

Invasive Breast Carcinoma

General Background

Most common cancer in women and leading cause of female cancer death worldwide.

Presenting signs and symptoms:

Unscreened populations—mass, skin erythema and edema due to cancer in dermal lymphatics.

Screened populations—spiculated mass, architectural distortion, MRI enhancement

Three pillars of diagnosis: physical exam, imaging, needle biopsy/cytology

When these are concordant, the risk of missing cancer is extremely low, but must do careful correlation.

General Risk factors:

Increased estrogen—seen with early menarche, fewer children, less lactation, and obesity.

Increased Alcohol

Pathogenesis/Molecular

Two main pathways separated by Estrogen Receptor (ER) status:

ER-Positive: ER+, HER2-, Diploid with specific chromosomal gains/losses (e.g., gain 1q, loss of 16q)→ usually low to intermediate-grade cancers

ER-Negative: ER-, HER2+/-, Aneuploid with complex karyotypes, Frequent TP53 mutations → frequently high-grade tumors with high proliferation

Both pathways show PIK3CA mutations, but it is more common in ER-positive tumors.

Molecular classification: (based on hierarchical cluster analysis of gene expression)

	Lumina A	Luminal B	HER2-Positive	Basal type
Percent of all tumors	~50%	~20%	~15%	~15%
Classic ER/HER2 status	ER+, HER2-	ER+, HER2-	ER-, HER2+	ER-, HER2-
Ki67	Low	Intermediate	High	High
Actual ER/HER2	ER+ HER2+			Triple Negative
Grade	Low High			
Recurrence Risk	Low, but long term High, but short term			
Therapies used	Hormone Rx		HER2 Rx	Chemotherapy

Note: Other molecular classifications exist and include additional/alternate groupings. This is just the most well-established frequently utilized.

General Considerations

Precursor lesions:

ER(+) cancers → FEA, ADH, Low-grade DCIS are non-obligate precursors

ER(-) cancers → Microglandular adenosis and High-grade DCIS are non-obligate precursors

Grading

Grade using the **Nottingham system** (see below) with its 3 characteristics.

Tubules formation: Assessed throughout the whole tumor at low magnification. Only structures with central lumina surrounded by polarized tumor cells are counted.

Nuclear pleomorphism: Assessed in the area showing the worst cytologic atypia

Mitotic count: Assessed in mitotic "hot spot." Remember to factor in your field area!

Feature	Score
Tubule formation	
Majority of tumor (>75%)	1
Moderate degree (10-75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small, regular, uniform (<1.5x the size of normal nucleus)	1
Moderate increase in size and variability (1.5-2x cell size)	2
Marked variation (>2x cell size) vesicular chromatin, often prominent nucleoli	3
Mitotic Count (<u>per 10 HPF</u>) Olympus, 10x oculars [most attending scopes], 40x objective	
0-8	1
9-17	2
≥18	3

Add the scores for gland formation, nuclear pleomorphism, and mitotic count:

Total Score	Final Grade
3-5	Grade 1
6 or 7	Grade 2
8 or 9	Grade 3

NOTE: Remember, the size of an HPF varies depending on your scope/magnification, so be sure to factor this in when counting mitoses!

Mitotic Count (per 10 HPF) Olympus, 15x oculars [most resident scopes], 40x objective

0-3	1
4-7	2
≥8	3

General Immunohistochemistry

Invasive cancers usually stain with low-molecular weight cytokeratins (including CK7 and CK19), EMA, and GATA-3.

Some cancers (often the well-differentiated ones) stain with GCDPF-15 (BRST2) and mammaglobin.

Some cancers (often the higher-grade triple-negative ones) stain with basal markers including high-molecular weight cytokeratins (e.g., CK5/6).

A subset of cancers (often metaplastic and/or triple negative) express S100, SOX10, and/or p63.

Is it invasive?

Invasive breast cancer is defined by the **absence of peripheral myoepithelial cells**.

Stains for myoepithelial cells (see below) should be employed as part of a **panel or cocktail** with at least one nuclear **and** one cytoplasmic stain (e.g., p63 and SMMHC).

However, do not rely solely on negative myoepithelial stains to diagnose invasion. The H&E findings must be concordant. Nests of in situ carcinoma may well be surrounded by reduced numbers of myoepithelial cells and those present may stain weakly.

Table 2. Summary of Commonly Used Myoepithelial Cell Markers

Markers	Clone	Pattern	Comments
p63	4A4	N	One of the most sensitive and specific myoepithelial cell markers, showing continuous dotlike pattern in normal ducts; focally discontinuous dotted line in in situ carcinomas; nonreactive or attenuated in invasive or papillary CAs; best used in combination with SMMHC or calponin
SMMHC	SMMS-1	C	More sensitive than p63; however, cross-reactivity with stromal myofibroblasts and vascular smooth muscle cells, although less-frequency than calponin; linear cytoplasmic with gaps in in situ CA
Calponin	EP798Y	C	A good myoepithelial cell marker with linear cytoplasmic pattern with gaps in in situ CAs; higher frequency of cross-reaction to stromal myofibroblasts and vascular smooth muscle cells; reactive to a small proportion of tumor cells
SMA	1A4	C	Sensitive but not specific myoepithelial marker; marked cross-reaction to stromal myofibroblasts and vascular smooth muscle cells
Maspin	G167-70	N, C	Very sensitive myoepithelial marker; no cross-reaction to stromal myofibroblasts or vascular smooth muscle cells; limited utility because of its frequent reactivity to tumor cells
CD10	56C6	C, M	A relatively sensitive myoepithelial marker; cross-reactivity to myofibroblasts; nonspecific reactivity to epithelial cells; no reactivity to vascular smooth muscle cells

Abbreviations: C, cytoplasmic; CA, carcinoma; CD10, cluster of differentiation 10; M, membranous; maspin, mammary serine protease inhibitor; N, nuclear; SMA, smooth muscle actin; SMMHC, smooth muscle myosin heavy chain.

