

**HISTOPATHOLOGY DEPARTMENT
NICOSIA GENERAL HOSPITAL**



HER-2 AND BREAST CANCER

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10th Marianna Lordos Symposium



**MARIANNA LORDOS
CANCER MEMORIAL FUND**

HER2 and BREAST CANCER



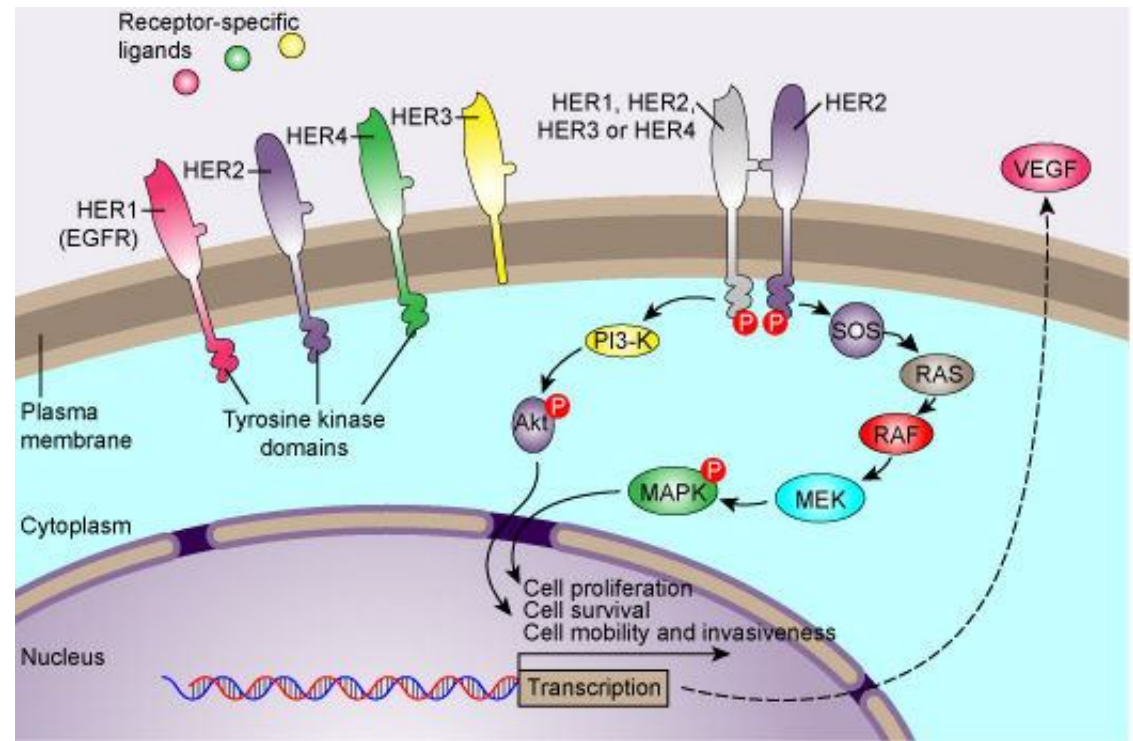
- The human epidermal growth factor receptor 2 gene HER 2/neu is amplified in approximately 20-25% of breast cancer.
- Women with HER2-positive breast cancer have a more aggressive disease, compared to women with HER2-negative breast cancer.
- Several studies have shown that agents that target HER2 are remarkably effective in both metastatic and adjuvant therapy. Trastuzumab improves response rates, time to progression and survival when used alone or added to chemotherapy.

HER2 and BREAST CANCER



The HER-2/neu gene is localised to chromosome 17 and encodes a transmembrane tyrosine kinase receptor protein that is a member of the epidermal growth factor receptor family, that mediates cell growth, differentiation and survival.

This family is involved in major pathways in signal transduction and ultimately affect cell proliferation, survival motility and adhesion.

