Interpreting the Pathology Report
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Disclosure
Ms. Zitella has acted as a consultant for Teva, Gilead, and On Q Health.

Learning Objectives
- Describe common techniques for pathologic evaluation of tumor samples including Immunohistochemistry, flow cytometry, cytogenetics, fluorescence in situ hybridization, and molecular testing
- Interpret pathology reports including the implications for prognosis and treatment selection
- Discuss and compare small-group reviews of provided examples facilitated by faculty to interpret pathology reports for treatment selection and prognosis
Overview

- Review basic pathology terms
- Describe the following
  - Immunohistochemistry
  - Flow cytometry
  - Cytogenetics
  - FISH: fluorescence in situ hybridization
  - Molecular diagnostics/PCR
- Examples of pathology reports
- Clinical significance of pathology report items

Pathology

- Malignancy can arise in any organ or tissue in the body
  - Exceptions: Fingernails, hair, and teeth
- Diagnosis of cancer requires a biopsy or surgical resection of tissue for histopathologic or cytologic examination of tissues or cells to identify:
  - Malignancy
  - The anatomical site of origin of the malignancy
  - The type of cells involved

Terms

- Histopathology
- Immunohistochemistry
- Cytopathology
- Flow cytometry
- Cytogenetics
- Fluorescence in situ hybridization (FISH)
- Polymerase chain reaction (PCR)
- Gene assay
**Histopathology**

- Examination of surgical specimen/whole pieces of tissue under a microscope by a pathologist
- Assessment that categorizes the tumor according to its corresponding normal tissue type or the cell type that it most closely resembles

For example:
- Hepatocellular carcinoma is derived from liver tissue
- Breast carcinoma from breast tissue
- Osteosarcoma from bone

How Is Tissue Processed After a Biopsy or Surgery?

- Tissue must be cut into thin sections and placed on slides
  - Frozen sections
    - prepared by freezing and slicing the tissue sample
    - process takes 15 to 20 minutes and can be done while the patient is in the operating room to give surgeon immediate diagnosis to guide the operation
  - Paraffin-embedded (permanent) sections
    - tissue placed in fixative (usually formalin) to preserve the tissue followed by processing with other solutions
    - tissue placed in paraffin wax and after wax has hardened, the tissue is cut into very thin slices
    - slices of tissue placed on slides and stained
    - process normally takes several days
    - provides the best quality for examination by the pathologist and produces more accurate results than a frozen section

Hematoxiln & Eosin (H&E stain)

- Cells are transparent so H&E stain is used to examine under a microscope.
  - Hematoxylin stains nuclear structures blue
  - Eosin stains eosinophilic structures (cytoplasm, intracellular/extracellular proteins) reddish-pink
Definitions

- **Hyperplasia**
  - Increased number of cells
  - May occur due to inflammation or normal response to stimuli

- **Dysplasia**
  - Abnormal cells with loss of normal cellular/tissue characteristics (architecture, orientation, size)
  - Some genetic abnormalities present with loss of some normal regulatory processes
  - May precede malignancy

- **Carcinoma-in-situ**
  - Localized malignant cells
  - "Pre-cancer"

- **Carcinoma**
  - Invasive (beyond basement membrane)

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Immunohistochemistry (IHC)

- IHC is used to help diagnose cancer, identify biomarkers, and to detect the presence of microorganisms
- Technique used to identify specific antigens in different kinds of tissue
  - Tissue is treated with an antibody that binds the specific antigen
  - The antibody is tagged with a radionuclide, a fluorescent dye, or an enzyme that produces a color reaction so that the antibody-antigen complex is visible under a microscope
- Examples of proteins detected by IHC:
  - Estrogen receptors, Ki-67, HER2
  - Viruses (HSV, CMV)
- For most specimens, microscopy with immunohistochemical stain is adequate to make a diagnosis

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Immunohistochemistry

• Examination of individual cells under a microscope by a pathologist
• Can be used to evaluate cervical specimens (Pap), fine needle aspirate, CSF, pleural fluid, urine, sputum, etc
• Flow cytometry in conjunction with cytology can further refine the diagnosis

Flow cytometry
§ method of measuring properties of cells in a sample, such as the number of cells, percentage of live cells, cell size and shape, and presence of proteins (such as tumor markers or antigens) on the cell surface
§ often used in the diagnosis, classification, and management of hematologic malignancies such as acute leukemia, chronic lymphoproliferative disorders, and non-Hodgkin lymphoma

Cytopathology

• Examination of individual cells under a microscope by a pathologist
• Can be used to evaluate cervical specimens (Pap), fine needle aspirate, CSF, pleural fluid, urine, sputum, etc
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Which of the following is a cancer that arises from epithelial tissue such as skin or the tissues that line or cover internal organs?

A. Carcinoma JL081
B. Sarcoma JL082
C. Leukemia JL083
D. Lymphoma JL084
Main Types of Cancer

- Carcinoma - arises from epithelial tissue such as skin or the tissues that line or cover internal organs. Subtypes of carcinoma include:
  - adenocarcinoma
  - basal cell carcinoma
  - squamous cell carcinoma
  - transitional cell carcinoma
- Sarcoma - cancer that arises from bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.
- Leukemia - cancer that arises from blood-forming tissue such as the bone marrow and results in malignant blood cells
- Lymphoma and myeloma - cancers that arises from lymphocytes
- Central nervous system cancers - cancers that arise from tissues of the brain and spinal cord.