From: Liu H. Application of Immunohistochemistry in Breast Pathology: A Review and Update. Archives of Pathology & Laboratory Medicine: December 2014, Vol. 138, No. 12, pp. 1629-1642.

Morphologic features of Cancer vs Mimics:

Feature	Invasive cancer	Complex sclerosing lesions	DCIS involving sclerosing adenosis
Stroma	Desmoplastic	Dense	Dense
Cytology	Atypical	Bland	Atypical
Gland profile	Angulated	Compressed	Solid/cribriform
Architecture	Infiltrative	Lobulated	Lobulated
Myoepithelial cells	Absent	Present	Present

Modified From: Peng et al. Update on Immunohistochemical Analysis in Breast Lesions. Archives of Pathology & Laboratory Medicine: August 2017, Vol. 141, No. 8, pp. 1033-1051.

Subtypes

Tumors showing a special histologic pattern in ≥90% of the tumor are designated as pure special tumor. Otherwise, they are designated as NST, which accounts for the majority of cases, including mixed patterns.

“No Special Type” (“NST”)

Older name: **Invasive Ductal Carcinoma (IDC)**;

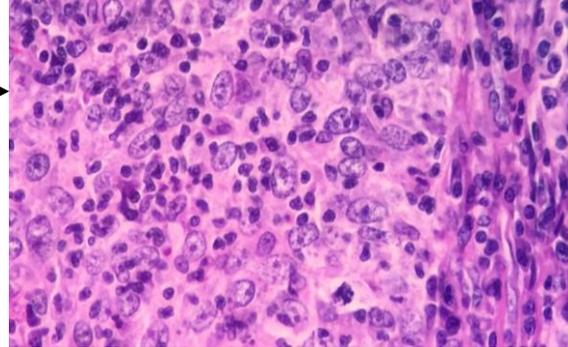
Now say: “Invasive Breast Carcinoma (IBC) of no special type (NST)”

A large and heterogeneous group that is essentially a “waste basket” including all cancers that don’t fit into one of the specific groups.

When a special type makes up 10-90% of tumor: report as “mixed” IBC-NST and special subtype

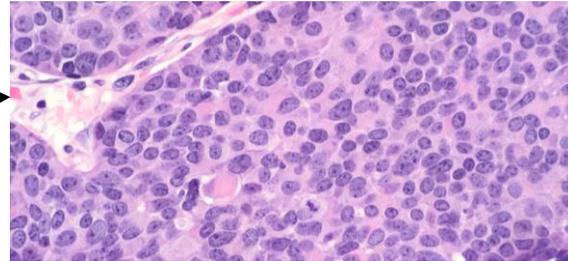
Special morphologic patterns (as opposed to subtypes):

Medullary pattern: Well-circumscribed, high-grade, pushing margins, syncytial architecture, and prominent tumor infiltrating lymphocytes (TIL). Better outcome than other stage-matched high-grade cancers (likely due to TILs). Usually triple-negative (Basal-like). Associated with BRCA1-related tumors.



Invasive carcinoma with Neuroendocrine differentiation:

Some degree of neuroendocrine differentiation by immunohistochemistry. Not currently of any clinical significance. More common in mucinous and solid papillary carcinomas. Must be sure to consider a much rarer primary neuroendocrine tumor/carcinoma and metastasis



Other rare subtypes: Carcinoma with Osteoclast-like giant cells, Pleomorphic pattern, Choriocarcinomatous pattern, Melanotic pattern, Oncocytic pattern, Lipid-rich pattern, Glycogen-rich clear cell pattern, and Sebaceous pattern

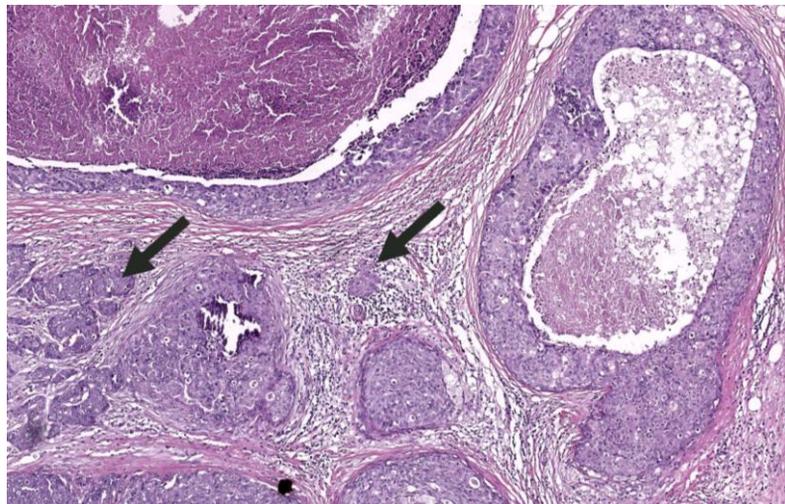
Microinvasive Carcinoma

Invasive breast carcinoma **≤1mm in size**
Usually **adjacent to an areas of DCIS**, often high-grade.

Earliest recognizable form of invasive carcinoma

- Invasion beyond myoepithelium
- Small, angulated clusters of tumor cells infiltrating stroma
- Often desmoplastic stromal changes

Better prognosis than larger invasive tumors



Often multifocal → if any single invasive focus is larger than 1 mm → invasive carcinoma (not micro)

Be cautious diagnosing this on core biopsy, as could be more invasion on excision. Often good to get levels to exclude larger foci of invasion

Invasive Lobular Carcinoma

Invasive breast carcinoma composed of **discohesive cells that are often individually dispersed or arranged in a single-file linear pattern.**

~10% of all invasive breast carcinomas

Most are Luminal A (**ER and PR positive**, HER2 negative)

CDH1 mutations → Loss of E-cadherin function → cellular discohesion.

