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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

NCI, 2012

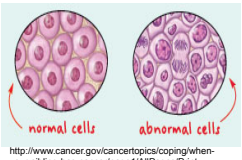
Terms

- 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



http://www.cancer.gov/cancertopics/coping/when-your-sibling-has-cancer/page1/AllPages/Print

Compton et al. (2012). AJCC cancer staging atlas: A companion to the seventh editions of the AJCC cancer staging manual and handbook.

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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

National Cancer Institute. (2010). Pathology reports. Retrieved from: http://www.cancer.gov/cancertopics/factsheet/detection/pathology-reports

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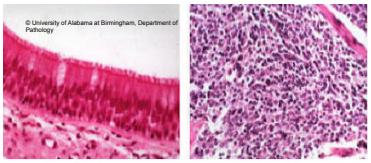
Hematoxilyn & Eosin (H&E stain)



Normal Airway | Lung Cancer

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

National Cancer Institute. (2010). Pathology reports. Retrieved from: http://www.cancer.gov/cancertopics/factsheet/detection/pathology-reports

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Definitions

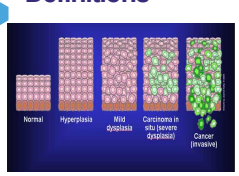
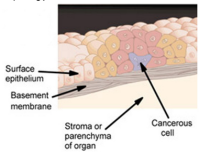


Image from: National Cancer Institute. (2009). Understanding Cancer Series. Retrieved from: http://www.cancer.gov/cancertopics/understandingcancer/can



- 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)

Image from: Wikimedia Commons
Retrieved from: http://en.wikipedia.org/wiki/File:In_situ_carcinoma.jpg

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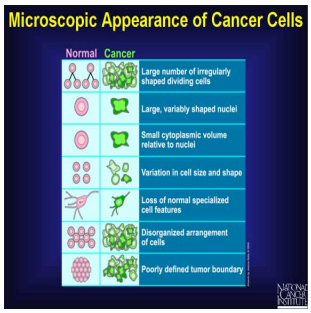
Cancer Cells Under the Microscope



National Cancer Institute. (2009). Understanding Cancer Series. Retrieved from: http://www.cancer.gov/cancertopics/understandingcancer/cancer/page18

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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 radioisotope, 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

Olsen, M. & Zitella, L. (Eds). (2013). *Hematologic malignancies in adults.*

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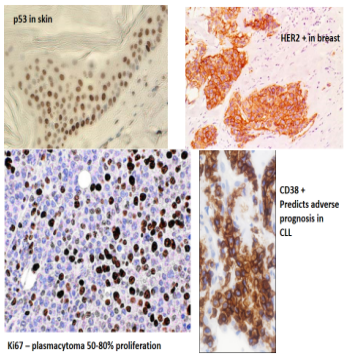
Immunohistochemistry



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
- 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

Image from Wikimedia Commons http://commons.wikimedia.org/wiki/File:Fluorescence_Assisted_Cell_Sorting_(FACS)_B.jpg

Which of the following is a cancer that arises from epithelial tissue such as skin or the tissues that line or cover internal organs?

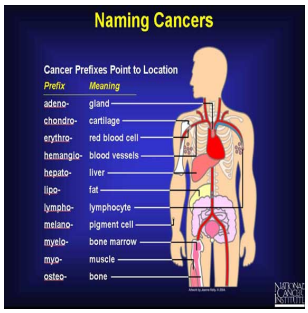
A. Carcinoma **JL081**
B. Sarcoma **JL082**
C. Leukemia **JL083**
D. Lymphoma **JL084**

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.

