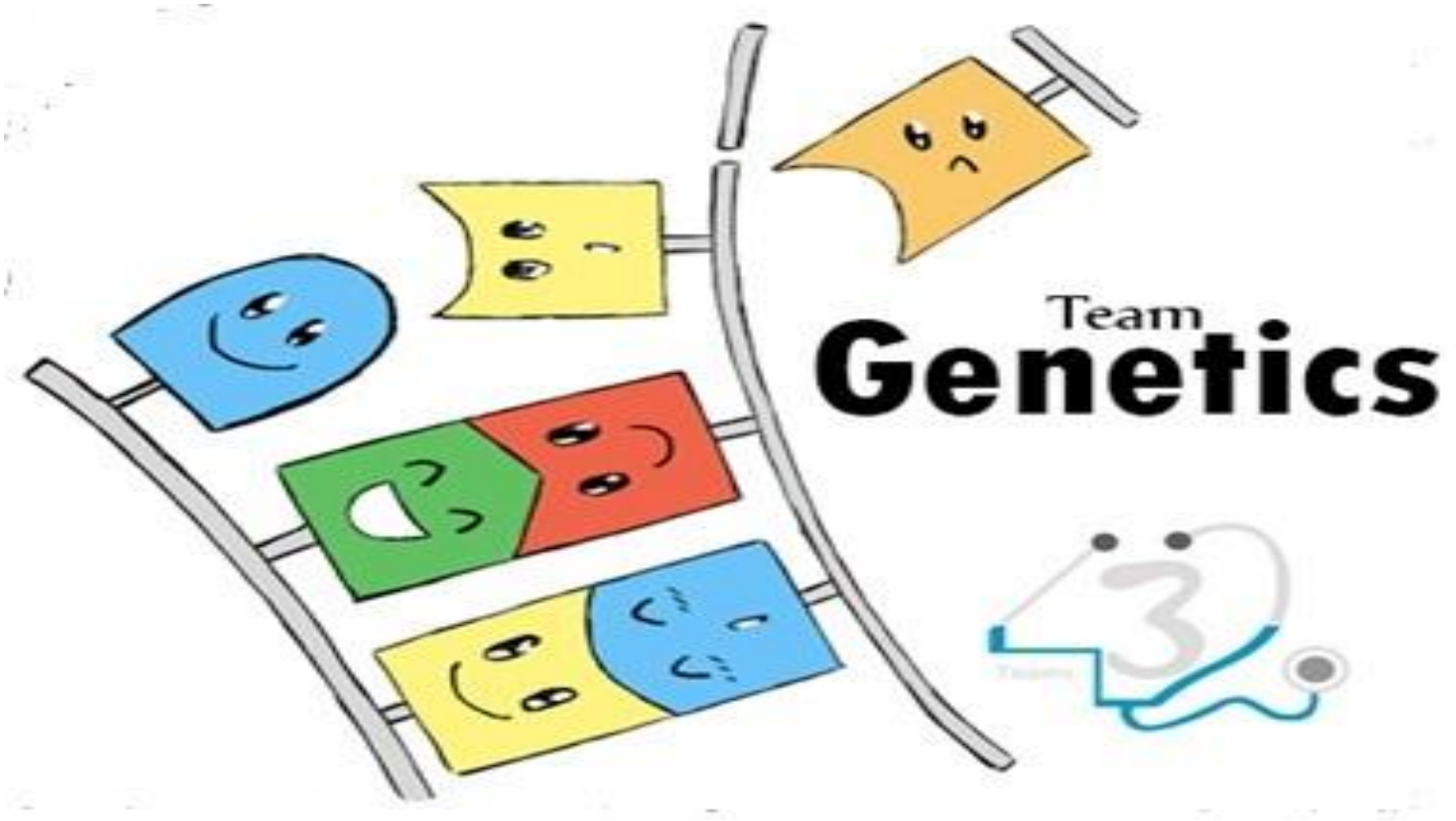

Lecture 2

Genetics Of Breast Cancer

*432 **Genetics** Team*

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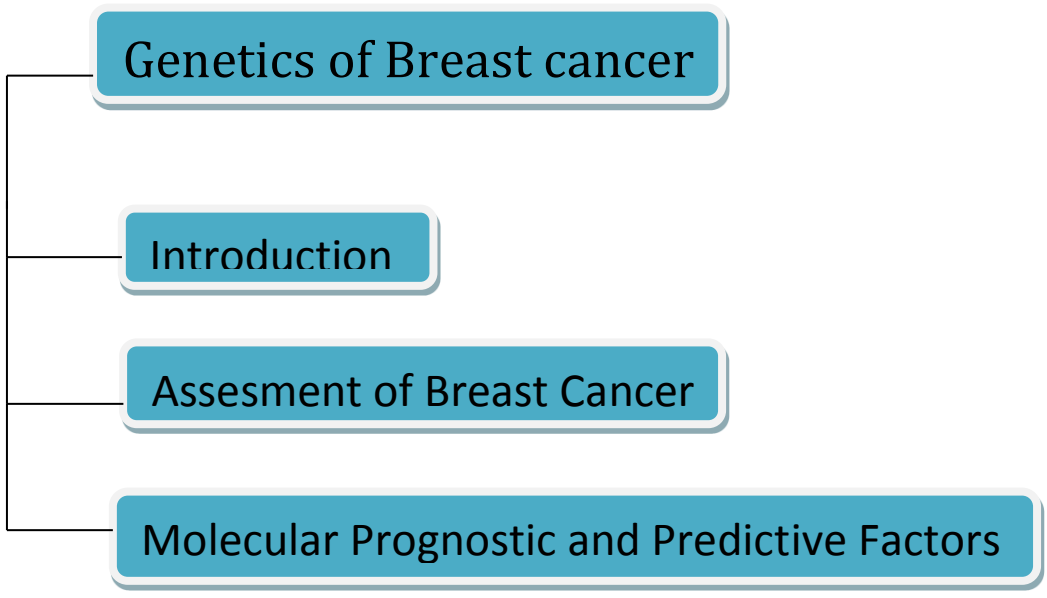
Reproductive Block

Color Index: Female notes are in Green. Male notes are in Blue. Red is important. Orange is explanation.

Objectives

- Understand that 5-10 % of breast cancers are related to specific inheritid gene mutations.
- Is aware of the factors which affect the prognosis of breast cancers like morphology of tumers and the status of steroid hormone receptors including oestrogen and progesterone.
- Understand the function and prognestic singnificance of HER2 gene on chromosome 17.
- Is aware that Herceptin is the drug used against HER2 positive breast tumour cells.

Mind Map:



Introduction

Genetics and Family History of breast cancer: About **5-10 %** 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 significance family history (i.e. multiple relative 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 can develop both Breast and ovarian cancer while BRCA2 Only breast. 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 mutations and the second by a subsequent somatic mutations. However, most carriers of the mutations will develop breast cancer by 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 disease 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 ataxia-telangiectasia gene.

REMEMBER :

Both BRCA1 and BRCA2 are tumor suppressor genes.

- BRC A1 (17q21.3) [½ of women with hereditary breast cancer]
- BRC A2 (13q12-13) [⅓ of women with hereditary breast cancer]

Assesment Of Breast Cancer

Traditional Morphologic prognostic factors

The Validated pathological metric that have been demonstrated to provide clinically useful prognostic informations to predict the prognosis and the recurrence in breast cancer include:-

1. Tumor Size.[Tumors >2.5 cm behave worse than tumor <2.5 cm]
2. Tumor Grade.
3. Histologic Type.
4. Lymph node staging. [80% Survival rate if it Negative]
5. Evidence of Vascular or Lymphatic invasion. [Only15-20% live to 5 Years] (80% Die before 5 years)

Hormone Receptors

The first of the prognostic and predictive biomarkers in breast cancer in clinical use, the steroid hormone receptors.

