Asia-Pacific Journal of Clinical Oncology

WILEY

REVIEW OPEN ACCESS

Trastuzumab Deruxtecan for HER2-Low Metastatic Breast Cancer: Practical Considerations for Medical Oncologists and Pathologists in Australia

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Revised: 19 September 2025 | **Accepted:** 13 November 2025

Keywords: HER2-low | immunohistochemistry | metastatic breast cancer | T-DXd | trastuzumab deruxtecan

ABSTRACT

Trastuzumab deruxtecan (T-DXd) is a third-generation, HER2-targeting antibody-drug conjugate that has been shown to significantly prolong overall survival, compared with standard chemotherapy, when used to treat patients who have "HER2-low" (HER2 immunohistochemistry [IHC] score 1+; or HER2 IHC 2+ plus in situ hybridization-negative) unresectable or metastatic breast cancer and who have received prior chemotherapy in the metastatic setting or developed disease recurrence during, or within 6 months of completing, adjuvant chemotherapy. The broad aims of this paper are: (a) To draw attention to some of the challenges associated with identifying whether patients have HER2-low metastatic breast cancer (mBC), in an environment where healthcare professionals have previously only needed to determine whether mBC is HER2-positive and therefore likely to respond to traditional HER2-targeted therapies; and (b) to indicate, where possible, what might be done to help overcome specific challenges in this regard. Advice regarding the management of specific T-DXd-related side effects of interest, including interstitial lung disease and pneumonitis, left ventricular dysfunction and emesis, is also offered.

1 | Introduction

For more than two decades, human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) scoring and/or the presence of HER2 (*ERBB2*) gene amplification have been employed to routinely classify breast cancers as either HER2-positive (HER2+; i.e. HER2 IHC 3+ or 2+/in situ hybridization [ISH]-positive) or HER2-negative (HER2−; i.e. HER2 IHC 0, 1+ or 2+/ISH-negative [ISH−]) [1–6]. This has been necessary to identify patients who have classically HER2+ disease, which is

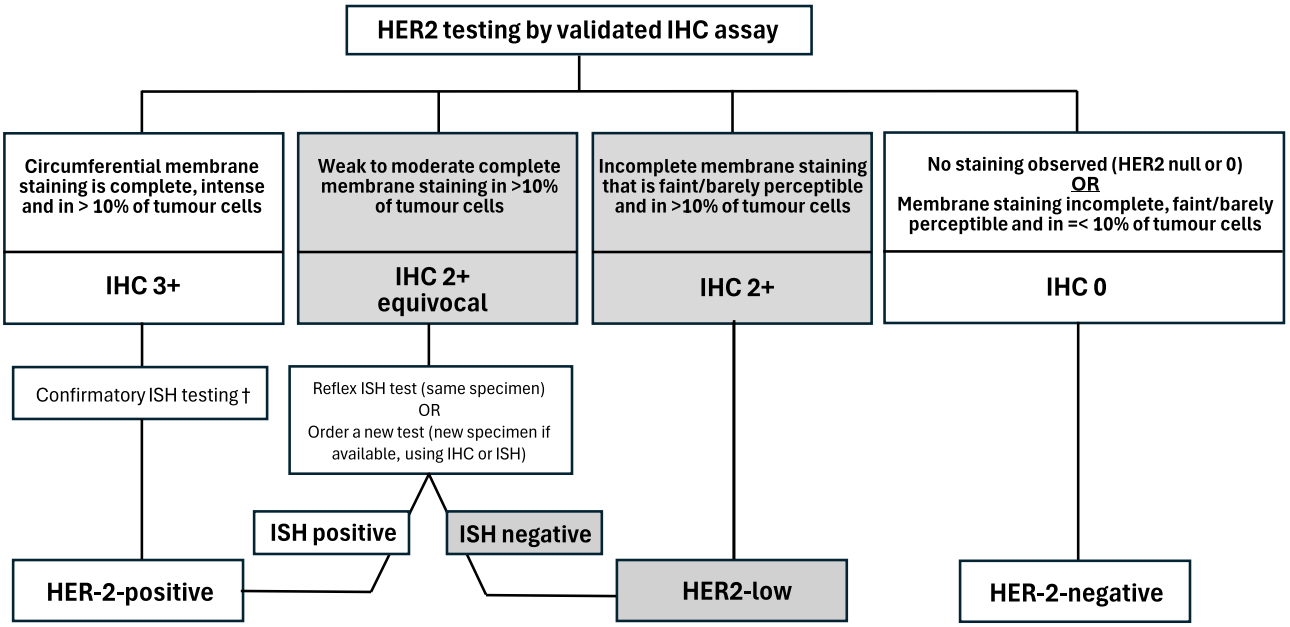


FIGURE 1 | How pathologists in Australia currently identify HER2-low breast cancer [3, 4, 14]. †In Australia, ISH testing is routinely performed when there is HER2 IHC 3+ staining, as well as in cases of HER2 IHC 2+ staining; this is because HER2+ patients in Australia can currently access Government-subsidized anti-HER2 therapy (via the PBS) only if the presence of HER2 gene amplification has first been confirmed [14, 15]. IHC, immunohistochemistry; ISH, in situ hybridization; PBS, pharmaceutical benefits scheme.

dependent on the activity of the HER2 signaling pathway, and who may therefore be eligible for therapy targeted against HER2 signaling. However, due to recent therapeutic developments, it has become important for healthcare professionals who treat metastatic breast cancer (mBC) to also ascertain whether such cancers are "HER2-low" (HER2 IHC 1+ or HER2 IHC 2+/ISH−; Figure 1) [6–15].