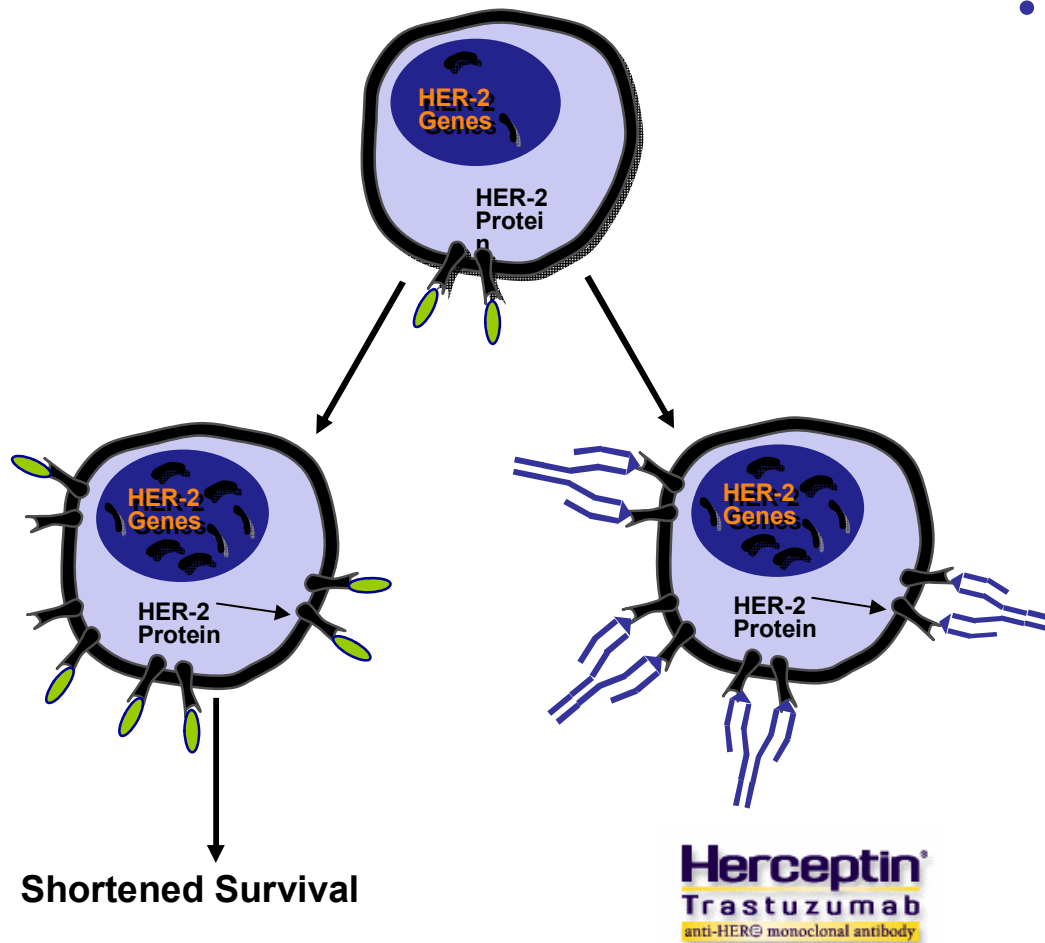


HER2 and BREAST CANCER



- In approximately 20-25% of women with breast cancer, there is a genetic alteration in the HER2 gene that produces an increased amount of the growth factor receptor protein on the tumor cell surface. HER2 overexpression can cause cells to divide, multiply, and grow more rapidly than normal.
- HER2 gene abnormality is only present in the breast cancer cells, not in normal breast cells, and cannot be passed onto other family members.
- Evaluation of HER2 amplification or protein overexpression in breast carcinomas is an important task in breast cancer patient management. Molecular targeting against HER2 protein such as the use of anti-HER2 therapy Trastuzumab (Herceptin) is now approved for clinical application.

HER2 and BREAST CANCER



- **Trastuzumab (Herceptin)** is a monoclonal humanised antibody that binds the extracellular domain of **HER-2** and interferes with the signal transduction cascade initiated by Her-2 overexpression and possibly stimulates an immune response to tumour cells overexpressing this receptor. Trastuzumab is often used with chemotherapy. But it may also be used alone or in combination with hormone-blocking medications.

HER2 and BREAST CANCER



- Because of HER2 prognostic role as well as its ability to predict response to Trastuzumab (Herceptin), breast tumors are routinely checked for overexpression of HER2/neu.
- Herceptin only works in people who have HER2 amplification.
- Accurate assessment of HER2 status is essential to ensure that all patients who are likely to benefit from Herceptin are correctly identified, while avoiding unnecessary treatment of patients who are unlikely to benefit. (side effects).

HER2 and BREAST CANCER

HER2 Service at Nicosia General Hospital



- **Routine testing for HER2 is recommended for most women diagnosed with breast cancer. Whenever breast cancer recurs or spreads, the cancer cells should be retested for HER2 as well as for hormone receptor status, as these can change from the original cancer in up to 20 to 30% of cases.**
- **Testing will usually be done at the same time as the initial biopsy or breast cancer surgery. Samples of cancer tissue from previous biopsies or an earlier surgery also may be used if they have not already been tested.**

HER2 Testing in BREAST CANCER



- **Immunohistochemistry (IHC):** measures the overexpressed protein coded by HER2 gene
- **Fluorescent in-situ Hybridisation (FISH):** measures gene amplification
- **Chromogenic in-situ Hybridisation (CISH):** measures gene amplification
- **Silver-enhanced in-situ Hybridisation (SISH):** measures gene amplification

The tissue-based assays are most commonly used to establish a patient's tumor HER2 status

