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Dynamic HER2-low status among patients with triple negative breast cancer (TNBC) and the impact of repeat biopsies

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Trastuzumab deruxtecan (T-DXd) is approved for HER2-low (HER2 immunohistochemistry (IHC)1+ or 2+ with non-amplified in situ hybridization (ISH)), but not HER2-0 (IHC 0) metastatic breast cancer. The impact of repeat biopsies (Bxs) in identifying new potential candidates with triple negative breast cancer (TNBC) for T-DXd treatment remains unknown. 512 consecutive patients with TNBC at diagnosis were included in the study cohort. Bxs were categorized as core, surgical, or metastatic based on the timing and method of biopsy (Bx) acquisition, and the total number of Bxs was determined for each patient. Additionally, matched biopsies were identified, and the rate of discordance in HER2 status was calculated. The proportion of patients with at least one HER2-low result increased as the number of successive Bxs increased [59%, 73%, 83%, 83%, and 100% when 1 (196 patients), 2 (231 patients), 3 (48 patients), 4 (29 patients), and ≥ 5 (8 patients) Bxs were obtained, respectively]. Among patients without a prior HER2-low result, approximately one-third demonstrated HER2-low status with each additional successive Bx. HER2 status exhibited variability between matched Bxs, with observed discordance rates of 26%, 44%, and 33% between matched core-surgical, early-metastatic, and two metastatic matched Bxs, respectively. Our findings indicate that HER2 status can vary between different Bxs taken during the disease course of patients with TNBC with the highest discordance rate observed between the primary and metastatic Bxs. For patients with metastastic HER2-0 TNBC, repeat Bxs can increase the chance of obtaining a HER2-low result, thereby offering patients a promising therapeutic option.

Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer, and metastatic triple-negative breast cancer (mTNBC) remains an incurable disease with limited therapeutic options[1]. Historically, systemic chemotherapy has been the mainstay of treatment for this patient population. However, significant progress has been made in recent years with the introduction of novel therapies such as immunotherapy[2] and antibody-drug conjugates (ADCs)[3,4].

Trastuzumab deruxtecan (T-DXd) is a novel ADC that consists of the anti-HER2 antibody trastuzumab, a cleavable linker, and a topoisomerase 1 inhibitor chemotherapy payload (deruxtecan)[5]. In the pivotal DESTINY-Breast04 study, T-DXd demonstrated significant improvements in overall survival (OS) for patients with HER2-low tumors (defined as immunohistochemical (IHC) HER2 scores of 1+, or 2+ with non-amplified in situ hybridization (ISH)) who had metastatic hormone receptor-positive (HR+) or TN breast cancer, compared to treatment of physician choice (TPC)[4]. An exploratory analysis of the 58 patients with HER2-low mTNBC who participated in the study revealed a highly clinically meaningful improvement in OS for this population (17.1 months with T-DXd versus 8.3 months with TPC; HR 0.58 (95% CI, 0.31–1.08))[6]. Consequently, T-DXd was approved by regulatory agencies and recommended in clinical guidelines for patients with HER2-low but not HER2 IHC score 0 (HER2-0) mTNBC[7–9]. Recently, the phase 3 DESTINY-Breast06 study confirmed the efficacy of T-DXd in patients with HER2-low metastatic HR+ breast cancer and suggested that this drug might be

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effective in populations with lower levels of HER2 expression (HER2-ultralow, defined as IHC 0 with membrane staining)[10].

Previous studies have shown variability in the proportion of HER2-low tumors among patients with TNBC[11–13]. A large study including over 150,000 patients with TNBC showed that approximately half the patients had a HER2-low result in their diagnostic biopsy (Bx), while the remaining half had a HER2-0 tumor[12]. However, several studies have demonstrated the dynamic nature of HER2 status over time, showing discordance (HER2-low to HER2-0 and vice versa) between matched biopsies (Bxs) taken at different time points throughout the disease course of patients with TNBC[14–16]. Furthermore, a recent autopsy study revealed that HER2 status may vary between Bxs of distinct metastatic sites or even within the same site of the same patient at a given time point, demonstrating significant heterogeneity in HER2 expression levels[17]. Given the demonstrated dynamic and heterogeneous nature of HER2 status among patients with TNBC, it is reasonable to hypothesize that repeat Bxs could detect new HER2-low results for patients with TNBC who previously had only HER2-0 results, rendering them eligible for T-DXd. Indeed, recently published guidelines addressing the management of HER2-low breast cancer recommend considering a repeat Bx in this context[8,18], and this approach is increasingly being performed in the real-world clinical setting. However, the clinical value of repeat Bxs in identifying new potential candidates for T-DXd among patients with TNBC remains unknown.

In this study, we evaluated the correlation between the total number of Bxs conducted per patient and the probability of detecting a HER2-low result for patients with TNBC. Furthermore, we assessed the specific additive value of each successive Bx in the detection of new HER2-low results for patients with prior HER2-0 only results. Additionally, we examined the discordance in HER2 status between matched Bxs from different time points throughout the disease course of patients with TNBC.

