

Research article

HER2 Testing in Breast Cancer: Correlation between Immunohistochemistry and FISH Using the Updated 2023 ASCO/CAP Guidelines

Thivaratana Sinthuwiwat¹, Somboon Kittikongwat³, Kanthida Jangyubol¹,
Bongkod Petcharat¹, Jutamas Yimnoon¹, Priyavudh Herabutya¹,
Pakaporn Tosirisuk², Archara Supavavej^{4*}

¹Cytogenetics Research Laboratory, Bureau for Research and Innovation Management,
Chulabhorn Royal Academy, Bangkok, Thailand,

²Department of Surgical Pathology, Chulabhorn Hospital, Bangkok, Thailand.

³Institute of Pathology, Ministry of Public Health, Bangkok, Thailand.

⁴Department of Medical Oncology, Chulabhorn Hospital, Bangkok, Thailand.

*Correspondence Author, email: archara.sup@cra.ac.th

Received: 13 January 2026; Revised: 22 May 2026; Accepted: 2 June 2026

Abstract

Background Human epidermal growth factor receptor 2 (HER2) is an important prognostic and predictive marker in breast cancer patients. HER2 amplification status is a prognostic factor for the response to HER2-targeted therapy. HER2 status can be screened via immunohistochemistry (IHC). HER2 gene amplification can be detected by fluorescence in situ hybridization (FISH) according to ASCO/CAP 2018 guidelines. In 2023, the ASCO/CAP guidelines for HER2 testing by FISH were updated. We analyzed the correlation between HER2 IHC status and FISH-HER2 expression according to updated guidelines. **Methods** Female patients with histologically confirmed invasive breast cancer were enrolled at Chulabhorn Hospital. HER2-IHC staining was performed and interpreted according to the 2023 ASCO/CAP guidelines. Equivocal (2+) or positive (3+) IHC results were confirmed with FISH testing. Results A total of 263 patients was classified into 192 patients in the HER2-IHC 2+ group and 71 patients in the HER2-IHC 3+ group. Among patients in the HER2-IHC 2+ group, 138 had negative FISH results, and 54 had positive FISH. **Results** All 71 patients with IHC 3+ had positive HER2 FISH results. Our study revealed a statistically significant relationship between positive (3+) IHC and FISH for HER2, with a correlation rate of 100% in the IHC 3+ group ($rs=0.639$, $p<0.001$) and 28% in the equivocal (2+) IHC group. **Conclusion** In accordance with the 2023 ASCO/CAP recommendations, our findings support that FISH testing is not required for patients with HER2 IHC 3+ breast cancer, which may help expedite therapeutic decision-making and treatment initiation.

Keywords: ASCO/CAP 2023, Breast cancer, Dual-probe ISH, FISH, HER2, IHC

Introduction

According to GLOBOCAN 2023, breast cancer remains the primary cause of cancer-related fatalities and the most prevalent malignancy among women globally, with a rising trajectory expected in the coming years.¹ Low and middle-income nations,

such as Thailand, account for roughly 60% of these deaths. Domestic data from 2023 indicates that breast cancer is the most frequent diagnosis in Thai women (23.2%) and ranks third among all cancers nationally (11.8%).² Over two decades, Thailand's incidence rates have climbed, reaching a peak age-standardized

rate (ASR) of 28.5 per 100,000 person-years.³ Crucial to treatment planning are biomarkers like Estrogen receptor (ER), Progesterone Receptor (PR), and Human Epidermal Receptor 2 (HER2), which serve as essential prognostic indicators.⁴ Notably, research has shown high levels of biomarker consistency between primary tumors and metastatic lymph nodes, specifically for HER2 (84.1%), estrogen receptor (ER) (77.6%), and progesterone receptor (PR) (82.2%).⁵

