Int. J. Curr. Res. Med. Sci. (2025). 11(5): 17-28

International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

(A Peer Reviewed, Indexed and Open Access Journal)

www.ijcrims.com

Review Article

Volume 11, Issue 5 -2025

DOI: http://dx.doi.org/10.22192/ijcrms.2025.11.05.003

"Immunohistochemistry and FISH in HER2-positive breast cancer: Diagnostic techniques and clinical implications"

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Abstract

HER2-positive breast cancer is a separate molecular subtype that bears significant implications for prognosis and therapy. The precise identification of HER2 status is paramount to the administration of targeted therapies, including trastuzumab and pertuzumab, with which the patients have gained substantial benefits. The two principal techniques for the assessment of HER2 status are Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH). The IHC test measures HER2 overexpression, while FISH detects HER2 gene amplification; this provides complementary information on which to base the treatment decision. This review specifically discusses the underpinning principles, strengths and weaknesses, and clinical applications of HER2 testing via IHC and FISH methods, including their implications for treatment selection and prognosis. The article also discusses problems with accuracy of testing, variances in laboratory practices, and future technologies that may further allow refinement of

HER2 testing. By integrating both IHC and FISH, the clinician can secure a more reliable diagnosis that leads to better defined therapeutic strategies and ultimately better outcome for their patients.

Keywords: HER2-positive breast cancer, Immunohistochemistry (IHC), Fluorescence In Situ Hybridization (FISH), HER2 testing, targeted therapies, gene amplification, diagnostic techniques, clinical implications, breast cancer prognosis, personalized treatment.

Introduction

The HER2 gene is for a protein set on the cell surface and has the vital function in regulating the growth and division of cells. In normal cells, the HER2 protein functions to maintain balance. In cancers such as breast cancer, however, the HER2 gene may be amplified or overexpressed, resulting in an excess of HER2 receptors on the cell surface. The overexpression results in the growth and proliferation of cancer cells in an uncontrolled manner and leads to the formation of HER2-positive breast cancer. The excess HER2 receptors on the cancer cells make cancer more aggressive and predestined to spread.[1,2]

In HER2-positive breast cancer, high concentrations of HER2 receptors trigger tumour growth through signalling pathways that enhance cell proliferation and survival. This breast cancer variant is more prone to recurrence and metastasis than HER2-negative breast cancers. However, the identification of the HER2-positive tumours also resulted in therapeutic approaches, such as trastuzumab (Herceptin) and pertuzumab, which specifically target HER2 receptors and block the action of this receptor blocking the growth of cancer cells. As a result, the prognosis of such patients has tremendously improved with the introduction of these targeted approaches in HER2-positive breast cancer.[3]

Epidemiology

HER2-positive breast cancer is one of the subtypes of breast cancer that accounts for 15 to 20% of the global burden of this malignancy. It was found mostly among younger women, under the usual age of 50, and with more grade tumors. The incidence of HER2 overexpression is slightly higher among Caucasian women than it is in African and Asian women. Out of the total breast cancer cases diagnosed in the United States, approximately 60,000 are HER2-positive breast cancer. This condition is common also in invasive ductal carcinoma (IDC), the predominant type of breast cancer. Although HER2-positive breast cancer affects a smaller proportion of all breast cancer cases, the development of targeted therapies has improved outcomes tremendously in the affected patients, resulting in increased survival rates and reduced recurrence.[4]

HER2 in breast cancer

Biological Role of HER2

With respect to the human epidermal growth factor receptor 2 (HER2), this receptor is placed in the epidermal growth factor receptor (EGFR) superfamily. It is a transmembrane tyrosine kinase receptor responsible for cell growth, survival, and differentiation. In a physiologically normal situation, HER2 expects to regulate cellular functions by transmitting signals from the extracellular environment to the cell nucleus, steering the hypersensitive processes of cell proliferation and cell repair. In contrast to most others in the EGFR family, HER2 is typically activated by dimerization with other receptor partners, without having any known natural ligand. This capacity to form heterodimers readily serves to enhance and amplify growth-promoting signal pathways, particularly the PI3K/Akt and MAPK pathways. While on the correct route, HER2 maintains normal tissue development and maintenance, deviations in HER2 expression levels may disrupt this stochasticity and may in turn allow for oncogenic transformation and aggressive of tumor.[5,6]