Results

Patients

Of the 789 patients included in the institutional database as of the data cutoff date, we identified 512 patients with TNBC at the time of diagnosis and at least one Bx with a known HER2 result. Patient characteristics are presented in Table 1. The median age at diagnosis was 52, with 238 patients (46%) being younger than 50. Among the patients, 423 (83%) were White, 34 (7%) were Black, 25 (5%) were Asian, and 14 (2%) were Hispanic. Nearly half the patients (48%) had stage 2 TNBC, and 8% of the patients had metastatic disease (stage 4) at diagnosis. Thirteen percent of patients had low ER expression (ER 1–10%, defined as ER-low) in their diagnostic Bx. Sixty percent of patients had a HER2-low result in their first eligible Bx. The majority of patients (83%) had only 1 or 2 eligible Bxs for the analysis. Forty-eight patients (9%) had 3 eligible Bxs and 29 patients (6%) had 4 eligible Bxs. Only 8 patients had 5 or more eligible Bxs (Table 1). Figure 1 presents the study flow diagram, illustrating the different cohorts included in the various parts of the analysis. The entire cohort (512 patients with 960 eligible Bxs) was included in the first part of the analysis, which assessed the impact of repeat Bxs (results described below and in Fig. 2). For patients with more than one included Bxs, the median time interval between repeat Bxs per patient was 5 months (range: 0.2-189). The median time interval between the first and last Bxs per patient was 5.4 months (range: 0.2-207).

Subsets of the study cohort were included in the matched Bxs analysis (Fig. 1). Of the total study population, 426 patients had a core Bx, 281 patients had a surgical Bx, and 114 patients had at least one metastatic Bx that was eligible for our analysis. Among them, a total of 242 patients had matched core-surgical Bxs, of whom 116 (48%) received neoadjuvant therapy, while the remaining patients underwent surgery as their primary intervention. Additionally, 71 patients had matched early-metastatic Bxs, and 49 patients had two matched metastatic Bxs. Only 35 patients had matched core-surgical-metastatic Bxs. The median time intervals between matched Bxs were 2.8 months (range: 0.3–15.4) for matched core-surgical Bxs, 22 months (range: 2.1–189) for early-metastatic Bxs, and 10.7 months (range: 0.1–88.4) for two metastatic Bxs.

Role of repeat Bxs in the detection of new HER2-low results

We first examined the likelihood of obtaining a HER2-low result based on the total number of Bxs conducted per patient (Fig. 2a). The proportion of patients with a detected HER2-low result increased as the number of Bxs per patient increased. While 59% (116/196, 95% CI 52–66%) of patients with only one eligible Bx had a detected HER2-low result, 74% (170/231, 95% CI 67–79%) of patients with a total of 2 Bxs and 83% of patients with a total of 3 (40/48, 95% CI 68–93%) or 4 (24 /29, 95% CI 64–94%) Bxs had a detected HER2-low result. All 8 patients (100%) with 5 or more Bxs in our dataset had a detected HER2-low result.

Next, we analyzed the pattern and timing of the detection of the first HER2-low result, based on the order in which the Bxs were conducted. As illustrated in Fig. 2b, new HER2-low results continued to be detected with each subsequent Bx. Among patients who underwent at least one Bx, a HER2-low result was detected in the first Bx for 60% (306/512, 95% CI 55–64%) of patients. Among those who had two or more Bxs, 13% (41/316, 95% CI 9–17%) had their first HER2-low result in their second Bx, and 73% (231/316, 95% CI 68–78%) had a known HER2-low result by their second Bx. Similarly, 9% (8/85, 95% CI 4–18%) of patients with three or more Bxs, and 8% (3/37, 95% CI 2–22%) of patients with four or more Bxs, had their first HER2-low result detected in their third or fourth Bx, respectively. For all patients who underwent five or more Bxs, the first HER2-low result was detected in one of the earlier Bxs.

Subsequently, we assessed the incremental value of each successive Bx in detecting new HER2-low results for patients who previously had only HER2-0 results and were deemed ineligible for T-DXd. With each successive Bx, a new HER2-low result was identified for one-third of patients who previously had only HER2-0 results (Fig. 2c). Among the 512 patients who underwent at least one Bx, 206 patients had a HER2-0 result in their first Bx. Out of those, 126 patients proceeded to a second Bx, and 41 (33%) had a detected HER2-low result in the second Bx. Among the 24 patients without prior HER2-low results who underwent a third Bx, 8 (33%) had their first detected HER2-low result in the third Bx. Similarly, out of the 8 patients with prior only HER2-0 results who underwent a fourth Bx, 3 (38%) had a first HER2-low result in the fourth Bx. The remaining 5 patients, who had undergone four Bxs and had no previously detected HER2-low results, did not undergo additional Bxs (Fig. 2c).

