Breast Cancer: Targets and Therapy

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REVIEW

HER2-Low Breast Cancer: Current Landscape and Future Prospects

Yelena Shirman, Shlomit Lubovsky, Ayelet Shai

Division of Oncology, Rambam Health Care Campus, Haifa, Israel

Correspondence: Ayelet Shai, Division of Oncology, Rambam Health Care Campus, HaAliya HaShniya St 8, PO Box 9602, Haifa, 3109601, Israel, Tel +972-47776400, Email a_shai@rambam.health.gov.il

Abstract: More than 50% of breast cancers are currently defined as "Human epidermal growth factor receptor 2 (HER2) low breast cancer (BC)", with HER2 immunohistochemistry (IHC) scores of +1 or +2 with a negative fluorescence in situ hybridization (FISH) test. In most studies that compared the clinical and biological characteristics of HER2-low BC with HER2-negative BC, HER2-low was not associated with unique clinical and molecular characteristics, and it seems that the importance of HER2 in these tumors is being a docking site for the antibody portion of antibody drug conjugates (ADCs). Current pathological methods may underestimate the proportion of BCs that express low levels of HER2 due to analytical limitations and tumor heterogeneity. In this review we summarize and contextualize the most recent literature on HER2-low breast cancers, including clinical and translational studies We also review the challenges of assessing low HER2 expression in BC and discuss the current and future therapeutic landscape for these tumors.

Keywords: HER2-low, ERBB2 low, breast cancer, HER2 targeted therapy, trastuzumab, trastuzumab-deruxtecan, T-DXd

Introduction

HER2 belongs to the epidermal growth factor (EGF) tyrosine kinase receptor family. HER2 is unique for both its functional characteristics as an orphan receptor that heterodimerizes with other tyrosine kinase receptors (TKIs) and its high oncogenic potential.[1] The HER2 gene is amplified in 15–20% of breast cancers, resulting in markedly increased HER2 protein content on the cell surface and enhanced signal transduction through HER2 heterodimers.[2] These tumors were associated with a higher risk of relapse and shorter survival before anti-HER2 treatments were available;[3,4] however, the development of trastuzumab, pertuzumab and other HER2 targeting agents revolutionized the treatment and improved the prognosis of patients with advanced[5,6] and early[7–9] HER2-positive breast cancer.

HER2 positivity is defined by a circumferential, complete, and intense membrane immunohistochemistry (IHC) staining in >10% of the tumor, defined as IHC +3, or a weak to moderate complete membrane staining in >10% of tumor cells (IHC +2) with a positive ISH test.[10] Trials designed to test the benefit of trastuzumab and of trastuzumab-emtansine in breast cancers with lower HER2 expression failed to show the benefit of these HER2 targeting agents,[11,12] and these tumors were defined as "HER2-negative". However, post-hoc analyses of the seminal adjuvant trastuzumab trials did not find an association between the degree of HER2 amplification or chromosome 17 polysomy and benefit from trastuzumab.[13,14] In addition, analyses of the NSABP B-13 and the NCCTG N9831 trials suggested that some patients with tumors that were defined as HER2-positive by local pathology and HER2-negative by central pathology derived benefit from trastuzumab,[13,15] raising questions about the impact of heterogeneity and analytical aspects of HER2 testing.

The success of clinical trials testing treatment with the HER2-directed antibody–drug conjugate T-DXd in breast cancers with a lower expression – IHC+1 and IHC+2 with a negative fluorescence in situ hybridization (FISH) test,[16,17] previously considered "HER2-negative", created a new terminology for these tumors, and they are now referred to as "HER2-low". However, it remains unclear whether HER2 oncogenic signal transduction plays a role in the progression of HER2-low breast cancer, and if the mechanism underlying the anti-tumor effects of trastuzumab-deruxtecan in HER2-low cancers involves disruption of HER2 signaling or merely better cytotoxic drug delivery.[18]

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In this review we will focus on 4 main topics: the molecular biology of HER2 in breast cancer and its relevance to HER2-low cancers, the clinical and prognostic significance of HER2-low status in local and metastatic breast cancers treated with standard regimens, the current and future landscape of HER2 targeted therapies for these tumors and the challenges of pathologic assessment of HER2-low BC.