Determining whether patients with HER2− disease have "HER2-low" disease has previously been of no therapeutic value. This is because agents targeting HER2 signaling directly, as well as earlier-generation HER2-directed antibody-drug conjugate (ADC) therapy, each of which have been established to be beneficial in the treatment of HER2+ disease, lack efficacy in patients with classically HER2− disease [6, 7]. This situation changed when trials of more potent HER2-targeted ADCs were extended to patients with HER2-low mBC, particularly when the DESTINY-Breast04 study [16] demonstrated that trastuzumab deruxtecan (T-DXd; ENHERTU [AstraZeneca and Daiichi Sankyo]) could significantly prolong the survival of these patients compared with standard chemotherapy [6–10, 16].

T-DXd is a third-generation ADC, comprised of an anti-HER2 monoclonal antibody (trastuzumab) bound via a cleavable linker to a cytotoxic topoisomerase I inhibitor payload [17–19]. T-DXd binds to the HER2 protein on the outer membrane of breast cancer cells and is then internalized, enabling subsequent intracellular delivery of the cytotoxic payload [18, 19]. Intracellular linker cleavage allows the topoisomerase I inhibitor to enter the nucleus, causing DNA damage and apoptotic cell death [18, 19].

1.1 | T-DXd Efficacy in HER2-Low Breast Cancer

The DESTINY-Breast04 study [16] was an open-label, multicenter, Phase III clinical trial, that compared T-DXd with chemotherapy in 557 patients who had HER2-low unresectable or mBC and had previously received chemotherapy (one or two lines) or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy [16]. Patients with hormone receptor-positive (HR^+) disease ($n = 494$) also needed to have received ≥ 1 line of endocrine therapy [16]. Compared with chemotherapy, the use of T-DXd (5.4 mg/kg, every 3 weeks) was associated with:

- *A significant overall survival (OS) benefit* among patients with HR^+ disease (median OS = 23.9 vs. 17.5 months; hazard ratio = 0.64, 95% confidence interval [CI] = 0.48–0.86, $p = 0.003$), as well as across all study participants (median OS = 23.4 vs. 16.8 months; hazard ratio = 0.64, 95% CI = 0.49–0.84, $p = 0.001$) [16];
- *A significantly reduced risk of disease progression or death* among patients with HR^+ disease (median progression-free survival [PFS] = 10.1 vs. 5.4 months [primary endpoint]; hazard ratio = 0.51, 95% CI = 0.40–0.64, $p < 0.001$), as well as

across all study participants (median PFS = 9.9 vs. 5.1 months; hazard ratio = 0.50, 95% CI = 0.40–0.63, $p < 0.001$) [16].

Based on these findings, T-DXd became the first approved HER2-targeted therapy for eligible patients with HER2-low mBC [7, 8, 10, 20], initially in the United States of America (USA) in August 2022 [21], and subsequently in more than 60 other countries around the world [22], including in the European Union [20] and Asia-Pacific region (e.g., in Australia [17, 23], Japan [24], and Singapore [25]). In Australia, T-DXd was already approved and Government-subsidized (via the national Pharmaceutical Benefits Scheme [PBS]) for use in eligible adult patients with previously treated unresectable or metastatic HER2+ breast cancer [23, 26]. In January 2023, the Australian Therapeutic Goods Administration also approved the use of T-DXd in adult patients with unresectable or metastatic HER2-low breast cancer, provided they have received prior chemotherapy in the metastatic setting or developed disease recurrence during, or within 6 months of completing, adjuvant chemotherapy [17, 23]. Those with HR^+ disease must also have received—and no longer be considered eligible for—endocrine therapy [17, 23]. For those meeting the requisite eligibility criteria, the treatment became Government-subsidized (via the PBS) in September 2024 [26].

1.2 | T-DXd Safety Profile

Reported adverse events among T-DXd-treated DESTINY-Breast04 study participants were consistent with the previously established safety profile for T-DXd [7, 8, 16, 27, 28]. Across clinical trials, the most common T-DXd-associated adverse events have been hematologic and gastrointestinal toxicities, including frequent emesis, as well as fatigue and alopecia [17, 28]. Other potential side effects for which it is important to screen patients and initiate early management strategies include interstitial lung disease (ILD), particularly pneumonitis, and cardiotoxicity (Figure 2) [7, 8, 17, 27, 28]. The locally approved Product Information for T-DXd [17], as well as an increasing range of other published documents, provide specific guidance regarding the detection and management of these and other possible toxicities [17, 28–32].

ILD/pneumonitis, which may be severe and potentially life-threatening, occurred in ~12% of T-DXd-treated patients during the DESTINY-Breast04 study (10% Grade 1–2, 1.3% Grade 3, 0% Grade 4, 0.8% Grade 5) [16, 17]. The median time to onset was 129 days (range, 26–710 days) [16], illustrating the need for vigilance through screening both early in therapy and also over longer follow-up. The concern is that pneumonitis may lead to pulmonary fibrosis, which may in turn result in permanent functional impairment and even death.

