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Examining "Her2 Low" patient populations for prediction to Her2-targeting antibody-drug conjugate response in Her2 negative patients using a novel high sensitivity immunohistochemistry assay

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Abstract

The positive results for trastuzumab deruxtecan (DS-8201) in Her2+ breast cancer from the pivotal phase II DESTINY-Breast01 trial announced in May 2019 represents a pivotal change in the approach to anti-Her2 therapy. Specifically, this antibody-drug conjugate (ADC) utilizes an exatecan derivative (Dxd) which targets Topoisomerase I and is thought to have a key benefit in mechanism of action over its comparator anti-Her2 ADC, Kadcyla (T-DM1, or ado-trastuzumab emtansine; a microtubule inhibitor). Data from the phase 1 trial for DS-2801 has demonstrated tumor shrinkage even in patients with low Her2 expression (Her2 IHC 2+/1+) by Her2 immunohistochemistry using the Ventana HER2 (4B5) assay (Lancet Oncol. 2017 Nov;18(11):1512-1522). This is thought to be due to a strong bystander effect of deruxtecan (Int J Cancer. 2019 May 14. doi: 10.1002/ijc.32408) and a resulting enhancement of anti-tumor immunity (Mol Cancer Ther. 2018 Jul;17(7):1494-1503). Thus, prediction of patient response to trastuzumab deruxtecan goes well beyond the past approaches of evaluating only Her2 over-expression or amplification, and potentially involves complex tumor and immune interactions, which drive a potent response even in patients who do not have high Her2 expression.

This represents an emerging "Her2 low" strategy in drug development which seeks to use antibodies against Her2 to direct cytotoxic or immune-stimulating payloads to tumor cells which do not overexpress Her2, but still retain some Her2 expression, even if considered diagnostically "Her2 negative" by the existing Her2 companion diagnostic strategies. Currently, there is no marketed approach to stratifying "Her2 low" patients for these therapies, as such an approach would require an improved method of Her2 detection or IHC interpretation strategy that allows better segmentation of patients with low Her2 expression. This demand is unlikely to be met through changing Her2 IHC interpretation, as the existing Her2 companion diagnostic strategy still remains challenging in clinical practice after nearly 15 years of improvements in scoring interpretations led by ASCO/CAP for improved testing performance.

This study demonstrates how we can meet this challenge using a novel approach to Her2 protein detection in human formalin-fixed, paraffin embedded (FFPE) tissues, called Quanticell ™. Quanticell relies on Konica Minolta's Phosphor-Integrated Dot (PID) technology for ultra-sensitive, in situ detection of Her2 protein in FFPE tissues. This approach is capable of detecting Her2 expression in clinical tissues that are characterized as "Her2 negative" by current IHC approaches, facilitating the determination of a new approach to a diagnostic cutpoint to stratify patients between "her2 negative" and "Her2 low"; which is distinct from the current "Her2 high" paradigm.

Methods

A breast tumor tissue FFPE microarray with 104 cases/208 cores (US Biomax, BR20810) was stained using anti-Her2/neu (clone 4B5) immunohistochemistry (Ventana i-VIEW detection system) to recapitulate the staining expected with existing Her2/neu companion diagnostic tests. The same microarray (serial section) was stained with Quanticell, Konica-Minolta's Phosphor Integrated Dot (PID) fluorescent nanoparticle technology as the detection system instead of DAB, while using the same primary assay conditions. The DAB stained TMA was scored using the ASCO/CAP guidelines for Her2 scoring to stratify patients into the standard 0+/1+/2+/3+ classes. The Quanticell stained TMA was scored using a novel quantitative approach, and the scoring methods compared.

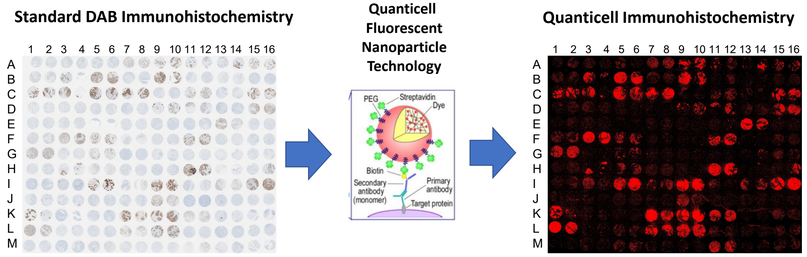


Figure1: The comparison approach. A standard DAB assay used in the companion diagnostic setting (Ventana 4B5) to predict response to anti-Her2 therapy was converted into a Quanticell-based assay using a novel fluorescent immunohistochemistry technology from Konica-Minolta. In comparison to DAB, the Quanticell approach has a much higher sensitivity and larger dynamic range, allowing clearer detection and precise quantification of Her2 across all patients regardless of the range of Her2 expression.

Comparison of Quanticell to DAB for Her2 Scoring

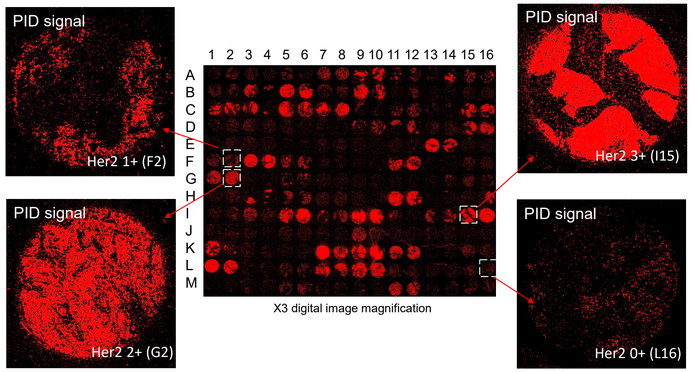


Figure 2: The Quanticell-based assay using a novel fluorescent immunohistochemistry technology from Konica-Minolta. The Quanticell approach shows much higher sensitivity compared to DAB, allowing clearer detection of "Her2 Low" examples. The assay also has a very large dynamic range, allowing the simultaneous detection of Her2 overexpressing (3+) examples as well as low Her2 expressing (1+) examples. Importantly, the Quanticell assay is still able to detect and quantify very low Her2 expression even in samples which would be classified as having no Her2 expression (0+).

