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Concordance between the DESTINY-Breast04/06 VENTANA 4B5 HER2 IHC clinical trial assay and other comparator assays for HER2-low breast cancer: Overall results of a large-scale, multicenter, global ring study

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Objective

- To evaluate concordance between the Ventana PATHWAY anti-HER2/neu (4B5) immunohistochemistry (IHC) assay (PATHWAY 4B5) and comparator assays in identifying human epidermal growth factor receptor 2 (HER2)-low breast cancer samples

Conclusions

- There was variability between PATHWAY 4B5 and comparator assays in identifying HER2-low versus HER2 IHC 0 (with or absent membrane staining)
- Positive percentage agreement (PPA) tended to be higher than negative percentage agreement (NPA), suggesting more consistent identification of HER2-low compared with HER2 IHC 0
- Awareness of available treatment options and careful choice, validation, and optimization of assay methods should be prioritized for pathologists and laboratories assessing tumor samples for HER2 status

Plain Language Summary

Why did we perform this research?
Breast cancer can be categorized based on the amount of a protein called human epidermal growth factor receptor 2 (HER2) that can be found on tumor cells.[1] Tumors that only express low levels of HER2 are categorized into a subset called HER2-low (immunohistochemistry [IHC] 1+ or IHC 2+/in situ hybridization not amplified). Trastuzumab deruxtecan (T-DXd) is an anticancer therapy called an antibody-drug conjugate that targets the HER2 protein on the surface of breast cancer cells. In the clinical trials DESTINY-Breast04 and DESTINY-Breast06, T-DXd was shown to be an effective treatment for patients with HER2-low breast cancer.[2,3] However, classification of tumors as HER2-low remains an ongoing challenge because pathologists and laboratories around the world use different IHC assays and we do not have a clear understanding of how the various assays may affect patient identification.[4-6] This study aimed to characterize the agreement between the Ventana PATHWAY anti-HER2/neu (4B5) IHC assay (PATHWAY 4B5), used in DESTINY-Breast04 and DESTINY-Breast06 and approved as the companion diagnostic for T-DXd for HER2-low breast cancer,[7] and other assays used around the world.

How did we perform this research?
Laboratories in the Americas, Europe, and the Asia-Pacific regions were included in this global study. A central laboratory categorized the HER2 IHC status of 50 breast cancer samples using PATHWAY 4B5. Sections of these samples were sent to participating laboratories where pathologists stained the samples using their routine assays and assessed their IHC score per the American Society of Clinical Oncology-College of American Pathologists 2018 guidelines. The pathologists then received virtual guideline alignment on HER2 IHC scoring, after which they rescored the samples. Following virtual alignment, pathologists' scores were compared with the central laboratory's PATHWAY 4B5 scores to determine the agreement in identifying HER2-low versus HER2 IHC 0.

What were the findings of this research?
The agreement between PATHWAY 4B5 and the comparator assays in categorizing a sample as HER2-low varied based on the comparator assay used. Comparator assays generally agreed with PATHWAY 4B5 in identifying samples as HER2-low, whereas agreement in identifying HER2 IHC 0 tended to be lower.

What are the implications of this research?
The IHC assay methods used by laboratories to assess HER2 status in breast cancer samples should be carefully chosen, validated, and optimized to identify patients who may benefit from treatment with T-DXd.

Where can I access more information?
Data for the first stage of the ring study presented at the AACR Annual Meeting 2024 can found here: https://datasourcebydaiichisankyo.com/congresses/-/view/congress/22001/AACR+2024

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Poster

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Introduction

- Historically, breast cancer has been categorized as either HER2-positive or HER2-negative (IHC 0, 1+, 2+/in situ hybridization not amplified [ISH−]) based on the American Society of Clinical Oncology-College of American Pathologists guidelines; approximately 80% of cases are considered HER2-negative[1-3]
- Some HER2-negative tumors may express low levels of HER2, with as many as 65% of these tumors meeting the criteria for HER2-low (IHC 1+, 2+/ISH−)[4,5]
- Trastuzumab deruxtecan (T-DXd), a HER2-directed antibody-drug conjugate, has demonstrated improved efficacy over the standard of care for HER2-low metastatic breast cancer in DESTINY-Breast04 and DESTINY-Breast06[6-9]
  - In DESTINY-Breast06, 24% of samples locally determined to be HER2 IHC 0 were centrally determined as HER2-low and 40% as HER2-ultralow[10]
  - These results underscore the need to reliably distinguish between HER2-low and HER2 IHC 0
- Ventana PATHWAY 4B5, approved in Australia, Canada, the European Union, Japan, and the United States as the companion diagnostic for HER2-low classification for T-DXd eligibility, was used in both trials to assess HER2 IHC status[11,12]
- Real-world differentiation between HER2-low and HER2 IHC 0 breast cancer is still an ongoing challenge, with a variety of HER2 IHC assays in clinical use globally
- This global ring study was conducted to analyze concordance between PATHWAY 4B5 and comparator assays in identifying HER2-low breast cancer
- Data for stage 1 were presented at the AACR Annual Meeting 2024. We report the overall results of the global ring study, combining data from stages 1 and 2