Identified potential risk factors for ILD/pneumonitis in the context of T-DXd therapy include the presence of lung comorbidities, specifically asthma, chronic obstructive pulmonary disease, prior ILD/pneumonitis, pulmonary fibrosis, pulmonary emphysema, and/or radiation pneumonitis [27, 30]. Patients with moderate or severe renal impairment, as well as those who have received prior abemaciclib therapy, may also be at increased risk [17, 27, 30, 33]. Baseline pulse oximetry and pulmonary function tests should be considered for patients with such risk factors. In addition, proactive monitoring for evidence of ILD/pneumonitis while on treatment is essential for all patients, to allow early inception of appropriate management should it manifest (Figure 2) [7, 17, 28–30, 34]. It is recommended that monitoring of patients on T-DXd therapy include computed tomography (CT) scanning, preferably high-resolution, to optimize early ILD/pneumonitis detection, ideally while still Grade 1 (asymptomatic) [7, 28–30, 34]. Care should also be taken to ensure that radiologists and other members of a patient's health care team understand the importance of monitoring T-DXd-treated patients for ILD/pneumonitis and of immediately alerting the treating oncologist should it manifest [28–30]. T-DXd therapy should be either interrupted or stopped as soon as possible after an ILD/pneumonitis diagnosis (Figure 2), with early initiation of corticosteroid therapy being the mainstay of ILD/pneumonitis management [7, 17, 28–30, 34]. Early involvement of respiratory physicians is also recommended when ILD/pneumonitis is suspected or confirmed.

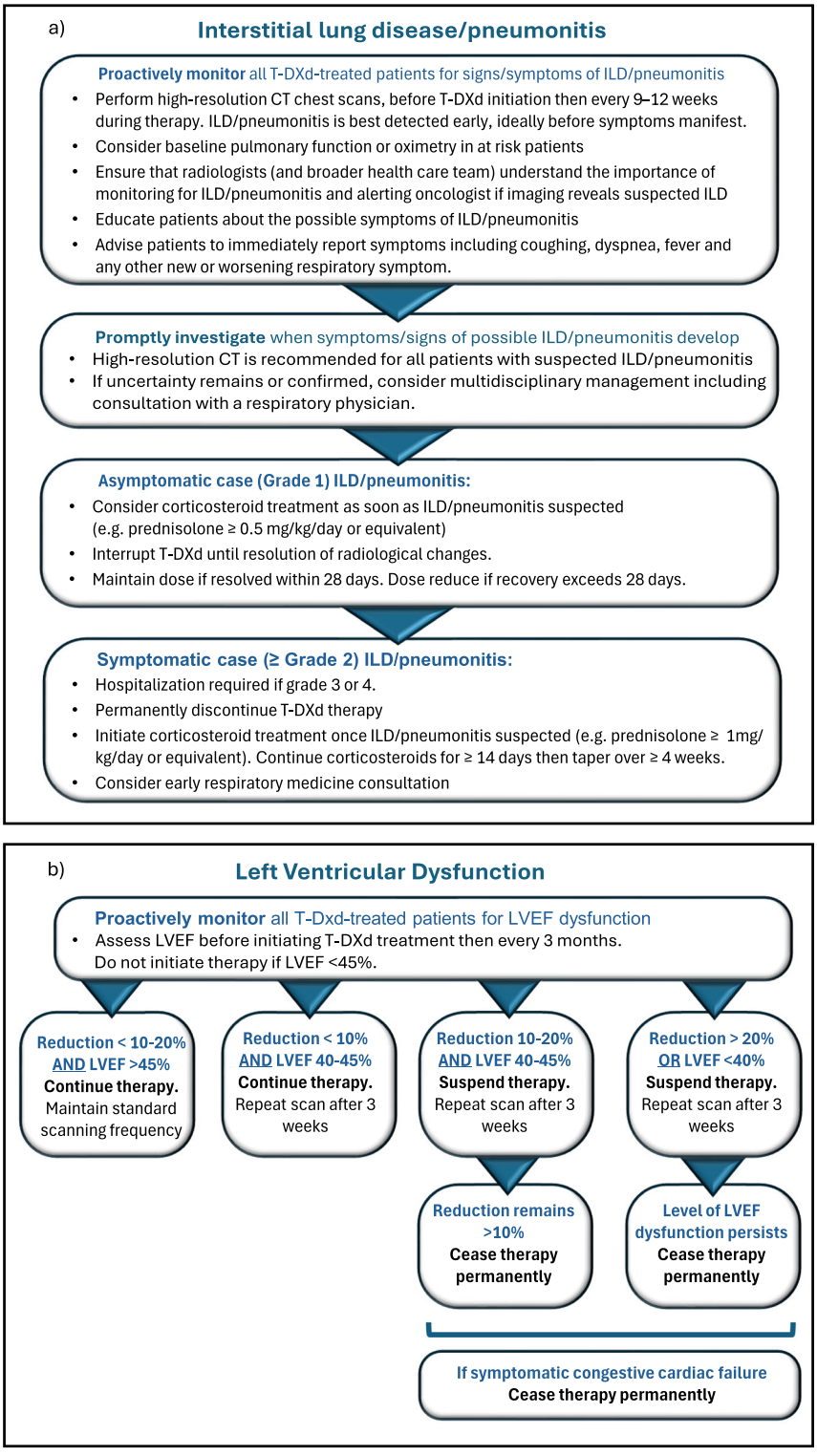
As T-DXd is a HER2-directed therapy, cardiotoxicity may occur. The rate of left ventricular dysfunction among T-DXd-treated patients during the DESTINY-Breast04 study was 4.6% ($n = 1$ Grade 3 event) [16]. As a reference, the rate of cardiac dysfunction among patients treated with trastuzumab monotherapy in the metastatic setting has been 6%–9% (depending on the criteria used to define cardiac dysfunction) [35]. For this reason, patients' cardiac function must be regularly assessed and local guidelines/protocols followed in the event of any cardiac adverse event (Figure 2) [7, 17, 28].

