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(REVIEW ARTICLE)

# HER-2 low in breast cancer: A new perspective for anti-HER-2 therapy

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## Abstract

HER-2 is a transmembrane receptor of the Epidermal Growth Factor Receptor (EGFR) subfamily that functions physiologically in normal cell proliferation and differentiation. HER-2 overexpression is found in 20–30% of breast cancer patients. Thus, HER-2 is established as a prognosis marker and therapeutic target for anti-HER-2. The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) issued guidelines for HER-2 classification based on immunohistochemical (IHC) and in situ hybridization (ISH) examinations. Before ASCO/CAP issued the latest update to their guidelines, HER-2 classification was divided into HER-2 negative, equivocal, and positive. Only patients with HER-2 positive (HER-2 overexpression) received anti-HER-2 therapy. After the DESTINY-Breast04 trial demonstrated the efficacy of trastuzumab deruxtecan in breast cancer with low HER-2 levels, ASCO/CAP officially updated the guidelines by introducing a low HER-2 classification, which is HER-2 expression with an immunohistochemical (IHC) score of 1+ or 2+ without HER-2 gene amplification on in situ hybridization (ISH). Trastuzumab deruxtecan exerts a substantial cytotoxic burden on HER-2-expressing tumor cells, significantly improving progression-free survival and overall survival compared with conventional chemotherapy. Reclassification of low HER-2 tumors bridges a critical gap in breast cancer management, providing a new perspective for patients with low HER-2 expression with anti-HER-2 therapy, thus highlighting the importance of accurate identification of HER-2 for prognosis determination and therapeutic reference in breast cancer. This review highlights HER-2 classification, the importance of IHC and ISH assays for HER-2 expression, and anti-HER-2 therapy options for HER-2 positive and HER-2 low based on the latest guidelines.

**Keywords:** breast cancer; HER-2 low; trastuzumab-deruxtecan; dish-testing

## 1. Introduction

Breast cancer is well recognized as a heterogeneous disease consisting of multiple molecular subtypes that exhibit distinct behaviours, therapeutic responses, and clinical outcomes. The expression of Human Epidermal Growth Factor Receptor-2 (HER-2) serves as one of the key biomarkers in defining these molecular subtypes. HER-2, or ErbB2/neu, is a transmembrane glycoprotein receptor belonging to the Epidermal Growth Factor Receptor (EGFR) subfamily of the Receptor Tyrosine Kinase (RTK) family. Under normal physiological conditions, HER-2 regulates critical cellular processes such as cell proliferation, survival, and differentiation (Rubin et al., 2024). The HER-2 gene was first identified in 1985, but its clinical correlation with breast cancer was confirmed in 1987 (Maadi et al., 2021).

HER-2 activation occurs through dimerization, which can take the form of homodimerization or heterodimerization with other HER family members. This activation triggers key downstream signalling pathways, including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K/Akt) pathways. These pathways are essential for maintaining cellular homeostasis; however, dysregulation or overexpression of HER-2 leads to uncontrolled cell proliferation, resistance to apoptosis, and eventual oncogenesis (Rubin et al., 2024). HER-2 overexpression is observed

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in approximately 20–30% of breast cancer cases and is strongly associated with aggressive tumor behaviour, increased risk of metastasis, and poor prognosis.

The clinical relevance of HER-2 overexpression in breast cancer has positioned it as a critical target for therapeutic intervention. HER-2 expression testing is essential for predicting whether a patient is eligible for anti-HER-2 therapies, such as monoclonal antibodies. HER-2 status can be assessed using immunohistochemistry (IHC) to evaluate protein expression or in situ hybridization (ISH) techniques, including fluorescence in situ hybridization (FISH) and dual in situ hybridization (DISH), to determine HER-2 gene amplification. The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) released updated guidelines in 2023, which introduced a new HER-2 classification: HER-2 negative, HER-2 low, and HER-2 positive. HER-2 low, a recently recognized category, identifies tumors with an IHC score of 1+ or 2+ without HER-2 gene amplification on ISH. This reclassification necessitates new therapeutic perspectives for HER-2 low tumors, as emerging evidence supports the efficacy of novel HER-2-targeted therapies in this subset.

