



**Comparison of Anti-HER2 Antibodies
Clone 4B5 and Clone EP3
Immunohistochemical Assay at the Low
End of HER2 Immunoreactivity**



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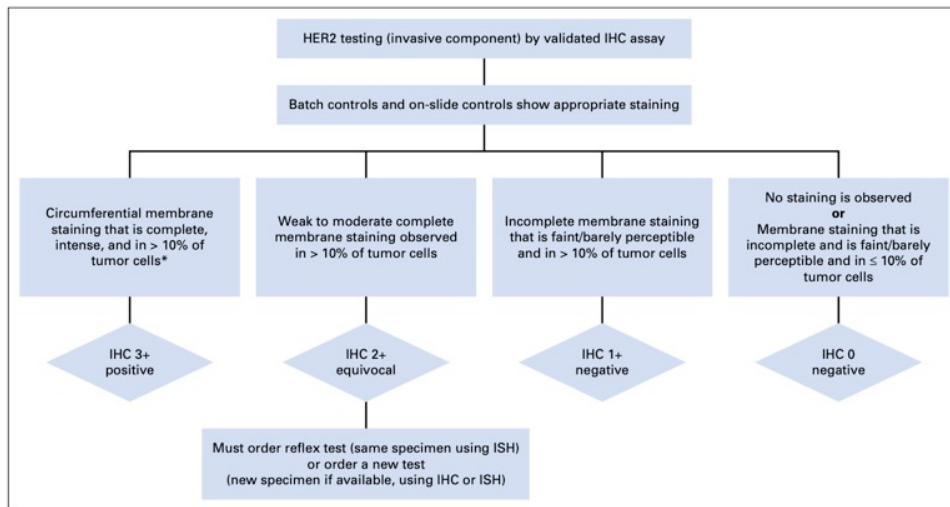
Background

In the DESTINY-04 clinical trial, a novel anti-HER2 antibody-drug conjugate (Trastuzumab-Deruxtecan, *Enhertu*) has shown improved survival in patients with HER2-low expression status.

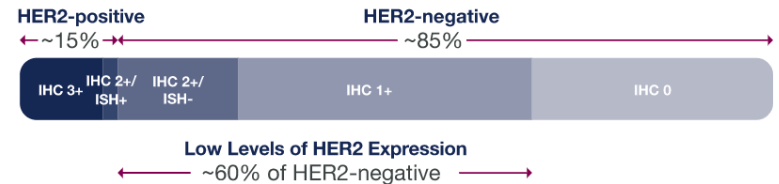
The Roche-Ventana 4B5 Rabbit Monoclonal anti-HER2 antibody assay has been approved by the FDA as the companion diagnostic for establishing HER2-low status as a pre-requisite for *Enhertu* treatment and has served to introduce the HER2-low spectrum concept.

Overview

The American Society of Clinical Oncology/ College of American Pathologist (ASCO/CAP) 2018/2023

