Human Genetics
434 team

MED 434 ICINE

Lecture 2:

Genetic of breast cencer

دعاء قبل المذاكرة
(اللهم إني أسألك فهم النبيين و حفظ المرسلين و الملائكة المقربين، اللهم اجعل ألسنتنا عامرة بذكرك و قلوبنا بخشيتك، إنك على كل شيء قدير و حسبنا الله و نعم الوكيل)

Lecture Objectives:

Understand that 5 to 10% of breast cancers are related to specific inherited gene mutations.

Is aware of the factors which affect the prognosis of breast cancer like the morphology of the tumour and the status of steroid hormone receptors including estrogen and progesterone.

Understand the function and prognosis significant of the HER2 gene on the chromosome 17.

Is aware that Herceptin is the drug used against HER2 positive breast tumour cells.

Introduction

- Carcinoma of the breast is one of the leading causes of cancer morbidity and mortality among women worldwide.
- In the USA alone, , there are more than 200,000 newly diagnosed cases of invasive breast cancer and in excess of 40,000 cancer-related deaths each year.
- When a new diagnosis of breast cancer is made, the most immediate issues for each patient involve :

1- What the diagnosis means for her future, whether or not she will survive.

2- Whether therapies beyond primary surgery might be of additional benefit.

- There has been an encouraging decline in mortality from breast cancer over the past years, which can be attributed to several factors like :

1- Largely related to public education.

2- Screening programs that lead to the discovery of the disease at an earlier and more treatable stage.

- In addition, there have been several significant and important treatment advances with **improvements in hormonal therapies**, the development of more effective combination chemotherapy regimens and the development of biologic therapeutics such as the targeted therapy against the human epidermal growth factor receptor 2(HER2) receptor tyrosine kinase.
- This evolution of therapeutic modalities for breast cancer has yielded an increasingly complex array of treatment options, both local and systemic sitating the development of some rational way of stratifying patients as to the most appropriate treatment regimen based on an assessment of the likelihood for disease recurrence after completion of local-regional therapy.

Generic and family history of breast cancer

- About 5% to 10% of breast cancers are related to specific inherited mutations.
- Women are more likely to carry a breast cancer susceptibility gene if they develop breast cancer **before** menopause, have bilateral cancer, have other associated cancers (e.g., ovarian cancer), have a significant family history (i.e., multiple relatives affected before menopause), or belong to certain ethnic groups.
- About half of women with hereditary breast cancer have mutations in gene BRCA1(on chromosome 17q21.3), and an additional one-third have mutations in BRCA2(on chromosome 13q12-13).
- Although their exact role in carcinogenesis and their relative specificity for breast cancer are still being elucidated, **both of these genes are thought to function in DNA repair**.
- They act as tumor suppressor genes, since cancer arises when both alleles are inactive or defective - one caused by a germ-line mutation and the second by a subsequent somatic mutation.
- However, most carriers "70-80%" of the mutations will develop breast cancer by the age of 70 years (postmenopause), as compared with only "7-8%" of women who do not carry a mutation and might develop breast cancer by the age of 70.
- The role of these genes in nonhereditary sporadic breast cancer is less clear, because mutations affecting BRCA1 and BRCA2 are infrequent in these tumors.
- Less common genetic diseases associated with breast cancer are :
- 1- the Li-Fraumeni syndrome(caused by germ-line mutations in p53).
- 2- Cowden disease(caused by germ-line mutations in PTEN).
- 3- Carriers of the ataxia-telangiectasia gene.

Recall:

- DNA → RNA→ Protein →normal cell function.
- The cell is protected by genes that encode protein which repair the damaged DNA or suppress the oncogenes. When there is a mutation in these proteins tumor my develop because the protection is impaired.

Traditional morphologic prognostic factors:

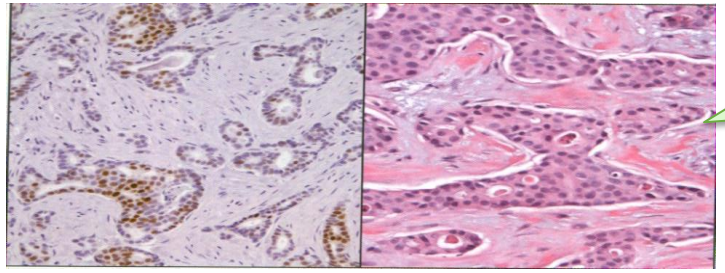
A major challenge in the treatment of breast cancer is to identify those patients more likely to develop recurrence so that the most appropriate therapy can be provided.

