GENETICS Team

Genetics of Breast Cancer

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Red = Important **Green = Extra information** **Blue= Doctor**

Objectives:

At the end of this lecture, the student should be able to:

a. Understands that 5 to 10% of breast cancers are related to specific inherited gene mutations.
b. Is aware of the factors which affect the prognosis of breast cancer like the morphology of the tumor and the status of steroid hormone receptors including estrogen and progesterone.
c. Understand the function and prognostic significance of the HER2 gene on chromosome 17.
d. Is aware that Herceptin is the drug used against HER2 positive breast tumor cells.

Introduction:

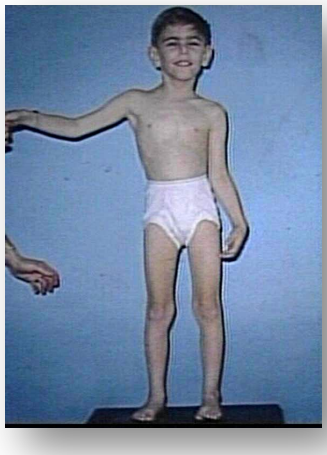
Carcinoma of the breast is one of the leading causes of cancer morbidity and mortality among women worldwide. In the United States alone, there are more than 200,000 newly diagnosed cases of invasive breast cancer and in excess of 40,000 cancer-related deaths each year. When a new diagnosis of breast cancer is made, the most immediate issues for each patient involve what the diagnosis means for her future, whether or not she will survive, and 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, likely largely related to public education and 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, necessitating 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.
Generics 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.

The age of 70 years, as compared with only 7% of women who do not carry a mutation. 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 the Li-Fraumeni syndrome (caused by germ-line mutations in **p53**; Cowden disease (caused by germ-line mutations in PTEN) and carriers of **the ataxia-telangiectasia gene.**

Ataxia-telangiectasia :- DNA breakage syndrome

patients with this syndrome have CNS problems and may develop breast cancer and lymphoma.



Ataxia telangiectasia (A-T) (also referred to as **Louis–Bar syndrome**) is a rare, neurodegenerative, inherited disease causing severe disability. Ataxia refers to poor coordination and telangiectasia to small dilated blood vessels, both of which are hallmarks of the disease.
It affects many parts of the body:

- It impairs certain areas of the brain including the cerebellum, causing difficulty with movement and coordination.
- It weakens the immune system causing a predisposition to infection.
- It prevents repair of broken DNA, increasing the risk of cancer. Which is why it is involved here.

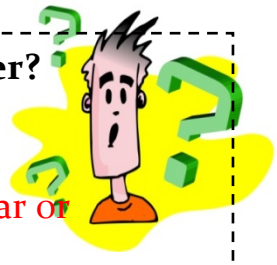
Predisposing factors:

- ✓ Age over 50.
- ✓ This is in general but might happen in younger age.
- ✓ More common in nulliparous women.
- ✓ Postmenopausal women are more prone to develop breast cancer than premenopausal women.
- ✓ Family history (more strong family history in first degree relatives. They have increase incidence for developing cancer)
- ✓ They are more prone to breast cancer if one of their relatives (1st degree relatives) had a breast cancer before age of 50 (premenopausal) and was bilateral.
- ✓ Proliferative breast diseases
- ✓ (Hyperplasia might progress to a breast cancer in 10%)

ASSESSMENT OF BREAST CANCER

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, tumor grade, lymph node staging, and evidence of vascular Or lymphatic invasion.



What should be done after receiving a biopsy of breast cancer?

1- Diagnosis (histological type)
2- Prognostic indicators (Grade and stage) and evidence of vascular or lymphatic invasion.

- The grade is related to the degree of differentiation of the cells, it is done according to a score (Grade 1, 2 and 3 or well, moderate and poorly differentiated), Women with poorly differentiated cancer are likely to have more aggressive breast cancer.
- Stage depends on 3 things (tumor size, lymph nodes and the presence or absence of metastasis)
- Breast cancer usually goes to axillary and mediastinal lymph nodes.

