ROSWELL PARK CANCER INSTITUTE

Pathology *and* The Pathology of Neoplasia

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Department of Pathology

Outline

What is Pathology?

What is a Pathology Department?

The pathologist's "tools"

Pathology Definitions and Concepts

How do we approach a pathology specimen?

A General Classification of Neoplasia.

The ugly histologic face of cancer (recognizing malignancy).

Grading and Staging Malignancy.

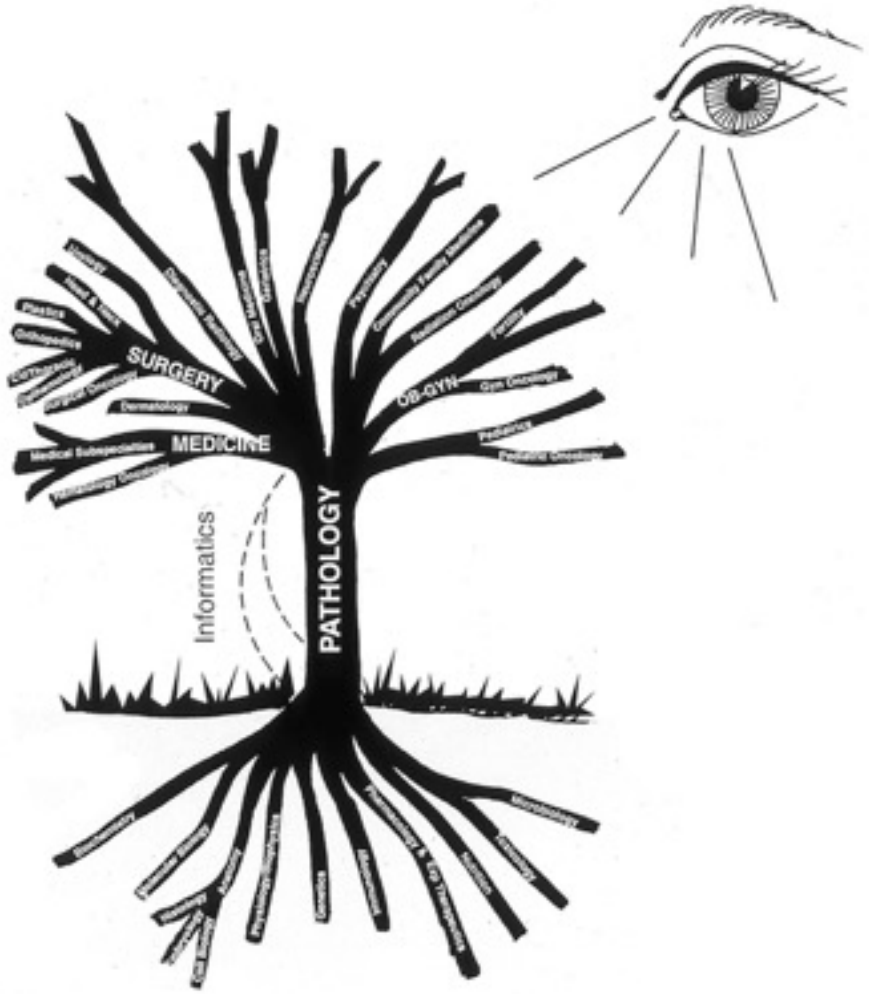
The Changing World of Pathology

Pathology and Research

Pathology

- "...branch of medicine which treats (studies) of the essential nature of disease, especially of the structural and functional changes in tissues and organs of the body which cause or are caused by disease."
  - Dorland's Medical Dictionary 24th Ed.
- "...something abnormal- the anatomic and physiologic deviations from the normal that constitute disease or characterize a particular disease."
  - Webster's Seventh New Collegiate Dictionary

Biomedical Science Tree



A Department of Pathology

Patient Care

ANATOMIC PATHOLOGY

CLINICAL PATHOLOGY

Experimental (Research)

TISSUE PROCUREMENT

TRANSLATIONAL

BENCH

Anatomic Pathology

- Surgical Pathology
  - Frozen Section (Intra-operative consultations)
  - Biopsy and Resection specimens
- Cytopathology
  - Pap smears (Gynecologic specimens)
  - Fine needle aspirates, washes, brushes
- Autopsy

Clinical Pathology

- Hematology
- Chemistry
- Microbiology
- Blood Bank (Transfusion Medicine)
- Tissue Typing (Transplant Pathology)
- Molecular Diagnostics
- Flow Cytometry

- Cytogenetics

Anatomic Pathology in Cancer

Patient has signs or symptoms of cancer

Clinician/Radiologist performs biopsy

Pathologist diagnoses cancer on biopsy

Clinician performs resection/excision

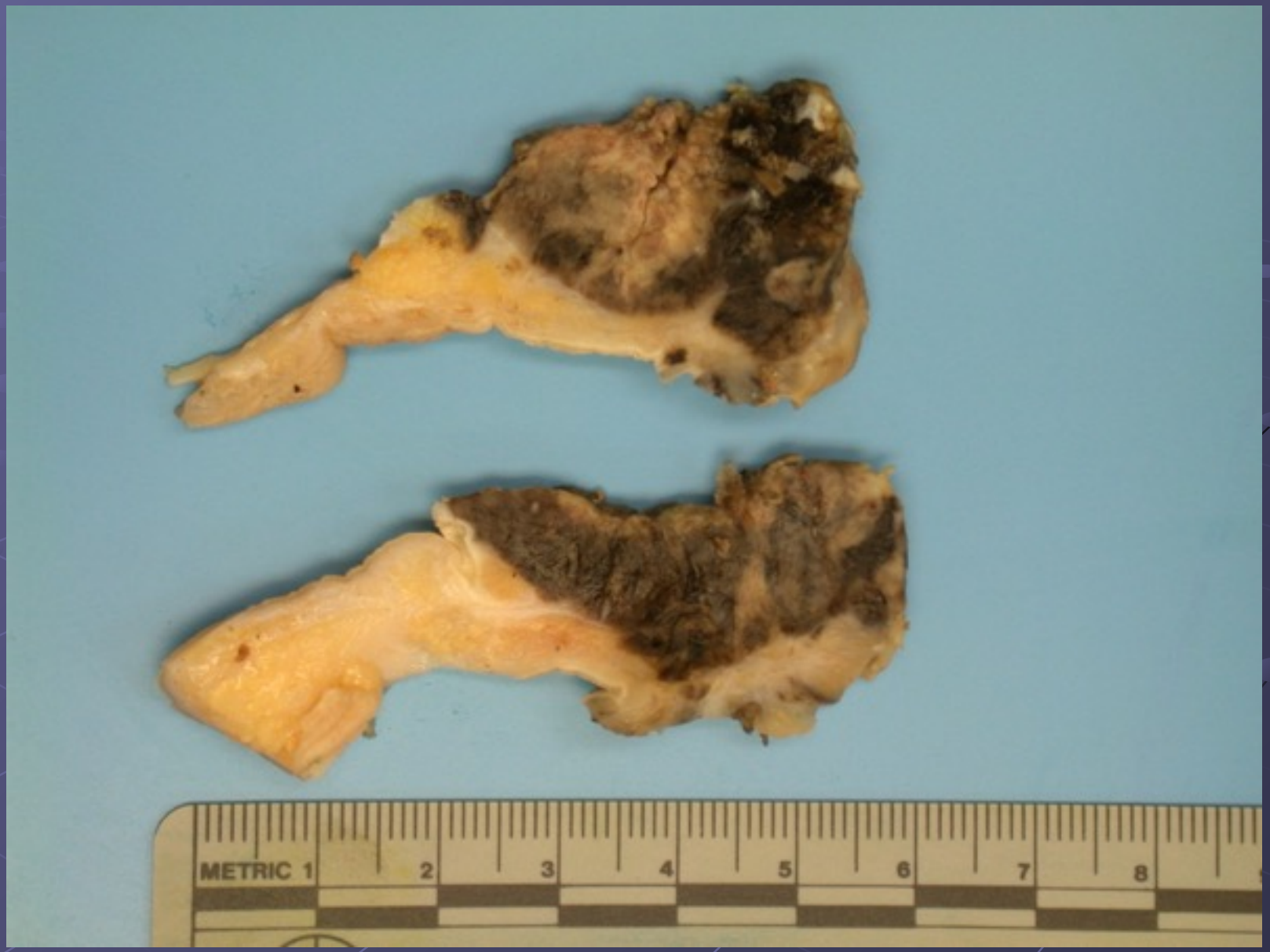
Pathologist stages and evaluates cancer

Molecular Characteristics of Tumor Evaluated for use in “Personalized Medicine”

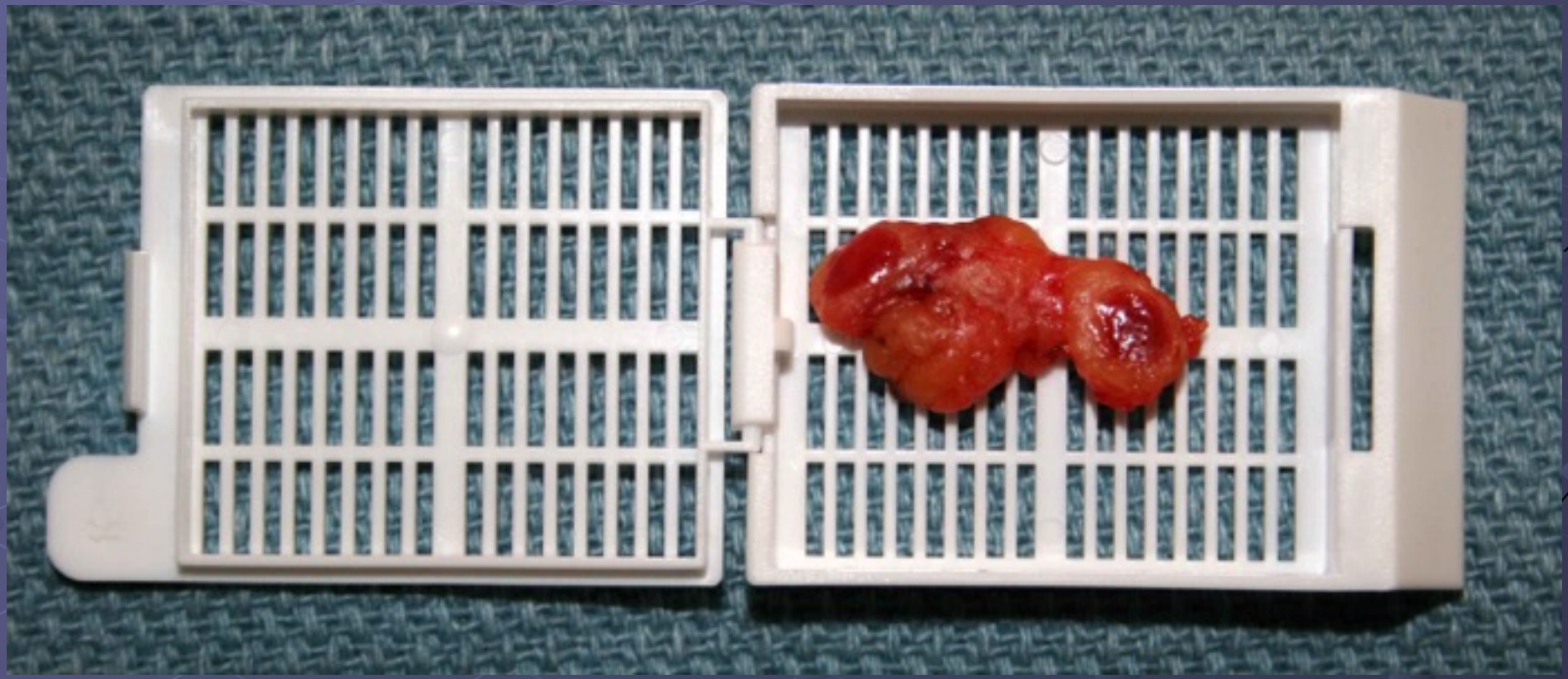
The Pathologist's Tools

- The "Grossing Station"
- Fixation (usually with Formalin- 37% formaldehyde)
  - Preserve tissue, enhance detail for microscopy
- Tissue Processing (usually overnight)
  - Remove tissue water gradually, replace with paraffin wax
- Paraffin Embed and cut tissue sections
- Hematoxylin and Eosin Stain
- The Light Microscope

Gross Examination



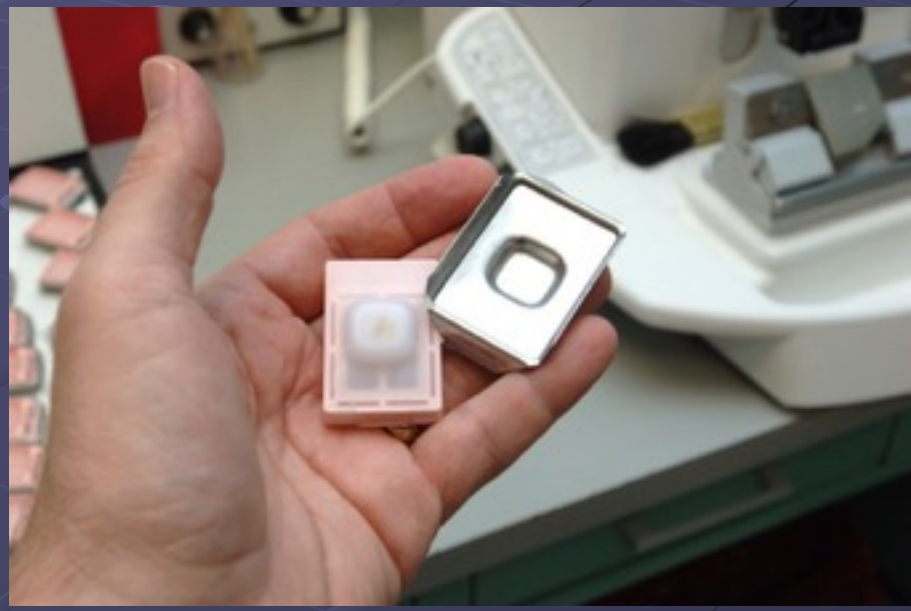
Cassettes and Tissue Sections



Tissue Processor

1. Dehydrate Tissue
   - Progressive series of alcohols removes water
2. Clear Ethanol with xylene
3. Replace with paraffin

Tissue Embedded in Liquid Paraffin



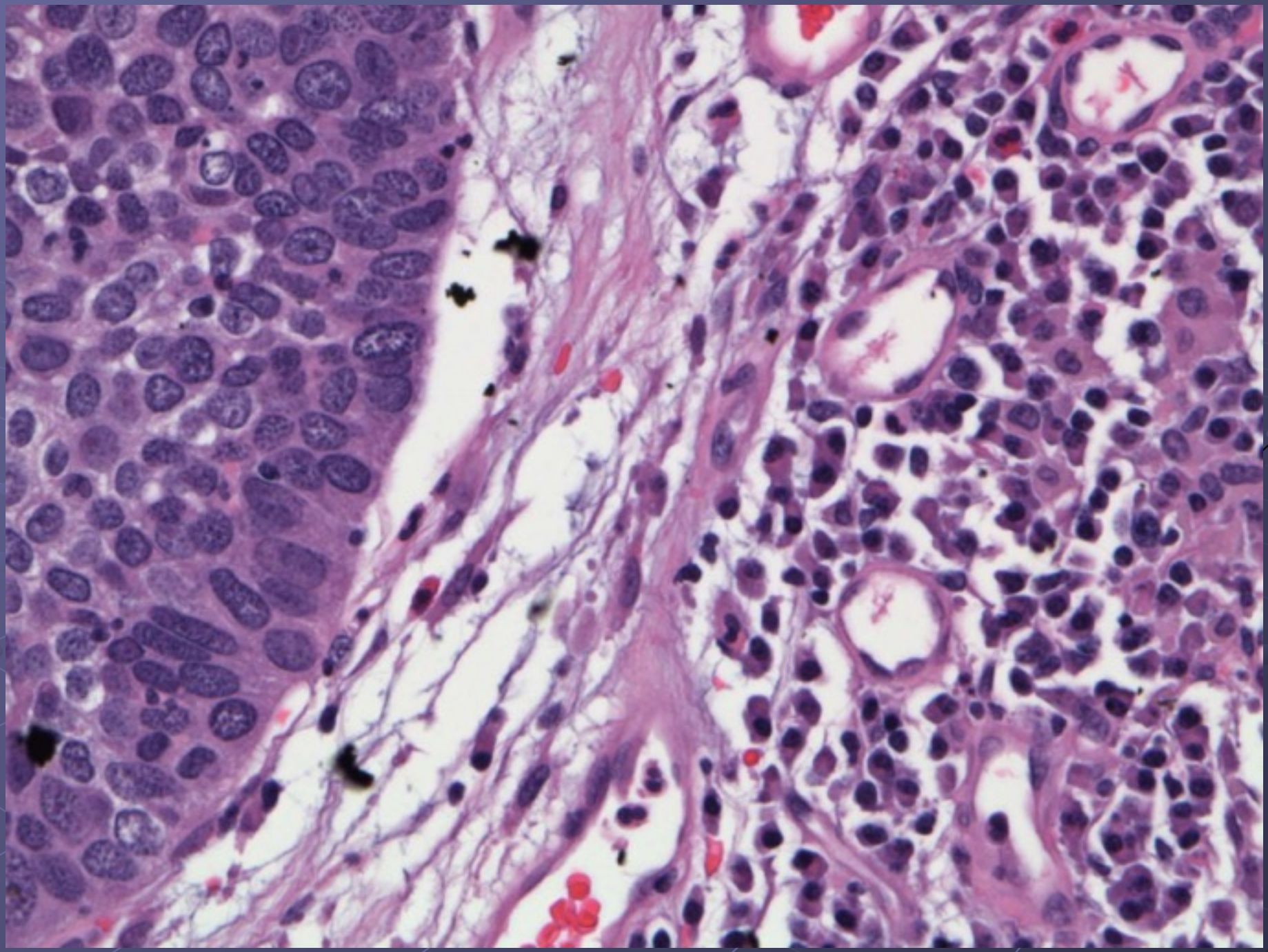
Pictures courtesy of www.protocolsonline.com

Sections Cut (~ 5µm) by microtome

Hematoxylin and Eosin (the H&E)

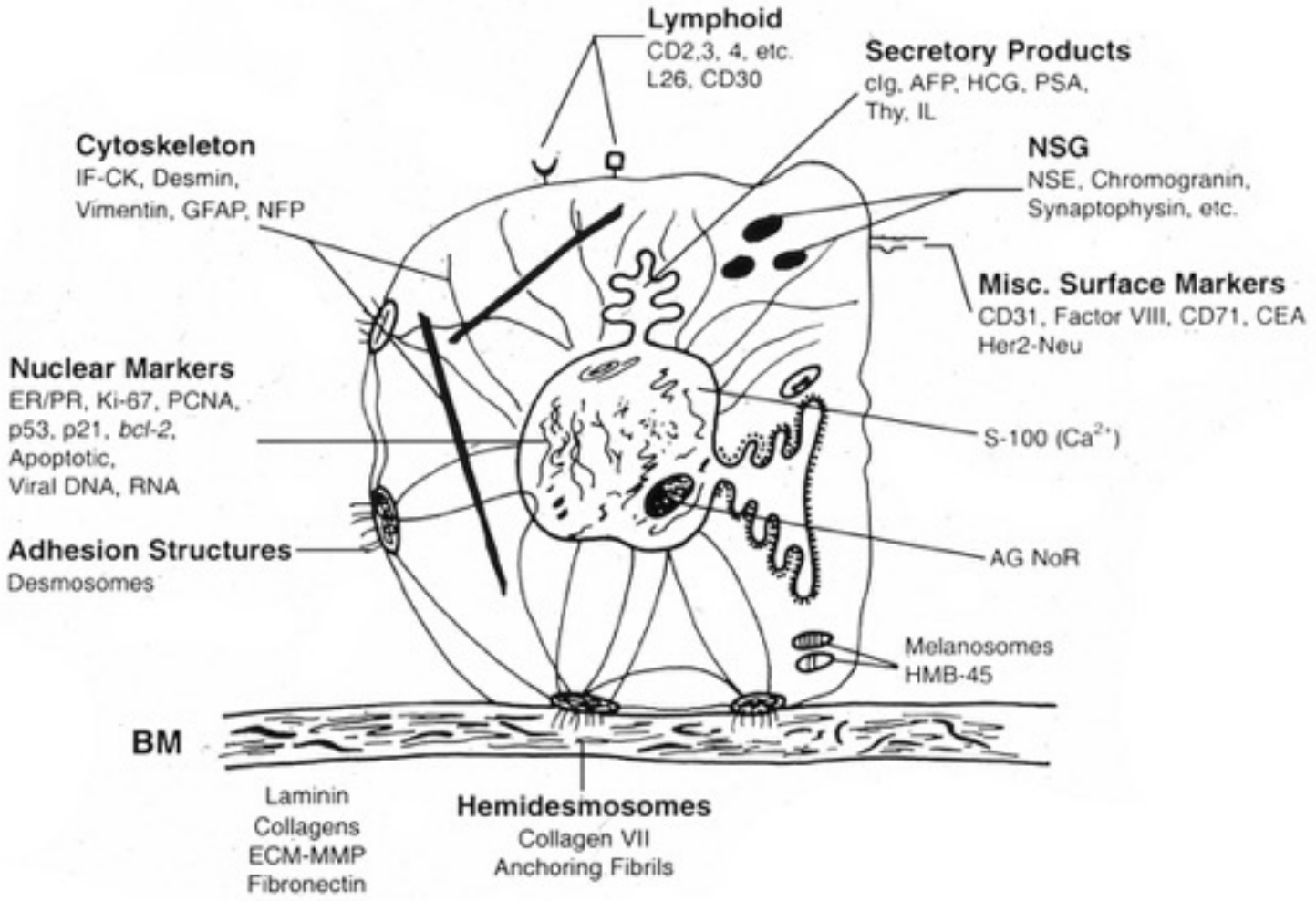
- The pink and purple foundation of pathology

- Hematoxylin
  - Purple/blue stain (BASOPHILIC)
  - Stains acidic materials
  - Stains nuclear contents
- Eosin
  - Pink stain (EOSINOPHILIC)
  - Stains most cytoplasmic contents



- Ancillary Studies
  - Flow cytometry
  - Immunohistochemical stains
  - Cytochemical stains
  - Cytogenetics
  - Electron microscopy

Generic IHC Cell



Immunohistochemistry (IHC)

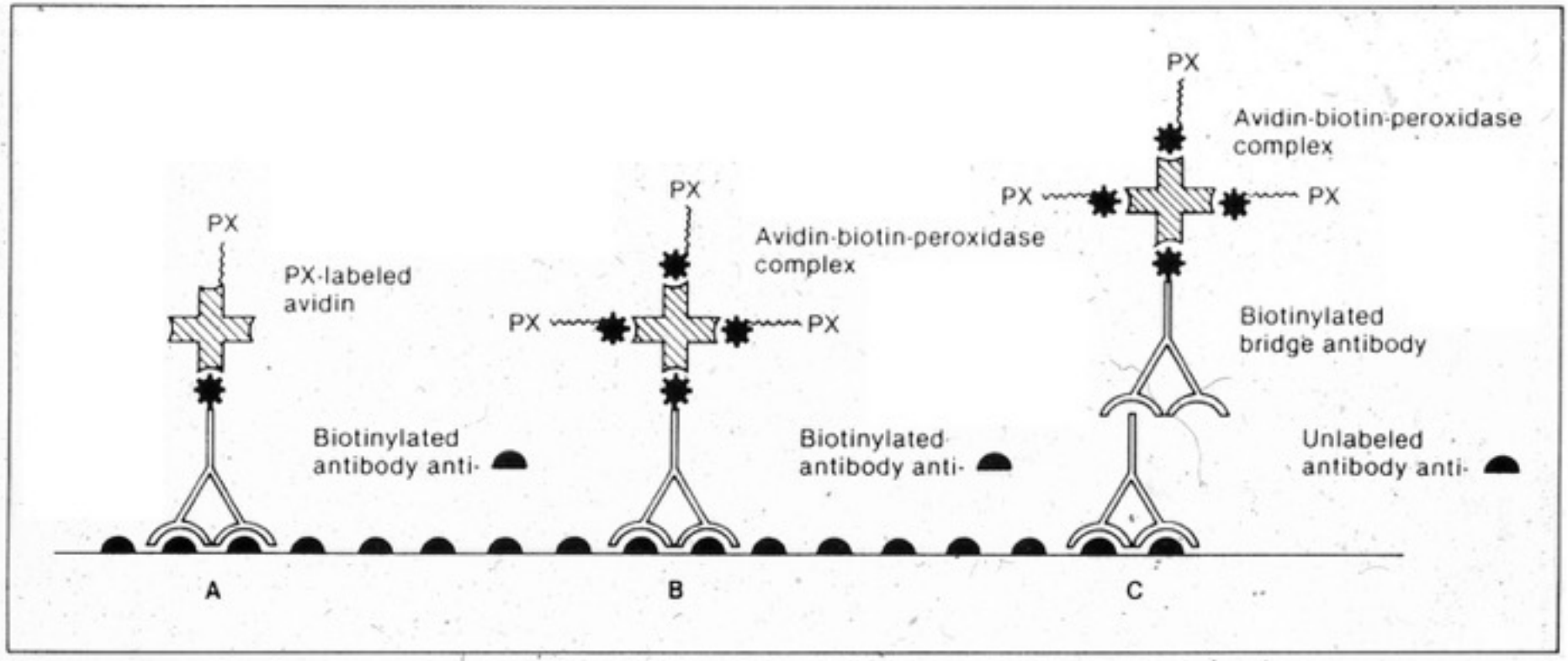


Fig. 3-2 Biotin-avidin immunoenzymatic techniques. Solid semicircle indicates antigen; PX, peroxidase; *, biotin; and shaded open cross, avidin. *A*, biotinylated primary antibody method; *B*, biotinylated peroxidase method; *C*, avidin-biotin-peroxidase complex method. (From Falini B, Taylor CR: New developments in immunoperoxidase techniques and their application. Arch Pathol Lab Med **107**:105-117, copyright 1983, American Medical Association.)