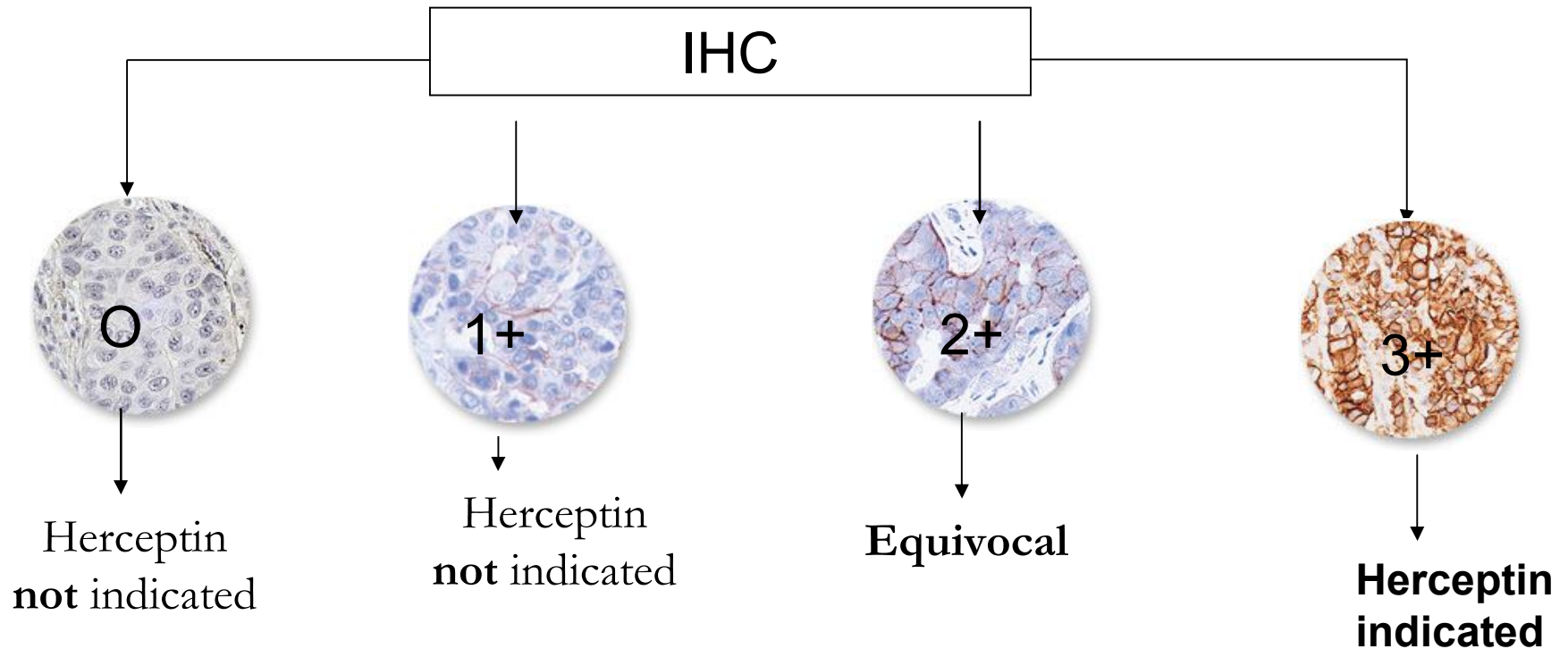
The tests commonly used to determine HER2 status:
Immunohistochemistry and FISH

HER2 and BREAST CANCER IMMUNOHISTOCHEMISTRY



IHC detects HER2 overexpression at the protein level

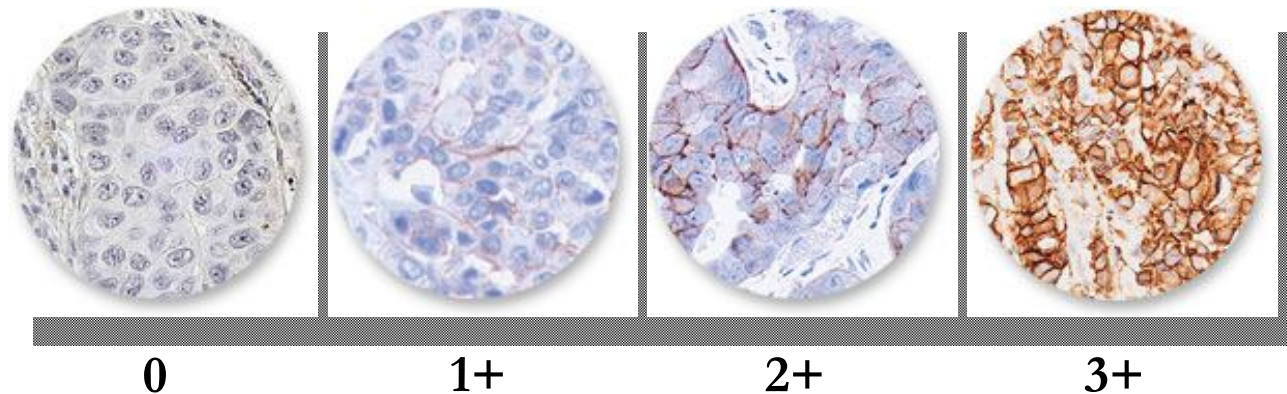
A tumor biopsy is scored as 0 (negative), 1+ (negative), 2+ (borderline), or 3+ (positive) on an IHC test based on the reviewer's interpretation of staining intensity and completeness of membrane staining.



HER2 and BREAST CANCER IMMUNOHISTOCHEMISTRY



- Familiar technology, well accepted
- Inexpensive
- Subjective scoring
- Semi quantitative
- Protein target may be effected by pre-analytical tissue processing (not proper formalin fixation of tissue)