Naming Cancers

Grading Cancer
Case study: BB

- 45-year-old female in usual state of good health
- Presented to ED after a syncopal episode and epistaxis
- 4-5 months ago, she began having large clots in her menses. She typically has regular cycles with normal menses.
  - Labs: WBC 10.6, Hb 9.1, platelets 36.
  - She received 1 unit of platelets
  - CT head: no acute bleed but with evidence of paranasalitis with air/fluid levels
  - CT abd/pelvis: moderate splenomegaly and a left ovarian cyst.

Bone Marrow Biopsy

Pathology

- Scattered cellular areas appear hypercellular at 90%.
- Erythroid cells are decreased in number with mild dyserythropoiesis.
- Myeloid cells are increased in number with left-shifted maturation and scattered blasts.
- The M:E ratio is 10:1.
- Megakaryocytes are adequate in number and normal in appearance.
- Trabecular bone is normal.
- A small subcortical lymphoid aggregate is identified.

Bone marrow cellularity

- Normal 30-70%
- Hypercellular > 70%
- Hypocellular < 30%

Bone marrow cellularity by age (hematopoietic cells/fat)

- Neonate (All bones, liver, spleen) 100:0
- Child (Most bones) 70:30
- Adult (Axial skeleton) 50:50
- Old age (Axial skeleton) 30:70
Bone Marrow Aspirate

The aspirate smears contain adequate cellular clumps. Erythrocyte cells are relatively decreased in number with mild dyserythropoiesis. Myeloid cells are relatively increased in number with a small subset of granulocytes showing Pelgeroid nuclei and hypogranular forms. A blast population is identified (15%) with smooth, open chromatin, moderate basophilic cytoplasm, occasionally cytoplasmic granules, and perinuclear hofs.

Eosinophils are mildly increased (8%). Megakaryocytes are adequate in number and normal in appearance.

Bone Marrow Aspirate Manual differential (cell count = 200)
- Blasts 15%
- Promyelocytes 1%
- Myelocytes 18%
- Metamyelocytes 8%
- Segs/bands 24%
- Erythroid 12%
- Lymphs 12%
- Plasma cells 2%
- Monos 0%
- Eos 8%
- Basos 0%

ANCILLARY STUDIES: Iron stain on the bone marrow shows scattered iron deposition. No ringed sideroblasts are identified.

Flow Cytometry

- Measures cellular properties as the individual cells move in a fluid stream through an electronic detector that is capable of rapidly analyzing multiple physical and chemical properties.
- Cells can be sorted so that additional analysis can be performed on a subset of cells using monoclonal antibodies that bind to intracellular proteins or proteins expressed on the cell surface.
- For example, if a monoclonal population of cells is identified, a panel of antibodies can be used to identify the proteins expressed by those cells to determine their phenotype and assist with diagnosis.
- When flow cytometry is used to determine the phenotype of a population of cells, it is also referred to as Immunophenotyping.

Flow Cytometric Immunophenotyping

- Flow cytometry was performed on the bone marrow to evaluate blasts.
- Blasts are increased, accounting for 15.9% of leukocytes, and show a myeloid phenotype expressing CD117, CD15, dim CD33, dim CD13, dim CD11C, partial CD19, and cytoplasmic MPO.
- The lymphocyte gate contains a heterogeneous population of lymphocytes, mostly T cells with fewer B- and NK cells. B cells unremarkable B cell antigen expression. T cells show unremarkable T cell antigen expression. NK cells show expected reactivity with panel antibodies.
Cytogenetics

Analytic Date: Cells Counted: 21 Karyotypes
Prepared: 4
Analyzed: 21

ISCN Description:
46,XX,t(8;21)(q22;q22)[19]/46,idem,del(9)(q?)[2]

Chromosome Analysis:
Bone marrow aspirate was cultured, and chromosomes were analyzed using the GTG banding method. Twenty-one metaphases with a balanced translocation between chromosomes #8 and #21 [t(8;21)]. In addition, two cells also demonstrated a chromosome #9 long arm deletion of undetermined breakpoint (del(9q)).

Comments:
The t(8;21) is clonal in nature and, as such, consistent with a neoplastic process. Specifically, the t(8;21) defines a distinct AML subtype by the WHO classification system: acute myeloid leukemia with t(8;21)(q22;q22); RUNX1/RUNX1T1.

Interpretation:
[t(8;21)] clone observed, consistent with AML.

G-banded karyotype: 47,XY, +
4, t(8;21)(q22;q22)

Chromosomes and Genes

Image adapted from: National Human Genome Research Institute.

Philadelphia Chromosome

Example: Translocation of Bcr-Abl Genes

http://www.cancer.gov/cancertopics/understandingcancer/cancergenomics/AllPages
Cells spend most of their time in interphase.