The HER2 gene, situated on chromosome 17 (17q12 - 21.32), plays a fundamental role in various malignancies, most notably breast cancer.⁶ It is responsible for regulating vital cellular functions, including proliferation, differentiation, motility, and programmed cell death.⁷ Approximately 15 - 20% of invasive breast cancers exhibit HER2 gene amplification⁸, a status that serves as both a prognostic and predictive indicator. Specifically, overexpression is linked to a higher likelihood of systemic metastasis and reduced survival rates.⁹ However, HER2 status also informs therapeutic strategies, as it determines sensitivity to anthracycline-based chemotherapy and identifies candidates for specialized targeted therapies.⁶

Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are the standard established HER2 testing methods used to determine the HER2 status of patients with breast cancer receiving anti-HER2 therapy. Recently, FISH was approved by the U.S. Food and Drug Administration (FDA) to assess HER2 amplification in HER2 testing in breast cancer.¹⁰ The FISH technique can be used to evaluate HER2 gene amplification in terms of the HER2/CEP17 ratio and HER2/nucleus ratio, according to the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) guidelines for breast cancer.¹¹ Many laboratories have adopted IHC as primary testing, followed by FISH as confirmation

for tissue with an equivocal IHC result, such as IHC 2+¹², given the higher failure rate, longer procedure time, and higher reagent cost of FISH compared with IHC. The ASCO/CAP 2018 version of breast cancer guidelines improved the analytic validity of HER2 testing by standardizing and ensuring accurate detection of HER2 gene amplification or protein overexpression in breast cancers and enhanced the clinical utility of HER2 as a predictive biomarker for potential responsiveness to therapies targeting the HER2 protein.¹² In HER2 2+ IHC or equivocal cases, the ASCO/CAP 2018 guidelines recommend reflex FISH testing on the same sample or ordering a new test if both IHC and FISH are available. The criteria of the 2023 “Human Epidermal Growth Factor Receptor 2 (HER2) Breast Testing Guideline Update” reaffirmed the 2018 “HER2 Breast Testing Guideline Focused Update” and remained unchanged.

The ASCO/CAP 2023 guidelines categorize HER2 testing by dual-probe FISH into 5 distinct groups based on the HER2/CEP17 ratio and the average HER2 copy number per cell. Group 1: HER2/CEP17 ratio ≥ 2 and HER2/nucleus >4 , group 2: HER2/CEP17 ratio ≥ 2 and HER2/nucleus <4 , group 3: HER2/CEP17 ratio <2 and HER2/nucleus ≥ 6 , group 4: HER2/CEP17 ratio <2 and HER2/nucleus ≥ 4 or <6 and group 5: HER2/CEP17 ratio <2 and HER2/nucleus <4 . as shown in Table 1. Group 1 cases are HER2-positive, and Group 5 cases are HER2-negative; neither type requires IHC correlation. Groups 2 - 4 include equivocal or borderline cases, and the guidelines specify that IHC should be performed on the same tissue section or, when available, that a new specimen be tested with both IHC and FISH to generate an IHC score and determine HER2 status.^{13,14}

Table 1 presents the 2023 “Human Epidermal Growth Factor Receptor 2 (HER2) Breast Testing Guideline Update” regarding HER2/neu assessment by fluorescence in situ hybridization (FISH) in breast cancer.

Group	HER2 testing		IHC	FISH
1	HER2/CEP17 ratio ≥ 2.0	Average HER2 copy number ≥ 4.0 signals/cell	-	Positive
2	HER2/CEP17 ratio ≥ 2.0	Average HER2 copy number < 4.0 signals/cel	3+	Positive
			2+	Negative*
3	HER2/CEP17 ratio < 2.0	Average HER2 copy number ≥ 6.0 signals/cell	2+,3+	Positive
4	HER2/CEP17 ratio < 2.0	Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell	3+	Positive
			2+	Negative*
5	HER2/CEP17 ratio < 2.0	Average HER2 copy number < 4.0 signals/cell	-	Negative

* If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low HER2 copy number by FISH and lack of protein overexpression.