Methods

- Stage 1 included laboratories in Belgium, Canada, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Switzerland, and the United States
- Stage 2 included laboratories in Australia, Brazil, Chile, China, Hong Kong, Malaysia, New Zealand, the Philippines, and Taiwan
- Laboratories eligible to participate in the study were actively scoring HER2 IHC for breast cancer in a clinical setting, had 2 independent pathologists, and were not routinely using PATHWAY 4B5
- The study was conducted under the guidance of a steering committee comprising expert pathologists
  - The steering committee chose 50 formalin-fixed paraffin-embedded clinical breast cancer samples for each stage from a cohort of 300 for scoring
  - Samples were stained using PATHWAY 4B5 and centrally scored as: IHC 0 (n = 15), IHC 1+ (n = 17), IHC 2+ (n = 13), and IHC 3+ (n = 5)
  - Reference slides were used to control for potential biological heterogeneity throughout the sample cores and ensure the consistency of HER2 status
- Participating laboratories were instructed to score samples per American Society of Clinical Oncology-College of American Pathologists 2018 guidelines using their routine protocols and assays (HercepTest [Omnis or Link 48], Leica Oracle, non-4B5 laboratory-developed tests [LDTs], or 4B5 LDTs) within 14 days of receipt of samples
- Following virtual alignment on interpretation of HER2 IHC scoring guidelines, pathologists rescored the samples after a 2-week washout period
- Pre- and postalignment scores were analyzed centrally to determine concordance with the nonreference scores from the PATHWAY 4B5 assay in identifying HER2-low cases
  - Scores were excluded if a technical failure was confirmed by the control slides
- The primary endpoint was the PPA (HER2-low as positive) and NPA (HER2 IHC 0 as negative) between the PATHWAY 4B5 and comparator assay scores for HER2-low versus HER2 IHC 0 (with or absent membrane staining) based on postalignment results
  - Additional analyses included deriving Cohen κ coefficient to assess strength of agreement, and preparing receiver operating characteristic curves to assess the effect of guideline alignment
- The exploratory endpoint was an estimation of the variability between and within multiple factors that might influence concordance between assays, conducted via a general linear mixed-model analysis

Results

Study Disposition

- A total of 6270 postalignment scores from 129 pathologists at 68 laboratories were available for analysis (**Table 1**)
  - 135 pathologists from 70 laboratories recorded 6580 scores before virtual alignment

Table 1. Overview of participating laboratories/pathologists

	Laboratories, n		Pathologists, n		Evaluable Scores, n		
	Prealignment	Postalignment	Prealignment	Postalignment	Prealignment	Postalignment	Overall
Total	70	68	135	129	6580	6270	12,850
France	5	4	10	8	484	391	875
Germany	4	4	7	7	339	340	679
Italy	4	4	8	8	394	395	789
Spain	6	6	12	12	564	567	1131
Europe, other[a]	8	8	16	16	780	776	1556
United States and Canada	12	11	23	21	1128	1029	2157
Australia and New Zealand	5	5	9	8	439	395	834
Latin America[b]	4	4	7	7	330	335	665
China	16	16	32	32	1588	1593	3181
Asia-Pacific[c]	6	6	11	10	534	449	983

[a]Includes Belgium, Greece, the Netherlands, Norway, and Switzerland.
[b]Includes Brazil and Chile.
[c]Includes Hong Kong, Malaysia, the Philippines, and Taiwan.

