Poster P3-09-20

Re-evaluation of human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) 0 or 1+ in metastatic breast cancer (mBC) samples to characterize the proportion of HER2-ultralow (IHC 0 with membrane staining)

Savitri Krishnamurthy,[1] Clara Lam,[2] Linlin Luo,[2] Rosalind Baverstock,[3] Nick Pyrih,[2] Stella Redpath,[2] Michele Sue-Ann Woo,[4] Simon M. Collin,[5] Caryn Davies,[6] Victoria Gannon,[6] Simone T. Sredni[2]

[1]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]AstraZeneca Pharmaceuticals LP, Gaithersburg, MD, USA; [3]AstraZeneca, Macclesfield, UK; [4]Daiichi Sankyo Inc., Basking Ridge, NJ, USA; [5]AstraZeneca, Cambridge, UK; [6]NeoGenomics, Fort Myers, FL, USA

Objective

- To estimate the proportion of samples which are HER2-ultralow (IHC 0 with membrane staining; with faint or incomplete membrane staining in >0 and ≤10% of tumor cells) among mBC tissue samples originally scored as IHC 0 (with or absent of membrane staining combined) and to evaluate inter- and intraobserver concordance and concordance with original scores

Conclusions

- Of the 400 tissue samples originally scored as HER2 IHC 0, 31.3%, 28.3%, and 37.5% had consensus rescores of HER2 IHC 0 absent of membrane staining, HER2-ultralow, and IHC 1+, respectively (3.0% did not have consensus rescore); these reclassified samples suggest a clinically actionable level of HER2 expression and may represent a patient population that could benefit from HER2-targeted therapies
- Interobserver concordance based on pathologists' first readings in rescoring was moderate to substantial[1] (overall percentage agreement [OPA], 61.2%-86.5%; Cohen κ, 0.427-0.774) while intraobserver concordance was high[1] (OPA, 84.7%-88.2%; Cohen κ, 0.765-0.805)
- Agreement between the consensus scores of pathologists and the original scores was moderate[1] (OPA, 70.8%; Cohen κ, 0.439)
- Median review times were shorter for samples rescored as HER2 IHC 0 absent of membrane staining (5.7 minutes) compared with samples rescored as HER2-ultralow (7.5 minutes) or IHC 1+ (7.0 minutes)
- Discrepancies in scoring and a trend to extended review times for HER2-ultralow and IHC 1+ highlight the need for standardized protocols, additional training, and digital tools for decision support
- Increased awareness of available therapies for HER2-ultralow and IHC 1+ cases may motivate pathologists to improve consistency in scoring for optimal patient care and treatment selection

Plain language summary

Why did we perform this research?

HER2 is a protein that can be found on the surface of tumor cells. Breast cancer (BC) can be characterized based on the amount of HER2 that is present on the cell surface.[1] Some subsets of BC, categorized as HER2-low (immunohistochemistry [IHC] 1+ or IHC 2+/in situ hybridization negative) or HER2-ultralow (with membrane staining; with faint or incomplete membrane staining in >0% and ≤10% of tumor cells), have low or very low levels of HER2 expression, respectively.[2-4] In the DESTINY-Breast04 clinical trial, trastuzumab deruxtecan (T-DXd), an anticancer therapy that was designed to target cells expressing HER2, was shown to be effective against HER2-low metastatic BC (mBC; cancer that has spread from its original site).[5-7] Results from the DESTINY-Breast06 clinical trial supported this and also suggested effectiveness against HER2-ultralow mBC.[7] However, the overall prevalence and characterization of HER2-ultralow among patients with mBC is still unclear. This study aimed to estimate the proportion of HER2-ultralow samples among mBC tissue samples originally scored as IHC 0 (no detectable level of HER2 protein) or IHC 1+ per 2018 American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) guidelines. Accurate identification of patients who express low levels or very low levels of HER2 who would be eligible for T-DXd treatment would improve patient care and treatment selection in clinical practice.

How did we perform this research?

