ARTHUR PURDY STOUT SOCIETY COMPANION MEETING: DIFFICULT NEW DIFFERENTIAL DIAGNOSES IN PROSTATE PATHOLOGY

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The Johns Hopkins Medical Institutions
High Grade PIN vs. PIN-Like Ductal Adenocarcinoma

Most prostatic ductal adenocarcinomas of the prostate are characterized by cribriform and/or papillary architecture lined by columnar pseudostratified malignant epithelium. PIN-like ductal adenocarcinomas closely resembles high-grade prostatic intraepithelial neoplasia (HGPIN) composed of simple glands with predominantly flat or tufting architecture. Cytologically, tumors are characterized by tall columnar atypical cells, basally located nuclei, and amphophilic cytoplasm. The tumors lack marked pleomorphism, necrosis, solid areas, cribriform formation, or true papillary fronds. No basal cells are present on p63 and/or high molecular weight cytokeratin staining. PIN-like ductal adenocarcinoma differs from HGPIN by the presence of cystically dilated glands, occasionally more crowded glands, a greater predominance of flat architecture, and less frequently prominent nucleoli. Verification often requires the immunohistochemical documentation of the absence of basal cells in numerous atypical glands. Although usual ductal adenocarcinoma is considered comparable to Gleason score 8, PIN-like ductal adenocarcinoma is accompanied by Gleason score 6 acinar carcinoma and behaves similar to Gleason score 6 acinar cancer. Recognition of this entity is critical to differentiate it from both HGPIN and conventional ductal adenocarcinoma.

High Grade PIN vs. Intraductal Carcinoma (IDC-P)

IDC-P is defined as malignant epithelial cells filling large acini and prostatic ducts, with preservation of basal cells. Its distinction from HGPIN is that it has:

- Solid or dense cribriform pattern

or

- Loose cribriform or micropapillary pattern with either:
  - Marked nuclear atypia: nuclear size 6 x normal
  - Non-focal comedonecrosis

IDC-P on prostate biopsies is frequently associated with high-grade cancer and poor prognostic parameters at radical prostatectomy as well as potentially advanced disease following other therapies. These findings support prior studies that IDC-P represents an advanced stage of tumor progression with intraductal spread of tumor. Consideration should be given to treat patients with IDC-P on biopsy aggressively even in the absence of documented infiltrating cancer.
<table>
<thead>
<tr>
<th>Cribriform Acinar Adenocarcinoma</th>
<th>Cribriform IDC-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of contour or branching architecture of prostatic ducts</td>
<td>Contour or branching architectures of prostatic ducts</td>
</tr>
<tr>
<td>Irregular, infiltrating borders</td>
<td>Rounded, circumscribed borders</td>
</tr>
<tr>
<td>Absence of basal cells</td>
<td>Basal cells present</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ductal Adenocarcinoma</th>
<th>IDC-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cribriform with large slit-like lumina</td>
<td>Cribriform with small rounded lumens</td>
</tr>
<tr>
<td>Tall columnar cells</td>
<td>Cuboidal cells</td>
</tr>
<tr>
<td>Papillary fronds with fibrovascular cores</td>
<td>Micropapillary tufts lacking fibrovascular cores</td>
</tr>
<tr>
<td>Basal cells usually absent</td>
<td>Basal cells always present</td>
</tr>
</tbody>
</table>

Cribriform Gleason Pattern 3 Adenocarcinomas

vs.

Cribriform Gleason Pattern 4 Adenocarcinomas

With the exception of cases of cribriform acinar prostate cancer with comedonecrosis, which is Gleason pattern 5, all cribriform acinar prostate cancer should be graded as Gleason pattern 4 for the following reasons.

- Even in a highly selected set of images thought to be the best candidates for cribriform pattern 3, most experts interpret the cribriform patterns as pattern 4.
- Most of the cribriform foci thought to be candidates for cribriform pattern 3 (73%) are associated with more definitive pattern 4 elsewhere on the needle biopsy specimen.
- Conceptually, one would expect the change in grade from pattern 3 to pattern 4 to be reflected in a distinct architectural paradigm shift where cribriform as opposed to individual glands are formed, rather than merely a subjective continuum of differences in size, shape and contour of cribriform glands.
- The only reason why cribriform pattern 3 even exists is because of the original Gleason schematic diagram, although Gleason never specifically studied the prognostic difference between what he called cribriform Gleason pattern 3 compared to Gleason pattern 4, and many of Gleason’s cribriform Gleason pattern 3 cancers may not even have been infiltrating carcinomas due to the lack of availability of immunohistochemistry for basal cell markers.
- There is poor reproducibility amongst experts differentiating cribriform pattern 3 vs. pattern 4 due to:
  1) Disagreement as to what are the key diagnostic features in a given case (ie. irregular distribution of lumina & variable slit-like lumina favor pattern 4 vs. small glands & regular contour favor pattern 3.
2) Disagreement as to assessment of given criteria: regular vs. irregular distribution of lumina

p63 Positive Prostate Adenocarcinoma

vs.

Basal Cell Carcinoma

Rarely, prostate cancer can aberrantly express diffuse p63 staining in a nonbasal cell distribution leading to the erroneous diagnosis of atrophy or basal cell carcinoma. Over 90% show a distinctive morphology composed predominantly of glands, nests, and cords with atrophic cytoplasm, hyperchromatic nuclei, and visible nucleoli. The diagnosis of prostate cancer is based on the morphology and confirmed by the absence of high molecular weight cytokeratin staining and positivity for alpha-methylacyl-CoA racemase (AMACR) in the atypical glands.

Basal cell carcinoma usually differs from p63 positive cancer in its architectural patterns. Patterns unique to basal cell carcinoma include: 1) adenoid cystic pattern; 2) large solid basaloid nests with comedonecrosis; 3) nests surrounded by a rim of collagen where the there is a dual cell population of cells consisting of inner cells with eosinophilic cytoplasm and outer basaloid cells with scant cytoplasm; and 4) irregular sized and shaped basaloid nests often with a desmoplastic stromal response. There is one pattern of basal cell carcinoma that consists of individual small glands with multilayering that resembles basal cell hyperplasia and p63 positive prostate cancer. The lack of high molecular weight cytokeratin positivity (HMWCK) and positivity for PSA and AMACR rules out this pattern of basal cell carcinoma.

There are several other situations where there is immunohistochemical labeling of prostate cancer with basal cell markers. Prostate cancer may show scattered cells positive for basal cell markers. The positivity is not in a basal cell distribution and represent aberrant staining of cancer cells. This phenomenon is more typically seen with HMWCK as compared to p63. More rarely, one can see adenocarcinoma of the prostate with focal retention of its basal cell layer. Only cases that are the most unequivocal prostate cancer based on architecture and cytology should be diagnosed in the face of basal cell staining.


Partial Atrophy

vs.

Prostate Cancer

Partial atrophy is the most common mimicker of prostate cancer. Partial atrophy may still retain the lobular pattern of post-atrophic hyperplasia, or have more of a disorganized diffuse appearance. Partial atrophy lacks the basophilic appearance of fully developed atrophy as the nuclei are more spaced apart. The presence of crowded glands with pale cytoplasm may lead to an overdiagnosis of low-grade adenocarcinoma. At higher power, however, the glands have benign features characterized by undulating luminal surfaces with papillary infolding. Most carcinomas have more straight, even luminal borders. In addition, the glands are partially atrophic with nuclei in areas reaching the full height of the cytoplasm. The nuclear features in partial atrophy tend to be relatively benign without prominent nucleoli, although nuclei may appear slightly enlarged with small nucleoli. One should hesitate diagnosing cancer when the nuclei occupy almost the full cell height and the cytoplasm has the same appearance as surrounding more obvious benign glands. Partial atrophy typically has a patchy basal cell layer and may express racemase and in small foci on needle biopsy no basal cells may be present, mimicking the staining pattern seen with prostate cancer.


Gastrointestinal Stromal Tumors (GIST)

vs.

Other Prostatic Stromal Tumors

Gastrointestinal stromal tumors (GISTs) are typically not included in the differential diagnosis of spindle cell tumors seen on prostate needle biopsy. However, their recognition is critical due to their unique clinical management. Rectal or extraintestinal GIST can result in a clinical impression of a prostatic lesion. One should consider CD117 (c-kit) in the immunohistochemical panel to exclude GIST before diagnosing a solitary fibrous tumor, leiomyosarcoma, or specialized prostatic stromal tumor on prostate needle biopsy.

Large Cell Neuroendocrine Versus Small Cell Carcinoma: When, Whether and How to Make the Distinction

Jeffrey L. Myers, M.D.
A. James French Professor and Director, Division of Anatomic Pathology
The University of Michigan, Ann Arbor, MI

Objectives
At the end of this presentation attendees will understand, 1) current criteria for separating large cell neuroendocrine from small cell carcinoma, and 2) the clinical, biological, histologic and phenotypic overlap between the two.

Key Points
- Small cell carcinoma (SCLC) is defined on basis of cytologic criteria.
- Large cell neuroendocrine carcinoma (LCNEC) is defined by a combination of histologic (i.e. neuroendocrine morphology, necrosis) and cytologic (i.e. large cell size, cytoplasm, nucleoli, coarse chromatin, high mitotic rate) criteria as well as the presence of neuroendocrine differentiation demonstrated using immunohistochemistry.
- LCNEC is separated from atypical carcinoid tumors based primarily on consistent presence of necrosis and higher mitotic rate.
- SCLC and LCNEC overlap in their clinical, biological, histopathologic, immunophenotypic, and genetic characteristics making separation difficult, and perhaps unimportant, in some patients.

