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Novel approach to HER2 quantification: digital pathology coupled with AI-based image and data analysis delivers objective and quantitative HER2 expression analysis for enrichment of responders to trastuzumab deruxtecan (T-DXd; DS-8201), specifically in HER2-low patients

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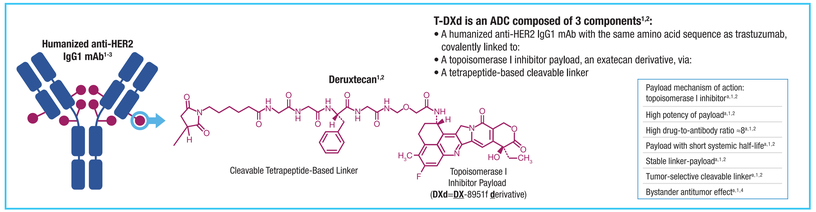
BACKGROUND

- T-DXd is an antibody-drug conjugate (**Figure 1**) that has shown antitumor activity in patients with metastatic breast cancer with HER2-overexpression (IHC 3+ or IHC 2+/ISH–) and those with low HER2 expression (IHC 1+ or IHC 2+/ISH–)[1-6]
- Current HER2 protein expression assessment is based on manual pathologist scoring that classifies tumors by the percentage of tumor cells with highest intensity and completeness of staining
- However, a critical need exists for more objective and quantitative methods to assess HER2 expression, specifically to better identify patients with low-level expression if T-DXd proves to be efficacious in this patient population

Figure 1. Structure and Characteristics of T-DXd



T-DXd is an ADC composed of 3 components[1,2]:

- A humanized anti-HER2 IgG1 mAb with the same amino acid sequence as trastuzumab, covalently linked to:
- A topoisomerase I inhibitor payload, an exatecan derivative, via:
- A tetrapeptide-based cleavable linker

Payload mechanism of action: topoisomerase I inhibitor[a,1,2]
High potency of payload[a,1,2]
High drug-to-antibody ratio ≈8[a,1,2]
Payload with short systemic half-life[a,1,2]
Stable linker-payload[a,1,2]
Tumor-selective cleavable linker[a,1,2]
Bystander antitumor effect[a,1,4]

ADC, antibody-drug conjugate; HER2, human epidermal growth factor receptor 2; IgG1, immunoglobulin G1; mAb, monoclonal antibody; T-DXd, trastuzumab deruxtecan.
[a] The clinical relevance of these features is under investigation.

METHODS

- Deep learning (DL)–based image analysis (IA) was used to generate a novel HER2 quantitative continuous score (QCS; **Figure 2**)
- Data analytic techniques determined optimal HER2 QCS for a phase 1 trial (J101, NCT02564900) of 151 patients with varying HER2 expression levels (ranging from IHC 0 to IHC 3+)
  - The HER2 QCS consists of DL models trained on commercial breast cancer samples to detect membrane, cytoplasm and nuclei of all tumor cells
- QCS was extensively trained using pathologists' annotations, and the performance was validated on unseen data to ensure its generalization and robustness
- QCS was blindly applied to J101 data. The optical density (OD; level of brown stain intensity) was computed on detected membranes to derive features that could be linked to survival prediction
- QCS features were selected to maximize objective response rate (ORR) in the positive group while maintaining high prevalence

RESULTS

- Analytical verification was demonstrated by showing high correlation (R=0.993) between the optical density (level of stain intensity) of the HER2 QCS cell membrane detection and expert pathologist annotation, which was comparable with correlation (R=0.995) between membrane annotations from 3 pathologists (**Figure 2**)
- HER2 QCS enables cell-by-cell HER2 expression analysis; differential distribution of cellular HER2 expression showed variable clinical response with a 3+ case with a majority of negative cells not responding to T-DXd (**Figure 3**)
- By using the HER2 QCS, we can further stratify patients compared to manual scoring. Even though the ORR in both groups seems similar, we have a positive prevalence of 80% and identified 68 of 75 responders. The conventional IHC score missed 27 patients (**Figure 4**)
- In the HER2 low population (IHC 2+/ISH–, IHC 1+/0; n=65) that would not be treated according to current guidelines, 42% of patients responded to T-DXd with a median progression-free survival (mPFS) of 13.7 months. Using HER2 QCS, we were able to further stratify this population into a subgroup of QCS-high patients with response and outcome increased to 53% and 14.5 months respectively, while the QCS-low group showed only 26% ORR and 8.6-month mPFS (**Figure 5**)
- Generally, the best performing QCS cutoffs were driven by a majority of tumor cells expressing a minimal amount of HER2, in contrast to current clinical guidelines that are driven by a minority of cells expressing higher levels of HER2 (**Figure 6**, top)
- We also examined spatial heterogeneity by characterizing cells as either bearing membrane stain above a determined OD threshold (positive cell) or lying within a certain distance from a positive cell. We observed similar efficacy with best performing cutoffs, again, being found when a minimal level of HER2 expression (OD) was examined (**Figure 6**, bottom)