Three things determine grade of a tumor :-

1-Tubular differentiation: increase tubular differentiation > well differentiated.

2-pleomorphism: (variation in size and shapes) increase pleomorphism > poorly differentiated.

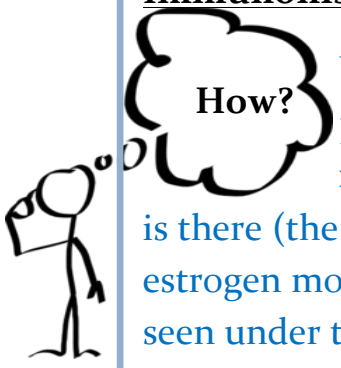
3-mitosis: increase mitosis > poorly differentiated.

Tumor size range between 2-5 cm

Hormone Receptors

The first of the prognostic and predictive biomarkers in breast cancer to enter routine clinical use, **the steroid hormone receptors** are in fact not new. It has been known for more than a century that oophorectomy increases the survival for some patients with advanced breast cancer. In addition, it has been known for some time that around 60% to 70% of breast carcinomas express estrogen receptors (ERs) and progesterone receptors (PRs). Furthermore, tumors that express these receptors depend on estrogen, progesterone, or both for growth. Thus, 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.

We can see estrogen and progesterone receptors by **Immunohistochemstry**



We do Immunohistochemistry for estrogen and progesterone (**it depends on antigen antibody reaction**) if the reaction was there, it means the antigen is there (the antigen in this case is the estrogen receptor, or the estrogen molecules which encode for this receptor). For this to be seen under the microscope; it should be labeled by a dye which will stain **brown** in estrogen positive tumor cell. In this case, the patient will benefit from ***anti-estrogenic drugs*** such as ***Tamoxifen*** and ***aromatase inhibitors***.

Molecular Prognostic and Predictive Factors HER2

The next major advance in the evolving role of prognostic and predictive markers in the diagnosis and therapeutic decision making for breast cancer came with the discovery of the importance of the *HER2* receptor tyrosine kinase in the biology and the clinical course of disease in breast cancer. **Normal cells have one copy of the *HER2* gene on each chromosome 17 (CHR17)**, and when this gene is expressed in normal breast epithelial cells, it **transmits signals regulating cell growth and survival**. In approximately **15% to 25% of breast cancers, the *HER2* gene is found to be amplified 2-fold to greater than 20-fold in each tumor cell nucleus relative to CHR17**, and 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, and higher proliferative index. In addition to the prognostic significance, retrospective studies have suggested that 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 tumor cells) has been shown to demonstrate a high specificity and affinity for the HER2 protein and 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.

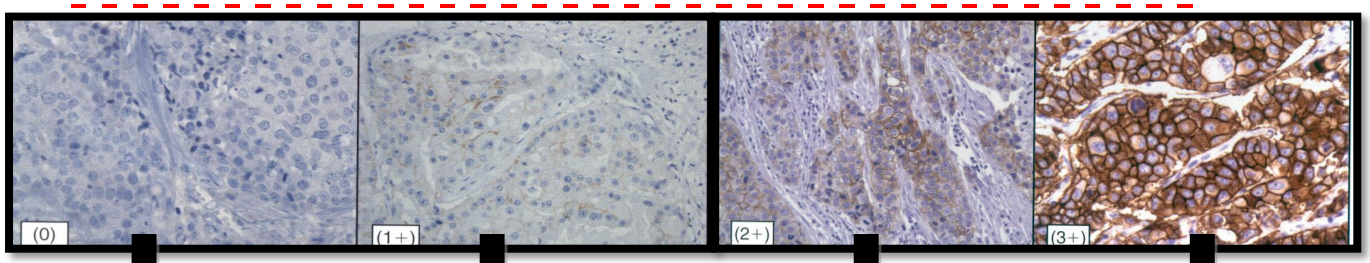
- **(Herceptin molecule (Trastuzumab) is known as biological targeted therapy.)**

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

1. Immunohistochemistry antibodies against Her2 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 the antibody.

2. 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, hybridization with a probe, and washing. First, a probe specific for the target of interest 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.

