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Concordance Between the DESTINY-Breast04 Clinical Trial Assay (4B5[CDx]) and Other HER2 IHC Assays for HER2-low Breast Cancer in Real-World Practice: First Phase of a Large-Scale, Multicenter Global Ring Study

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Objective

- To assess the concordance between the VENTANA PATHWAY anti-human epidermal growth factor receptor 2 (HER2)/neu (4B5) clinical trial assay (4B5[CDx]) and comparator assays (CAs) in differentiating between HER2-low (immunohistochemistry [IHC] 1+ and IHC 2+/in situ hybridization not amplified [ISH–]) and HER2 IHC 0 breast cancer (BC) samples following virtual scoring guideline alignment for HER2 IHC scoring that focused on HER2-low identification

Conclusions

- Concordance between the 4B5(CDx) and CAs in the ability to categorize HER2-low versus HER2 IHC 0 varied among assay types
  - The positive percent agreement (PPA) in identifying HER2-low tended to be numerically high across assay types, with 4B5 laboratory-developed test (LDT) assays having the highest PPA
- Virtual scoring guideline alignment did not substantially alter agreement between assays, suggesting that the observed discordance is due to inherent differences in the assay and was not confounded by inconsistent adherence to scoring guidelines

Plain Language Summary

Why did we perform this research?
Breast cancer (BC) can be characterized based on the amount of a protein called human epidermal growth factor receptor 2 (HER2) found on tumor cells.[1] A subset of tumors only express low levels of HER2, and these are categorized as HER2-low (immunohistochemistry [IHC] 1+ or IHC 2+/in situ hybridization not amplified). In the clinical trial DESTINY-Breast04, trastuzumab deruxtecan (T-DXd), an anticancer therapy called an antibody-drug conjugate, was established as an effective treatment for HER2-low breast cancer.[2] However, successful identification of HER2-low tumor samples remains an ongoing challenge, with pathologists using several different testing methods to identify HER2 expression levels.[3-5] The study presented here sought to characterize the agreement between the assay used in DESTINY-Breast04 (4B5 [CDx]) and other assays used around the world.

How did we perform this research?
This first phase of the study included laboratories in the United States, Canada, and Europe. The HER2 IHC status of 50 BC samples was determined by a central laboratory using the 4B5(CDx). Sections of these samples were then sent to different laboratories around the world where pathologists stained the samples, using their standard assays, and scored them for HER2 expression levels. The pathologists then received virtual guideline alignment on HER2 IHC scoring, after which they rescored the samples. All scores were compared with the reference 4B5(CDx) scores to determine the agreement in identifying HER2-low versus HER2 IHC 0.

What were the findings of this research and what are the implications?
The agreement between the 4B5(CDx) and the comparator assays (CAs) in identifying HER2-low varied based on the type of CA used, with the ability of CAs to correctly identify HER2-low tending to be generally high. There tended to be low agreement in correctly identifying HER2 IHC 0 samples, suggesting the need for further improvement in methods.

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Introduction

- More than half of all BCs express HER2 at low levels, defined as an IHC score of 1+ or IHC 2+ and ISH– per the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines[1,2]
  - Clinically, these tumors have been reported as HER2 negative, and patients with this subtype have limited targeted therapy options[1,3]
- In the pivotal phase 3 DESTINY-Breast04 trial, trastuzumab deruxtecan (T-DXd), a HER2-directed antibody-drug conjugate (ADC), demonstrated a robust response with a manageable safety profile in patients with HER2 IHC 1+ and IHC 2+/ISH– metastatic BC (mBC) and led to approval by the US Food and Drug Administration (FDA) and the European Medicines Agency for the use of T-DXd in patients with HER2-low mBC[3-5]
  - These results support the clinical need for a subclassification of HER2-negative BC into HER2-low, defined as IHC 1+ or IHC 2+/ISH–, and HER2 IHC 0
  - DESTINY-Breast04 assessed HER2 IHC scoring using the VENTANA PATHWAY anti-HER2/neu (4B5) assay, which has now been approved by the FDA as a companion diagnostic test (CDx)[3,6]
- However, real-world differentiation between HER2-low and HER2 IHC 0 mBC is still an ongoing challenge[7-9]
  - We present an assessment of the concordance between the 4B5(CDx) and various CAs in clinical use