T-DXd is considered highly emetogenic [7, 31, 32]. The rate of nausea (any grade) in the T-DXd arm of the DESTINY-Breast04 study was 76.0%, while the rate of vomiting (any grade) was 40.4% [17]. Use of two or three prophylactic antiemetic medications is recommended from the first dose (e.g., dexamethasone plus 5-HT_3 receptor antagonist and a neurokinin-1 receptor antagonist) [7, 17, 31, 32]. In Australia, both the approved Product Information [17] and current Australian eviQ guidelines [32] offer advice about how to help prevent and manage nausea in T-DXd-treated patients.

1.3 | Current Real-World Implications

T-DXd is now a standard-of-care treatment option for patients who have HER2-low mBC and have received prior chemotherapy in the metastatic setting or developed disease recurrence within 6 months of adjuvant chemotherapy [6, 7]. This has implications for the practice of both medical oncologists and pathologists, in particular:

- Medical oncologists who might wish to use T-DXd when indicated now need to ensure that testing to identify patients with HER2-low breast cancer is performed when required;
- Pathologists should now report whether breast cancer patients have HER2-low disease, in line with current clinical practice guidelines [11–14] (e.g., American Society of Clinical Oncology [ASCO]/College of American Pathologists [CAP] [11], Royal College of Pathologists of Australasia [RCPA] [13, 14]), which now indicate that pathologists need to distinguish between HER2 IHC 0 and HER2 IHC 1+ stains (the RCPA's structured



CT, computed tomography; ILD, interstitial lung disease; LVEF, left ventricular ejection fraction.

FIGURE 2 | Addressing the risks of ILD/pneumonitis and left ventricular dysfunction in the context of T-DXd therapy: a guide to patient monitoring and management. CT, computed tomography; ILD, interstitial lung disease; LVEF, left ventricular ejection fraction.

reporting protocol for invasive carcinoma of the breast now formally recommends use of the term "HER2-low" [14]).

Based on current HER2 classification/reporting guidelines [11, 13, 14], up to two-thirds of all breast cancers previously classified as HER2⁻ may now be identified as HER2-low cancers (HER2 IHC 1+ or 2+/ISH−) [7–9, 12, 36, 37, 38]. Being able to reliably and reproducibly identify patients who have HER2-low mBC ensures that all those who are eligible are not denied the opportunity to receive T-DXd, a potentially life-extending therapy. Accurate exclusion of patients who do not have HER2-low disease also allows clinicians to avoid exposing those for whom the benefits

of T-DXd are unproven to the risk of T-DXd-related toxicities or adverse events.

2 | Identifying HER2-Low mBC in Australia

HER2 testing in Australia to establish eligibility for conventional HER2-targeted agents continues to entail HER2 IHC to detect and assess the expression of HER2 protein, followed by ISH testing if/when the HER2 IHC score is either 2+ or 3+ (to detect/confirm whether HER2 gene amplification is present; Figure 1) [4, 13, 14]. ISH testing is routinely performed when there is HER2 IHC 3+ staining, as well as in cases of HER2 IHC 2+ staining, because patients in Australia can only access Government-subsidized anti-HER2 therapy via the PBS when HER2 gene amplification has been confirmed [14, 15]. In this way, mBC may be defined as being HER2− (HER2 IHC 0, IHC 1+ or 2+/ISH−) or HER2+ (HER2 IHC 3+ or 2+/ISH-positive).

Until recently, pathologists had no reason to focus on distinguishing between HER2 IHC 0 and HER2 IHC 1+ staining, as both characterize HER2− breast cancer, and there was no approved treatment specifically for the "HER2-low" subset of HER2−disease [6, 7]. However, with the advent of approved therapy for patients with HER2-low mBC (HER2 IHC 1+ or 2+/ISH−) [17], being able to distinguish between these two expression states has become clinically important. Pathologists are now routinely being asked to distinguish between HER2 IHC 0 or IHC 1+ stains, to identify the HER2 IHC 1+ cases and thus ensure that suitable candidates do not miss out on the opportunity to receive T-DXd, with the attendant benefits [11–14].

2.1 | Which Tissue Sample(s) to Test When Assessing the HER2 Status of mBC

Numerous pre-analytical, analytical, and post-analytical factors can influence the outcome of HER2 IHC staining, potentially affecting the sensitivity, as well as the accuracy/reproducibility, of HER2 IHC testing, particularly when specimens have a low level of HER2 expression (HER2 IHC 0–1+) [2, 8, 9, 14, 39–42]. Important pre-analytical factors include the type of biopsy sample used and the way it has been prepared for testing (e.g., time to fixation, duration/type of fixation). In general, when performing HER2 IHC for the purpose of identifying HER2-low breast cancer, use of a core biopsy or surgical resection specimen is recommended (Table 1), although cytology specimen testing is acceptable if other sample types are unavailable [13, 14]. Bone biopsies should only be used if no other disease site can be assessed, as the decalcification process required prior to testing such samples may lead to inaccurate results [5, 7, 42–44].