Molecular Biology of HER2 in Breast Cancer

Mechanisms of HER2 Overexpression in Breast Cancer

To date, several molecular mechanisms of HER2 overexpression in breast cancer have been described, of these, amplification of the HER2 gene (also called *erb*B-2 and *neu*) is the most common mechanism.[19] Breast cancers can have up to 25–50 copies of the HER2 gene and up to 40–100-fold increases in HER2 protein expression, resulting in up to 2 million receptors expressed at the tumor cell surface.[20]

Furthermore, it has been shown that transcriptional upregulation results in HER2 mRNA levels of 4- to 8-fold in breast cancer cells without HER2 gene amplification and of 64–128 fold in cells with HER2 gene amplification.[21] Interplay between various promoters, promoter 1[22] and promoter 2,[23] enhancers and transcription factors such as ESX,[24] TFAP2,[25,26] YY1,[27] EGR2[28] is involved in HER2 transcription, resulting in overexpression.

Epigenetic mechanisms also contribute to transcriptional regulation of HER2. Histone modifications such as acquisition of H3K4me3 and H3K9ac increase ErbB2 transcription independently from gene amplification.[29] It was shown that DNA hypomethylation of the HER2 gene body enhancer enables binding of the transcription factor TFAP2C, resulting in increased transcription.[30] Furthermore, in vitro studies have shown that disruption of ubiquitination of HER2 by chaperone-interacting protein (CHIP)[30,31] results in reduced degradation.[32] A recent study reported that HER2 neddylation, the process of removing the ubiquitin-like protein (NEDD8) from HER2, is a post- translational modification that results in reduced degradation and stabilized HER2 expression.[33]

Finally, anti-cancer therapy may affect the expression of the HER2 receptor. In ER+/HER2− breast cancer cells following endocrine treatment, defects in MutL mismatch repair complex (*MLH1/3, PMS1/2*) result in interference with lysosomal protein trafficking. Studies suggested that HER2 levels increase with MutL loss even before endocrine therapy, uncovering another mechanism of HER2 overexpression.[34,35] Radiation therapy can induce HER2 expression through radiation-induced NF-κB activity. As NF-κB activation through the PI3K/Akt pathway is a major downstream event of HER2 overexpression, a loop-like HER2-NF-κB-HER2 pathway in radiation-induced adaptive resistant breast cancer cells has been proposed.[36]

Of note, close inspection of the literature disclosed that some of the mechanisms were described in HER2-low tumors as well, including gene amplification,[19] HER2 gene body enhancer,[30] radiation therapy and endocrine therapy.[35,36]

Molecular Biology of the Carcinogenic Effect of HER2

HER2, a member of the HER receptor family consisting of 4 cell surface receptor tyrosine kinases (EGFR/erbB1/HER1, erbB2/HER2, erbB3/HER3, erbB4/HER4),[37–39] was found to be a key player in the pathogenesis of breast cancer in 1987.[23] Since then, an abundance of experimental evidence on the molecular mechanisms underlying HER2 tumorigenesis has accumulated.[40] Signal transduction via the HER receptors is initiated upon ligand-induced[41] dimerization. HER2 does not bind a ligand and is activated by heterodimerization with other HER family members. This results in transphosphorylation of their intracellular domains,[42] which in turn activates signaling pathways, principally the PI3K/Akt, mitogen-activated protein kinase (MAPK), protein kinase C (PKC), RAS/RAF/MEK. These events lead to deregulation of the cell cycle through upregulation of various cyclins such as cyclin D1, E and CDK6, and degradation of cell cycle inhibitors such as p27Kip1 and results in proliferation, survival, and differentiation.[43–46]

Additionally, crosstalk with other signaling pathways further promotes the carcinogenic effect of HER2. About 50% of HER2-amplified tumors express the estrogen receptor. HER2/ER crosstalk, through ligand-independent phosphorylation of estrogen-receptor (ER), results in activation and consequent enhancement of ER activity at the DNA level, stimulating cell proliferation and mutagenesis. Moreover, phosphorylation of co-repressors results in disruption of regulation of ER transcriptional complexes in the nucleus.[47]

Although HER2 overexpression alone, that is, independent from mutational activation, holds tumorigenic potential, breast carcinomas can harbor HER2-activating mutations that act as oncogenic drivers.[48] Interestingly, HER2 somatic mutations occur in only about 2.7% of breast cancer patients[49] and they more frequently occur in HER2-negative or HER2-low breast carcinomas.[48]

Molecular Biology of HER2-Low Breast Cancer: What is the Significance of the HER2 Level of Expression?