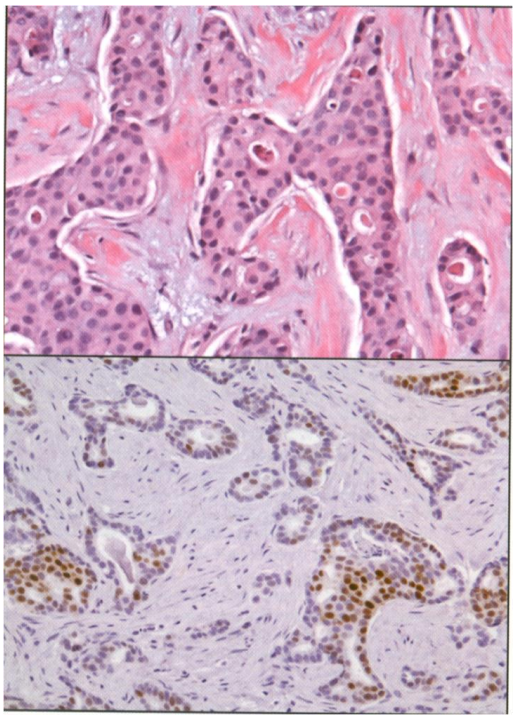
It has been known for some time that around 60-70% of breast carcinomas express Estrogen receptors (ERs) and progesterone receptors (PRs). Thus, the estrogen receptor became the first target for either treatment by therapeutic hormonal manipulations with ER antagonists such as

1- Tamoxifen [Esterogen receptor antagonist]
2- Aromatase inhibitors[Decrease The concentration of esterogen either the local or metastatic]

The presene 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.

Prognostic and management indicator

Immunohistochemistry [Anti estrogen and anti progesterone receptors Antibody] for the evaluation of estrogen receptors in Invasive ductal carcinoma of the breast (Brown color)



Note:

If I see the positive staining in 30% of tumor cells 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 and Predictive Factors

HER2

Normal cells have one copy of the HER 2 gene on each chromosome 17 and when this gene is expressed in normal epithelial cells, it is transmits signals regulating cell growth and survival and produce protoncogenes. In approximetly 15% to 25% of breast cancers, the HER2 gene is found to be Amplified2-Fold to greater than 20-Fold so that epidermal growth factor increased producing more proto-Oncogenes in each tumor nucleus relative to chromosome17, and this amplification drives gene expression, generating up to 100 times the normal number of HER2 receptors proteins at the cell surface. Breast tumors that have HER2/neu gene amplification are more aggressive.

HER2 Positive breast cancers is significantly correlated with several unfavorable pathological tumor charactrastics, including larger tumor size, positive axillary lymph nodes, higher nuclear grades, and higher proliferative index.

REMEMBER:

Molecular genetic studies in Breast Cancer

- BRCA1 & BRCA2.
- Estrogen and Progesterone receptors.
- HER/neu.

Note:

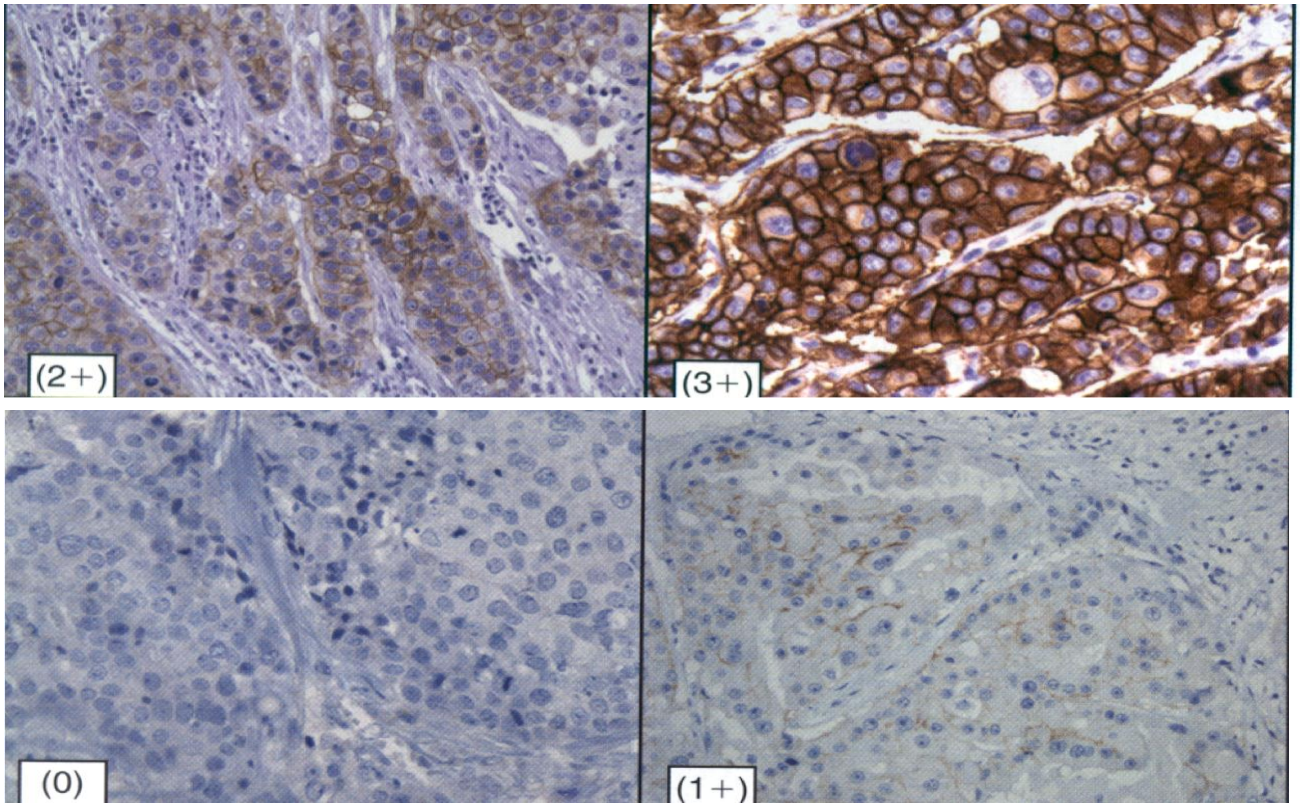
HER2 Over expression may have a predictive role for response to adjuvant chemotherapy and endocrine therapy.

Herceptin [TRANSTUZUMAB]

It is the drug used against H2r2 Positive tumour cells. Herceptin is a molecular genetic targeted therapy so I don't have to give Cytotoxic drugs.

With high affinity and specifity for HER2 protein(It will block them), and in preclinical studies was shown to be most effective against tumor cells with HER2 Overexpression, It is remarkably effective theraputic agent in both the metastatic and in combination with cytotoxic chemotherapy.

So we predicting the prognosis and helping in the management by giving herceptin to selectid patients that's why we do HER2/neu status.



Immunohistochemistry (Antibody HER2/neu) is incubated with breast cancer cells 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)
At least 10% of tumor cells Positive and Complete membrane stain so that I can use Herceptin.

Patient 0: Negative HER/neu, She will no benefit from Herceptin, <u>Most likely</u> have Estrogen progesterone receptors positive and Herceptin negative [Luminal A (OnlyTamoxifen)].

Patient +1: Negative HER/neu , She has brown staining because she has the receptors but it is scattered and not complete (need to be at least 10% of the cells).

Patient +2: May be Positive may be Negative HER/neu, Some cells are positive and nuclear staining is not complete, I need to do more tests. Need to do FISH stain.

Patient +3: Positive, Need to give Herceptin, <u>Most likely</u> Estrogen progesterone receptors negative and Herceptin Positive [Luminal C].

Note:

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)

Gene expressing profiling can separate breast cancer into subtypes:

1 – Luminal A (estrogen Receptors positive, HER2/NEU negative) so Tamoxifen will be beneficial to the patient.

2 – Luminal B (estrogen Receptors positive, HER2/NEU positive) so Tamoxifen and Herceptin will be beneficial to the patient.

3 – Luminal C (estrogen Receptors negative, HER/NEU positive) so Herceptin will be beneficial to the patient.

4 – Luminal D (basal-like) (estrogen Receptors negative, HER/NEU negative) bad tumor and aggressive.

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.