Often little host reaction or disturbance of background architecture.

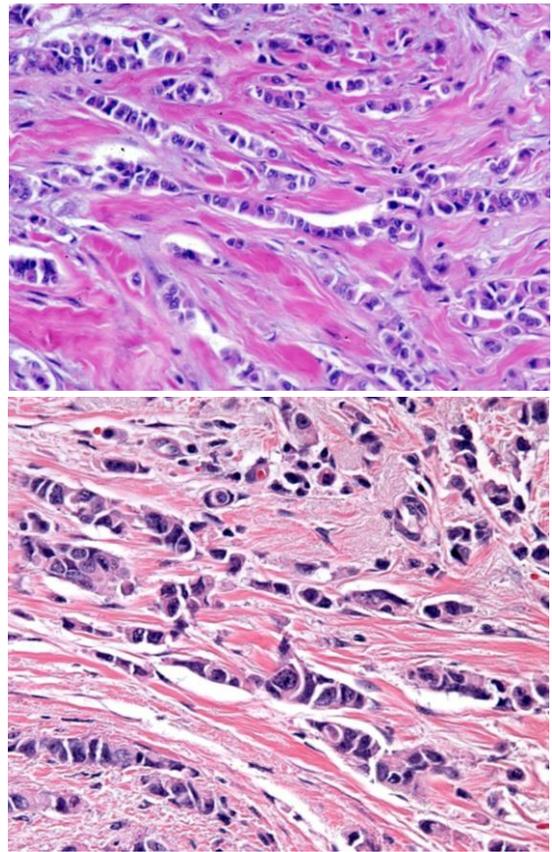
Occasional intracytoplasmic lumina.

Can have signet-ring cells.

Often low-grade nuclei.

Pleomorphic lobular carcinoma: Same discohesive growth, but with marked nuclear pleomorphism (>4x size of lymphocyte = high-grade DCIS cytology)

Immunohistochemical stains can confirm loss of E-cadherin and are therefore helpful in confirming the diagnosis, but morphology is most important



IHC Stain	Normal Epithelium	Lobular Carcinoma	No Special Type
E-Cadherin	Membrane staining	Negative	Membrane staining
P120 catenin	Membrane staining	Cytoplasmic	Membrane staining
β -catenin	Membrane staining	Absence of membrane staining	Membrane staining

Tubular Carcinoma

Low-grade invasive carcinoma composed of **well-formed tubules with open lumina lined by a single layer of neoplastic cells.**

Often old women. ~1.5% of Invasive carcinomas.

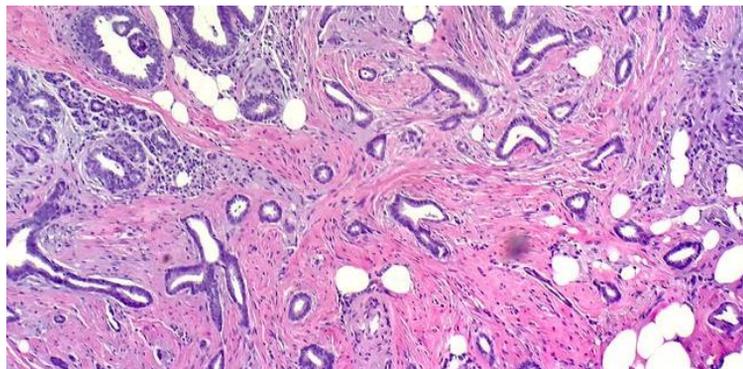
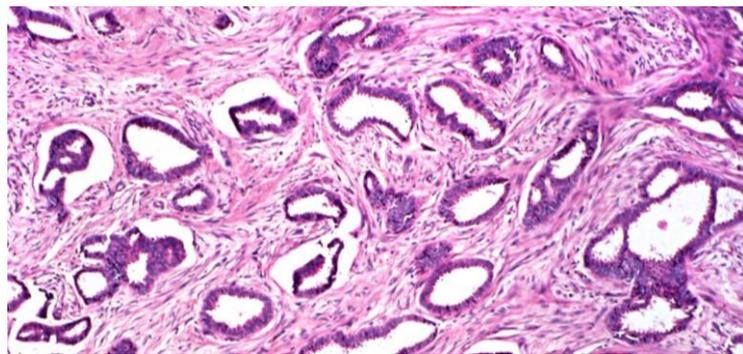
Luminal A subtype (ER and PR positive, HER negative)

Small, **angulated to ovoid glands** and tubules with open lumina set in fibrous, desmoplastic stroma.

Relatively **low-grade** nuclei.

>90% of tumor must have this morphology (as is the rule for all special types)

Good prognosis.



Cribriform Carcinoma

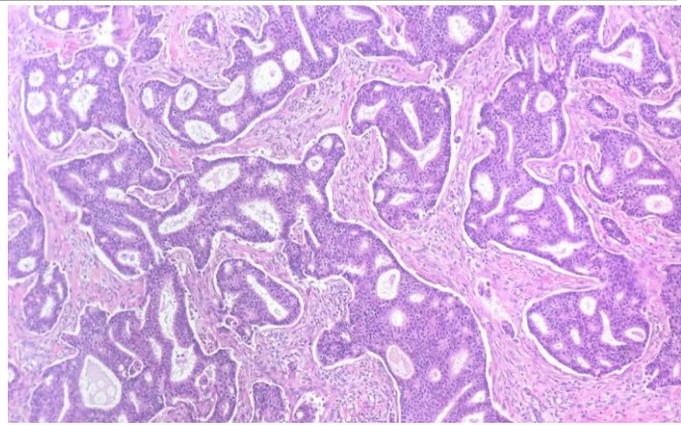
Low-grade invasive carcinoma composed of islands of tumor cells with **well-defined cribriform spaces**.