**Chromosome Analysis**

Conventional cytogenetics

FISH: Interphase or molecular cytogenetics


Translocation 8;21

FISH

Cytogenetics

Image from: [http://www.biologia.uniba.it/rmc/TUMORS/project/REARRANGEMENTS/ETO-AML1.html](http://www.biologia.uniba.it/rmc/TUMORS/project/REARRANGEMENTS/ETO-AML1.html)
### Cytogenetics

**FISH (molecular cytogenetics, interphase cytogenetics)**

- **Description**: Can detect abnormalities of chromosomes.
- **Time to Complete**: 2 weeks
- **Pros**: Identifies the complete karyotype including the number of chromosomes and chromosomal abnormalities (translocations, inversions, and deletions, etc.).
- **Cons**: Cells must be cultured for 48-96 hr and harvested when actively dividing and in metaphase.

### Real-time PCR

- **Description**: Detects specific genes and number of gene copies on chromosomes.
- **Time to Complete**: 2-3 days
- **Pros**: Generally sensitive to 1/200 cells. Cells do not have to be actively dividing.
- **Cons**: Specific for 1 target and doesn’t detect other mutations that might be present.

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### Fluorescence in situ Hybridization and PCR Results

- **FISH analysis**: Interphase fluorescence in situ hybridization (FISH*) analysis was performed with the dual-fusion RUNX1T1(ETO)/RUNX1(AML1) probe (Abbott) which identifies the AML-associated translocation, t(8;21). Two hundred interphase nuclei were scored for fusion signals consistent with rearrangement between the RUNX1T1/RUNX1 genes. 157 (78.5%) of which were positive for double fusion signals.

- **Interpretation**: Positive by FISH for RUNX1T1/RUNX1 gene rearrangement.

- **PCR tests**: FLT3-ITD mutation and NPM1 mutation negative.

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### Polymerase Chain Reaction (PCR)

- **Laboratory technique used to amplify DNA sequences**.
- **Short DNA sequences called primers are used to select the portion of the genome to be amplified**.
- **The temperature of the sample is repeatedly raised and lowered to help a DNA replication enzyme copy the target DNA sequence**.
- **Can produce a billion copies of the target sequence in just a few hours**.
Specific PCR Tests in AML

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3-ITD</td>
<td>Prognostic for poorer prognosis</td>
</tr>
<tr>
<td>CEBPA</td>
<td>Prognostic for more favorable prognosis in setting of normal cytogenetics and absence of FLT3-ITD</td>
</tr>
<tr>
<td>NPM1</td>
<td>Prognostic for more favorable prognosis in setting of normal cytogenetics and absence of FLT3-ITD</td>
</tr>
<tr>
<td>KIT</td>
<td>Predictive for anti-KIT therapy (imatinib) Prognostic for poorer prognosis if present with t(8;21), inv(16), or t(16;16) mutations</td>
</tr>
</tbody>
</table>

WHO Classification of Acute Myeloid Leukemia

- **AML with recurrent genetic abnormalities**
  - AML with t(8;21); RUNX1-RUNX1T1
  - AML with inv(16)
  - AML with t(15;17); PML-RARA
  - AML with mutated NPM1
  - AML with mutated CEBPA

  Patients with these genetic abnormalities generally have a high rate of remission and a better prognosis compared to other types of AML.

- **AML with myelodysplasia-related changes**
  - AML transformed from prior myelodysplastic syndrome (MDS) or myeloproliferative disease (MPD). Occurs most often in elderly patients and generally has a poorer prognosis.

- **AML and MDS, therapy-related**
  - AML/MDS that develops after chemotherapy and/or radiation; often characterized by specific chromosomal abnormalities and generally has a poorer prognosis.

- **AML, not otherwise categorized**
  - Includes subtypes of AML that do not fall into the above categories.

Case Study

45-year-old female with a 1.6 x 1.4 x 1.3 cm mass in the breast at 1:00 position, 4 cm from nipple. Family history of breast cancer, (maternal aunt, age 55; maternal aunt, age 45). Left core biopsy, 2 years prior was benign.

**MAMMOGRAM:** The breast tissue is extremely dense, which lowers the sensitivity of mammography. There is a developing density in the upper outer left breast at the posterior depth at the 1 o’clock position which is round, high density, and has spiculated margins. An M-shaped metal biopsy marker is seen adjacent to this lesion. Fine calcifications are also seen adjacent to the biopsy marker on the CC view. Within the right breast, no new focal dominant mass, architectural distortion, or suspicious microcalcifications are identified.

**ULTRASOUND:** Targeted ultrasound reveals a 1.6 x 1.4 x 1.3 cm hypoechoic lesion with internal heterogeneous echogenicity and irregular margins at the 1 o’clock position, 4 cm from the nipple.

**IMPRESSION:**
1. RIGHT BREAST: BI-RADS 1, Negative.
Pathology Report of Biopsy

DIAGNOSIS: INVASIVE DUCTAL CARCINOMA

Sections of the biopsy demonstrate invasive ductal carcinoma with grade 2 features. Hormone studies have been ordered on this specimen and will be reported in an addendum.