Recent studies have sought to investigate the molecular biology of HER2-low breast cancer. In the past decade, "Molecular portraits" of human breast tumors have been developed through hierarchical clustering methods, in which genes are grouped based on similarity in their expression pattern.[50] The most widely accepted molecular portraits, known as intrinsic subtypes, ie luminal A, luminal B, HER2-enriched, and basal-like, provide insight on the molecular biology of breast tumors and have been translated into clinical assays that are now widely implemented in clinical practice.[51,52] Schettini et al and Agostinetto et al studied the molecular characteristics of HER2-low breast cancer using the PAM50 assay, a qRT-PCR based, 50-gene molecular profile assay.[51] Both have demonstrated that HER2-low breast cancers are a heterogeneous group of tumors comprising primarily of luminal A (50.8–56.9%) and luminal B (22.8–28.8%), with a minority being HER2-enriched (3.5–3.6%) and basal-like (13.3–17.7%).[52,53] Zahng et al evaluated HER2-low genomic profile using MammaPrint testing, a microarray-based, 70-gene expression assay[54] and Blueprint testing, a microarray-based, 80-gene molecular subtyping assay[55] and showed similar distributions of these molecular subtypes.[56] Additional studies which used the PAM50-based Prosigna assay[57,58] and IHC-based molecular subtype distribution[59] found that molecular subtype distribution of the HER2-low subgroup was comprised predominantly of luminal B tumors (58.9–76% %) and 20–28.6% of luminal A tumors. While these studies used different assays, they consistently found a high prevalence of luminal subtypes and a low prevalence of HER2-enriched and basal-like subtypes among HR+/HER2-low tumors. Along these lines, in the studies by both Schettini et al and Agostinetto et al, proliferation-related genes and tyrosine-kinase receptor genes were more frequently over-expressed in HER2-0 tumors compared to HER2-low tumors, whereas HER2-low tumors had higher expression of luminal-related genes.

When tested separately, hormone receptor (HR) negative/ HER2-low (HR-/HER2-low) tumors were mostly basal-like, with a slightly higher proportion being HER2-enriched compared to HR-/HER2-negative tumors.[60] Zahng et al used a 520-gene panel and did not find genes or signaling pathways that were differentially expressed between HR-/HER2-low and HR-/HER2-negative tumors. Denkert et al reported that, in 556 tumors sequenced in the GeparSepto trial, there was no difference in the frequency of PIK3CA and TP53 mutations between HER2-low and HER2-negative tumors when analyzed differentially by HR expression.[61] Additionally, Schettini et al emphasized the lack of difference in gene expression between the two HER2 subgroups within the triple-negative (TN) breast tumors. Taken together, these findings suggest that HR status and luminal genes, and not HER2 expression, are the key determinants of the biology of HER2-negative and HER2-low breast cancers.

Several studies evaluated the mRNA levels of ERBB2 and found them to be directly proportional to HER2 protein expression, with HER2-low mRNA levels being closer to HER2-negative levels.[53,58,60] Higher ERBB2 expression was observed in HR+/HER2-low tumors compared with HR−/HER2-low tumors, without corresponding enrichment of the HER2-enriched subtype. This finding was explained by the fact that the HER2-enriched phenotype is not defined solely by expression of *ERBB2*.[53,60]

Zhang et al analyzed the genomic data of 523 breast cancers by next generation sequencing using a 520-gene panel. Pathway analysis demonstrated that HER2-low tumors had significantly more mutations involved in PI3K-Akt signaling than HER2-positive and HER2-negative breast tumors, and less mutations in checkpoint genes, Fanconi anemia, and p53 signaling and cell cycle pathway compared to HER2-negative breast tumors. However, when analyzing HR-positive and HR-negative tumors separately, they could not detect significant differences between HER2-low and HER2-negative tumors.[59]

Finally, Denkert et al analyzed germline mutation data from 549 patients and reported higher rates of germline BRCA1/2 mutations or other breast cancer predisposition genes (26.8% vs 18.9%) in patients with HER2-negative breast cancers compared to HER2-low breast cancers.