IHC: what is it good for?

- Identify or confirm the source of a tumor
  - CK7, CK20, other markers “specific” to tumor type
- Increase the sensitivity of tumor detection
  - eg find individual metastatic melanoma cells in a lymph node using Melan-A
- Predict tumor behavior/prognosis
  - proliferative index (Ki-67)
- Identify potential therapeutic targets
  - Estrogen receptor – Tamoxifen (breast cancer)

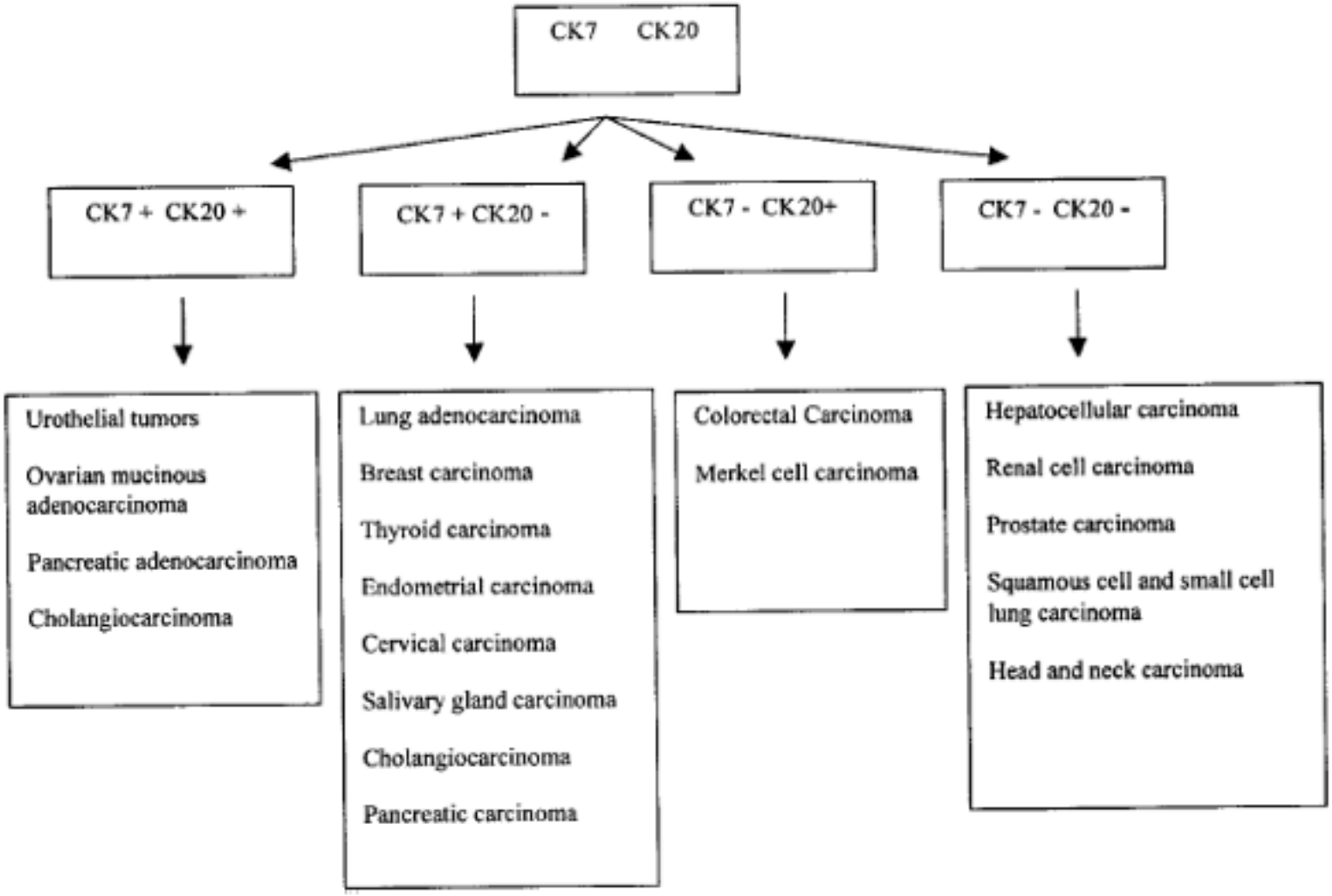


FIGURE 1. Approach to immunohistochemical markers used in unknown primary cancer.

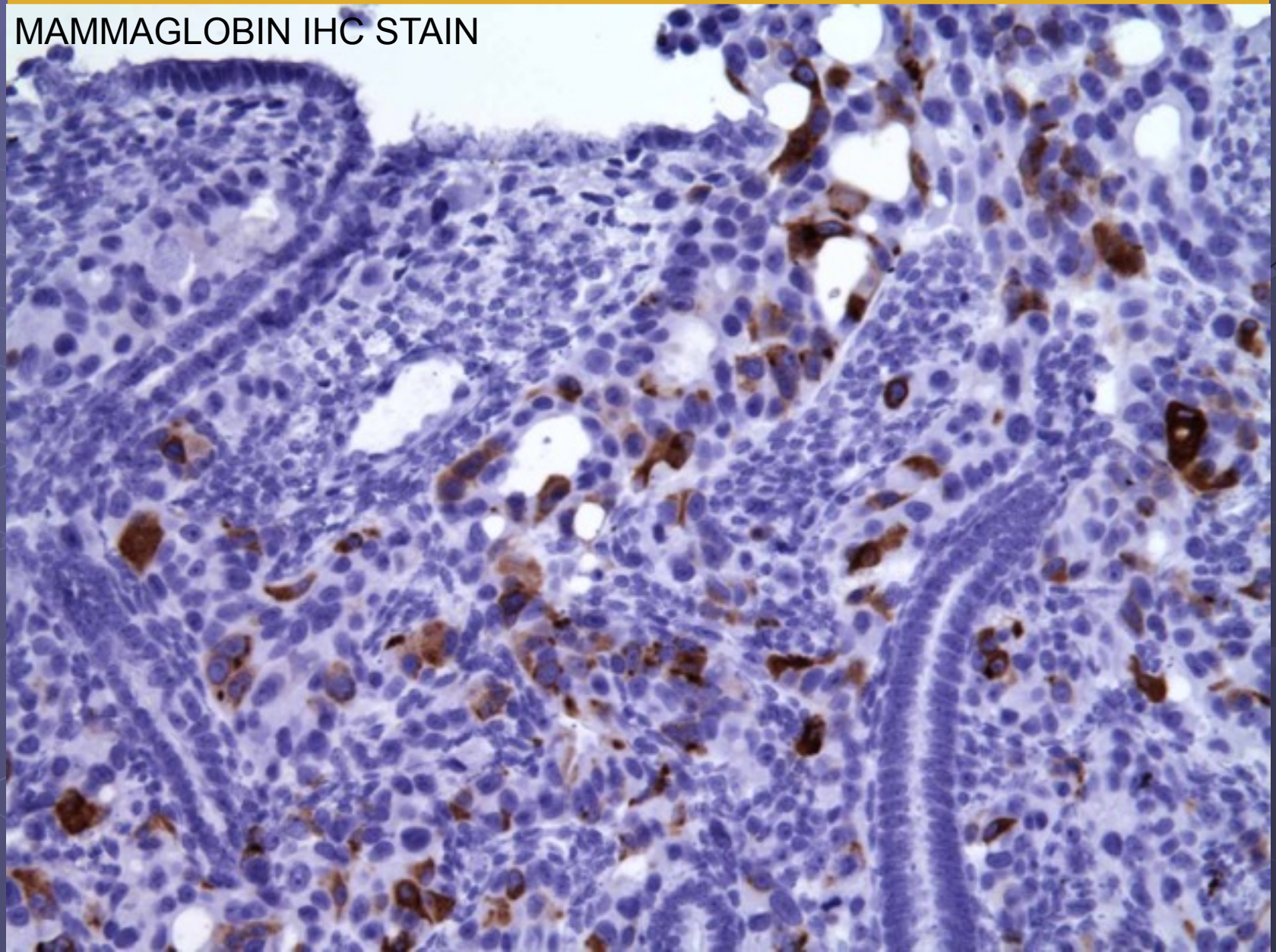
from Varadhachary, GR. *et al.*, Cancer **100(9):**1776 (2004).

Additional Markers Used as Suggested by Clinical Data (after a Preliminary Workup with CK7 and CK20)

Tumor	Marker
Urothelial carcinoma	UROIII, THR, HMWCK
Breast carcinoma	GCDFP-15, ER, PR
Lung (mainly adenocarcinoma)	TTF-1, surfactant A and B
Medullary thyroid carcinoma	TTF-1, Calcitonin
Merkel cell carcinoma	CD117
Hepatocellular carcinoma	Hep par-1
Prostate carcinoma	PSA, PAP
Cholangiocarcinoma	CK19
Mesothelioma	Calretinin

Breast Cancer Metastatic to Uterus

MAMMAGLOBIN IHC STAIN



Immunohistochemistry

- DISADVANTAGES
  - FALSE Negative
    - Antigen- not present in tissue, degraded
    - Antibody- too dilute, expired, wrong antibody
  - FALSE Positive
    - Cross reactive or non-specific antibody binding
    - Endogenous peroxidase or avidin/biotin binding
    - Entrapped normal cells
    - Tumor uptake of normal cell antigens

Definitions and Concepts

Definitions

- TUMOR

Swelling.

- CANCER

“crab”, tumor with fatal course and association with formation of secondary tumors (metastasis)

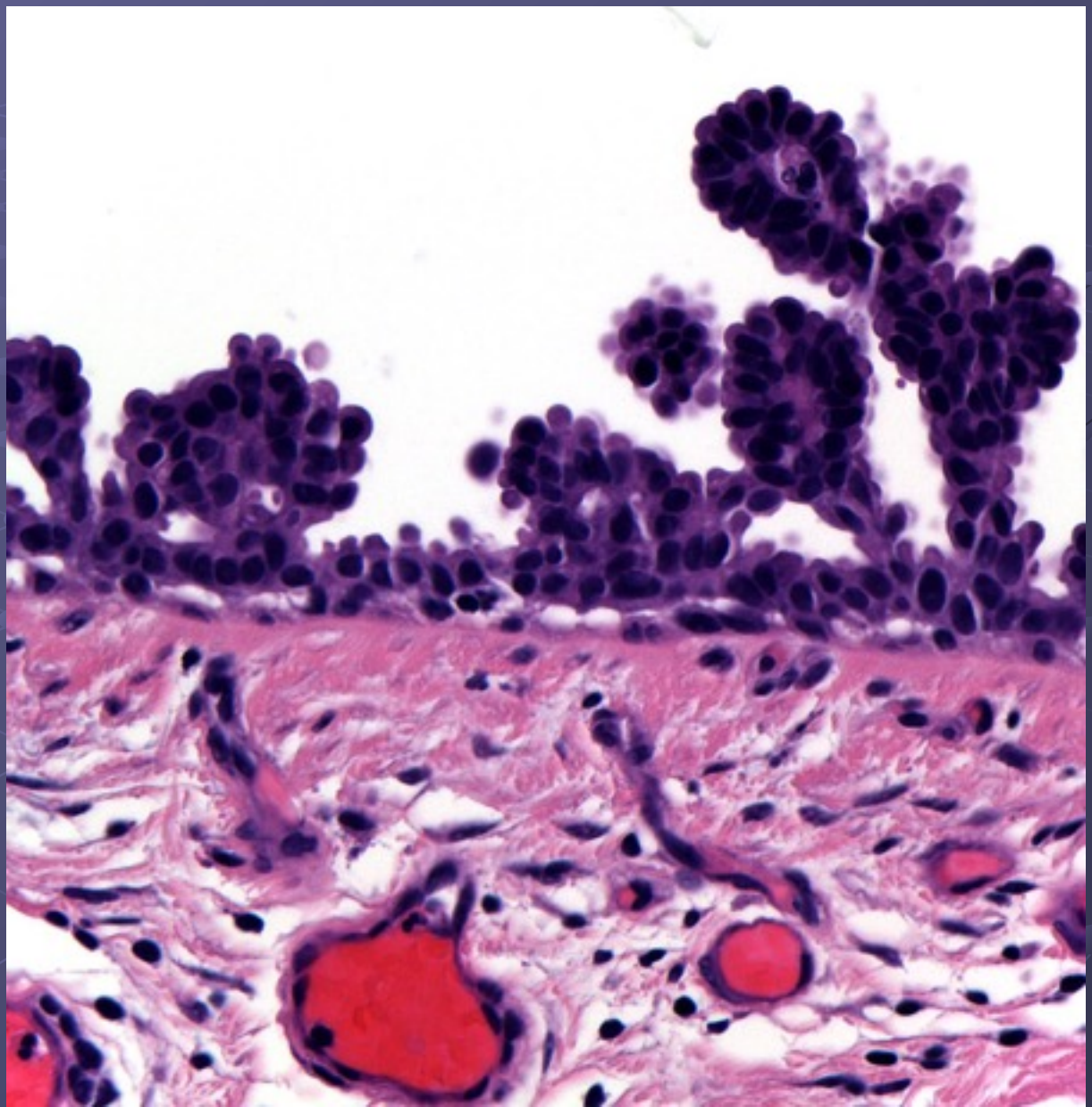
Definitions

- EPITHELIUM (epithelial) → when malignant it is called CARCINOMA

Derived from ectoderm or endoderm: the protective, absorptive and secretory lining of the body both externally (e.g. skin), and internally (e.g. lining of colon, pancreatic glands)

Common types of epithelium are: squamous and columnar/glandular

Epithelium and Basement Membrane



Definitions

- MESENCHYME (mesenchymal) → when malignant it is called SARCOMA

Largely derived from mesoderm: structural and functional elements of the body- “connective tissue” (cartilage, bone, collagen, nerve, blood vessels, etc...)

Definitions

- NEOPLASIA

Any new or abnormal growth (typically implies a clonal population of cells).

- NEOPLASM

Mass of new tissue which persists and grows independently of its surrounding structures and which has no physiologic use

Definitions

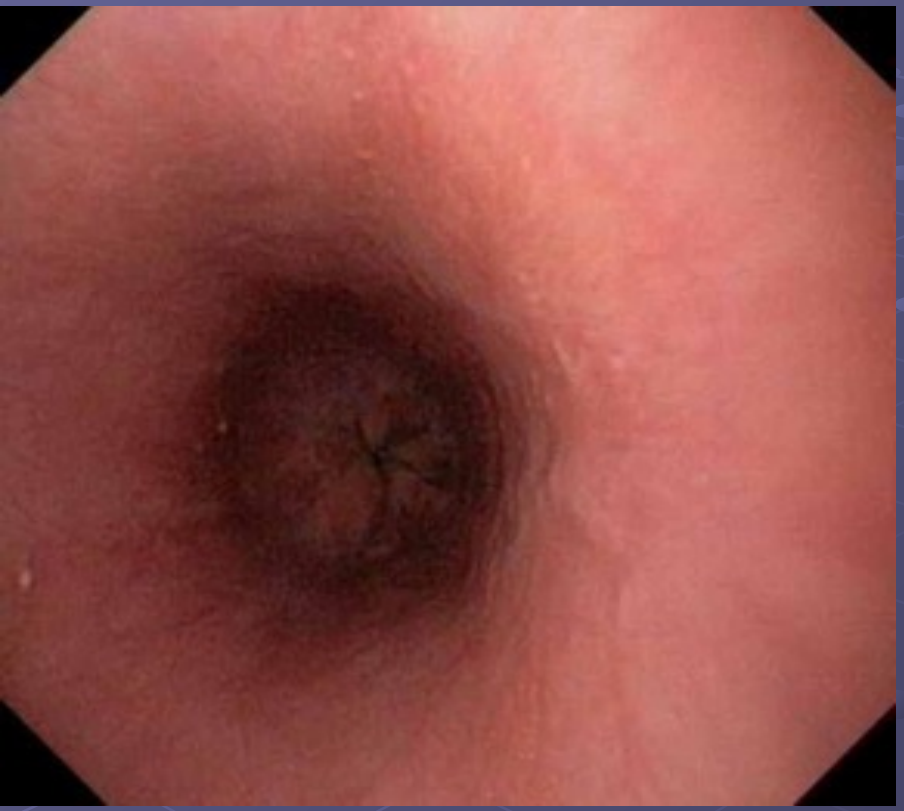
- METAPLASIA

A change in tissue cells to a form not normal for that tissue, usually reversible and protective

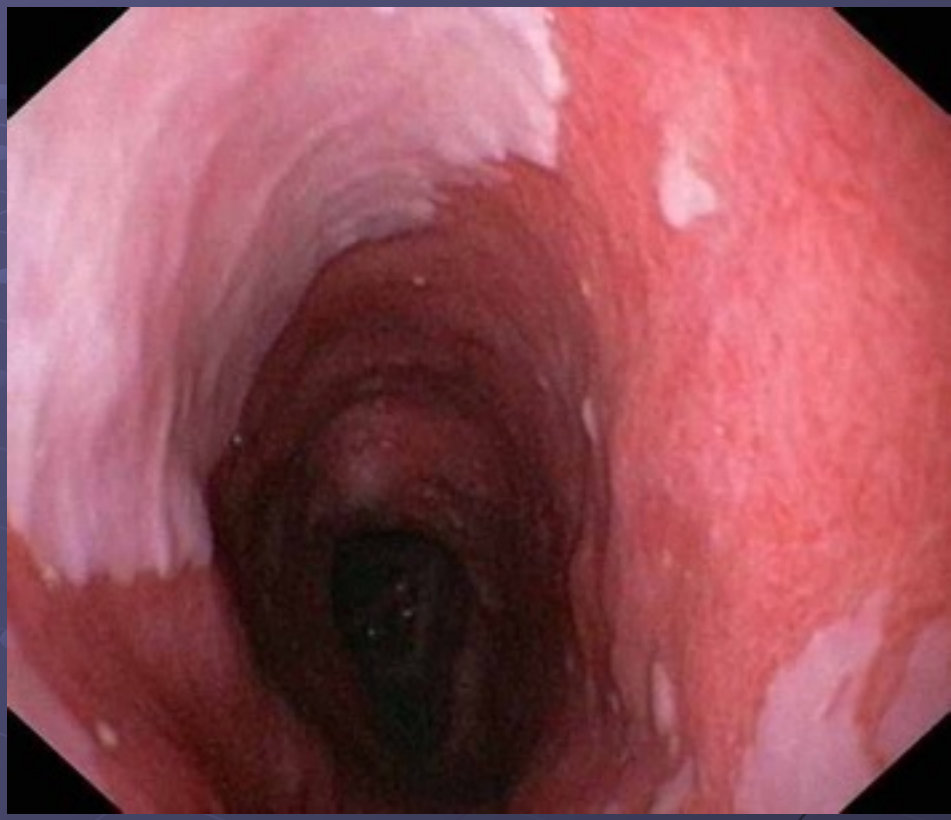
- HYPERPLASIA

An increase in the NUMBER of cells, while maintaining normal tissue architecture

Normal esophagus

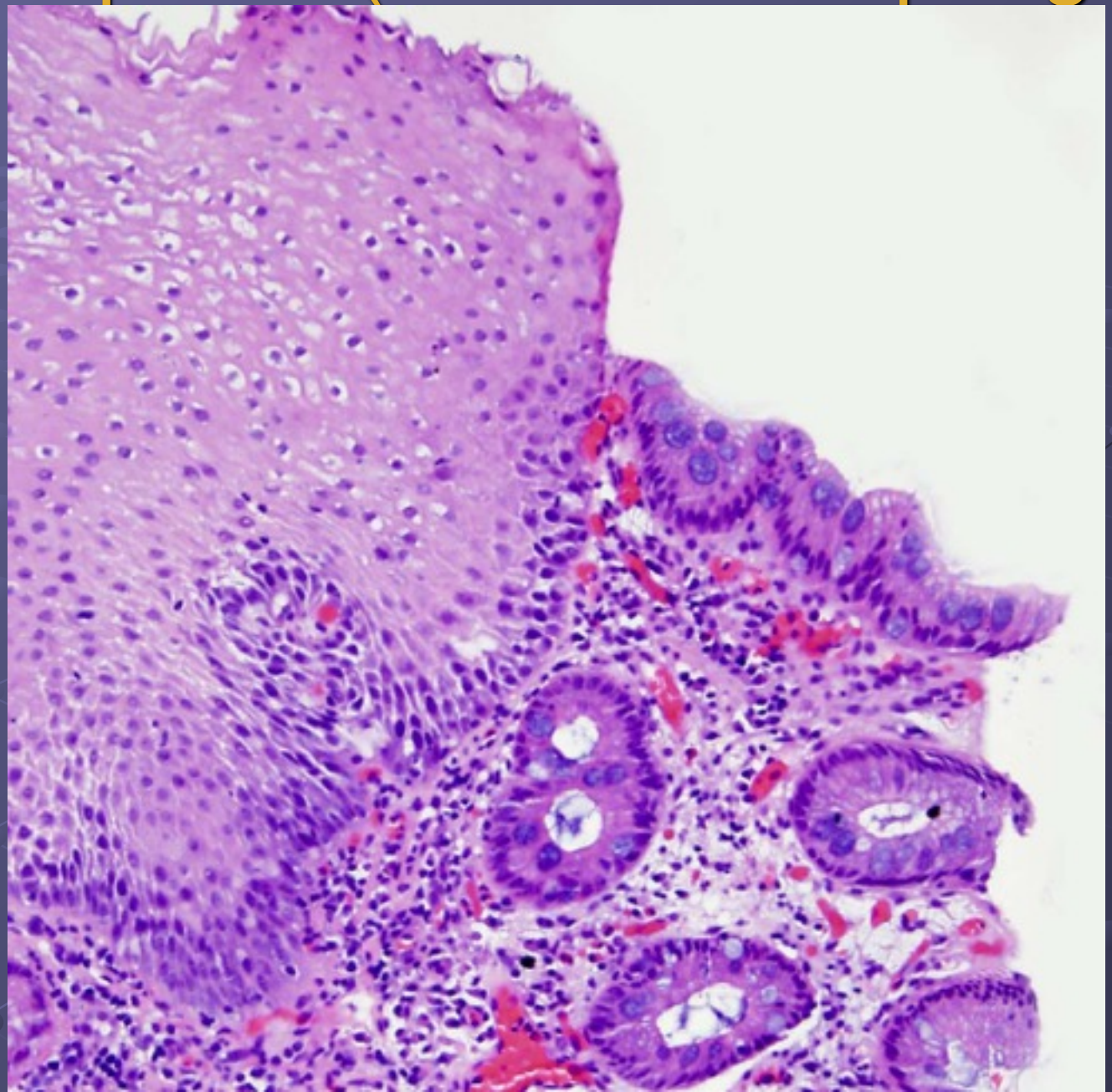


Barrett's metaplasia



Images taken from WWW: "The GastroLab Endoscopy Archive"