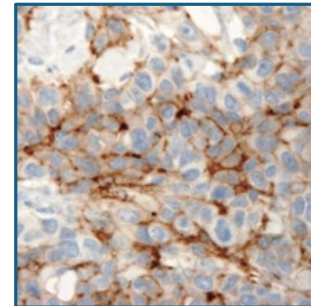


Prevalence of HER2 expression across the spectrum¹²

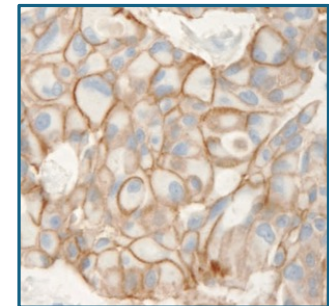


HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

HER2 IHC Score 1+

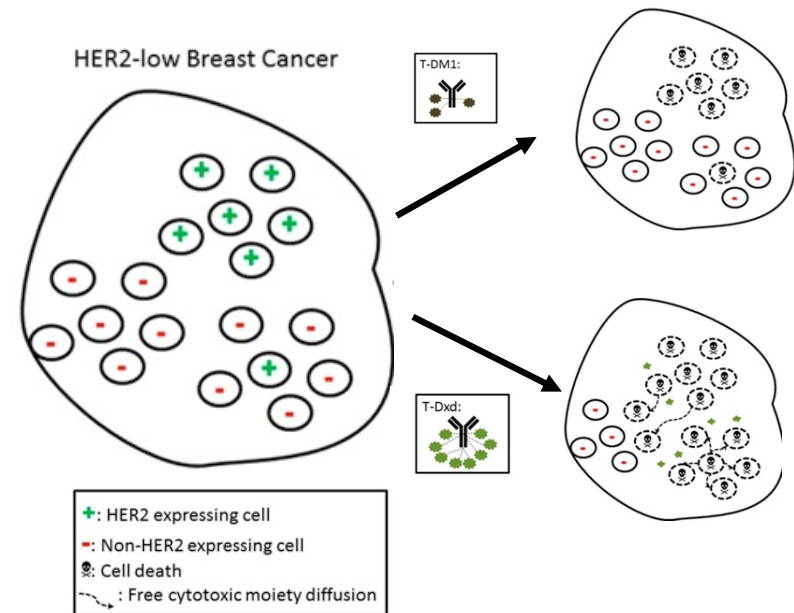
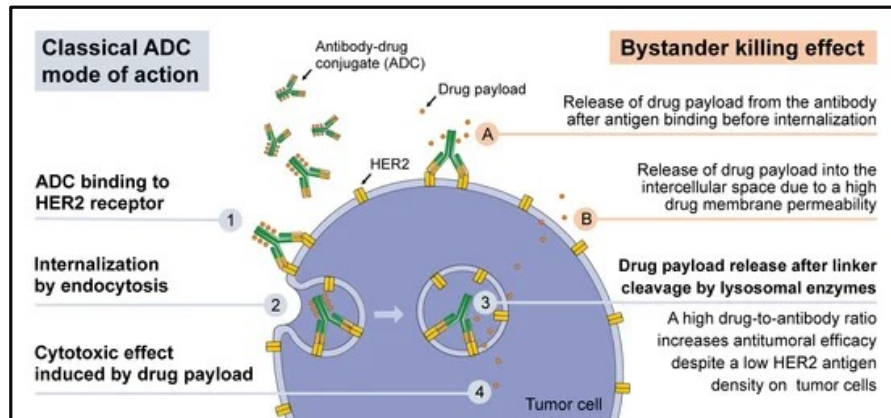


HER2 IHC Score 2+



Overview

Mechanisms of Action of the Novel Anti-HER2 drugs Trastuzumab-Deruxtecan



Background

- Identifying patients with HER2-low expression is important, as these patients have shown benefit from *Enhertu* treatment.
- Our goal in this study:
 - To evaluate two different anti-HER2 IHC assays.
 - **4B5 Clone Rabbit Monoclonal anti-HER2 antibody (Roche-Ventana) and EP3 Clone anti-HER2 antibody (Epitomics-Abcam on Leica platform)**
 - And compare their immunoreactivity, **particularly at the 0 and 1+ HER-2 IHC scores.**

Methods and Materials

64 cases of invasive breast carcinoma
(from 2018 to 2022)
Scores 0, 1+ and 2+/
negative-ISH, assessed by
Roche-Ventana 4B5 anti-
HER2 antibody assay kit

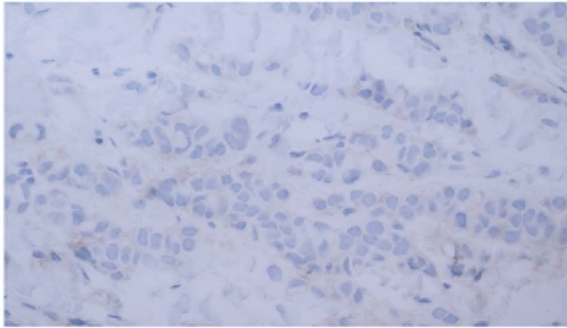
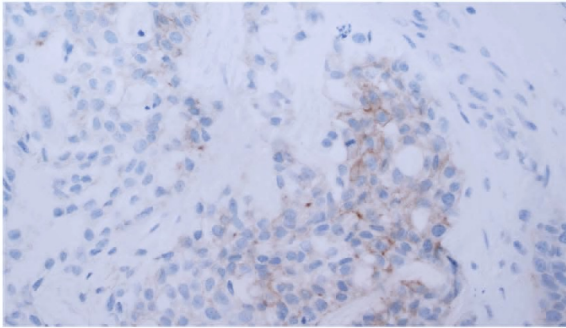
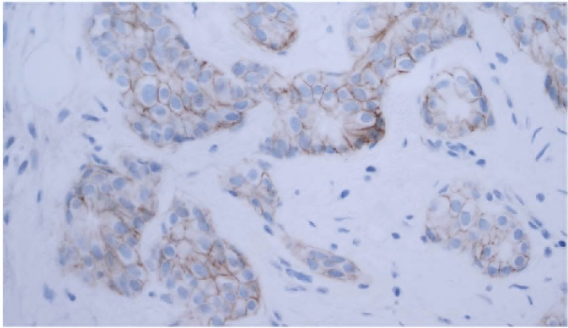
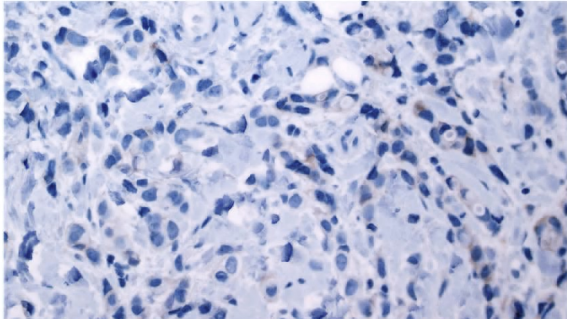
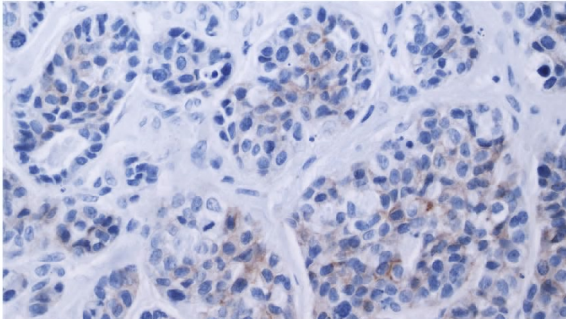
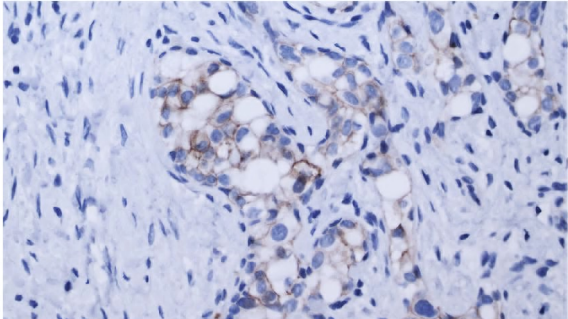