CLINICAL HISTORY: 45-year-old female with a 1.6 x 1.4 x 1.3 cm mass in the breast at 1 o'clock position, 4 cm from nipple. Family History of breast cancer, (two maternal aunts).

OPERATION: 10-gauge ultrasound guided core biopsy, left breast 1 o'clock

CLINICAL DIAGNOSIS: Suspicious for malignancy

OTHER STUDIES SENT: Immunohistochemical studies for estrogen receptor, progesterone receptor, and Ki-67 and fluorescence in situ hybridization (FISH) studies for HER2 gene amplification.
Contents of a Pathology Report

- Patient information: Name, birth date, biopsy date
- Gross description: Color, weight, and size of tissue as seen by the naked eye
- Microscopic description: How the sample looks under the microscope and how it compares with normal cells
- Diagnosis: Type of tumor/cancer and grade determined by examining cells and tissues under a microscope
- Tumor size: Measured in centimeters
- Tumor margins
  - Positive margins mean that cancer cells are found at the edge of the material removed
  - Negative, not involved, clear, or free margins mean that no cancer cells are found at the outer edge
  - Close margins are neither negative nor positive
- Notes about samples that have been sent for other tests or a second opinion

College of American Pathologists (CAP) Cancer Protocols

- Pathology reports critical for accurate staging and treatment decisions
- Research in the 1990s
  - Pathologists frequently omitted elements in their diagnostic reports that were recommended for optimal patient care
- Cancer Protocols developed by CAP to:
  - Ensure standardized and comprehensive pathology reporting
  - Reduce ambiguity in terminology
  - Allow data to be more easily communicated, compared, and retrieved, which fosters research efforts

Left Breast Lumpectomy

SPECIMEN SUBMITTED:
A. RIGHT BREAST WIRE LOCALIZATION BIOPSY
B. LEFT BREAST WIRE LOCALIZATION BIOPSY
C. LEFT AXILLA SENTINEL LYMPH NODE #1 COUNT 15,900 (TPC)
D. LEFT AXILLA SENTINEL LYMPH NODE #2 (TPC)
E. LEFT AXILLA SENTINEL LYMPH NODE #3 COUNT 680 (TPE)
F. LEFT AXILLA SENTINEL LYMPH NODE #4 COUNT 499 (TPF)
G. LEFT BREAST WIRE LOCALIZATION BIOPSY
H. NEW INFERIOR MARGIN LEFT BREAST
I. NEW DEEP MARGIN LEFT BREAST
J. NEW ANTERIOR SUPERIOR MARGIN LEFT BREAST

DIAGNOSIS:
A. BREAST TISSUE WITH COLUMNAR CELL CHANGE AND ASSOCIATED MICROCALCIFICATIONS
B. BREAST TISSUE WITH COLUMNAR CELL CHANGE AND MILD HYPERPLASIA
C. ONE LYMPH NODE NEGATIVE FOR CARCINOMA (0/1)
D. ONE LYMPH NODE NEGATIVE FOR CARCINOMA (0/1)
E. POSITIVE FOR METASTATIC CARCINOMA IN ONE OF TWO LYMPH NODES, 0.5 CM WITH EXTRACAPSULAR EXTENSION (1/2)
F. ONE LYMPH NODE NEGATIVE FOR CARCINOMA (0/1)
G. INVASIVE DUCTAL CARCINOMA WITH MINOR COMPONENT OF ASSOCIATED DUCTAL CARCINOMA IN SITU
H. USUAL DUCTAL HYPERPLASIA
I. BENIGN BREAST TISSUE
J. USUAL DUCTAL HYPERPLASIA
In Situ Carcinoma (Type): Minor component of ductal carcinoma in situ
DCIS Size: Associated with < 10% of invasive carcinoma mass
Extracapsular Extension (not 25%) No
Nuclear Grade Intermediate
Margins Status, DCIS > 0.5 cm
Lymph Node Status 1/5 positive for carcinoma
Largest Metastatic Focus 0.05 cm
Extracapsular Extension 0.05 cm
Distant Metastasis Unknown
Other Lesions Atypical ductal hyperplasia, usual ductal hyperplasia
TNM Stage (AJCC, 7th Edition) pT2 pN1a

Additional Tests in Breast Cancer

All specimens

<table>
<thead>
<tr>
<th>ER, PR</th>
<th>Tumor protein</th>
<th>IHC</th>
<th>Diagnostic</th>
<th>Prognostic [positive]</th>
<th>Predictive for hormonal therapy (tamoxifen, aromatase inhibitors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2</td>
<td>Tumor protein or gene amplification</td>
<td>FISH, IHC</td>
<td>Diagnostic</td>
<td>Prognostic [negative]</td>
<td>Predictive for anti-HER2 therapy (trastuzumab, lapatinib, pertuzumab)</td>
</tr>
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For early-stage breast cancer only

<table>
<thead>
<tr>
<th>Oncotype Dx®</th>
<th>Tumor RNA</th>
<th>21-gene RT-PCR expression assay</th>
<th>Prognostic Predictive</th>
</tr>
</thead>
<tbody>
<tr>
<td>MammaPrint®</td>
<td>Tumor RNA</td>
<td>70-gene microarray expression assay</td>
<td>Prognostic</td>
</tr>
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</table>
Addendum to report immunohistochemical studies for estrogen receptor, progesterone receptor, Ki-67

Cold ischemia time: Not provided. Total fixation time: 17 hours, 30 minutes

Unless otherwise specified, fixation is in 10% neutral buffered formalin, as specified in the 2007 and 2010 ASCO/CAP guidelines (see References below). All antibodies and probes have been appropriately validated according to the relevant guidelines.