This study aimed to determine the concordance rate between IHC and FISH for HER2 testing in breast cancer patients at Chulabhorn Hospital, based on the 2023 ASCO/CAP recommendations. Specifically, the study evaluated whether HER2 IHC 3+ results could be considered definitively positive without

requiring confirmatory HER2-FISH testing, thereby supporting the indication for anti-HER2 therapy. This approach may reduce testing turnaround time by at least 14 days, lower costs, and facilitate earlier treatment initiation.

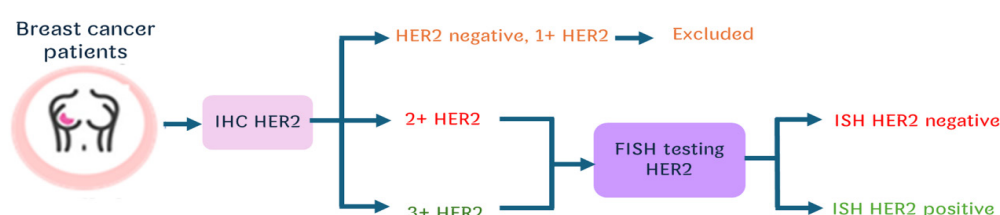
Method

Study population.

This single-center retrospective study enrolled female patients who were diagnosed with breast cancer between 1 January 2022 and 31 December 2023. This study protocol was reviewed and approved by the Human Research Ethics Committee of Chulabhorn Royal Academy (EC074/2567). Patients with histologically confirmed invasive breast carcinoma and IHC staining were interpreted per the 2023 CAP/ASCO update recommendations. After that, the IHC staining results of equivocal (2+) or positive (3+)

samples were confirmed with FISH for HER2 testing. Patients with negative results for HER2-IHC result (scores 0 and 1+) were not required for FISH for HER2 testing and were excluded. Patient demographic data and pathologic details, including hormonal status, HER2 status, the marker of proliferation, proliferative index Kiel 67 (Ki-67), and tumor staging, were collected at the time of diagnosis on the basis of the 8th AJCC classification. The workflow of the research procedure for the patients included in the study is shown in Figure 1.

Figure 1. Flow diagram of the patients included in the study



Immunohistochemistry

IHC staining was performed on 3- μ m-thick, formalin-fixed, paraffin-embedded (FFPE) sections from the same sample blocks used for HER2 FISH testing. IHC was performed using an anti-HER2/neu (4B5) rabbit monoclonal antibody (Ventana Medical Systems, Inc.). The reliability and validity of HER2 IHC results rely on the principles of the VENTANA anti-HER2/neu (4B5) assay. Pre-analytical variables heavily impact clinical validity; specifically, strict adherence to tissue fixation in 10% neutral buffered formalin (NBF) for 4 to 8 hours is critical to prevent antigen degradation and ensure accurate membrane staining. To minimize human error and maximize reproducibility, all assays were performed using automated slide stainers with pre-diluted reagents and were continuously validated against standardized cell line controls (PATHWAY HER2 4-in-1). Two breast pathologists reviewed the IHC slides. The first pathologist has 5 years of specialized experience and evaluates 120 cases annually in the Department of Surgical Pathology, Chulabhorn Hospital. The second pathologist had 6 years of experience and had assessed more than 1,000 cases annually at the Institute of Pathology, Ministry of Public Health. The results were interpreted according to the 2018 ASCO/CAP guidelines.

HER2 FISH testing.

FISH was performed on 2 μ m sections of formalin-fixed, paraffin-embedded samples via dual-color HER2/CEP17 DNA probes (PathVysion Her2/neu DNA probe Kit; Abbott). The DNA probe contains HER2 at a specific locus (red signal) and a control probe specific for the centromere of chromosome 17 (green signal). HER2 amplification signals were detected using a fluorescence microscope (Olympus BX63). Images were captured at 60X magnification, analyzed

by ASI (Applied Spectral Imaging), and processed via the GENASIS program. The interpretation of the results was based on the 2023 ASCO/CAP guidelines.