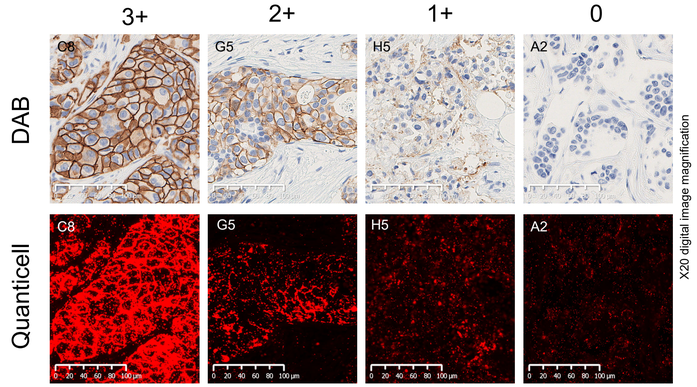
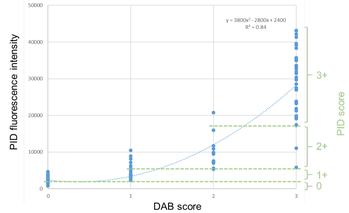


Figure 3: Comparison of DAB staining to Quanticell staining. The Quanticell approach creates the potential to develop a new patient stratification approach for newer anti-Her2 Antibody-drug Conjugate (ADC) therapies which have been shown to be efficacious in patients normally considered diagnostically negative by the existing Her2 companion diagnostics.

Her2 Classification by Assay Type



Her2 Score	0+	1+	2+	3+
PID score (mean)	2360	5510	9608	29066
# samples s	103	14	14	37
Mann-Whitney U test	N/A	0+ / 1+ difference p=2.03*10-e8	1+/2+ difference p=2.07*10-e3	2+ / 3+ difference p=1.47*10-e7

Figure 4: Comparison of DAB scoring to Quanticell scoring. The current Her2 ASCO/CAP scoring approach using traditional DAB staining techniques require the pathologist to report the 0+/1+/2+/3+ classification based on the percent of cells which have staining of a certain type. The most critical determination is in classifying patients as either 0+/1+ or 2+/3+ for ultimate determination of "Her2 positive" vs "Her2 negative" for classic anti-Her2 therapy. Here, even patients classified as 2+ may still receive therapy if they are determined to be positive by Her2 FISH testing.

However, modern anti-Her2 directed ADCs have been shown to have efficacy in patients traditionally classified as "Her2 negative" (0+/1+). While examination of the clinical reason for this continues, a leading hypothesis is that a more sensitive or calibrated Her2 test which has the ability to classify "Her2 Low" patients could direct these patients to Her2 ADC therapy optimally. Quanticell can meet this demand as it does not require the use of the typical 0/1/2/3 Her2 score classifications, but rather provides a quantitative, linear score that can be used to determine the precise amount of Her2 expression. The Quanticell approach can be use to create new patient response classifications in the way that accurately reflects response to anti-Her2 ADCs. The graph showing the relationship between the Her2 Quanticell score and the DAB Her2 classifications are shown to illustrate this potential.

DAB score \ PID score	3+	2+	1+	0	Total
3+	36	2	0	0	38
2+	1	12	0	0	13
1+	0	7	9	0	16
0	0	0	49	54	103
Total	37	21	58	54	170

=0+ by DAB IHC but considered 1+ by Quanticell

=0+ by DAB IHC and Quanticell

Figure 5: Comparison of DAB classifications to presumptive Quanticell classifications in a confusion matrix. The Quanticell scores were assigned into novel 0+/1+/2+/3+ classifications based on a statistical approach to reflect how they may be used for therapy determination:

- For traditional anti-Her2 therapy, only the patients assigned to the Quanticell 3+ category would be considered "Her2 positive". This approach matches to the traditional DAB-based 3+ classification well.
- For traditional anti-Her2 therapy, the patients assigned to the Quanticell 2+ category would be reflex tested using Her2 FISH. There is some disagreement between the traditional DAB-based 2+/1+ classifications and the Quanticell 2+/ 1+ classifications that highlights the potential ability to define a differential Quanticell classification for anti-Her2 ADCs here.
- For traditional anti-Her2 therapy, the patients assigned to 1+ or 0+ classes are considered "Her2 negative". However, there is significant discrepancy between the 1+ and 0+ classifications between the two methods. Patients classified as 0+ by DAB can be bifurcated into two novel Quanticell 0+ and 1+ categories which may represent a key bifurcation of "Her2 Low" and "Her2 negative" classes appropriate for anti-Her2 ADC therapy using the Quanticell method.

Discussion

Based on clinical data being presented from novel anti-Her2 therapies, such as DS-8201, which show efficacy in "Her2 negative" patients, it may be necessary to redefine patient classes from the classical "Her2 positive" and "Her2 negative" assignments and include a novel "Her2 Low" classification. Based on the available clinical studies, the existing DAB IHC approach is not able to correctly identify a "Her2 Low" classification. Here, we introduce the Quanticell IHC method as an approach which may enable ideal patient stratification for the novel anti-Her2 ADC therapies by creating new classes of "Her2 high", "Her2 low", and "Her2 negative" which can be used to predict patient response for these therapies.