HER2 status distribution according to the type of Bx and metastatic location

HER2-low was detected in 58% of core, 63% of surgical, and 54% of metastatic Bxs. The distribution of HER2 status (HER2-low vs. HER2-0) did not significantly vary according to Bx type ($p = 0.26$ for core vs. surgical Bxs, $p = 0.2$ for core vs. metastatic Bxs and $p = 0.43$ for surgical vs. metastatic Bxs; Fig. 3a). The proportion of HER2-low was numerically higher in Bxs with ER-low (ER 1–10%) compared to those with ER < 1%, but the difference was not statistically significant, regardless of the Bx type (63% vs. 57%, 66% vs. 62%, and 56% vs. 54%, of patients with HER2-low vs. HER2-0 results in core, surgical, and metastatic Bxs, respectively; $p > 0.05$ for all comparisons; Fig. 3a). The distribution of HER2 status according to the metastatic site is presented in Supplementary Fig. 1. In total, 187 metastatic Bxs from 114 patients were included in this analysis. The incidence of a HER2-low result was significantly lower in Bxs from lung or pleural origin compared to non-lung/pleural origin (34% compared to 65%; $p < 0.001$). However, these results should be interpreted with caution due to the relatively small number of Bxs from each location.

HER2 status discordance between matched Bxs

HER2 status discordance was evaluated in three different sets of matched Bxs: core-surgical matched Bxs, early-metastatic matched Bxs, and two metastatic matched Bxs. Among 242 patients with core-surgical matched Bxs, HER2 status discordance was observed in 64 (26%) patients. Of those 64, HER2 status changed from 0 to low in 55% of the patients, from low to 0 in 44%, and from low to 3+ in 1% of the patients (Fig. 3b). When evaluating patients separately based on whether they received neoadjuvant therapy or

Table 1 | Clinicopathological characteristics of the overall study population

Characteristic	Overall study population (*N* = 512)
Age, *median (range)*	52 (25–97)
Age groups, *no*. (%)	
<50	238 (46%)
>50	274 (54%)
Race, *no*. (%)	
White	423 (83%)
Black/African American	34 (7%)
Asian	25 (5%)
Hispanic	14 (3%)
Other	8 (2%)
Not reported	8 (2%)
Stage at Dx, *no*. (%)	
Stage 1	145 (28%)
Stage 2	247 (48%)
Stage 3	74 (14%)
Stage 4	43 (8%)
Unknown	3 (1%)
ER status at Dx, *no*. (%)	
<1%	447 (87%)
1–10%	65 (13%)
HER2 in 1st eligible Bx, ***no.*** **(%)**	
HER2-low	305 (60%)
HER2-0	207 (40%)
NA therapy, *no*. (%)	
Yes	278 (54%)
No	238 (45%)
unknown	4 (1%)
No. of Bxs, *no*. (%)	
1	196 (38%)
2	231 (45%)
3	48 (9%)
4	29 (6%)
>5	8 (1%)

Dx diagnosis, *ER* estrogen receptor, *NA* neoadjuvant.

had surgery as their primary intervention, similar proportions of HER2 status discordance were observed between core-surgical matched Bxs (25% and 28% of cases, respectively; Fig. 3c). Among 71 patients with early-metastatic matched Bxs, HER2 status discordance was seen in 31 (44%) patients. For the majority of those patients, HER2 status changed from low to 0. Finally, among 49 patients with two metastatic matched Bxs, HER2 status discordance was observed in 16 (33%) patients (Fig. 3b), with HER2 status changing from 0 to low for the majority of patients. HER2 status switch from HER2-low to HER2-positive, was observed in one patient (1%) in the core-surgical matched biopsies analysis, two patients (6%) in the early-metastatic matched biopsies analysis, and one patient (6%) in the matched two metastatic biopsies (Fig. 3b).

While the observed discordance in HER2 status between core-surgical matched Bxs or between two metastatic matched Bxs did not reach statistical significance ($p = 0.45$ and $p = 0.3$, respectively), significant discordance in HER2 status was observed between early-metastatic matched biopsies ($p = 0.026$; Fig. 3b). HER2 status discordance between core-surgical matched biopsies remained nonsignificant, regardless of whether neoadjuvant therapy was administered or not ($p = 0.78$ and $p = 0.58$, respectively; Fig. 3c).

Among 35 patients with matched core-surgical-metastatic Bxs, HER2 status discordance was observed for nearly half the patients. HER2 status discordance was mainly observed between early (core/surgical) and metastatic Bxs (Fig. 3d).

Discussion

As demonstrated in the DB-04 study, T-DXd is a potentially life-prolonging medication for patients with HER2-low metastatic HR+ or TNBC[4]. Since only up to half of the patients with TNBC have a detected HER2-low result based on a single Bx[12], the detection of new HER2-low results and thus identification of new candidates for T-DXd treatment is an unmet need for this patient population. Our analysis reveals two main related findings. Firstly, we observed significant discordance in HER2 status between matched Bxs, underscoring the highly dynamic nature of HER2 status throughout the disease course of patients with TNBC, in agreement with prior studies[19]. While discordance in HER2 status was observed in all matched Bxs comparisons, a significant discordance was found only between the early and metastatic stages of the disease, resulting in a change in HER2 status for 44% of the patients. Secondly, we demonstrate the novel finding that a repeat Bx is an effective strategy for detecting new HER2 low results, thereby identifying additional potential candidates for T-DXd treatment.