Mechanisms of HER2 Overexpression

HER2 overexpression is usually due to the amplification of gene copies in tumor cells, which may be categorized as the classical mechanism of gene amplification leading to the production of multiple copies of the HER2 gene. This, in turn, results in the overexpression of HER2 so that there are huge numbers of HER2 proteins found on the cell surface, significantly increasing the signaling for cell growth and division. Less common mechanisms, which include transcriptional upregulation or post-translational modifications, can also contribute to HER2 protein overexpression. Gene amplification can be identified by techniques such as fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC), measuring in the first instance the number of HER2 genes and in the second the amount of HER2 protein. The massive presence of HER2 proteins leads to activating downstream signaling pathways even in the absence of the growth signal and promotes the hallmarks of malignancy: uncontrolled proliferation, resistance to apoptosis (programmed cell death), and enhanced tumor invasiveness. These mechanisms make HER2-positive tumors even more aggressive and consequently ordeal competent clinically without an appropriate form of targeted treatment.[7,8]

Significance of HER2 Amplification in Breast Cancer Prognosis

The amplification of the HER2 gene in breast cancer definitively impacts patient prognosis. Prior to HER2-targeted therapies being made available, HER2-positive breast cancer was considered to be associated with a poor prognosis due to increased recurrence and metastasis and decreased overall survival in comparison to HER2-negative disease. Therefore, the poorly differentiated tumors, which are aggressive in nature, especially in growth rates, have a higher incidence of lymphatic involvement. The evolution and availability of HER2-targeted therapy have completely transformed this outcome, as patients with HER2-positive disease treated in the present era with targeted therapy, such as trastuzumab (Herceptin), pertuzumab, and the newer approaches like T-DM1 and neratinib, often have survival rates that are comparable to or even better than some of their HER2-negative counterparts. Thus, HER2 amplification is still a marker of biologically aggressive cancer, but it has become the most important predictive tumor marker that impacts the direction toward effective, personalized treatment strategies that have markedly enhanced prognosis.[9,10]

Immunohistochemistry (IHC) for HER2 testing

With the availability of immunohistochemistry (IHC) as a laboratory technique, HER2 status in breast cancer-diagnosed tissues may be assessed. With IHC, antibodies specific to the HER2 protein on the surface of tumour cells are applied. Once bound, the antigen-antibody complex undergoes a visible color change (most often brown staining), which allows measuring HER2 protein expression by considered observation under a microscope. The method is relatively rapid, inexpensive, and extensively available; therefore it is first-second line for testing HER2 in pathology laboratories. It is important to make the correct assessment of HER2 expression because it is the prerequisite for being an eligible patient for anti-HER2 therapy such as trastuzumab (Herceptin) and pertuzumab that yield better outcomes in HER2-positive breast cancer treatment.[11,12]

Principles of IHC

Immunohistochemistry (IHC) involves the specific detection of target proteins in tissue sections using specific antibodies. In the case of HER2 testing, IHC involves specific antibodies directed against the HER2 protein that is expressed on the surface of breast cancer cells. In this instance, the reactive binding of the antibodies on their HER2 proteins is subsequently visualized via an external chemical reaction characterized by a color change, which is generally brown. The brown staining indicates the presence and the quantity of HER2 protein expression in tumor tissue. Such factors, including the degree of staining intensity and the proportion of stained cells, assist in making the

designation HER2-positive or HER2-negative. Accordingly, those designations will guide treatment decisions.[13]

IHC is initiated by harvesting tissues, usually obtained by core needle biopsy or surgical specimen, fixating them in formalin, and embedding them in paraffin to preserve cellular structures. Thin tissue sections are cut and placed on microscope slides for data collection. Subsequently, the slides are deparaffinized and rehydrated, subjected to antigen retrieval, which entails subjecting slides to heat or enzyme treatment for the exposure of HER2 protein epitopes for antibody binding. Subsequently, a specific primary antibody against HER2 is applied, followed by secondary antibody that is conjugated an enzyme such as HRP. After washing steps to remove unbound antibodies, a chromogenic substrate like DAB is added, which produces visible brown staining in sites containing HER2 proteins. Finally, the slides are counterstained (typically with hematoxylin to stain nuclei blue), mounted, and microscopically examined by a pathologist. Careful control and standardization of each reagent and step are absolutely critical to producing accurate, reproducible HER2 test results.[14,15]

IHC Scoring System for HER2

The HER2 IHC scoring system defines how much and what type of HER2 protein is expressed in the tissue of breast cancer. The scale is 0 to 3+, with 0 indicating no staining, and 3+ indicates stains that are very intense or stain all tumor cells on the membrane. Its scoring is:

- **Score 0:** No staining or faint/incomplete membrane staining in <10% of tumor cells. Interpretation: HER2-negative.
- **Score 1+:** Faint/barely perceptible membrane staining in >10% of tumor cells, with incomplete membrane staining. Interpretation: HER2-negative.
- **Score 2+:** Weak to moderate complete membrane staining in over 10% tumor cells. Interpretation: Equivocal - additional testing with ISH techniques such as FISH are required to determine HER2 gene amplification.
- **Score 3+:** Strong, complete, and uniform membrane staining in over 10% of tumor cells. Interpretation: HER2-positive.[16,17]

Advantages:

1. **Accessibility and Widespread Use:**The immunohistochemistry (IHC) process is ubiquitous to all hospital and diagnostic pathology laboratories across the development spectrum. The means of IHC normally includes standard lab equipment; thus, it can also be done in small centers that possess no specialized molecular testing facilities.
2. **Cost-Effectiveness:** Immunohistochemistry is far cheaper than other methods, such as fluorescence in situ hybridization; thus, it becomes the best choice as a screening test, particularly in low-resource settings where healthcare costs become a real issue.
3. **Quick Turnaround Time:**This technique from IHC allows report generation from 24 to 48 hours once the tissue is prepared, making it faster for diagnosis and treatment planning. That is quite critical in aggressive cancers, as in HER2-positive breast cancer, where immediate initiation of treatment improves prognosis.
4. **Visualization of Tissue Architecture:** The presence of HER2 protein overexpression is not only detected by IHC but also the position of expression in respect of tissue morphology, which could help in differentiating true tumor staining from that of non-tumor cells or necrotic areas: the true-tumor versus non-tumor staining.
5. **Standardized Scoring Systems:** The ASCO/CAP guidelines provide clear criteria for scoring HER2 IHC results (0, 1+, 2+, 3+), assisting in improving consistency in different laboratories and pathologists.[18,19]

Disadvantages:

Subjectivity and Variability: IHC interpretation will vary according to the observer, especially in borderline or equivocal cases (e.g., those scored 2+). Even experienced pathologists can differ in their scoring due to such subjectivity.

1. **Technical Sensitivity to Pre-Analytical Factors:** IHC is sensitive to many important aspects of tissue handling, fixation time, and processing techniques: all impact on the results of IHC. Poor fixation may lead to weak or inconsistent staining, contributing to false-negatives or false-positives.
2. **Equivocal Results Requiring Further Testing:** About 15-20% of cases receive 2+ scores (-equivocal), meaning that they cannot be diagnosed through IHC. These cases need to be tested further, generally with FISH or some other in situ hybridization method, which ends up prolonging the diagnostics and raising costs.
3. **Risk of Misinterpretation:** This non-specific background staining, tissue artifacts, or variability in antibody performances could lead to misinterpretation. There might be over- or underestimation of HER2 expression leading to an incorrect classification of patients and con sequently affect their eligibility for lifesaving targeted therapies.
4. **Does Not Detect Gene Amplification Directly:** IHC assesses HER2 protein overexpression, but does not measure the amplification of the HER2 gene at the DNA level directly. Some tumors with HER2 amplification typically also express very little HER2, which can lead to false-negatives by IHC alone.[20,21]

Fluorescence In Situ Hybridization (FISH) for HER2 testing

Fluorescence in situ hybridization (FISH) is a molecular cytogenetic approach aimed at identifying and assessing HER2 gene amplification within tissues affected by breast cancer. Contrary to IHC, which investigates protein expression, FISH directly measures the copy number of the HER2 gene (ERBB2) within tumor cells. FISH is invaluable in ambiguous IHC results (score 2+) where the protein expression is not conclusive, leading to a clear diagnosis of HER2 status. The FISH test is regarded as the gold standard for ascertaining HER2 positivity because of its high sensitivity, specificity, and reproducibility.[22]

Mechanism of FISH in Detecting HER2 Gene Amplification

In FISH testing for HER2 the two probes being utilized are:

- One for the HER2 (ERBB2) gene that gives red fluorescence.
- A control probe, which usually targets the centromere of chromosome 17 (CEP17) that gives green fluorescence, serving as the reference for normalizing gene copy number.