Archived tissue samples from patients with mBC were rescored for HER2 expression level by 3 independent pathologists who were not told what the original HER2 IHC scores of the samples were and were not provided specific training to identify HER2-ultralow. The samples originated from community practices and were assessed twice by each pathologist with a gap of 2-10 weeks between assessments. The new HER2 IHC scores were compared with the original IHC scores. Scoring reproducibility of each pathologist (first reading versus second reading) and between pathologists was also assessed.

What were the findings of this research?

Tissue samples originally scored as HER2 IHC 0 were reclassified as either HER2 IHC 0 (absent of staining), HER2-ultralow, or HER2 IHC 1+. The reproducibility between first and second readings was high for each pathologist, and the alignment in rescoring between the different pathologists was moderate to substantial.[8] Alignment between the pathologists' new scores and the original scores was moderate.[8]

What are the implications of this research?

The findings of this study highlight the need for standardized protocols, additional training, and possible digital tools to enhance accuracy and reliability in HER2 IHC scoring, especially with tissue samples with lower HER2 IHC scores, to optimize patient care and treatment outcome. Additionally, patients historically categorized as HER2 IHC 0 may be classifiable as HER2-ultralow, making them eligible for T-DXd treatment.

References

1. Tarantino P et al. *J Clin Oncol.* 2020;38:1951-1962.
2. Schettini F et al. *NPJ Breast Cancer.* 2021;7:1.
3. Tarantino P et al. *J Clin Oncol.* 2023;34:645-659.
4. Bardia A et al. *N Engl J Med.* 2024;doi: 10.1056/NEJMoa2407086.
5. Nakada T et al. *Chem Pharm Bull.* 2019;67:173-185.
6. Ogitani Y et al. *Cancer Sci.* 2016;107:1039-1046.
7. Modi S et al. *N Engl J Med.* 2022;387:9-20.
8. Landis JR, Koch GG. *Biometrics.* 1977;33:159-174.

Please scan this quick response (QR) code with your smartphone camera or app to obtain a copy of this poster.

Poster

This study was sponsored by AstraZeneca. In March 2019, AstraZeneca entered into a global development and commercialization collaboration agreement with Daiichi Sankyo for trastuzumab deruxtecan (T-DXd; DS-8201).

Poster presented at the 2024 San Antonio Breast Cancer Symposium (SABCS); December 10-13, 2024; San Antonio, Texas by Savitri Krishnamurthy, MD.

Corresponding author email address: skrisna@mdanderson.org.

Introduction

- The efficacy of trastuzumab deruxtecan (T-DXd) in patients with HER2-low mBC was established with the results of the phase 3 DESTINY-Breast04 trial (median number of lines of previous treatment = 3)[2] and substantiated by those of DESTINY-Breast06 in the intention-to-treat population (patients with hormone receptor–positive, HER2-low mBC and HER2-ultralow mBC; median number of lines of previous treatment = 2)[3]
- T-DXd efficacy in the HER2-ultralow population in DESTINY-Breast06 was consistent with the HER2-low population, with a numerically greater median progression-free survival (PFS; 13.2 months) and objective response rate (ORR; 61.8%) compared with chemotherapy of physician's choice (8.3 months and 26.3%, respectively)[3]
- However, published evidence on the prevalence and characterization of HER2-ultralow in patients with mBC is limited
- The primary objective of this study was to assess the proportion of archival tissue samples from patients with mBC originally scored as HER2 IHC 0 (with and absent of staining combined) and IHC 1+ that were rescored as HER2-ultralow per American Society of Clinical Oncology–College of American Pathologists (ASCO-CAP) guidelines
- Secondary objectives included evaluating inter- and intraobserver concordance, evaluating observer concordance with original scores, and assessing the fields analyzed and the median time to review samples