Immunohistochemistry Comment:
The estrogen and progesterone receptor staining is reported in the diagnosis section below. The FDA approved Ventana monoclonal rabbit antibody clone SP1 is used for estrogen receptor immunohistochemistry with polymer detection method. The FDA approved Ventana monoclonal rabbit antibody clone 1E2 is used for progesterone receptor immunohistochemistry with polymer detection method. Internal control benign breast tissue is present. External control tissue (negative, 1+, and 3+ intensity) stains appropriately. An estimate of the overall percentage of cells staining is provided below.

Addendum Diagnosis:
Left breast, wire localized lumpectomy
-- Estrogen receptor positive (3+, 95%)
-- Progesterone receptor positive (3+, 95%)
-- Ki67 positive (20%)

All breast cancer pathology specimens should be tested for which of the following?

A. Estrogen receptor JL085
B. Progesterone receptor JL086
C. Her2neu gene overexpression JL087
D. A and B JL088
E. All of the above JL089

ASCO and NCCN Guidelines: Breast Cancer Testing

- Biologic information plays an important role in accurate breast cancer staging
  - The most significant biomarkers for prognosis and treatment decisions
    - ER, PR, and HER2
      - ASCO and NCCN recommend that all newly diagnosed and recurrent invasive breast cancers are assessed by immunohistochemistry (IHC) for tumor ER and PR status
  - ASCO, NCCN, and the College of American Pathologists (CAP) recommend testing for HER2 status in all patients diagnosed with invasive primary, recurrent, or metastatic breast cancer
    - The threshold for an IHC score of 3+ changed from uniform, intense staining of >30% of tumor cells to greater than 10% of tumor cells in 2013
    - If the IHC score is 2+: FISH should be performed on the same specimen, if possible
What is HER2 Amplification?

Immunohistochemistry HER2

Fluorescence in situ hybridization (FISH)

- Uses fluorescence-tagged probes to detect specific DNA sequences in tissue samples
- Normal cell has 2 copies of HER2
- Amplification is based on the gene copy number and the ratio between the numbers of HER2 and CEP17 sequences.
Comparison of IHC and FISH for HER2

Green is chromosome 17 centromere; red is HER2/neu.

Addendum to report fluorescence in situ hybridization (FISH) studies for HER2 gene amplification

Cold ischemia time: Not provided. Total fixation time: 17 hours, 30 minutes

Unless otherwise specified, fixation is in 10% neutral buffered formalin, as specified in the 2007 and 2010 ASCO/CAP guidelines (see References below). All antibodies and probes have been appropriately validated according to the relevant guidelines.

FISH DESCRIPTION:
Analytic Data: Block G12: (HER2/D17Z1)
Cells Scored: 25
Total HER2 target signals: 74
Total D17Z1 control signals: 54
Ratio HER2/D17Z1: 1.37

Fluorescence in situ hybridization was performed using the dual-color PathVysion® Her-2 DNA probe kit containing a HER2 locus-specific probe (LSI HER2) and a control probe specific for the pericentromeric region of chromosome 17 (D17Z1). A total of 25 interphase nuclei were scored for the number of HER2 and D17Z1 signals. The observed HER2:D17Z1 signal ratio is reported in the addendum diagnosis section below. A ratio <1.8 is considered to be negative, between 1.8 and 2.2 ambiguous, and >2.2 positive for HER2 gene amplification based on FDA-approved criteria. Cells with innumerable (>10) HER2 signals are scored as having 10 signals. HER2 FISH analysis includes participation by at least three observers. Results from the PathVysion® HER2 DNA probe kit (Vysis) are intended for use as an adjunct to other clinical and pathological information. This kit has been approved by the U.S. Food and Drug Administration for in vitro diagnostic use.

ADDENDUM DIAGNOSIS:
LEFT BREAST, WIRE LOCALIZED LUMPECTOMY
-- HER2 NEGATIVE FOR AMPLIFICATION BY FISH (RATIO: 1.37)

What Does This All Mean?

- Site: Left breast
- Specimen Type: Wire localized lumpectomy
- Invasive Carcinoma Type: Ductal with component of DCIS
- Histologic Grade Composite (Nottingham)
  - Grade 2 of 3 (Tubules=3, Nuclear grade=2, Mitotic activity <2)
- Estrogen receptor: positive (3+, 95%)
- Progesterone receptor: positive (3+, 95%)
- Ki67: positive (20%)
- HER2: negative for amplification by fish (ratio: 1.37)

TNM Stage (AJCC, 7th Edition): pt2 pN1a
- T: pathologic
- Staging studies showed no evidence of distant metastases

Final Stage: Stage IIB, pT2pN1aM0
ER+, PR+, HER2 neg
Which of the following is true about immunohistochemistry as a technique to identify antigens?