INTRODUCTION

A revised WHO classification of lung tumors was published in 1999 and again in 2004, building on the popular foundation provided in the 1981 version. Like its predecessors, the updated WHO classification scheme relies heavily on routine light microscopy for tumor classification. Immunohistochemical stains have been established as an important diagnostic adjunct for certain tumor types, particularly tumors with neuroendocrine differentiation. The current scheme does not specifically categorize neuroendocrine neoplasms together, separating carcinoid tumors (typical and atypical) from more conventional forms of carcinomas. Small cell carcinoma (SCLC) remains a distinct carcinoma category, while large cell neuroendocrine carcinomas (LCNEC) are a subset of the larger group of large cell carcinomas. This review focuses on the two high grade variants of neuroendocrine neoplasms and those features that separate SCLC from LCNEC.

SMALL CELL CARCINOMA

Small cell lung cancer (SCLC) accounts for around 15% of all bronchogenic carcinomas, and is considered a high grade representative of the family of neuroendocrine lung tumors. Small cell carcinoma is strongly associated with cigarette smoking. Men are affected more commonly than women by a ratio of about 2:1, and most patients present in the 6th or 7th decade of life. Nearly all patients have advanced stage disease at presentation, and for that reason surgery is reserved for rare patients in whom tumor is
confined to the lung. In a review limited to patients who underwent some sort of surgical procedure, survival for stage I and II (“limited”) disease was 50% at two years, but only 14% at 5 years. The survival at 5 years was not significantly different from that seen in patients with “extensive” (stage III and IV) disease. Combination chemotherapy with or without radiation remains the mainstay of therapy in most patients.

Small cell carcinoma is a highly malignant epithelial neoplasm composed of relatively small cells with distinctive round to oval nuclei characterized by a diffuse (“salt and pepper”) chromatin pattern and inconspicuous nucleoli. Cell size is variable, however, and comprises a range that merges with that seen in non-small cell carcinomas. In addition, a minor population of larger cells with prominent but small nucleoli is common in surgical specimens and does not, by itself, exclude the diagnosis. The cells generally have only scant cytoplasm and are arranged in broad sheets which frequently show necrosis. Focal areas demonstrating various growth patterns associated with neuroendocrine neoplasia (i.e. nested/organoid, peripheral palisading, trabecular, rosettes) occur in a substantial number of sufficiently preserved tumors. Extensive crush artifact and basophilic staining of blood vessel walls (Azzopardi phenomenon) are characteristic but not pathognomonic of this tumor. Cytogenetic and molecular studies fail to demonstrate a single specific abnormality, although abnormalities in p53, bcl2/bax, cyclin D1, RB loss and LOH at 3p occur in a high percentage of both SCLC and large cell neuroendocrine carcinomas (see below).

Three variants of SCLC were recognized by the 1981 WHO classification: 1) oat cell (“lymphocyte-like”) carcinoma which corresponds to the classically described small cell carcinoma, 2) intermediate cell type which differs in that the cells tend to have more cytoplasm, are less regular in contour, and are often polygonal or fusiform, 3) combined small cell carcinoma in which definite small cell carcinoma is admixed with a clearly identifiable squamous cell, adenocarcinoma or large cell component. The Pathology Committee of the International Association for the Study of Lung Cancer (IASLC) proposed separating SCLC into, 1) SCLC (pure or classical type), 2) mixed small cell/large cell carcinoma, and 3) combined small cell/non-small cell (i.e. squamous cell or adeno-) carcinoma. Although SCLC can be distinguished from non-small cell carcinomas with a great deal of consistency by light microscopy, subclassification using previously proposed categories was subject to frequent interobserver disagreement. Furthermore, a number of studies demonstrated no significant clinical, therapeutic, or prognostic differences between subtypes. The revised WHO classification scheme includes only combined SCLCs as a distinct variant.

Diagnosis of SCLC can be made with confidence and a high degree of interobserver agreement in greater than 90% of cases. In difficult cases the differential diagnosis includes other forms of intermediate and high grade neuroendocrine (i.e. atypical carcinoid tumors and large cell neuroendocrine carcinomas – see below) and non-neuroendocrine (i.e. squamous cell, adeno-, and large cell) carcinomas. Immunostains for neuroendocrine-associated proteins (e.g. chromogranin, synaptophysin) are of limited value since a substantial subset of SCLCs lack immunohistochemical evidence of neuroendocrine differentiation, and a minor subset of non-small cell carcinomas are
positive. Keratin profiles can be helpful in selected cases. In the end, however, there is no single stain and no combination of stains that consistently and categorically allows separation of these entities, a differential diagnosis that still hinges primarily on a combination of cytologic and histologic findings.

**LARGE CELL NEUROENDOCRINE CARCINOMA**

Small cell carcinoma and atypical carcinoid tumors can usually be diagnosed on the basis of light microscopy alone. Immunohistochemical staining for neuropeptides (i.e. neuron specific enolase, chromogranin, synaptophysin, serotonin, bombesin) can be helpful in difficult cases but are not required for diagnosis. Application of these techniques to non-small cell carcinomas will reveal neuroendocrine differentiation in two additional groups of tumors -- so-called *large cell neuroendocrine carcinomas (LCNEC)* and *non-small cell carcinomas with neuroendocrine differentiation*.

Large cell neuroendocrine carcinoma refers to a subset of high grade neuroendocrine tumors characterized by, 1) a "neuroendocrine" histological growth pattern (i.e. organoid, palisading, trabecular, rosette-like); 2) "large" polygonal cells with lower N:C ratio than SCLC, coarse vesicular chromatin, and conspicuous nucleoli; 3) high (> 10/2 mm²) mitotic rate; 4) necrosis; 5) immunophenotypic and/or ultrastructural evidence of neuroendocrine differentiation. Historically tumors of this type were referred to by a variety of terms (e.g. atypical carcinoid tumors, intermediate variant of SCLC, large cell neuroendocrine tumor, and large cell carcinoma with neuroendocrine differentiation), indicating the difficulty in identifying these poorly differentiated carcinomas as a distinct nosological entity. Nonetheless, LCNECs appear to be highly aggressive bronchogenic carcinomas with a prognosis similar to that for SCLC.

Rates of interobserver agreement for the diagnosis of LCNEC are low, a feature that sets it apart from SCLC. This likely reflects the considerable histologic and cytologic overlap between LCNEC and SCLC at one end of a spectrum, and overlap with other forms of non-small cell lung carcinoma at the other. Perhaps the most difficult criteria to apply is the presence of a “neuroendocrine” growth pattern, something that frequently resides within the eye of the beholder. Cytologic features by themselves do not reliably separate LCNEC from SCLC; several studies have shown substantial overlap in cell size. Immunohistochemical studies are of limited value in that certain proteins (e.g. CD117, bcl-2, PAX-5, CRMP5) are expressed more frequently in high grade rather than lower grade neuroendocrine lung tumors but do not consistently separate LCNEC from SCLC.

While difficulty in separating LCNEC from SCLC may be the bad news, the good news is that large retrospective case series suggest that there may be limited value in making the distinction at all. A number of studies have shown survival rates for patients who undergo surgery for early stage LCNEC that are superimposable on those reported for rare patients with early stage SCLC treated surgically. The survival rates for both are lower than that observed in patients with other forms of early stage but high grade non-neuroendocrine lung carcinoma. Comparisons of patients with late stage disease also show a similar survival experience that is different from lower grade forms of neuroendocrine lung tumors but similar to that seen in other types of late stage non-neuroendocrine lung carcinoma.
<table>
<thead>
<tr>
<th></th>
<th>typical carcinoid</th>
<th>atypical carcinoid</th>
<th>SCLC</th>
<th>LCNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45</td>
<td>55</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>M:F</td>
<td>1:1</td>
<td>1:1</td>
<td>2:1</td>
<td>4:1</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>30-50%</td>
<td>60-70%</td>
<td>&gt;95%</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Central</td>
<td>75%</td>
<td>60%</td>
<td>&gt;90%</td>
<td>40%</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>90%</td>
<td>60%</td>
<td>&lt;10%</td>
<td>45%</td>
</tr>
<tr>
<td>≥ II</td>
<td>10%</td>
<td>40%</td>
<td>≥90%</td>
<td>55%</td>
</tr>
</tbody>
</table>

Table: Summary of Neuroendocrine Lung Neoplasms
REFERENCES


47. Marchevsky AM, Gal AA, Shah S, Koss MN. Morphometry confirms the presence of considerable nuclear size overlap between "small cells" and "large cells" in high-grade pulmonary neuroendocrine neoplasms.[see comment]. American Journal of Clinical Pathology 2001;116(4):466-72.