To date, there are no published studies that assess if HER2 is an active oncogene in HER2-low breast cancers, and it is currently unknown if activation of HER2 signaling contributes to malignant transformation and progression of these tumors. It is possible that HER2 signaling is active in the small fraction of HER2-low tumors that are defined as "HER2 enriched" by gene expression analysis,[60] but the role of HER2 in the pathogenesis of most HER2-low breast cancers is unknown.

Clinical Significance of HER2-Low Status

HER2-low status is more frequent in HR+ than HR- breast cancer,[62] and rates of HER2-low staining increase as levels of expression of HR increase.[63] HER2-low status was found to be associated with better prognostic pathological tumor characteristics compared with HER2-negative, both in HR-positive and HR-negative tumors.[62,64,65] Among HR-negative tumors, HER2-low cancers are less often "basal like" when classified by the PAM50 gene array[60] compared to HER2-negative tumors, and in another study on HR-negative tumors, HER2-low status was more frequently seen in low-grade tumors (35% vs 18%) and in tumors with apocrine IHC markers (57% vs 36%) compared to HER2-negative tumors.[66] Thus, it seems that among cancers without HER2 amplification, low HER2 expression is found more often in tumors that have markers of better prognosis.

Multiple retrospective studies looked at the association of HER2-low status and prognosis among HER2 non-amplified cancers, with conflicting results. In localized HR-positive disease, a study looking at 23,000 patients from 6 Asian centers reported better disease-free survival (DFS) and overall survival (OS) for HER2-low compared to HER2-negative breast cancers.[67] Xu et al reported that, among 678 patients with ER positive, HER2 non-amplified tumors, HER2-low cancers had similar DFS as HER2-negative tumors in the initial 5 years after diagnosis, and better DFS from year 5 onwards.[64] A study from Japan with 2890 patients found that HER2-low status was not associated with 5-year DFS and OS.[63] Along these lines, a recent cohort study of 1,136,016 patients from the National Cancer Database in the US similarly found that patients with HER2-low breast cancers have a similar prognosis to those with HER2-negative breast cancer.[68] Similar results were reported in additional cohort studies in several countries.[60,63,65,69,70] Mutai et al reported that in tumors with a high 21-gene recurrence score (RS), those with HER2-low status had better DFS rates than those with HER2-negative status, despite similar clinical-pathological features. Better DFS and OS were also reported in HER2-low node-negative BC patients who did not receive any systemic adjuvant treatment.[71]

Several retrospective studies did not find an association between HER2-low status and DFS or OS in localized HR-negative breast cancer.[60,63,64,66] However, a nationwide study from Korea with a median follow-up of 12 years reported better breast cancer-specific survival in patients with HR-negative HER2-low breast cancer compared with HER2-negative HR-negative breast cancer,[65] and Tan et al also reported better OS for HR-negative HER2-low breast cancer patients.[67] Jacot et al reported that, in HR-negative breast tumors, HER2 +2, ISH negative tumors had worse DFS and OS compared to HER2-negative and HER2 +1, combined.[66]

The effect of HER2-low status on the prognosis of patients undergoing neoadjuvant chemotherapy was reported in several studies. A pooled analysis of neoadjuvant trials from Germany reported that HER2-low status was associated with lower pathological complete response (pCR) rated in HR-positive[61] but not HR-negative breast cancer. In this study, patients with HR-negative HER2-low breast cancer had better DFS and OS, and in an exploratory analysis this difference was seen only in patients without a pCR after neoadjuvant chemotherapy. Patients with HR-positive BC had similar outcomes regardless of HER2 status. A single-center retrospective study from Korea similarly reported that in patients with HR-negative tumors, but not in those with HR-positive tumors, HER2-low status was associated with higher DFS rates after neoadjuvant chemotherapy.[72] In contrast, 4 retrospective studies found no association between HER2-low status and prognosis in HR-positive and HR-negative BC patients undergoing neoadjuvant chemotherapy.[73–76]