Methods

- Whole-slide images (WSIs; N = 600) of mBC tissue samples from primary sites and a range of metastatic sites originating from community practices and obtained from patients in January 2020 or later were used in this study
- The WSIs had been stained with VENTANA PATHWAY anti-HER2/neu (4B5) assay (Roche) and originally scored as HER2 IHC 0 (n = 400) or IHC 1+ (n = 200) per ASCO-CAP 2018 guidelines
- During the study, the WSIs were randomized and reassessed (blind) by 3 independent pathologists who were also blinded to the original score of the samples
- Each WSI was reassessed twice by each pathologist, with a washout period of 2-10 weeks
- The pathologists were aware of the 2023 ASCO-CAP guidelines but were not provided specific training for HER2-ultralow scoring
- At each reading, the pathologists reported the percentage of membrane staining on each WSI and the time to review each WSI
- Using pathologists' first readings, WSIs were centrally recategorized as either IHC 0 absent of membrane staining, HER2-ultralow, IHC 1+, or IHC 2+ and consensus rescores were determined (scores with staining percentages that 2/3 or 3/3 pathologists were aligned on; in cases without a majority, the consensus score was recorded as missing)
- Inter- and intraobserver concordance, as well as pathologists' concordance with original scores, were described by overall percentage agreement (OPA) and Cohen κ

Results

Consensus Rescoring Results

- Of the 600 WSIs assessed, 2 of 3 pathologists showed consensus in 250 cases (41.7%) and 3 of 3 showed consensus in 336 cases (56.0%)
- Overall, 255 WSIs were classified as IHC 0 per consensus rescore, with 129 (21.5%) showing no membrane staining and 126 (21.0%) being classified as HER2-ultralow (**Table 1**)
- Of the 400 WSIs originally scored IHC 0, 263 (65.8%) were rescored as HER2-ultralow (113/400, 28.3%) or IHC 1+ (150/400, 37.5%), and 125 (31.3%) were rescored as IHC 0 absent of membrane staining

Table 1. Consensus HER2 IHC Rescores[a] for WSIs Initially Scored as IHC 0[b] and IHC 1+

n (%)	Original HER2 IHC Score Per 2018 ASCO/CAP Guidelines		
	IHC 0[b] N = 400	IHC 1+ N = 200	Overall N = 600
HER2 IHC 0 absent of staining	125 (31.3)	4 (2.0)	129 (21.5)
HER2-ultralow	113 (28.3)	13 (6.5)	126 (21.0)
HER2 IHC 1+	150 (37.5)	175 (87.5)	325 (54.2)
HER2 IHC 2+	0 (0.0)	6 (3.0)	6 (1.0)
No consensus	12 (3.0)	2 (1.0)	14 (2.3)

Cells highlighted in blue indicate reclassification changes between IHC 1+ and IHC 0, as well as IHC 0 to HER2-ultralow and IHC 1+.
[a]Consensus HER2 IHC score was defined as a score that most pathologists (either 2 of 3 or 3 of 3) agreed upon, based on the first rescore from each pathologist.
[b]With or absent of membrane staining combined (membrane staining not distinguished).

Concordance Analyses

- The mean interobserver OPA was 69.9%
- Concordance between pathologists A and B was substantial[1] (OPA, 86.5%; Cohen κ, 0.774) (**Table 2**)
  - In comparison, concordance between pathologists A and C and between pathologists B and C was moderate[1] (OPA, 62.0% and 61.2%, respectively; Cohen κ, 0.431 and 0.427, respectively) (**Table 2**)

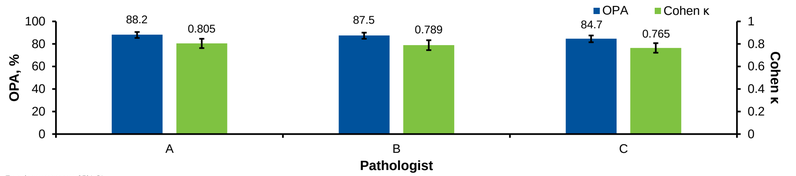
Table 2. Concordance of HER2 IHC Scores Between Pairs of Pathologists[a]

	Pathologist Pairing		
	A and B	A and C	B and C
OPA (95% CI), %	86.5 (83.5-89.1)	62.0 (58.0-65.9)	61.2 (57.1-65.1)
Cohen κ (95% CI)	0.774 (0.729-0.818)	0.431 (0.381-0.482)	0.427 (0.378-0.476)

[a]Each pathologist's first IHC score on each slide.