National Cancer Institute. 2014. What is Cancer? http://www.cancer.gov/cancertopics/cancerlibrary/what-is-cancer

Naming Cancers

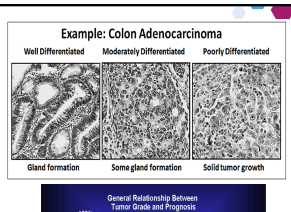


National Cancer Institute. (2009). Understanding Cancer Series. Retrieved from: http://www.cancer.gov/cancertopics/understandingcancer/cancer/page3

Grading Cancer

- **GX**: Grade cannot be assessed
  - undetermined grade
- **G1**: Well differentiated
  - low grade
- **G2**: Moderately differentiated
  - intermediate grade
- **G3**: Poorly differentiated
  - high grade
- **G4**: Undifferentiated
  - high grade

There are tumor-specific grading systems for breast cancer (Nottingham score) and prostate cancer (Gleason score).



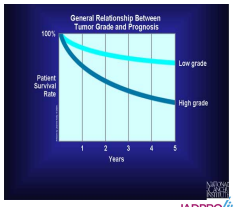


Image from: National Cancer Institute. (2009). Understanding Cancer Series. Retrieved from: http://www.cancer.gov/cancertopics/understandingcancer/cancer/page22

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 parasinusitis 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.

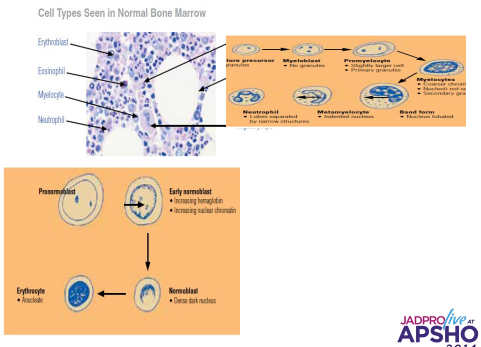
Bone marrow cellularity

- 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



Bone Marrow Aspirate

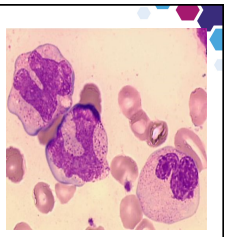
The aspirate smears contain adequate cellular spicules. Erythroid cells are relatively decreased in number with mild dyserythropoiesis.

Myeloid cells are relatively increased in number with a small subset of granulocytes are dysplastic 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%, erythroids 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.



BONE MARROW, ASPIRATE AND BIOPSY
-- ACUTE MYELOID LEUKEMIA WITH t(8;21), 15%
-- HYPERCELLULAR MARROW WITH MYELOID HYPERPLASIA AND SUBSET DYSPLASTIC FORMS

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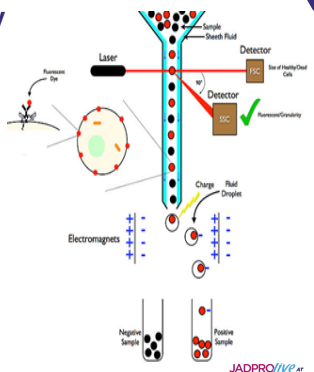
Flow Cytometry

- 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.



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Flow Cytometric Immunophenotyping

	Blast gate(%)	Lymphocyte gate (%)
% Gated/CD45+	16	8
B LINEAGE		
CD10	<1	<1
CD19	46dim	6
CD20	<1	6
CD22	1	6
cCD79a	1	7
TdT	3	<1
mKappa (% of gated)	4.5	3.2
mLambda (% of gated)	0.2	2.0
T/NK LINEAGE		
CD7	<1	86
CD5	<1	88
cCD3	1	91
sCD3	<1	91
CD4	16	58
CD8	1	31
CD56	62	10
CD16	<1	2
CD2	1	93
CD11c	20	7
MYELOID/MISC		
HLA-DR	80	31
CD34	94	2
CD117	95	2
CD13	2dim	<1
CD33	2dim	<1
CD11	16	6
MPO	99	1
CD14	3	<1
CD64	8	1
CD61	<1	<1
MISCELLANEOUS		
CD5+/CD8+		58
CD4+/CD8+		31
CD57/CD8		1.9
CD21/CD117	31	1
CD38/CD34	90	1

Specimen type: Bone marrow
% Viability by 7-AAD: blast gate: >99 %, lymphocyte gate: >99 %
Cell count: 47.2 K/uL

Gates:
Blast: dim/ moderate CD45, low SSC
Lymphocyte: bright CD45, low SSC

Flow cytometry was performed on the bone marrow primarily to evaluate blasts.

