  - Genetic associations
  - Trophic stimuli (e.g., gastric NETs)
- Functional status
- Range of aggressiveness
- Histologic patterns

- Carcinoid heart disease
- Glucagonoma rash
**Neuroendocrine Tumors: Nomenclature Issues**

- Multiple different systems
- “Carcinoid tumor” – archaic but entrenched
- “Neuroendocrine” *versus* “Endocrine”
- “Tumor” *versus* “Neoplasm”
- When to use “Carcinoma”
  - Tumor type specific
  - Grade specific
  - Stage specific
- Goal for nomenclature:
  - Pathologically reproducible
  - Clinically relevant

**Terminology for Neuroendocrine Tumors: Synonyms**

- Well differentiated neuroendocrine tumor
- Low / intermediate grade NET
- Grade 1 / 2 NET
- Carcinoid tumor / atypical carcinoid tumor
- Islet cell tumor
- Well differentiated neuroendocrine carcinoma
### Pulmonary Neuroendocrine Tumors: W.H.O.

<table>
<thead>
<tr>
<th></th>
<th>Mitotic Rate</th>
<th>Necrosis</th>
<th>Cell Size</th>
<th>Cytoplasm</th>
<th>Nucleoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical Carcinoid</td>
<td>0-1/10 hpf</td>
<td>No</td>
<td>Variable</td>
<td>Variable</td>
<td>Small</td>
</tr>
<tr>
<td>Atypical Carcinoid</td>
<td>2-10/10 hpf</td>
<td>Punctate</td>
<td>Variable</td>
<td>Variable</td>
<td>Small</td>
</tr>
<tr>
<td>Small Cell Carcinoma</td>
<td>&gt;10/10 hpf</td>
<td>Abundant</td>
<td>Small</td>
<td>Minimal</td>
<td>Absent</td>
</tr>
<tr>
<td>Large Cell NE Ca.</td>
<td>&gt;10/10 hpf</td>
<td>Abundant</td>
<td>Large</td>
<td>Moderate</td>
<td>Present</td>
</tr>
</tbody>
</table>

### Grading of Pulmonary Neuroendocrine Tumors

<table>
<thead>
<tr>
<th>Grade</th>
<th>Well Differentiated</th>
<th>Poorly Differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Grade</td>
<td>Carcinoid Tumor</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>Atypical Carcinoid Tumor</td>
<td></td>
</tr>
<tr>
<td>High Grade</td>
<td>Small Cell Carcinoma</td>
<td>Large Cell NE Carcinoma</td>
</tr>
</tbody>
</table>

### Survival of Pulmonary Neuroendocrine Tumors: 158 Cases (AFIP)

![Survival Curve Graph]

- TC: Typical Carcinoid
- AC: Atypical Carcinoid
- LCNEC: Large Cell NE Carcinoma
- SCLC: Small Cell Carcinoma
Grading of Pancreatic Neuroendocrine Tumors Using Mitotic Rate and Necrosis

Low grade (n= 36)
No necrosis AND < 2 mitoses per 50 HPF

Intermediate grade (n = 33)
Presence of necrosis OR >/= 2 mitoses per 50 HPF


Survival in Pancreatic Neuroendocrine Tumors

Midgut NET Survival:
Stage IV Disease

van Eeden et al., Hum Pathol 2002; 33: 1126

ENETS Grading of GEP-NETs

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitoses</th>
<th>Ki-67 Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt;2 / 10 H.P.F.</td>
<td>&lt;= 2%</td>
</tr>
<tr>
<td>G2</td>
<td>2-20 / 10 H.P.F.</td>
<td>3-20%</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20 / 10 H.P.F.</td>
<td>&gt;20%</td>
</tr>
</tbody>
</table>

Poorly Differentiated (High Grade) Neuroendocrine Carcinoma
**WHO 2010 Grading of GEP-NETs**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitoses</th>
<th>Ki-67 Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt;2 / 10 H.P.F.</td>
<td>&lt;= 2%</td>
</tr>
<tr>
<td>G2</td>
<td>2-20 / 10 H.P.F.</td>
<td>3-20%</td>
</tr>
<tr>
<td>G3</td>
<td>&gt;20 / 10 H.P.F.</td>
<td>&gt;20%</td>
</tr>
</tbody>
</table>