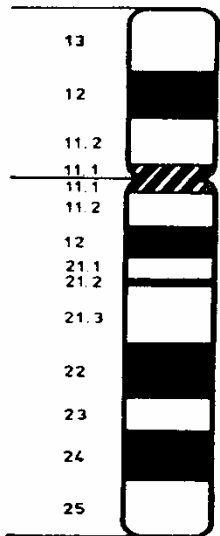


HER2 and BREAST CANCER FLUORESCENT IN-SITU HYBRIDIZATION



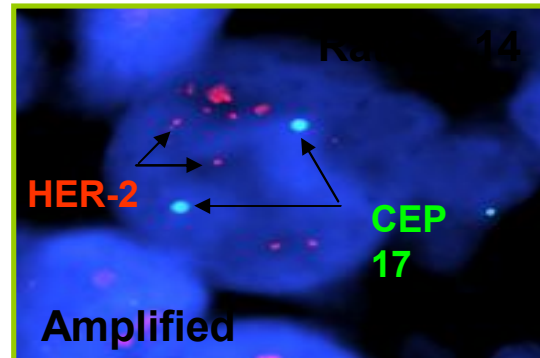
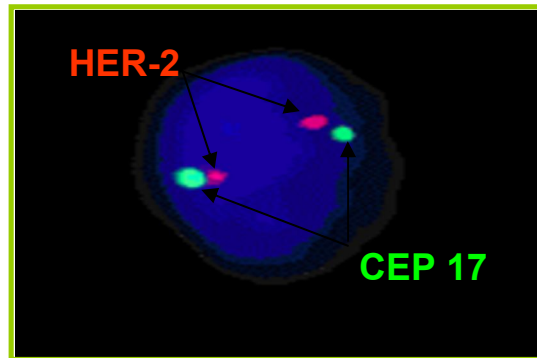
FISH testing measures the amount of the HER2/neu gene in each cell. It measures the HER2/neu gene copy number against a standard internal chromosomal control (CEP 17).

CHROMOSOME MAP



17

← CEP 17
← LSI HER-2



FISH Reporting
Count Her 2 signals and CEP 17 signals and determine ratio of HER 2/cep 17

<2 not amplified

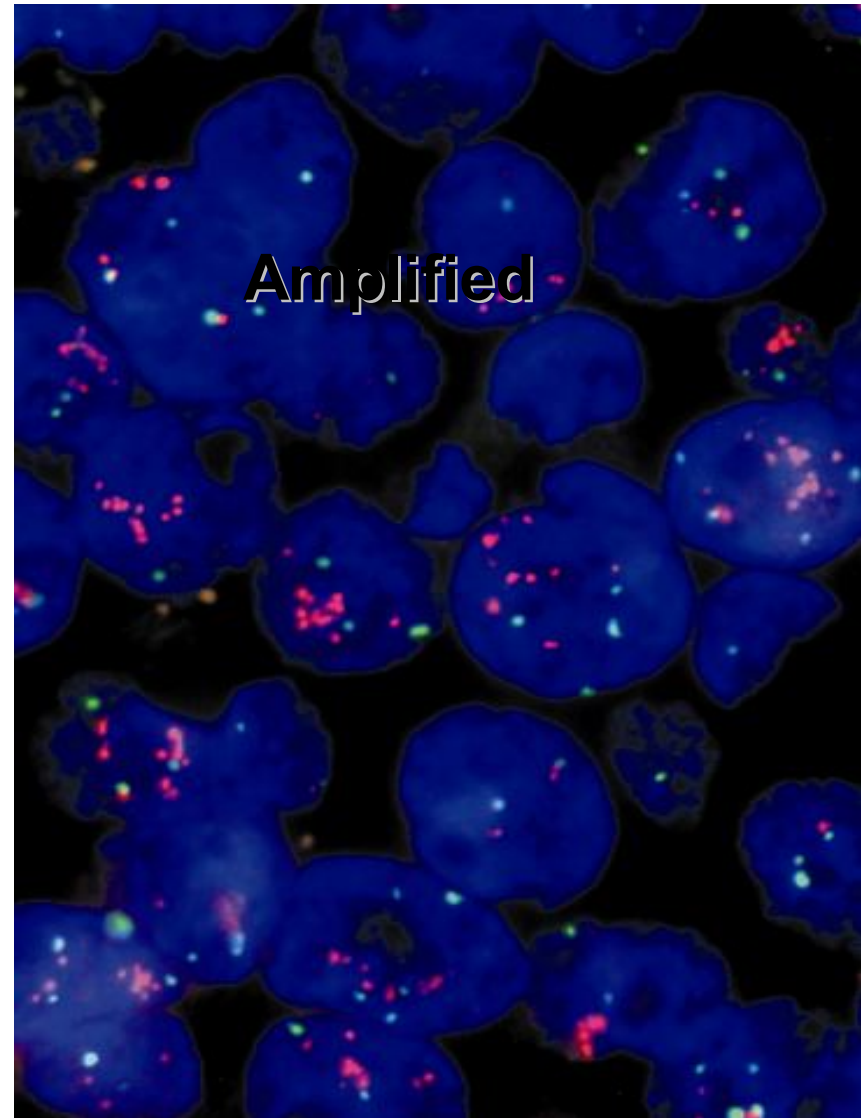
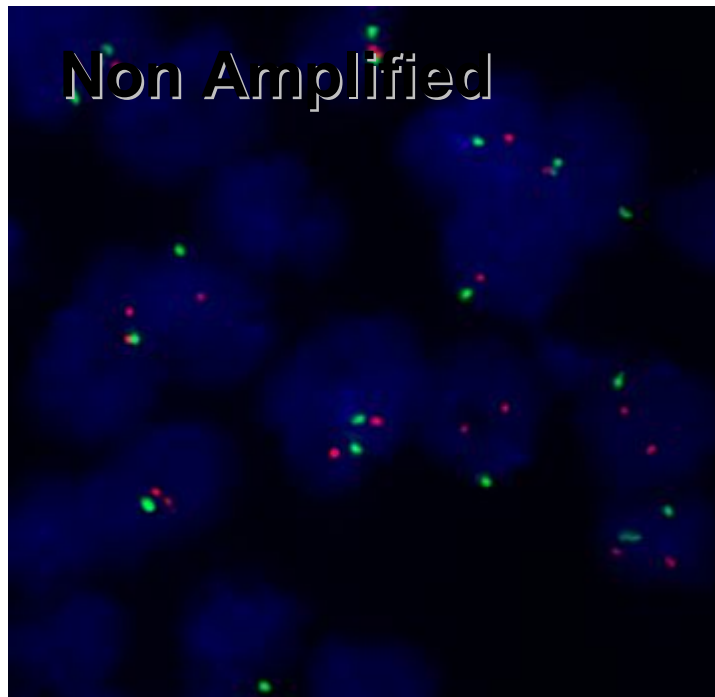
>2 amplified

HER2 and BREAST CANCER

FLUORESCENT IN-SITU HYBRIDIZATION FOR HER2



FISH interpretation of HER2 status in breast cancer tissue specimens.