Blasts are increased, accounting for 15.5% of leukocytes, and show a myeloid phenotype, expressing CD56, CD117, CD15, dim CD13, dim CD33, 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.

BONE MARROW, ASPIRATE, FLOW CYTOMETRY IMMUNOPHENOTYPING
-- ABNORMAL CD34+ BLAST POPULATION, 15.5%, EXPRESSING CD56, CD117, CD15, DIM CD13, DIM CD33, DIM CD11C, PARTIAL CD19, AND YTOPLASMIC MPO
-- HETEROGENEOUS B, T, AND NK CELLS WITHOUT ABERRANT ANTIGEN EXPRESSION

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Cytogenetics

Analytic Data: Cells Counted: 21 Karyotypes prepared: 4
Analyzed: 21 Band resolution: <400
Imaged: 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 GTW banding method. Twenty-one metaphase cells were analyzed, all of which were abnormal and characterized by 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)

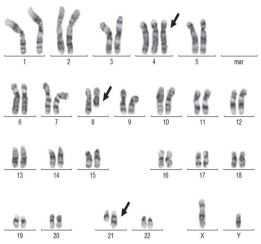

Image from: Parihar, M. et al. (2012). Leukemia & Lymphoma, 53(1), 103-109
http://informahealthcare.com...doi/full/10.3109/10428194.2011.603447

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Chromosomes and Genes

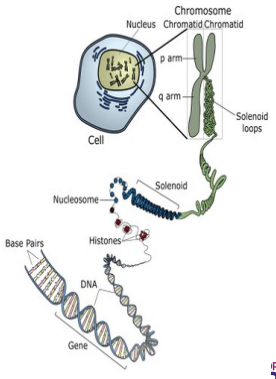

Image adapted from: National Human Genome Research Institute.

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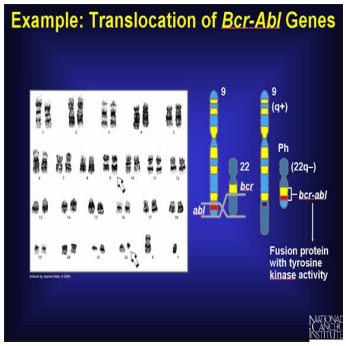
Philadelphia Chromosome



http://www.cancer.gov/cancertopics/understandingcancer/cancergenomics/AllPages

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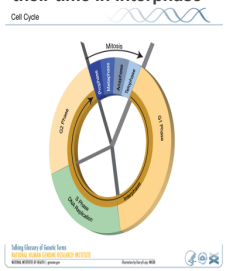
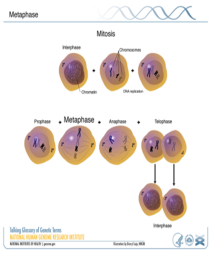
Cytogenetics

Cells spend most of their time in interphase



Metaphase



http://www.genome.gov/glossary/index.cfm?id=26

Chromosome Analysis

Conventional cytogenetics

Image from: National Institutes of Health. National Human Genome Research Institute. "Talking Glossary of Genetic Terms." http://www.genome.gov/glossary

FISH: Interphase or molecular cytogenetics

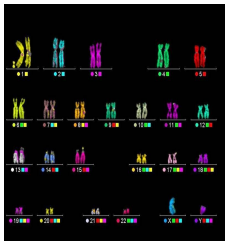
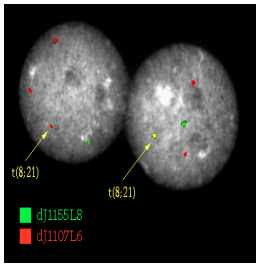


Image from: http://www.nasa.gov/mission_pages/station/research/experiments/Chromosome-14.jpg