The unstained slide from the
same tissue block was cut
Tested using the EP3 anti-
HER2 antibody on the Leica
Bond III platform
(1:50, H1 for 20 min).



The results were
evaluated in a blinded
fashion by breast-
pathologist.

Results

Antibodies	HER2 SCORE		
	0	1+	2+
4B5 clone			
EP3 clone			

Results

Her-2 Score by Antibody		4B5 (Roche)		
		0	1+	2+
EP3 (Cell-Marque)	0	9	2	0
	1+	5	19	2
	2+	0	14	13

- **2** out of **35** cases (5.7%) which expressed HER2 score 1+ immunoreactivity with the 4B5 antibody showed no immunoreactivity using the EP3 antibody.

Results

Her-2 Score by Antibody		4B5 (Roche)		
		0	1+	2+
EP3 (Cell-Marque)	0	9	2	0
	1+	5	19	2
	2+	0	14	13

- **5** out of **14** cases (35.7%) which expressed HER2 score 1+ immunoreactivity with the EP3 antibody showed no immunoreactivity using the 4B5 antibody.

Results

Her-2 Score by Antibody		4B5 (Roche)		
		0	1+	2+
EP3 (Cell-Marque)	0	9	2	0
	1+	5	19	2
	2+	0	14	13

- There was a **64.2%** concordance rate for HER2 score 0

Results

Her-2 Score by Antibody		4B5 (Roche)		
		0	1+	2+
EP3 (Cell-Marque)	0	9	2	0
	1+	5	19	2
	2+	0	14	13

- There was a **54.2%** concordance rate for HER2 score 1+

Results

Her-2 Score by Antibody		4B5 (Roche)		
		0	1+	2+
EP3 (Cell-Marque)	0	9	2	0
	1+	5	19	2
	2+	0	14	13

- There was an **86.6%** concordance rate for HER2 score 2+

Conclusion

- Anti-HER2 antibody clone EP3 demonstrated similar immunoreactivity to clone 4B5 at the low end of immunoreactivity (HER2 IHC score 0, 1+ and 2+).

Her-2 Score by Antibody		4B5 (Roche)		
		0	1+	2+
EP3 (Cell-Marque)	0	9	2	0
	1+	5	19	2
	2+	0	14	13

- Anti-HER2 antibody clone EP3 trended towards a higher degree of immunoreactivity across all cases.

Discussion

- Her-2 IHC staining interpretation has many variables starting from tissue sampling, pre-analytical and analytical.
- Her-2 IHC assay is design to distinguish score 3+ from non-3+;
- The correlation between immunoreactivity and expression is not linear.
- Her-2 low status has not yet been proven predictive.
- The score 0 versus Her-2 low by 4B5 or EP3 clones may not wholly represent physiologic expression at low end of Her-2. Further study by other assay may be necessary (i.e. mass spectrometry).
- The most recent CAP/ASCO guidelines appropriately has not designated the Her-2 low as a predictive status. It recommends reporting it as negative, score “1+ or 0”.

Thank you

References

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