A. Tissue is treated with an radioactive-tagged antibody that binds the specific antigen so that the antibody-antigen complex is visible under a microscope

B. Method of measuring properties of cells in a sample, such as the number of cells, percentage of live cells, cell size and shape, and presence of proteins (such as tumor markers or antigens) on the cell surface as the individual cells move in a fluid stream through an electronic detector

C. Detects specific genes and number of gene copies on chromosomes

D. Detects abnormalities of chromosomes

Oncotype Dx

The Oncotype DX assay requires one tumor block and an H&E slide from the same block. When blocks are submitted, typically 35 to 65 microns of tissue will be used.

- 21-gene assay
- predicts chemotherapy benefit and 10-year distant recurrence to inform adjuvant treatment decisions in certain women with early-stage invasive breast cancer

Oncotype Dx Report

- Breast cancer recurrence score of 13 = average rate of distance recurrence 9%
- Positive estrogen receptor score: 8.2
- Positive progesterone receptor score: 9.2
- Negative HER2 score: 8.9

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<td>&lt;18</td>
<td>6.8%</td>
<td>None</td>
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<tr>
<td>18-30</td>
<td>14.3%</td>
<td>None</td>
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<tr>
<td>&gt; 30</td>
<td>30.5%</td>
<td>28%</td>
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Female, stage III, node-negative, ER/PR-positive breast cancer treated with 5 years of tamoxifen


Oncotype Dx Report

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Gene Microarray

- Used to study the expression of many genes at once
- Thousands of gene sequences are placed in known locations on a glass slide called a gene chip
- Sample containing DNA or RNA is placed in contact with the gene chip
- Complementary base pairing between the sample and the gene sequences on the chip produces light that is measured
- Areas on the chip producing light identify genes that are expressed in the sample

Colorectal Carcinoma

- Histology: primarily adenocarcinoma (96%)
  - Most adenocarcinomas are thought to arise from adenomatous polyps over a long period of time
- Other histopathological classifications of colorectal tumors
  - adenocarcinoma in situ
  - medullary carcinoma
  - mucinous carcinoma (colloid type)
  - signet cell carcinoma, squamous cell (epidermoid) carcinoma, adenosquamous
  - small cell carcinoma
  - undifferentiated carcinoma, or carcinoma, NOS
- Histologic grade is assigned from G1 to G4

Case study

- 64-year-old Filipina physician presents for routine colonoscopy
- Past medical history: None
- Social history: Married with 2 children
- Alcohol use: None
- Tobacco use: 24-pack-year smoking history, quit 5 years ago
- Colonoscopy showed biopsy-proven adenocarcinoma
- Staging studies revealed no evidence of distant metastases
- Hemicolectomy
### Colon Cancer: Gross Specimen

![Colon Cancer: Gross Specimen](http://en.wikipedia.org/wiki/File:Colon_cancer.jpg)

### Colon Cancer Stained With H&E

![Colon Cancer Stained With H&E](http://en.wikipedia.org/wiki/File:Colonic_carcinoid_(1)_Endoscopic_resection.jpg)

### Pathology Report

<table>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Site:</strong> Sigmoid</td>
</tr>
<tr>
<td><strong>Specimen:</strong> Tumor or lymph node</td>
</tr>
<tr>
<td><strong>Depth of Invasion:</strong> Submucosa</td>
</tr>
<tr>
<td><strong>Distant Metastasis:</strong> Unknown</td>
</tr>
<tr>
<td><strong>Tumor Deposits:</strong> Absent</td>
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**ASCENDING COLON, POLYPECTOMY**
- Tubular Adenoma

**ASCENDING COLON, BIOPSY**
- Tubular Adenoma

**SIGMOID COLON, COLECTOMY**
- Invasive Colonic Adenocarcinoma, Moderately Differentiated, Arising in a Tubular Adenoma, Two Lymph Nodes Negative for Malignancy
Which molecular test should be ordered for all cases of metastatic colon cancer?

A. EGFR JL090  
B. RAS JL091  
C. BRAF JL092  
D. ALK JL093  
E. All of the above JL094

Molecular Diagnostics in Colorectal Cancer

- **EGFR** overexpressed ~20% of colorectal cancers  
  - Polymerase chain reaction (PCR) test  
  - No predictive value for anti-EGFR therapy so routine EGFR testing not recommended

- **RAS** mutations present ~40% of colorectal cancers  
  - PCR test  
  - RAS/RAF/MAK pathway is downstream from EGFR and KRAS mutations predict a lack of benefit from anti-EGFR therapy (cetuximab, panitumumab)  
  - All patients diagnosed with metastatic colorectal cancer should have RAS genotyping performed  
  - Anti-EGFR therapy should not be administered if a RAS mutation is present

- **BRAF** mutations occur only in patients without RAS mutation  
  - PCR test  
  - Also appear to confer resistance to anti-EGFR therapy so identify another subset of patients unlikely to benefit from cetuximab or panitumumab  
  - Strong prognostic marker that is associated with decreased overall survival