This review article aims to comprehensively discuss the significance of HER-2 testing in breast cancer patients as both a prognostic biomarker and a therapeutic guide. It highlights HER-2 classification, the importance of IHC and ISH assays for HER-2, and anti-HER-2 therapy options for HER-2 positive and HER-2 low based on the latest guidelines.

## 2. Review

### 2.1. Human Epidermal Growth Factor Receptor-2 (HER-2)

#### *2.1.1. HER-2 in Normal Cell Proliferation*

Normal human cells possess a variety of receptors that facilitate signal transduction from the extracellular environment, one of which is Human Epidermal Growth Factor Receptor-2 (HER-2). HER-2, a member of the Epidermal Growth Factor Receptor (EGFR) subfamily within the Receptor Tyrosine Kinase (RTK) family, regulates normal cell proliferation, differentiation, and tissue maintenance. In breast tissue, HER-2 is expressed at low levels under physiological conditions and is essential for mammary gland development (Gutierrez and Schiff, 2011). During key stages such as puberty, pregnancy, and lactation, HER-2 signalling regulates ductal morphogenesis and alveolar development, ensuring proper formation and branching of mammary ducts and the development of milk-producing lobules in preparation for breastfeeding. These processes are tightly controlled to maintain epithelial cell homeostasis, structural integrity, and functional adaptability of breast tissue (Rubin et al., 2024)

Structurally, HER-2 consists of three domains: extracellular, transmembrane, and intracellular. The intracellular domain catalyzes catalytic activity, mediating signalling through tyrosine residue phosphorylation. The transmembrane domain facilitates structural signal transduction into the intracellular domain via dimerization involving protein kinases. The extracellular domain binds to ligands, triggering downstream signalling cascades (Qi, Yu et al., 2024). Unlike other HER family members, HER-2 is unique because it remains structurally primed for dimerization, even without ligand binding.

In normal breast epithelial cells, HER-2 facilitates signal transduction primarily through heterodimerization with other HER family receptors to activate downstream pathways like mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/Akt (Raghav and Moasser, 2022). These pathways mediate cell proliferation, survival, and repair, ensuring tissue regeneration and wound healing after minor injuries or physiological stress. HER-2 signalling allows epithelial cells to respond efficiently to physiological demands while maintaining cellular balance. Tight regulation of HER-2 expression prevents excessive signalling, ensuring mammary gland homeostasis and tissue function are maintained under normal conditions (Hayat et al., 2023).

#### *2.1.2. HER-2 Overexpression in Breast Cancer*

In a study by Martínez-Sáez and Aleix (2021), it was reported that more than 20–30% of breast cancer patients had HER-2 expression. HER-2 overexpression in breast cancer not only leads to hyperactivated signalling but also disrupts the regulatory balance between cell proliferation and apoptosis. The aggressive behaviour of HER-2-overexpressing tumors is often driven by its ability to bypass cell cycle checkpoints, promoting uncontrolled mitotic activity and tumor expansion. Breast cancer cells can harbor 25–50 copies of this gene, leading to a 40–100 times increase in HER-2 protein expression (Iqbal and Naveed. 2014).

Overexpressed HER-2 amplifies the activation of downstream pathways, particularly the PI3K/Akt and MAPK cascades, which inhibit pro-apoptotic signals and enhance cellular survival mechanisms (Raghav and Moasser, 2022). It enables

cancer cells to evade programmed cell death, a critical hallmark of malignancy. Heterodimerization in HER-2 overexpression is more commonly found with HER-3. This heterodimerization forms the most potent signalling pair that drives aggressive tumor growth. Moreover, HER-2 overexpression induces increased genomic instability, further driving tumor heterogeneity and resistance risk to conventional therapies (Pan et al., 2024).

In addition to amplification and overexpression, HER-2 activity can be influenced by complex regulatory mechanisms. One such mechanism is its crosstalk with estrogen receptors (ER), where ER activation can induce HER-2 expression, thereby enhancing it signalling activity (Iqbal and Naveed, 2014). Epigenetic factors, such as DNA methylation, transcriptional regulation via promoter regions, and neddylation also contribute to HER-2 upregulation. Elevated HER-2 expression is further associated with enhanced angiogenesis and increased cellular migration which facilitating metastasis to distant tissues (Raghav and Moasser, 2022). These cumulative effects of HER-2 overexpression underscore its role in tumorigenesis and disease progression, highlighting the importance of anti-HER-2 therapies for improved patient outcomes.