Thus, although controversial, several studies suggest that HER2-low status may be associated with better prognosis compared with HER2-negative status in high-risk tumors like HR-negative tumors without a pCR and HR-positive tumors with a high 21-gene RS, or those who did not get any adjuvant treatment. This better prognosis is achieved without anti-HER2 therapy, suggesting that HER2-low status may be a marker rather than an oncogenic driver in these tumors.

In patients with metastatic breast cancer, HER2-low status is more often found in HR-positive disease. When adjusted to other variables such as ER expression, visceral disease and age, patients with HER2-low metastatic BC were found to have a slightly better prognosis than those with HER2 -negative status in a study involving more than 15,000 patients diagnosed between the years 2000–2015 (HR 0.95, CI 0.91–0.99; P = 0.02).[77] In a more recent series of patients treated with the CDK 4/6 inhibitor Palbociclib, HER2-low status was not associated with outcomes.[78] Others have also reported similar outcomes for HER2-low and HER2-negative metastatic BC patients.[79]

Current Treatments and Ongoing Trials of HER2 Targeted Therapy for HER2-Low Breast Cancer

Trastuzumab deruxtecan (T-Dxd) is a potent antibody drug conjugate (ADC) that has proved to be superior to trastuzumab emtansine in the 2nd line treatment of patients with HER2-positive MBC.[80] T-Dxd is the 1st ADC to be approved for use in patients with HER2-low breast cancer, based on the Destiny breast −04 trial. This Phase III trial randomized 557 patients with metastatic breast cancer and centrally confirmed HER2-low (1+ or 2+ with negative FISH) expression to receive T-Dxd vs standard chemotherapy (capecitabine, eribulin, gemcitabine, paclitaxel or nab-paclitaxel). All patients had received one or more prior lines of chemotherapy for metastatic breast cancer and those with HR-positive disease were endocrine refractory. At a median follow-up of 18.4 months, a 49% reduction in the risk of progressive cancer and a 36% reduction in the risk of death were observed in patients treated with T-DXd vs standard chemotherapy. Median progression-free survival was 10.1 months for the T-DXd-treated patients vs 5.4 months for those treated with standard chemotherapy. In the patients with HR-positive disease, median overall survival was 23.9 months vs 17.8 months, respectively. Median overall survival in the total study population was 23.4 months for T-DXd recipients vs 16.8 months for standard chemotherapy recipients—a significant gain of 6.6 months in median survival favoring the antibody-drug conjugate (ADC).[16] In the neoadjuvant setting, t-Dxd treatment achieved a high response rate of 75% when administered alone and 63% when administered with endocrine therapy in HR-positive, HER2-low localized breast cancer in the Phase II TRIO-US B12 TALENT trial.[81]

The activity of T-Dxd is achieved by its potent payload and by the high ratio of payload molecules per ADC molecule.[82] It is the 1st anti-HER2 agent that has shown activity in tumors that express a low amount of HER2. This has led the oncology and research community to realize that any amount of HER2 expression may be sufficient to elicit an anti-tumor response by potent ADC targeting HER2 and has further strengthened the understanding of the bystander effect, by which the chemotherapy portion of ADCs enters and kills nearby tumor cells—even those that have little or no HER2 expression. It is unclear, however, if the ability of the antibody portion of T-Dxd to block HER2 signaling is part of its mechanism of action in HER2-low tumors, or if it merely serves to deliver the potent payload into HER2-expressing tumor cells.