Biomarkers in Colon Cancer

| **RAS** mutations (NRAS, HRAS) | Tumor DNA PCR, multiplex assays, or direct sequencing | Recommended for all metastatic colon cancer cases  
Predicative for lack of response to anti-EGFR therapy (cetuximab, panitumumab)  
Prognostic (poor) |
|---|---|---|
| **MSI** and/or MMR protein loss | Tumor DNA for MSI testing with PCR or tumor IHC for MMR proteins | Screening for Lynch syndrome  
Predictive for lack of benefit with adjuvant single-agent fluoropyrimidine therapy  
Prognostic (poor) |
| **CEA** | Serum protein immunoassay | Surveillancce |
| **Coxsylt** | 32-gene tumor microarray assay | Prognostic in Stage II  
Not FDA-approved |
| **OncoPrint Colorect** | 32-gene RT-PCR gene expression assay | Prognostic in Stage II and IIIA/B  
Not FDA-approved |
| **BRAF** mutations | Tumor DNA PCR, multiplex assays, or direct sequencing | Prognostic (poor)  
Predictive for lack of benefit for anti-EGFR therapy |

Which cancer treatment may be prescribed for patients with metastatic colon cancer and a RAS mutation?

A. Cetuximab JL095  
B. Panitumumab JL096  
C. A or B JL097  
D. None of the above JL098

Case Study (cont)

- Stage 1A moderately differentiated invasive adenocarcinoma of the sigmoid colon  
- Treated with hemicolectomy  
- 1 year later, presents with R lower lobe pulmonary nodule

Lung Biopsy

- Right lung needle core biopsy  
  - adenocarcinoma positive for CK7, CK20 (focal) and TTF-1, consistent with a lung primary  
- She underwent R lower lobectomy and staging studies which showed no distant metastases.  
- CEA within normal limits and no evidence of colon cancer recurrence
**Lung Cancer**

- The two major classes
  - Non-small cell (NSCLC): 85% of cases
    - Adenocarcinoma: most common
    - - premedetrex preferred
    - Squamous cell carcinoma
      - unlikely to benefit from premedetrex
      - higher risk for life threatening hemorrhage from bevacizumab (Travis et al., 2011)
  - Small cell lung cancer (SCLC)

**IHC Staining**

- Used to differentiate various types of NSCLC
- Two main IHC tests
  - TTF-1
    - present in 70-85% of pulmonary adenocarcinomas
    - can also be useful to distinguish primary pulmonary adenocarcinoma from pulmonary metastases from another primary adenocarcinoma
  - p63
    - best diagnostic marker for squamous cell

**R Lower Lobectomy Pathology Report**

**LUNG CARCINOMA SUMMARY**

- Site right lower lobe
- Breath Pathology
- Tumor Type Adenocarcinoma (acinar, lepidic and micropapillary patterns)
- Size 2.5 cm
- Histologic Grade Moderately differentiated
- Pathology
  - invasive adenocarcinoma with a predominantly acinar and lepidic growth pattern, and a focal area with micropapillary architecture
  - positive for CK7 and TTF1, but negative for CK20 and CDX2 and is most compatible with lung primary
  - EGFR deletion in exon 19 is detected by PCR
  - FISH NEGATIVE FOR ALK GENE REARRANGEMENT
  - FISH NEGATIVE FOR ROS1 GENE REARRANGEMENT
  - No Kras mutation by PCR
- Stage IB adenocarcinoma of the lung

- TNM (AJCC, 7th Edition) pT2 pN0
Adenocarcinoma Classification

- Classification updated in 2011 by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society to address advances in oncology, molecular biology, pathology, radiology, and surgery
- Based on the 2004 World Health Organization (WHO) classification
- Eliminates the terms bronchioloalveolar carcinoma (BAC) and mixed subtype adenocarcinoma.
- The subtypes that replace BAC and mixed subtype adenocarcinoma are:
  - adenocarcinoma in situ (AIS)
  - minimally invasive adenocarcinoma (MIA)
  - invasive adenocarcinoma, predominant growth pattern: lepidic, acinar, papillary, micropapillary, or solid with mucin
  - invasive mucinous adenocarcinoma (formerly mucinous BAC)

Case Study (cont)

- Stage 1B adenocarcinoma of lung treated with R lower lobe lobectomy
- Adjuvant chemotherapy for close margins and visceral pleural invasion: 4 cycles of carboplatin and pemetrexed
- Follow-up CT scans and CEA levels every 3 months
- One year later, CEA levels increased
- PET scan: 1 cm node in the retrocaval and 1 cm R paratracheal node
- Mediastinoscopy performed for biopsy

Pulmonary Nodule Biopsy Pathology Report

- OPERATION: Flexible bronchoscopy with mediastinoscopy and biopsy
- FROZEN SECTION DIAGNOSIS:
  A. lymph node, right level IV, biopsy
  -- Lymph node, no tumor seen
  B. lymph node, right level II, biopsy
  -- Metastatic adenocarcinoma