Luminal A subtype (ER and PR positive, HER negative)

Well-defined rounded to angulated cribriform spaces (like cribriform DCIS), but without surrounding myoepithelial cells, set in desmoplastic stroma.

Low nuclear grade.

Good prognosis. Very rare



Mucinous Carcinoma

Invasive breast cancer characterized by **clusters of epithelial cells suspended in pools of abundant extracellular mucin**.

Well-circumscribed grossly (mimicking benign process).

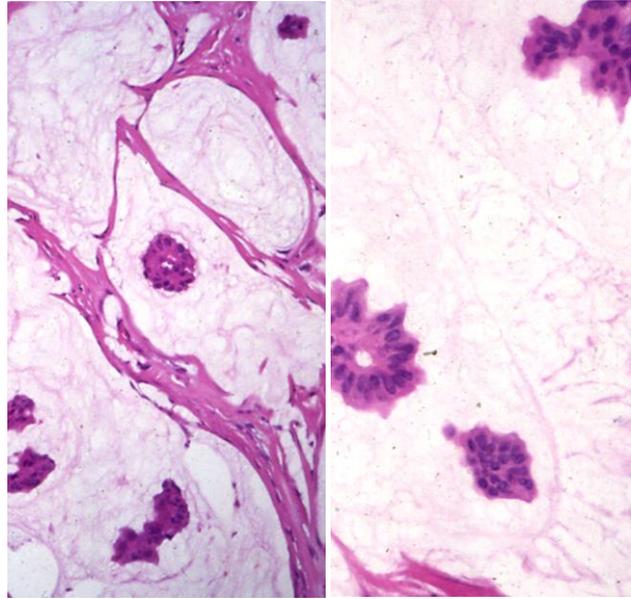
Uncommon. **Luminal A** molecular type (ER & PR +, HER -)

Low to intermediate nuclear grade.

Frequent neuroendocrine differentiation.

Good prognosis.

Mucinous cystadenocarcinoma: Invasive breast cancer characterized by cystic structures lined by tall columnar cells with intracytoplasmic and intracystic mucin, like pancreatic IPMNs or ovarian mucinous carcinomas



Invasive Micropapillary Carcinoma

Invasive breast carcinoma composed of **small, hollow, or morula-like clusters of malignant cells, surrounded by clear spaces with inside-out growth pattern**.

Pure form is uncommon, often mixed with other patterns.

Luminal A or B (ER and PR +, HER – usually)

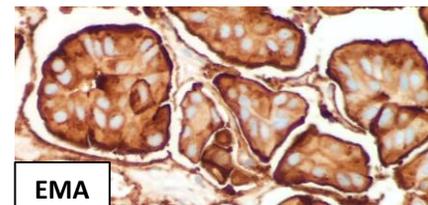
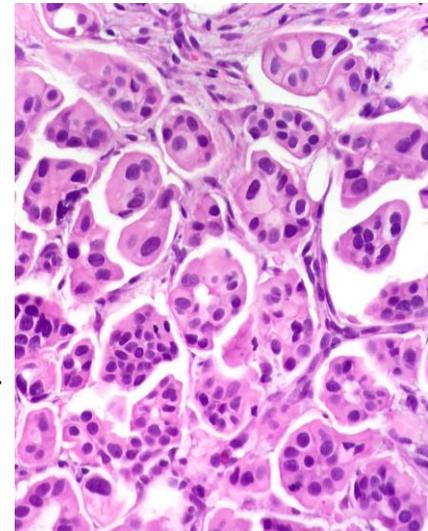
No fibrovascular cores (as is the case with all micropapillary tumors!)

Characteristic empty spaces around cells with delicate stromal framework.

Show **reverse polarity** → Apical surface faces outward stroma (can see on **EMA stain** where it stains the outside more strongly)

Often eosinophilic, granular cytoplasm and intermediate to high-grade nuclei. Cuboidal to columnar cells.

Significantly more lymphovascular invasion and positive lymph nodes, but when stage-matched with NST tumors, not significantly worse survival.



Metaplastic Carcinoma

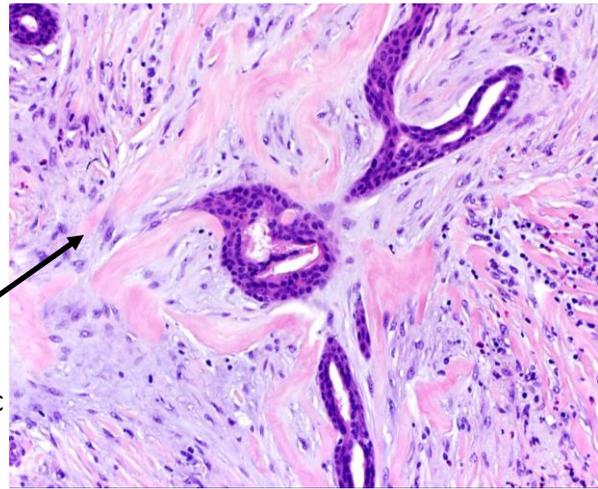
Invasive Breast Cancers with differentiation of epithelium towards squamous or mesenchymal-looking elements.

Usually present as a mass; **Rare**, <1% of all breast cancers.