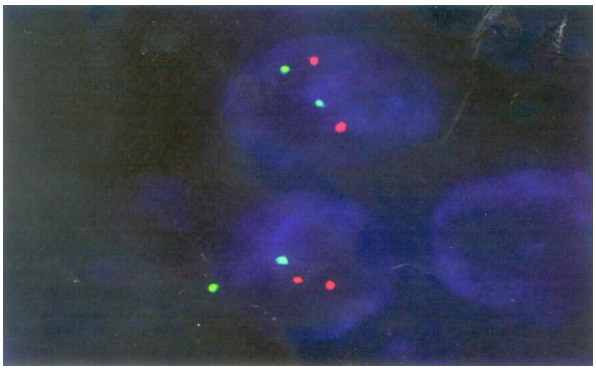
First, a probe specific for the target of interest (eg. Cancer cells) is applied to the slide, along with a nuclear counterstain and reagents or heat that enhance denaturation of target DNA and reduce background. The slides are sealed and incubated in a humid environment under conditions that denature 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.

Note:

[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 producea 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.

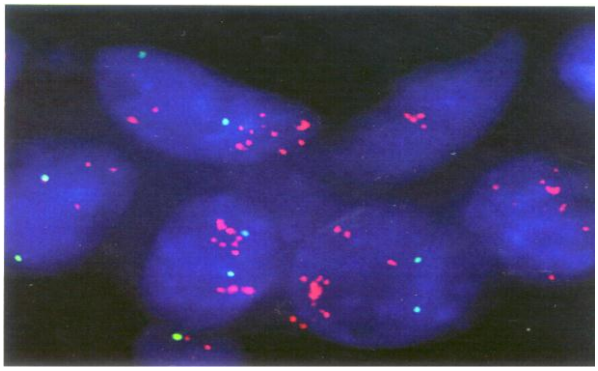


This patient doesn't have HER2/neu gene mutation and amplification. Either Luminal A or D

Only Treated with Tamoxifen

HER2/neu gene Red

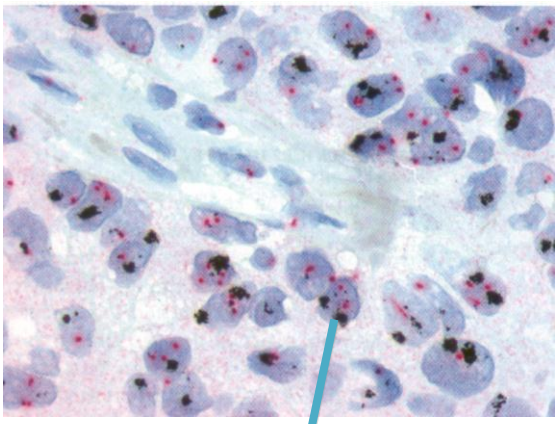
Chromosome 17 Green.



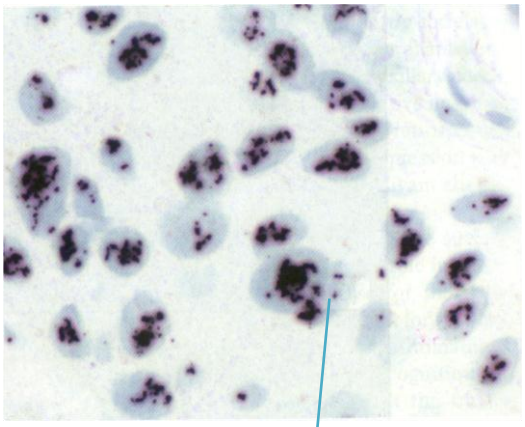
This patient has HER2/neu gene mutation and amplification. She has >2 Copies of HER2/neu

She most likely has lymph node involvementand metastasis, because this tumor is aggressive.