### 2.2. HER-2 Detection in Breast Cancer

HER-2 detection is crucial to breast cancer patients because the overexpression result will be significant in prognostic evaluation and selection of the most effective targeted therapies. The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) first developed comprehensive guidelines for HER-2 detection in breast cancer in 2007 (Wolff et al., 2023). Since then, the guidelines have changed to incorporate newer advances in the research and science concerning HER-2.

#### *2.2.1. Immunohistochemistry/IHC As a Tool for Evaluating HER-2 expression*

HER-2 overexpression is characterized by the increased expression of the HER-2 protein, which can be detected using immunohistochemistry (IHC). IHC is a widely used immunological technique for detecting specific antigens in tissue samples through antibodies. These antibodies target antigens and are visualized via enzyme or fluorochrome-based signals, providing clear histological evaluations (Janardhan et al., 2018). Although immunohistochemical testing has been in use since 1942, it was not until 1998 that it became the gold standard for HER-2 assay in breast cancer diagnostics (Jorgensen et al., 2021). The current standard operating procedure recommends using the PATHWAY anti-HER-2/neu (4B5) antibody for HER-2 testing (Garrido et al., 2024).

The IHC scoring system combines staining intensity and the percentage of positively stained cells to quantify protein expression levels, resulting in scores of 0, 1+, 2+, or 3+ (Wolff et al., 2023). The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have standardized guidelines for interpreting HER-2 expression. Previously, HER-2 expression was classified into three groups: HER-2 negative, HER-2 equivocal (ambiguous), and HER-2 positive (Bianchi et al., 2015). Equivocal cases (1+ or 2+) required in situ hybridization (ISH) confirmation to assess HER-2 gene amplification.

The 2023 ASCO/CAP update introduced a new classification, HER-2 low, which includes tumors with IHC scores of 1+ or 2+ without gene amplification as determined by ISH testing (Ivanova et al., 2023). An IHC score of 0 reflects no staining or incomplete membrane staining in <10% of tumor cells. A score of 1+ indicates faint or incomplete staining in >10% of tumor cells, while a score of 2+ corresponds to weak-to-moderate staining in >10% of tumor cells or intense staining in ≤10%. A score of 3+ signifies complete and intense staining in >10% of tumor cells, confirming HER-2 positivity (Ivanova et al., 2024).

IHC's primary advantage lies in its ability to directly evaluate histological protein expression rapidly, simply, and cost-effectively (Janardhan et al., 2019). However, its limitations include interpretative subjectivity, particularly in borderline cases, and pre-analytical variables such as tissue fixation, processing, sample quality, and antibody affinity. Intratumoral heterogeneity poses a challenge, as HER-2 expression may vary within the same tumor, leading to inconsistent results (Pellas et al., 2023). These limitations underscore the need for high-quality samples, comprehensive tissue sampling, and meticulous pathologist evaluation to achieve accurate IHC results.

#### *2.2.2. In Situ Hybridization/ISH (FISH and DISH) As a Tool for Evaluating HER-2 Amplification*

The recommended molecular technique used to detect HER-2 gene amplification at the DNA level is in situ hybridization (ISH). Before the ASCO/CAP guideline was updated, ISH assay was only recommended in patients with equivocal IHC (score 2+). After the guideline update, ISH is recommended for patients with IHC scores of 1+ or 2+, where further confirmation of HER-2 status is required (Schlam et al., 2023). Among the available ISH methods, the most widely used are FISH (fluorescence in situ hybridization) and DISH (dual in situ hybridization).

Fluorescence In Situ Hybridization (FISH) employs fluorescent-labelled probes to identify HER-2 gene amplification on chromosome 17. As the gold standard for HER-2 detection, FISH offers high sensitivity and accuracy (Mansfield et al., 2013). However, it requires specialized fluorescence microscopy and is expensive. Furthermore, fluorescence signals are prone to fading over time, necessitating prompt analysis after staining, which limits long-term result storage (Rathi et al., 2023).