Immunohistochemistry (IHC) for the assessment of the level of HER2 protein expression at the tumor cell membrane.



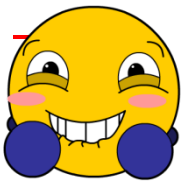
(0) → no stain → **negative** → won't response to Herceptin

(1+) → Granular staining on the cytoplasm → regarded as **negative** → no Herceptin treatment is given

(2+) → membranous staining but staining is not complete → could be **negative** or **positive** (will be discussed later).

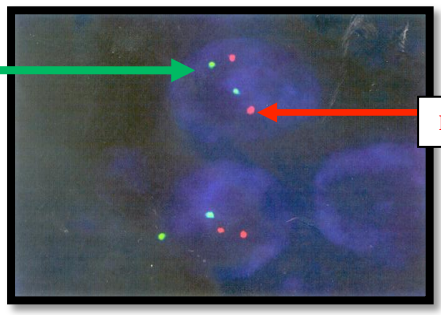
(3+) → very clear staining → **Positive** → give Herceptin → usually estrogen and progesterone negative.

What is the next step in patient with (2+) result for HER-2 receptors?

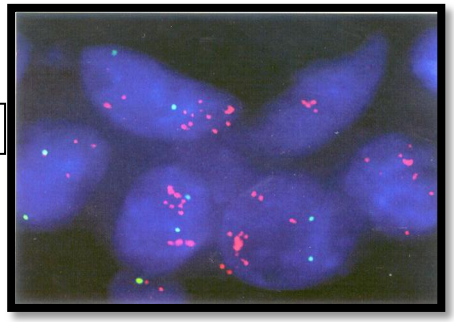


- We do Fluorescent or silver in situ hybridization (FISH or SISH), which is a molecular genetic technique with higher specify and sensitivity to sort out these patient to either negative or positive.

Green = **chromosome 17**



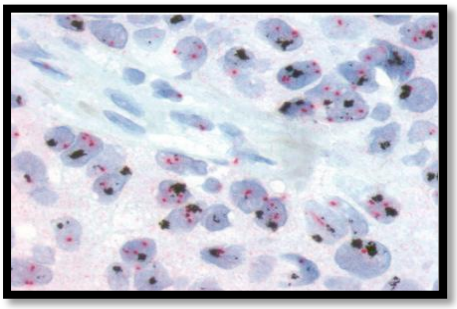
Red = HER-2 neu



Fluorescence in situ hybridization image in normal cell, **negative for HER-2 receptor.**

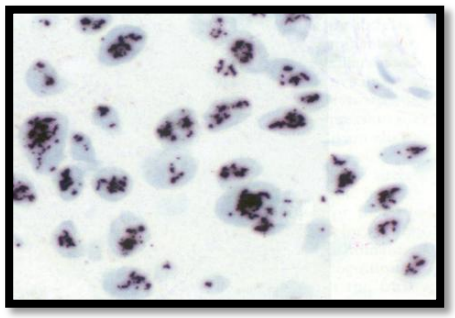
Fluorescence in situ hybridization image showing **amplified** reaction, **positive for HER-2**

Silver in situ hybridization (SISH)



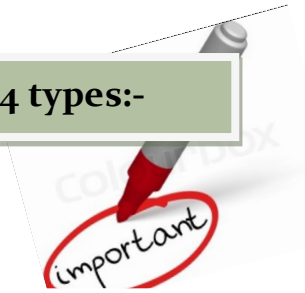
Black→ HER-2,

Red → Chromosome 17



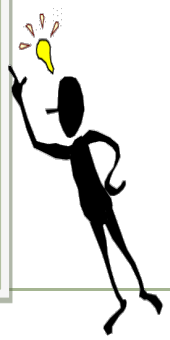
Negative **Positive**

Robbins 9th edition page 709 , they divided breast cancer into 4 types:-



1-luminal A	2-luminal B	3-luminal C	4-basal cancer
Estrogen ,Pro gesterone Positive	Estrogen And Progesterone Negative	Estrogen ,Pro gesterone Positive	Estrogen And Progesterone Negative
Herceptin Negative.	Herceptin Positive	Herceptin Positive	Herceptin Negative.
(ER+,PR+,HER2-)	(ER-,PR-,HER2+)	(ER+,PR+,HER2+)	(ER-,PR-,HER2-)

This type will benefit from Tamoxifen and Aromatas inhibitors.



this type doesn't respond for any therapy (Tamoxifen , Herceptin), we treat them by chemotherapy, and because of this he have a bad prognosis.