Translocation 8;21

FISH



Cytogenetics

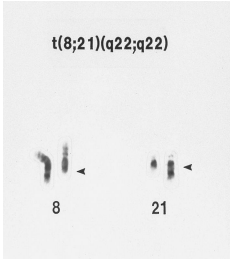


Image from: http://www.biologia.uniba.it/rmc/TUMORS/project/REARRANGEMENTS/ETO-AML1.html

	Cytogenetics	FISH (molecular cytogenetics, interphase cytogenetics)	Real-time PCR
Description	Can detect abnormalities of chromosomes	Detects specific genes and number of gene copies on chromosomes	Detect only 1 type of mutation in 1 gene or 1 type of DNA
Time to Complete	2 weeks	2-3 days	1-2 days
Pros	Identifies the complete karyotype including the # of chromosomes and chromosomal abnormalities (translocations, inversions, and deletions, etc)	Generally sensitive to 1/200 cells Cells do not have to be actively dividing	Sensitive—can detect 1/100,000 cells Cells do not have to be actively dividing
Cons	Cells must be cultured for 48-96 hr and harvested when actively dividing and in metaphase Unable to detect small chromosomal abnormalities or genetic mutations	Specific for 1 target and doesn't detect other mutations that might be present	Specific for 1 target and doesn't detect other mutations that might be present

My Cancer Genome. (2013). Retrieved from: http://www.mycancergenome.org/content/other/molecular-medicine/types-of-molecular-tumor-testing/

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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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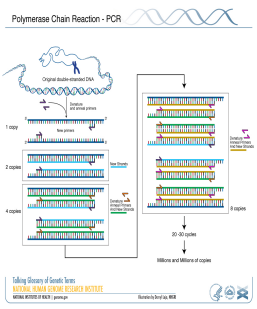
Polymerase Chain Reaction (PCR)



- 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.

National Institutes of Health. National Human Genome Research Institute. "Talking Glossary of Genetic Terms." http://www.genome.gov/glossary/index.cfm?id=159

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Specific PCR Tests in AML

FLT3-ITD mutation	Prognostic for poorer prognosis
CEBPA mutation	Prognostic for more favorable prognosis in setting of normal cytogenetics and in absence of FLT3-ITD
NPM1 mutation	Prognostic for more favorable prognosis in setting of normal cytogenetics and in absence of FLT3-ITD
KIT mutation	Predictive for anti-KIT therapy (imatinib) Prognostic for poorer prognosis if present with t(8;21), inv(16), or t(16;16) mutations

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WHO Classification of Acute Myeloid Leukemia

AML with recurrent genetic abnormalities	Includes: • 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.

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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.

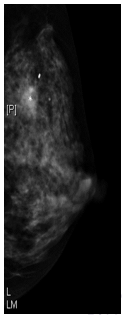
Within the right breast, no new focal dominant mass, architectural distortion, or suspicious microcalcifications are identified.

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

IMPRESSION:

1. RIGHT BREAST: BI-RADS 1, Negative.

2. LEFT BREAST: BI-RADS 4B, Intermediate suspicion for malignancy.



Bi-RADS® Categories for Breast Imaging Using Mammography, Ultrasound or MRI

Category	Findings
0	Incomplete examination (e.g. additional mammography views, ultrasound, or previous studies are necessary to assign a final assessment category)
1	Negative
2	Benign (e.g lipomas, calcified fibroadenomas)
3	Probably Benign (e.g. findings that have <2% chance of malignancy, such as a noncalcified circumscribed solid mass)
4	Suspicious Abnormality (e.g findings that are not obviously malignant and have a wide range of probability of malignancy) • 4A: low suspicion for malignancy • 4B: intermediate suspicion of malignancy • 4C: moderate concern, but not classic for malignancy
5	Highly Suggestive of Malignancy (e.g. findings that have > 95% chance of malignancy)
6	Known Biopsy – Proven Malignancy