The validated pathologic metric that have been demonstrated to provide clinically useful prognostic information in breast cancer include:

- **Tumor size**
- **Histologic type**
- **Evidence of vascular or lymphatic invasion**
- **Tumor grade**
- **Lymph node staging**

Hormone receptors

- The first of the prognostic and predictive biomarkers in breast cancer to enter routine clinical use.
- 60% to 70% of breast carcinomas express estrogen receptors (ERs) and progesterone receptors (PRs).
- tumors that express these receptors depend on estrogen, progesterone, or both for growth
- the ER became the first target for either treatment by therapeutic hormonal manipulations with ER antagonists such as **tamoxifen** or treatment with **aromatase inhibitors**, which will decrease the local concentrations of estrogen within the tumor microenvironment of mammary tissue or within metastatic deposits.
- The presence of ERs in breast cancer is a weak prognostic factor; however, it is optimally useful as a predictive factor for the benefit of adjuvant (additional or supportive) tamoxifen or aromatase inhibitors therapy.



If I see the positive staining (brown color) in 30% of tumor cell the tumor is estrogen positive, And they need to treat her with Tamoxifen also for progesterone.
And there is no need to remove the adrenal or ovaries [To decrease the amount of Estrogen].
Usually these Estrogen and Progesterone tumors have better prognosis than the others.

Molecular prognostic predictive factors HER2

-Normal cells have one copy of the *HER2* gene on each chromosome 17 (CHRl 7), it transmits signals regulating cell growth and survival.

-In approximately 15 to 25% of breast cancers , the HER2 gene is found to be amplified.

-this amplification drives gene expression, generating up to 100 times the normal number of *HER2* receptor proteins at the cell surface.

-*HER2*- positive breast cancer is significantly correlated with several unfavorable pathologic tumor characteristics including: larger tumor size, positive axillary nodes, higher nuclear grade, higher proliferative index

-*HER2* over expression may have a predictive role for response to adjuvant chemotherapy and endocrine therapy.

-The Herceptin molecule (Herceptin is the drug used against H2r2 positive tumour cells) has been shown to demonstrate a high specificity and affinity for the HER2 protein .

-in preclinical studies was shown to be most effective against tumor cells with *HER2* over expression. The therapeutic efficacy and tolerability of Herceptin therapy has been investigated in several clinical trials, and this drug has proved to be a remarkably effective therapeutic agent in both the metastatic and, more recently, the adjuvant setting, particularly in combination with cytotoxic chemotherapy.

Demonstration of HER2 neu receptors can be done by using the following techniques:-

1-Immunohistochemistry antibodies against Her1 receptors are applied to the tissue and if the antigen (HER2) is present a reaction is visualized by means of a dye or a color producing enzyme, which is used to label antibody.

2-Fluorescent or silver in situ hybridization (FISH or SISH)

Immunohistochemistry of(anti HER2)

o\+1

- Counseling.
- Herceptin is not required neither effective.
- If estrogen (+) and show (0) result on Immunohistochemistry reaction it is luminal A.(tamoxifen)

+2 border line

Require more sensitive testing method→ (FISH)

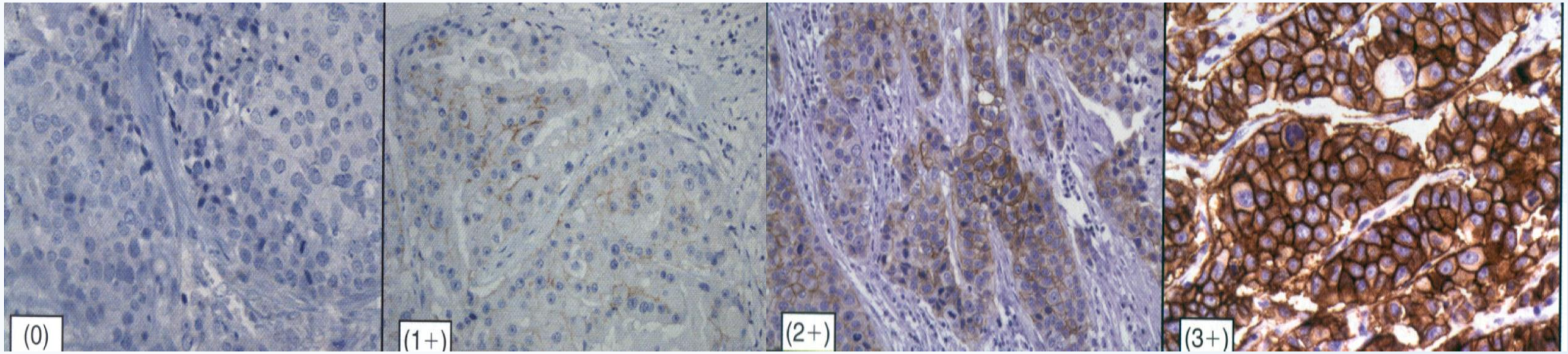
- If (FISH) results were **negative** for HER2.it is just as 0\+1