Summery;

1-a well-differentiated tumor might metastasis to brain, lung, lymph nodes or anywhere else. So in this case the stage is more important than grade.

2- breast cancer can be familial in 10-15%

3-the most common presentation in breast diseases is (breast lump), may be with or without pain and nipple discharge

4-Women having mutation with family history almost 100% will develop breast cancer.

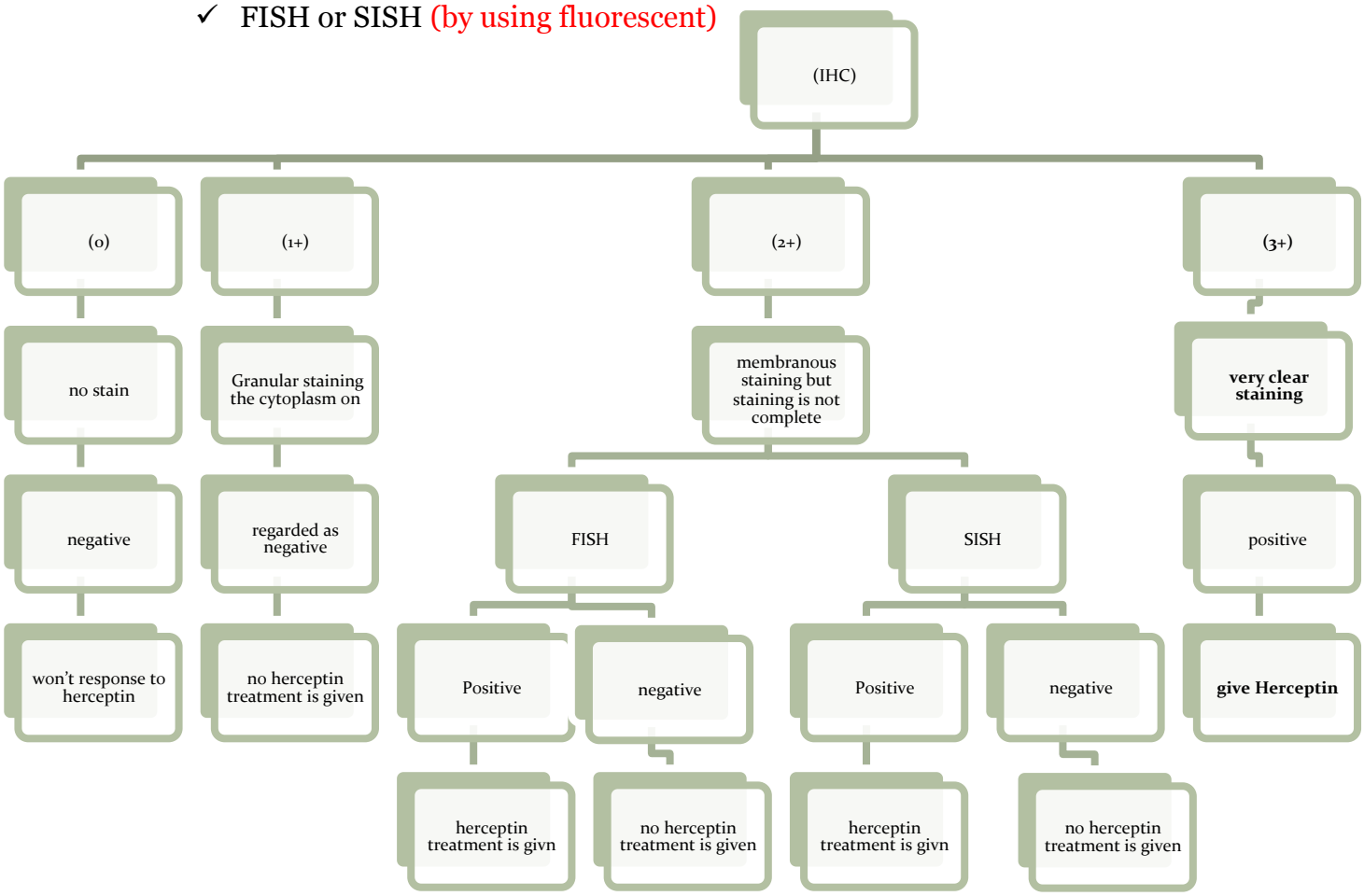
5-we have to predict the prognosis by detecting the grade, stage, TNM (tumor size, lymph node, metastasis) , hormonal status, Herceptin status.