In contrast, dual In Situ Hybridization (DISH) uses chromogenic probes that allow HER-2 gene detection under a standard light microscope. DISH utilizes two probes: one specific to the HER-2 and the second for chromosome 17 (CEP17), serving as an internal control to calculate the ratio of HER-2/CEP17. DISH has become a preferred method in many laboratories due to its cost-effectiveness, better signal stability, and accessibility in resource-limited settings. Unlike FISH, DISH allows for prolonged result analysis and direct tissue morphology evaluation, enhancing diagnostic value (Horii et al., 2013; Mansfield et al., 2013). The ASCO/CAP guidelines recommend DISH as a complementary test to immunohistochemistry for determining HER-2 expression.

The ASCO/CAP 2023 guidelines classify ISH results into five distinct categories. The first category is for patients with ≥ 2 HER-2/CEP17 ratio and ≥ 4 signals per cell HER-2 copy number. The result is classified as ISH amplified, indicating eligibility for HER-2 targeted therapy. Same as the first category, the second category only differs because it has < 4 signals per cell. Further evaluation with IHC is required. If the result is 3+, it is classified as a positive HER-2 expression. If the result is 2+, then a repeat ISH involving an additional observer unaware of the previous result is required. Using +2 staining, the observer should count at least 20 cells covering the invasive area of the cancer. If the second result changes, the result should be reviewed according to internal procedures to determine the conclusion. If the result remains unchanged, it is classified as a negative HER-2 expression, similar to cases where the result is 1+.

The ratio of HER-2/CEP17 for the third to fifth category is <2. The third category is for the patient with ≥ 6 signals per cell of average copy number and ≥ 4, but < 6 is for the fourth category. Further evaluation with IHC is required. If the result is 3+, it is classified as a positive HER-2 expression. If the result is 2+, then a repeat ISH involving an additional observer unaware of the previous result is required. Using +2 staining, the observer should count at least 20 cells covering the invasive area of the cancer. If the second result changes, the result should be reviewed according to internal procedures to determine the conclusion. If the result remains unchanged, it is classified as positive for the third category and negative HER-2 expression for the fourth category. It is classified as a positive HER-2 expression if the result is 1+ for the third category but negative for the fourth category. The fifth category is for patients with < 4 signals per cell of average copy number. The result is classified as non-amplified HER-2, indicating it is not eligible for HER-2 targeted therapy.

In addition to classification, ASCO/CAP guidelines outline specific criteria for test validation or repeat analysis. Invalid tests occur under the following conditions: when controls fail to produce appropriate results, fewer than two invasive tumor areas are identified and analyzed, more than 25% of signals are unscorable due to weak signals, more than 10% of signals occur over cytoplasm, poor nuclear resolution, and strong autofluorescence. If even one of these criteria is met, the test is considered invalid and must be repeated for reliable assessment (Ivanova et al., 2023).

Despite standardized guidelines, interpreting HER-2 results can be complex, particularly in cases of HER-2 protein overexpression or borderline ISH findings. Factors such as monosomy of chromosome 17 can skew HER-2/CEP17 ratios, leading to false-positive results (Brunelli et al., 2015). Additionally, intratumoral heterogeneity may result in inconsistent HER-2 amplification across different tumor regions, complicating diagnosis and classification. Routine clinical practice lacks molecular tests, such as RNA expression profiling or protein array analysis, that could provide complementary data for HER-2 testing.

### 2.3. Update for HER-2 Assay Reporting in Breast Cancer

#### *2.3.1. Previous Reporting System*

HER-2 classification is essential for breast cancer, as HER-2 serves as a critical biomarker for prognosis and therapeutic decision-making. Accurate classification of HER-2 expression is pivotal in guiding targeted treatment strategies and predicting clinical outcomes. HER-2 determination is primarily performed using immunohistochemistry (IHC) as the initial assay. For equivocal cases, further confirmation is done through in situ hybridization (ISH) techniques such as dual in situ hybridization (DISH) (Memon et al., 2022). The ASCO/CAP guidelines are the widely accepted reference for interpreting HER-2 status.

Before the amendments introduced by ASCO/CAP, HER-2 status was classified into three categories: HER-2 negative, HER-2 equivocal, and HER-2 positive (Farshid et al., 2020). Patients with HER-2 negative status were defined as those with an IHC score of 0 or 1+. HER-2 equivocal status applied to patients with an IHC score of 2+, requiring further testing via ISH to assess HER-2 gene amplification. If ISH results were not amplified, HER-2 expression was interpreted as HER-2 negative. Conversely, if ISH results showed HER-2 amplification, HER-2 expression was confirmed positive (Ahn et al., 2020). Patients classified as HER-2 positive exhibited an IHC score of 3+ or an IHC score of 2+ with an amplified ISH result. Only HER-2-positive patients are eligible for treatment, as they have been shown to respond well to anti-HER-2 monoclonal antibody therapies.