**Gastrointestinal Carcinoid Tumors:**

Five-Year Survival Rates by Site and Stage

<table>
<thead>
<tr>
<th></th>
<th>Localized</th>
<th>Regional</th>
<th>Distant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>68%</td>
<td>35%</td>
<td>10%</td>
</tr>
<tr>
<td>Small Bowel</td>
<td>57%</td>
<td>67%</td>
<td>40%</td>
</tr>
<tr>
<td>Appendix</td>
<td>91%</td>
<td>81%</td>
<td>28%</td>
</tr>
<tr>
<td>Colon</td>
<td>74%</td>
<td>51%</td>
<td>25%</td>
</tr>
<tr>
<td>Rectum</td>
<td>87%</td>
<td>41%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Data from SEER registry, 1973-1999; modified from Modlin et al., *Gastroenterology* 2005; 128: 1717

**ENETS TNM Staging System for Lower Jejunal and Ileal NETs**

**T – PRIMARY TUMOR**

<table>
<thead>
<tr>
<th>TX</th>
<th>Primary tumor cannot be assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor in mucosa / submucosa and &lt;= 1cm</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor in muscularis propria or &gt;1cm</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor in subserosa</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor invades peritoneum or other organs</td>
</tr>
</tbody>
</table>

**N - REGIONAL LYMPH NODES**

<table>
<thead>
<tr>
<th>NX</th>
<th>Regional lymph nodes cannot be assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>No regional lymph node metastases</td>
</tr>
<tr>
<td>N1</td>
<td>Regional lymph node metastases present</td>
</tr>
</tbody>
</table>

**M- DISTANT METASTASIS**

<table>
<thead>
<tr>
<th>MX</th>
<th>Distant metastases cannot be assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>No distant metastases</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastases present</td>
</tr>
</tbody>
</table>
Staging of NETs

- ENETS system – published 2007
- AJCC/UICC system – published 2009
- Both largely based on carcinoma staging
- Some differences (pancreas, appendix)
- System used must be stated
- Clinical validation data forthcoming

Classification Systems for NETs

- Predominantly WHO 2000 (GI tract) and WHO 2004 (Pancreas)

WHO Classification of Pancreatic NETs (2004)

- Well differentiated endocrine tumor
  - Benign behavior: confined to pancreas, <2 cm, non-angioinvasive, ≤ 2 mitoses per 10 HPF, ≤ 2% Ki67-positive cells
  - Uncertain behavior: confined to pancreas ≥ 2 cm, >2 mitoses per 10 HPF, > 2% Ki67-positive cells, OR angioinvasive
- Well differentiated neuroendocrine carcinoma
  - Low grade malignant: invasion of adjacent organs or metastases
- Poorly differentiated neuroendocrine carcinoma
  - High grade malignant: >10 mitoses per 10 HPF

WHO Classification of GEP-NETs (2010)

- Well differentiated NETs
  - Well differentiated neuroendocrine tumor, Grade 1 (NET G1)
  - Well differentiated neuroendocrine tumor, Grade 2 (NET G2)
- Poorly differentiated NETs
  - Poorly differentiated neuroendocrine carcinoma, Grade 3 (NEC G3)
- TNM should be performed in all cases
**Ki67 Labeling Index of NETs**

- Strong predictor of prognosis
- Basis for grading systems
- Correlates well with mitotic index
- Sharp separation of well differentiated and poorly differentiated neuroendocrine neoplasms

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**Mitotic Counting versus Ki67 Staining**

Stronsberg et al., Hum Pathol 2009; 40: 1262-8

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**Ki67 Staining**

- Low Grade
- High Grade

---

**Well Diff. NET**

**Small Cell Carcinoma**
Ki67 Labeling Index of NETs

- Strong predictor of prognosis
- Basis for grading systems
- Correlates well with mitotic index
- Sharp separation of well differentiated and poorly differentiated neuroendocrine neoplasms
- Methods of Assessment
  - Manual counting (2000 cells per ENETS)
  - “Eyeballed” estimate
  - Digital image analysis

Calculating Ki67 in Neuroendocrine Tumors

“How often do you use Ki67 in NETs?”
- For every neuroendocrine tumor
- For most neuroendocrine tumors
- Only when there is a diagnostic challenge
- Rarely
- I never heard of Ki67

Straw Poll

“How do you determine the Ki67 percentage in NETs?”
- Counting 2000 cells
- Digital image analysis
- “Eyeballed” estimate
- Have the resident do it