Deparaffinization and rehydration of the tissue section must be done first, along with treatment to expose the DNA before applying labeled probes for hybridization to their specific DNA sequences. After excess unbound probes are thoroughly washed, the slides are counterstained (normally with DAPI, which stains the nuclei blue), and examined under fluorescence microscopy. HER2 amplification is assessed by counting red (HER2) and green (CEP17) signals in at least 20 tumor cell nuclei.[23]

HER2/CEP17 Ratio and Its Clinical Significance:

The heart of FISH interpretation in HER2 testing hinges around determining the HER2/CEP17 ratio, which compares the signal number of the HER2 gene versus those of chromosome 17 centromere (CEP17) in nuclei of tumor cells. The ratio is used to determine whether the HER2 gene is amplified, availing it as a major indicator of more aggressive forms of breast cancer while being predictive concerning the response to HER2-targeted treatments like trastuzumab and pertuzumab.

• HER2/CEP17 ratio - 2.0
→ Amplification of the HER2 gene
→ HER2 positive breast cancer
→ Applicable for treatment with HER2 targeted therapy

• HER2/CEP17: ratio < 2.0 AND Average HER2 copy number < 4.0 signals/cell
→ HER2 negative
→ Not suitable for HER2 targeted therapy

• HER2/CEP17 ratio < 2.0 AND Average HER2 copy number within 4.0-6.0 signals/cell
→ Equivocal
→ Further evaluation or repeat testing needed

• HER2 copy number = or > 6.0 signals/cell (irrespective of ratio)
→ HER2 positive, even if ratio is < 2.0

This dual-criteria (both ratio and absolute copy number) is used to avoid misclassification due to chromosomal abnormalities 17, since such abnormalities may distort the ratio but not absolute copy number.[24,25]

Guidelines for scoring FISH results:

Fish Results Scoring Guidelines According to American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP), organizations that periodically update the recommendations for scoting FISH results, the following are some broad guidelines:

1. Count at least 20 invasive tumor nuclei in areas with signals that are well visualized and evenly distributed.
2. Report both the HER2/CEP17 ratio and the average HER2 copy number per nucleus.
3. Define thresholds for result classification:
Positive: HER2/CEP17 ratio ≥ 2.0 or HER2 copy number ≥ 6.0
Negative: HER2/CEP17 ratio < 2.0 and HER2 copy number < 4.0
Equivocal: HER2 copy number between 4.0 and 6.0 (when ratio is < 2.0)
4. Other repeat tests or alternate test methods like by using a different tissue blocks or IHC should be considered in case of equivocal or borderline cases.

Correct scoring as well as following these well defined criteria, ensure that patients are accurately diagnosed for those therapies, and that the treatments are accordingly directed to maximize benefits and avoid unnecessary treatments.[26,27]

Comparison of IHC and FISH tests

Feature	IHC (Immunohistochemistry)	FISH (Fluorescence In Situ Hybridization)
Principle	Detects **HER2 protein expression** on the tumor cell membrane	Detects **HER2 gene amplification** at the DNA level
Detection Target	HER2 **protein**	HER2 **gene copy number** and chromosome 17 (CEP17)
Methodology	Uses **antibodies** and **chromogenic detection**	Uses **fluorescent DNA probes** to hybridize with HER2 and CEP17
Staining/Signal	Brown color (visible under light microscope)	Fluorescent signals (visible under fluorescence microscope)
Scoring System	0, 1+, 2+ (equivocal), 3+	HER2/CEP17 ratio and/or HER2 copy number per cell
Interpretation Criteria	Based on **staining intensity** and **percentage of stained cells**	Based on **ratio ≥2.0** or **HER2 copies ≥6.0 per cell**
Equivocal Range	IHC 2+ (requires confirmatory FISH)	HER2/CEP17 ratio < 2.0 with HER2 copies between 4.0–6.0
Turnaround Time	Rapid (1–2 days)	Longer (2–4 days or more)
Cost	Relatively **low**	Relatively **high**
Availability	Widely available in most pathology labs	Requires **specialized equipment and training**

Subjectivity	Somewhat **subjective**; prone to inter-observer variability	**More objective**; quantitative assessment
Best Use	**Initial screening** method	**Confirmatory test**, especially for IHC 2+ cases
Tissue Requirement	Formalin-fixed, paraffin-embedded tissue	Same, but **higher quality fixation** required
Advantages	Quick, affordable, maintains tissue architecture	High specificity and sensitivity, direct gene measurement
Limitations	May yield equivocal or false results due to technical variability	More expensive, longer processing time, needs fluorescent microscopy[28,29]

Clinical applications of IHC and FISH in HER2 testing

1. Initial Diagnosis and Prognosis:

Immunohistochemistry, or IHC, and fluorescence in situ hybridisation (FISH), are two equally mandatory tests that one can perform at diagnosis for assessment of HER2 status in breast cancer patients. HER2 overexpressed detected via IHC places a patient in the high-risk group for more aggressive tumors and poor prognosis. IHC assists in identifying the level of HER2 protein on cell surface tumor cells. Thus, a 3+ result score is indicative of strong HER2 positivity and implies the tumors are likely to respond to HER2-targeted therapies such as trastuzumab.