Large Cell Neuroendocrine vs Small Cell Carcinoma
When, Whether and How to Make the Distinction

March 8, 2009

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and
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At the end of this talk attendees will understand,

- current criteria for separating large cell neuroendocrine from small cell carcinoma, and
- the clinical, biological, histologic and phenotypic overlap between the two
WHO Classification of Lung Tumors*
Neuroendocrine Lung Neoplasms

✓ small cell carcinoma

large cell carcinoma
✓ large cell neuroendocrine carcinoma

carcinoid tumor
✓ typical carcinoid tumor
✓ atypical carcinoid tumor

# Neuroendocrine Lung Tumors Comparison*

<table>
<thead>
<tr>
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<th>LCNEC</th>
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<td>≥90%</td>
<td>55%</td>
</tr>
</tbody>
</table>

SMALL CELL CARCINOMA

General

- cigarette smoking
- \( \sim 15\% \) of incident cases
- men \( \geq \) women (\( \sim 1-2:1 \))
- central >> peripheral
SMALL CELL CARCINOMA

small cell ca rates in US

• ↓ in men
• ↑ in white women
• ↓ in black women (since 1990)

from Devesa Int J Cancer 2005; 117: 294
## SMALL CELL CARCINOMA

### Survival*

<table>
<thead>
<tr>
<th>cStage</th>
<th>% of pts</th>
<th>1 year</th>
<th>5 years</th>
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<tr>
<td>I</td>
<td>7%</td>
<td>41%</td>
<td>28%</td>
</tr>
<tr>
<td>II</td>
<td>4%</td>
<td>73%</td>
<td>21%</td>
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<tr>
<td>III</td>
<td>32%</td>
<td>54%</td>
<td>11%</td>
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<tr>
<td>IV</td>
<td>57%</td>
<td>22%</td>
<td>1%</td>
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</table>

*from Shepherd et al. J Thorac Oncol 2007*
SMALL CELL CARCINOMA
WHO Definition

malignant epithelial tumor consisting of,

- small cells with scant cytoplasm, ill-defined cell borders

“usually less than the size of three small resting lymphocytes”
WHO “size rule”
\[ \leq 3 \times \text{lymphocyte diameter} \]
SMALL CELL CARCINOMA

WHO Definition

- small cells with scant cytoplasm, ill-defined cell borders
SMALL CELL CARCINOMA
WHO Definition

- small cells with scant cytoplasm, ill-defined cell borders
- finely granular nuclear chromatin, and absent or inconspicuous nucleoli
• finely granular chromatin
• absent/inconspicuous nucleoli
“In 29 cases, a varying percentage of cells demonstrated nucleoli that were conspicuous but small.”

Nicholson et al. AJSP 2002
SMALL CELL CARCINOMA

WHO Definition

• small cells with scant cytoplasm, ill-defined cell borders
• finely granular nuclear chromatin, and absent or inconspicuous nucleoli
• cells are round, oval and spindle-shaped; prominent nuclear molding
round, oval and spindle-shaped
prominent nuclear molding
high mitotic count (>10/2 mm²)
SMALL CELL CARCINOMA
WHO Definition

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- finely granular nuclear chromatin, and absent or inconspicuous nucleoli
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SMALL CELL CARCINOMA
Immunohistochemical Profile

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<thead>
<tr>
<th>small cell ca</th>
<th>squamous cell ca</th>
<th>adca</th>
</tr>
</thead>
<tbody>
<tr>
<td>keratin</td>
<td>+</td>
<td>+</td>
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</table>

dot-like, perinuclear staining pattern
### SMALL CELL CARCINOMA

**Immunohistochemical Profile**

<table>
<thead>
<tr>
<th></th>
<th>small cell ca</th>
<th>squamous cell ca</th>
<th>adca</th>
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</thead>
<tbody>
<tr>
<td>keratin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CRG</td>
<td>+/−</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td>SYN</td>
<td>+/−</td>
<td>−/+</td>
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"NE" Differentiation in Non-small Cell Lung Carcinomas Using TMAs*

<table>
<thead>
<tr>
<th></th>
<th>Sq cell ca</th>
<th>Adenocarcinoma</th>
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<tbody>
<tr>
<td>CRG</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>SYN</td>
<td>10 (4.3%)</td>
<td>23 (11.2%)</td>
</tr>
<tr>
<td>CD56</td>
<td>29 (12.4%)</td>
<td>11 (5.1%)</td>
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**“NE” Differentiation in Non-small Cell Lung Carcinomas**

<table>
<thead>
<tr>
<th></th>
<th>Sq cell ca</th>
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<tr>
<td><strong>CRG</strong></td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td><strong>SYN</strong></td>
<td>28%</td>
<td>25%</td>
</tr>
<tr>
<td><strong>CRG/SYN/CD57 (leu 7)</strong></td>
<td>41%</td>
<td>35%</td>
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*Data collected from 10 peer reviewed publication, 1990 – 2005
### SMALL CELL CARCINOMA

**Immunohistochemical Profile**

<table>
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<tbody>
<tr>
<td>CRG</td>
<td>+/−</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td>SYN</td>
<td>+/−</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td>34βE12</td>
<td>−</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>p63</td>
<td>−</td>
<td>+</td>
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<tr>
<td>TTF-1</td>
<td>+</td>
<td>−</td>
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</table>
combined small cell + squamous cell ca

TTF-1

p63

34βE12 (ker903)
<table>
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<tr>
<th></th>
<th>SMALL CELL CARCINOMA</th>
<th>Squamous Cell Ca</th>
<th>Adca</th>
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<tbody>
<tr>
<td>Small Cell Ca</td>
<td>+</td>
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</tr>
<tr>
<td>Keratin</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>CRG</td>
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</tr>
<tr>
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<td>−/+</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>−</td>
</tr>
<tr>
<td>TTF-1</td>
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Large Cell Carcinoma
WHO 2004

- poorly differentiated NSCLC that lacks cytologic and architectural features of SCLC and glandular or squamous differentiation

- 5 variants:
  - large cell neuroendocrine
  - basaloid carcinoma
  - lymphoepithelioma-like carcinoma
  - clear cell carcinoma
  - large cell ca with rhabdoid phenotype
Large Cell Neuroendocrine Carcinoma

Definition

- *neuroendocrine* morphology
- necrosis (extensive)
Large Cell Neuroendocrine Carcinoma

Definition

• neuroendocrine morphology
• necrosis (extensive)
• >10 mitosis/2 mm² (10 hpf)
• cytologic features of NSCLC:
  – large size, low N:C, nucleoli, coarse chromatin
large cell neuroendocrine carcinoma

vs.

atypical carcinoid tumor
ATYPICAL CARCINOID TUMOR

Definition*

- “neuroendocrine” growth pattern
- uniform cytology ± “atypia”
- 2-10 mits/2 mm²
- ± necrosis

Large Cell Neuroendocrine Carcinoma
Comparison with Atypical Carcinoid

Asamura et al. J Clin Oncol 2006; 24: 70-6

<table>
<thead>
<tr>
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<th>atypical carcinoid</th>
<th>LCNEC</th>
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<tr>
<td>NE morphology</td>
<td>✓</td>
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<tr>
<td>necrosis</td>
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<tr>
<td>atypia</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>mitotic rate</td>
<td>2-10/2 mm²</td>
<td>&gt;10/2 mm²</td>
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ATYPICAL CARCINOID TUMOR

Definition*

• "neuroendocrine" growth pattern
• uniform cytology ± "atypia"
• 2-10 mits/2 mm²

9 (8.5%) of 106 cases had < 2 mits/2 mm²
Beasley et al Hum Pathol 2000; 31: 1255

• 2-10 mits/2 mm²

ATYPICAL CARCINOID TUMOR

Definition*

- "neuroendocrine" growth pattern
- uniform cytology ± "atypia"
- 2-10 mits/2 mm²
- ± necrosis

71 (67%) of 106 cases had necrosis
Beasley et al Hum Pathol 2000; 31: 1255

- 2-10 mits/2 mm²
- ± necrosis

Large Cell Neuroendocrine Carcinoma
Comparison with Atypical Carcinoid

atypical carcinoid  LCNEC

10 mits/2 mm²
Large Cell Neuroendocrine Carcinoma

Definition

- neuroendocrine morphology
- necrosis (extensive)
- >10 mitosis/2 mm² (10 hpf)
- cytologic features of NSCLC:
  - large size, low N:C, nucleoli, coarse chromatin
- immunohistochemical confirmation
Chromogranin
Large Cell Neuroendocrine Carcinoma

Definition

- neuroendocrine morphology
- necrosis (extensive)
- >10 mitosis/2 mm² (10 hpf)
- cytologic features of NSCLC:
  - large size, low N:C, nucleoli, coarse chromatin
- immunohistochemical confirmation

LCNEC vs other NSCLC

LCNEC vs SCLC
Is cell size a reliable criterion for separating large cell neuroendocrine carcinoma from small cell carcinoma?
SCLC vs LCNEC
Nuclear Size Overlap*

- n = 12 LCNEC and 16 SCLC
- measured tumor cell (TC) and lymphocyte (L) nuclear areas
- histograms for each peak TC/L:
  A = 2
  B = 3
  C = 4
  D = 5
  E = 6
  F no peak

SCLC vs LCNEC

Nuclear Size Overlap*

5 (31%) of 16 “SCLC” had predominant population of cells 4-6 times larger than lymphocytes

SCLC vs LCNEC

Nuclear Size Overlap*

The frequency distribution of tumor nuclear diameter/lymphocyte size ratios in SCLC (2.75 ± 0.86) overlaps with LCNEC (3.22 ± 0.86)

*Hiroshima et al. Mod Pathol 2006; 19: 1358
Is cell size a reliable criterion for separating large cell neuroendocrine carcinoma from small cell carcinoma? NO!
Is immunohistochemistry useful for separating large cell neuroendocrine carcinoma from small cell carcinoma?
**SCLC vs LCNEC**

Role of Immunohistochemistry*

*Hiroshima et al. Mod Pathol 2006; 19: 1358
SCLC vs LCNEC
Role of Immunohistochemistry*

SCLC vs LCNEC
Role of Immunohistochemistry*

PAX-5 expression in SCLC & LCNEC

Neither cell size nor immunohistochemistry are useful for separating large cell neuroendocrine carcinoma from small cell carcinoma?
## NEUROENDOCRINE LUNG TUMORS

### Diagnostic Reproducibility*

<table>
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<tr>
<th></th>
<th>Unanimous (5 of 5)</th>
<th>Majority (4 of 5)</th>
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<tr>
<td>typical carcinoid</td>
<td>58%</td>
<td>92%</td>
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<tr>
<td>atypical carcinoid</td>
<td>50%</td>
<td>75%</td>
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<tr>
<td>SCLC</td>
<td>70%</td>
<td>90%</td>
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<tr>
<td><strong>LCNEC</strong></td>
<td><strong>40%</strong></td>
<td><strong>50%</strong></td>
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</table>

*Travis et al. Hum Pathol 1998; 29: 272*
Does separating LCNEC from SCLC have value?
If so, when?
LCNEC versus SCLC?