- If the results of (FISH) were **positive** .it is just as +3.

+3

- It responses to **Herceptin**
- Most likely :
- Estrogen (-) , HER(+) → luminal C.

- HER2 cancers is the most aggressive technically , Herceptin is very specific and can give the patient better prognosis compared to the the type which has no effective treatment,



- Immunohistochemistry (antibody HER2/neu) is incubated with breast cancer and look for change in the color of the membrane because epidermal growth factor act on those receptors located in the cytoplasm and stimulate the propagation (انتشار)
  - If at least 10% of tumor cells positive and complete membrane stain so we can use Herceptin.
  - Patient(0): negative HER2/neu, she will have no benefit from Herceptin, most likely have estrogen-progesterone receptors positive and Herceptin negative.”Luminal A only tamoxifen”
  - Patient(+1): negative HER2/neu, she has receptors but it is scattered and not complete(a little brown) “need to be at least 10% of the cells”
  - Patient(+2): may be positive may be negative HER2/neu, some cells are positive but nucleur staining is not complete, we need to do more tests”FISH staining”
  - Patient(+3): positive HER2/neu, give Herceptin, most likely estrogen-progesterone receptors negative and Herceptin positive”Luminal C”

Fluorescent or silver in situ hybridization(FISH or SISH)

- In FISH, fluorescently tagged DNA or RNA probes are used to identify genomic sequences of interest.
- FISH may be used to identify sequences of interest in tissue sections, an advantage that permits correlation of probe hybridization with tissue morphology.
- When coupled to conventional cytogenetics, FISH provides high resolution for identification of specific abnormalities, e.g., gene amplification, deletions, and translocations.
- FISH requires denaturation (transfer double stranded DNA into single stranded DNA), hybridization with a probe, and washing.
- First, a probe specific for the target of interest (eg. Cancer cells) is applied to the slide, along with a nuclear counterstain and reagents or heat that enhance denaturation of target DNA and reduce background. The slides are sealed and incubated in a humid environment under conditions that denature the DNA, allowing hybridization to occur between the probe and its cDNA sequence. The unbound probe is then removed by washing, and patterns of fluorescence are interpreted by fluorescence microscopy.

In another words we take a sample from the breast tissue and we do denaturation (by Heating or Alkalinizing substance and the results is separation of the strands of DNA) and hybridization with probes (strands) and these strands have marker for HER2/neu gene on chromosome 17 and we see these markers on adding fluorescence, and if HER2/neu amplified it will bind with it and produce a light which I can see in the Immunofluorescence

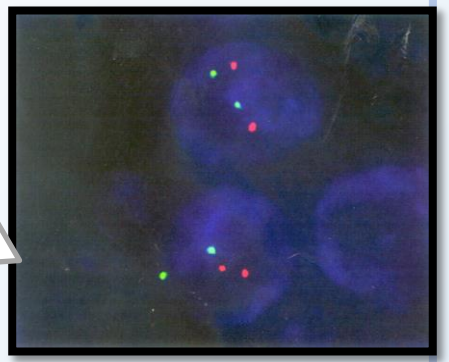
Principle of hybridization

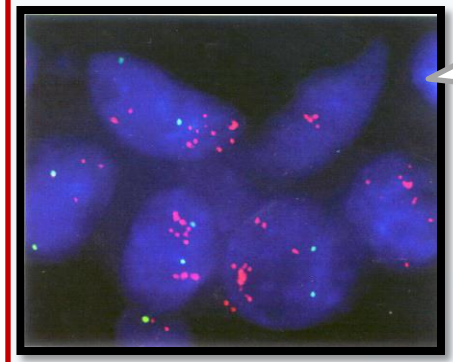
- DNA is double stranded.
- Bonds between complementary bases hold stands together
- (Cytosine ⟷ Guanine;

Adenine ⟷ Thymine).