The underlying cause of the observed discordance between matched Bxs (Fig. 3b) is not fully understood. Potential explanations include biological changes over time, intra-tumor heterogeneity, and/or analytical variations. Previous studies have demonstrated changes in HER2 expression over time, with approximately 10–15% of cases showing transitions from HER2-positive to HER2-negative or vice versa between primary and metastatic tumors[20–24]. Based on these findings, current guidelines recommend performing a repeat Bx of a metastatic site to reassess HER2 and ER status at the time of disease recurrence[25–27]. In the aforementioned reports, HER2 status discordance was primarily attributed to clonal evolution under therapeutic pressure and intra-tumor heterogeneity[22,23], rather than technical analytical limitations. This is because the available HER2 expression quantification methods, namely the use of IHC together with ISH-based assays in accordance with the ASCO/CAP clinical recommendations, were specifically designed to differentiate between these two distinct levels of HER2 expression[28]. However, HER2 IHC was not designed and optimized specifically to differentiate between HER2-low and HER2-0 tumors within the lower range of HER2 expression[28]. As a result, using IHC in this context may encounter technical analytical variations that can contribute to the observed shifts between HER2-0 and HER2-low over time. Such analytical variations may arise from inter-observer variability among pathologists in determining HER2 status[29,30], changes in tissue handling during the pre-analytical phase that can impact the assay sensitivity[31,32], and evolving guidelines over time such as the ASCO/CAP recommendations[33].

Regardless of the underlying cause of the observed HER2 expression dynamics over time, this finding may justify obtaining repeat Bxs to detect new HER2 low results, thus identifying new patients eligible for T-DXd treatment. Our study demonstrates that the probability of detecting a HER2-low result increases with the total number of Bxs, reaching 83% with 3 Bxs and 100% in a small group of patients who underwent 5 or more Bxs. Furthermore, we found that in each successive Bx, a new HER2-low result is detected for one-third of patients without a previously known HER2-low result. Given the eventually very high detection rate of HER2-low with repeat Bxs, one might question the necessity of these additional Bxs and instead spare patients from the risk involved in this minimally invasive approach. However, the current approval framework[18] and the potential toxicity associated with administering this drug[34] make it unadvisable to be used outside of its approved indication.

Our findings highlight the value of repeat Bxs in detecting new HER2-low results. However, the question of whether and to what extent the expression of the target impacts T-DXd efficacy is currently under