Therapies in HER-2 positive are very diverse. Some are trastuzumab, lapatinib, pertuzumab, and Trastuzumab emtansine (T-DM1). Trastuzumab is a first-line monoclonal antibody used in HER-2 positivity for a long time and is reported to have effective clinical outcomes. It selectively binds to extracellular domain IV to inhibit receptor dimerization. The inhibition blocks the downstream signalling pathways PI3K/AKT, MAPK, and so on to control cancer cell proliferation. Trastuzumab also destroys HER-2-expressing tumor cells by increasing the activation of antibody-dependent cellular cytotoxicity (ADCC) that recruits immune cells (Pereira et al., 2018).

Lapatinib is a tyrosine kinase inhibitor (TKI) whose mechanism blocks downstream signalling by targeting the intracellular kinase domain. Lapatinib is an alternative for patients who experience resistance to trastuzumab. Lapatinib can also be combined with other drugs in certain circumstances, such as advanced HER-2 positive can be combined with capecitabine and letrozole combination in the luminal subtype (Cheng. 2024).

Pertuzumab is another type of monoclonal antibody that targets extracellular domain II, inhibiting heterodimerization with other receptors, especially HER-3 as strongest heterodimer of HER-2. Pertuzumab will significantly improve treatment effectiveness when combined with trastuzumab and chemotherapy (Pan et al., 2024). T-DM1 is a drug option that selectively delivers cytotoxic agents to HER-2 that can reduce systemic toxicity. T-DM1 will release DM1 to disrupt microtubule function so that apoptosis of cancer cells can occur.

*2.3.2. Updated Reporting System*

The DESTINY-Breast04 study conducted in 2022 demonstrated the effectiveness of treatment for HER-2 low breast cancer, particularly in metastatic cases. In response, ASCO/CAP introduced the HER-2 low classification in 2023. The updated classification now distinguishes HER-2 status into three categories: HER-2 negative, HER-2 low, and HER-2 positive (Wolff et al., 2023). HER-2 negative is for the patient with a 0 IHC score. HER-2 low is for the patient with a 1+ or 2+ IHC score accompanied by a non-amplified ISH result. This group corresponds to the second, fourth, or fifth categories of ISH, as defined by the HER-2/CEP17 ratio and the average HER-2 signal copy number per cell. HER-2 positive is for patients with a 3+ IHC score or patients with a IHC score 1+ or 2+, confirmed by amplified ISH result.

Before the HER-2 low classification was established, there was no clear boundary between IHC scores of 0 and 1+. This ambiguity often led to misclassification and inconsistent evaluation of HER-2 status, particularly in cases of very low HER-2 protein expression detectable by IHC. Consequently, patients with an IHC score of 1+ were previously grouped with an IHC score of 0 despite emerging evidence of significant biological and clinical differences between the two groups.

The introduction of the HER-2 low classification has provided a clearer demarcation, with an IHC score of 1+ now recognized as indicating low HER-2 expression with potential biological significance. Recent studies have shown that over 50% of breast cancer patients fall into the HER-2 low category as of 2023 (Shirman et al., 2023). This heterogeneous population primarily includes luminal A and luminal B subtypes, as well as other hormone receptor (HR)-positive breast cancer subtypes (Schettini et al., 2022).

Trastuzumab deruxtecan is reported to be effective in HER-2 low, so it is the main choice of therapy in this classification (Shirman et al., 2023; Schettini et al., 2022). Trastuzumab deruxtecan has a cytotoxic topoisomerase I inhibitory mechanism specifically targeted at HER-2 cancer cells that can preserve healthy tissue. After binding to the receptor, the drug undergoes internalization by releasing deruxtecan intracellularly to induce DNA damage and apoptosis. The proof of trastuzumab was done last 2022 in the phase III DESTINY-BREAST04 trial. In the trial, trastuzumab deruxtecan significantly increased the survival rate of HER-2 patients, making it a breakthrough for low-grade HER-2 breast cancer, especially in low HER-2 patients with metastasis (Yang et al., 2023; Tarantino et al., 2022).