Metaplasia (Barrett's Esophagus)



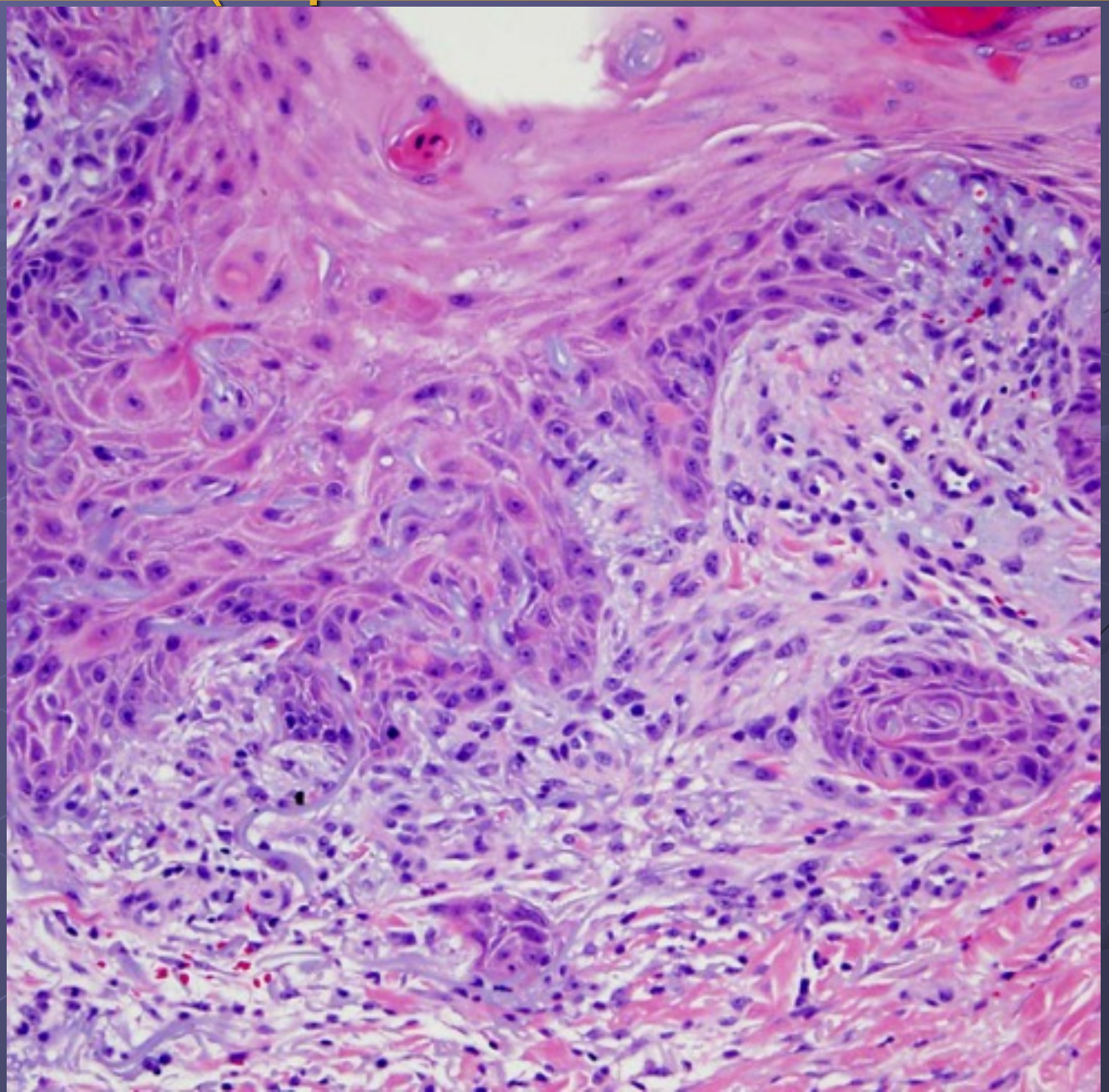
Definitions

- INVASION

The penetration of neoplastic epithelial cells through the basement membrane.

Also used less specifically to refer to cells (epithelial or mesenchymal) penetrating any adjacent existing structure.

Invasion (Squamous Cell Carcinoma)

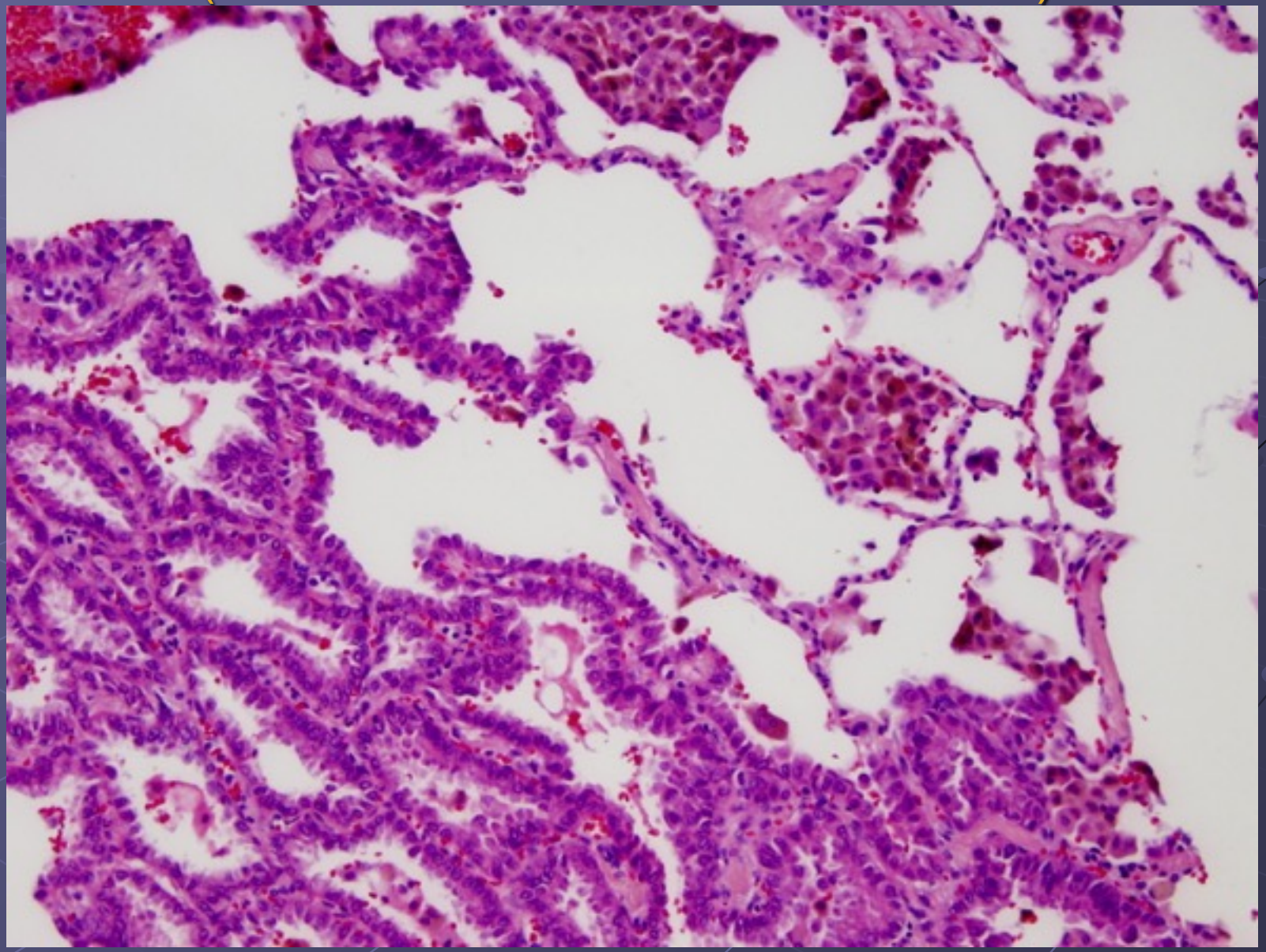


Definitions

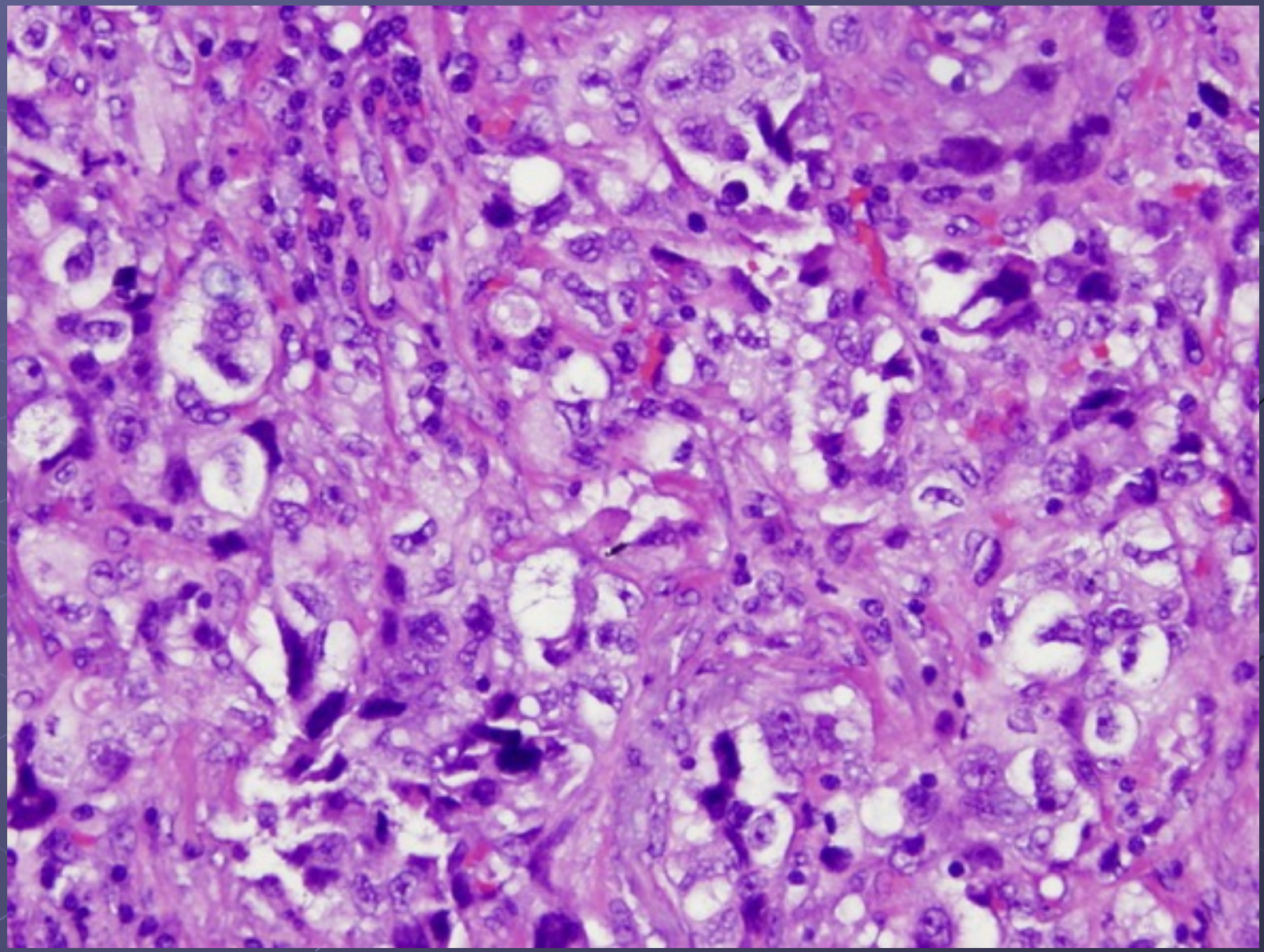
- DIFFERENTIATION

Degree to which a neoplasm recapitulates normal tissue in all its characteristics (often assessed as a GRADE)

Well Differentiated Lung Adenocarcinoma (Bronchioloalveolar Carcinoma 200x)



Poorly differentiated lung adenocarcinoma (200x)



Definitions

- ATYPIA

The condition of being irregular, refers generally to abnormalities of histologic appearance- not always neoplastic in nature (can be “reactive”, as in setting of radiation therapy or inflammation)

- ANAPLASIA

A loss of normal differentiation and form.

Definitions

- DYSPLASIA

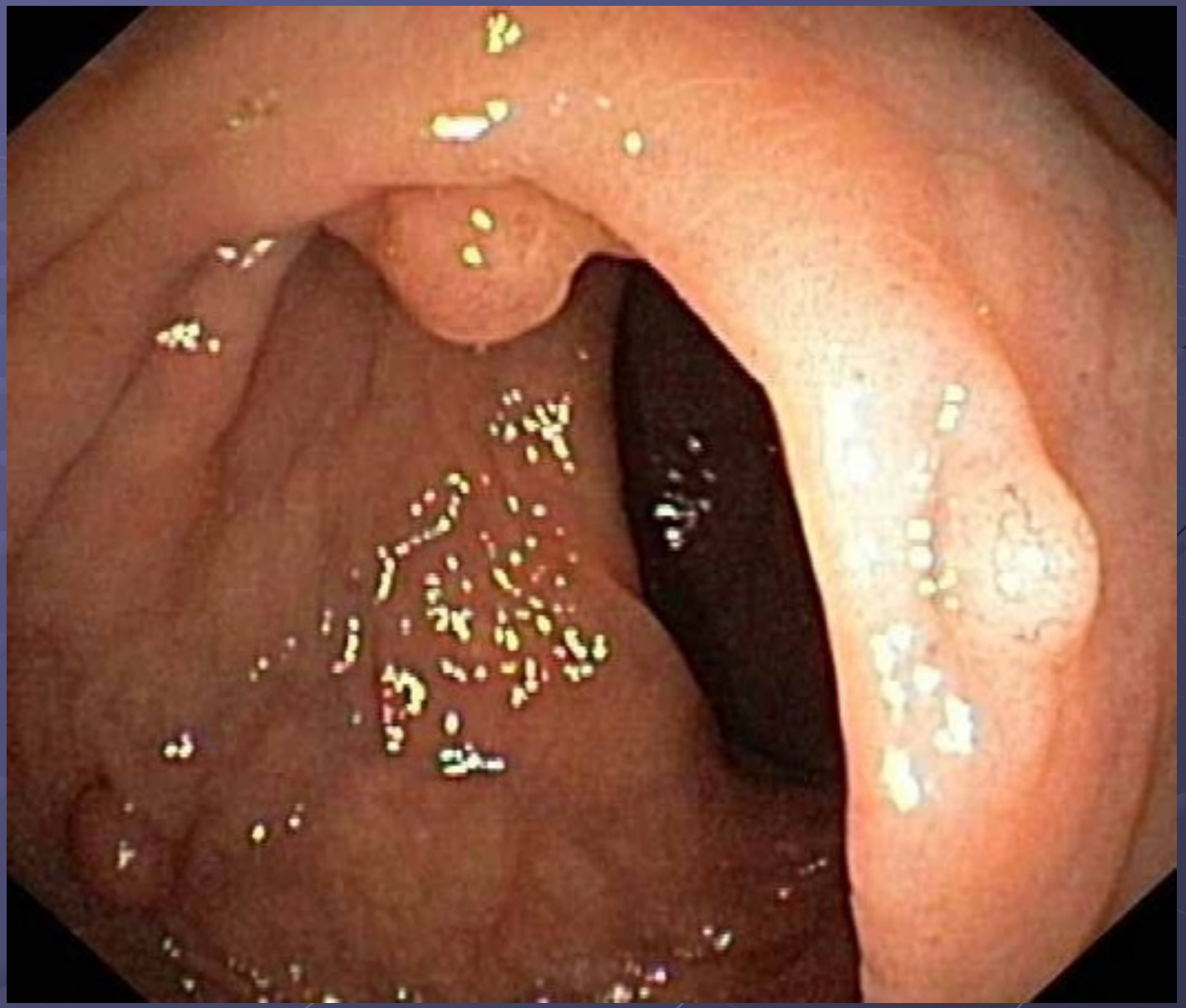
Abnormality of maturation and development, manifesting as architectural, organizational and cytologic atypia

MILD → MODERATE → SEVERE

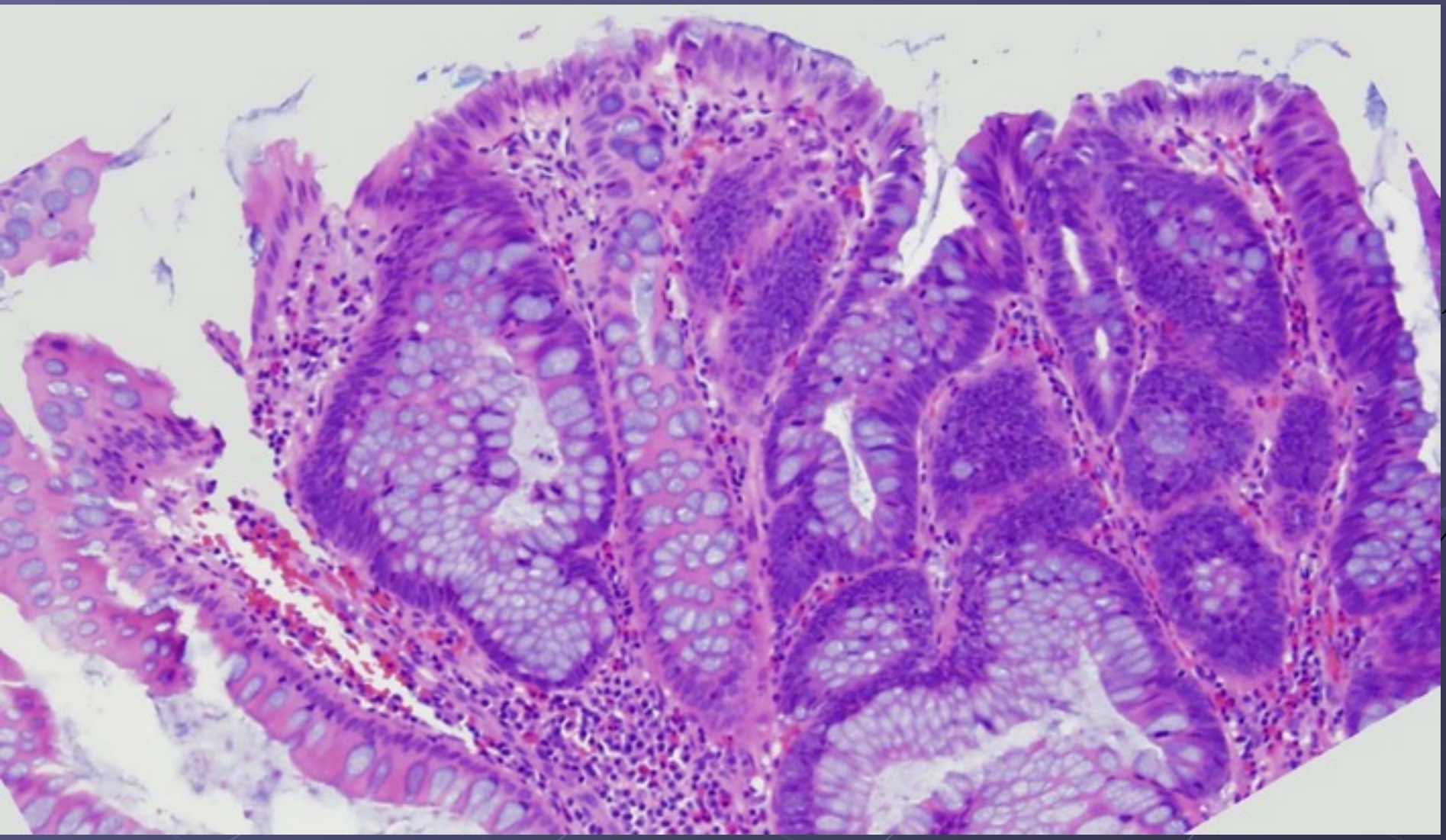
(SEVERE usually is Carcinoma in-situ)

DYSPLASIA IS ALWAYS ATYPICAL, ATYPIA IS NOT ALWAYS DYSPLASTIC!