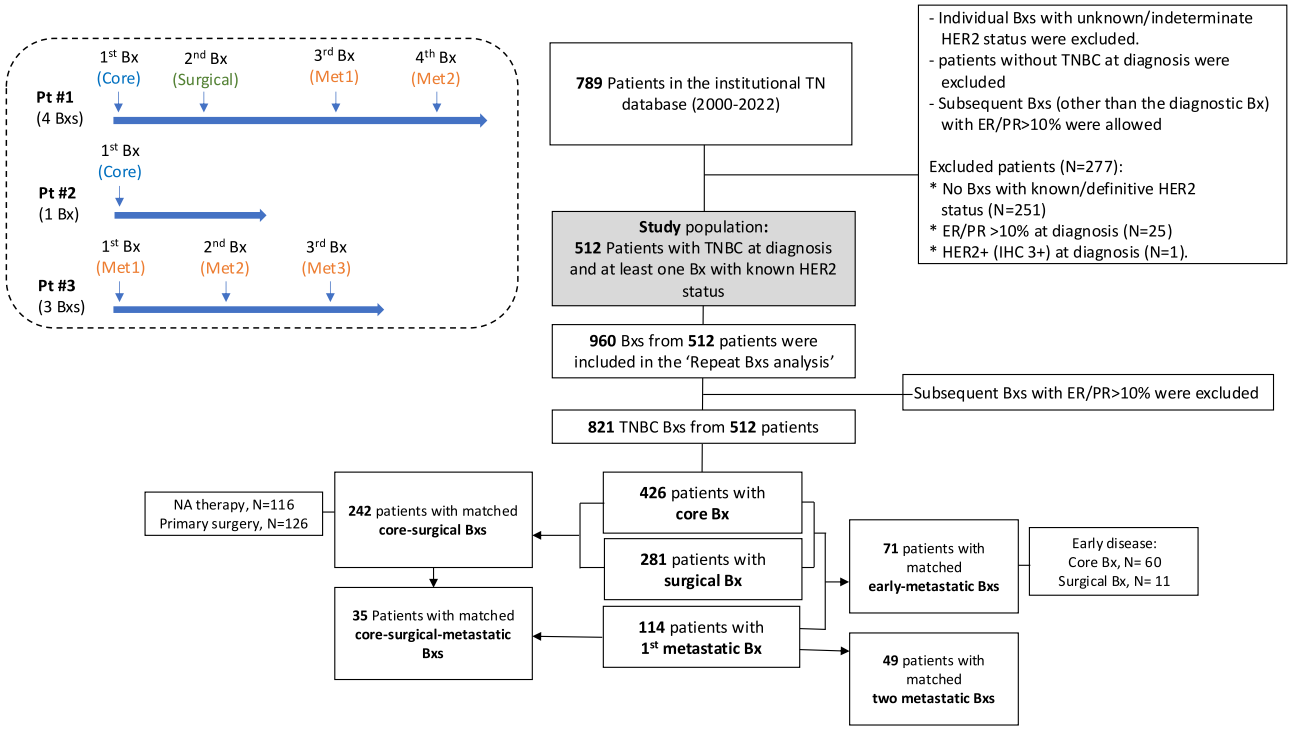

Fig. 1 | Study design and flow diagram. In the dashed box on the left are three representative patients. For each patient, the total number of Bxs was determined. Bxs were classified as core, surgical or metastatic and were ordered chronologically. Met metastatic, TN triple negative, ER estrogen receptor, PR progesterone receptor, Bxs biopsies, NA neoadjuvant, IHC immunohistochemistry.

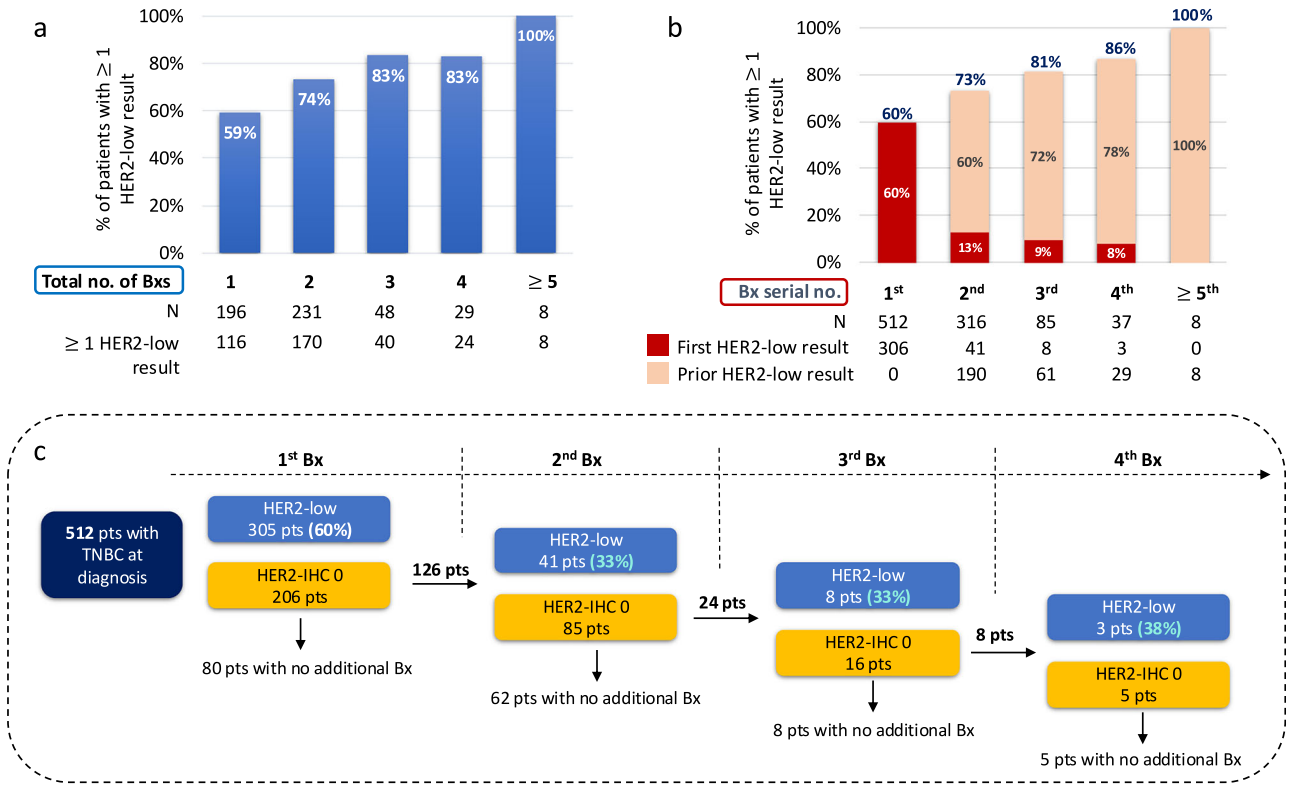

Fig. 2 | The impact of repeat Bxs in the detection new of HER2-low results. a The probability of detecting a HER2-low result according to the total number of Bxs conducted per patient. **b** The probability of detecting a first HER2-low result according to the Bx serial number. **c** The probability of detecting a first HER-low result for patients with prior only HER2-IHC 0 results. Bxs biopsies, pts patients, TNBC triple negative breast cancer.