Adapted from: D'Orsi CJ, Bassett LW, Berg WA, et al. BI-RADS: Mammography, 4th edition in: D'Orsi CJ, Mendelson EB, Ikeda DM, et al: Breast Imaging Reporting and Data System: ACR BI-RADS – Breast Imaging Atlas, Reston, VA, American College of Radiology, 2003. Retrieved from http://www.acr.org/Quality-Safety/Resources/BIRADS/Mammography

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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
GROSS DESCRIPTION: One specimen is received labeled with the patient's name and medical record number. The specimen labeled "left breast 1 o'clock" is received in formalin and consists of multiple, pale, tan fragments of tissue that measure 1.8 x 1.4 x 0.4 cm in aggregate. The specimen is entirely submitted between sponges in one cassette labeled A1.
OTHER STUDIES SENT: Immunohistochemical studies for estrogen receptor, progesterone receptor, and Ki-67 and fluorescence in situ hybridization (FISH) studies for HER2 gene amplification.

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Nottingham Histologic Score

(also known as Elston-Ellis modification of the Scarff-Bloom-Richardson grading system)

Glandular Acinar)/ Tubular Differentiation (how much of the tumor tissue has normal breast (milk) duct structures)	Nuclear Pleomorphism (assessment of the size and shape of the nucleus in the tumor cells)	Mitotic Rate (number of mitoses /10 hpfs, which is a measure of how many cells are diving and growing)	Overall Grade
Score 1: >75% of tumor area forming glandular/tubular structures Score 2: 10% to 75% of tumor area forming glandular/tubular structures **Score 3: <10% of tumor area forming glandular/ tubular structures**	Score 1: Nuclei small with little increase in size in comparison with normal breast epithelial cells, regular outlines, uniform nuclear chromatin, little variation in size Score 2: Cells larger than normal with open vesicular nuclei, visible nucleoli, and moderate variability in both size and shape Score 3: Vesicular nuclei, often with prominent nucleoli, exhibiting marked variation in size and shape, occasionally with very large and bizarre forms	Score 1: ≤ 3 mitoses per mm^2 Score 2: 4-7 mitoses per mm^2 Score 3: ≥ 8 mitoses per mm^2	**Grade 1 (Low):** scores of 3, 4, or 5 **Grade 2 (Intermediate):** scores of 6 or 7 **Grade 3 (High):** scores of 8 or 9 ___ Only microinvasion present (not graded) ___ No residual invasive carcinoma after presurgical (neoadjuvant) therapy ___ Score cannot be determined

College of American Pathologists. (2013). Invasive Breast Carcinoma Protocol. Retrieved from: http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2013/BreastInvasive_13protocol_3200.pdf

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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

National Cancer Institute. (2010). Pathology reports. Retrieved from: http://www.cancer.gov/cancertopics/factsheet/detection/pathology-reports

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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

http://www.cap.org/

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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 (TPD)
E. LEFT AXILLA SENTINEL LYMPH NODE #3 COUNT 680 (TPE)
F. LEFT AXILLA SENTINEL LYMPH NODE #4 COUNT 499 (TPF)
G. LEFT BREAST WIRE LOC LUMPECTOMY
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

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Breast Invasive Carcinoma Summary

LEFT BREAST, 1 O'CLOCK, 5cm FROM NIPPLE

Site: Left breast

Specimen Type Wire localized lumpectomy
Invasive Carcinoma Type Ductal
Histologic Grade Composite (Nottingham)
Grade 2 of 3 (Tubules=3, Nuclear grade=2, Mitotic activity =2)
Size (Invasive, cm) 2.1cm
Lymphovascular Invasion Not seen
Paget's Disease (Absent or Present) Unable to assess (no nipple)
Margin Status, Invasive As below
Superior >0.5cm
Inferior: >0.5cm (invasive cancer was within 0.1 cm of the inferior margin in the initial lumpectomy but was not present in the additional inferior margin)
Medial >0.5cm
Lateral >0.5cm
Anterior (Superficial) >0.5cm
Posterior (Deep): >0.5cm (invasive cancer was at the posterior margin in the initial lumpectomy but was not present in the additional posterior margin)
Skeletal Muscle Involvement Unable to assess