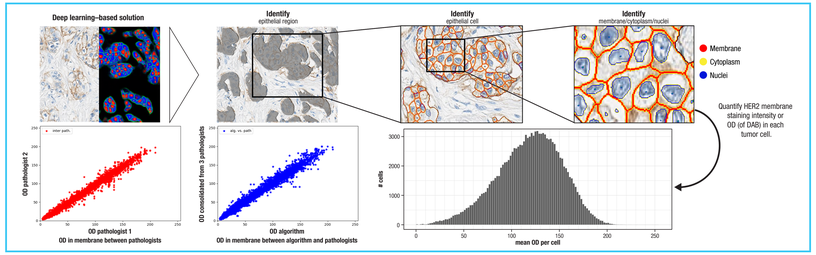
CONCLUSIONS

- Taken together, these data establish a clinical proof-of-concept demonstrating that use of HER2 QCS can potentially enhance prediction of patient outcome with T-DXd by increasing the sensitivity and specificity of identification of patients with high and low HER2 expression, especially in the HER2-low population
- The ability to identify patients in the HER2-low group who will benefit from HER2-targeted treatment is critical for a patient population that would otherwise not be treated with anti-HER2 therapy
- Further clinical verification and validation is ongoing

RESULTS

Figure 2. Quantitative Continuous Score (QCS) Analytical Development and Verification

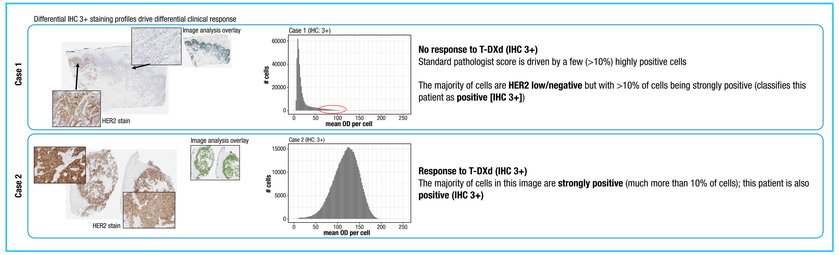
Image Analysis Enables Objective and Continuous HER2 Scoring by Quantifying HER2 Expression in Each Individual Tumor Cell



DAB, 3,3'-diaminobenzidine; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; OD, optical density.

Figure 3. Individual IHC 3+ Histogram Comparisons

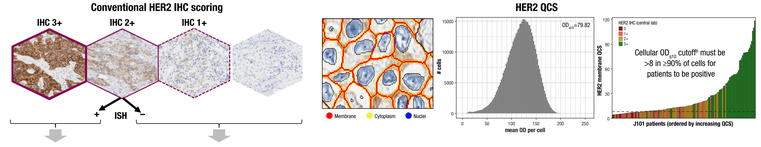
Two Examples of How Clinical Response Varies With Respect to Pattern of HER2 Expression Across the Whole Tumor Sample



HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; OD, optical density of brown DAB, after computational color separation; T-DXd, trastuzumab deruxtecan.