The efficacy of trastuzumab deruxtecan highlights the importance of accurately identifying HER-2 low tumors through IHC and DISH testing. This classification bridges a previously unmet clinical need, offering targeted therapy to a

population historically excluded from HER-2-directed treatments. Moreover, its precise mechanism of action underscores the potential of deruxtecan trastuzumab to target tumor cells while minimizing systemic toxicity selectively (Corti et al., 2023).

## 3. Conclusion

Scientific evidence supports that anti-HER-2 monoclonal antibody therapy, particularly trastuzumab deruxtecan, demonstrates significant clinical benefits for breast cancer patients with low HER-2 expression. This breakthrough has paved the way for updated HER-2 classification guidelines, emphasizing the importance of accurate HER-2 testing. These advancements provide new therapeutic opportunities and renewed hope for patients previously excluded from HER-2-targeted treatments.

## Compliance with ethical standards

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The authors declare that there is no conflict of interest related to this publication.

## References

[1] Ahn, S., Woo, J.W., Lee, K. and Park, S.Y. (2020). HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. Journal of Pathology and Translational Medicine, [online] 54(1), pp.34–44. doi: https://doi.org/10.4132/jptm.2019.11.03.

[2] Ballard, M., Jalikis, F., Krings, G., Schmidt, R.A., Chen, Y.-Y., Rendi, M.H., Dintzis, S.M., Jensen, K.C., West, R.B., Sibley, R.K., Troxell, M.L. and Allison, K.H. (2016). 'Non-classical' HER2 FISH results in breast cancer: a multi-institutional study. Modern Pathology, 30(2), pp.227–235. doi:https://doi.org/10.1038/modpathol.2016.175.

[3] Bianchi, S., Saverio Caini, Paglierani, M., Saieva, C., Vezzosi, V., G. Svegliati Baroni, Simoni, A. and Palli, D. (2014). Accuracy and Reproducibility of HER2 Status in Breast Cancer Using Immunohistochemistry: A Quality Control Study in Tuscany Evaluating the Impact of Updated 2013 ASCO/CAP Recommendations. Pathology & Oncology Research, 21(2), pp.477–485. doi:https://doi.org/10.1007/s12253-014-9852-0.

[4] Brunelli, M., Alessia Nottegar, Bogina, G., Caliò, A., Cima, L., Eccher, A., Vicentini, C., Marcolini, L., Scarpa, A., Pedron, S., Brunello, E., Sakari Knuutila, Sapino, A., Marchiò, C., Bria, E., Molino, A., Carbognin, L., Tortora, G., Jasani, B. and Miller, K. (2015). Monosomy of chromosome 17 in breast cancer during interpretation of HER2 gene amplification. American Journal of Cancer Research, [online] 5(7), p.2212. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC4548332/

[5] Cheng, X. (2024). A Comprehensive Review of HER2 in Cancer Biology and Therapeutics. Genes, [online] 15(7), pp.903–903. doi:https://doi.org/10.3390/genes15070903.

[6] Chhikara, B.S. and Parang, K. (2022). Global Cancer Statistics 2022: the trends projection analysis. Chemical Biology Letters, [online] 10(1), p.451. Available at: https://pubs.thesciencein.org/journal/index.php/cbl/article/view/451.

[7] Farshid, G., Dhatrak, D., Gilhotra, A., Koszyca, B. and Nolan, J. (2020). The impact of 2018 ASCO-CAP HER2 testing guidelines on breast cancer HER2 results. An audit of 2132 consecutive cases evaluated by immunohistochemistry and in situ hybridization. Modern Pathology, 33(9), pp.1783–1790. doi:https://doi.org/10.1038/s41379-020-0555-7.

[8] Gutierrez, C. and Schiff, R. (2011). HER2: biology, detection, and Clinical Implications. Archives of Pathology & Laboratory Medicine, [online] 135(1), pp.55–62. doi:https://doi.org/10.1043/2010-0454-RAR.1.

[9] Horii, R., Matsuura, M., Iwase, T., Ito, Y. and Akiyama, F. (2013). Comparison of dual-color in-situ hybridization and fluorescence in-situ hybridization in HER2 gene amplification in breast cancer. Breast Cancer, 21(5), pp.598–604. doi:https://doi.org/10.1007/s12282-012-0436-0.