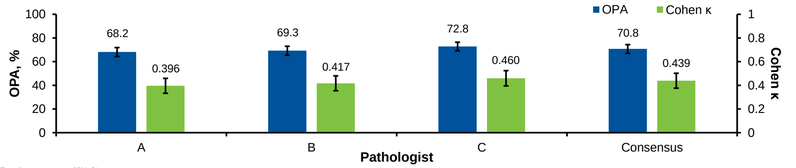
- All pathologists showed high concordance between their respective scoring assessments, suggesting consistency within pathologists
  - For all 3 pathologists, intraobserver OPA was >80% and Cohen κ ranged from 0.765 to 0.805 (**Figure 1**)
- Agreement between pathologists' consensus scores and original scores was moderate[1] (OPA, 70.8% [95% CI, 67.0%-74.4%]; Cohen κ, 0.439 [95% CI, 0.376-0.502])
  - For individual pathologists, OPA with the original score varied (range, 68.2%-72.8%), with Cohen κ showing fair to moderate agreement (range, 0.396-0.460)[1] (**Figure 2**)

Figure 1. Intraobserver Concordance[a] According to OPA and Cohen κ



Error bars represent 95% CI.
[a]Concordance between each pathologist's first and second rescores.

Figure 2. Concordance Between First Rescores and Original Scores[a] According to OPA and Cohen κ



Error bars represent 95% CI.
[a]Samples recategorized as HER2 IHC 0 absent of staining and HER2-ultralow were pooled together to calculate concordance of rescores with original scores, which did not discriminate HER2 IHC 0 absent of staining from HER2-ultralow.

Review Times for First Rescore

- Median time to review WSI rescored as HER2-ultralow and IHC 1+ tended to be longer than for IHC 0 absent of staining (**Table 3**)
- The number of fields analyzed ranged from 1 to 48 across all pathologists and rescore values

Table 3. Median Time to Review Slides

	Consensus HER2 IHC score				
	HER2 IHC 0 Absent of Staining N = 129	HER2-Ultralow N = 126	HER2 IHC 1+ N = 325	HER2 IHC 2+ N = 6	Total N = 586[a]
Fields analyzed, median (range), n	9.7 (2.7-19.0)	14.0 (3.5-32.0)	13.5 (1.5-38.0)	15.0 (8.0-17.7)	12.7 (1.5-38.0)
Time to review, median (range), min	5.7 (3.3-8.5)	7.5 (5.0-11.5)	7.0 (3.0-10.7)	7.3 (6.3-8.5)	7.0 (3.0-11.5)

[a]14 slides have not been included as there was no consensus score for those slides.

Abbreviations and Definitions

ASCO-CAP, American Society of Clinical Oncology–College of American Pathologists; HER2, human epidermal growth factor receptor 2; HER2-ultralow, IHC 0 with membrane staining (with faint or incomplete membrane staining in >0% and ≤10% of tumor cells); IHC, immunohistochemistry; mBC, metastatic breast cancer; OPA, overall percentage agreement; ORR, objective response rate; PFS, progression-free survival; T-DXd, fam-trastuzumab deruxtecan-nxki; WSI, whole-slide images.

Acknowledgments

Under the guidance of the authors and in accordance with Good Publication Practice, medical writing and editorial support was provided by Jennifer Lau, PhD, Selene Jarrett, PhD, and Vishal Gor, PhD, of ApotheCom, and was funded by AstraZeneca.

Disclosures

Dr Savitri Krishnamurthy serves on scientific advisory boards for Daiichi Sankyo and AstraZeneca.

References

1. Landis JR, Koch GG. *Biometrics.* 1977;33:159-174.
2. Modi S et al. *N Engl J Med.* 2022;387:9-20.
3. Bardia A et al. *N Engl J Med.* 2024;doi: 10.1056/NEJMoa2407086.