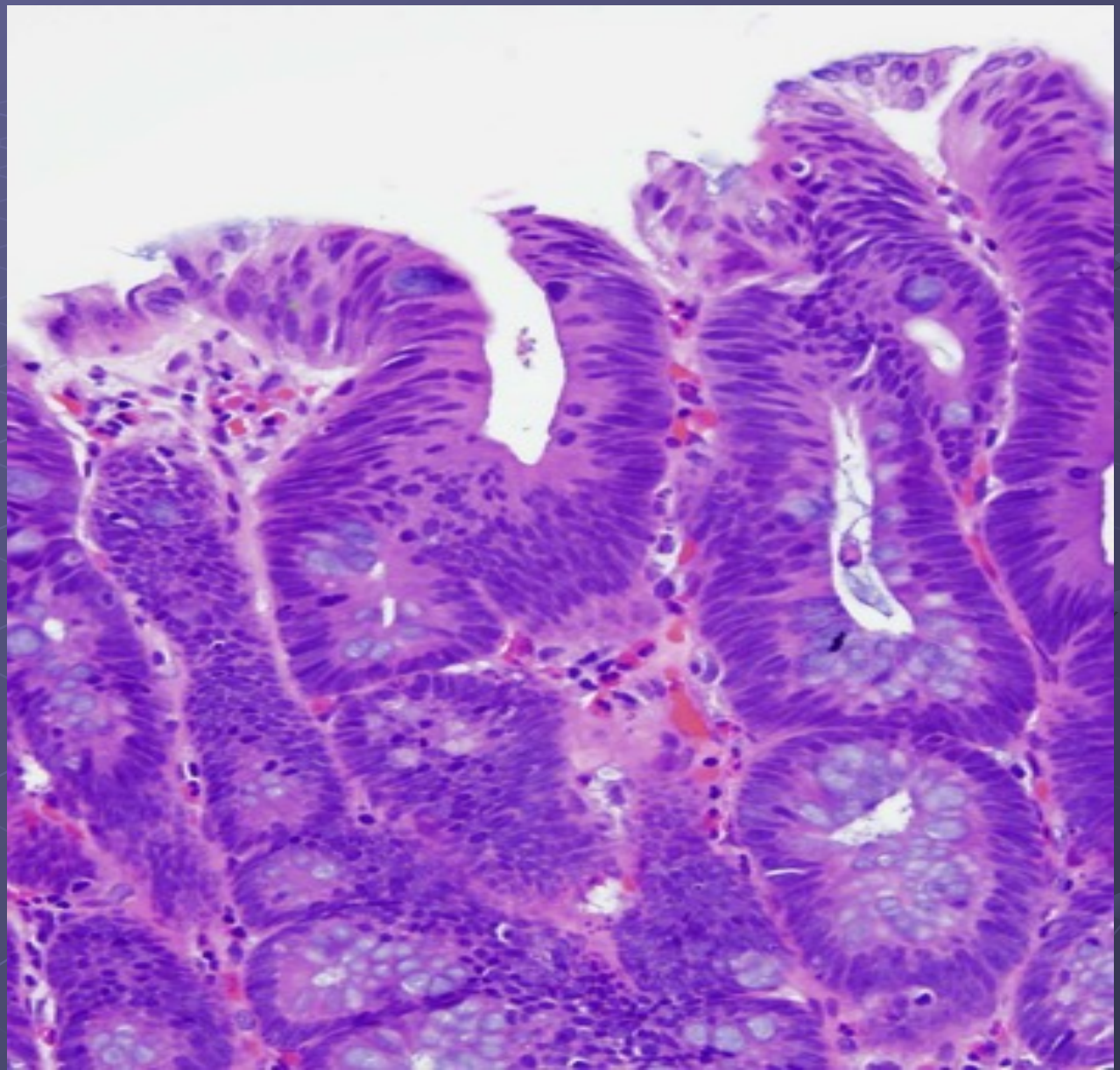
Colonoscopy



Colonic Adenoma (Dysplasia 100x)



Colonic Adenoma (Dysplasia 200x)



Definitions

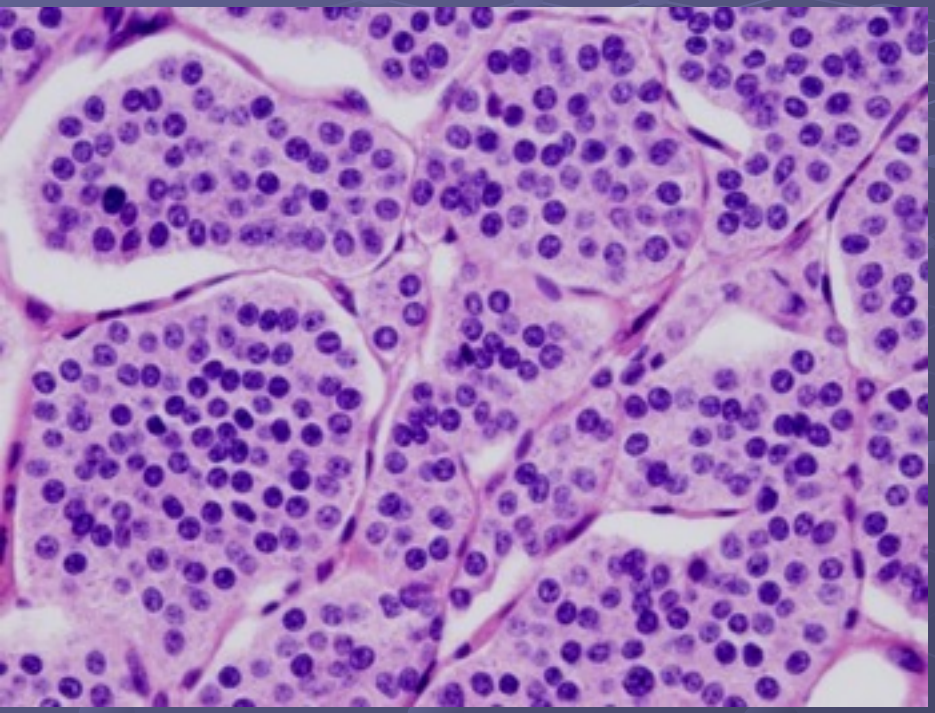
- PLEOMORPHISM

Variation in cellular size and shape

- MALIGNANT

A neoplasm with invasive and metastatic capacity.

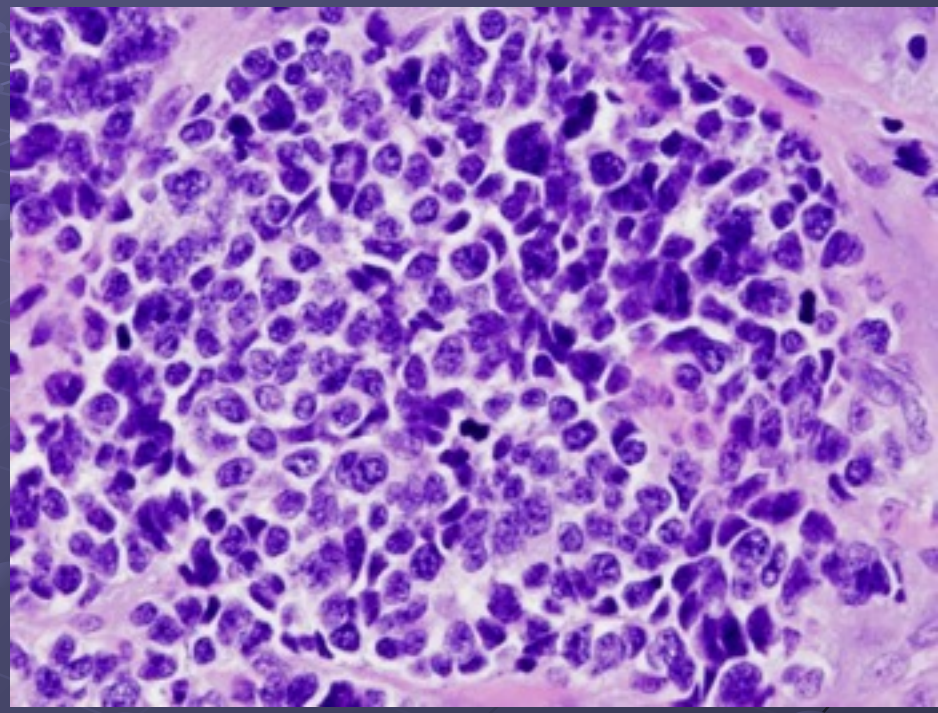
Pulmonary Neuroendocrine Carcinoma



Carcinoid (400x)

LOW GRADE, MINIMAL TO NO ATYPIA

MONOMORPHIC

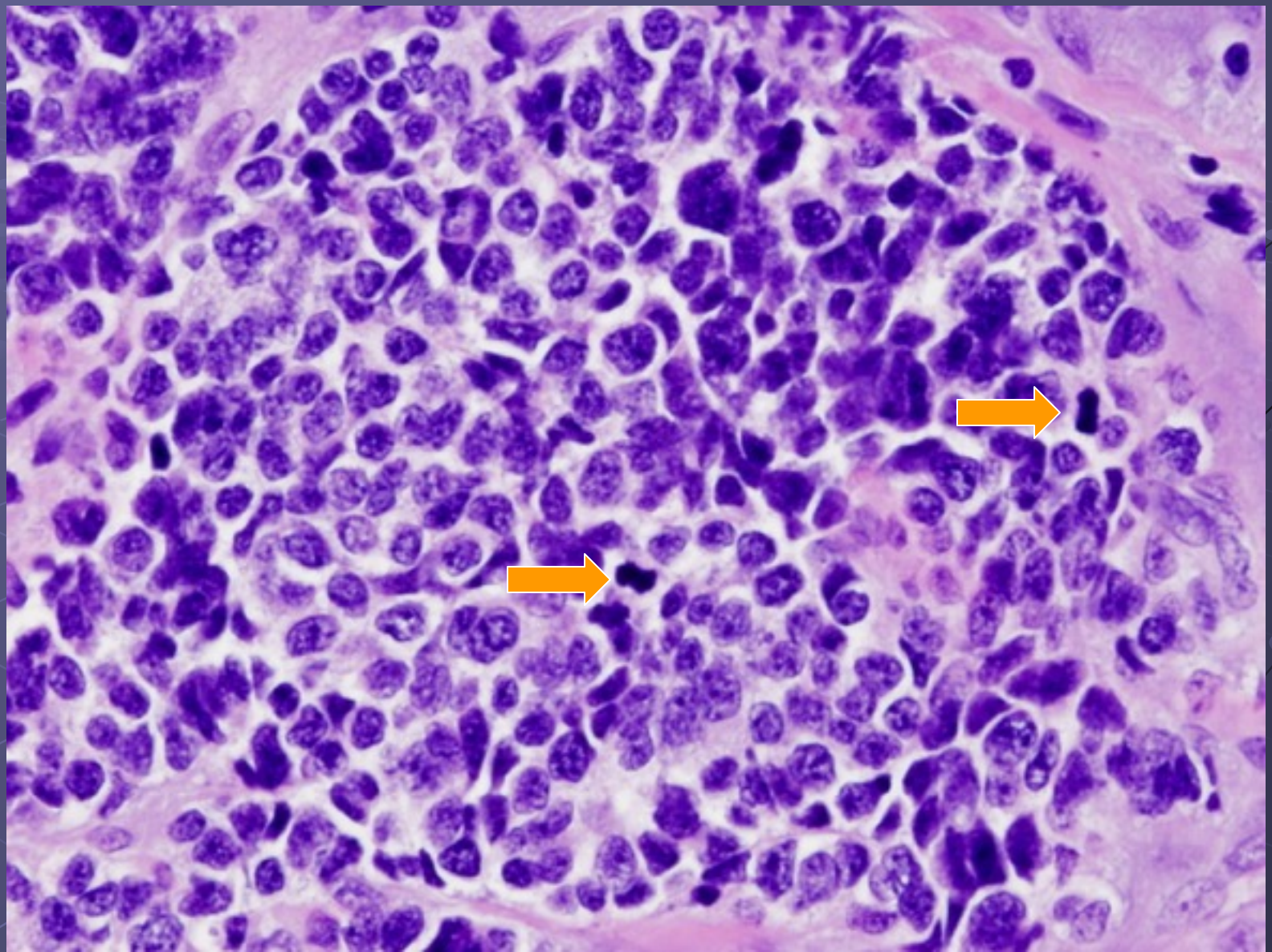


Small Cell Carcinoma (400x)

HIGH GRADE, SEVERE ATYPIA

PLEOMORPHIC

NEC: Small Cell Carcinoma (400x)



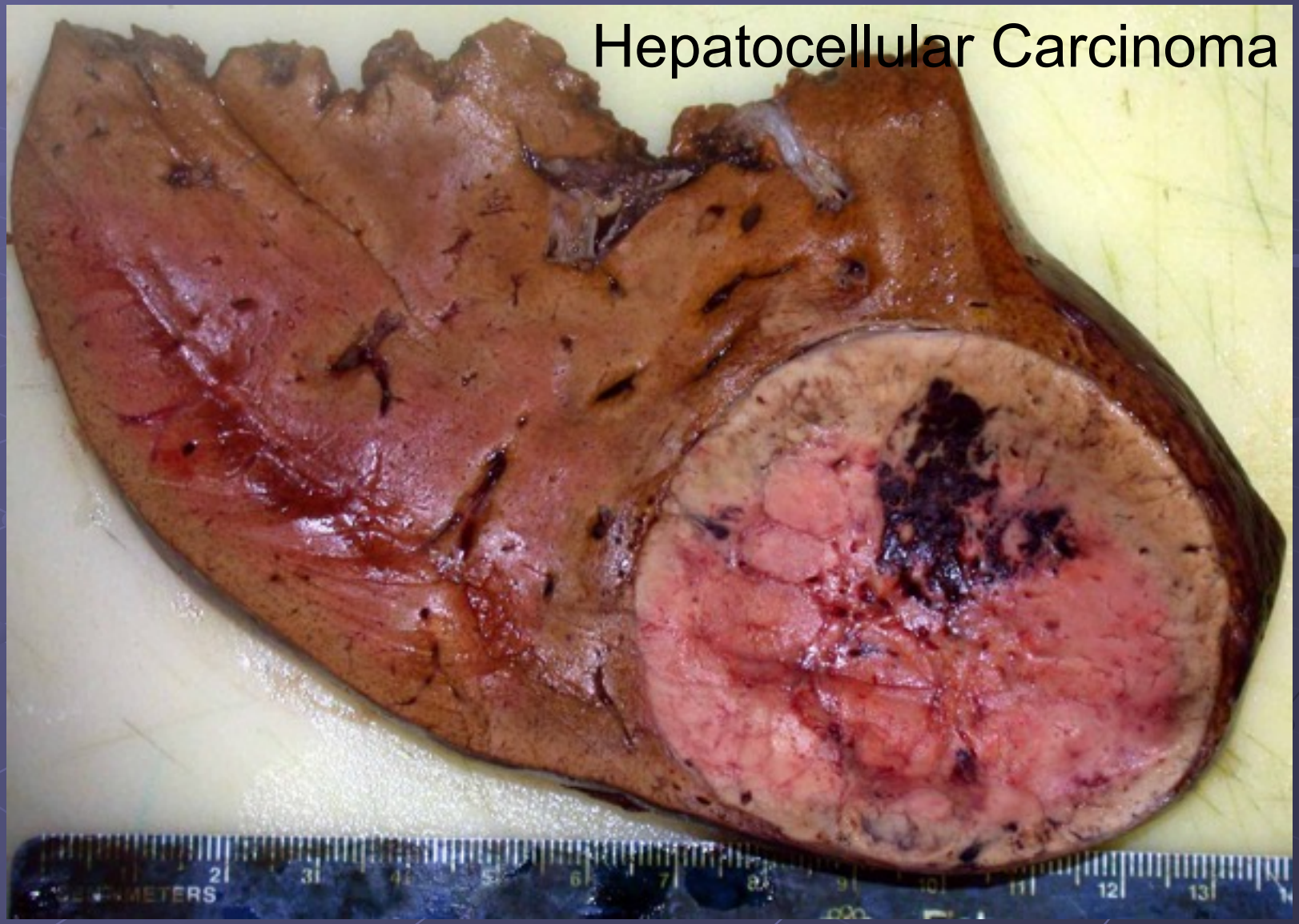
Approaching a pathologic specimen.

Beware: gross photos ahead

Approaching a pathologic specimen

- GROSS APPEARANCE
  - "dissect" and describe the specimen
  - Take carefully selected portions of tissue for subsequent examination under the microscope
- MICROSCOPIC APPEARANCE
  - Routine Hematoxylin and Eosin stained slides
  - Special histologic techniques (stains, immunohistochemistry)

Gross Specimen...



Another Gross Specimen

Adenocarcinoma of colon

Approaching a pathologic specimen

- Is there pathology present?
- What is the general nature of the pathology?
  - Infectious, neoplastic, inflammatory/autoimmune
- If it IS neoplastic, what parameters must be collected?
  - Benign or malignant?, tumor type, grade, stage, resection margin status

General Types of Neoplasia

- EPITHELIAL (eg. Carcinoma)
  - Generally derived from ectoderm or endoderm
- MESENCHYMAL (eg. Sarcoma)
  - Generally derived from mesoderm (neural tissue is an exception → it's ectodermal)
- HEMATOPOEITIC (eg. Lymphoma)
  - Generally derived from mesoderm
- MIXED (eg. Carcinosarcoma)
- OTHER (eg. Germ cell tumor)

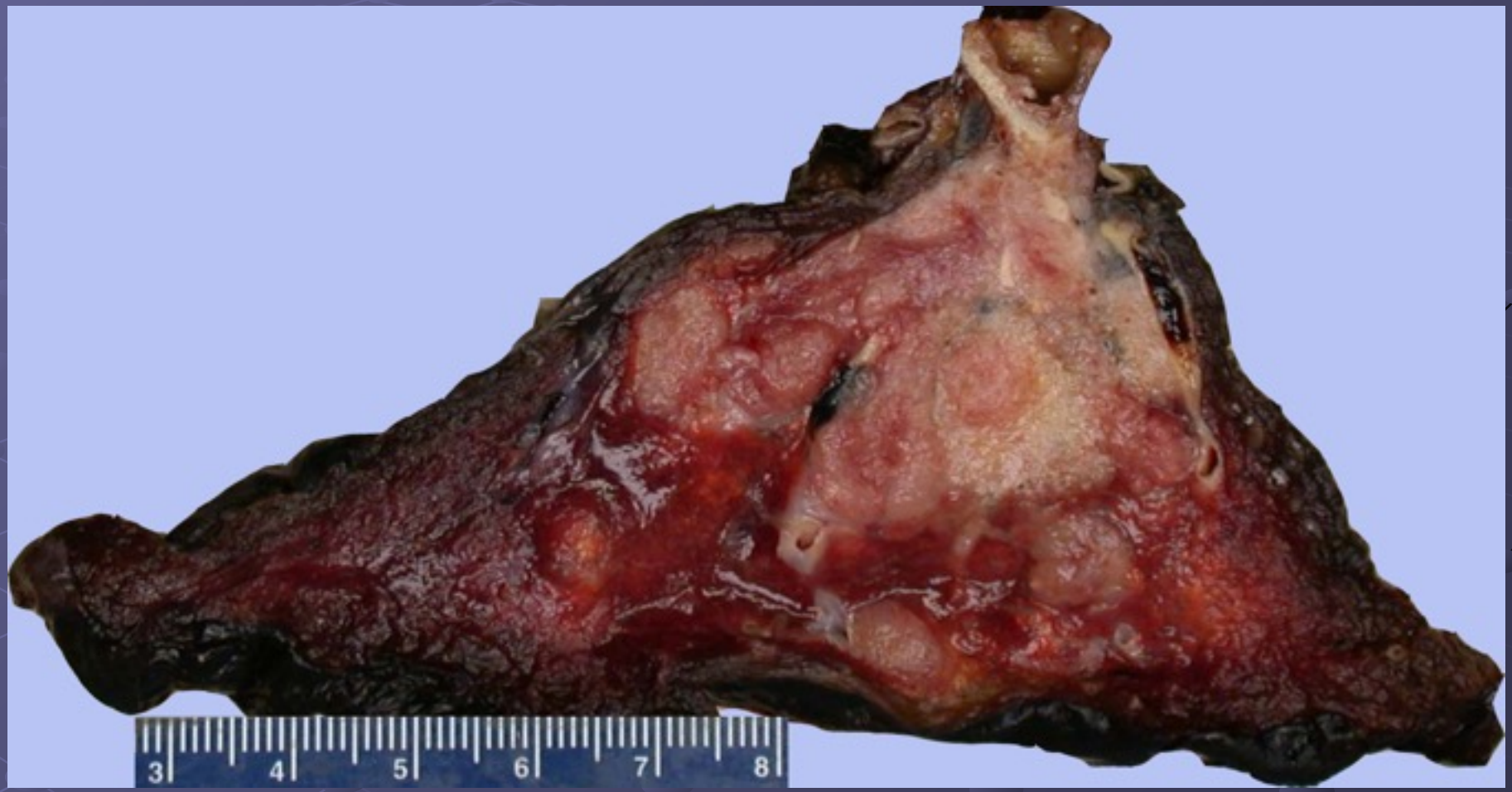
EPITHELIAL TUMORS

adenoma

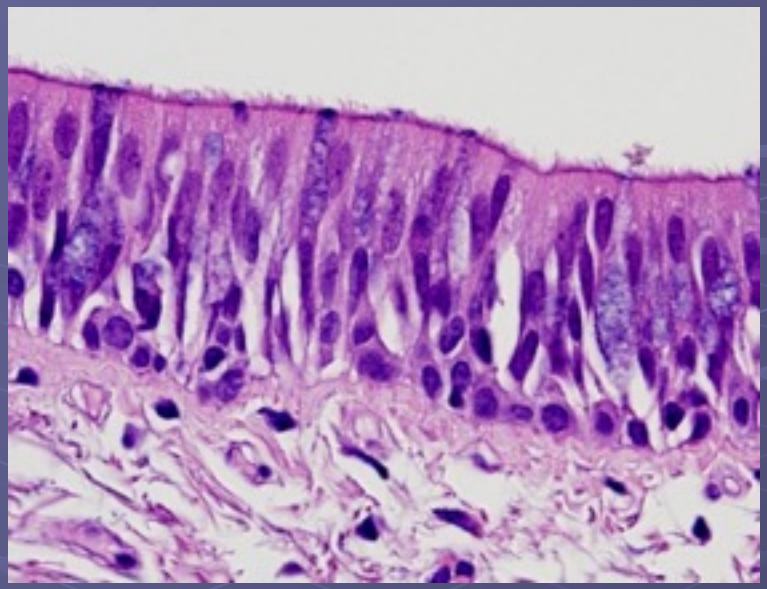
Carcinoma

- Malignant tumor of epithelium
- Comes in different types:
  - Squamous cell carcinoma
  - Adenocarcinoma (glandular)
  - Neuroendocrine carcinoma
- Often preceded by a pre-invasive lesion: severe dysplasia (carcinoma *in-situ*)

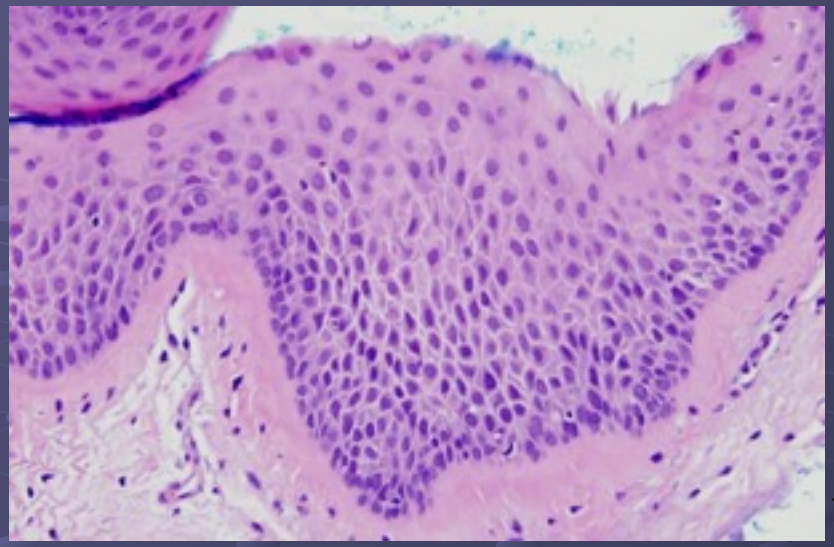
Adenocarcinoma of Lung

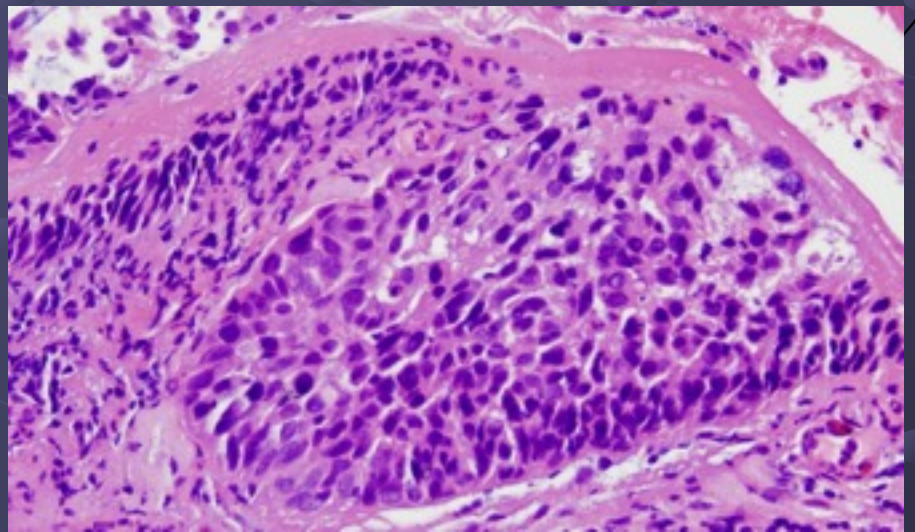


Normal Bronchial Epithelium (400x)