[10] Ivanova, M., Francesca Maria Porta, D'Ercole, M., Pescia, C., Elham Sajjadi, Giulia Cursano, Elisa De Camilli, Pala, O., Mazzarol, G., Venetis, K., Guerini-Rocco, E., Curigliano, G., Viale, G. and Fusco, N. (2023). Standardized pathology report for HER2 testing in compliance with 2023 ASCO/CAP updates and 2023 ESMO consensus statements on HER2-low breast cancer. Virchows Archiv, 484(1). doi:https://doi.org/10.1007/s00428-023-03656-w.

[11] Janardhan, K.S., Jensen, H., Clayton, N.P. and Herbert, R.A. (2018). Immunohistochemistry in Investigative and Toxicologic Pathology. Toxicologic Pathology, [online] 46(5), pp.488–510. doi:https://doi.org/10.1177/0192623318776907.

[12] Jørgensen, M.S., Storebø, O.J., Stoffers-Winterling, J.M., Faltinsen, E., Todorovac, A. and Simonsen, E. (2021). Psychological therapies for adolescents with borderline personality disorder (BPD) or BPD features—A systematic review of randomized clinical trials with meta-analysis and Trial Sequential Analysis. PLOS ONE, 16(1), p.e0245331. doi:https://doi.org/10.1371/journal.pone.0245331.

[13] Maadi, H., Soheilifar, M.H., Choi, W.-S., Moshtaghian, A. and Wang, Z. (2021). Trastuzumab Mechanism of Action; 20 Years of Research to Unravel a Dilemma. Cancers, [online] 13(14), p.3540. doi:https://doi.org/10.3390/cancers13143540.

[14] Mansfield, A.S., Sukov, W.R., Eckel-Passow, J.E., Sakai, Y., Walsh, F.J., Lonzo, M., Wiktor, A.E., Dogan, A. and Jenkins, R.B. (2013). Comparison of Fluorescence In Situ Hybridization (FISH) and Dual-ISH (DISH) in the Determination of HER2 Status in Breast Cancer. American Journal of Clinical Pathology, 139(2), pp.144–150. doi:https://doi.org/10.1309/ajcp13gjaojayjmw.

[15] Martin, M. and López-Tarruella, S. (2016). Emerging Therapeutic Options for HER2-Positive Breast Cancer. American Society of Clinical Oncology Educational Book, 36(36), pp.e64–e70. doi:https://doi.org/10.1200/edbk_159167.

[16] Memon, R., Prieto Granada, C.N., Harada, S., Winokur, T., Reddy, V., Kahn, A.G., Siegal, G.P. and Wei, S. (2021). Discordance Between Immunohistochemistry and In Situ Hybridization to Detect HER2 Overexpression/Gene Amplification in Breast Cancer in the Modern Age: A Single Institution Experience and Pooled Literature Review Study. Clinical Breast Cancer, 22(1). doi:https://doi.org/10.1016/j.clbc.2021.05.004.

[17] Modi, S., Jacot, W., Yamashita, T., Sohn, J., Vidal, M., Tokunaga, E., Tsurutani, J., Ueno, N.T., Prat, A., Chae, Y.S., Lee, K.S., Niikura, N., Park, Y.H., Xu, B., Wang, X., Gil-Gil, M., Li, W., Pierga, J.-Y., Im, S.-A. and Moore, H.C.F. (2022). Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. New England Journal of Medicine, 387(1). doi:https://doi.org/10.1056/nejmoa2203690.

[18] Iqbal, N. and Iqbal, N. (2014). Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Molecular Biology International, 2014(1), pp.1–9. doi:https://doi.org/10.1155/2014/852748.

[19] Pan, L., Li, J., Xu, Q., Gao, Z., Yang, M., Wu, X. and Li, X. (2024). HER2/PI3K/AKT pathway in HER2-positive breast cancer: A review. Medicine, [online] 103(24), pp.e38508–e38508. doi:https://doi.org/10.1097/md.0000000000038508.

[20] Pellas, U., Bauer, A., Ilija Vladimir Baroš, Fattorini, C. and Tibor Tot (2023). HER2-low metastases of HER2-negative primary tumors: a single institution analysis of intertumoral and internodal heterogeneity in node-positive breast cancer. Frontiers in Oncology, 13. doi:https://doi.org/10.3389/fonc.2023.1167567.