Survival

P = .9147

J Clin Oncol 24: 70-6
“Large cell neuroendocrine histology has a significant adverse prognostic impact on pathologic stage Ia non-small cell carcinoma.”

Iyoda 2006
LCNEC versus SCLC?
Survival in Stage I Disease

Takei 2002

Asamura 2006

Survival Rate

Years

TC (n = 50)
AC (n = 4)
LCNEC (n = 63)
SCLC (n = 58)

$P = .1851$
Fig 5. Kaplan-Meier curves for overall survival stratified according to chemotherapeutic protocols in the adjuvant setting and tumor stage

LCNEC is more likely to respond to chemotherapeutic strategies targeting SCLC

“SCLC-based” = platinum-etoposide
of cytological “criteria” for LCNEC, only the presence of cytoplasm is useful
of cytological “criteria” for LCNEC, only the presence of cytoplasm is useful.

no consistent difference at level of protein expression
of cytological “criteria” for LCNEC, only the presence of cytoplasm is useful.

no consistent difference at level of protein expression

low rates of interobserver agreement among experts
of cytological “criteria” for LCNEC, only the presence of cytoplasm is useful

no consistent difference at level of protein expression

low rates of interobserver agreement among experts

no difference in therapeutic response or natural history
Large Cell Neuroendocrine Carcinoma
Practical Approach?

any way to make this SCLC?

- finely dispersed chromatin?
- inconspicuous nucleoli?
- scant cytoplasm?
- is cell size the only issue?
- clinical context?
  - central mass in smoker with mediastinal adenopathy?
Large Cell Neuroendocrine Carcinoma
Practical Approach?

any way to make this SCLC?

YES!
Large Cell Neuroendocrine Carcinoma
Practical Approach?

any way to make this SCLC?

compelling reason to acknowledge neuroendocrine differentiation?

• LCNEC already diagnosed
• IHC stains and it really, really looks neuroendocrine but ≠ atypical carcinoid
• been called SCLC but it isn’t
Large Cell Neuroendocrine Carcinoma
Practical Approach?

any way to make this SCLC?

compelling reason to acknowledge neuroendocrine differentiation?

NO

YES!
Large Cell Neuroendocrine Carcinoma
Practical Approach?

any way to make this SCLC?

LCC, sq cell ca, adca

compelling reason to acknowledge neuroendocrine differentiation?

NO

NO
SCLC vs LCNEC

Key Points

• SCLC is defined on the basis of cytologic criteria
• LCNEC is defined by a combination of histologic and cytologic criteria + “proof” of NE differentiation (immunohistochemistry)
• LCNEC is separated from atypical carcinoid based on consistent presence of necrosis and higher mitotic rate
• SCLC and LCNEC overlap in clinical, biological, histopathologic, immunophenotypic and genetic characteristics
Triple negative and basal-like breast cancer: one or many diseases?

Implications for surgical pathologists

Jorge S Reis-Filho, MD PhD FRCPath
The Breakthrough Breast Cancer Research Centre
Institute of Cancer Research
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Take home messages
- The majority of basal-like breast cancers have a triple negative phenotype and the vast majority of triple negative cancers display a basal-like transcriptome, however the two are not synonymous.
- Basal-like and triple negative breast cancers comprise a heterogeneous group of diseases, which are characterised by a constellation of morphological features.
- Not all basal-like and triple negative breast cancers have a poor outcome.
- The precursors of invasive breast carcinomas of basal-like and triple negative phenotype include ductal carcinoma in situ harbouring a similar phenotype and, possibly, a subgroup of microglandular adenosis.
- A subgroup of basal-like and triple negative breast cancers displays an exquisite sensitivity to anthracycline-based neoadjuvant chemotherapy.
- Defects in the p53, pRB and p16 pathways are found in a significant proportion of basal-like and triple negative cancers.
- BRCA1 pathway dysfunction is found in a substantial proportion of basal-like and triple negative breast cancers and can be exploited therapeutically (e.g. inhibitors of the PARP enzyme and cross-linking agents)

Introduction
Breast cancer is a heterogeneous disease, encompassing a plethora of entities which not only have distinct morphological features but also clinical behaviour. In recent years, it has become apparent that this diversity may be underpinned by distinct patterns of genetic, epigenetic and transcriptomic aberrations 1-4. In fact, the marriage of pathology and genetics has led to the establishment of clear examples of genotypic-phenotypic correlations. For instance, secretory carcinomas of the breast consistently harbour a t(12;15)(p13;q25) translocation, leading to the formation of a fusion transcript ETV6-NTRK31,5-8. Lobular carcinomas have been shown to be characterised by inactivation of the CDH1 gene, which encodes E-cadherin, a transmembrane adhesion molecule that mediates homophilic-homotypic adhesions. A recent conditional mouse model has provided strong circumstantial
evidence to suggest that CDH1 gene inactivation is not only involved in the characteristic discohesiveness of lobular lesions, but may be also involved in the peculiar metastatic pattern of invasive lobular carcinomas\cite{9}.

Although morphology is often associated with the pattern of molecular aberrations in breast cancers, it is also clear that tumours of the same histological type display remarkably different clinical behaviour. This is most evident in invasive ductal carcinomas of no special type (IDC-NST), where even tumours of the same histological grade may have distinct outcome and remarkably different responses to systemic therapy \cite{2,3}. With the boom of high throughput technologies and the apocalyptic promise of microarray analysis, several groups endeavoured in devising a new taxonomy solely based on the molecular features of breast cancers. The gene expression microarray-based class discovery studies pioneered by the Stanford group have led to the identification of at least five subgroups of breast cancer: luminal A, luminal B, normal breast-like, HER2 and basal-like breast cancer \cite{10-14}. This taxonomy was devised based on the analysis of IDC-NSTs and a limited number of lobular carcinomas and has proven to be of prognostic significance. Although based on the analysis of a rather limited number of samples and with varying definitions in each study, this classification has captured the attention of oncologists, pathologists and scientists alike, with some authorities in the field claiming that the gold standard for the classification of breast cancers is microarray-based gene expression profiling.

It should be noted, however, that this taxonomy identified subgroups of breast cancer that were to some extent already known. In fact, the most robust distinction observed by microarray analysis is between the transcriptome of oestrogen receptor (ER)-positive and ER-negative breast cancers. Luminal tumours are described as those that show expression patterns reminiscent of normal luminal epithelial cells of the mammary gland, including consistent expression of low molecular weight cytokeratins 8/18, ER and genes associated with an active ER pathway \cite{11,12,15,16}. At least two subgroups of luminal tumours have been identified: luminal A, which are usually of low histological grade, have an excellent prognosis and show high levels of expression of ER-activated genes; and luminal B, which are more often of higher histological grade, have higher proliferation rates and a significantly worse prognosis than luminal A tumours \cite{11,12,14-16}. Normal breast-like cancers are rather poorly characterised; one of the defining features of these tumours is that they consistently cluster together with samples of fibroadenomas and normal breast. The clinical significance of normal breast-like tumours is yet to be determined \cite{11,12,14-16} and some have suggested that this subgroup may be a mere artefact of expression profiling (i.e. disproportionately high content of stromal cells). HER2 tumours are usually ER-negative and characterised by
overexpression of the human epidermal growth factor receptor type 2 (HER2) and genes associated with HER2 pathway and/or HER2 amplicon on 17q12. HER2 cancers have a very aggressive clinical behaviour, however they are amenable to novel tailored therapies using either humanised monoclonal antibodies against HER2 or HER2 tyrosine kinase inhibitors. Although the vast majority (>80%) of HER2 cancers as defined by microarrays harbour HER2 gene amplification or HER2 3+ immunohistochemical expression, not all tumours that are HER2 amplified fall into the HER2 cluster by expression arrays analysis. There is also evidence to suggest that some HER2 amplified, ER-positive cancers fall within the luminal B subtype rather than the HER2-microarray subtype. Basal-like cancers, another group of ER-negative cancers, are so named because the neoplastic cells of this tumour type consistently express genes usually found in normal basal/myoepithelial cells of the breast, including cytokeratins 5 and 17. It should be emphasised that basal-like breast cancers, unlike ‘basal’/myoepithelial cells of normal breast, almost uniformly express cytokeratins 8 and/or 18, calling into question the initial histogenetic implications of this microarray-based taxonomy of breast cancers.

What is a basal-like breast cancer?

The characteristics of basal-like breast cancer have been extensively reviewed in the last 18 months. It should be noted that there is still no internationally accepted definition for basal-like breast cancers. Some groups have employed microarray-based expression profiling to define basal-like breast cancers, whereas others have used immunohistochemical surrogates. In fact, there are several surrogate markers for basal-like breast cancers already described, the most used of which are i) the panel based on the mRNA expression profiling of basal-like breast cancers defined by Nielsen et al. and Cheang et al., ii) lack of ER, progesterone receptor (PR) and HER2 (triple negative phenotype), or iii) expression of high-molecular weight cytokeratins.