Statistical analysis

To determine the concordance rate between HER2 IHC and FISH for HER2 testing in breast cancer patients, in accordance with the ASCO/CAP 2023 guidelines at Chulabhorn Hospital, we used descriptive statistics to identify baseline clinical characteristics of HER2 alterations in breast cancer patients. The results are shown as correlations with percentages and were subsequently analyzed via Spearman's rank-order correlation.

Results

Between January 2022 and December 2023, we enrolled 916 patients who were diagnosed with breast cancer at Chulabhorn Hospital. Among these, 263 patients exhibited equivocal or HER2 IHC3+ staining and required confirmed FISH studies, which were included in this study.

Demographic data

Among the 263 patients with HER2 IHC2+ or HER2 IHC3+ breast cancer, the mean age was 56.93 ± 11.81 (SD) years. All invasive breast carcinoma samples were tested for estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 expression via immunohistochemistry (IHC). ER was negative in 21.76% (57 of 263) of patients and positive in 74.52% (196 of 263) of patients. PR was negative in 36.5% (96 of 263) of patients and positive in 59.7% (157 of 263). Ki-67 expression was less than 20% in 21.29% (56 of 263) of the tumors and 20% or more in 66.16% (174 of 263) of the tumors. The clinicopathologic characteristics of HER2 alterations among female patients with breast cancer are shown in Table 2.

Table 2 Clinicopathological characteristics of HER2-positive breast cancer patients in each ICH group at Chulabhorn Hospital, Thailand.

Characteristics		Total (n=263)	HER2 IHC Equivocal (2+) (n = 192)	HER2 IHC Positive (3+) (n = 71)
Median age, years		56.93 ± 11.81	55.97 ± 10.30	57.28 ± 12.33
Stage				
0		1		
1		42		
2		94		
3		69		
4		57		
Hormonal status				
Estrogen Receptor (ER)	negative	57 (21.76%)	26 (13.54%)	31 (43.66%)
	positive	196 (74.52%)	164 (85.42%)	32 (45.07%)
	no data	10 (3.80%)	2 (1.04%)	8 (11.27%)
Progesterone Receptor (PR)	negative	96 (36.50%)	56 (29.17%)	40 (56.34%)
	positive	157 (59.70%)	134 (69.79%)	23 (32.39%)
	no data	10 (3.80%)	2 (1.04%)	8 (11.27%)
Marker of Proliferation Kiel 67 (Ki-67)	<20%	56 (21.29%)	51 (26.56%)	5 (7.04%)
	≥20%	174 (66.16%)	120 (62.50%)	54 (76.06%)
	no data	33 (12.55%)	21 (10.94%)	12 (16.90%)

The concordance rate between HER2 IHC and FISH

The patients were categorized into two groups according to their HER2 IHC staining status. A total of 192 patients were classified into the equivocal or 2+HER2 IHC staining group, and 71 were classified into the positive or 3+HER2 IHC staining group. All patients were confirmed by FISH.

As shown in Table 3, of the 192 patients, 118

had negative FISH results (group 5), and 53 were confirmed to be FISH-positive (group 1). Furthermore, 21 patients were assigned to groups 2, 3, and 4 based on dual-probe FISH per the ASCO/CAP 2023 guidelines, necessitating additional workup. In those groups, blind observers performed FISH, and at least 20 cells were counted. Furthermore, 71 patients had positive scores, 3+ HER2 IHC staining, and FISH confirmation, all of which were positive.

Table 3 Genomic HER2 alteration clone characteristics by dual-probe FISH and IHC for HER2 based on the 2023 ASCO/CAP guidelines among patients with breast cancer at Chulabhorn Hospital, Thailand.