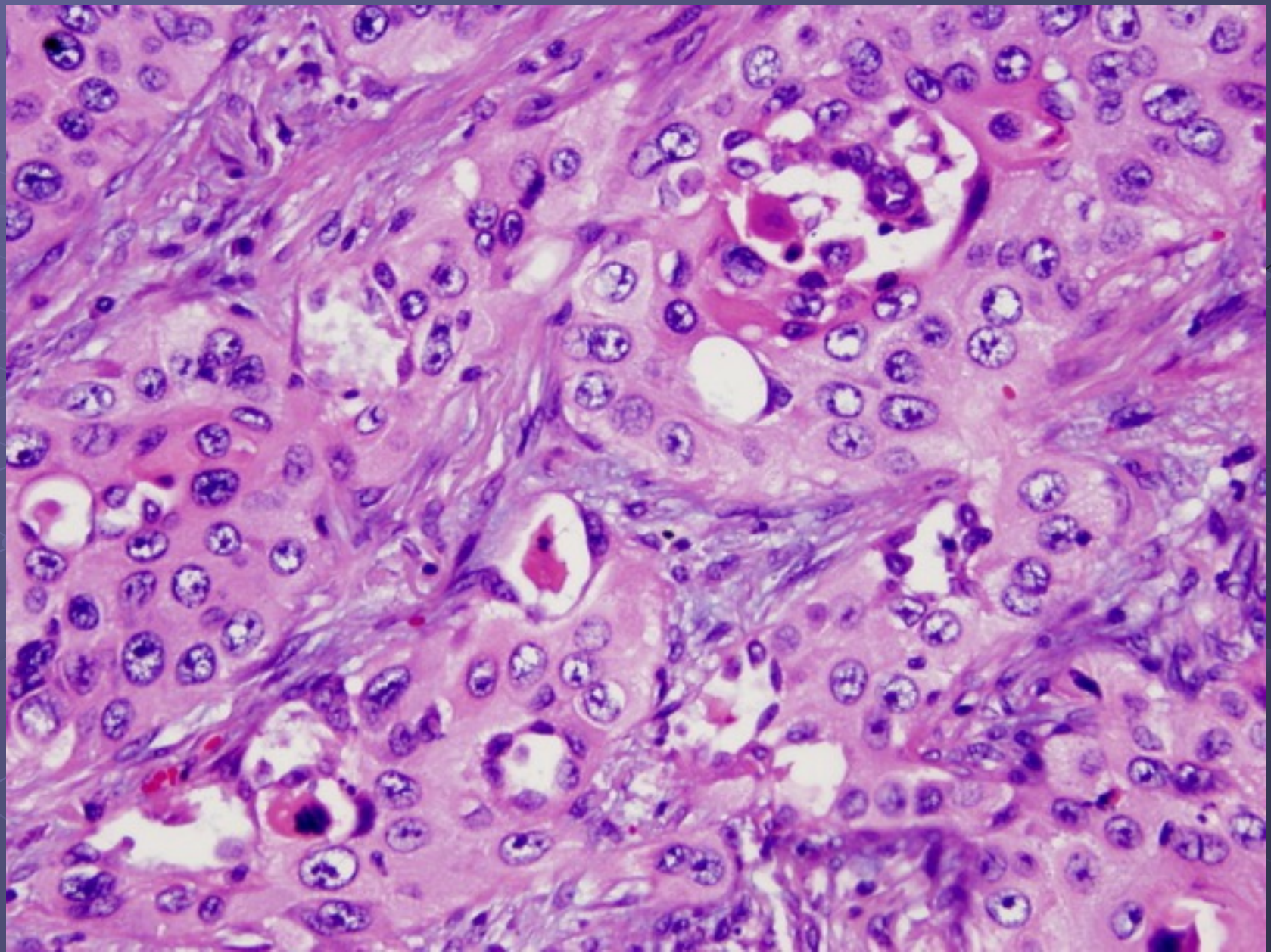
Bronchial Squamous Metaplasia (200x)





Severe Bronchial Squamous Dysplasia/ Carcinoma in-situ (200x)

Invasive Squamous Cell Carcinoma (400x)



Carcinoma

- One hallmark of an epithelial neoplasm is cellular expression of cytokeratin (CK) intermediate filaments

- Cytokeratin expression profile can also help identify the primary site/source of a carcinoma (immunohistochemistry)
  - CK 7, CK 20, CK 5/6

MESENCHYMAL TUMORS

- OMA

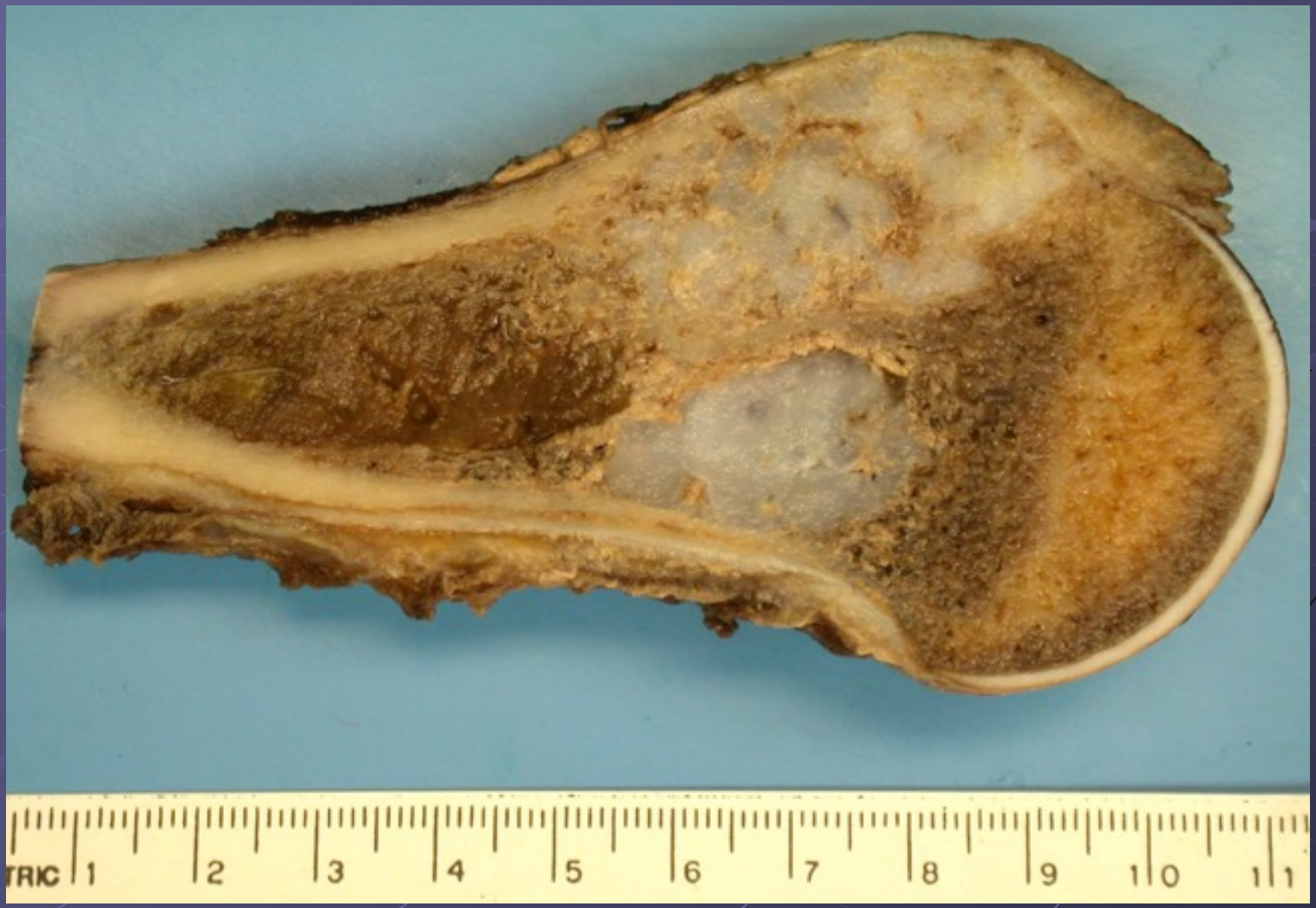
Mesenchymal tumors

- Tumors of connective/mesenchymal tissue

Named according to their putative origin and differentiation:

Tissue Type	Malignant	Benign
Smooth muscle	leiomyosarcoma	leiomyoma
Skeletal muscle	rhabdomyosarcoma	rhabdomyoma
Blood vessels	angiosarcoma	angioma
Cartilage	chondrosarcoma	chondroma
Bone	osteosarcoma	osteoma
Adipose (fat)	liposarcoma	lipoma

Chondrosarcoma (Humerus)



Mesenchymal Tumors

- *Usually* express the intermediate filament vimentin, and NOT cytokeratins

- May also express markers of differentiation:

Smooth Muscle →	smooth muscle actin
Nerve →	S100
Vascular →	Cluster of Differentiation 31 (CD31)

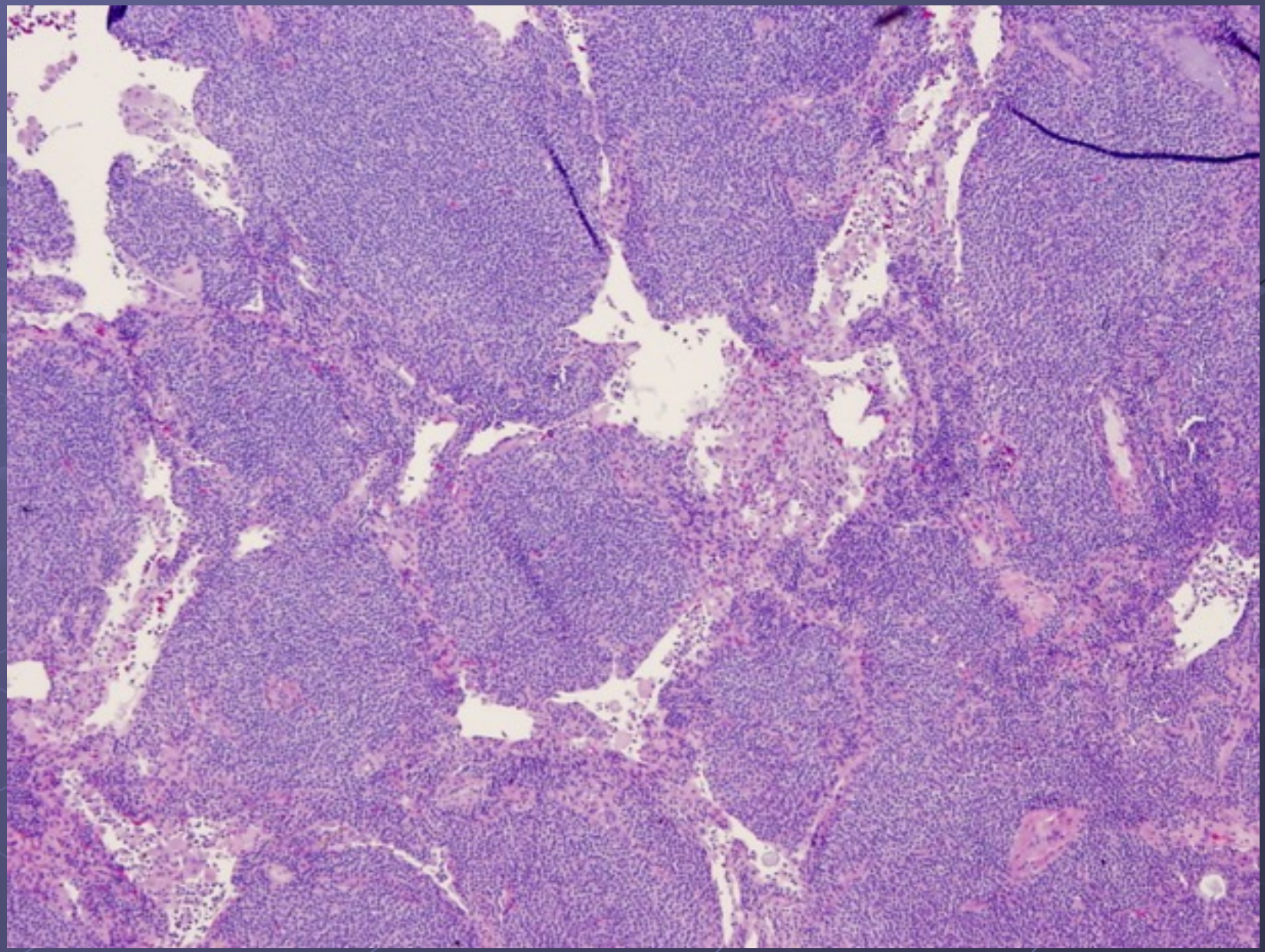
HEMATOPOETIC NEOPLASIA

leukemia

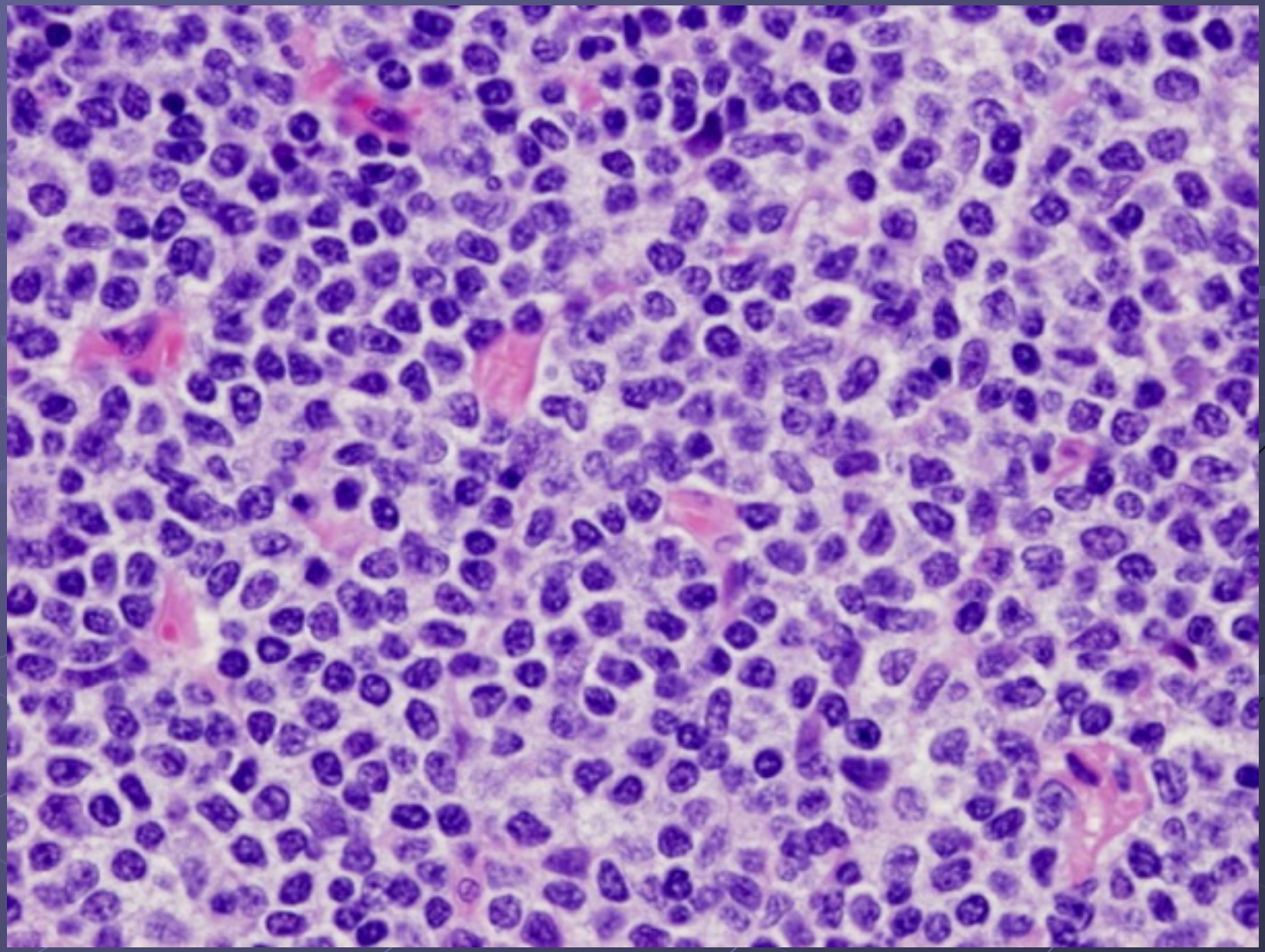
Hematopoetic Neoplasia

- Derived from bone marrow cells (red, white)
- Lymphoma (cells usually involve lymph node or other lymphoid tissue like spleen)
  - T-cell (CD3+, CD20-)
  - B-cell (CD20+, CD3-)
  - NK cell (CD56+)
- Leukemia (cells circulate in blood)
  - Myeloid
  - Lymphoid

MALT Lymphoma of Lung (40 x)



MALT Lymphoma (400 x)



So how do we know if a bunch of cells is benign or malignant?

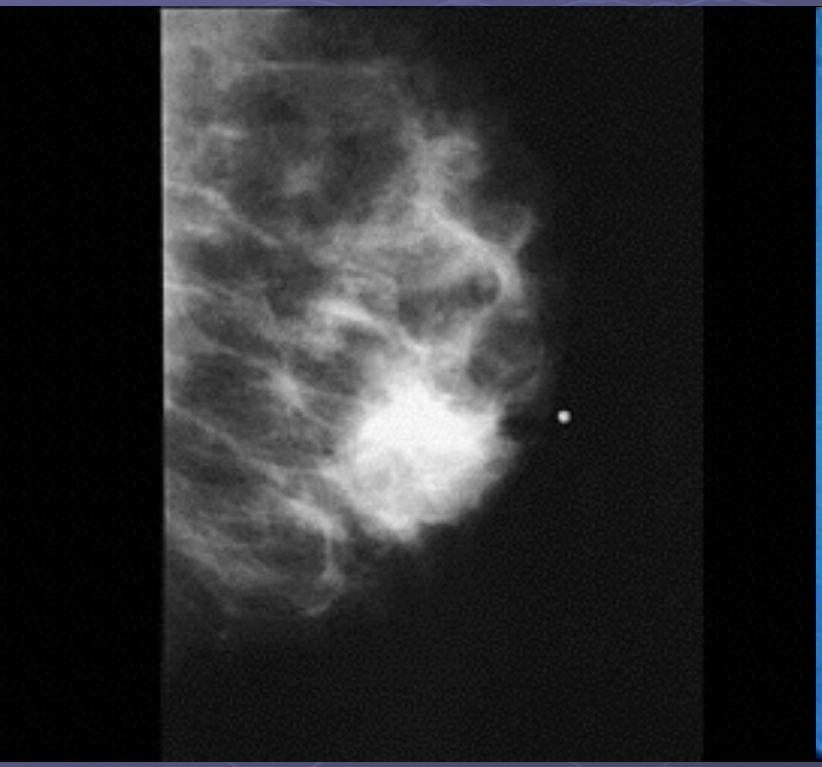
Recognizing Malignancy

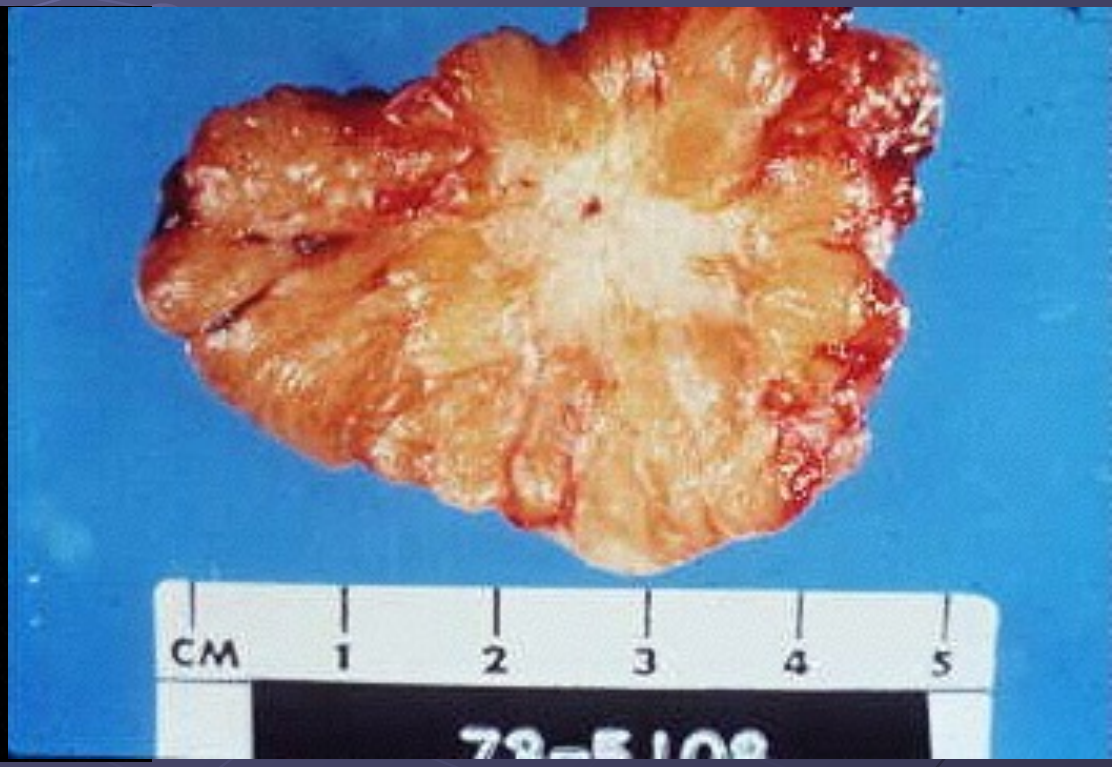
1. Architecture and Behavior
2. Proliferation (mitotic rate, antigenic markers)
3. Necrosis
4. Cytology (nuclear and cytoplasmic)

Recognizing Malignancy

- Architecture and behavior
  - How closely does tumor resemble normal tissue?
  - What is the contour of the tumor?
  - Is there invasion of adjacent tissue?
  - Is there invasion of blood vessels or nerves?
  - Does it have a capsule?