Straw Poll
Assessment of Ki67 Labeling Index:
Digital Image Analysis

Entire section with tumor is scanned

Manual selection of “hot spots” with highest proliferative activity

Manually check accuracy of positive cell identification
- inclusion of most tumor cells
- exclusion of non-tumor cells
- counts positive tumor cells
- does not count positive non-tumor cells
Manual exclusion of positive non-tumor cells (e.g., lymphocytes)

\[ \text{Ki67\%} = 1.7 \]

**Correlation between Digital Image Analysis and Manual Cell Count (2000 cells)**

\[
\text{ICC, 0.981; CI, 0.966-0.991}
\]


**Ki67 Index Scored by 20 Observers on 45 Images with “Eyeballed” Estimate**

**Correlation between Digital Image Analysis and Median Observer Count by “Eyeballed” Estimate**

\[
\text{ICC, 0.88; CI, 0.80-0.93}
\]

Consistency of Ki67 Scores by Digital Image Analysis (DIA), Manual Cell Count (MC), and “Eyeballed” Estimate (EE)

<table>
<thead>
<tr>
<th></th>
<th>Intraclass Correlation (ICC)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIA vs. MC</td>
<td>0.98</td>
<td>0.97-0.99</td>
</tr>
<tr>
<td>DIA vs. EE (Mean of 20 observers)</td>
<td>0.88</td>
<td>0.80-0.93</td>
</tr>
<tr>
<td>EE interobserver (n=20)</td>
<td>0.13</td>
<td>0.05-0.37</td>
</tr>
</tbody>
</table>

Intra-observer Consistency by “Eyeballed” Estimate on Three Repeat (Flipped) cases

Ki67 Labeling Index of NETs

- Strong predictor of prognosis
- Basis for grading systems
- Correlates well with mitotic index
- Sharp separation of well differentiated and poorly differentiated neuroendocrine neoplasms
- Methods of Assessment
  - Manual counting (2000 cells per ENETS)
  - Digital image analysis
  - “Eyeballed” estimate
- Intratumoral heterogeneity
Heterogeneity of Ki67 Labeling in NETs: Impact on Prognostic Significance of Grading

- 47% of cases with G1 vs. G2 heterogeneity
- Define grade based on highest Ki67:
  - G2 identified in 48% of core biopsies (3 cores)
  - G2 identified in 35% of core biopsies (1 core)
  - Predictive value of G1 on core biopsy:
    - 65% (3 cores); 59% (1 core)

Survival based on Ki67 Labeling - Whole Sections

<table>
<thead>
<tr>
<th>Survival</th>
<th>OS</th>
<th>DFS</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Ki67</td>
<td>p=0.0402</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Highest Ki67</td>
<td>p=0.0005</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Survival based on Ki67 Labeling – Core Biopsies

<table>
<thead>
<tr>
<th>Survival</th>
<th>OS</th>
<th>DFS</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three core</td>
<td>p&lt;0.0001</td>
<td>p=0.002</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Single core</td>
<td>p=0.0038</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>
Ki67 Immunohistochemistry: Issues

- More sensitive for proliferation than mitoses
- Method of quantification
  - Counting 2000 cells
  - "Eyeballing"
  - Image analysis
- Regional variation within tumor
  - Resection vs. biopsy sampling
  - Increase with disease progression
- Potential discordance with mitotic rate

Ki67 and Mitotic Rate Discordance in PanNETs

McCall et al., Mod Pathol 2012; 25 (Suppl 1):
Ki-67 grade 2 / mitotic grade 1 tumors have more aggressive histologic features

McCall et al., *Mod Pathol* 2012; 25 (Suppl 1):

Ki-67 G2/mitotic G1 PanNETs have decreased overall survival

*p* < 0.01

Ki-67 G2/mitotic G1 PanNETs are not significantly different from concordant G2

*p* = 0.13

Ki-67 G3/mitotic G2 PanNETs have decreased overall survival

Basturk et al., *Mod Pathol* 2013; submitted
Prognostic Significance of Grade in NETs

![Graph showing the relationship between grade and survival over months.](image)

Strosberg et al., Hum Pathol 2009; 40: 1262-8

The Good News About Different Classification Systems for NETs:

*They ALL Work!!*

Genetic Progression in Neuroendocrine Tumors

- Continuous clonal evolution of established neoplasms
- Poly/oligoclonal ➔ Monoclonal
- Increasing aggressiveness with clinical progression
- (?) Low grade to High grade transformation (?)