		Group	Conditions		N (%)
IHC	2+	1	HER2/CEP17 ratio ≥ 2.0	Average HER2 copy number ≥ 4.0 signals/cell	53 (20.2)
		2		Average HER2 copy number < 4.0 signals/cel	12 (4.6)
		3	HER2/CEP17 ratio <2.0	Average HER2 copy number ≥ 6.0 signals/cell	1 (0.4)
		4		Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell	8 (3.0)
		5		Average HER2 copy number < 4.0 signals/cell	118 (44.9)
	3+	1	HER2/CEP17 ratio ≥ 2.0	Average HER2 copy number ≥ 4.0 signals/cell	70 (26.6)
		2		Average HER2 copy number < 4.0 signals/cel	0
		3	HER2/CEP17 ratio <2.0	Average HER2 copy number ≥ 6.0 signals/cell	1 (0.4)
		4		Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell	0
		5		Average HER2 copy number < 4.0 signals/cell	0
Total					263 (100)

Regarding the genomic characteristics of HER2 alterations, this study revealed that of 71 IHC-positive (3+) patients, 70 were in group 1 (classical HER2 amplification) based on dual-probe FISH, per the ASCO/CAP 2018 guidelines. However, one patient was in group 3 (Co-amplification, previously characterized as polysomy 17). The 2+ IHC group or equivocal share of varieties of HER2 clones may interfere with the IHC results.

The correlation rate between the HER2 IHC 2+ group and HER2 FISH amplification was 28.12%,

which was not statistically significant. However, the correlation rate between the HER2 IHC 3+ group and HER2 FISH amplification was 100%, with a Spearman's rank-order correlation of 0.639 and statistically significant (Table 4). This study also evaluated the diagnostic performance of the IHC method for determining HER2 status, with FISH as a confirmatory test. The positive predictive value for positive IHC 3+ cases was 100.0% (95% CI: 94.9 - 100.0).

Table 4 Correlation between IHC and FISH results for HER2 expression according to the 2023 ASCO/CAP guidelines.

Testing modalities		FISH		Total	Correlation (%)
		Negative	Positive		
IHC	2+	138	54	192	28.12
	3+	0	71	71	100*
Total		138	125	263	

*rs=0.639, p<0.001

Discussion

Focused updates to the ASCO/CAP guidelines for HER2 testing in breast cancer were published in 2023¹⁴ to address several concerns raised following publication of the 2013 ASCO/CAP guidelines¹⁵ and to refine the definitions of HER2 status in specific situations. The 2018 and 2023 updated ASCO/CAP guidelines for HER2 testing by fluorescence in situ hybridization (FISH) currently classify HER2 amplification into five groups¹², and FISH results must be interpreted in conjunction with immunohistochemistry (IHC) results.¹⁶ Determination of HER2 amplification status using the FISH technique can significantly reduce the number of equivocal results and increase the number of truly negative HER2 IHC results.¹⁴

In Thailand, many laboratories use immunohistochemistry (IHC) as the primary method for assessing HER2 status, reserving fluorescence in situ hybridization (FISH) to confirm equivocal cases (IHC 2+) and HER2 IHC3+. As the US FDA-approved method for determining HER2 amplification, FISH is the gold standard and reference standard against which emerging technologies are benchmarked. It is also poised to remain the definitive method for HER2 testing in low- and middle-income countries, which carry a disproportionate burden of global breast cancer cases.¹⁷ Furthermore, the Comptroller General's Department of Thailand updated the diagnostic criteria in November 2022. This amendment omitted the requirement for FISH confirmation in IHC 3+ cases, thereby accelerating patient access to targeted anti-HER2 regimens.