- Heat/alkalinize DNA – separation of strands ('denaturation') occurs.
- Cool separated strands – complementary double strands re-form.
- Labelled complementary single-strand DNA can identify a DNA sequence (e.g. a gene) in intact cells or disrupted cell preparations.

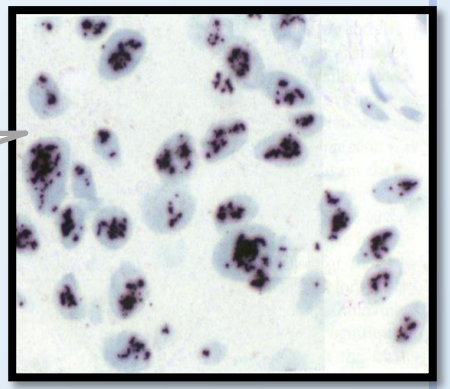
This patient doesn't have HER2/neu gene mutation and amplification. Either Luminal A or D
Only Treated with Tamoxifen
HER2/neu gene Red
Chromosome 17 Green.

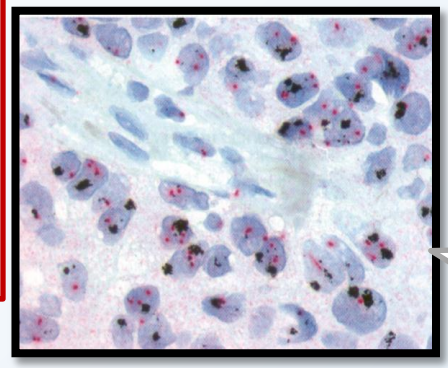




This patient has HER2/neu gene mutation and amplification. She has >2 Copies of HER2/neu
She most likely has lymph node involvement and metastasis, because this tumor is aggressive
Treated with Herceptin

Dual-color silver in situ hybridization (SISH) image.





This is SISH (silver in situ hybridization) here instead of marking the strands with fluorescence; I mark them with silver nitrate. Also I use the common microscope instead of Immunofluorescence microscope.

Classification of breast cancer:

• Gene expression profiling separates breast cancer into four molecular subtypes:

1-Luminal A: (Estrogen receptor-positive, HER2/NEU-negative).

2-Luminal B: (Estrogen receptor-positive, HER2/NEU Overexpressing).

3-HER2/NEU positive: (Estrogen receptor-negative, HER2/NEU Overexpressing).

3-Basal-like: (Estrogen receptor-negative, HER2/NEU-negative).

• Medullary carcinoma, a rare subtype of invasive ductal carcinoma is often associated with the following:

1-BRCA 1 mutations.

2-Lacks the estrogen and progesterone receptors and do not overexpress HER2/NEU, a combination that is often referred as **triple-negative**.

Prognosis of breast cancer is influenced by:

•Tumor invasion and size. In situ carcinomas carry an excellent prognosis, as do invasive carcinomas LESS than 2 cm in size.

•Extent of lymph node involvement.