Genomic Imbalances in Pancreatic NETs

- 28 nonmetastatic PanNETs
- 17 paired primary and metastatic PanNETs
- Comparative genomic hybridization
- Genomic alterations in all metastasizing but only 58% of nonmetastatic PanNETs
- Clonality demonstrated in 95% of pairs
- Genomic changes enriched in metastases compared to paired primaries

Zhao et al., Genes Chromosomes Cancer 2001; 32: 364
Genomic Imbalances in PanNETs

- 34 “benign” and malignant PanNETs (females)
- X chromosome inactivation
- 50% with polyclonal pattern (!)
  - 29% of “benign” monoclonal
  - 61% of malignant monoclonal
- PanNETs are initially poly/oligoclonal with development of more aggressive monoclonal component

Genomic Imbalances in PanNETs

Gastrointestinal Carcinoid Tumors: Genetic Progression

Clonality in PanNETs

Clonality in PanNETs
Ileal Carcinoid Tumor: Primary

Liver Metastasis

Lymph Node Metastasis

- Primary and LN metastasis - < 1 mitosis / 10 HPF
- Liver metastasis - 3 mitoses / 10 hpf

Mitosis <1/50 HPF

Mitosis 65/50 HPF
Progression of Low Grade to High Grade Neuroendocrine Tumors

- Fits idea of genetic progression in neuroendocrine tumors
- Anecdotal examples exist
- Probably an uncommon pathway for development of HG NEC
- Rarely (?)ever) gives rise to small cell carcinoma
- Biological behavior ???

Clinical-Pathological Features of Well differentiated HGNET and Poorly Differentiated HGNECa

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Mitoses/ 10 HPF</th>
<th>Ki67</th>
<th>Mets</th>
<th>Fu (mo)</th>
<th>NED/AWD/DOD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD-LG/M NEN</td>
<td>56</td>
<td>0-7</td>
<td>1-10%</td>
<td>28%</td>
<td>44</td>
<td>63/17/20</td>
</tr>
<tr>
<td>NE Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WD-HGNET (n=8)</td>
<td>51</td>
<td>16</td>
<td>30-60%</td>
<td>100%</td>
<td>30</td>
<td>1-580/6</td>
</tr>
<tr>
<td>PD-HGNECa  (n=8)</td>
<td>62</td>
<td>36</td>
<td>50-95%</td>
<td>100%</td>
<td>14</td>
<td>20/mk/55</td>
</tr>
</tbody>
</table>

NED = no evidence of disease
AWD = alive with disease
DOD = died of disease

Tang et al., Mod Pathol 2010; 23 (Suppl 1): 133A

Two Pathways to the Development of High Grade NETs
Well differentiated HGNETs exhibit a different molecular phenotype from Poorly differentiated HGNECa.

<table>
<thead>
<tr>
<th>IHC</th>
<th>p53</th>
<th>β-catenin</th>
<th>Rb</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD-HGNET</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD-HGNECa</td>
<td>56%</td>
<td>61%</td>
<td>72%</td>
</tr>
<tr>
<td>(n=34)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Progression of Gastroenteropancreatic Neuroendocrine Tumors: Implications for Grading

- Grade may increase with disease progression – grade heterogeneity may complicate grading
- Sampling issues inherently limit accuracy, especially when 1 – 2 mitoses can change grade
- Incorporate radiographic assessment of tumor growth rate into therapeutic decisions
- Document proliferative rates to generate better data about prediction of outcome
NET Pathology Consensus Meeting: Methods

- Delphic consensus methodology
- Series of “Yes” or “No” questions
- Rigorous group discussions
- Voting for 80% agreement
- Re-discussion if <80% agreement
- Revoting
- Final tabulation: “agreement” vs. “no agreement”

Is “neoplasm” preferable to “tumor”?

<table>
<thead>
<tr>
<th>Agreement Level</th>
<th>Percentage</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agree strongly</td>
<td>25%</td>
<td>4</td>
</tr>
<tr>
<td>Agree with minor reservation</td>
<td>18.75%</td>
<td>3</td>
</tr>
<tr>
<td>Agree with major reservation</td>
<td>6.25%</td>
<td>1</td>
</tr>
<tr>
<td>Disagree with minor reservation</td>
<td>6.25%</td>
<td>1</td>
</tr>
<tr>
<td>Disagree with major reservation</td>
<td>12.50%</td>
<td>2</td>
</tr>
<tr>
<td>Disagree strongly</td>
<td>31.25%</td>
<td>5</td>
</tr>
</tbody>
</table>