In Situ Carcinoma (Type): Minor component of ductal carcinoma in situ
DCIS Size: Associated with < 10% of invasive carcinoma mass
Extensive (>25%) No
Nuclear Grade Intermediate
Necrosis Not identified
Margin Status, DCIS > 0.5 cm

Lymph Node Status 1/5 positive for carcinoma
Largest Metastatic Focus 0.5 cm
Extracapsular Extension 0.1 cm
Distant Metastasis Unknown
Other Lesions Atypical ductal hyperplasia, usual ductal hyperplasia
TNM Stage (AJCC, 7th Edition) pT2 pN1a

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Pathology Report (cont)

Several pages listing:

- each specimen received
- gross description
- inked margins

Hooray for the summary section!

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Additional Tests in Breast Cancer

All specimens			
ER, PR	Tumor protein	IHC	Diagnostic Prognostic (positive) Predictive for hormonal therapy (tamoxifen, aromatase inhibitors)
HER2	Tumor protein or gene amplification or overexpression	FISH, IHC	Diagnostic Prognostic (negative) Predictive for anti-HER2 therapy (trastuzumab, lapatinib, pertuzumab)
For early-stage breast cancer only			
Oncotype Dx®	Tumor RNA	21-gene RT-PCR expression assay	Prognostic Predictive
MammaPrint®	Tumor RNA	70-gene microarray expression assay	Prognostic

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%)

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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**

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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 tumors cell 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

National Comprehensive Cancer Network. (2014). *NCCN clinical practice guidelines in oncology: Breast cancer [v.3.2014].* Retrieved from http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf ; Wolff, A. C., et al. (2013). Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Journal of Clinical Oncology*

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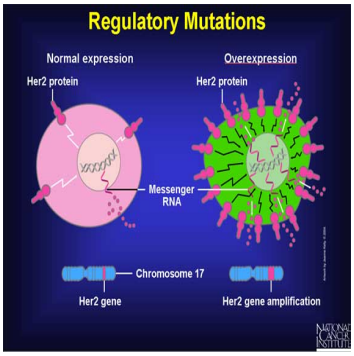
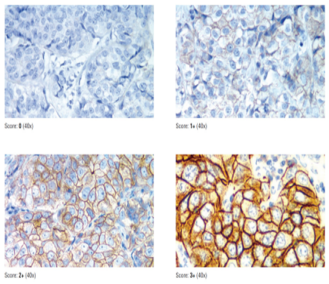
What is HER2 Amplification?



http://www.cancer.gov/cancertopics/understandingcancer/cancergenomics/AllPages

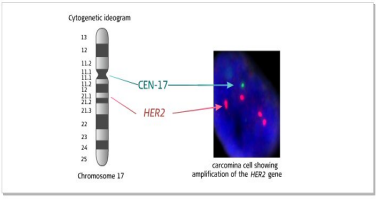
Immunohistochemistry HER2



In HER2 and EGFR, only membranous – not cytoplasmic reactivity, counts as specific staining

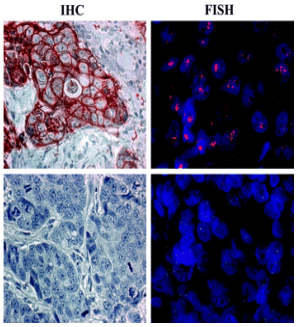
Note the different scores for differing intensities of HER2 membranous staining. Only 3+ is treated with Herceptin. 2+ scores are "equivocal" and are retested with FISH.

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*.

Xia W, et al. Clin Cancer Res 2004;10:3815-3824

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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 uidelines (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(r) 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 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(r) HER2 DNA probe kit (Vysis) are intended for use as an adjunct to other clinical and pathologic 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)

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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
- P = pathologic
- Staging studies showed no evidence of distant metastases

Final Stage: Stage IIB, pT2pN1aM0
ER+, PR+, HER2 neg

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Which of the following is true about immunohistochemistry as a technique to identify antigens?