- Assays in use varied by country/region (**Table 2**)
  - Non-4B5 LDTs were the most common comparator assays used (44 laboratories, 3794 scores)
  - The Leica Oracle–Leica Bond III comparator assay was only used in 2 laboratories for a total of 196 scores, limiting the conclusions drawn for this assay

Table 2. Overview of comparator assay use by country/region

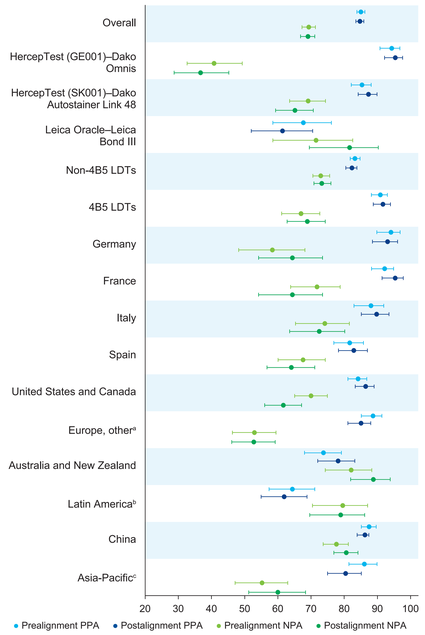
Comparator Assays Used	Overall Cases/Laboratories	Overall Postalignment Scores
HercepTest (GE001)–Dako Omnis	**5**	467
Spain	1	
Europe, other[a]	3	
United States and Canada	1	
HercepTest (SK001)–Dako Autostainer Link 48	**10**	929
Italy	3	
Spain	2	
United States and Canada	3	
Latin America[b]	1	
Asia-Pacific[c]	1	
Leica Oracle–Leica Bond III	**2**	196
Spain	1	
Asia-Pacific[c]	1	
Non-4B5 LDTs	**44**	3794
France	3	
Germany	4	
Italy	1	
Spain	2	
Europe, other[a]	4	
United States and Canada	6	
Australia and New Zealand	4	
Latin America[b]	3	
China	13	
Asia-Pacific[c]	4	
4B5 LDTs	**10**	884
France	3	
Europe, other[a]	1	
United States and Canada	2	
Australia and New Zealand	1	
China	3	

[a]Includes Belgium, Greece, the Netherlands, Norway, and Switzerland.
[b]Includes Brazil and Chile.
[c]Includes Hong Kong, Malaysia, the Philippines, and Taiwan.

Concordance Between Comparator Assays and PATHWAY 4B5

- Overall, the postalignment PPA and NPA were 84.8% (95% CI, 83.6%-86.0%) and 69.2% (95% CI, 67.0%-71.2%), respectively (**Figure 1**)
- Across different comparator assays, there was substantial variability in PPA and NPA:
  - PPA ranged from 61.6% (Leica Oracle) to 95.5% (HercepTest Omnis)
  - NPA ranged from 36.9% (HercepTest Omnis) to 81.7% (Leica Oracle)
- Postalignment PPA generally tended to be higher than NPA (**Figure 1**)
- Variability of PPA and NPA between country/region subgroups may be related to the assays used most commonly in each region

Figure 1. PPA and NPA by assay and country/region subgroup



PPA and NPA values were calculated based on HER2-low and HER2 IHC 0 scores only.
[a]Includes Belgium, Greece, the Netherlands, Norway, and Switzerland.
[b]Includes Brazil and Chile.
[c]Includes Hong Kong, Malaysia, the Philippines, and Taiwan.

- The overall postalignment area under the receiver operating characteristic curve (AUROC) was 0.77
  - AUROC ranged from 0.70 with Leica Oracle–Bond III to 0.83 with 4B5 LDTs
- Postalignment Cohen κ for comparator assays tended towards a moderate to substantial concordance with PATHWAY 4B5