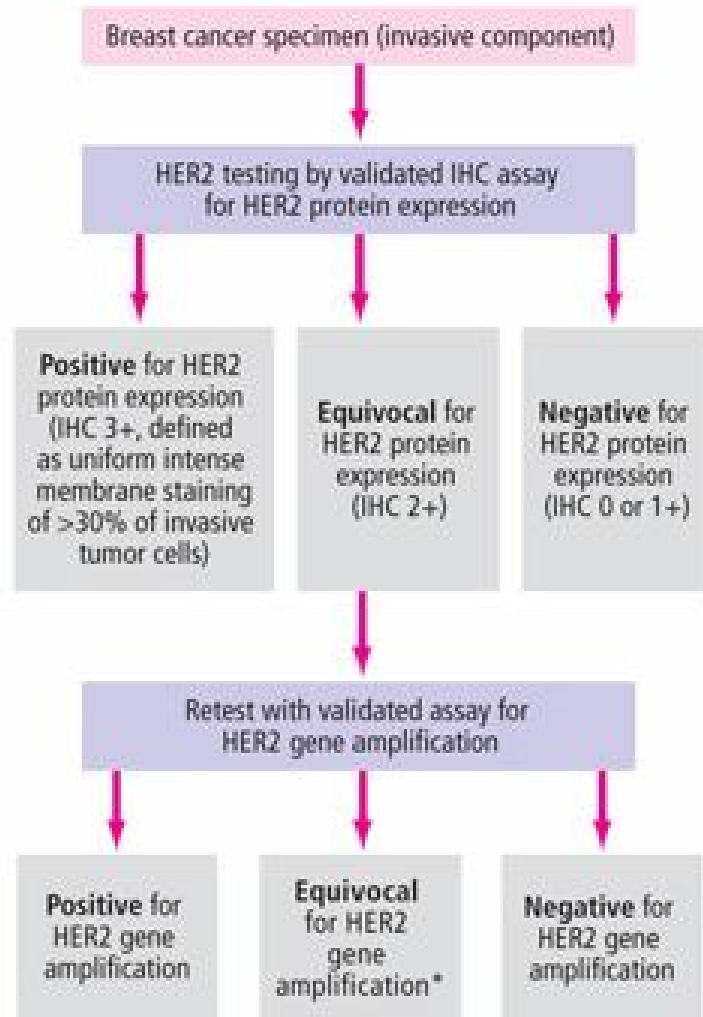
HER2 and BREAST CANCER



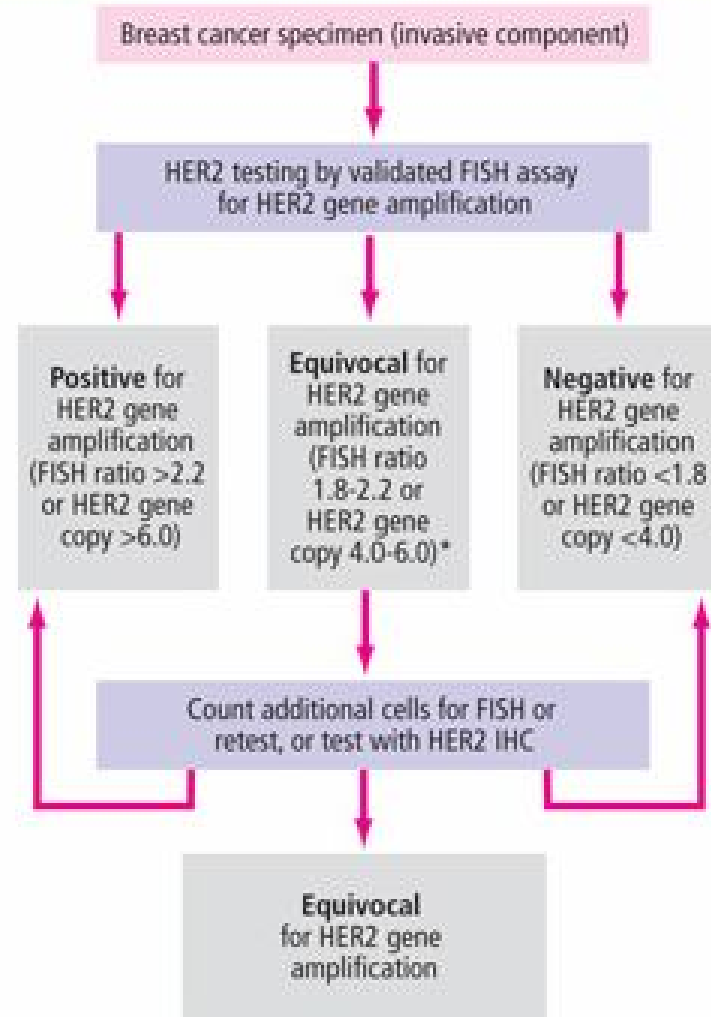
HER2 Testing Algorithm

ASCO/CAP guidelines for HER2 testing

IHC testing algorithm

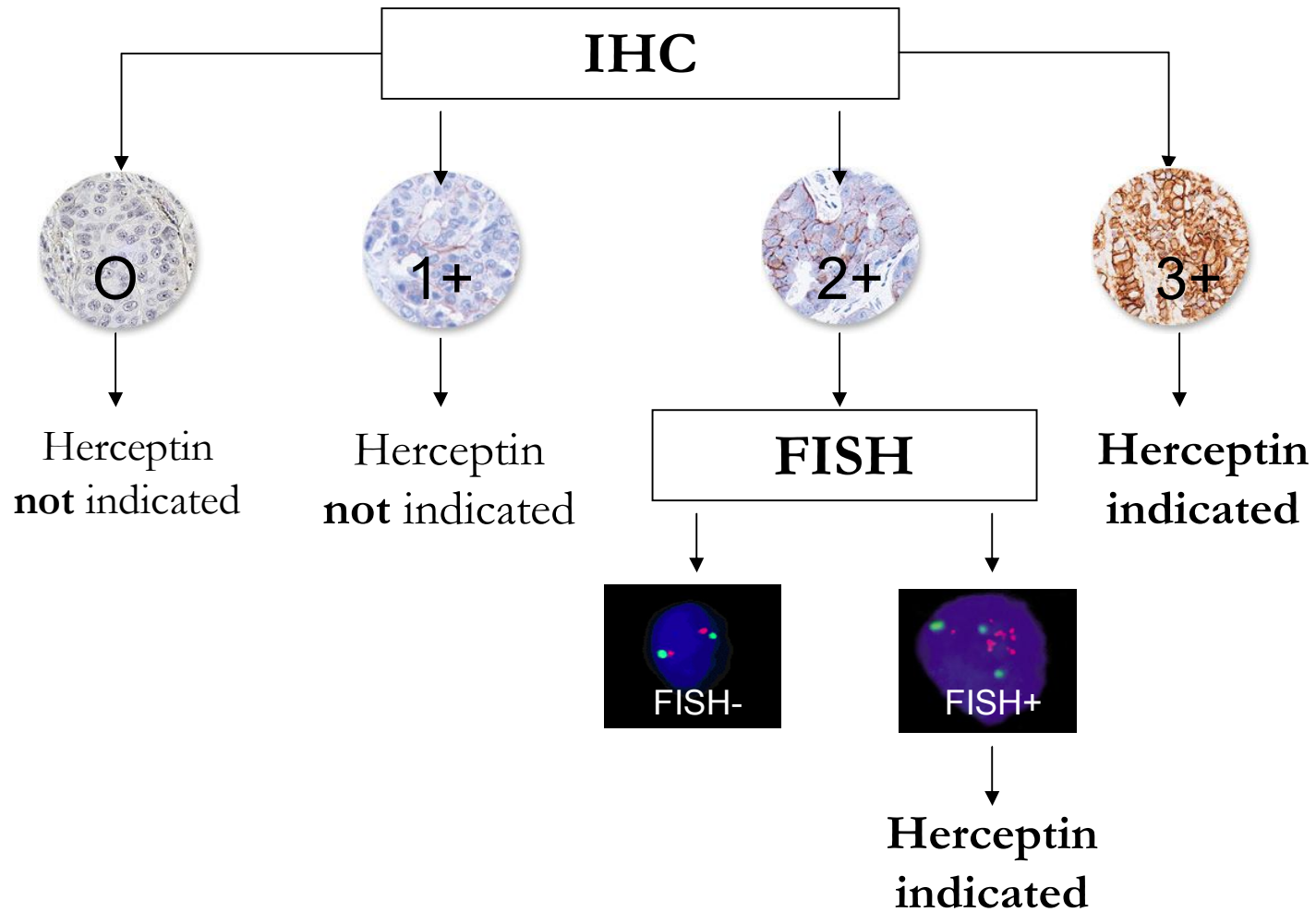


FISH testing algorithm



HER2 and BREAST CANCER

HER2 Testing Algorithm



HER2 and BREAST CANCER

HER2 Service at Nicosia General Hospital



- **HER2 team**
 - **3 Pathologists Consultants (Dr. I. Zouvani, Dr. E. Iacovou, Dr. M. Vassiliou)**
 - **3 Clinical Scientists (Dr. N. Anastasiadou, Mrs M. Demosthenous, Mrs S. Iosif)**
- **Provide a service to all public hospitals and private sector at Cyprus**
- **Currently test approximate 600 cases per year**
- **IHC with A0485 Her2 Antibody (DAKO) and FISH with PathVysion (Vysis, Abbott)**

HER2 and BREAST CANCER

HER2 Service at Nicosia General Hospital



Accurate assessment of HER2 status is essential thus laboratory and Pathologists are evaluated continuously

- **Quality Assurance**
 - **External QA (UK-NEQAS). Four mandatory testing events per year for IHC and FISH with satisfactory performance**
 - **Internal QA including competency assessment of Pathologists and Clinical Scientists**
- **Apply recommended methods to increase accurate interpretation of HER2 status**
- **Continuously training of Pathologists and Clinical Scientists**