The findings of this study reinforce the diagnostic reliability of Immunohistochemistry (IHC) in identifying HER2-positive breast cancer. Per the

consensus guidelines provided by ASCO/CAP and the College of American Pathologists (CAP), and the Royal College of Pathologists in the United Kingdom, we defined a HER2 IHC 3+ result as intense, complete membrane staining in more than 10% of tumor cells. Given that the National Institute for Health and Care Excellence (NICE) guidelines recommend a 2-week turnaround time for HER2 assessment, streamlining the diagnostic workflow is essential to prompt initiation of anti-HER2 therapies¹⁸. The high concordance between strong immunohistochemical expression and gene amplification is well-documented. Gordian-Arroyo et al demonstrated that 100% of HER2 IHC 3+ cases were FISH-positive, with 97.8% (134/137) exhibiting classic Group 1 amplification (HER2/CEP17 ratio ≥2.0 and an average HER2 copy number ≥4.0 signals/cell). Mirroring these findings, 98.6% (70/71) of the IHC 3+ patients in our cohort who underwent reflex FISH testing were categorized as Group 1, while a negligible 14% (1/71) were categorized as Group 3. This strong association was further validated by a statistically significant Spearman's rank-order correlation of 0.639 (p < 0.05). The representative IHC and FISH images shown in Figure 2 provide a visual perspective of the relationship between the IHC score and the FISH results. Our findings are consistent with international evidence. Prior concordance studies comparing dual-probe ISH (FISH and DISH) and IHC 3+ results, based on the 2018 and 2023 ASCO/CAP guidelines, are shown in Table 5.

Collectively, these data suggest that an IHC 3+ designation serves as a sufficiently robust indicator to justify immediate targeted therapy without the need for routine gene amplification verification. This aligns with findings by Thambamroong¹⁹, which

suggested that routine reflex FISH testing for all HER2 IHC-positive patients is unnecessary. Omitting compulsory FISH confirmation for IHC 3+ cases successfully reduces patient waiting times and

alleviates financial burdens, while ensuring that patients receive therapeutic benefits from anti-HER2 regimens.

Figure 2. Representative immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) images (60×magnification). (A). Case with a 2+HER2 IHC score (incomplete and heterogeneous membrane staining) (B). HER2 non-amplification by FISH. (C) Case with a 3+HER2 IHC score (complete membrane staining that is intense and detected in >10% of tumor cells. (D) HER2 amplification by FISH.