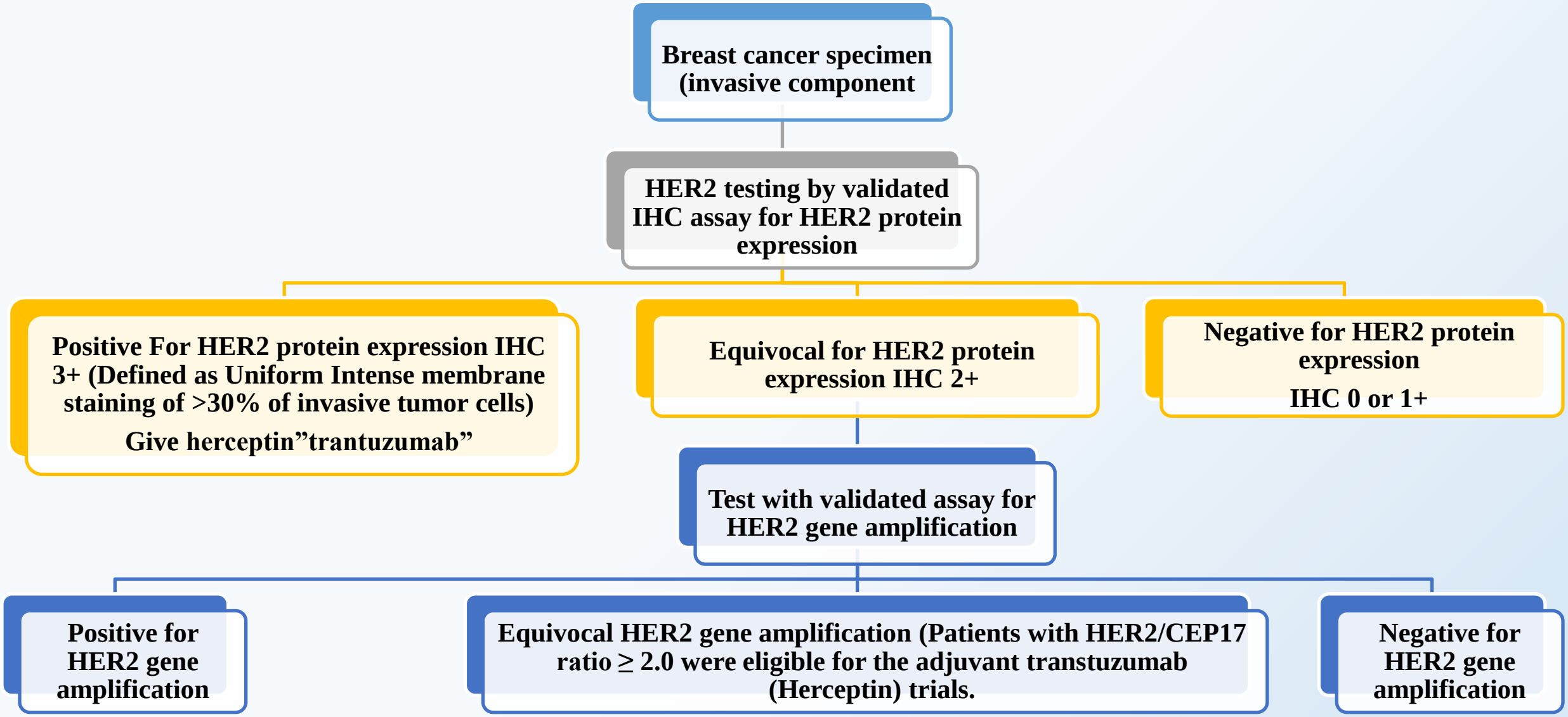
•Distant metastases.

•Histologic grade. Well-differentiated carcinomas are associated with better prognosis.

•The presence or absence of estrogen or progesterone receptors. The presence confers a slightly better prognosis.

•Overexpression of HER2/NEU. Overexpression is associated with a poorer prognosis.

ASCO/CAP Guideline recommendations for the optimal algorithm for HER2 testing by IHC



1- Which one of the following is an estrogen receptor antagonist?

a. Trastuzumab (Herceptin).

b. Tamoxifen.

c. Mifepristone.

d. Anastrozole.

2- On which chromosome does the BRCA 1 mutation gene resides?

a. 13.

b. 10.

c. 21.

d. 17.

3- On which chromosome does the HER2 gene resides?

a. 13.

b. 10.

c. 21.

d. 17.

4- What do you call a breast cancer that's ER(+) and HER2(+) ?

a. Luminal A.

b. Luminal B.

c. Basal-Like.

1-List two laboratory techniques that are used to detect HER2/NEU Overexpression?

a.Immunohistochemistry antibodies against HER2 receptors.

b.Fluorescent or silver in situ hybridization (FISH or SISH).

2-List two known drugs that are used in some types of breast cancer?

a.Trastuzumab (Herceptin). b.Tamoxifen.

3-List the four molecular subtypes in breast cancer?

a.Luminal A. b.Luminal B. c.HER2/NEU positive.

d.Basal-like.

Ans: 1-B 2-D 3-D 4-B

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(اللهم إني استودعتك ما قرأت وما حفظت وما تعلمت فرده لي عند حاجتي إليه إنك على كل شيء قدير وحسبنا الله و نعم الوكيل)