A. Tissue is treated with an radioisotope-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

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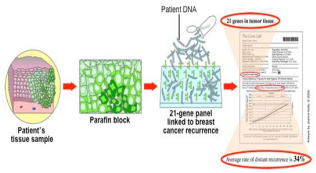
Oncotype Dx

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



National Cancer Institute. (2009). Understanding Cancer Series. Retrieved from: http://www.cancer.gov/cancertopics/understandingcancer/genomewideprofiling/page13

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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

Female, stage I/II, node-neg, ER-pos breast cancer treated with 5 years of tamoxifen

Recurrence score	10-year rate of distant recurrence	Benefit of adjuvant chemotherapy
<18	6.8%	None
18-30	14.3%	None
> 30	30.5%	28%

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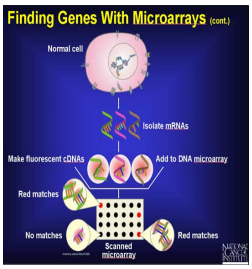
Gene Microarray



http://www.cancer.gov/cancertopics/understandingcancer/moleculardiagnostics/page11

- 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

National Institutes of Health. National Human Genome Research Institute. "Talking Glossary of Genetic Terms." http://www.genome.gov/glossary/index.cfm?id=125

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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 GX to G4

ACS, 2011; Edge et al., 2010.

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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

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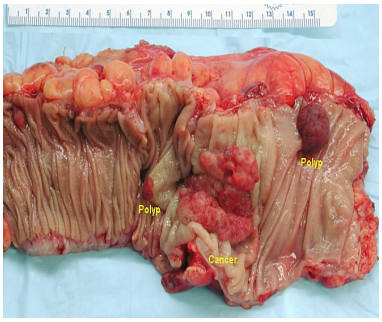
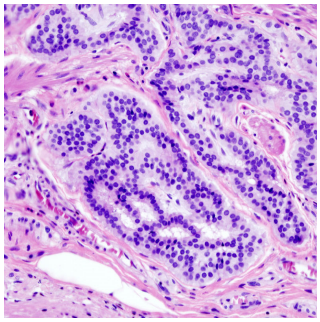
Colon Cancer: Gross Specimen



http://en.wikipedia.org/wiki/File:Colon_cancer.jpg

Colon Cancer Stained With H&E



http://en.wikipedia.org/wiki/File:Colonic_carcinoid_(1)_Endoscopic_resection.jpg

Pathology Report

COLORECTAL CARCINOMA SUMMARY
Site: Sigmoid

Specimen Type Partial colectomy
Tumor Type Adenocarcinoma
Size (cm) 1.2 cm
Grade Moderately differentiated
Tumor Regression Grade (0-3), If Applicable N/A
Depth of Invasion (Specify) Submucosa
Proximal Margin (cm) Negative
Distal Margin (cm) 7.2 cm from closest margin
Circumferential Resection Margin (cm) 4.8 cm
Lymphovascular Invasion Present
Perineural Invasion Not identified
Large Venous Invasion Not identified
Lymph Node Status Negative (0/2)
Largest Metastatic Focus N/A
Extracapsular Extension N/A
Tumor Deposits (Absent/Present) Absent
Total Number
Largest Size (cm) N/A
Distant Metastasis (Specify) Unknown
KRAS Status (Normal/Abnormal) Unknown

TNM Stage (AJCC, 7th Edition)
pT1 pN0

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 occurs 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

National Comprehensive Cancer Network. (2014). *NCCN clinical practice guidelines in oncology: Colon cancer [v. 1.2014].* Retrieved from http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