Despite using distinct definitions for basal-like breast cancers, we and others have demonstrated that basal-like tumours have distinctive clinical presentations, histological features, response to chemotherapy and outcome. In brief, basal-like tumours comprise a heterogeneous group of cancers that account for up to 15% of all breast cancers. These tumours affect younger patients, are more prevalent in African-American women and more often present as interval cancers. Histologically, as a group, the vast majority of basal-like breast cancers are IDC-NSTs of high histological grade and characterised by high mitotic indices, the presence of central necrotic zones, pushing borders, conspicuous lymphocytic infiltrate and typical/atypical medullary features. However, the vast majority of medullary and atypical medullary, metaplastic,
secretory, myoepithelial and adenoid cystic carcinomas of the breast also display a basal-like phenotype. More recently, a subgroup of lobular carcinomas has been shown to express high molecular weight cytokeratins, however it remains to be determined whether these cases truly display a basal-like transcriptome. At the immunohistochemical level, the majority of basal-like breast cancers lack or display low levels of ER and PR, lack HER2 gene amplification and express genes usually found in ‘basal’ myoepithelial cells of the normal breast including high molecular weight cytokeratins, P-cadherin, caveolins 1 and 2, nestin, αB crystallin and epidermal growth factor receptor (EGFR) and, in a minority of cases, harbour EGFR gene amplification or aneusomy. p53 immunohistochemical expression or TP53 gene mutations in up to 85% of cases and alterations of the pRB and p16 G1/S cell cycle checkpoint are remarkably prevalent in these cancers. In fact, a recent study demonstrated that approximately 30% of basal-like breast cancers concurrently display lack of pRB expression, p16 overexpression and p53 immunoreactivity, whereas this profile was rarely seen in tumours of other molecular subgroups. Basal-like display remarkably high proliferation indices as defined by mitotic counting or by MIB1 (Ki67) labelling index.

Basal-like cancers, as defined by microarrays or by immunohistochemical surrogates, have been shown to have a more aggressive clinical behaviour. In fact, some studies have demonstrated that expression of basal keratins is a prognostic factor independent of tumour size, grade and lymph node status. However, when compared to either ER-negative non-basal-like cancers or to grade-matched non-basal-like cancers, carcinomas with a basal-like phenotype do not seem to be associated with a poorer outcome. In addition, the pattern of metastatic spread of tumours with a basal-like phenotype seems to be different from that of non-basal-like cancers: they are reported to less frequently disseminate to axillary nodes and bones and to favour a haematogenous spread, with a peculiar proclivity to develop metastatic deposits in the brain and lungs.

What is a triple negative breast cancer?
Triple negative cancers are defined as tumours that lack ER, PR and HER2 expression, accounting for 10-17% of all breast carcinomas, depending on the thresholds used to define ER and PR positivity and the methods for HER2 assessment. It should be noted that different studies have employed different methods and thresholds to define lack of expression of these markers. Furthermore, future studies are likely to produce slightly different results given the change in the definition of HER2 positivity according to the new ASCO/ CAP guidelines. Despite the issues with the definitions of triple negative cancers,
the interest in these tumours stems from the lack of tailored therapies for this group of breast cancer patients and the overlap with the profiles of basal-like cancers.

The main characteristics of triple negative cancers that have emerged from the literature illustrate the similarities between basal-like and triple negative tumours, including the fact that they are more frequently affect younger patients (<50 years)\(^\text{32,61,63,64}\), are more prevalent in African-American women\(^\text{64-66}\), often present as interval cancers and are significantly more aggressive than tumours pertaining to other molecular subgroups\(^\text{32,34,61,63-65}\). This aggressiveness is best translated by the fact that the peak risk of recurrence is between the 1\(^{\text{st}}\) and 3\(^{\text{rd}}\) years and the majority of deaths occur in the first 5 years following therapy\(^\text{61,63}\). On the other hand, the differences in outcome between triple negative cancers and tumour with other phenotypes are reduced at 10 years of follow up. Interestingly, patients with basal-like\(^\text{42}\) or triple negative cancers\(^\text{61,65}\) have a significantly shorter survival following the first metastatic event when compared to those with non-basal-like/ non-triple negative controls.

From a pathologist point of view, the differences between triple negative and non-triple negative breast cancers are not surprising, given that the majority of triple negative cancers are of histological grade III\(^\text{61,62}\) and basal-like phenotype. It should be noted, however, that the group of cancers defined by triple negativity is more heterogeneous than that defined by basal-like phenotype\(^\text{26,69,70}\). Apart from the more heterogeneous transcriptome, triple negative cancers also show more varied histological features. In fact, up to 10% of triple negative tumours were shown to be of grade I in one study\(^\text{61}\). Furthermore, apart from medullary, metaplastic, secretory, myoepithelial and adenoid cystic carcinomas\(^\text{1,4,39,40,52}\) which are preferentially triple negative tumours, several other histological special types of breast cancer may display a triple negative phenotype, including apocrine carcinomas, pleomorphic lobular carcinomas and duct-lobular cancers\(^\text{13,52,70}\).

There are conflicting results on the prevalence of lymph node metastasis at diagnosis in patients with triple negative cancers: whilst in one study, there was a higher prevalence of lymph node metastasis in triple negative cancers when compared to controls\(^\text{61}\), others found no difference\(^\text{32,62}\) or an inverse association between triple negative phenotype and lymph node metastasis\(^\text{63}\). Interestingly, it has been described that unlike non-triple negative cancers, no correlation between tumour size and presence of lymph node metastasis was observed in the triple negative group\(^\text{61}\). A similar dissociation between tumour size and prevalence of lymph node metastasis at diagnosis was identified by Foulkes et al.\(^\text{71}\) in tumours arising in \textit{BRCA1} germline mutation carriers.
Are basal-like and triple negative cancers synonymous?

Given that there is no internationally accepted definition for basal-like breast cancers, it is not surprising that there has been a great deal of confusion as to whether triple negative and basal-like breast cancers are synonymous. Although several groups have used these terms interchangeably, it should be noted that not all basal-like cancers lack ER, PR and HER2 and not all triple negative cancers display a basal-like phenotype. The vast majority of triple negative cancers are of basal-like phenotype. Likewise, the vast majority of tumours expressing ‘basal’ markers are triple negative. It should be noted, however, that there is a significant number of triple negative cancers that do not express basal markers and a small, but still significant, subgroup of basal-like cancers that express either hormone receptors or HER2. Bertucci et al. have addressed this issue directly and confirmed that not all triple negative tumours when analysed by gene expression profiling were classified as basal-like cancers (i.e. only 71% were of basal-like phenotype) and not all basal-like breast carcinomas classified by expression arrays displayed a triple negative phenotype (i.e. 77% were of triple negative phenotype). Taken all together, these results are in accord with the concept that the triple negative phenotype is not an ideal surrogate marker for basal-like breast cancers and call for caution in the interpretation of ongoing therapeutic trials whose selection of patients was made on the basis of lack of ER, PR and HER2 expression. Furthermore, there are several lines of evidence to suggest that the group of triple negative cancers is substantially more heterogeneous than the group encompassed by basal-like breast cancers.

Precursors of basal-like and triple negative cancers: getting beyond the high grade ductal carcinoma in situ (DCIS)

Despite the multiple definitions of basal-like breast cancer, a group of high grade DCIS lacking ER, PR and HER2 and expressing ‘basal’ markers has been identified. However, it should be noted that its prevalence is lower than that of invasive triple negative and basal-like breast cancer and that triple negative and basal-like cancers often lack an overt in situ component.

It has more recently been established a link between microglandular adenosis and triple negative and basal-like cancers. In fact, the vast majority of invasive cancers developing in the context of microglandular adenosis have been shown to be of triple negative phenotype. Interestingly, invasive carcinomas developing in the context of microglandular adenosis not uncommonly display metaplastic elements or are of adenoid cystic morphology. Shin et al. have recently demonstrated that microglandular adenosis may be a non-obligate precursor of triple negative and basal-like breast cancers.
Microglandular adenosis was shown to harbour the expected pattern of genetic aberrations of basal-like and triple negative cancers $^{85}$. Furthermore, similar patterns of genetic aberrations were found in matched microglandular adenosis, atypical microglandular adenosis and invasive carcinomas and a stepwise progression in the number of gross chromosomal changes from microglandular adenosis to invasive carcinoma was observed $^{85}$. Our group has recently identified examples of basal-like and triple negative cancers arising in the context of microglandular adenosis, including one case in a BRCA1 germline mutation carrier (Geyer F, Jones RL and Reis-Filho JS, unpublished observations).

**Basal-like and tumours arising in BRCA1 germ-line mutation carriers**

There is increasingly more coherent evidence to suggest a link between BRCA1 pathway and basal-like breast cancers $^{90,91}$. In fact, the vast majority of tumours arising in BRCA1 germline mutation carriers, in particular those diagnosed before 50 years of age, have morphological features similar to those described in basal-like cancers $^{92,93}$ and display a basal-like phenotype as defined by immunohistochemistry $^{94,95}$ or expression arrays $^{16}$.