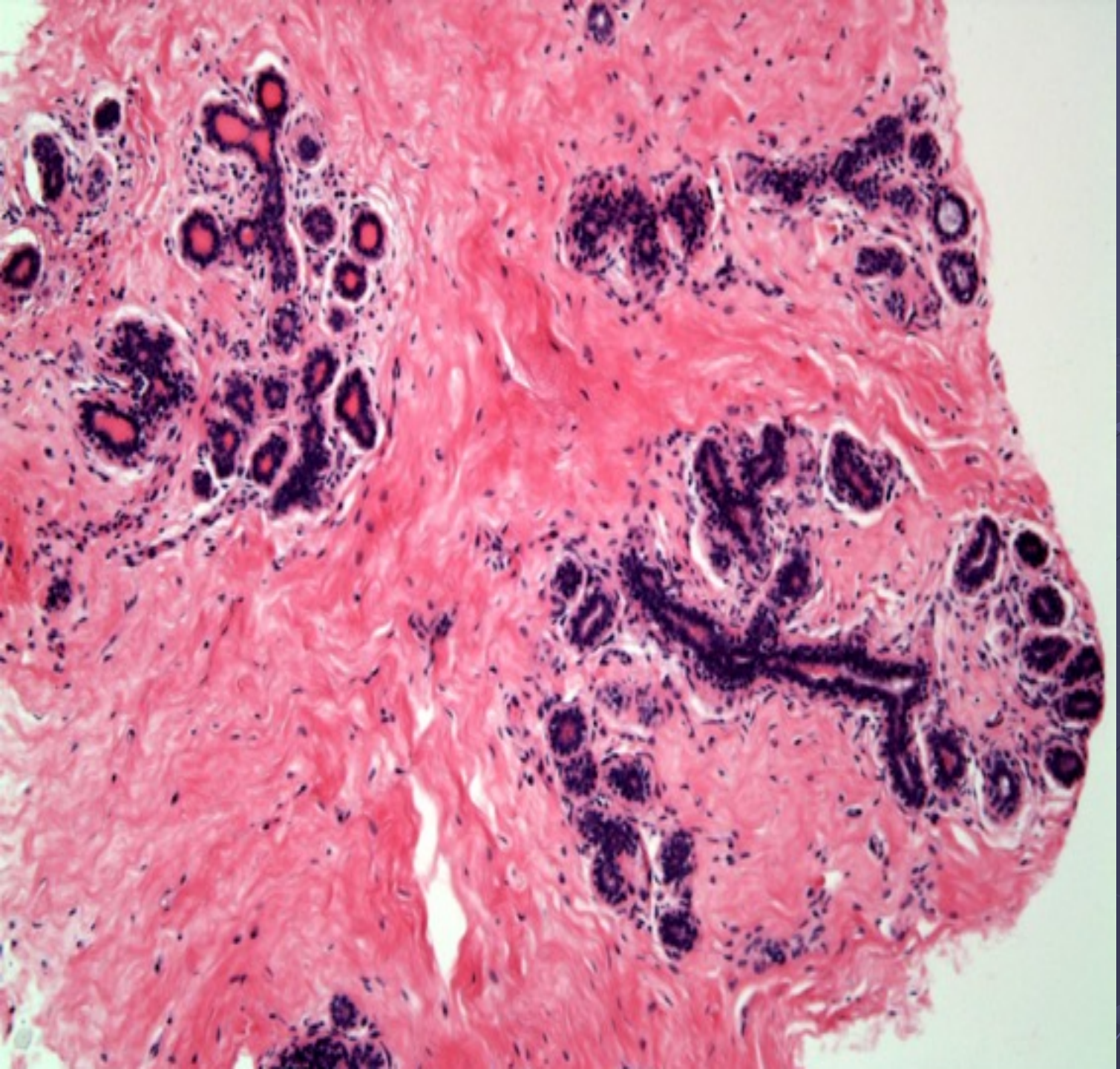
Mammogram of Ductal Carcinoma





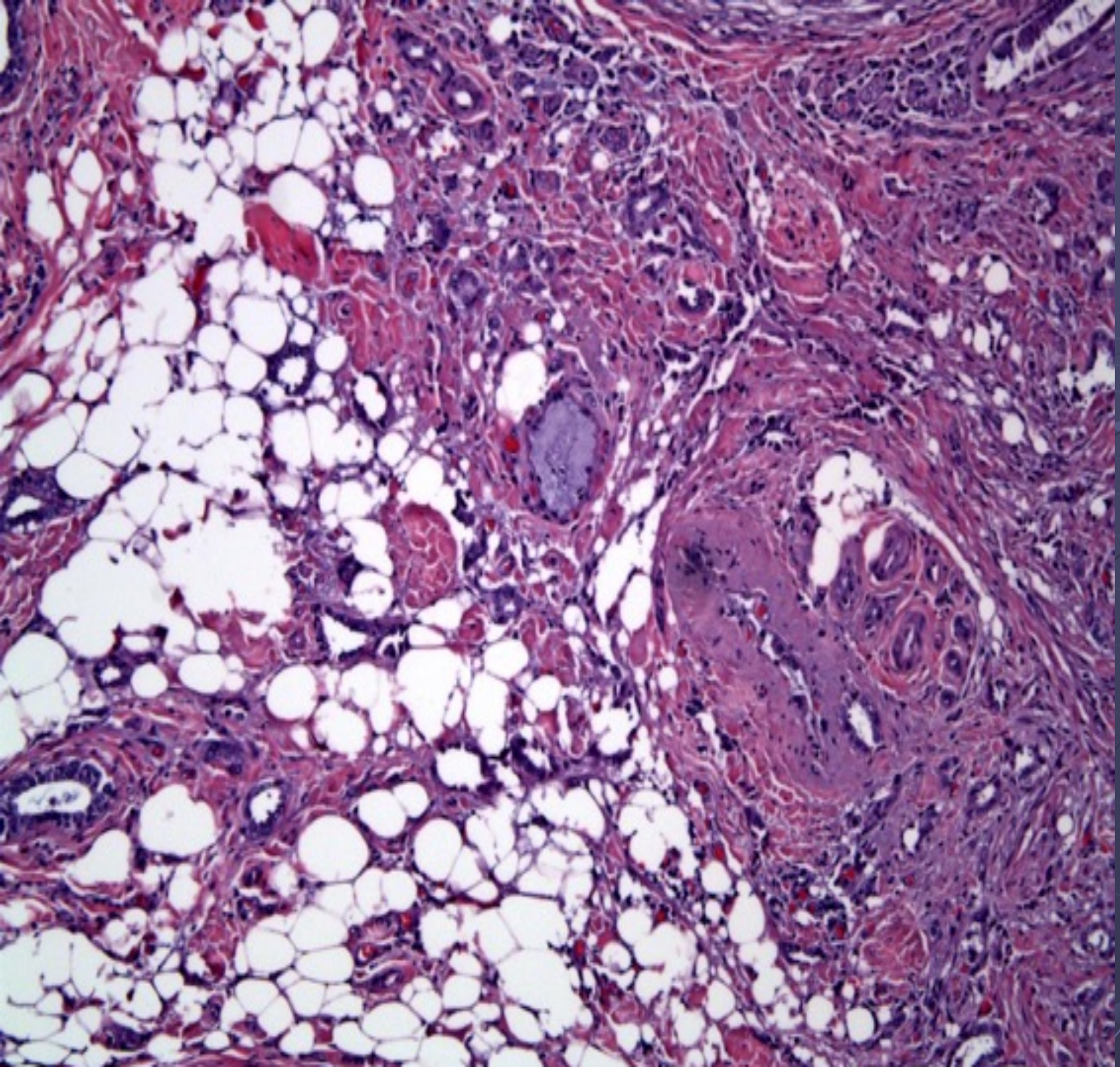
ISBN: 0-7216-8462-9

Image from: medic.med.uth.tmc.edu



BENIGN

- Organized
- Well circumscribed
- No infiltration of adjacent tissue.
- Normal stroma



MALIGNANT

-Disorganized.

- Poorly circumscribed.

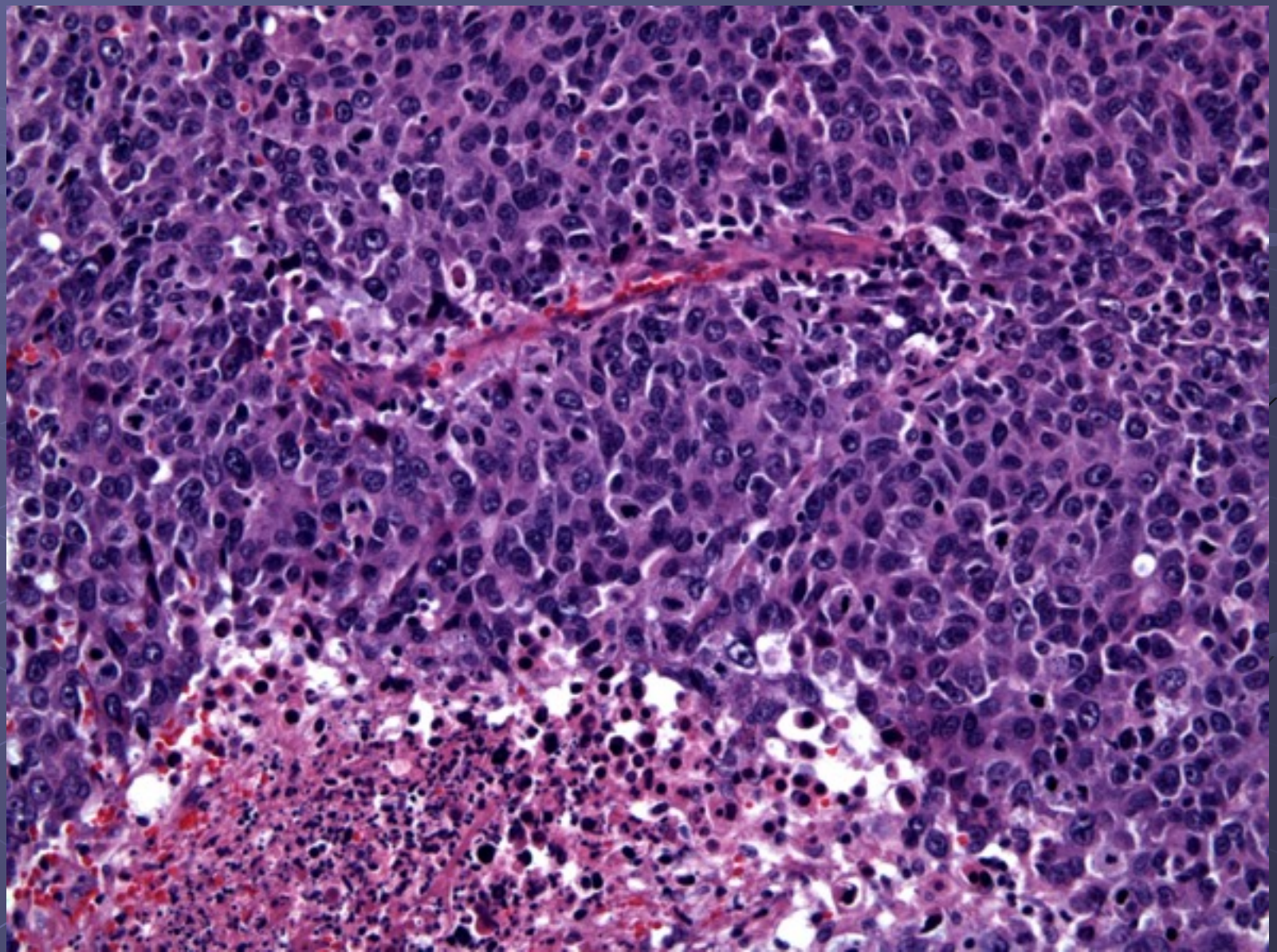
- Infiltrates adjacent fat.

- Desmoplastic stroma.

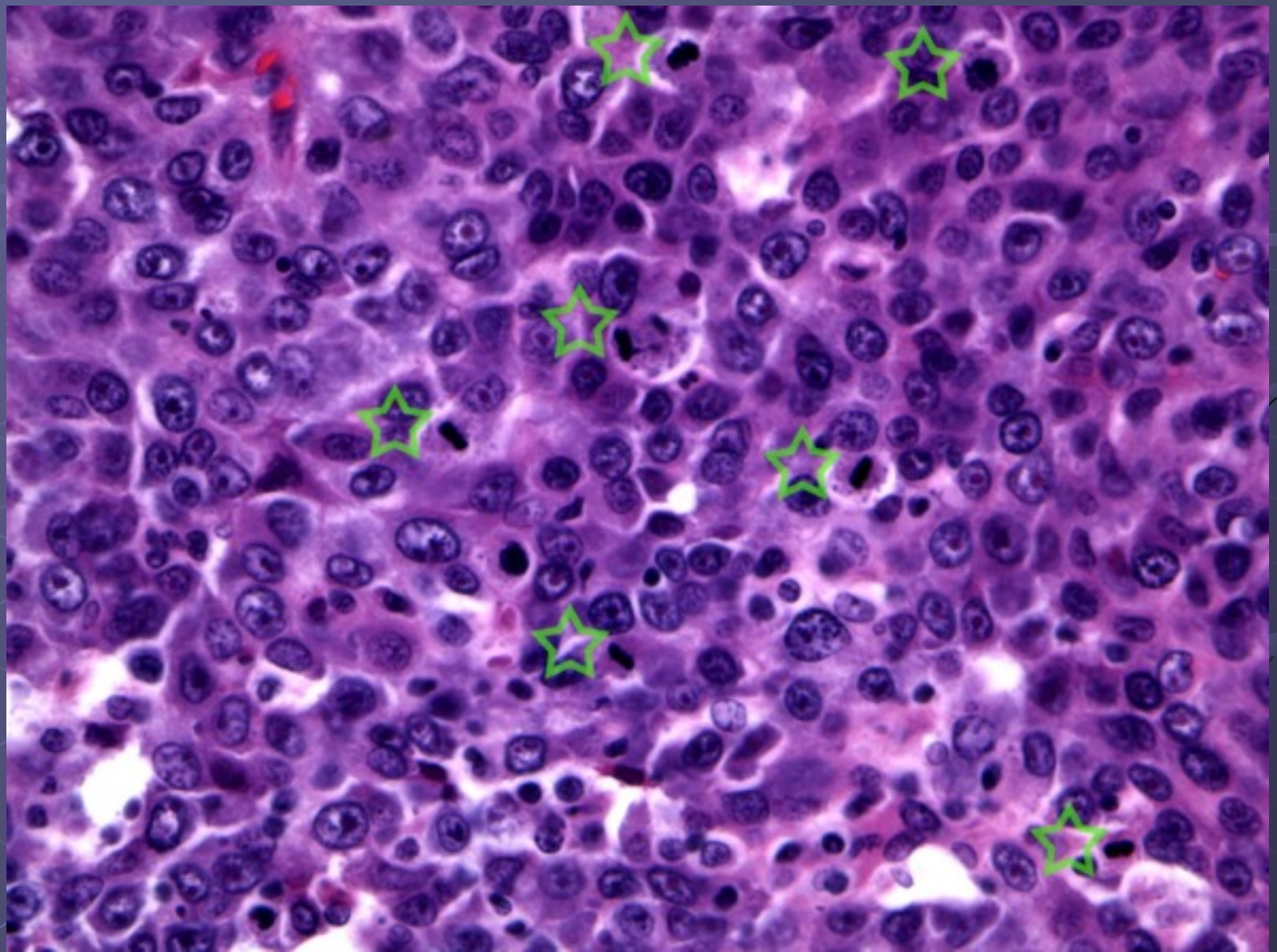
Recognizing Malignancy

- Proliferative Rate
  - What is the mitotic count? (number of mitoses per 10 high-power 400x microscopic fields)
  - Are mitoses normal in form?
  - What is the percentage of cells expressing markers of proliferation? (Ki-67/Mib-1)
- Necrosis
  - Is there tumor necrosis? (function of rapid uncontrolled proliferation, loss of cell cycle control)

Hepatocellular Carcinoma with Necrosis



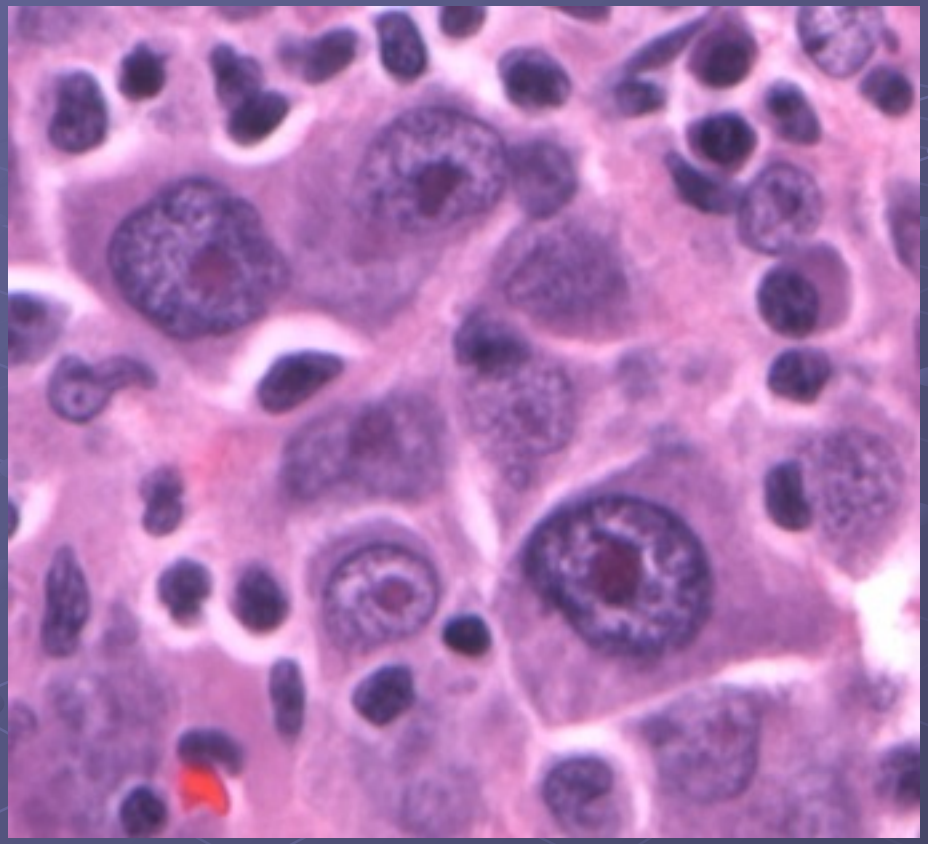
Hepatocellular Carcinoma with Mitosis

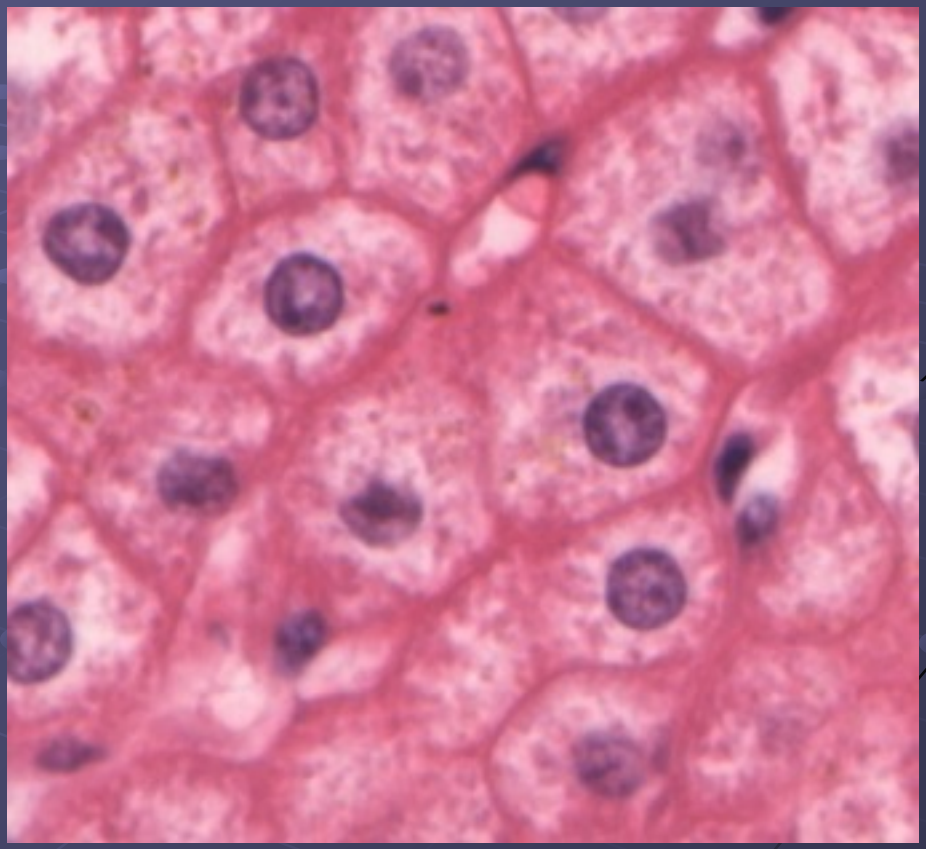


Recognizing Malignancy

- Cytology (a function of rapid proliferative rate and abnormal, active cellular “machinery”)
  - What is the character of the nuclear chromatin?
  - Are there abnormal nucleoli present?
  - What is the contour of the nuclear membrane?
  - What is the nucleus:cytoplasmic ratio? (N:C ratio)
  - Is the cytoplasm mature?

Benign or malignant nuclei (400x)?





#1

MALIGNANT

#2

BENIGN

GRADING AND STAGING MALIGNANCY

Grading Malignancy

- Grading is based on cytologic and/or architectural characteristics of the tumor

- Attempts to predict future behavior of a tumor
  - High, Severe or Poor essentially means Bad behavior

- Generic systems for many tumors

Well → Moderate → Poor → Undifferentiated

Severe → Moderate → Mild

High → Low

Grading Malignancy

- Some tumor types have specific, well validated grading systems:
  - Prostate → Gleason Grade
    - Primarily based on tumor architecture
  - Breast → Nottingham Grade
    - Tubule formation, Nuclear features, Mitotic rate
  - Endometrium → FIGO Grade
    - Architecture with nuclear feature modifier

Gleason Grading of Prostate Carcinoma

Figure Taken from Epstein J. *et al.* Am J Surg Pathol 29(9): 1228 (2005).

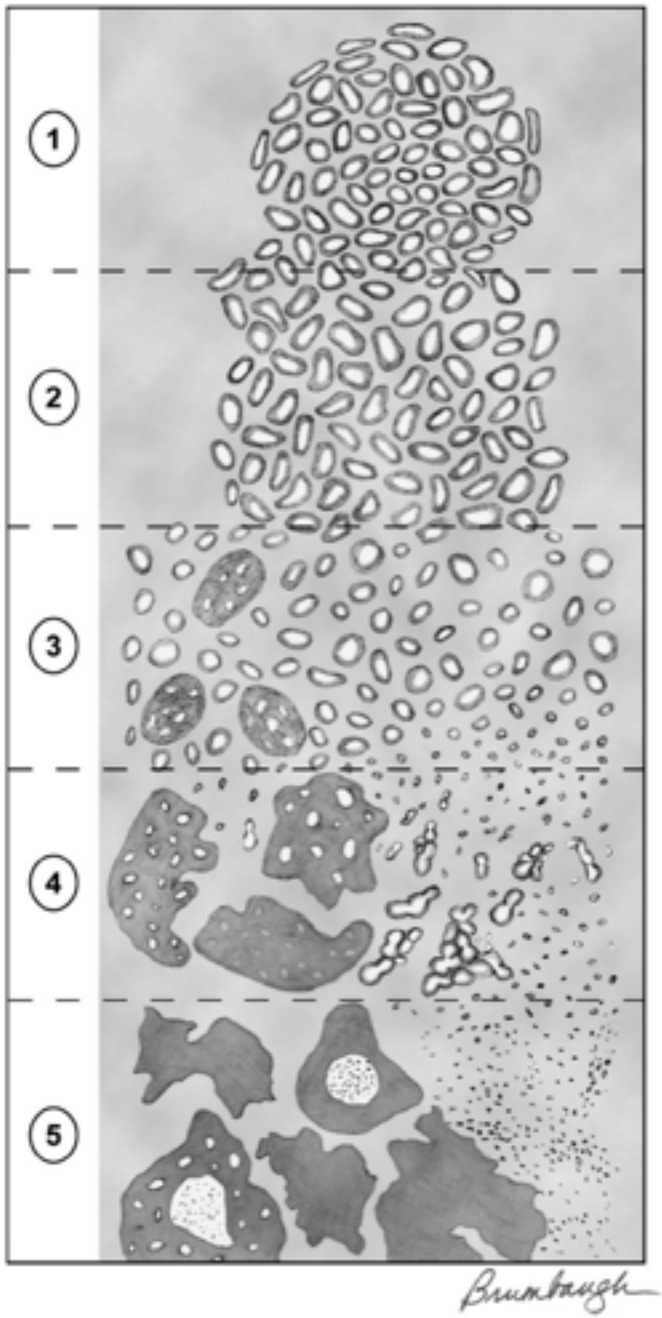


FIGURE 12. Schematic diagram of modified Gleason grading system.