Several distinct patterns (with some overlap, often mixed):

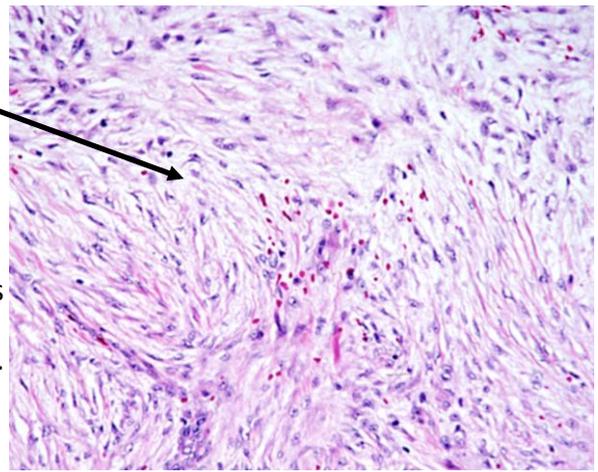
Low-grade Adenosquamous Carcinoma

Well-developed, rounded glands and tubules associated with solid squamous nests infiltrating through desmoplastic stroma. Sometimes associated “cannon ball” lymphoid aggregates. Good prognosis.



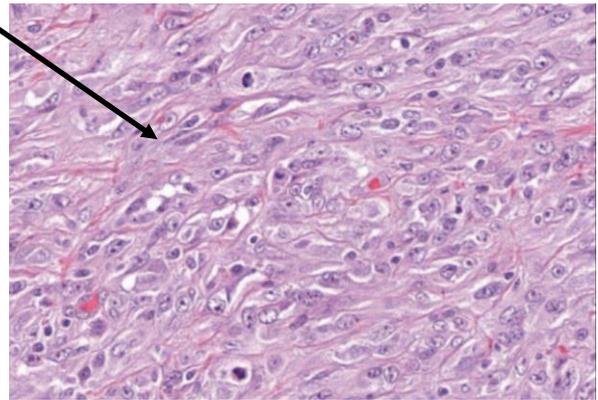
Fibromatosis-like Metaplastic Carcinoma

Bland spindled cells with pale eosinophilic cytoplasm and slender nuclei in stroma with variable collagen. Only mild nuclear atypia. Often arranged in fascicles. Some cells may be plumper/epithelioid. Good prognosis.



Spindle Cell Carcinoma

Atypical spindle cells with a variety of architectural patterns (e.g., fascicles, herringbone, etc...). Elongate to plump spindled cells with moderate to high-grade cytologic atypia. Often associated inflammation. Includes a spectrum of tumors from sarcomatoid SCC to myoepithelial carcinoma. Worse prognosis.

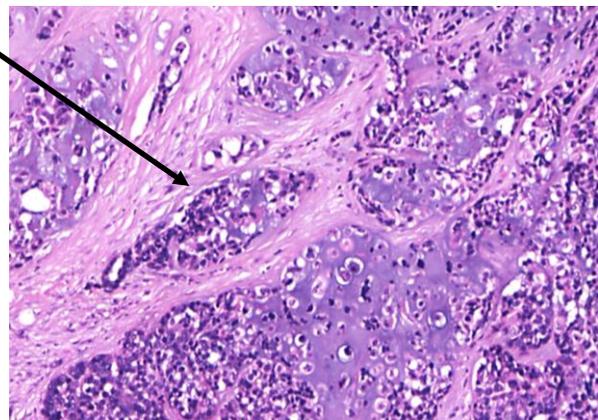


Squamous Cell Carcinoma

Pure squamous cell carcinoma. Often cystic. Must exclude a metastasis. Worse prognosis.

Metaplastic Carcinoma with Heterologous Mesenchymal Differentiation

Essentially a carcinosarcoma. Heterologous elements may include chondroid, osseous, and rhabdoid components. Epithelial and mesenchymal components can have variable atypia. Sometimes extensive sampling is necessary to find the epithelial component (and exclude a primary sarcoma).



IHC: Vast majority **do not express ER, PR, or HER2** (Triple Negative). However, they **do express some epithelial markers**:

(+) p63, HMWCKs (e.g., CK5/6), CK AE1/AE3

(-) CK7, CD34,

(+/-) SMA, CD10, Desmin, β -catenin

Molecular: Frequent TP53, PIK3CA, and WNT pathway mutations. May be derived from late dedifferentiation or basal-like stem cells.

Clinical: Much fewer LN metastases.

Carcinoma with Apocrine Differentiation

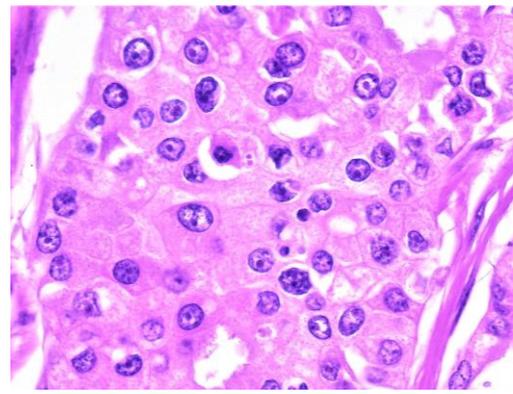
(sometimes just called "Apocrine Carcinoma")

An invasive carcinoma with large cells with abundant eosinophilic, granular cytoplasm and large nuclei with prominent nucleoli (resembling apocrine sweat glands)

Androgen receptor (AR)-positive; Often triple negative.