HER2 and BREAST CANCER

HER2 Service at Nicosia General Hospital



- **IHC and FISH study for 200 Breast Cancer cases**
- **Results available on 194/200 cases**
- **6 slides not included in the results**
 - **Inadequate (Very bad fixation of tissue)**
 - **Sections floated off**
- **Analysis performed on 194 cases**

HER2 and BREAST CANCER

HER2 Service at Nicosia General Hospital



- IHC and FISH available for 194 cases

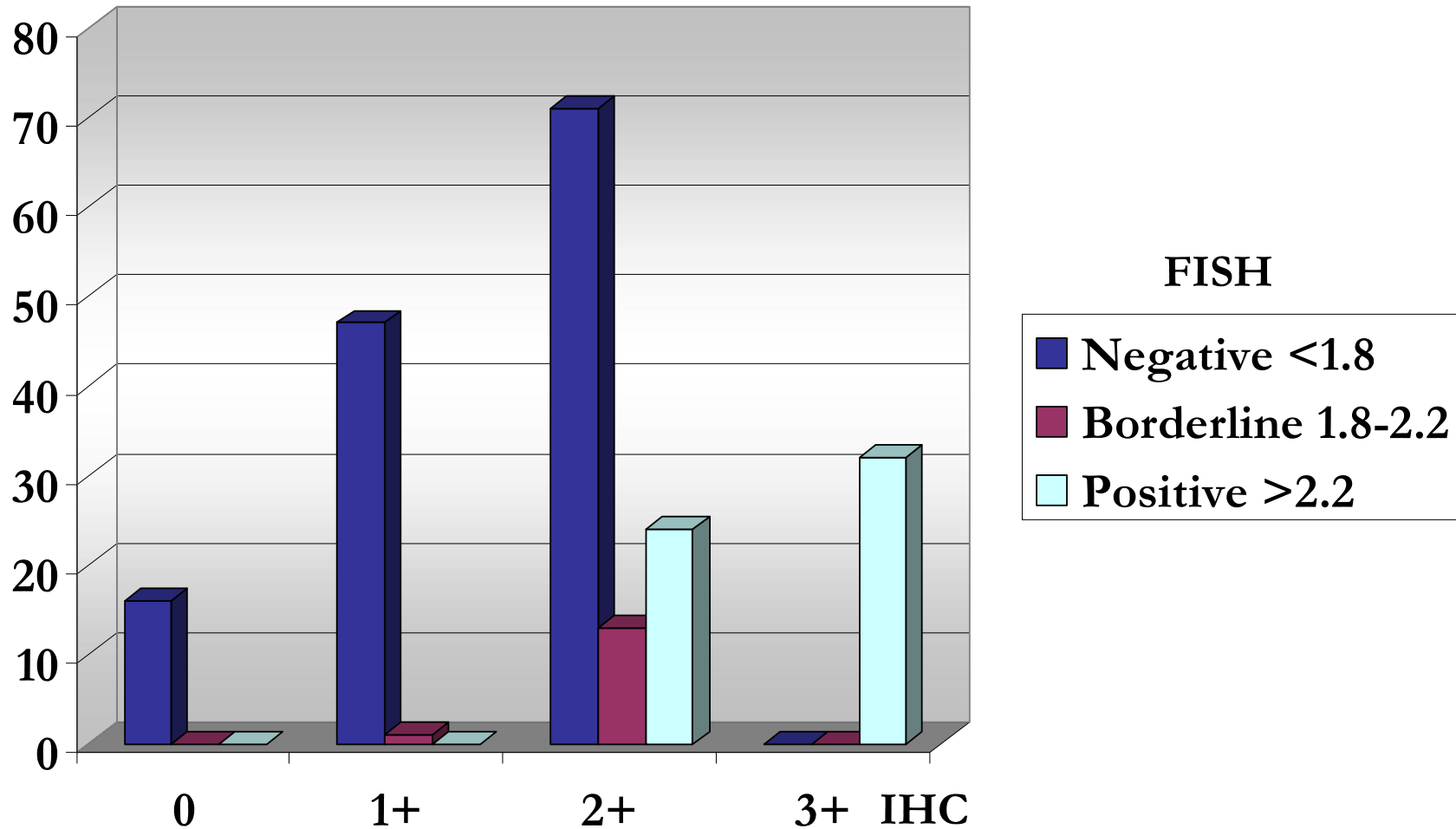
		FISH PathVysion		
		Negative <1.8	Borderline 1.8-2.2	Positive >2.2
IHC Dako A0485	0	16	0	0
	1+	47	1	0
	2+	71	13	14
	3+	0	0	32

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Comparison of IHC vs FISH on Breast Cancer Cases



High concordance between FISH and 0, 1+ and 3+ IHC

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Comparison of IHC vs FISH on Breast Cancer Cases

ASCO HER2 guidelines:

Concordance between methods must be

95% for positive cases and

95% for negative cases

HER2 and BREAST CANCER

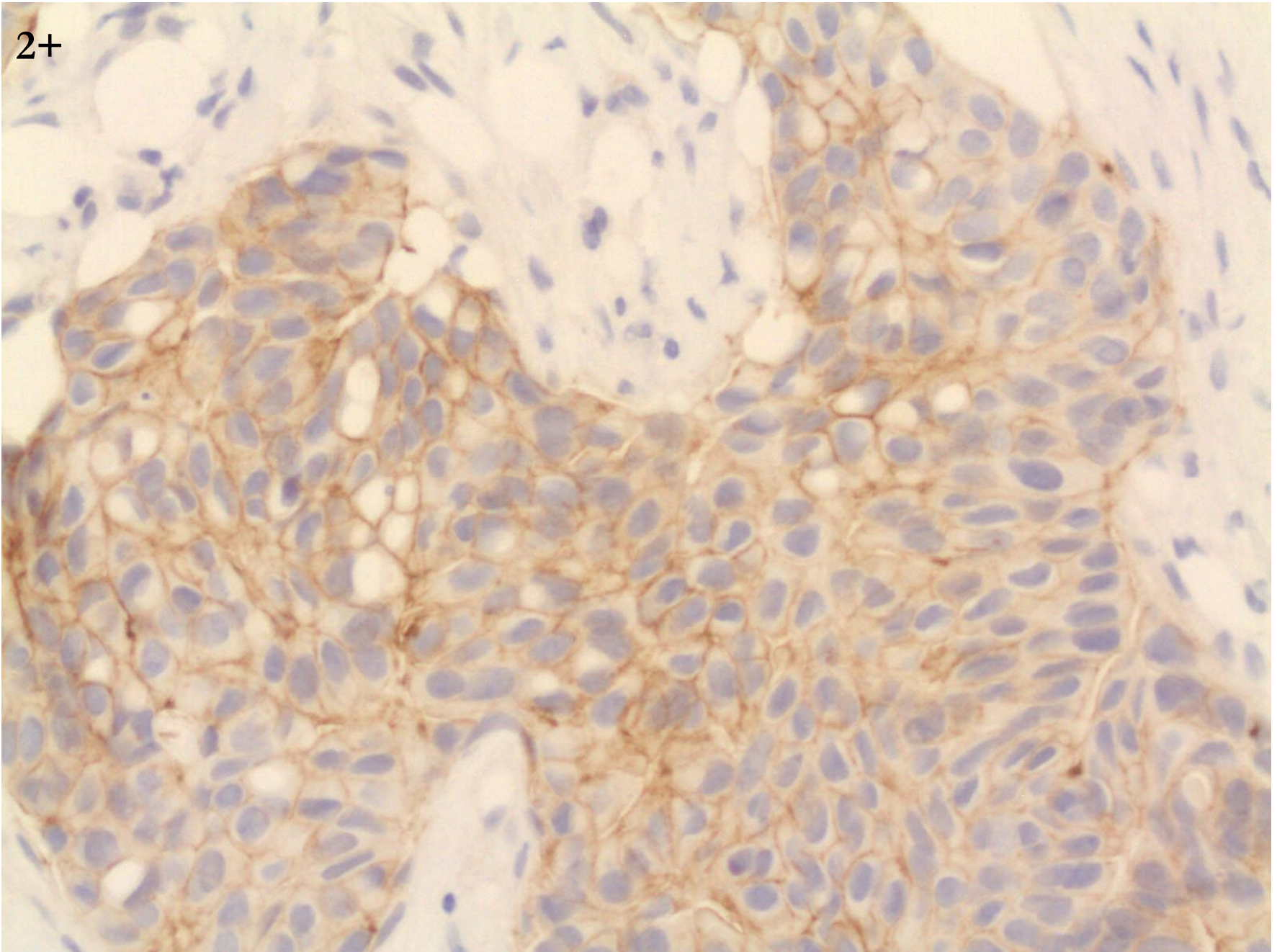
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Comparison of 2+ IHC vs FISH on Breast Cancer Cases

- Modification of the scoring system for HER2 IHC by obtaining a normalised IHC score for the breast cancer. This modification had a 50% reduction in the number of equivocal cases.
- Non-proper fixation can lead to incorrect IHC status
- Some 2+ IHC cases have polysomy

2+



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Comparison of 2+ IHC vs FISH on Breast Cancer Cases

- **Assessment of HER2 IHC status by an FDA approved system**
- **The use of a quantitative image analysis system can reduce slide scoring variability in 2+ cases**

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Comparison of IHC vs FISH vs RT-PCR on Breast Cancer Cases

IHC, FISH and RT-PCR results available for 64 cases

IHC Dako A0485	PathVysion FISH Result			RT-PCR Roche Result		
	Negative <1.8	Bordeline 1.8-2.2	Positive >2.2	Negative <1.8	Bordeline 1.8-2.2	Positive >2.2
0	6	0	0	6	0	0
1+	12	0	0	11	0	0
2+	10	6	8	15	3	5
3+	0	0	22	3	5	14

High concordance between FISH, RT-PCR and 0 and 1+ IHC

Some of the confirmed HER2 positive cases were found negative with RT-PCR

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Comparison of IHC vs FISH vs PT-PCR on Breast Cancer Cases

- High concordance rate between the three methods for the cases that are IHC 0 and 1+
- But some of the 3+ cases, confirmed with FISH, were found non-amplified with RT-PCR
- Dilution of DNA from non-malignant cells
but this problem can be overcome by use of precise tissue extraction.

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Conclusions



- **Accurate assessment of HER2 status is essential to ensure that all patients who are likely to benefit from Herceptin are correctly identified**
- **Proper sample handling-fixation (Formalin-fixation and fixation time 6-48h) is very important**
- **High concordance between IHC and FISH for positive and negative HER2 breast cancer cases**
- **SISH or CISH or RT-PCR on paraffin-embedded tissue may represent in the future an alternative approach for HER2 equivocal cases in our Lab**



Thank you