Table 3. AUROC data for HER2-low versus HER2 IHC 0

	HER2-Low vs HER2 IHC 0 Breast Cancer			
	AUROC		Cohen κ (95% CI)	
	Prealignment	Postalignment	Prealignment	Postalignment
Overall	0.78	0.77	0.55 (0.53-0.57)	0.54 (0.52-0.57)
Comparator Assay				
HercepTest (GE001)–Dako Omnis	0.77	0.78	0.40 (0.31-0.49)	0.37 (0.28-0.46)
HercepTest (SK001)–Dako Autostainer Link 48	0.78	0.78	0.55 (0.49-0.61)	0.54 (0.48-0.60)
Leica Oracle Bond III	0.68	0.70	0.37 (0.23-0.51)	0.39 (0.26-0.51)
Non-4B5 LDTs	0.78	0.77	0.56 (0.53-0.59)	0.55 (0.52-0.58)
4B5 LDTs	0.82	0.83	0.60 (0.54-0.66)	0.63 (0.57-0.69)
Country/Region				
France	0.85	0.86	0.66 (0.59-0.74)	0.64 (0.55-0.73)
Germany	0.83	0.83	0.57 (0.46-0.67)	0.61(0.51-0.70)
Italy	0.82	0.83	0.63 (0.54-0.71)	0.64 (0.55-0.72)
Spain	0.75	0.74	0.49 (0.41-0.57)	0.48 (0.40-0.56)
Europe, other[a]	0.75	0.71	0.44 (0.37-0.51)	0.40 (0.32-0.47)
United States and Canada	0.77	0.76	0.54 (0.49-0.60)	0.50 (0.44-0.56)
Australia and New Zealand	0.75	0.80	0.52 (0.43-0.60)	0.62 (0.54-0.71)
Latin America[b]	0.70	0.68	0.39 (0.29-0.49)	0.36 (0.26-0.46)
China	0.82	0.83	0.65 (0.61-0.69)	0.66 (0.62-0.70)
Asia-Pacific[c]	0.73	0.71	0.43 (0.35-0.52)	0.41 (0.31-0.51)

[a]Includes Belgium, Greece, the Netherlands, Norway, and Switzerland.
[b]Includes Brazil and Chile.
[c]Includes Hong Kong, Malaysia, the Philippines, and Taiwan.

κ Color Coding: 0.61-0.80 | 0.41-0.60 | 0.21-0.40
AUC Color Coding: AUC >0.80 | AUC 0.70-0.80 | AUC <0.70

Results of General Linear Mixed-Model Analysis

- Samples were less likely to be scored as HER2 IHC 0 with HercepTest (GE001) Omnis and more likely to be scored as HER2 IHC 0 with non-4B5 LDTs (**Table 4**)
- In artifact-free samples, the odds for a HER2 IHC 0 assessment over HER2-low assessment were 1.53 times higher (**Table 4**)

Table 4. Effect of contributing factors on variance of scoring HER2 IHC 0 and HER2-low

Parameter	*P*	OR (95% CI)
Training status (reference category: prealignment)		
Postalignment	0.5827	1.049 (0.884-1.246)
Assay type (reference category: 4B5 LDTs)		
HercepTest Omnis (GE001)	<0.0001	0.060 (0.023-0.156)
HercepTest Link 48 (SK001)	0.5528	1.237 (0.613-2.495)
Non-4B5 LDTs	<0.0001	3.172 (1.803-5.580)
Leica Oracle Bond III	<0.0001	24.015 (8.014-71.967)
Free from artifacts (reference category: no)		
Yes	0.0526	1.534 (0.995-2.363)
Highest magnification used (reference category: ≤5×)		
10×	0.1028	0.443 (0.167-1.178)
20×	0.0014	0.207 (0.079-0.542)
40×	0.1792	0.518 (0.198-1.353)
Time spent on HER2 IHC scoring (reference category: <1 min)		
1-5 min	<0.0001	0.105 (0.069-0.160)
>5-10 min	<0.0001	0.026 (0.015-0.045)
>10 min	<0.0001	0.033 (0.014-0.078)

Dependent variable: the categorized HER2 scorings (HER2 IHC 0 and HER2-low) provided by the pathologists.
Independent variables: fixed effects (reference category): training status (prealignment), assay type (4B5 LDTs), free from artifacts (no), highest magnification used (≤5×), time spent (<1 min).

- Among the random effects examined, the main contributing factor (estimate of 28.4) is nested and crossed effect of sample, pathologist, and laboratory, indicating that the sample constitution and the assessing pathologists in the laboratories are contributing the most to the assessment for HER2-low and HER2 IHC 0
  - Note that pathologists were limited to the equipment of their laboratory, including available assays and staining methods
  - Further investigation would be required to analyze the detailed relationships and effects of these factors

Abbreviations

AUC, area under the curve; AUROC, area under the receiver operating characteristic curve; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; LDTs, laboratory-developed tests; NPA, negative percentage agreement; OR, odds ratio; PPA, positive percentage agreement; T-DXd, trastuzumab deruxtecan.

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