The immunohistochemical similarities between BRCA1 tumours and basal-like breast carcinomas are deeper than those illustrated by the expression of high molecular weight cytokeratins and other proteins expressed by myoepithelial cells. In fact, both basal-like breast cancers and tumours arising in BRCA1 germline mutation carriers show a peculiar pattern of cell cycle protein expression $^{92,96-98}$, both rarely harbour CCND1 gene amplification $^{96,97}$, however they express significantly lower levels of p27 $^{92,98}$, and higher levels of Skp2 $^{92,96}$, cyclin E $^{92,98}$ and caspase 3 $^{98}$ when compared to sporadic breast carcinomas and BRCA2 mutation tumours.

Although, even at the genetic level, sporadic basal-like cancers and tumours arising in BRCA1 mutation carriers show similar molecular genetic profiles $^{99-103}$, they differ by the lack of BRCA1 somatic mutations in sporadic basal-like cancers. Despite the lack of BRCA1 mutations, it has been recently demonstrated that BRCA1 pathway may be dysfunctional in sporadic basal-like tumours $^{35,90,91}$. BRCA1 protein expression levels have been shown to be significantly lower in tumours of high histological grade, lacking ER and PR expression and of basal-like phenotype $^{104}$. We and others have hypothesised that this downregulation would be mediated by epigenetic mechanisms, such as gene promoter methylation and/ or transcriptional silencing of BRCA1. In fact, BRCA1 gene promoter is methylated in $>$60% of medullary $^{105,106}$ and metaplastic $^{35}$ breast cancers of basal-like phenotype. However, despite the significantly lower levels of BRCA1 mRNA expression in sporadic basal-like cancers than grade matched controls $^{35}$, both sporadic invasive ductal carcinomas with and without basal-
like phenotype showed a similarly low prevalence of BRCA1 gene promoter methylation\textsuperscript{35,107}. We therefore investigated alternative epigenetic mechanisms of BRCA1 pathway inactivation and found that sporadic invasive ductal carcinomas with basal-like phenotype expressed ID4, a negative regulator of BRCA1\textsuperscript{108,109}, at significantly higher levels than grade-matched controls\textsuperscript{35}. This mechanism may account for the low levels of BRCA1 expression in sporadic basal-like carcinomas of ductal morphology. Interestingly, a recent study has suggested that BRCA1 plays a critical role in the differentiation of ER-negative stem/progenitor cells to ER-positive luminal cells\textsuperscript{110}, however it remains to be determined whether BRCA1 inactivation in luminal epithelial cells cannot lead to de-differentiation or acquisition of a stem-like phenotype.

Based on the fact that the majority of basal-like breast cancers show a dysfunctional BRCA1 pathway\textsuperscript{35,90,91} and harbour TP53 gene mutations\textsuperscript{12,16,76}, we have engineered the conditional mouse \textit{BLG-Cre;Brca1\textsuperscript{F22-24/F22-24};p53\textsuperscript{+/—}}, where the \textit{Brca1} gene is inactivated in β-lactoglobulin-expressing cells (i.e. luminal epithelial cells of the mouse mammary gland) and all cells of the animal have only one wild-type allele of p53\textsuperscript{111}. Consistent with our findings in human tumours, pathological analysis of the tumours arising in the above mice revealed that 78% lacked hormone receptors and HER2 and expressed basal markers (cytokeratins 14 and/or EGFR) and 88% showed homologous metaplastic elements. This mouse model provides another line of evidence for the link between basal-like phenotype and BRCA1 pathway dysfunction and may prove useful for testing novel therapies for basal-like cancers\textsuperscript{111}. Interestingly, another conditional mouse model \textit{K14cre;Brca1\textsuperscript{F/F};p53\textsuperscript{F/F}}, where \textit{Brca1} and \textit{Trp53} were inactivated in basal cells of the mouse mammary gland, has been shown lead to the development of tumours whose morphological and phenotypic characteristics are remarkably similar to those observed in our study\textsuperscript{111}. Taken together, these findings provide circumstantial evidence to suggest that despite the cell of origin, \textit{Brca1} inactivation may lead to basal-like breast cancers. This is not surprising, given that BRCA1 has been shown to play a pivotal role in the regulation of ER expression and that RNA interference-mediated silencing of BRCA1 in breast cancer cell lines leads to a marked reduction in expression of endogenous ER protein levels\textsuperscript{112}.

**Basal-like and triple negative breast cancers are heterogeneous**

Recent studies have clearly demonstrated that, contrary to the initial idea that basal-like breast cancers would constitute a homogeneous group of cancers with aggressive clinical behaviour, basal-like and triple negative cancers are heterogeneous in their histological features, immunohistochemical profiles, outcome and response to therapy.
As discussed above, although the majority of basal-like breast cancers are high-grade tumours characterised by a constellation of morphological features, there are low-grade lesions that display a triple negative and basal-like phenotype. It is rather arguable to classify under the same term high grade IDC-NST of triple negative phenotype with secretory and adenoid cystic carcinomas, given that their genetic features and clinical behaviour are remarkably different. However, these lesions consistently display a triple negative immunophenotype and harbour a basal-like transcriptome.

Detailed analysis of the immunophenotype of basal-like breast cancers demonstrates that, again, these tumours are heterogeneous. Although defects of p53, pRB and p16 are significantly more frequently found in triple negative and basal-like breast cancers, these proteins are concurrently expressed in an abnormal pattern in 30% and 50% of basal-like and non-basal-like triple negative cancers, respectively. BRCA1 pathway appears to be dysfunctional in a significant proportion of triple negative and basal-like breast cancers, however the proportion of cases that lack competent homologous recombination DNA repair due to BRCA1 pathway abnormalities remain to be determined.

Contrary to the widely held belief that basal-like and triple negative breast tumours would be chemotherapy resistant, several studies have demonstrated that a subgroup of these cancers display a remarkable sensitivity to conventional chemotherapy regimens. In fact, 17% to 58% of patients with triple negative breast cancers have been shown to evolve to pathological complete response after anthracycline- or anthracycline+taxane-based neoadjuvant chemotherapy and 17% of triple negative cancers evolved to pathological complete response after neoadjuvant platinum-based chemotherapy. However, when followed up, despite the higher prevalence of pathological complete response, patients with basal-like and triple negative cancers have been shown to have a worse outcome than those with non-triple negative or non-basal-like tumours. This apparent paradox has been recently resolved. There are several lines of evidence to suggest that patients with triple negative or basal-like cancers that evolve to pathological complete response after neoadjuvant chemotherapy have an excellent prognosis, whereas those who fail to achieve pathological complete response have a dismal outcome. These results provide another level of evidence of the heterogeneity of triple negative and basal-like breast cancers and suggest that a subgroup of these cancers is sensitive to genotoxic agents. It should be noted, however, that markers for the identification of patients with triple negative and basal-like cancers that benefit most from chemotherapy remain to be defined. Furthermore, several groups have recently identified a subgroup of good prognosis ER-
negative cancers, encompassing a subgroup of triple negative and basal-like tumours, that is characterised by the expression of an immune response module. This transcriptomic profile may prove helpful for the identification of patients with triple negative and basal-like cancers that have a better outcome and, once more, illustrates the heterogeneity of this group of tumours.

Finally, a subgroup of triple negative and basal-like breast carcinomas have been shown to have a dysfunctional BRCA1 pathway and this subgroup may be amenable to specific therapeutic strategies. Given that tumours that have a dysfunctional BRCA1 pathway lack competent homologous recombination DNA repair, our group and others have hypothesised that these cancers would be exquisitely sensitive to cross-linking agents and inhibitors of the PARP enzyme. Reassuringly, in vitro studies and animal models have demonstrated that tumours with BRCA1 or BRCA2 loss of function are indeed sensitive to these agents. Consistent with this hypothesis, results of PARP inhibitor phase I clinical trials that included patients with BRCA deficient tumours have been encouraging and sustained responses in patients with BRCA1/2 deficient breast or ovarian metastatic cancers have been observed. Given these exciting results, several clinical trials testing cross-linking agents (e.g. carboplatin and cisplatin) and PARP inhibitors in patients with BRCA1 germline mutations and sporadic basal-like breast cancers are currently ongoing (for a list of clinical trials, please see). If positive, these studies may render the identification of tumours lacking competent homologous recombination compulsory in our diagnostic practice.

Conclusions
Basal-like breast cancer is a heterogeneous group of tumours that is more prevalent in young, African-American patients. The majority of these cancers are of triple negative phenotype and have a poor outcome. Despite their sensitivity to chemotherapy agents, basal-like and triple negative cancers have a relatively poor outcome. Currently, triple negative cancers are routinely identified in clinical practice, whereas there is no internationally accepted definition for basal-like cancers, there is still no clear clinical indication for the routine identification of these tumours, and microarray-based gene expression profiling is far from becoming the 'gold standard' for breast cancer classification in clinical practice.

Given that basal-like breast cancers are still heterogeneous, regardless of the definition employed, it is possible that in the next few years markers that identify subgroups of basal-like or triple negative cancers that respond to specific agents, rather than the identification of
basal-like cancers *per se*, will become part of our diagnostic armamentarium. Perhaps then, the contribution of microarray-based gene expression profiling will come full circle and deliver on the promise of individualised therapies for every cancer patient. With the advent of massively parallel sequencing $^{130}$, which allows for the genome-wide quantitative and qualitative genomic and transcriptomic characterisation of cancers, and the imminent death of microarrays $^{131,132}$, it is likely that the taxonomy of breast cancers will be revisited again $^{1,24}$. This time, it is possible that more homogeneous molecular subgroups, their biological drivers and therapeutic targets will be identified. Until then, we, pathologists, have to strive for providing optimal assessment of the histological features, and ER, PR and HER2 status of breast cancers.