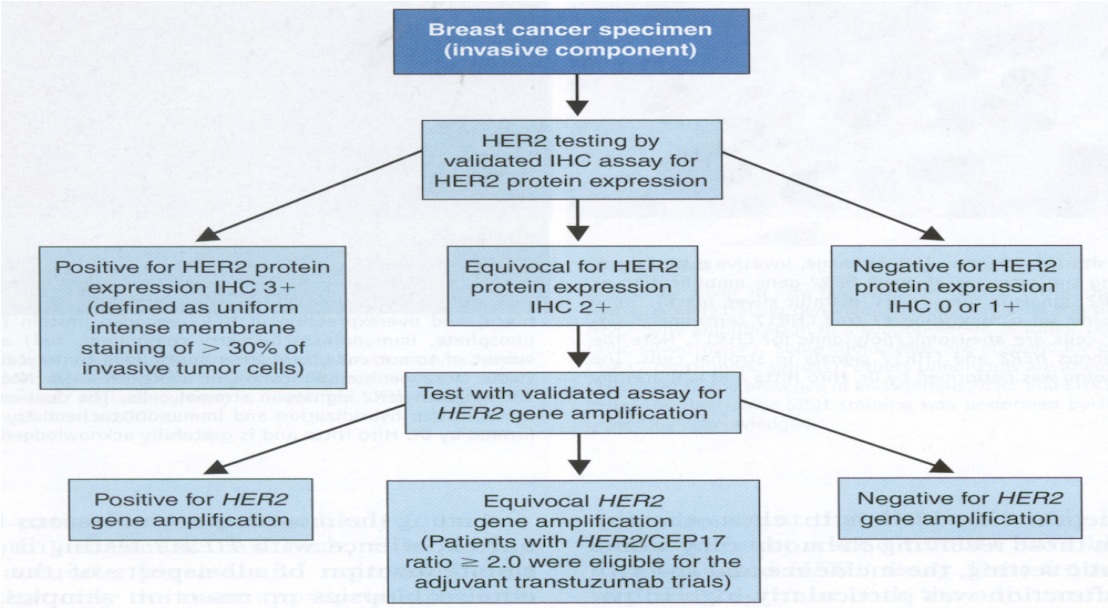
6- When we face breast cancer we have to determine the prognosis

- **Demonstration of HER-2 neu receptors can be done by:**

✓ Immunohistochemistry antibodies against HER-2 receptors (by using a dye)

✓ FISH or SISH (by using fluorescent)





QUESTIONS

1. **The most diagnostic feature of breast cancer is :**
   a. Negative HER2 gene
   b. Positive HER2
   c. Equivocal HER2
   d. None of the above

2. The prognosis of tumor with HER 2 gene :
a. Good prognosis
b. Treated by Tamoxifen
c. Treated by Herceptin
d. Highly metastasized

3. Regarding the presence of Estrogen and Progesterone receptors on tumor cells :
a. Poor prognosis
b. The tumor responds well to Tamoxifen
c. The tumor responds well to Herceptin
d. Highly metastasized

4. If breast cancer express Her2 receptor that mean ?
A- high grade (poorly differentiated)
B- low mitotic rate
C- small size tumor

Answers: B, C , B, A