Often mostly solid growth with high mitotic index → Grade 2 or 3

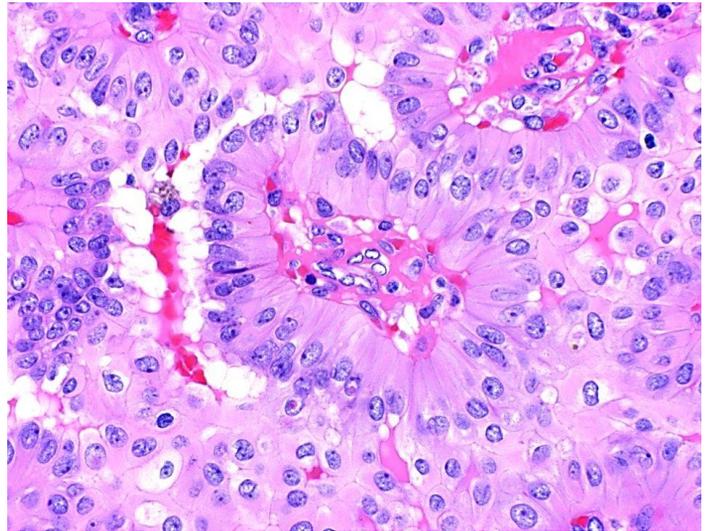
Favorable clinical prognosis. Often older women, low-stage. Androgen deprivation therapy a potential treatment option.



Rare Types

Tall Cell Carcinoma with Reverse Polarity:

Rare subtype of breast carcinoma with tall columnar cells with reverse nuclear polarity, arranged in solid and solid papillary patterns, most commonly associated with IDH2 mutations. (Resembles tall cell papillary thyroid carcinoma). Express both high and low molecular weight cytokeratins (e.g., CK7 + CK5/6) and caretinin. Triple negative. Indolent.



Salivary gland tumors

Most salivary gland tumors can occur in the breast, where there are usually relatively more indolent than their head and neck counterparts. They are often triple-negative.

Acinic Cell Carcinoma: Clear to granular epithelial cells containing zymogen granules arranged in glands and solid sheets. Triple negative. Intermediate behavior.

Adenoid Cystic Carcinoma: An invasive carcinoma composed of epithelial and myoepithelial cells arranged in tubules, cribriform, and solid patterns associated with basophilic matrix and basement membrane material. Frequent MYB-NFIB fusions. Triple negative, but generally good prognosis (unlike in head and neck), cured by surgery alone.

Secretory Carcinoma: Epithelial cells with intracytoplasmic secretory vacuoles and extracellular, eosinophilic, bubbly secretions, arranged in a variable architecture. Frequent ETV6-NTRK3 fusions. Triple negative. Generally indolent.

Mucoepidermoid Carcinoma: Composed of a mixture of 1) mucinous cells, 2) squamous cells, and 3) "intermediate" cells, arranged in a solid and cystic pattern. Frequent MAML2 fusions. Triple negative. Good prognosis if low-grade.

Polymorphous Adenocarcinoma: Monotonous neoplastic cells with a variety of architectures, including large nests surrounded by cords and single-file growth. Triple negative.

Familial Syndromes

Breast cancer shows more familial clustering than most tumors.

BRCA1/2

BRCA-genes are tumor suppressors involved in the homologous recombination repair pathway (repairs DNA breaks using sister chromatids as a template) → mutations in BRCA → genomic instability → oncogenesis

Highest risks: **Breast and ovarian cancer**

~3.5% of all breast cancers; More common in certain populations, like Ashkenazi Jews

Treatment: patients may opt for **prophylactic bilateral mastectomy and salpingo-oophorectomy**

before 40 yrs → Must submit entire FT and ovary looking for STIC

Carcinomas can be treated with **PARP inhibitors** (PARP helps with single-strand DNA breaks, so when combined with BRCA mutations → cancer cells can't repair breaks at all → "synthetic lethality")

Characteristic	BRCA1	BRCA2
Risk of breast cancer	40-90%	45-85%
Risk of ovarian/fallopian tube high-grade serous carcinoma	40-50%	10-20%
Male breast cancer risk	Lower	Higher
Other Cancer risk	Possibly pancreatic and colon	Pancreatic cancer, prostate cancer
Morphology	Circumscribed growth pattern with pushing borders, dense lymphocytic infiltrate. High-grade.	Variable morphology and grade
Molecular cancer type	Basal-like (triple-negative)	Luminal A (ER/PR +; HER2 -)

Li Fraumeni Syndrome

TP53-associated:

Autosomal dominant TP53 mutation (one of the most prominent tumor suppressors)

Early onset of a broad spectrum of cancers. Most common is breast (>90% lifetime risk), but also soft tissue, brain (esp. choroid plexus carcinoma), adrenal cortical, bone, etc...

CHEK2-associated:

Germline mutations in CHECK2, moderately penetrant. CHECK2 is a tumor suppressor activated by double strand DNA breaks (upstream of TP53 and BRCA1). Mutation → disrupt DNA repair → more errors → carcinogenesis. ~30% lifetime risk of breast cancer. Also increased risk of a variety of cancers.

Peutz-Jeghers Syndrome

Autosomal dominant polyp and cancer syndrome. Germline mutations in tumor suppressor STK11.

Characteristic hamartomatous polyps in >95% of patients, often in small bowel.

Also frequent mucocutaneous melanin pigmentation.

Increased risk of many cancers including Breast, colon, stomach, pancreas, ovary (SCTATs), etc...

CDH1-associated Breast Cancer

Inactivating germline mutations in **CDH1 (gene for E-cadherin)** resulting in characteristic **lobular carcinoma** of the breast.

Most (but not all) CDH1 mutations are associated **with Hereditary diffuse gastric cancer (HDGC)**, which also has germline mutations in CDH1 and can have lobular carcinoma of the breast also.