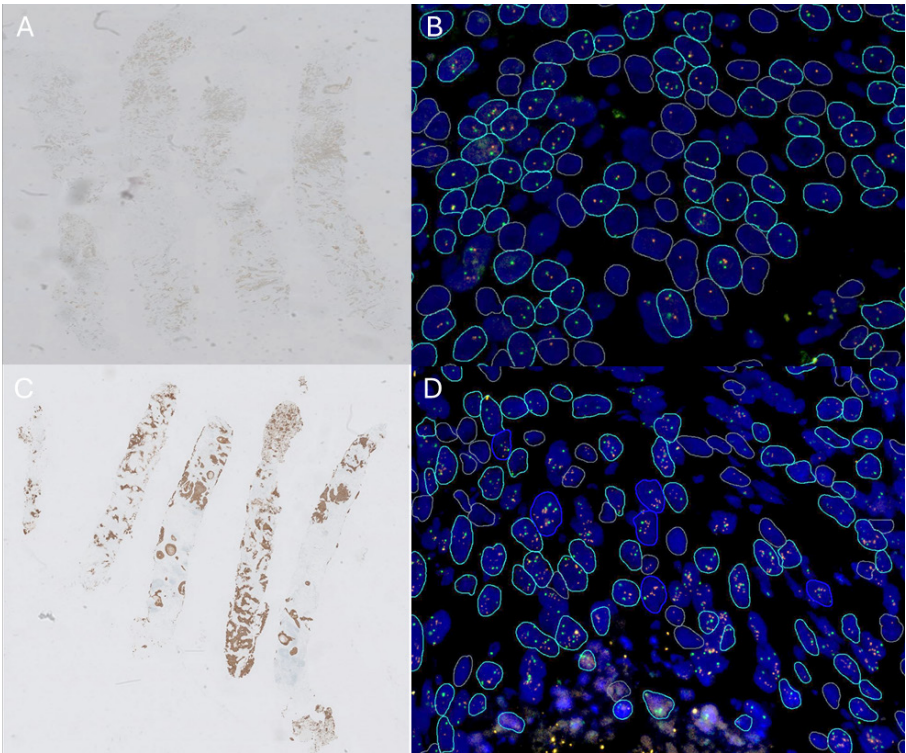


Table 5 Prior concordance studies comparing dual-probe ISH (FISH, SISH, and DISH) and IHC 3+ results, based on the 2018 and 2023 ASCO/CAP guidelines.

No	Author	Sample sizes	Concordance rates	Population	Methodology	ASCO/CAP guidelines.
1	Gordian-Arroyo et al. [8]	1350	100%	American	FISH	2018
2	Farshid et al. [20]	1815	93.4%	Australia	Silver ISH	2018
3	Li et al. [21]	2514	10.6%	China	FISH	2018
4	Thambamroong [19]	141	91.76%,	Thai	FISH	2018
5	Current author	263	100%	Thai	FISH	2023
6	Lampenkhoe et al. [22]	1,307	92%	Thai	DISH	2023

Conclusion

Our study adds valuable evidence by providing real-world confirmation and providing implementation evidence supporting the selective use of reflex FISH testing primarily in HER2 equivocal (2+) cases, together with previously published data showing a high concordance rate between HER2 IHC 3+ results and HER2 amplification determined by FISH according to the 2023 ASCO/CAP guidelines. These findings have important implications for clinical management and support previous evidence suggesting that reflex FISH testing in patients with HER2 IHC 3+ results may not be required and could be omitted to streamline treatment.

Acknowledgements

We would like to thank Thitiphong Suntharayuth and Kerati Jirawattanapalin from the Biostatistics and Research Data Management Division, Bureau for Research and Innovation Management, Chulabhorn Royal Academy.

References

1. Arnold M, Morgan E, Rumgay H, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast*. 2022;66:15-23. doi:10.1016/j.breast.2022.08.010
2. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229-263. doi:10.3322/caac.21834
3. Insamran W, Sangrajang S. National Cancer Control Program of Thailand. *Asian Pac J Cancer Prev*. 2020;21(3):577-582. doi:10.31557/APJCP.2020.21.3.577
4. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst*. 2014;106(5):dju055. doi:10.1093/jnci/dju055
5. Virani S, Sriplung H, Rozek LS, Meza R. Escalating burden of breast cancer in southern Thailand: analysis of 1990-2010 incidence and prediction of future trends. *Cancer Epidemiol*. 2014;38(3):235-243. doi:10.1016/j.canep.2014.03.003
6. Ahn S, Woo JW, Lee K, Park SY. HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. *J Pathol Transl Med*. 2020;54(1):34-44. doi:10.4132/jptm.2019.11.03
7. Aman NA, Doukoure B, Koffi KD, et al. HER2 overexpression and correlation with other significant clinicopathologic parameters in Ivorian breast cancer women. *BMC Clin Pathol*. 2019;19:1. doi:10.1186/s12907-018-0081-4
8. Gordian-Arroyo AM, Zynger DL, Tozbikian GH. Impact of the 2018 ASCO/CAP HER2 Guideline Focused Update. *Am J Clin Pathol*. 2019;152(1):17-26. doi:10.1093/ajcp/aqz012
9. Exman P, Tolane SM. HER2-positive metastatic breast cancer: a comprehensive review. *Clin Adv Hematol Oncol*. 2021;19(1):40-50. <https://pubmed.ncbi.nlm.nih.gov/33493147/>
10. Shah MV, Wiktor AE, Meyer RG, et al. Change in Pattern of HER2 Fluorescent in Situ Hybridization (FISH) Results in Breast Cancers Submitted for FISH Testing: Experience of a Reference Laboratory Using US Food and Drug Administration Criteria and American Society of Clinical Oncology and College of American Pathologists Guidelines. *J Clin Oncol*. 2016;34(29):3502-3510. doi:10.1200/JCO.2015.61.8983
11. Wang B, Ding W, Sun K, Wang X, Xu L, Teng X. Impact of the 2018 ASCO/CAP guidelines on HER2 fluorescence in situ hybridization interpretation in invasive breast cancers with immunohistochemically equivocal results. *Sci Rep*. 2019;9(1):16726. doi:10.1038/s41598-019-53003-w
12. Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO-College of American Pathologists Guideline Update. *J Clin Oncol*. 2023;41(22):3867-3872. doi:10.1200/JCO.22.02864
13. Antolín S, García-Caballero L, Reboredo C, et al. Is there a correlation between HER2 gene amplification level and response to neoadjuvant treatment with trastuzumab