Totals 100% 16 AGREEMENT

NET Pathology Consensus Meeting: Results

- Consensus reached on 91/108 questions (84%)
- Most without consensus highly polarized
- Areas of agreement
  - Terminology
  - Immunohistochemistry
  - Staging parameters
  - Grading parameters
  - Metastasis-specific issues
  - Prognostic factors
  - Treatment-related biomarkers

NET Pathology Consensus Meeting: “No Agreement” Issues

- 17 Questions
- Some minor issues
  - How to quantify extent of disease
  - Terminology
- Some substantive issues
  - Use of routine immunohistochemical staining
  - Concept of tumor progression in NETs
  - Use of staining for Ki67
NET Pathology Consensus Meeting: Development of Minimal Pathology Data Set

- Information for diagnostic classification
- Information for grading
- Information for staging
- Immunohistochemical staining
- Margin assessment
- Other prognostic factors
- Therapeutic biomarkers
- Metastasis-specific information
- Biopsy vs. resection

Klimstra DS et al., Am J Surg Pathol 2010; 34: 300-313

Pancreatic Neuroendocrine Tumors: Medical Treatment

- “Conventional” cytotoxic chemotherapy ineffective
- Somatostatin analogs
  - Block SSTR2
  - Inhibit function
  - Oncologic responses
- Alkylating agents
  - Streptozocin
  - Dacarbazine
    - Temozolomide
- Everolimus (RAD001)

Treatment Related Biomarkers: SSTR2

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


Temozolomide for PanNETs

- Temozolomide trials
  - Objective response in 45% of PanNETs
  - Little response in midgut NETs (carcinoid tumors)
- Cytotoxic effect:
  - Induces DNA methylation at \textsuperscript{6}O position of guanine
  - DNA mismatch, apoptosis
  - Requires decreased levels of DNA repair enzyme, \textsuperscript{6}O-methylguanine DNA methyltransferase (MGMT)
- MGMT detection
  - Promoter methylation
  - Immunohistochemistry

MGMT Immunohistochemistry

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>n</th>
<th>MGMT deficient, n (%)</th>
<th>MGMT intact, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic neuroendocrine</td>
<td>37</td>
<td>19 (51)</td>
<td>17 (49)</td>
</tr>
<tr>
<td>Nonfunctional</td>
<td>24</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Insulinoma</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Gastrinoma</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Glucagonoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>60</td>
<td>0</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Lung</td>
<td>40</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Typical</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Atypical</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Small intestine</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>


Treatment Related Biomarkers: MGMT

<table>
<thead>
<tr>
<th>Protein retained by IHC</th>
<th>Protein lost by IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter unmethylated</td>
<td>18</td>
</tr>
<tr>
<td>Promoter methylated</td>
<td>1</td>
</tr>
</tbody>
</table>

Basturk, Zhang, et al.; unpublished data
**MGMT Assessment in Glioblastoma**

**Targeting the mTOR Pathway**

- PTEN
- PIK3CA
- TSC2

PanNET 31
PanNET 10
PanNET 93

- = Gene mutated
- = Therapeutically targetable (Everolimus)

**“Radiant-3 Trial”:**

**Everolimus (RAD001) for PanNETs**

- Randomized, phase 3 trial of 410 patients with advanced, well differentiated PanNETs (n=207 everolimus arm and n=203 for the placebo arm)

- The median progression-free survival was 11.0 months with everolimus as compared with 4.6 months with placebo, representing a 65% reduction in the estimated risk of progression or death

**PanNETs with mTOR Pathway Abnormalities**

- PTEN
- Phospho-S6K
- TSC2

Yao et al., NEJM; 2011; 364:514-523
pS6 Kinase is expressed in 67% PanNETs
<table>
<thead>
<tr>
<th>The Pathologic Evaluation of NETs: Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Nomenclature</td>
</tr>
<tr>
<td>• Coming together, recognizing synonyms</td>
</tr>
<tr>
<td>• Grading standardized</td>
</tr>
<tr>
<td>• For GEP-NETs</td>
</tr>
<tr>
<td>• Evaluating issues</td>
</tr>
<tr>
<td>• Staging established</td>
</tr>
<tr>
<td>• Two systems, need to test</td>
</tr>
<tr>
<td>• Minimal data set published</td>
</tr>
<tr>
<td>• Identify basic data for correlative studies</td>
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<tr>
<td>• Future: Biomarker Assessment</td>
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