E-cadherin is important for cell adhesion and tumor suppression

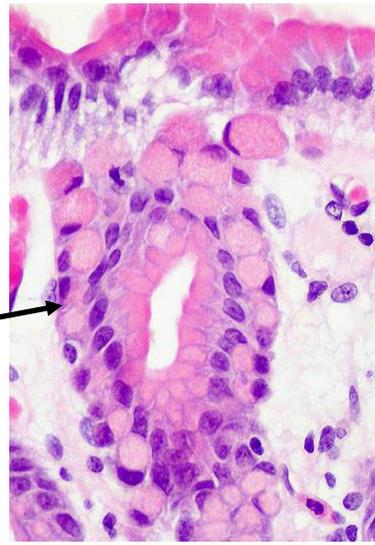
Classic HDGC finding: Signet ring carcinoma in situ

Signet ring cells above basement membrane

Pagetoid spread

Can then progress to invasive, diffuse gastric cancer

Often Multifocal



Ataxia-Telangiectasia

Autosomal recessive disorder with progressive cerebellar ataxia, oculocutaneous telangiectasia, variable immunodeficiency, sterility, and sinopulmonary infections.

Mutations in ATM gene (tumor suppressor → phosphorylates p53 and BRCA1 in response to DNA double-strand breaks

High risk of malignancy and sensitivity to ionizing radiation

Homozygotes have full disorder

Heterozygotes have a risk of breast cancer at a young age.

COWden's Syndrome

PTEN mutation. Autosomal dominant

Tumor suppressor → lots of different tumors

Other PTEN syndromes include: Bannayan-Riley

Ruvalcaba syndrome and Lhermitte-Duclos disease

At risk for:

Breast Cancer (*highest risk*)

Multiple **hamartomas** (mouth, GI tract)

Thyroid carcinoma (usually Follicular)

Endometrial Cancer

Trichile**MMOOOOO**mas

Lipomas

Esophagus: Glycogen acanthosis

Stomach: Polyps that often resemble **HP's**

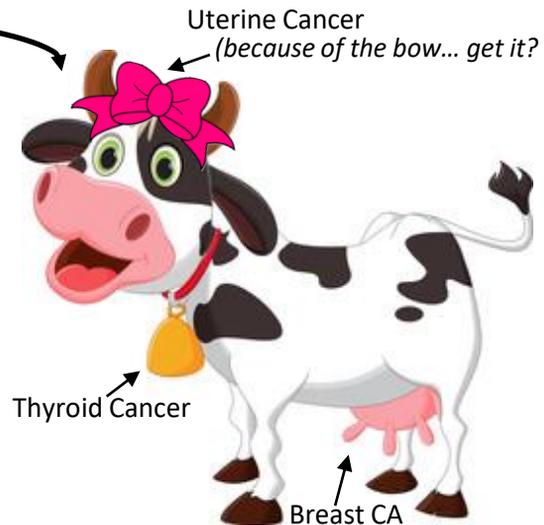
Colon: **Stroma-rich polyps** with cystically dilated glands

Can mimic JP's.

Can contain **Adipocytes** in lamina propria (relatively unique)

Can get **ganlgioneuromatous** polyps

Think of this cow



Prognostic Markers Testing

Estrogen Receptor (ER)

Step 1: Checklist for initial quality control*

- The sample is adequate for biomarker testing:
Receptor testing should not be interpreted on any specimen that has insufficient invasive cancer for interpretation or severe processing artifacts
- External and internal controls (if present) stain appropriately
If controls are not working as expected, the test should not be reported until the issue has been addressed
- Preanalytic variables (fixative type, time to fixation, time in fixation) are documented
If this information is not available to the laboratory, a comment should be added to the report that the results should be interpreted with caution

Step 2: Evaluate percentage of cancer cells staining and stain intensity

≤ 10% of cells staining OR intensity is weak

> 10% of cells staining AND intensity is moderate or strong

If result considered concordant with histology (Table 3)

Report as ER Positive

Take steps to confirm/ adjudicate result per lab-specific SOP* and correlate with histology (Table 3)

Steps to consider including in SOP (Supplement Figure 1):

- Re-review of controls
- A second reviewer to confirm interpretation
- Validated quantitative digital image analysis to confirm interpretation
- Comparison of result with any prior patient-specific results
- Retesting the same sample if analytic issues suspected (e.g., controls did not work as expected)
- Repeating the test on a different block or subsequent specimen if there are no internal controls, preanalytic issues are suspected, or result is unusual or unexpected

< 1% of cells staining

1%-100% of cells staining

Report as ER Negative
(reported data elements should include status of control[†])

ER Positive

1%-10% of cells staining

> 10% of cells staining (but weak)

Report as ER Low Positive
and add recommended comment[‡] (reported data elements should include percentage of cells staining, intensity, and status of control[†])

Report as: ER Positive
(reported data elements should include percentage of cells staining and intensity)