Methods

- The first phase of this 2-phase study encompassed the United States (US), Canada, and Europe
- Qualified laboratories were actively scoring HER2 IHC for BC in a clinical setting, were not using the 4B5(CDx), and had 2 pathologists available
  - Where possible, pathologists had not received any training in HER2-low scoring
  - Overall, 21.1% of pathologists enrolled in the study (16/76) disclosed that they had received previous training in HER2-low scoring
- 50 formalin-fixed paraffin-embedded BC samples were chosen from an initial cohort of 300 samples by a steering committee composed of expert pathologists
  - Samples were stained in a central laboratory using the 4B5(CDx) and scored using the 2018 ASCO/CAP breast scoring guidelines[10]
  - 15 samples were scored as IHC 0, 17 as IHC 1+, 13 as IHC 2+, and 5 as IHC 3+
  - Unstained slides from the selected cases were then sent to the comparator laboratories
- Within 14 days of sample receipt laboratories stained the samples following their routine protocols, and 2 pathologists independently scored them using their routine scoring algorithms
  - Following their baseline readings study pathologists received virtual guideline alignment on HER2 IHC scoring, with an emphasis on HER2-low identification
  - Alignment consisted of approximately 30 cases comprising biopsies and surgical specimens across a range of HER2 expression levels covering IHC scores of 0 to 3+ and included borderline cases that were difficult to interpret
  - The pathologists then rescored the samples following a 2-week washout period
- The pre- and postalignment scores were centrally analyzed for concordance with the 4B5(CDx) in identifying HER2-low cases
  - The primary endpoint was the positive percent agreement (PPA; considering HER2-low as positive) and the negative percent agreement (NPA; considering HER2 IHC 0 as negative) between the 4B5(CDx) and CA scores for HER2-low versus HER2 IHC 0 based on the postalignment score results

Results

Study Disposition

- Laboratories in several countries using various CA platforms participated in the study; 3449 postalignment scores were available for central analysis (**Table 1 and Table 2**)

Table 1. Overview of Laboratories Included in the Study

	Overall N	Guideline Alignment Status	
		Prealignment n	Postalignment n
Laboratories with CA HER2 IHC scores	**39**	**39**	**37**
Germany	4	4	4
France	5	5	4
Italy	4	4	4
Spain	6	6	6
US and Canada	12	12	11
Europe, other	8	8	8
Participating pathologists	**76**	**76**	**71**
Germany	7	7	7
France	10	10	7
Italy	8	8	8
Spain	12	12	12
US and Canada	23	23	21
Europe, other	16	16	16
Total evaluable CA HER2 IHC scores for central analysis	**7138**	**3689**	**3449**

Table 2. Overview of Antibody and Staining Platforms Used in the Study

Antibody and Staining Platforms Used	Overall Cases/Laboratories
HercepTest (GE001)—Dako Omnis	**5**
Spain	1
US and Canada	1
Europe, other	3
HercepTest (SK001)—Dako Autostainer Link 48	**8**
Italy	3
Spain	2
US and Canada	3
Leica Oracle—Leica Bond III	**1**
Spain	1
Non-4B5 LDTs	**20**
Germany	4
France	3
Italy	1
Spain	2
US and Canada	6
Europe, other	4
4B5 LDTs	**6**
France	3
US and Canada	2
Europe, other	1

Concordance Between the 4B5(CDx) and CAs

- Postalignment results are described as there was no statistically significant variation between pre- and postalignment scores (**Table 5**)
- The postalignment PPA and NPA for the overall scores were 87.5% (95% CI, 86.0%-89.0%) and 61.9% (95% CI, 58.9%-64.9%), respectively
  - The PPA and NPA varied across subgroups, with the highest PPA seen with 4B5 LDTs (96%) and the highest NPA seen with Leica Oracle Bond III (80.0%) (**Table 3**)
- The Cohen κ value for the comparison of the overall CA postalignment scores with the 4B5(CDx) scores was 0.51 (95% CI, 0.48-0.54) (**Table 4**)
  - The highest Cohen κ value was seen with 4B5 LDTs (κ = 0.59)
- The area under the receiver operating characteristic curve (AUROC) was generally between 0.7 and 0.8 for most subgroups (**Table 4**)
  - The AUROC showed agreement above 0.8 for the 4B5 LDTs

Table 3. HER2-low Versus HER2 IHC 0 Postalignment Assessments

		HER2-low vs HER2 IHC 0 BC Postalignment Scores	
		PPA (95% CI), %	NPA (95% CI), %
Overall postalignment CA test results (N = 3017)		87.5 (86.0-89.0)	61.9 (58.9-64.9)
CA subgroups	**HercepTest Omnis (GE001) n = 408**	95.5 (92.3-97.7)	36.9 (28.9-45.4)
	HercepTest Link48 (SK001) n = 707	88.5 (85.2-91.2)	64.3 (57.8-70.4)
	Leica Oracle Bond III n = 84	59.3 (45.0-72.4)	80.0 (61.4-92.3)
	Non-4B5 LDTs n = 1395	83.8 (81.2-86.1)	67.8 (63.5-72.0)
	4B5 LDTs n = 423	96.0 (93.0-98.0)	58.8 (50.4-66.8)
Country subgroups	**Germany n = 290**	93.1 (88.5-96.3)	64.4 (54.2-73.6)
	France n = 300	95.4 (91.5-97.9)	64.4 (54.4-73.6)
	Italy n = 347	89.9 (85.2-93.5)	72.5 (63.6-80.3)
	Spain n = 493	83.0 (78.4-86.9)	64.2 (56.6-71.3)
	US and Canada n = 912	86.6 (83.6-89.2)	61.8 (56.1-67.3)
	Europe, other n = 675	85.0 (81.3-88.2)	52.8 (46.2-59.3)