In the DESTINY-Breast04 study, each patient's tumor HER2 status was assessed by central HER2 testing conducted on an adequate archived or recent tumor biopsy (fine-needle aspiration cytology samples and bone biopsies were excluded) [16]. Reported study outcomes were similar regardless of whether HER2 IHC status had been determined using a primary sample or recurrent disease [16, 45]. This suggests that, in real-world practice, clinicians may use either an archived (e.g., primary) or recent tumor biopsy specimen to test for HER2-low mBC. However, it must be remembered that archived biopsy findings do not always reflect the biology of a patient's currently active disease [7, 46–49].

TABLE 1 | Optimizing HER2 IHC testing [7, 11—14].

Step	Recommendation
sample selection	Use of core biopsy or surgical resection specimen recommended
	Avoid use of bone biopsies if possible[a]
	Avoid using decalcified samples, if possible
sample fixation	Optimal time to fixation (cold ischemic time) ≤ 1 h[b]
	Optimal duration of fixation = 6–72 h, depending on specimen size[b]
	Use adequate volumes of neutral buffered formalin
Section preparation	Keep sections 5–10 microns thick, and cut within six weeks of HER2 staining
	Ensure optimal assay conditions (use the VENTANA 4B5 assay)
	Optimize HER2 assay controls (have validated controls for all HER2 IHC scores [0, 1+, 2+, 3+])

[a]If unavoidable, try to isolate soft-tissue core from bone metastasis (as decalcification is then not required) [42]. We recommend use of EDTA (ethylenediaminetetraacetic acid) as decalcification agent if need for decalcification is unavoidable [42–44.].
[b]Applies to core biopsies and resections.

Data show that a substantial proportion of patients with HER2 IHC 0 primary disease (~15% in one study [46]) may be HER2-low at relapse, and vice versa [46–50]. Such changes in HER2 status are more common when patients have previously been diagnosed with HER2-low or HER2− breast cancer than when they have initially been diagnosed with HER2+ disease [46, 48, 49]. In addition, the HER2 antigenicity of archived material also decreases over time, causing HER2 IHC staining to appear weaker and increasing the risk of a false-negative result when looking for HER2-low disease [51]. Consequently, although HER2 testing in cases of mBC may be performed using any previously archived sample, we recommend that recent metastatic disease samples be tested whenever possible to evaluate a patient's contemporary HER2 status and assess other biomarkers (consistent with current ASCO/CAP guidelines [11]).

2.2 | The Practical Challenges of IHC-Based Identification of HER2-Low mBC

Challenges when assessing whether a patient has HER2-low breast cancer include: (a) the lack of a universally employed HER2 IHC assay for this specific purpose, with current assays having been optimized for the detection of HER2+ disease, in terms of sensitivity and specificity, rather than to distinguish between HER2-low and HER2− (HER2 IHC 0) tumors [8, 39, 52, 53]; and (b) the lack of any universally agreed or implemented

criteria to score IHC stains at low levels of HER2 expression. These challenges exist largely because pathologists have not previously had to routinely identify HER2-low breast cancer.

When performing HER2 IHC testing, staining patterns and intensities can vary between assays and can depend on how individual laboratories implement each assay in terms of the specific assay conditions, the controls employed, etc. This can influence the outcome of HER2 IHC tests and contribute to inter-laboratory variability, even when laboratories are using the same assay. During DESTINY-Breast04, centralized HER2 IHC testing was conducted using a VENTANA anti-HER2/neu (clone 4B5) assay (Roche Diagnostics) [16]. For this reason, we recommend that pathology laboratories use this assay when assessing whether someone may have HER2-low breast cancer and might be a suitable candidate for T-DXd therapy. Most pathology laboratories in Australia already employ this assay.

There are many other sources of potential variability that can affect the results of HER2 IHC testing when assessing whether a patient has HER2-low breast cancer. For example:

- It can be inherently difficult to determine whether a HER2 IHC stain should be scored as "0" or "1+", given how subjective current criteria defining different HER2 IHC stains can be (Figure 1) and also given how subtle the differences between a HER2 IHC 0 and 1+ stain often are [39, 52];
- There can also be heterogeneity when it comes to HER2 expression (and/or HER2 gene amplification), within samples or between samples from different sites in the same patient, particularly at the lower end of the HER2 expression spectrum [7, 10, 39, 52, 54–58].