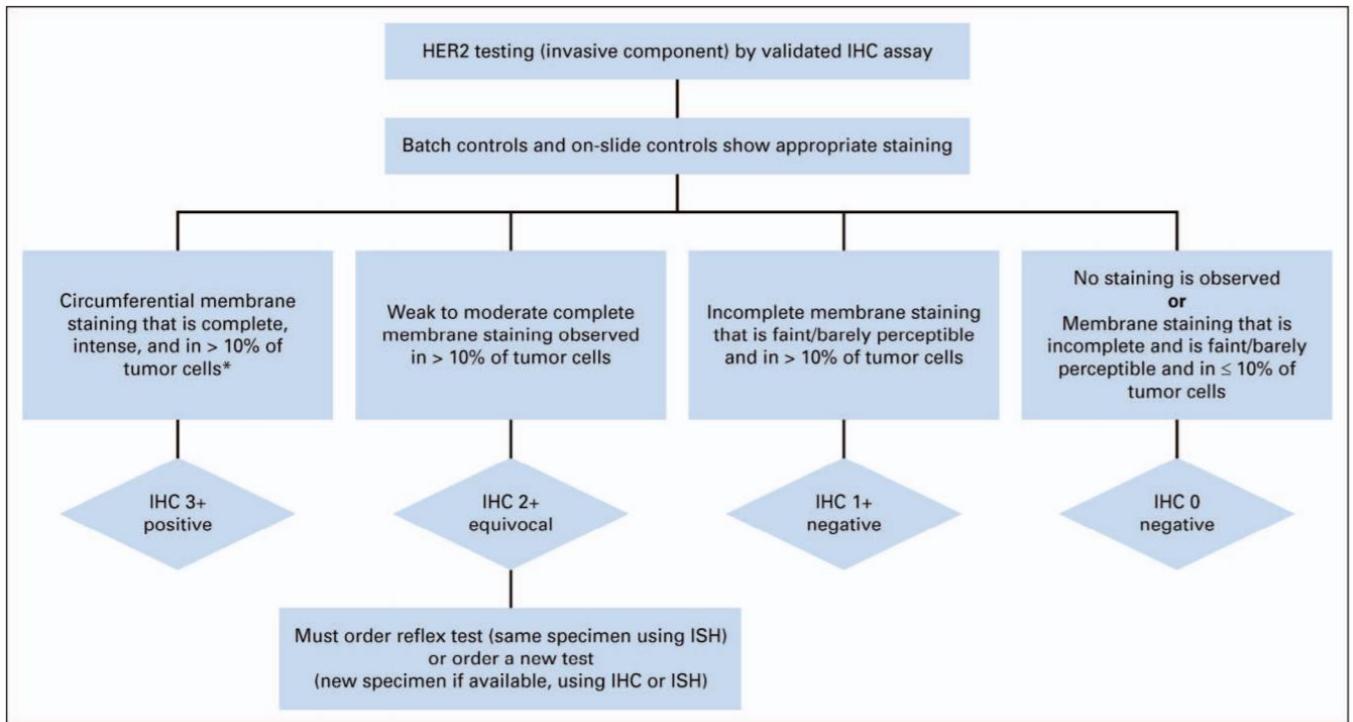
Table 3. Invasive Breast Cancer Histopathologic Concordance With ER Staining

Highly Unusual ER-Negative Results	Highly Unusual ER-Positive Results
Low-grade invasive carcinomas of no special type (also known as invasive ductal carcinoma)	Metaplastic carcinomas of all subtypes
Lobular carcinomas (classic type)	Adenoid cystic carcinomas and other salivary gland-like carcinomas of the breast
Pure tubular, cribriform, or mucinous carcinomas	Secretory carcinoma
Encapsulated papillary and solid papillary carcinomas	Carcinomas with apocrine differentiation

NOTE. If a result is considered highly unusual/discordant, additional steps should be taken to check the accuracy of the histologic type or grade as well as the preanalytic and analytic testing factors. This workup may include second reviews and repeat testing. If all results appear valid, the result can be reported with a comment noting that the findings are highly unusual and testing of additional samples may be of value to confirm the findings.

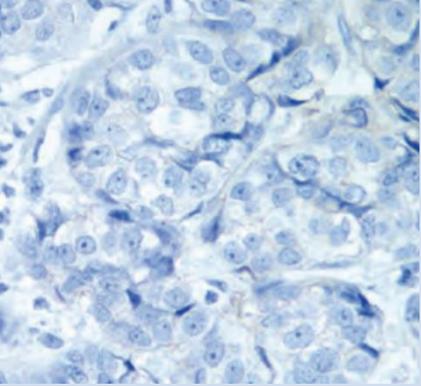
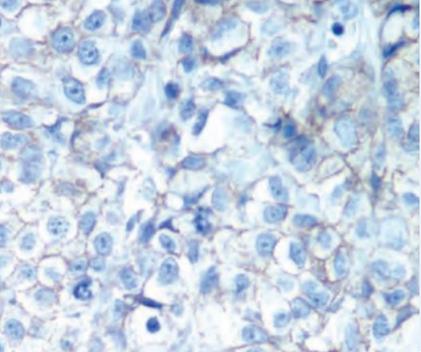
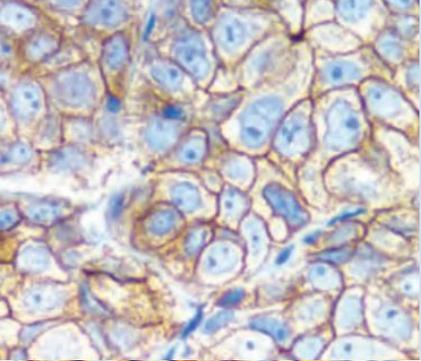
From: Allison KH et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. Arch Pathol Lab Med. 2020 May;144(5):545-563.

HER2



Wolf et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med. 2018 Nov;142(11):1364-1382.

HER2 Grading:

Score	Interpretation	Staining Pattern	Think	
0	Negative	<u>No staining</u> is observed, or membrane staining is observed in <10% of tumor cells	Essentially noting, like an eraser 	
1+	Negative	A <u>faint/barely perceptible membrane staining</u> is detected in >10% of tumor cells. The cells exhibit incomplete membrane staining	Slight pencil tracing 	
2+	Equivocal (order FISH)	A <u>weak to moderate complete, circumferential membrane staining</u> is observed in >10% of tumor cells.	Ballpoint pen 	
3+	Positive	A <u>strong complete membrane staining</u> is observed in >10% of tumor cells.	Sharpie marker 	