Cancer Staging

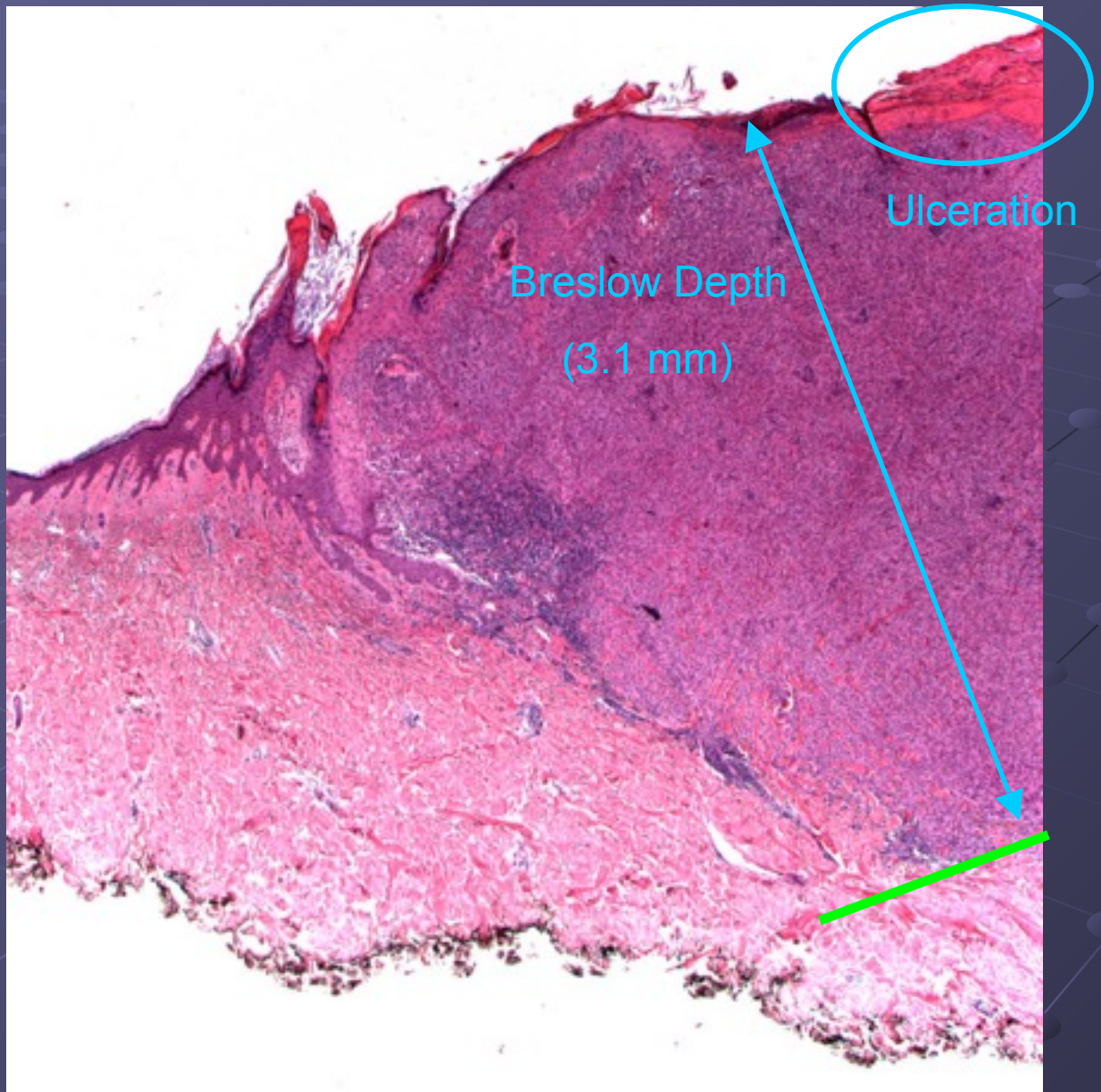
- Staging is a precise method for establishing the type and extent of tumor *at a specific point in time* during the course of the disease. Staging is critical to the modern management of cancer because it allows the clinical team to:

1. Plan treatment
2. Estimate prognosis
3. Group similar patients to evaluate results on treatment protocol studies
4. Facilitate information exchange between institutions
5. Otherwise contribute to the study of cancer

Staging

- Performed according to guidelines published by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC or Union Internationale Contre le Cancer)
- Stage is NOT the same thing as grade
- The staging system for each tumor type is different (see example for melanoma)

Staging Melanoma



Staging Melanoma

Tis Melanoma in-situ

T1 Melanoma measuring 1.0 mm or less

- a] no ulceration, mitosis <1 /mm^2
- b] ulceration or mitosis >=1 /mm^2

T2 Melanoma measuring 1.01 – 2.0 mm

- a] no ulceration
- b] ulceration

T3 Melanoma measuring 2.01 – 4.0 mm

- a] no ulceration
- b] ulceration

T4 Melanoma measuring >4.01 mm

- a] no ulceration
- b] ulceration

Staging Melanoma

NX	Lymph nodes not assessed
N1	One lymph node positive
N2	Two or three nodes positive OR In-transit/Satellite metastasis
N3	Four or more nodes positive OR In-transit/Satellite metastasis AND nodes positive

M0	No distant metastasis
M1	Distant metastasis

AJCC Stage Groups
Melanoma

Stage	0	Tis	N0	M0
Stage	IA	T1a	N0	M0
	IB	T1b	N0	M0
		T2a	N0	M0
Stage	IIA	T2b	N0	M0
		T3a	N0	M0
	IIB	T3b	N0	M0
		T4a	N0	M0
	IIC	T4b	N0	M0
Stage	III	any T	any N+	M0
Stage	IV	any T	any N+	M1

Melanoma Survival by AJCC Stage

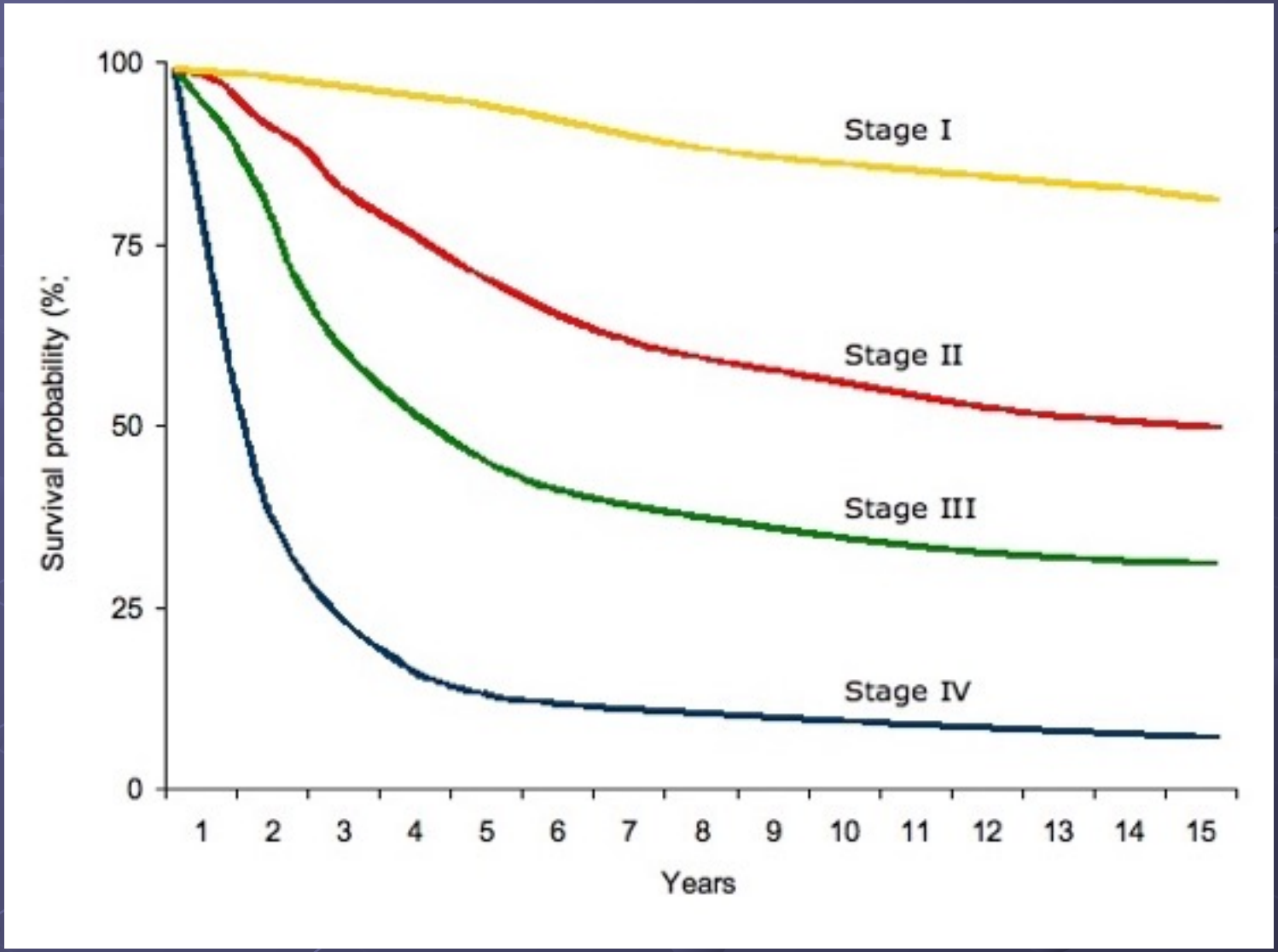
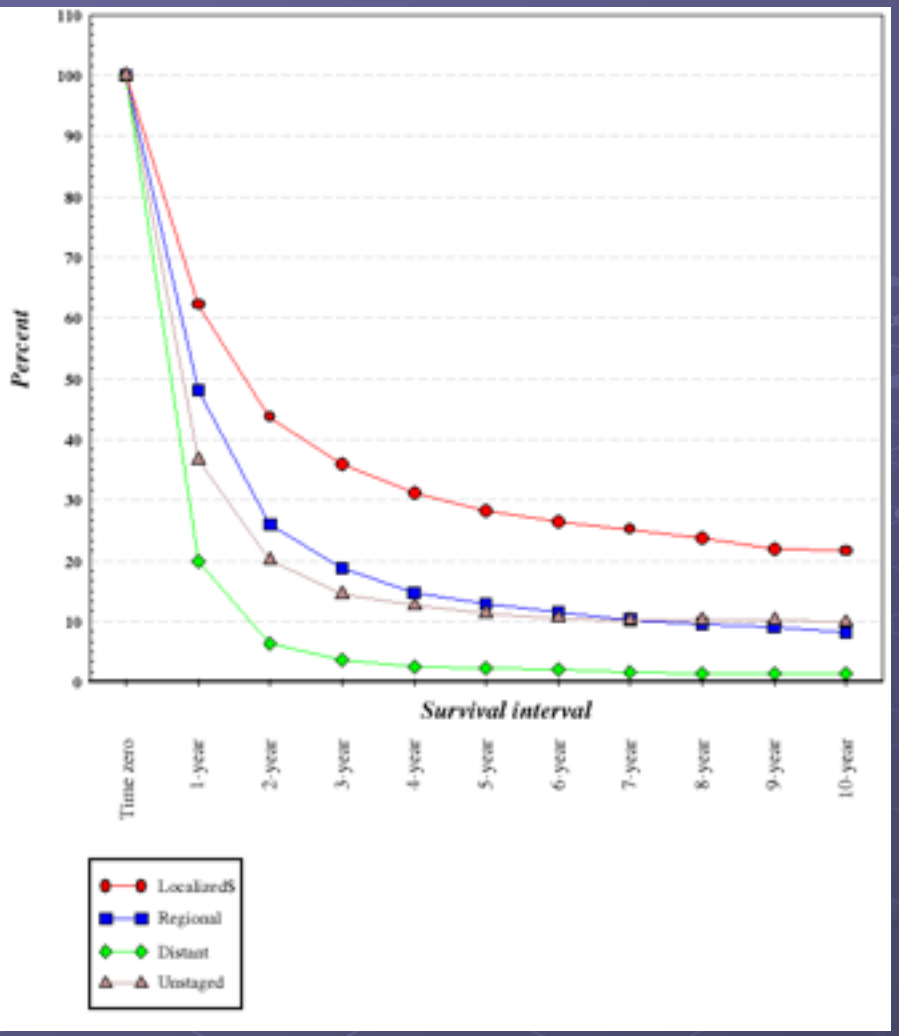
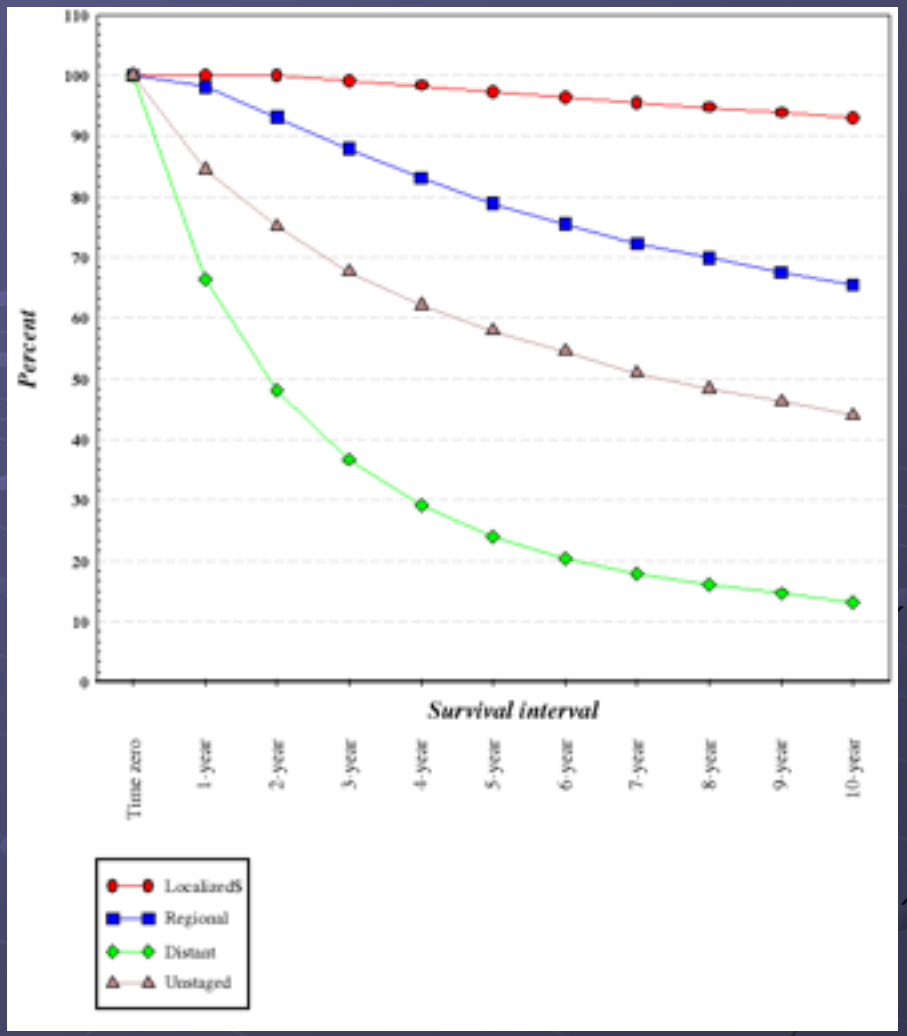


Figure taken from Melanoma Molecular Map Project (www.mmmp.org)





ESOPHAGUS

BREAST

National Cancer Institute SEER Data 1998-2001

And finally…

ADVANCES in pathology and pathology in research

The Changing World of Pathology

- Traditional histopathology merges with molecular and genetic evaluation:
  - Molecular or genetic classification of cancer.
  - Identify primary origin of metastasis
- Personalized therapy
  - Use biopsy tissue to plan best treatment
  - OmniSeq multigene platform

2008 WHO Classification of myeloid neoplasms and acute leukemia.

Acute myeloid leukemia and related neoplasms

Acute myeloid leukemia with recurrent genetic abnormalities

AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*

APL with t(15;17)(q22;q12); *PML-RARA*

AML with t(9;11)(p22;q23); *MLLT3-MLL*

AML with t(6;9)(p23;q34); *DEK-NUP214*

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*

AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*

Provisional entity: AML with mutated NPM1

Provisional entity: AML with mutated CEBPA

Molecular Classification

- Should we group tumors by genotype rather than conventional histology or origin site?

- *BRAF* mutated tumors:
  - Melanoma
  - Colorectal adenocarcinoma
  - Lung adenocarcinoma
  - Papillary thyroid carcinoma

The Future of Pathology

- Traditional histopathology merges with molecular and genetic evaluation:
  - Molecular or genetic classification of cancer.
  - Identify primary origin of metastasis
- Personalized therapy
  - Use biopsy tissue to plan best treatment
  - OmniSeq multigene platform

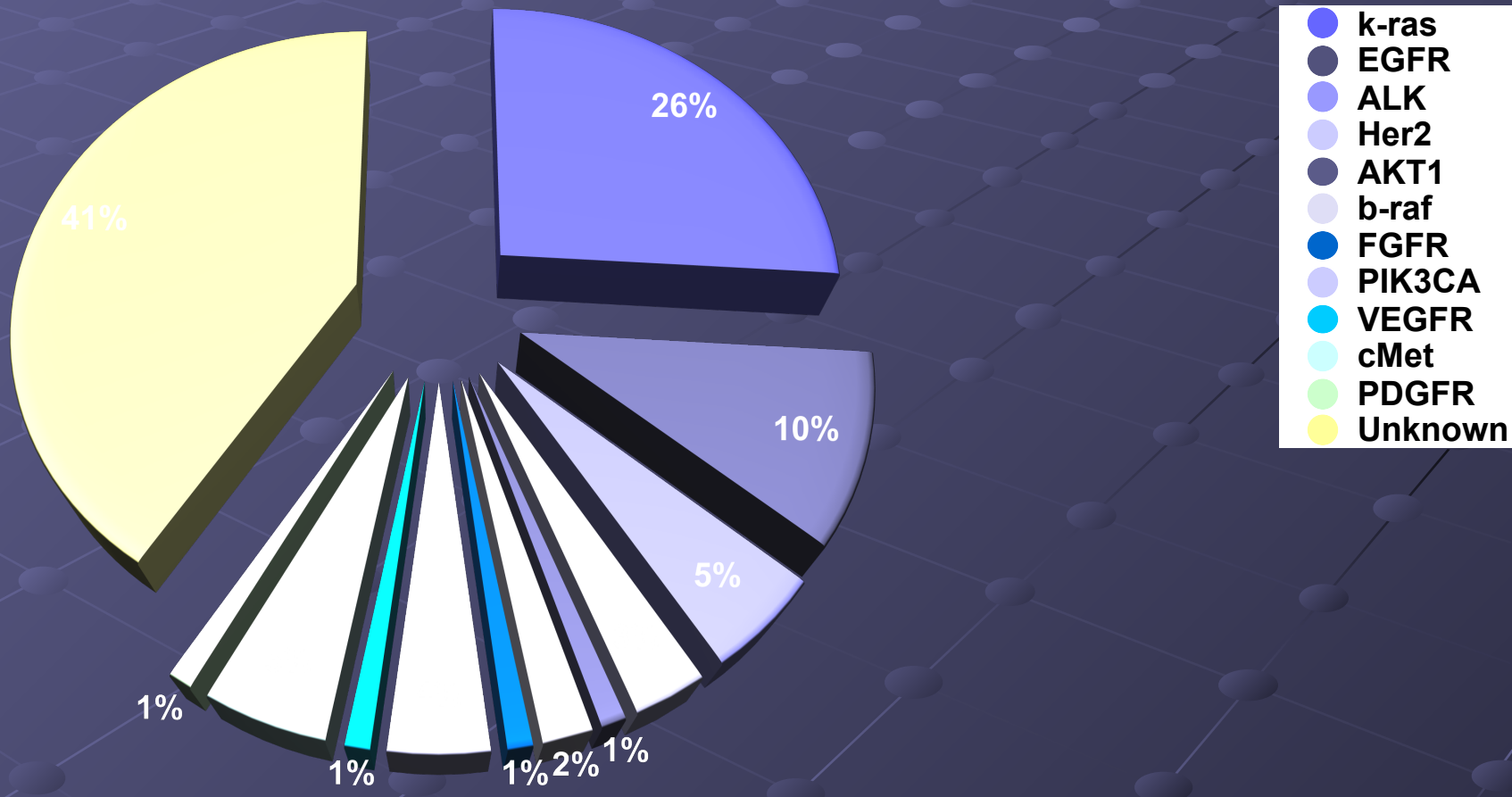
Lung Adenocarcinoma: Molecular targets

Based on data from:

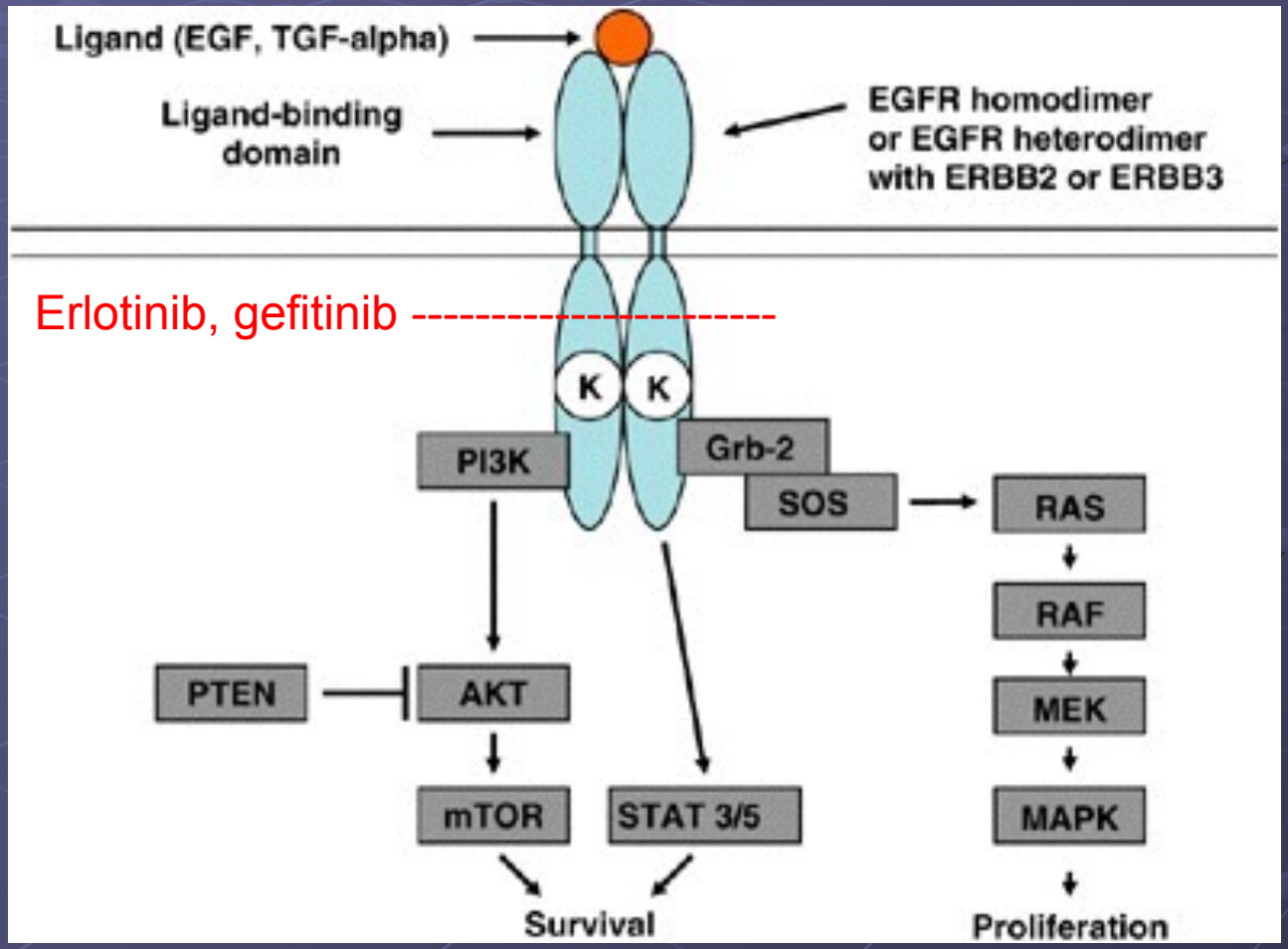
Ding *et al.*, Nature 455:1069, 2008

And modified from a figure developed by:

Dr. F. Hirsch, University of Colorado



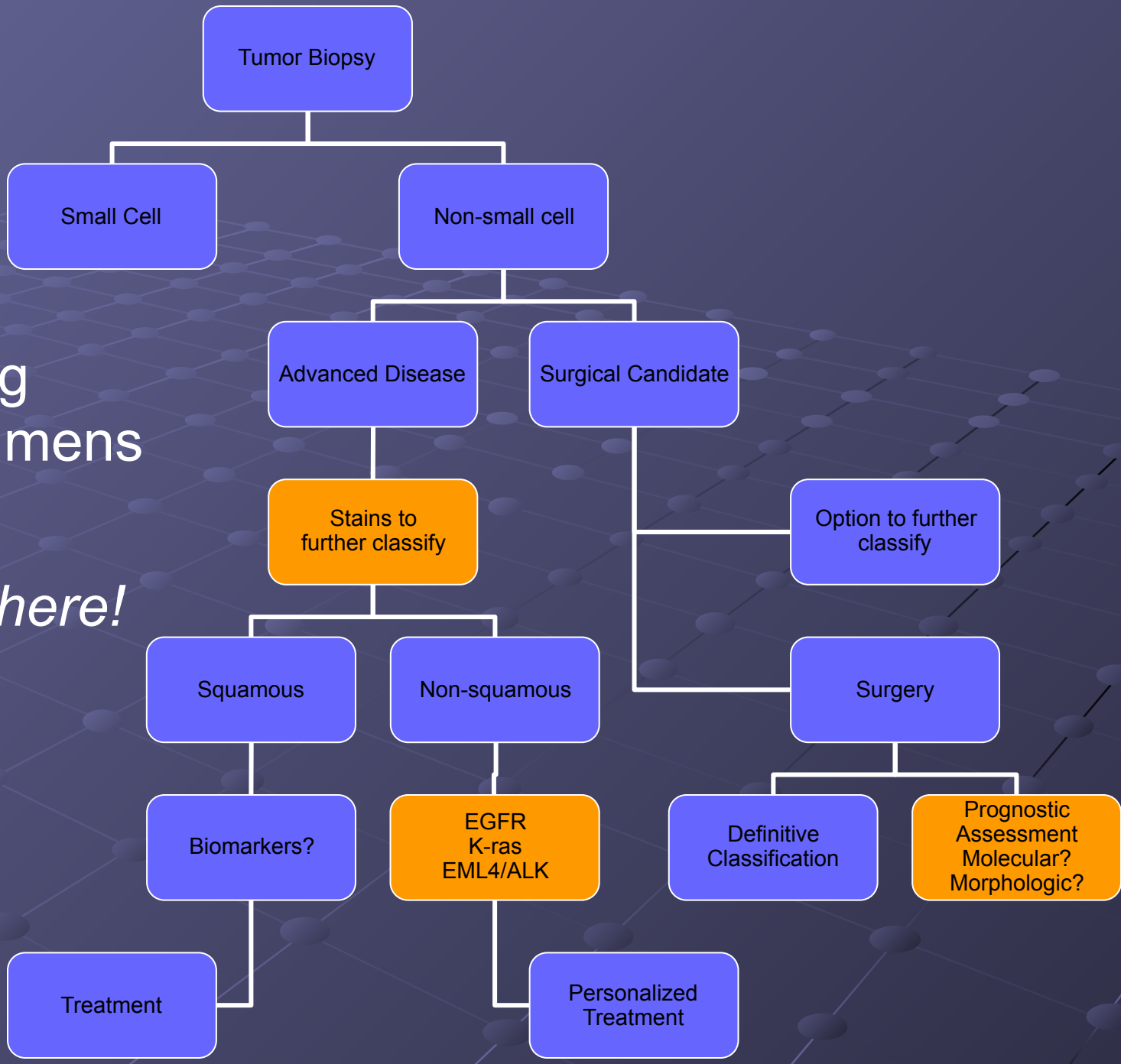
the EGFR Pathway



From: Ladanyi M, Pao, W. **Mod Pathol** 21 Supp2, S16-21, 2008.

Handling Lung Cancer Specimens in 2016.

The future is here!



RPCI Center for Personalized Medicine

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SEARCH

City & Region > Personalized medicine offers glimpse into the future

Carl Morrison, head of the Center for Personalized Medicine, says gene sequencing could be transformative. Harry Scull Jr./Buffalo News

Updated: 12/16/2012, 10:57 PM Published: 12/16/2012, 10:57 PM

Personalized medicine offers glimpse into the future

BY: HENRY DAVIS / NEWS MEDICAL REPORTER

Personalized Medicine

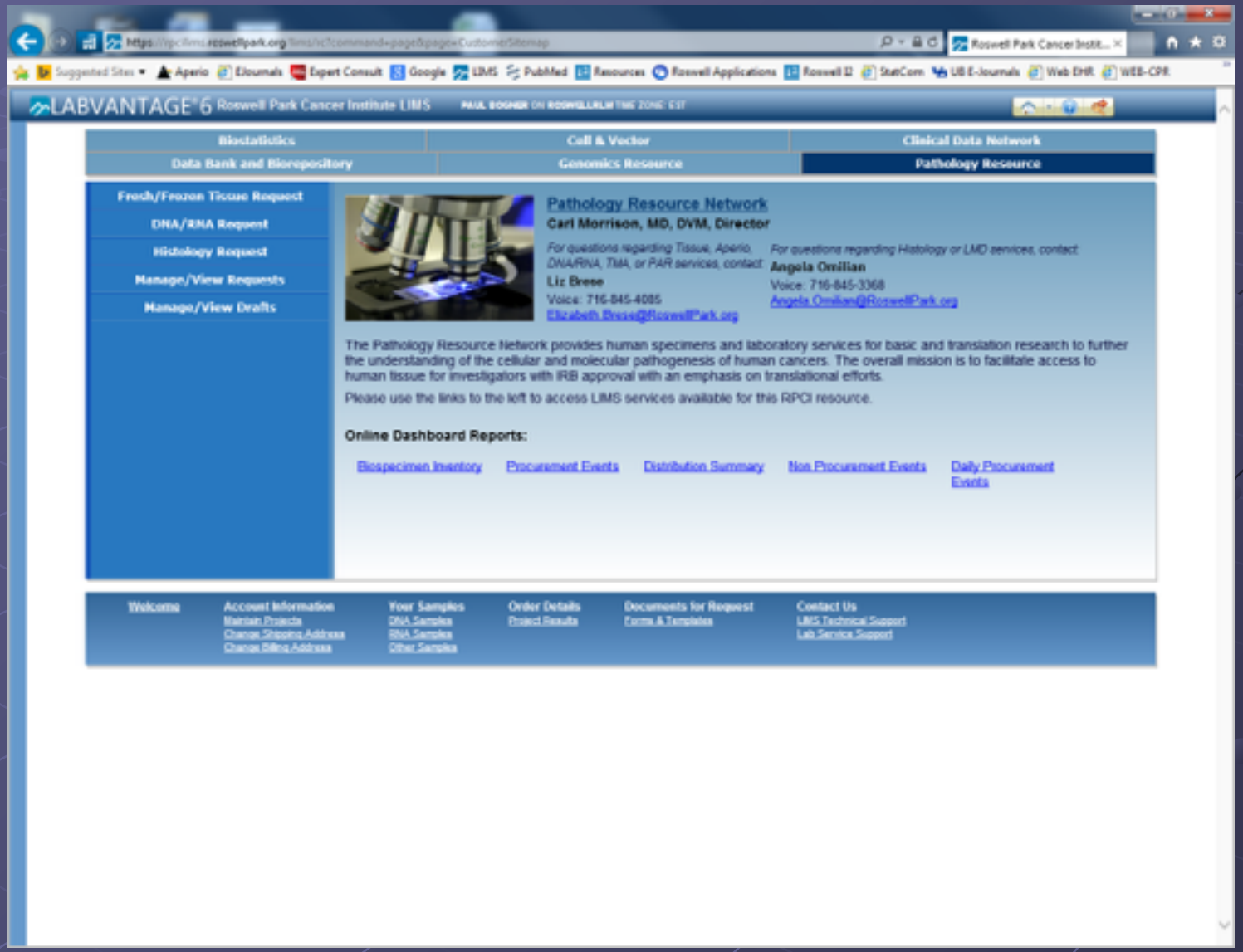
- OmniSeq Target™
  - Includes dual platform next gen mutation testing
  - 23 cancer associated "actionable" genes such as ALK, EGFR, PTEN, KRAS, NRAS, BRAF, etc
  - Detects mutations, translocations, copy number changes, etc.
  - Currently utilized for lung cancer and melanoma (others to follow)

Pathology and Research

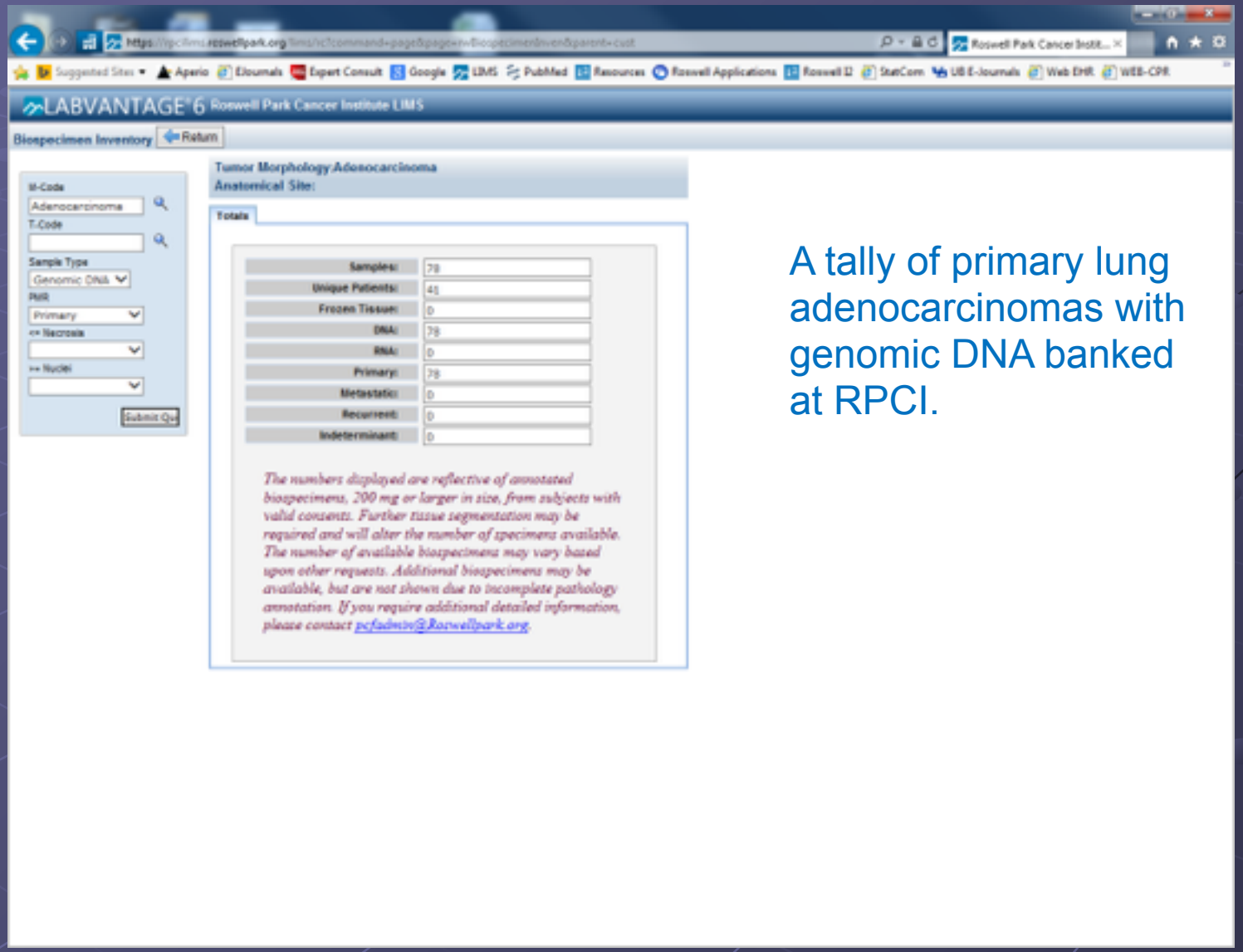
- Initiate both basic and translational research
- Tissue procurement and specimen archiving

- Bridge between clinic and research bench
  - Analyze human/animal experimental histology
  - Build and evaluate tissue based experiments
    - example: tissue microarray
  - Provide clinical perspective to scientific groups

Pathology Resource Network (PRN)

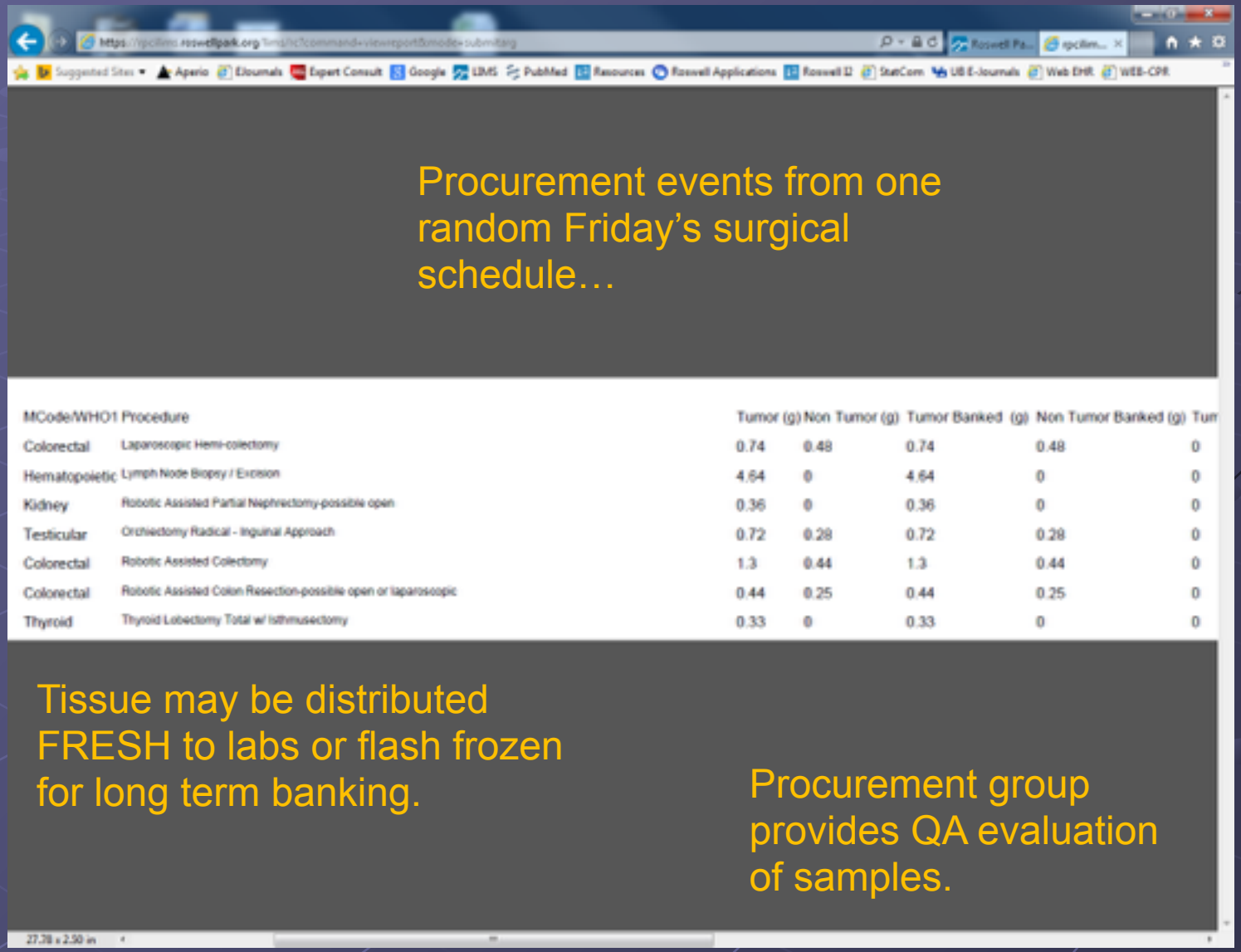


LIMS: Biospecimens



A tally of primary lung adenocarcinomas with genomic DNA banked at RPCI.

Tumor Procurement



Procurement events from one random Friday's surgical schedule…

MCode/WHO1	Procedure	Tumor (g)	Non Tumor (g)	Tumor Banked (g)	Non Tumor Banked (g)	Tum
Colorectal	Laparoscopic Hemi-colectomy	0.74	0.48	0.74	0.48	0
Hematopoietic	Lymph Node Biopsy / Excision	4.64	0	4.64	0	0
Kidney	Robotic Assisted Partial Nephrectomy-possible open	0.36	0	0.36	0	0
Testicular	Orchiectomy Radical - Inguinal Approach	0.72	0.28	0.72	0.28	0
Colorectal	Robotic Assisted Colectomy	1.3	0.44	1.3	0.44	0
Colorectal	Robotic Assisted Colon Resection-possible open or laparoscopic	0.44	0.25	0.44	0.25	0
Thyroid	Thyroid Lobectomy Total w/ Isthmusectomy	0.33	0	0.33	0	0

Tissue may be distributed FRESH to labs or flash frozen for long term banking.

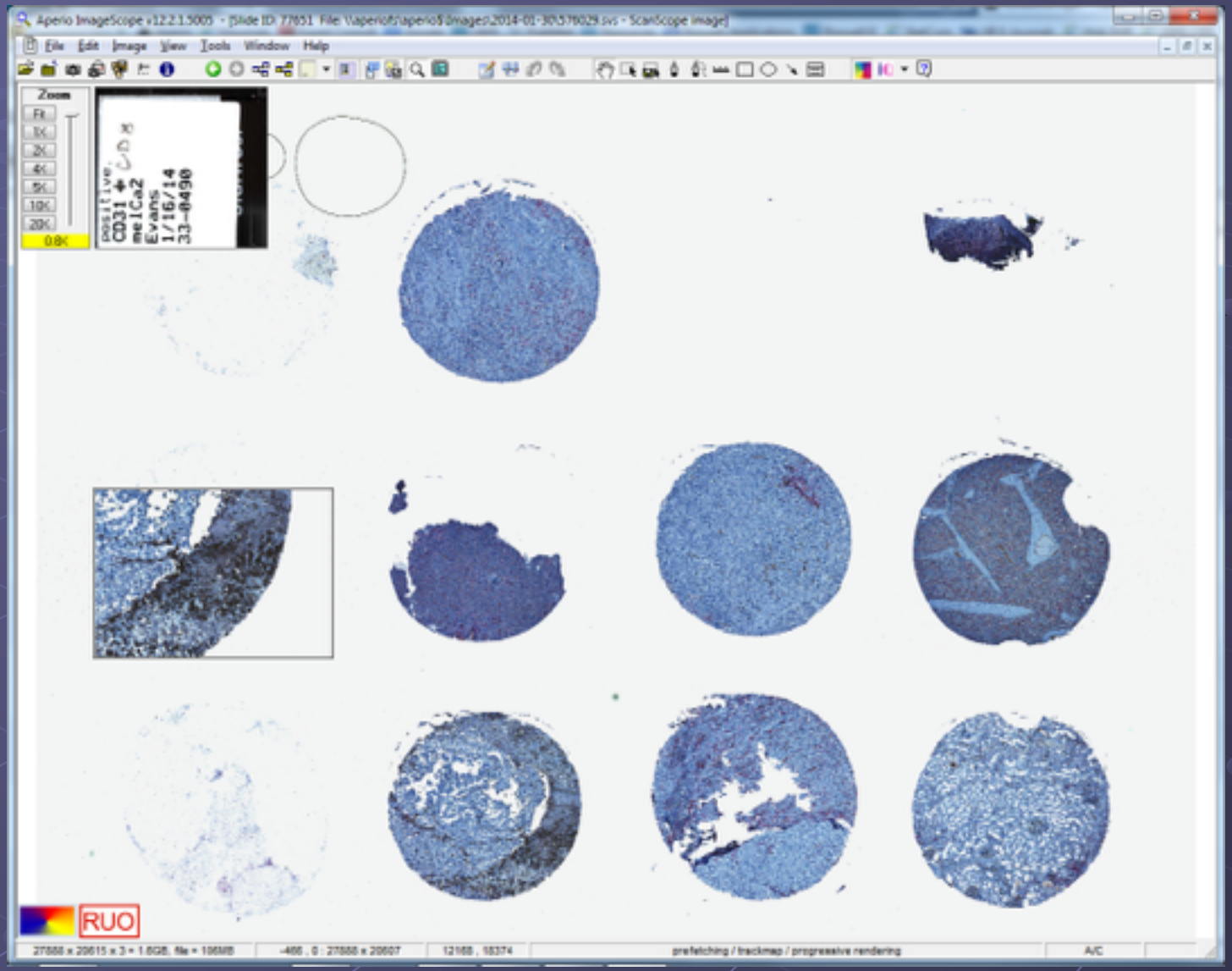
Procurement group provides QA evaluation of samples.

Pathology and Research

- Initiate both basic and translational research
- Tissue procurement and specimen archiving

- Bridge between clinic and research bench
  - Analyze human/animal experimental histology
  - Build and evaluate tissue based experiments
    - one example: tissue microarray
  - Provide clinical perspective to scientific groups

Tissue Microarray



Outline

What is Pathology?

What is a Pathology Department?

The pathologist's "tools"

Pathology Definitions and Concepts

How do we approach a pathology specimen?

A General Classification of Neoplasia.

The ugly histologic face of cancer (recognizing malignancy).

Grading and Staging Malignancy.

The Changing World of Pathology

Pathology and Research

The End

feel free to contact me with questions or comments:
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