Considering these variables, it is unsurprising that HER2 IHC results can vary between pathologists or laboratories, with reported concordance levels tending to be lowest when distinguishing between HER2 IHC 0 and IHC 1+ stains [7, 11, 37, 59, 60]. For example, in one concordance study [60], involving 18 experienced pathologists in the USA, 92 biopsy samples were scored as HER2 IHC 0 by at least one pathologist; however, in only 24 of these cases (26%) did 17 or 18 pathologists agree that the sample was HER2 IHC 0. It should be noted that HER2 IHC scoring in this study [60] was not only performed at a time when there was no clinical need to distinguish between HER2 IHC 0 and 1+ disease, but also that participating pathologists were unaware that their ability to distinguish between IHC 0 and 1+ stains—and their level of concordance in this regard—was going to be assessed; many said they would have examined the low-expressing cases more closely if they had known [60]. Regarding this latter point, there is evidence suggesting that a proportion of cases previously identified as HER2 IHC 0 may be rescored as HER2-low when pathologists are aware of the importance of distinguishing between HER2 0 and 1+ stains and/or have received specific training in how to do this [36, 61]. Illustrating this, during a multinational study in which pathologists first received training in low-end HER2 expression scoring, rescoring of 789 samples previously classified as HER2− unresectable/mBC resulted in 67% being rescored as HER2-low, including over 30% of cases originally scored as HER2 IHC 0 [36].

2.3 | Practical Implications for Medical Oncologists and Pathologists/Pathology Laboratories

2.3.1 | Sample Selection

The use of recently obtained biopsy samples is preferred when assessing whether a patient has HER2-low mBC. If a metastatic sample is not available, the results of previous HER2 testing performed on an earlier-obtained biopsy specimen may be used instead. Considering how patients were enrolled in the DESTINY-Breast04 study [16], it is certainly reasonable to initiate T-DXd therapy based on previously obtained HER2-low results if testing of a more recent sample is not possible; the validity of using T-DXd when a more recently obtained metastatic specimen has been scored as HER2 IHC 0 remains unknown.

Importantly, when checking whether patients have HER2-low mBC, medical oncologists should not rely on pathology reports prepared at a time when pathologists were not required to routinely distinguish between HER2 IHC 0 and IHC 1+ stains for therapeutic reasons and so may not have striven-Study of Trastuzumab Deruxtecan (T-DXd) vs Investigator's Choice Chemotherapy in HER2-low, Hormone Receptor Positive, Metastatic Breast Cancer (DB-06) to accurately determine whether individual patients had HER2 IHC 1+ disease. If a patient with mBC has previously been identified as having HER2− disease (or as being HER2 IHC 0), it is prudent to request that the HER2 IHC stain be rescored.

2.3.2 | Refinement of Scoring

Given that HER2-low mBC is now "therapeutically targetable," ensuring that pathologists in Australia can identify HER2-low breast cancer as accurately and reproducibly as possible, and with acceptable intra-/inter-observer concordance levels, has become a priority. Accordingly, pathologists are being encouraged to review, refine and seek to standardize the way they identify HER2-low breast cancer in terms of their pre-analytic, analytic, and post-analytic processes and procedures. In addition, they need to be mindful that a sizeable proportion of patients who may previously have been identified as having HER2 IHC 0 or HER2− mBC might now be re-classified as HER2-low mBC cases if their HER2 IHC stains were to be rescored today (i.e., by a pathologist who has a contemporary understanding of the importance of distinguishing between HER2 IHC 0 and IHC 1+) [36, 61]. In Australia, the availability of treatment for HER2-low mBC also increases the importance of accurately distinguishing between HER2 IHC 2+ and 3+ stains, and being as sure as possible that a sample is IHC 3+ if it is found to be ISH−. This is because, somewhat counterintuitively, IHC 3+/ISH−patients are currently ineligible for Government-subsidized T-DXd—or any other HER2-targeted therapy—via the PBS.

2.3.3 | Methodological Guidelines

Despite its limitations, IHC-based HER2 testing (± ISH) remains the approved and recommended way to identify whether patients

have HER2-low mBC and may, therefore, be suitable candidates for T-DXd therapy (Figure 1) [8, 11–14, 17]. Current RCPA guidelines now recommend that pathologists distinguish between HER2 IHC 0 and 1+ stains, specifying the HER2 IHC value in their reports and not simply classifying both HER2 IHC 0 and 1+ cases as HER2-negative [13, 14]. To ensure that they are well placed to do this, pathologists are also being encouraged to: [8, 10, 11, 14]

- Strive to optimize pre-analytic handling/processing of breast cancer tissue samples (Table 1);
- Adhere to up-to-date HER2 testing/HER2 IHC scoring guidelines [11];
- Examine HER2 IHC at high power (40x) when discriminating between 0 and 1+ staining (as partial membrane staining can be overlooked at lower powers) [11, 61];
- Consider having a second pathologist review stains when results are close to the "0" versus "1+" interpretive threshold (> 10% of cells with incomplete membrane staining that is faint/barely perceptible) [11];
- Use a separate, validated control for each possible HER2 IHC score (i.e., 0, 1+, 2+, and 3+) instead of using controls that have been optimized to dichotomize HER2 as either "positive" or "negative".