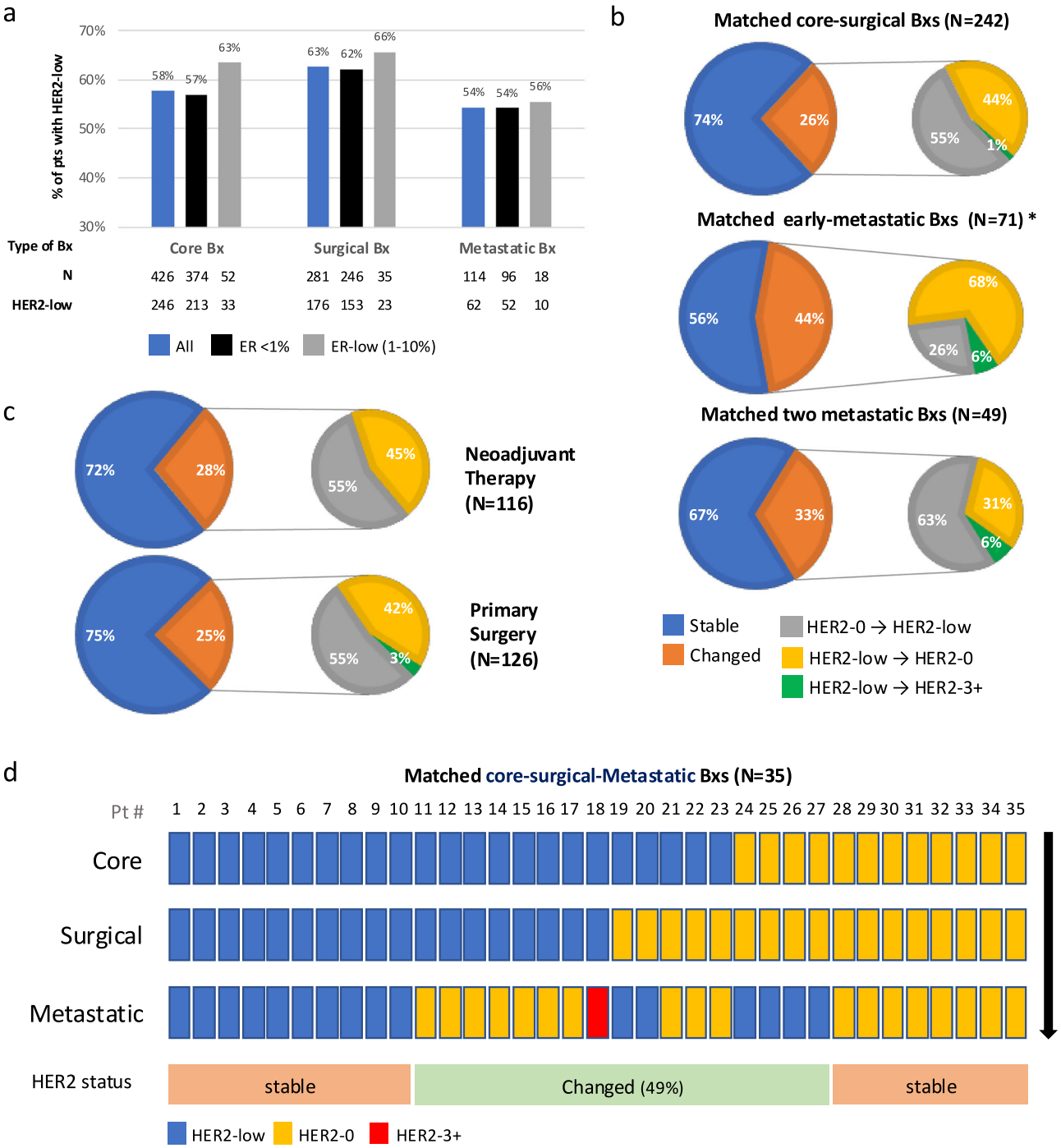

Fig. 3 | HER2 status discordance between matched Bxs of patients with TNBC. **a** HER2 status distribution according to the Bx type and ER status. **b** HER2 status discordance between core-surgical matched Bxs, early-metastatic matched Bxs and two metastatic matched Bxs. For the early-metastatic matched analysis, the core Bx was considered the early Bx, unless the core Bx was missing and then the surgical bx was used instead. * Significant discordance between matched Bxs. **c** The impact of neoadjuvant therapy on core-surgical HER2 status discordance. Matched Bxs are repeat biopsies taken from the same patient at specified time points throughout the disease course. **d** HER2 status discordance between matched core-surgical-metastatic Bxs. Bxs biopsies, ER estrogen receptor, TN triple negative.