- and chemotherapy in HER2-positive breast cancer? *Virchows Arch.* 2021;479(4):853-857. doi:10.1007/s00428-021-03104-7
14. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol.* 2018;36(20):2105-2122. doi:10.1200/JCO.2018.77.8738
 15. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol.* 2013;31(31):3997-4013. doi:10.1200/JCO.2013.50.9984
 16. Pasricha S, Menon V, Gupta G, et al. Impact of 2018 ASCO/CAP guidelines on HER-2 reporting categories of IHC and reflex FISH in breast cancer. *Breast J.* 2020;26(11):2213-2216. doi:10.1111/tbj.14031
 17. Morsberger L, Pallavajjala A, Long P, et al. HER2 amplification by next-generation sequencing to identify HER2-positive invasive breast cancer with negative HER2 immunohistochemistry. *Cancer Cell Int.* 2022;22(1):350. doi:10.1186/s12935-022-02761-1
 18. The Royal College of Pathologists. *Pathology Reporting of Breast Disease in Surgical Excision Specimens Incorporating the Dataset for Histological Reporting of Breast Cancer.* The Royal College of Pathologists. <https://www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html>. Accessed November 9, 2024.
 19. Thambamroong T. The concordance between IHC and ISH for HER-2 testing in breast cancer in Nakhon Pathom Hospital, Thailand, based on the ASCO/CAP 2018 guidelines: a retrospective study. *Ecancermedicalscience.* 2022;16:1370. doi:10.3332/ecancer.2022.1370
 20. Farshid G, Dhattrak D, Gilhotra A, Koszyca B, Nolan J. 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. *Mod Pathol.* 2020;33(9):1783-1790. doi:10.1038/s41379-020-0555-7
 21. Li A, Bai Q, Kong H, et al. Impact of the Updated 2018 American Society of Clinical Oncology/College of American Pathologists Guideline for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. *Arch Pathol Lab Med.* 2020;144(9):1097-1107. doi:10.5858/arpa.2019-0369-OA
 22. Iampengkhae K, Pisutpunya A, Vuthithammee C, Sodsoon P, Apornvirat S. Comparative Analysis of Equivocal (2+) and Positive (3+) HER2 Immunohistochemistry (IHC) and Bright-Field Dual-Color In Situ Hybridization (DISH) in Primary Breast Cancer From 1,307 Node-Positive Patients. *Breast Cancer (Auckl).* 2025;19:11782234251407914. doi:10.1177/11782234251407914

Citation

Sinhuwiwat T, Kittikongwat S, Jangyubol K, Petcharat B, Yimnoon J, Herabutya P, Tosirisuk P and Supavavej A. HER2 Testing in Breast Cancer: Correlation between Immunohistochemistry and FISH Using the Updated 2023 ASCO/CAP Guidelines. *J Chulabhorn Royal Acad.* 2026;8(4): 360-369. <https://he02.tci-thaijo.org/index.php/jcra/article/view/268386>

Online Access

<https://he02.tci-thaijo.org/index.php/jcra/article/view/268386>