Biomarkers in Colon Cancer

RAS mutation (KRAS, NRAS)	Tumor DNA PCR, multiplex assays, or direct sequencing	Recommended for all metastatic colon cancer cases Predictive 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; tumor IHC for MMR proteins	Screening for Lynch syndrome Prognostic (positive) Predictive for lack of benefit with adjuvant single-agent fluoropyrimidine therapy
CEA	Serum protein immunoassay	Surveillance
ColoPrint®	18-gene microarray assay	Prognostic in Stage II *Not FDA-approved
Oncotype Dx Colon®	12-gene RT-PCR gene expression assay	Prognostic in Stage II and IIIA/B *Not FDA-approved
BRAF mutation	Tumor DNA PCR, multiplex assays, or direct sequencing	Prognostic (poor) Predictive for lack of benefit for anti-EGFR therapy

NCCN 3.2014 http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

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)

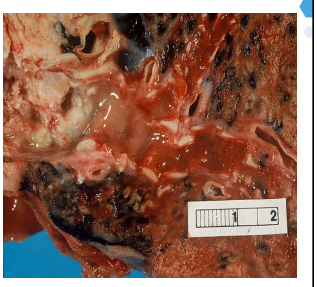
- Stage 1A moderately differentiated invasive adenocarcinoma of the sigmoid colon
- Treated with hemicolectomy
- 1 year later, presents with R lower lobe pulmonary nodule

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
      - premetrexed preferred
    - Squamous cell carcinoma
      - unlikely to benefit form premetrexed
      - higher risk for life threatening hemorrhage from bevacizumab (Travis et al., 2011)
  - Small cell lung cancer (SCLC)



Squamous cell carcinoma

http://en.wikipedia.org/wiki/File:Ca_bronchus.jpg

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

Histological type	Immunostain
Squamous-cell carcinoma	CK5/6 positive CK7 negative
Adenocarcinoma	CK7 positive TTF-1 positive
Large-cell carcinoma	TTF-1 negative
Small-cell carcinoma	TTF-1 positive CD56 positive Chromogranin positive Synaptophysin positive

NCCN, 2014; Travis et al., 2011

R Lower Lobectomy Pathology Report

LUNG CARCINOMA SUMMARY

Site right lower lobe
Specimen Type Lobectomy
Tumor Type Adenocarcinoma (acinar, lepidic and micropapillary patterns)

Size 2.5 cm

Histologic Grade Moderately differentiated
Separate Tumor Nodules (Absent/Present) Absent
Bronchial & Vascular Resection Margins Negative
Vascular Invasion (Venous Or Arteriolar) Absent
Pleural Involvement Involved (PL1)
Pleural/Elastic Layer Invasion Present
Visceral Pleural Involvement Absent
Parietal Pleural Involvement Absent
Lymph Node Status, Ipsilateral Negative (0/3)
Location (Specify) N/A
Largest Metastatic Focus N/A
Extracapsular Extension N/A
Lymph Node Status, Contralateral N/A
Location (Specify) N/A
Largest Metastatic Focus N/A
Extracapsular Extension N/A
Nonneoplastic Lung Unremarkable
Distant Metastasis (Specify) Unknown

TNM (AJCC, 7th Edition) pT2 pN0

- **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 REARRANGEMEN
  - FISH NEGATIVE FOR ROS1 GENE REARRANGEMENT
  - No Kras mutation by PCR

Stage IB adenocarcinoma of the lung

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)

Travis et al., 2011

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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 area and 1 cm R paratracheal node
- Mediastinoscopy performed for biopsy

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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.

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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 ant-EGFR therapy

NCCN. 3.2014. http://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

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Other Biomarkers in Lung Cancer

RAS mutation	PCR, multiplex assays, or direct sequencing	10%–30% Increased incidence in non-Asians, smokers, and in invasive mucinous adenocarcinoma	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
HER2 mutations	IHC, FISH	**2%–4%**	Predictive for response to trastuzumab, afatanib
MET amplification	IHC, PCR	**2%–4%**	Predictive for response to crizotinib
RET rearrangements	FISH	**1%**	Predictive for response to cabozantinib

http://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf; http://www.mycancergenome.org/content/disease/lung-cancer

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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**

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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 1,000× 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/

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