2.3.4 | Standard Setting and Training

There is a need for agreed reference standards for the interpretation of IHC stains at low levels of HER2 expression, to help pathologists distinguish between HER2 IHC 0, 1+, and 2+ specimens. Work conducted as part of the Australian HER2-Low Breast Cancer Concordance Study may assist in this regard [61]. This study utilized 60 deidentified breast cancer core biopsies (20 from each of three laboratories) that had initially been evaluated within the previous few months (using the VENTANA 4B5 HER2 assay) and scored locally as HER2 IHC 0, 1+ or 2+/ISH−. All were core biopsies with short ischemic times, no decalcification and good fixation to minimize pre-analytical confounders, and slides were scanned at 40 times magnification. Nine experienced breast pathologists from eight laboratories across Australia convened to specify agreed scoring conventions for cancers with low levels of HER2 protein expression and identify potential scoring pitfalls. Each pathologist then independently scored HER2 IHC expression for all samples. Cases were then jointly reviewed by the panel to establish consensus scores for each. Notably, 7 of 17 (41.2%) cases originally reported locally as HER2 IHC 0 were re-classified as HER2-low, while 7 of 32 cases (21.8%) originally reported locally as HER2 IHC 1+ were reclassified as HER2 IHC 0 (i.e., either "ultra-low" or null) [61]. Encouragingly, the consensus score matched the majority opinion of pathologists' independent scores in 93% of cases, and individual pathologists' scores matched the subsequently agreed consensus score ~81% of the time (range 73.3%–91.67%) [61]. This study has resulted in an established set of reference cases that will now be used for peer training; it is hoped that it might also lead to the development of guidelines for distinguishing between HER2 IHC 0 and 1+ stains, as well as a national quality assurance program (QAP) specifically for the assessment of HER2-low cancers.

Accumulating data suggest that appropriate training in HER2-low scoring can help pathologists identify HER2-low breast cancer with an acceptable level of accuracy [61–63]. In a multinational concordance study [62], > 70 pathologists underwent training in HER2-low scoring (ASCO/CAP 2018 criteria); among ~50 who used a VENTANA 4B5 HER2 assay, the post-training concordance rate for HER2 IHC 0 (positive percent agreement) was 89.2% (up from 74.6% pre-training; $p < 0.001$), and the post-training negative percent agreement for HER2-low was 91.1% (up from 80.6% pre-training; $p < 0.001$). In another study, three central pathologists received education in HER2 scoring (ASCO/CAP 2018 criteria), incorporating definitions of HER2-low and HER2 ultra-low [63]. They then assessed 30 test cases, differentiating between "IHC 0 absent membrane" staining from "IHC 0 with membrane" staining. When an 85% concordance threshold was not met, individual pathologists participated in a one-on-one discrepant case review session. Substantial scoring agreement was subsequently found upon assessment of a further 500 samples of varying HER2 status (overall percent agreement: 91%–94% for a HER2-low cutoff; 96%–97% for HER2 ultra-low) [63]. These findings [61–63] offer reassurance that, when appropriate quality assurance measures are implemented, and educational and training opportunities that focus on the assessment of HER2-low cancers (including QAPs) are provided, pathologists in Australia should be able to achieve acceptable levels of concordance when distinguishing between HER2 IHC 0 and 1+ stains.

As pathologists adjust to routinely distinguishing between HER2 IHC 0 and 1+ stains, something that has not been a focus in the past, there is a risk that significant inter-observer discordance will occur when it comes to what they designate as HER2 IHC 0 versus HER2 IHC 1+ breast cancer (due to the subjective nature of current criteria used to make this distinction), particularly when a pathologist does not specialize in breast pathology. Such discordance could theoretically result in T-DXd being administered to individual patients who would have been excluded from DESTINY-Breast04 (based on an IHC 0 result at the time of centralized HER2 IHC testing). This is a group for whom it has not yet been demonstrated that the potential benefits of T-DXd outweigh the potential risks. Mitigating this concern is the emergence of promising data regarding the efficacy of T-DXd in patients with HER2 "ultra-low" mBC (HER2 IHC > 0 and < 1+; see next section) [64]; we believe it is prudent to keep these data in mind when considering the risk-benefit profile for T-DXd in the HER2-low mBC setting.

3 | Looking to the Future

3.1 | T-DXd for Patients With HER2 "Ultra-Low" mBC

There is currently no conclusive evidence that HER2-low breast cancer (HER2 IHC 1+ or 2+/ISH−) is biologically distinct from breast cancer expressing no detectable levels of HER2, in terms of clinicopathologic phenotype or prognosis [6–9, 11, 36, 50, 65, 66]. Whether there is a specific minimum level of HER2 expression that reliably identifies mBC patients who are likely to benefit from T-DXd, and below which it can be reliably predicted that treatment is futile, is also yet to be determined

but warrants continued evaluation. In the Phase II DAISY study [67], involving patients who had received ≥ 1 prior line of chemotherapy for mBC, use of T-DXd was associated with an ~30% confirmed objective response rate among a subgroup of 36 patients with HER2 IHC 0 disease (vs. 37.5% among a cohort of 74 individuals with HER2-low disease). The study investigators suggest that these patients may have had HER2 "ultra-low" disease, with these very low levels of HER2 allowing uptake of T-DXd, and/or that the efficacy of T-DXd could be partially mediated by HER2-independent mechanisms [67].