References


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SERRATED POLYPS AND COLORECTAL ADENOCARCINOMA
Genetic Considerations, Microsatellite Instability and Lynch Syndrome
R.E. Petras, M.D.
Ameripath, Inc.

There may be at least five separate but overlapping molecular pathways to colorectal cancer (1). Approximately 20% of colorectal carcinomas appear to have a genetic basis (2,3). This latter group includes the 3% of cases related to Lynch syndrome (Hereditary Nonpolyposis Colon Cancer Syndrome [HNPCC]) and the 1% associated with familial adenomatous polyposis (FAP) and its variants. About 85% of colorectal cancers are thought to originate through the chromosomal instability pathway. These tumors typically demonstrate DNA aneuploidy and have abnormalities of chromosomes 5, 17, 18 and contain mutational changes in APC gene, K-ras proto-oncogene, DCC tumor suppressor gene and p53 tumor suppressor gene (4). Familial adenomatous polyposis colorectal carcinomas arise via this pathway. Approximately 15% of colorectal carcinoma appears to arise in the so-called “mutator phenotype”. These cancers tend to be DNA diploid and are associated with microsatellite instability. The Lynch syndrome cancers are associated with the “mutator phenotype”.

DNA integrity is essential for normal cell function. DNA insults can occur due to the direct effects of chemicals or radiation and are usually corrected through the excision repair system. DNA replication errors are of two types; 1) simple mispairing of nucleotides, the most common type, and 2) “slipping” errors, in which genes may contain too many or too few copies of repeat short DNA nucleotide sequences known as “microsatellites”. Normally, these errors are recognized, the cell cycle arrested and the mismatched segment corrected. For those errors not immediately corrected by DNA polymerase, the mismatch repair (MMR) system acts as a back-up for additional proofreading of DNA. Failure to repair mismatches allows the error (mutation) to persist and to become the template for subsequent DNA replication (5). The known mismatch repair genes and their relative frequency in Lynch syndrome are presented in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>GENE</th>
<th>FREQUENCY</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMLH1</td>
<td>49%</td>
<td>3p21</td>
</tr>
<tr>
<td>hMSH2</td>
<td>45%</td>
<td>2p15</td>
</tr>
<tr>
<td>hPMS2</td>
<td>4%</td>
<td>7p22</td>
</tr>
<tr>
<td>hPMS1</td>
<td>1%</td>
<td>2p32</td>
</tr>
<tr>
<td>hMSH6</td>
<td>1%</td>
<td>2p15</td>
</tr>
<tr>
<td>hMSH3</td>
<td>0%</td>
<td>5q11-13</td>
</tr>
</tbody>
</table>
Microsatellite instability (MSI) is best viewed as an epiphenomenon found in colorectal tumor DNA but not in non-neoplastic tissues. It indicates that extensive mutation exists in the non-encoding repetitive DNA sequences that are particularly prone to replication error, the microsatellites. The majority of MSI is linked to somatic inactivation of \textit{hMLH1} through hypermethylation inactivation of the promotor region; but it can also be detected in persons with germline mismatch repair gene mutations, the definition of Lynch syndrome (5,6). MSI is detected in 15% of colorectal cancers overall and is present in over 95% of the cancers found in patients with Lynch syndrome.

Lynch syndrome patients, because they have a germline mutation of a mismatch repair gene, are at increased lifetime risk for colorectal (up to 80%) and other cancers (3,6). These cancers develop at significantly younger ages (e.g., average age for colorectal carcinoma = 44 years) (6). Other Lynch syndrome related tumors include cancers of the endometrium, ovary, stomach, biliary tract, urinary tract, kidney, central nervous system, small bowel, and skin tumors (6).

Lynch syndrome patients and families can sometimes be identified by taking a careful patient and family medical history, can be suggested from the pathologic findings of excised tumors, and can be detected by direct evaluation of the mismatch repair system. Pathologic features of colorectal cancer that suggest MSI/Lynch syndrome include right-sided location, synchronous or metachronous large bowel cancers, large bulky polypoid tumors with circumscribed pushing margins, tumors showing prominent lymphoid infiltrate, cancers of poor differentiation (medullary or undifferentiated carcinoma) or mucinous and signet ring cell histology (2,3,7).

The diagnosis of Lynch syndrome is evolving. Originally, the Amsterdam criteria were used to clinically identify HNPCC including the Lynch syndrome patients (8). The original Amsterdam criteria include; a) three or more relatives with a colorectal cancer with at least one a first-degree relative; b) colorectal carcinoma in two generations; and c) one or more colorectal carcinomas occurring in a person less than 50 years of age. In order to increase the sensitivity, the Amsterdam criteria were modified (Amsterdam II criteria) to include; a) three or more relatives with any Lynch syndrome related carcinoma; b) colorectal carcinoma in two generations; and c) and one or more Lynch syndrome related carcinomas in a person younger than 50 years of age (9). There are many problems with detecting Lynch syndrome based upon the Amsterdam criteria alone. Patient histories are less useful now than in the past because of smaller family sizes. Excision of colorectal adenomas interrupts the adenoma-carcinoma sequence. Patients in whom the family history is unknown or incomplete limit the utility of these criteria. Physician history taking is often not thorough. More importantly, depending upon the cohort, up to 33% of persons having a germline mutation of a mismatch repair gene are Amsterdam criteria negative and only 60% of Amsterdam criteria positive kindred have a detectable mutation (9-16). These Amsterdam positive/gene mutation negative kindred are often referred to as familial colorectal cancer syndrome type X.

Special testing (MSI testing by polymerase chain reaction [PCR] or immunohistochemical stains) now augments the clinical criteria. Controversy over the use of MSI analysis has led to the development of the Bethesda guidelines for testing colorectal tumors for microsatellite instability. The latest iteration, the revised Bethesda guidelines (12) requires than just one of the following criteria be met: colorectal cancer before age 50, synchronous or metachronous colorectal or other Lynch-related tumor regardless of age, colorectal cancer with MSI-high pathology in a patient less than 60, person with colorectal cancer and a first-degree relative with colorectal adenoma or carcinoma or other Lynch-related tumor (cancer less than 50; adenoma less than 40), colorectal cancer with two or more relatives with colorectal or other Lynch-related tumor regardless of age.

The American Gastroenterological Association (AGA) position states that genetic testing should be performed on families meeting Amsterdam criteria, on any affected person meeting the modified Bethesda guidelines, and on any first-degree relative of those with known mutations of mismatch repair
genes (3). They suggest that following pre-test genetic counseling and written informed consent, immunohistochemistry for MMR gene products and/or MSI testing by PCR be performed on tumor tissue. The international guidelines for evaluation of MSI by PCR recommend use of consensus markers; BAT25, BAT26, D5S346, D2S123, D17S250. If two or more markers are abnormal, the carcinoma is considered MSI-High (MSI-H). If one marker is abnormal, the tumor is classified as MSI-Low (MSI-L). If no markers are abnormal the cancer is referred to as MSI-Stable (MSS). Laboratories using more than 5 loci modify this classification with ≥ 30% - 40% abnormal defined as MSI-H, < 30% - 40% as MSI-L and none abnormal as MSS. Immunohistochemistry can be used to detect MSI. Almost all MSI-H cancers can be identified if the antibody panel includes MLH1, MSH2, PMS2 and MSH6 (13,16). Immunohistochemistry and MSI analysis by PCR each have advantages and limitations. PCR requires a molecular laboratory and usually requires normal tissue for comparison. Immunohistochemistry is more widely available but can be limited by poor tissue fixation or poor technique rendering interpretation difficult. Immunohistochemistry may be superior because the findings can direct gene sequencing and MSI is not always seen in Lynch syndrome kindred with MSH6 germline mutation (14). Patients with MSI-H cancer should undergo additional genetic testing including gene sequencing. MSS and MSI-L tumors require no further testing (3). Additional genetic evaluation may be considered if the clinical history is compelling.

The clinical significance of identifying Lynch syndrome is that affected individuals and at risk persons are identified and can be screened and treated with correct surgery. Subtotal colectomy is usually recommended to treat Lynch related colon cancer because of the high likelihood of synchronous/metachronous cancers. Partial colectomy with colonoscopy every 1-2 years is a reasonable alternative (6). Furthermore, clinicians can institute proper screening such as colonoscopy at a young age, (beginning at age 25 or 5 years younger than the youngest cancer in the family), periodic endometrial sampling (every 1-2 years starting at age 25), pelvic ultrasound, CA125 serum testing and urine cytology or molecular testing for urinary tract carcinoma. Many experts screen all resected colorectal cancers for MSI initially by PCR. Immunohistochemistry is a useful alternative and some prefer this as the initial test because an abnormality in protein expression correlates almost invariably with MSI-H by PCR. In cases showing normal MMR proteins or equivocal staining by immunohistochemistry, MSI testing by PCR should be done in clinically suspicious cases to exclude a germline mutation that can yield an antigenic protein that is biologically inactive.

MSI testing in sporadic colorectal carcinoma is a subject of considerable contemporary interest and debate. Much like their Lynch syndrome counterparts, sporadic MSI-H carcinomas have a predilection for the right colon, mucinous histology and a prominent lymphoid infiltrate (17). There are strong arguments for routine testing for MSI in all resected colorectal carcinoma including the lower mortality rate independent of tumor stage (18). Sporadic MSI-H cancer can also be associated with an increased rate of metachronous tumors with subsequent clinical implications for cancer surgery, surveillance and follow-up. MSI status may also have implications for chemotherapy. There is improved survival in MSS and MSI-L stage II and stage III cancers treated with fluorouracil-based regimens (19,20). Finally, routine MSI testing could increase the detection of Lynch syndrome because 44% of probands were over age 50 and up to 22% of patients in Lynch syndrome did not fulfill Amsterdam or Bethesda guidelines (16).