Treated with Herceptin [TRANSTUZUMAB

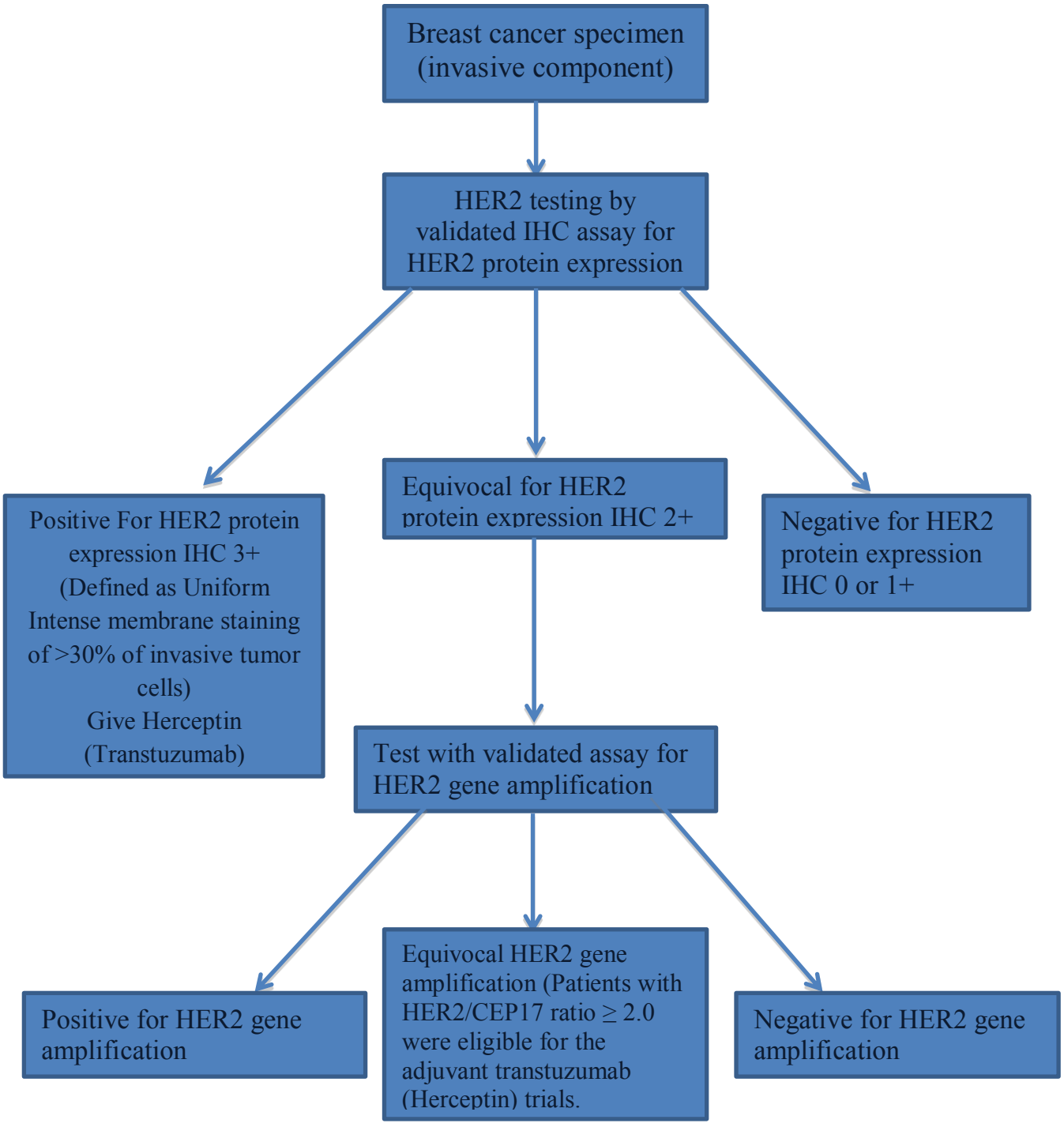


This is SISH (silver in situ hybridization) here instead of marking the strands with fluorescence; I mark them with silver nitrate. Also I use the common microscope instead of Immunofluorescence microscope.



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

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



QUESTIONS

1- The most diagnostic feature of breast cancer is:

a.Negative HER2.
b. Positive HER2.
c. Equivocal HER2.
d. None of the above.

2- The prognosis of tumor with HER2 gene is:

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 will respond to Tamoxifen.
c. The tumor will respond to Herceptin.
d. Highly metastasized.

4- If breast cancer express HER2 receptors that mean:

a. High grade (poor differentiated).
b. Low mitotic rate.
c. Small size tumor.

Answers:B,C,B,A

Special thanks to pathology team for providing us the template

For any questions, suggestions or problems, please contact us:
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