investigation. Acknowledging the limits of cross-trial comparisons, it is worth noting that trials enrolling patients with HER2-positive disease, such as the DESTINY-Breast02[35] and DESTINY-Breast03[36] trials, showed higher objective response rates (ORRs) with T-DXd compared to patients with HER2-low disease in the DESTINY-Breast04[4] study (70-80% compared to 50%, respectively). Similarly, in the phase 2 DAISY trial, which enrolled patients with various HER2 expression levels, the ORR for T-DXd among patients with HER2-positive tumors was significantly higher than for HER2-low tumors (70.6% vs. 37.5%, OR: 3.96, 95% CI 1.78–8.77, $p = 0.001$), suggesting a positive correlation between the target expression level and drug efficacy[37]. Additionally, progression-free survival was significantly higher as HER2 expression increased (11.1 vs 6.7 vs 4.2 months in the HER2-positive, HER2-low, and HER2-0 cohorts respectively). Finally, correlative studies from the DAISY trial demonstrated a positive correlation between T-DXd uptake and HER2 expression level, as well as a reduction in HER2 expression levels following T-DXd resistance, both of which suggest that the expression of HER2 plays a pivotal role in determining the efficacy of T-DXd[37].

However, there is also evidence supporting the idea that T-DXd efficacy is not solely dependent on HER2 target expression. Several studies have shown that the specific immunohistochemistry (IHC) score within the HER2-low group (IHC 1+ compared to IHC 2 +) did not seem to affect the efficacy of T-DXd[4,37,38]. Additionally, the DAISY trial demonstrated efficacy of T-DXd in patients with HER2-0 metastatic breast cancer, albeit to a lesser extent, with a 29.7% ORR in this population[37]. Moreover, responses for T-DXd were also observed in patients with HER2-0 and a very low T-DXd uptake. Interestingly, confirmed ORRs were 19% (4/21) in patients with a previous HER2-0 result (in the last Bx taken before study entry, as part of routine care) and a new HER2-low result in the study baseline Bx, suggesting some efficacy of T-DXd in this unique setting[37]. The recently published phase 3 DESTINY-Breast06 trial demonstrated a clinically meaningful benefit of T-DXd in patients with HER2-ultralow status (defined as IHC 0 < HER2 < IHC 1 +), providing further validation for the efficacy of this drug in tumors with very low expression of HER2[10]. The observed efficacy of T-DXd in cells with negative or very low HER2 expression is likely attributed, at least in part, to the bystander effect of T-DXd, in which the membrane permeable payload is cleaved from the antibody and can diffuse out of a targeted cell into adjacent cells with lower or null HER2 expression[39]. Finally, the impact of HER2 status switch on T-DXd efficacy remains unclear. In the RELIEVE study, the change in HER2 status between the primary and metastatic pre-T-DXd Bx significantly impacted time to next treatment (TTNT), with a longer TTNT observed in cases where HER2 status changed from 0 to low compared to from low to 0[40]. However, additional larger studies are needed to evaluate the impact of HER2 expression dynamics on T-DXd efficacy. Considering all of the above, it is reasonable to consider offering T-DXd in the setting of a newly detected HER2-low result after a prior HER2-0 result. However, this decision should take into account other available treatment options and be made after a thorough discussion of the potential risks and benefits with the patient.

Our study has several limitations. Firstly, this is a single institutional retrospective study which may limit the generalizability of the findings. Secondly, the HER2 status for each Bx was obtained retrospectively from the institutional database or archival pathology reports, and there was no centralized review of the specimens specifically for this study. Additionally, while the majority of the Bxs were analyzed by the breast pathology team in our institution, a minority of them were analyzed outside our institution. This introduces the possibility of biases related to analytical variations, but it also reflects real-world scenarios where patients undergo Bxs at multiple time points and across different healthcare centers. Thirdly, the proportion of HER2-low results observed in our dataset is relatively higher compared to previously reported cohorts (around 60% in our cohort compared to 30-50% in historical cohorts[11–13]). One possible explanation is the inclusion of patients with ER-low disease in our TNBC cohort. Figure 3a demonstrates a numerically higher prevalence of HER2-low among patients with ER-low disease compared to those with ER < 1%, which aligns with previous reports showing a positive correlation between ER expression and HER2-low status[15,29,41]. Finally, the treatments received between Bxs, either in the early phase of the disease or after disease recurrence, might impact HER2 status and contribute to the observed discordance in HER2 status over time and those were not analyzed. However, as also reported in a previous study[42], we demonstrated that neoadjuvant treatment does not affect core-surgical Bxs discordance. Nonetheless, this does not rule out other potential effects of therapeutic pressure on the evolution of HER2 status.