[21] Pereira, P., Sai Kiran Sharma, Carter, L.M., Edwards, K.J., Pourat, J., Ashwin Ragupathi, Janjigian, Y.Y., Durack, J.C. and Lewis, J.S. (2018). Caveolin-1 mediates cellular distribution of HER2 and affects trastuzumab binding and therapeutic efficacy. Nature Communications , 9(1). doi:https://doi.org/10.1038/s41467-018-07608-w.

[22] Qi, Yu, Shu-Min Deng, Kuan-Song W. (2024). Receptor tyrosine kinases in breast cancer treatment: unraveling the potential. American Journal of Cancer Research, [online] 14(9), pp.4172–4196. doi:https://doi.org/10.62347/kivs3169.

[23] Raghav K.P. and Moasser, M.M. (2022). Molecular Pathways and Mechanisms of HER2 in Cancer Therapy. Clinical Cancer Research, [online] 29(13), pp.2351–2361. doi:https://doi.org/10.1158/1078-0432.ccr-22-0283.

[24] Rathi, A., Sahay, A., Tanuja Shet, Patil, A. and Desai, S. (2023). Validation of Dual-Color Dual In Situ Hybridization for HER2/neu Gene in Breast Cancer. Archives of Pathology & Laboratory Medicine, 148(4). doi:https://doi.org/10.5858/arpa.2022-0543-oa.

[25] Santamaria, S., Gagliani, M.C., Grazia Bellese, Marconi, S., Lechiara, A., Dameri, M., Aiello, C., Tagliatti, E., Castagnola, P. and Cortese, K. (2021). Imaging of Endocytic Trafficking and Extracellular Vesicles Released Under Neratinib Treatment in ERBB2+ Breast Cancer Cells. Journal of Histochemistry & Cytochemistry, 69(7), pp.461–473. doi:https://doi. org/10.1369/ 00221554211026297.

[26] Shao, T., Wood, M., Wing, A., Hnatovska, M., Mendes, M., Brendan Mullen, J. and Chang, M.C. (2016). Comparison of HER2 Dual-Color and Fluorescence In Situ Hybridization in Breast Cancer. American Journal of Clinical Pathology, 146(3), pp.339–345. doi:https:// doi.org/10.1093/ajcp/aqw117.

[27] Schettini, F., Brasó-Maristany, F., Kuderer, N.M. and Prat, A. (2022). A perspective on the development and lack of interchangeability of the breast cancer intrinsic subtypes. npj Breast Cancer, [online] 8(1), pp.1–4. doi:https:// doi.org/10.1038/s41523-022-00451-9.

[28] Schlam, I., Tolaney, S.M. and Tarantino, P. (2023). How I treat HER2-low advanced breast cancer. Breast (Edinburgh, Scotland), [online] 67, pp.116–123. doi:https://doi.org/10.1016/j.breast.2023.01.005.

[29] Shirman, Y., Shlomit Lubovsky and Shai, A. (2023). HER2-Low Breast Cancer: Current Landscape and Future Prospects. Breast cancer, [online] Volume 15, pp.605–616. doi:https://doi.org/10.2147/bctt.s366122.

[30] Rubin, E., Shan, K.S., Dalal, S., Dieu, Milillo-Naraine, A.M., Guaqueta, D. and Ergle, A. (2024). Molecular Targeting of the Human Epidermal Growth Factor Receptor-2 (HER2) Genes across Various Cancers. International journal of molecular sciences, 25(2), pp.1064–1064. doi:https://doi.org/10.3390/ijms25021064.

[31] Wolff, A.C., Somerfield, M.R., Dowsett, M., Hammond, E.H., Hayes, D.F., McShane, L.M., Saphner, T.J., Spears, P.A. and Allison, K.H. (2023). Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO–College of American Pathologists Guideline Update. Journal of Clinical Oncology, 41(22). doi:https://doi.org/10.1200/jco.22.02864.

[32] Xia, X., Hu, T., He, X., Liu, Y., Yu, C., Kong, W., Liao, Y., Tang, D., Liu, J. and Huang, H. (2023). Neddylation of HER2 Inhibits its Protein Degradation and promotes Breast Cancer Progression. International Journal of Biological Sciences, [online] 19(2), pp.377–392. doi:https://doi.org/10.7150/ijbs.75852.