Table 4. Postalignment AUROC and Cohen κ Scores for HER2-low versus HER2 IHC 0

		HER2-low vs HER2 IHC 0 BC	
		AUROC[a]	Cohen κ (95% CI)
Overall		0.77	0.51 (0.48-0.54)
CA subgroups	**HercepTest Omnis (GE001)**	0.78	0.37 (0.28-0.46)
	HercepTest Link48 (SK001)	0.78	0.55 (0.48-0.61)
	Leica Oracle Bond III	0.68	0.35 (0.17-0.53)
	Non-4B5 LDTs	0.76	0.52 (0.47-0.57)
	4B5 LDTs	0.85	0.59 (0.51-0.68)
Country subgroups	**Germany**	0.83	0.61 (0.51-0.70)
	France	0.86	0.64 (0.55-0.73)
	Italy	0.83	0.64 (0.55-0.72)
	Spain	0.74	0.48 (0.40-0.56)
	US and Canada	0.76	0.50 (0.44-0.56)
	Europe, other	0.71	0.40 (0.32-0.47)

[a]Comparing CA scores to the nonreference 4B5(CDx) standard

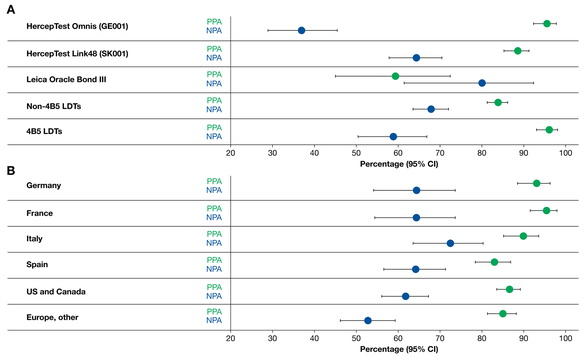
κ Color Coding: 0.61-0.80 | 0.41-0.60 | 0.21-0.40

AUC Color Coding: AUC >0.80 | AUC 0.70-0.80 | AUC <0.70

Concordance Across Subgroups

- PPA tended to be high across all subgroups (**Figure 1**)
  - The lowest PPA was observed with the Leica Oracle Bond III assay (**Figure 1A**)
- Postalignment NPA tended to be lower across subgroups (**Figure 1**)
  - The lowest NPA was observed with the HercepTest Omnis (GE001) assay (**Figure 1A**)
- NPA tended to show more variability between assay types compared with laboratory geographies (**Figure 1**)

Figure 1. Forest Plots for Postalignment PPA and NPA for (A) CA and (B) Country Subgroups



Factors Contributing to Scoring of HER2-low Versus HER2 IHC 0

- A generalized linear mixed model was used to analyze the possible factors contributing to variance in scoring of HER2-low versus HER2 IHC 0 (**Table 5**)
- HER2 IHC 0 classification was more likely when slides were free from artifacts (odds ratio [OR], 1.82 [95% CI, 1.01-3.27]; $P < 0.05$) (**Table 5**)
- The effect of assay type varied, with HER2 IHC 0 classification being more likely with the HercepTest Link48 (SK001), Leica, and non-4B5 LDTs (ORs, >1; $P < 0.01$) and less likely with HercepTest Omnis (GE001)(OR, <1; $P = 0.06$) (**Table 5**)
  - The exceptionally high OR for the Leica assay is due to the small number of test scores available for analysis

Table 5. Factors Contributing to Variance of Scoring for HER2-low Versus HER2 IHC 0

Parameter[a]	P	OR (95% CI)
Scoring guideline alignment status		
Postalignment	0.1221	0.83 (0.66-1.05)
Assay type		
HercepTest Link48 (SK001)	0.0032	4.60 (1.67-12.69)
HercepTest Omnis (GE001)	0.0615	0.33 (0.10-1.06)
Non-4B5 LDTs	<0.0001	10.19 (3.96-26.22)
Leica Oracle Bond III	<0.0001	282.08 (53.68-1482.32)
Free from artifacts		
Yes	0.0469	1.82 (1.01-3.27)
Highest magnification used		
10×	0.1382	0.31 (0.07-1.46)
20×	0.0167	0.15 (0.03-0.71)
40×	0.2862	0.43 (0.09-2.02)
Time spent on HER2 IHC scoring		
1-5 min	<0.0001	0.17 (0.09-0.30)
>5-10 min	<0.0001	0.05 (0.02-0.10)
>10 min	0.0146	0.24 (0.08-0.75)

[a]Reference categories for comparison of each parameter are as follows: scoring guideline alignment status, prealignment; assay type, 4B5(CDx); free from artifacts, no; highest magnification used, ≤5x; time spent on HER2 IHC scoring, <1 min.

Abbreviations

ADC, antibody-drug conjugate; ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; AUROC, area under the receiver operating characteristic curve; BC, breast cancer; CDx, companion diagnostic test; CTA, clinical trial assay; EMA, European Medicines Agency; FDA, US Food and Drug Administration; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; LDT, laboratory-developed test; mBC, metastatic breast cancer; NPA, negative percentage agreement; OR, odds ratio; PPA, positive percentage agreement; T-DXd, trastuzumab deruxtecan; US, United States.

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Disclosures

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