COLORECTAL SERRATED POLYPS AND THE SERRATED PATHWAY TO COLORECTAL CANCER

Colorectal Hyperplastic Polyps and Hyperplastic (Serrated) Polyposis Syndrome

Hyperplastic polyps are the most common benign polyp of the large intestine (2,21). These polyps are usually small (less than 5 mm), sessile and are often about the same color as the surrounding colonic
mucosa. Histologically, evenly distributed absorptive and goblet cells line crypts that are elongate and dilated. Inhibition of normal apoptosis is thought to be the underlying mechanism for polyp formation and because there are more epithelial cells per unit area than normal, the cells must pseudostratify, imparting a serrated or micropapillary appearance. Characteristically, the basement membrane under the surface epithelium is thickened and hyalinized. Regenerative epithelial changes, mitoses figures and active inflammation can be quite prominent at the crypt bases. This regenerative area can occasionally cause diagnostic confusion with dysplasia and carcinoma, especially in a variant referred to as inverted hyperplastic polyp (22,23). In this inverted variety, the regenerative epithelium of the crypt base is misplaced into or beneath the muscularis mucosae. Most examples of inverted hyperplastic polyp are now probably best classified as a sessile serrated polyp (see below) and are easily recognized if one is cognizant of its existence and also notes the overall architectural and cytologic similarity to hyperplastic polyp/sessile serrated polyp. The entity is distinguished from invasive adenocarcinoma by the lack of infiltration and tumor desmoplasia.

The differential diagnosis between hyperplastic polyp and tubular adenoma can be difficult, especially in a diminutive polyp that has been treated by hot biopsy (so-called “Thermal Polyp”). Useful features in the differential are found in Table 2.

**TABLE 2**

**HYPERPLASTIC POLYP VS. TUBULAR ADENOMA**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Hyperplastic Polyp</th>
<th>Tubular Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regenerative Zone</td>
<td>Basal</td>
<td>Surface</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Usually No</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyalinized basement membrane</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

In a “tight call”, as long as an adenoma diagnosis is not going to result in a surgical resection (e.g., right colonic adenoma incompletely excised), I err on the side of adenoma to insure that the patient will receive more frequent surveillance. Mixtures of hyperplastic polyp, sessile serrated polyp and adenoma occur (24,25). Mixed polyps and serrated adenomas are considered in more detail below.

**Hyperplastic (Serrated) Polyposis Syndrome**

Rare examples of patients with colons carpeted by hyperplastic-like polyps (so-called hyperplastic polyposis) have been described (2). The WHO defines hyperplastic polyposis as individuals with: a) 5 or more hyperplastic polyps proximal to the sigmoid colon of which 2 are \( \geq 1 \) cm, b) any number of hyperplastic polyps proximal to the sigmoid colon if the person has a first degree relative with hyperplastic polyposis and c) more than 30 hyperplastic polyps of any size and any location (26). The form with 30 or more small hyperplastic polyps without sessile serrated polyp morphology (seen below) has been called type 2 and probably does not predispose to adenocarcinoma (27). The type 1 associated with large (\( \geq 1 \) cm) polyps with sessile serrated polyp morphology is associated with MSI-H cancers in which there is methylation-induced loss of expression of \( hMLH1 \) (27,28). Indeed, hyperplastic polyposis may be a marker for the so-called “mutator phenotype”. Colectomy specimens typically show a spectrum
of serrated polyps with typical hyperplastic polyps, traditionally defined serrated adenomas (see below) and unusual hyperplastic polyps (sessile serrated polyps – see below). Serrated polyposis may be a better name for this syndrome. Hyperplastic polyposis patients are prone to colorectal carcinoma with a reported prevalence of up to 50%. Once diagnosed, careful consideration should be given to the clinical follow-up and prophylactic colectomy may be indicated (29). Some cases have shown evidence of inheritance presumably caused by a genetic predisposition to hypermethylation. The type and order of methylated genes varies and may account for MSS, MSI-L and MSI-H cancers described. Some patients with MYH-associated polyposis (MAP) also met criteria for hyperplastic polyposis syndrome and MAP should be considered when adenomas coexist with hyperplastic polyposis (30). When several cancers in hyperplastic polyposis syndrome families are MSI-H, the distinction from Lynch syndrome can be difficult. Features that favor hyperplastic polyposis include; background serrated adenomas and sessile serrated polyps, presence of some MSS or MSI-L cancers in the kindred, older age at onset of cancer, limited numbers of affected family members, methylation of \textit{hMLH1} and failure to detect germline mutation of mismatch repair genes.

\textbf{Serrated Polyps and Colorectal Adenocarcinoma}

Several lines of evidence link “hyperplastic polyps” with colorectal carcinoma. Investigators have reported individual cases and small series of carcinoma complicating “hyperplastic polyps” (31-39). The association between colorectal cancer and hyperplastic polyposis has already been noted above. There is a high rate of co-existing hyperplastic polyps but not adenomas in patients with MSI-H carcinoma (31). A large series of MSI-H colorectal carcinoma predated by biopsy proved “hyperplastic polyps” at the same site has been reported (36).

Molecular events involved in the serrated polyp family are now recognized. Methylation-induced inactivation of mismatch repair genes occurs in both hyperplastic polyps and carcinoma. As shown in Table 3, methylation inactivation of genes and certain gene mutations (especially BRAF) appear to be involved in the serrated pathway to carcinoma (40,41). These molecular events have been verified (42-47).

\begin{table}
\centering
\caption{Methylation/Mutations in Serrated Polyp Family}
\begin{tabular}{|l|c|c|c|}
\hline
 & HP (%) & SSP (%) & Mixed (%) \\
\hline
MINT 1 & 23 & 30 & 100 \\
MINT 2 & 32 & 70 & 100 \\
MINT 31 & 23 & 70 & 100 \\
hMLH1 & 0 & 13 & 70 \\
MGMT & 36 & 57 & 60 \\
KRAS (mutation) & 18 & 13 & 0 \\
BRAF (mutation) & 19 & 75 & 89 \\
\hline
\end{tabular}
\end{table}

HP = hyperplastic polyp; SSP = sessile serrated polyp; Mixed = mixed polyps and serrated adenomas.
“Hyperplastic polyps” associated with carcinoma have been unusually large and right-sided. They have been reported under a number of synonyms including giant hyperplastic polyp, sessile serrated adenoma, sessile serrated polyp, inverted hyperplastic polyp, and polyp with epithelial serrated proliferation.

It is becoming clear that there are several different pathological entities that have been called “hyperplastic polyps” in the past. This serrated polyp family includes conventional hyperplastic polyp, mixed hyperplastic/sessile serrated polyp/adenoma, serrated adenoma (epithelial dysplasia defined, usually pedunculated and left sided, having eosinophilic cytoplasm and showing gastric foveolar change and often referred to as the traditionally defined serrated adenoma) and hyperplastic-like polyps with unusual features that have been referred to as sessile serrated polyps or sessile serrated adenomas (29,32,34,37). Sessile serrated polyps appear related to serrated adenomas and mixed polyps and could be the specific precursor lesion to sporadic MSI-H carcinoma. Transitions from sessile serrated polyps to areas of dysplasia and carcinoma with loss of hMLH1 protein expression have been described (38,39).

Sessile serrated polyps as the name implies are sessile, large (frequently 1 cm or more), right-sided, and often show poor endoscopic circumscription. A number of cytological and architectural abnormalities have been reported in the sessile serrated polyp, especially those that have been associated with carcinoma (33,36,37,39). The abnormal proliferation/dysmaturation features include persisting nuclear atypia with large nuclei and nucleoli high (upper third) in the crypts, high (upper third of the crypt) mitoses figures and irregular distribution of dystrophic goblet cells. Architectural abnormalities include basal crypt dilatation, horizontally oriented crypts, crypt branching, an increased epithelial:stromal ratio (>50%), inverted crypts, prominent serration, increased surface villosity/papillations and the lack of a surface basement membrane thickening typical of convention hyperplastic polyps. Some authors suggest that a diagnosis of sessile serrated polyp requires the presence of at least four of the architectural and abnormal proliferation features mentioned above (43). Immunohistochemical differences between hyperplastic polyp and sessile serrated polyp have been described using Beta-catenin, CDX2 and MUC6 (48,49) but it is uncertain whether this will be useful clinically.

Once recognized, the sessile serrated polyp creates a patient management dilemma. Calling them “sessile serrated adenomas” may not be an appropriate default diagnosis because it can be confused by the clinician for serrated adenoma. It is unknown whether colonic resection which is typically done for incompletely excised adenomas should be recommended for sessile serrated polyps which are incompletely excised at endoscopy. Furthermore, endoscopic follow-up for serrated adenoma would typically be at three-years (if the clinician considers serrated adenoma or sessile serrated adenoma a variant villous adenoma) or in five-years. In a cohort of 91 patients with sessile serrated polyps preceding MSI-H carcinomas, 19 predated the carcinomas by less than three years (36). Sessile serrated polyps should be treated by complete excision if possible. Until more is known, a shorter surveillance interval (e.g., 1-2 years) seems prudent for these types of polyps that are incompletely excised or associated with additional similar endoscopically appearing polyps that have remained unsampled (34-37).
REFERENCES