The ongoing Phase III DESTINY-Breast06 study [64, 68] is evaluating T-DXd in patients with HR$^+$/HER2-low (n = 713) or HR$^+$/HER2 ultra-low (HER2 IHC > 0 and < 1+; n = 153) advanced or mBC, all of whom had either experienced disease progression within 6 months of starting first-line endocrine treatment in combination with a CDK4/6 inhibitor or received ≥ 2 previous lines of endocrine therapy in the metastatic setting. Notably, none of the DESTINY-Breast06 participants had received prior chemotherapy for advanced or metastatic disease. After 18.2-month (median) follow up, the use of T-DXd was not only associated with a statistically significant improvement in PFS among patients with HR$^+$/HER2-low mBC (median 13.2 vs. 8.1 months in the chemotherapy arm [primary endpoint]; hazard ratio = 0.62, 95% CI = 0.51–0.74, $p < 0.0001$), but also with a similar magnitude of improvement in median PFS among the patients with HER2 ultra-low disease (median 13.2 vs. 8.3 months in the chemotherapy arm [pre-specified subgroup analysis]; hazard ratio = 0.78, 95% CI = 0.50–1.21) [64]. The safety profile of T-DXd was reported to have been consistent with that observed in previous T-DXd trials. We keenly await further data from this and similar trials of T-DXd in patients with low/ultra-low HER2-expressing mBC. This includes data from the ongoing DESTINY-Breast15 study [69], which, by including a cohort of patients with HER2 IHC 0 mBC, will shed further light on whether T-DXd therapy might have clinical utility across the full spectrum of HER2 expression.

3.2 | Improving the Identification of Potential T-DXd Responders When HER2 Is in the Low Range

The use of semi-quantitative, IHC-based testing to distinguish between HER2 IHC 0 and HER2-low breast cancer is inherently challenging, and the challenge is likely to be exacerbated if pathologists are routinely required to distinguish between HER2 IHC 0 and HER2 ultra-low disease. However, to date, all clinical trials of T-DXd in patients with HER2-low breast cancer have enrolled patients whose HER2-low status was defined on the basis of IHC and, for this reason, the use of HER2 IHC—plus ISH (if/when required)—currently remains the only way to determine whether patients are potentially eligible for T-DXd therapy [8, 11–14, 17].

It would clearly be preferable to identify a more precise and easily reproducible way of distinguishing between potential responders to T-DXd and likely nonresponders. This could be via the use of a more quantitative or objective method of distinguishing between different levels of HER2 expression, especially at lower levels of HER2 expression, or even an entirely different biomarker or testing modality, based on the biological action of T-DXd. Several novel methods of HER2 quantification are currently being evaluated, including the use of real-time polymerase chain reaction to assess HER2 messenger RNA expression levels and the use of immunofluorescence-based assays [7–10, 39, 54, 70, 71]. The potential utility of digital image analysis and/or artificial intelligence (AI) as a way to enhance the precision of HER2 testing—and help reduce or remove existing subjectivity and variability in the identification of HER2-low breast cancer—is also being evaluated [61, 72–74]. Of course, the clinical utility of any new method of HER2 IHC scoring would first need to be demonstrated in appropriately designed clinical outcome-based validation trials before it could be recommended for use in real-world clinical practice.

Gaining a better understanding of the impact of differential or heterogeneous HER2 expression on responses to T-DXd (and other HER2-directed ADCs) may also help guide treatment decisions and lead to further refinement of HER2 IHC scoring and reporting [7]. For example, if it was demonstrated that the presence of intratumoral heterogeneity influenced the efficacy of T-DXd, pathologists would then need to further refine how they report heterogeneity. Further research is clearly required, including assessment of the impact of HER2 expression heterogeneity between metastatic sites or within the primary tumor. Heterogeneity could be assessed by standard IHC techniques or potentially with novel functional imaging modalities, such as HER2-targeted PET.

4 | Conclusion

In Australia, T-DXd is both an approved treatment option for eligible adult patients with previously treated HER2+ mBC and now also a standard-of-care treatment option for those with previously treated HER2-low mBC. Consequently, ensuring that patients with HER2-low mBC are reliably identified is now an essential part of clinical care. The challenge for pathologists—having not been previously required to routinely check and report whether patients have HER2-low breast cancer—will be ensuring that they are now identifying HER2-low breast cancer accurately and with an acceptable level of intra/inter-observer concordance. Both medical oncologists and pathologists should also be mindful that a proportion of breast cancer specimens previously identified as being HER2 IHC 0 or HER2− (when it was not necessary for pathologists to distinguish between HER2 IHC 0 and IHC 1+ status) might now be classified as HER2-low if rescored today by a pathologist who is aware of the importance of distinguishing between HER2 0 and 1+ stains and/or has received specific training in how to do this.

Acknowledgments

In November 2022 and April–May 2023, AstraZeneca Australia convened and funded a series of roundtable meetings around Australia (in Brisbane, Sydney, Melbourne, Adelaide and Perth, respectively), each attended primarily by pathologists and medical oncologists. The purpose of these meetings was: (a) To discuss the potential practical implications of the DESTINY-Breast04 study data, in terms of their potential impact on the future treatment paradigm for patients who have HER2-low metastatic breast cancer (mBC); and (b) to discuss the practical implications

of having to identify patients who have HER2-low mBC, both from pathologists' and medical oncologists' perspective. The authors' decision to prepare this manuscript was inspired by the discussions that took place during these meetings; each had participated in one of the aforementioned meetings (N.P. and E.L. in Sydney; N.M. and S.L. in Brisbane; B.Y. in Melbourne; G.F. and S.S. in Adelaide; B.D. and A.R. in Perth), and each was also directly involved in the production of this manuscript. Although AstraZeneca Australia supported the production of the manuscript, neither the company nor any of its representatives had any input into its content beyond the provision of requested factual information. The authors thank Tim Brereton for writing assistance.

Funding

The authors have nothing to report.

Consent

The authors have nothing to report.

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