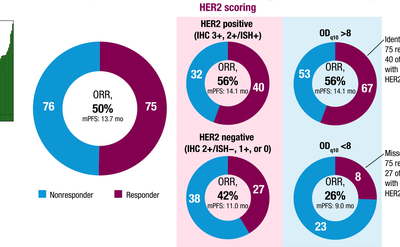
Figure 4. HER2 QCS Shows Broader Stratification of Efficacy Between HER2-High and -Low Expressors by Identification of a Greater Number of HER2 Expressors With Equivalent Efficacy

All patients with metastatic breast cancer from DS8201-A-J101 available for assessment (n=151)



Outcomes with T-DXd treatment[a]	HER2+ (IHC 3+ or 2+/ISH+) (n=72)	HER2– (IHC 2+/ISH–, 1+, or 0) (n=65)
ORR, %	56	42
mPFS, mo	14.1	11.0

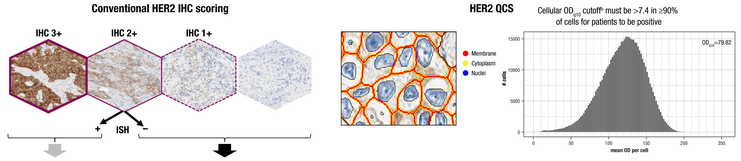
Outcomes with T-DXd treatment	HER2 QCS+ (OD_{10} >8) (n=120)	HER2 QCS– (OD_{10} <8) (n=31)
ORR, %	56	26
mPFS, mo	14.1	9.0



HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; mPFS, median progression-free survival; OD, optical density; OD_{10}, optical density in the 10% quantile; ORR, objective response rate; QCS, quantitative continuous score; T-DXd, trastuzumab deruxtecan.
[a] Analysis is based on cases that could be centrally confirmed by IHC and ISH. For 14 patients, no ISH data was available and therefore they could not be classified as HER2+ or HER2–.
[b] Cutoff determined through optimal cutoff analysis.

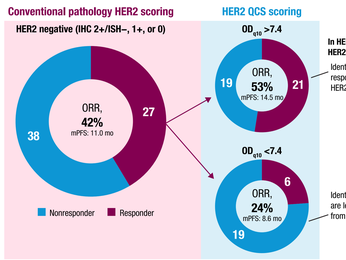
Figure 5. HER2 QCS Provides Greater Stratification of Efficacy Within the HER2-Low Population as Identified by Conventional IHC

Patients with HER2-negative (IHC 2+/ISH–, 1+, or 0) metastatic breast cancer from DS8201-A-J101 (n=65)



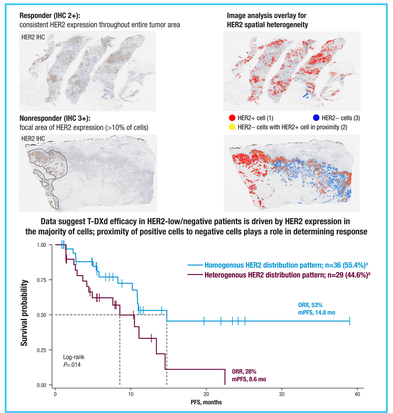
Outcomes with T-DXd treatment[a]	HER2+ (IHC 3+ or 2+/ISH+) (n=72)	HER2– (IHC 2+/ISH–, 1+, or 0) (n=65)
ORR, %	56	42
mPFS, mo	14.1	11.0

Outcomes with T-DXd treatment	HER2 QCS+ (OD_{10} >7.4) (n=40)	HER2 QCS– (OD_{10} <7.4) (n=25)
ORR, %	53	24
mPFS, mo	14.5	8.6



HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; mPFS, median progression-free survival; OD, optical density; OD_{10}, optical density in the 10% quantile; ORR, objective response rate; QCS, quantitative continuous score; T-DXd, trastuzumab deruxtecan.
[a] Analysis is based on cases that could be centrally confirmed by IHC and ISH. For 14 patients, no ISH data was available and therefore they could not be classified as HER2+ or HER2–.
[b] Cutoff determined through optimal cutoff analysis.

Figure 6. In HER2-Low/Negative Patients, Heterogeneous Expression May Be a Key Driver of T-DXd Response



HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; mPFS, median progression-free survival; OD, optical density; ORR, objective response rate; T-DXd, trastuzumab deruxtecan.
[a] At least 95% of cells need to have a certain OD value or be in close proximity to a positive cell.
[b] Less than 95% of cells have a certain OD value or are in close proximity to a positive cell.

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