On the other hand, in equivocal cases with positive IHC 2+ results, FISH testing is relied on to confirm the presence of amplification of the HER2 gene. In fact, the positive score from FISH testing (as in HER2/CEP17 ratio greater than 2) will fortify the diagnosis of HER2-positive breast cancers and, in its place, direct the most appropriate treatment that should be considered. Certainly, to an accurate HER2 status picture, also, this test predicts recurrence and metastasis, thus aiding the clinician in forming prognosis towards patients.[30]

2. Guiding Treatment Decisions:

The primacy of IHC and FISH in the clinical setting is to assist in determining treatment strategies. HER2-positive breast cancers respond extremely well to antitumor agents, particularly trastuzumab (Herceptin) and pertuzumab, which act by blocking HER2 receptors to inhibit growth of cancer cells. In cases where the tumor is HER2-positive, these targeted drugs will form an integral part of its treatment regimen, either as monotherapy or in conjunction with chemotherapy.

For borderline HER2 expression (IHC 2+), FISH is usually done to assess HER2 gene amplification. If FISH is positive for amplification, targeted treatment is recommended. On the contrary, HER2-negative tumors derive no benefit from these targeted agents, and treatment focuses mainly on standard chemotherapy or hormonal therapy in the case of hormone receptor-positive cancers.[31]

3. Monitoring Treatment Response:

IHC and FISH are also invaluable tools in the course of HER2-targeted therapy for monitoring responses to it. Once the treatment with trastuzumab has started, repeated testing can be done to determine whether the tumor overexpresses HER2 anymore or if there is any change in gene amplification.

Re-testing for HER2 status may indicate possible changes in a patient's HER2 expression in the case of therapy failure or development of resistance against HER2-targeted treatment. For instance, a tumor that was at first HER2 positive may convert to an HER2-negative phenotype; thus, a different regime of treatment is required.[32]

4. Assessment of Metastatic Disease:

In tumors of metastatic breast cancer, HER2 testing provides the clinician with evidence to determine if the metastatic tumors retain the HER-2 status of the primary tumor. If an HER-2-negative tumor metastasizes, it may become associated with HER-2 over-expression; hence WHO recommends retesting the HER-2 status of the metastases to assess whether HER-2-targeted therapy could be offered.

In cases of metastatic HER-2-positive disease, FISH testing on biopsies obtained from the metastatic site to confirm HER-2 amplification is possible. In such instances, HER-2-targeted therapies can tremendously improve overall survival through the control of tumor progression.

5. Evaluation of HER2-low Tumors:

Especially with newer studies demonstrating that tumors with low HER2 expression (IHC 1+ or 2+ without gene amplification) may be the candidates for HER2-targeted therapies, this concept of HER2-low breast cancer is broadening. IHC and FISH methods would then come into play to identify these patients, who were classically considered not to be candidates for HER2-targeted therapies. This new therapeutic approach may find application in trastuzumab deruxtecan, an antibody-drug conjugate effective in HER2-low tumors, potentially widening treatment access to a larger cohort of patients.

6. Predicting Tumor Behavior and Recurrence Risk:

HER2 testing is useful to predict the behavior of breast cancer tumors. HER2-positive cancers are aggressive cancer types and risk a higher recurrence rate. These patients can be classified into high and low-risk categories through the IHC and FISH biopsy tests, making possible different follow-up schemes. Patients with HER2-positive tumors may need more close supervision of recurrence despite lower levels in patients with HER2-negative tumors, although this group receives routine care and prevention measures.[33,34]

Challenges in HER2 Testing

1. Variability in Testing Methods:

HER2 testing can be done with many methods including IHC-immunohistochemistry or FISH-fluorescent in situ hybridization. Different antibodies or reagents used in the labs may result in discrepancies. For example, IHC is subjective since pathologists judge the staining intensity differently. FISH is quite objective, but the probe quality or technical faults may cause misinterpretation. This leads to discrepancy in test results especially when IHC shows borderline or equivocal scores (e.g. 2+), which are to be confirmed using FISH.[35]