In conclusion, our work highlights the impact of repeat Bxs in identifying new potential candidates with mTNBC for T-DXd treatment. While our study focused on patients with TNBC, the same rationale could apply to detecting HER2-low results in women with HR+ metastatic breast cancer, and repeat Bxs are likely to provide similar value in that large population. The high detection rate of HER2-low with repeat Bxs and emerging evidence on the efficacy of T-DXd among patients with very low HER2 expression raises questions about the utility of the current approval framework. However, until improved HER2 quantification methods are approved or the indication for T-DXd is broadened, repeat Bxs have the potential to expand the population that can benefit from this important new therapeutic option.

Methods

This study was performed in compliance with the Declaration of Helsinki. The retrospective analyses were performed with Institutional Review Board (IRB) approval from an institutional protocol (Massachusetts General Hospital). Per IRB regulations, individual patient consent was not required for this retrospective analysis

Study population

Consecutive patients with TNBC (defined as estrogen receptor (ER) and progesterone receptor (PR) < 10% and HER2-negative) at diagnosis were retrospectively identified from an institutional TNBC database. The database, which was established in 2017 under an IRB-approved protocol, includes patients with TNBC that were treated in a single large academic institute (Massachusetts General Hospital, Boston, MA) between 2000-2022. Demographics and clinicopathological information for patients with TNBC treated at the institution before the initiation of the database were retrospectively retrieved from electronic medical records at the time of database initiation. Information on patients treated after 2017 was prospectively collected into the database. Patients with HR+ (ER/PR > 10%) or HER2-positive (HER2 IHC 3+ or IHC 2+ and ISH amplified) breast cancer at diagnosis were excluded from the analysis. However, patients with TNBC at their initial diagnostic Bx but with subsequent later Bxs showing HR+ or HER2-positive results were allowed. For each patient, Bxs with unknown or indeterminate HER2 status were excluded. Notably, some of the Bxs analyzed in our institution before 2016, when HER2 status was reported as IHC 0/1+ instead of distinct HER2 IHC 0 or HER2 IHC 1 + , were also excluded from the analysis. Therefore, only patients with TNBC at their initial diagnosis and with at least one Bx with a known HER2 status (i.e., eligible Bx) were included in the study population.

Patient demographics and clinicopathological factors were extracted from the database. The ER, PR, and HER2 status of eligible Bxs were also obtained from the database or from the original pathological reports if absent in the database.

Repeat Bxs analysis

'Repeat biopsies' were defined as all the biopsies taken for a specific patient throughout the course of the disease. All eligible Bxs of the entire study population were included in the repeat Bxs analysis (Fig. 1). For each of the eligible Bxs, the HER2 status was defined as either HER2-low (HER2 IHC 1+ or HER2 IHC 2+ and ISH non-amplified) or HER2-0 (HER2 IHC 0). For each patient, Bxs were categorized and ordered as presented in Fig. 1. In summary, eligible Bxs were classified as core, surgical, or recurrent/metastatic, and were arranged chronologically based on the order in which they were performed (referred to as first Bx, second Bx, third Bx, etc.). Additionally, the total number of eligible Bxs was determined for each patient.

Matched Bxs analysis

Matched Bxs were defined as two biopsies taken from the same patient at specified time points throughout the disease course. In contrast to the repeat Bxs analysis described above, in which subsequent later Bxs (other than the diagnostic Bxs) with ER/PR > 10% were allowed, those Bxs were excluded from the matched Bxs analysis, and only Bxs with ER/PR < 10% were allowed. Of the entire study population, patients with matched core-surgical Bxs, matched early-metastatic Bxs, and matched two metastatic Bxs were identified, and HER2 status was compared between matched Bxs. The rate of HER2 status discordance and the direction of change (low to 0 or 0 to low) were determined. For the early-metastatic matched Bxs analysis, the core Bx was considered the early Bx, unless the core Bx was missing and then the surgical bx was used instead. For cases with several metastatic Bxs the first metastatic Bx was used. Finally, a subset of patients with matched core-surgical-metastatic Bxs was identified and analyzed.

Statistical analysis

Categorical variables were summarized using counts and percentages, while continuous variables were summarized with the median and range. For key variables, 95% binomial confidence intervals were estimated. Fisher's exact test was applied to compare differences in HER2 status (HER2-low vs. HER2-0) across core, surgical, and metastatic biopsies and to evaluate the relationship between ER status and HER2 status within each Bx type. For all paired data, McNemar's test was used to assess HER2 status (HER2-low vs HER2-0) discordance across matched Bxs. Due to the small number of HER2-positive cases observed, HER2-positive cases were ignored for analysis purposes. The chi-squared test was used to compare different Bxs locations. Statistical significance for all tests was declared at the 0.05 type I error. All statistical analyses were performed using R version 4.2.2.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available in order to protect patient privacy. Parts of the data will be available on reasonable request from the corresponding author.

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Author contributions

Y.B. and S.J.I designed the study. Y.B. and S.J.I wrote the first draft of the manuscript. Y.B., G.F., A.D., N.M. and S.I.J participated in data collection, analysis and interpretation. All authors participated in review and approval of the final manuscript.

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Additional information

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