DIAGNOSIS (MICROSCOPIC):
A. lymph node, right level IV, biopsy
  - ONE LYMPH NODE, NEGATIVE FOR CARCINOMA (0/1)
B. lymph node, right level II, biopsy
  - METASTATIC ADENOCARCINOMA
C. lymph node, right level IV, lymphadenectomy
  - METASTATIC ADENOCARCINOMA
D. lymph node, right level II, lymphadenectomy
  - METASTATIC ADENOCARCINOMA

COMMENT: I have reviewed the frozen sections and confirm the intra-operative diagnoses rendered. Permanent sections demonstrate metastatic adenocarcinoma in the level II lymph nodes as well as in the second submitted level IV lymph node. The carcinoma is positive for CK7 and TTF1, but negative for CK20 and CDX2 and is most compatible with lung primary.
Case Study (cont)

- Stage IIIA recurrent adenocarcinoma of lung with positive right level II and IV nodes and recurrent tumor in her bronchial stump
- Treated with chemoradiation
  - RT 66 Gy
  - Etoposide/cisplatin × 2 cycles
- Post chemoradiation PET: new liver lesion

CT-Guided FNA of Liver Lesion

- CYTOLOGIC DIAGNOSIS: LIVER, LEFT LOBE, CT-GUIDED FINE NEEDLE ASPIRATION AND CORE BIOPSY
- METASTATIC ADENOCARCINOMA, CONSISTENT WITH LUNG PRIMARY

DIAGNOSTIC COMMENTS: The aspirate smears are mildly cellular and show crowded clusters and sheets of cells with nuclear enlargement, pleomorphism, membrane irregularities, and variably prominent nucleoli. They have a moderate amount of delicate cytoplasm and occasional large cytoplasmic vacuoles. Scattered benign hepatic parenchyma is present. Sections of the core show similar findings. Immunohistochemical stains show the tumor cells to be positive for CK7 and TTF1. They are negative for CK20 and CDX2. The morphologic and immunohistochemical findings are most consistent with a metastatic adenocarcinoma of lung primary.

IMMEDIATE EVALUATION: Immediate evaluation of representative Diff-Quik stained slides via Nikon webcam showed adequate cellular material.

Which molecular test should be ordered for all cases of adenocarcinoma of the lung?

A. EGFR JL099
B. ALK JL100
C. RAS JL101
D. A and B JL102
E. All of the above JL103
Biomarkers for Lung Cancer

- Patients with adenocarcinoma of the lung should have tumors tested for EGFR and ALK mutations.

  - EGFR mutations (PCR, multiplex assays, or direct sequencing)
    - 10% in non-Asians and up to 50% in Asians
    - Increased incidence in never smokers, Asians, and non-mucinous tumors
    - Never occurs with RAS mutation
    - Predictive for response to anti-EGFR therapy (erlotinib, gefitinib, afatinib)

  - ALK mutations (EML4-ALK gene fusion) (FISH)
    - 2%–7%
    - Increased incidence in male, younger, have adenocarcinoma histology, and who are nonsmokers
    - Usually doesn’t occur with EGFR mutations
    - Predictive for anti-ALK therapy (crizotinib)
    - Predictive for lack of benefit for anti-EGFR therapy

Other Biomarkers in Lung Cancer

- RAS mutation
  - PCR, multiplex assays, or direct sequencing
  - 10%–20%
  - Increased incidence in non-Asians, smokers, and/or invasive mucinous adenocarcinomas
  - Predictive for poorer prognosis
  - Predictive for lack of benefit with anti-EGFR therapy
  - Predictive for poor response to platinum/vinorelbine chemotherapy

- ERCC1
  - IHC, FISH
  - Predictive for poor response to platinum chemotherapy
  - Predictive for poorer prognosis

- ROS1 mutation
  - FISH
  - 1%
  - More common in light smokers (< 10 pack-years) or never-smokers, younger age, and adenocarcinoma histology
  - Predictive for response to crizotinib

Which cancer treatment may be prescribed for patients with ALK-positive lung cancer?

A. Erlotinib JL104
B. Platinum drug JL105
C. Crizotinib JL106
D. Afatanib JL107
Future Directions

Next-generation sequencing
- Allow for whole exome sequencing, which examines all protein-coding regions in the human genome
- Allow for whole genome sequencing, which analyzes both protein-coding and non-coding regions in the human genome
- Currently there are custom panels that can detect up to thousands of mutations at 1,000x sensitivity
- Expensive
- Unclear significance of low levels of mutations

http://www.mycancergenome.org/content/other/molecular-medicine/types-of-molecular-tumor-testing/

Summary

- Diagnosis of cancer relies on biopsy and evaluation of the biopsy using multiple techniques
- Histopathology is mainstay of pathology review
- Molecular tests to detect genetic abnormalities increasingly important to determine prognosis and to direct targeted therapy
- To learn more about genetic alterations in cancer
  http://www.mycancergenome.org/

References

References (cont)


References (cont)

- American Cancer Society. 2011; Edge et al., 2010.
- Types of molecular tumor testing. http://www.mycancergenome.org/content/other/molecular-medicine/types-of-molecular-tumor-testing/
- National Human Genome Research Institute http://www.genome.gov/glossary/index.cfm?id=26