2. Inconsistent Sample Quality:

The characteristics of the tissue sample are critical to the accurate assessment of HER2. If the sample is poorly fixed or stored, any number of false-negative or false-positive results could be obtained. For instance, if the tissue is over-fixed or under-fixed, the cell-tissue architecture may be altered in such a way as to compromise HER2 protein detection. Another relevant consideration in testing accuracy of HER2 is the tumor content in the sample; it is an essential parameter that can give unreliable results if not assessed correctly, consequently delaying the diagnosis or definitive treatment.

3. Interpretation of Equivocal Results:

There are times when HER2 testing can yield indeterminate results especially in IHC scoring for which a score of 2+ is considered borderline. It means that a test is neither positive or negative and needs further confirmation usually undertaken with FISH. This can be annoying because it delays treatment decisions and consumes additional costs and time for confirmation of HER2 status. Often, the management of such equivocal cases lacks consistency in various clinical guidelines, and this adds to the complexity of the decision-making process by doctors and their patients.[36]

4. HER2 Heterogeneity in Tumor Samples:

Heterogeneity of the tumors indicates that different portions in the entire tumor can have different amounts of HER2 expression. It may lead to false-negative or false-positive results, particularly when small tissue samples are obtained, which would not represent the tumor entirely. Some tumor cells might overexpress HER2, while some do not overexpress it, which can introduce a problem in the testing. This defect will cause unpredictable results, particularly small and poorly distributed sample sizes.

5. Tumor Evolution and HER2 Status Changes:

HER2 status may also change over time. A tumor may be HER2-positive at first, but it may evolve to HER2-negative later, especially during the course of treatment. This is due to the selection of resistant cancer cells not expressing HER2. For example, HER2-negative cells may survive and continue to proliferate after HER2-directed therapy, leading to a relapse or metastasis. Rechecking HER2 status at recurrence or metastasis is quite important, as it may determine the next step in treatment.

6. Financial and Logistical Barriers:

Testing for HER2 can prove to be costly, particularly with FISH that requires particular instruments and expertise. These may not be available in some settings, thus risking delays in diagnosis and treatment. Some insurance companies may even deny coverage for confirmatory testing, particularly when the finding of the first test is inconclusive. These expenses may be prohibitive for some patients, delaying or preventing them from getting treatment using the right agents in a timely manner.[37]

Conclusion

Accurate HER2 testing serves as the keystone in the diagnosis and management of breast cancers that are HER2 positive. The Immunohistochemistry (IHC) test and the Fluorescence In Situ Hybridization (FISH) test are important diagnostic methods with specific strengths and specific drawbacks. While IHC is the most common, economical and reasonable method of HER2 protein overexpression assessment, FISH provides greater assurance in an amplification measure and is an important consideration when IHC results are equivocal. In combination, both approaches lead to a better understanding of HER2 status and guide personalized treatment decisions. While these have their inherent merits, problems-such as testing practice variation and resulting false-positive or false-negative results-still plague them. Further improvements in the characterisation and treatment approaches will be afforded by the continuing research and development of methods such as liquid biopsy and next-generation-sequencing for HER2 detection. Therefore, the future of treatment for HER2-positive breast cancer patients will rely on the confluence of robust tests, adherence to standard guidelines, and any further studies that may arise with these technologies. In the relentless evolution of this field, IHC and FISH would continue to play an elementary role in HER2 testing in clinical decision-making, patient outcome optimization, and, ultimately, breast cancer therapy advancement.

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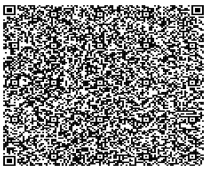	Website: www.ijcrims.com
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How to cite this article:
Bunny willies. Kona, Annie Rishitha Kiran Nalukurthi, Kamavarapu Bala Swaroop Rayal, Dr. Tanwir Ahamad, R.Roshinee, Priyanka Gadi, R.V.D Sai Sarvani, Nirmalkumar T, Sathishkumar S. (2025). "Immunohistochemistry and FISH in HER2-positive breast cancer: Diagnostic techniques and clinical implications". Int. J. Curr. Res. Med. Sci. 11(5): 17-28.
DOI: http://dx.doi.org/10.22192/ijcrms.2025.11.05.003