Ongoing and pending trials in the HER2-low setting include approximately 17 recruiting studies as of a search done in January 2023. DESTINY08 is a Phase Ib trial assessing T-Dxd with various combinations, including durvalumab in metastatic HER2-low breast cancer.[83] A Phase II trial is assessing the addition of pyrotinib, an oral inhibitor of the intracellular portion of HER2, to chemotherapy in the neoadjuvant treatment of HR-positive HER2-low breast cancer.[84]

Several trials are testing novel agents in HER2-low tumors. A Phase I trial is assessing safety and efficacy of a novel anti-HER2 antibody drug conjugate MRG002 in MBC. This molecule is composed of a humanized anti-HER2 IgG1 monoclonal antibody conjugated to a microtubule disrupting agent, monomethyl auristatin E (MMAE).[85] Another Phase I study is assessing the combination of an enhancer of zeste homolog (EZH) ½ dual inhibitor, valemotostat, in combination with t-Dxd.[86] Valemetostat targets epigenetic regulation by inhibiting both the EZH1 and EZH2 enzymes that act through histone methylation to regulate gene expression. In pre-clinical studies, reactivation of the silenced genes resulted in decreased proliferation of EZH2-expressing cancer cells.[87,88]

The I-spy-P1.01 trial is evaluating the safety of a novel ADC, trastuzumab duocarmazine with weekly paclitaxel in several tumor types, including HER2-low metastatic breast cancer.[89] Trastuzumab duocarmazine consists of trastuzumab conjugated to a highly potent duocarmycin payload through maleimide attachment to interchain disulfides.[90] The Phase III TULIP trial compared T-duocarmazine with anti-HER2 therapy and chemotherapy in patients with metastatic HER2-

positive BC that progressed on 2 prior anti HER2 therapies, and resulted in improvement in progression-free survival for patients treated with T-duocarmazine.[91]

A pending single-arm Phase II trial will be looking at ARX788 in HER2-low MBC.[92] ARX788 is a next-generation, site-specific anti-HER2 ADC that utilizes a unique non-natural amino acid-enabled conjugation technology and a non-cleavable amberstatin (AS269) drug-linker to generate a homogeneous ADC with a high drug-to-antibody ratio. The payload AS269 is conjugated by the synthetic amino acid para-acetylphenylalanine (pAF) to a humanized anti-HER2 mAb.[87] A recent Phase I trial demonstrated high stability and low serum exposer of pAf-AS269 resulting in low systemic toxicity.[93]

Another Phase I trial is investigating the safety and activity of another novel ADC, BL-M07D1, in patients with HER2-amplified and HER2-low MBC. This ADC is based on trastuzumab, with a wild type Fc portion that is expected to elicit an immune response and a toxic payload.[94] The DecipHER trial is investigating a dendritic cell (DC) vaccine given with chemotherapy in several breast cancer subsets, including HER2-low BC. DCs have been in clinical use for three decades for boosting anti-tumor immunity. They present antigens to naïve T cells and polarize them into effector or tolerogenic subsets.[95] The DecipHER trial utilizes HER2 and HER3 primed dendritic cells and delivers 8 intra-tumoral injections in order to assess maximal tolerated dose and efficacy.

Pathologic Assessment of HER2 Breast Cancer

While T-Dxd is approved for treatment of HER2-low breast cancer, a Phase II trial suggested that it is active in tumors that are defined as HER2-negative.[96] Since this ADCs mechanism of action involves binding to HER2, it is plausible that current immunohistochemistry does not accurately detect and quantify low levels of HER2 expression. Categorizing breast cancers as "HER2-low" carries inherent challenges,[97] and only a 26% concordance rate was found between 18 pathologists in designating breast tumors as HER2+1 or HER2-negative,[98] questioning the accuracy of the HER2-low diagnosis. An IHC score of +1 is defined as faint, barely perceptible membranous reactivity in >10% of tumor cells and an IHC score of HER2-negative is defined as no membrane staining or faint, barely perceptible membranous reactivity in <10% of cells,[99] thus distinguishing between these scores is understandably difficult. Discordance in scoring between the biopsy and the surgical specimen was seen in more than 20% of patients treated with neoadjuvant chemotherapy without anti-HER2 therapy,[100] and HER2-low IHC results change between primary tumors and metastatic relapses in more than 35% of patients.[100,101]

There are numerous pitfalls associated with the available preanalytical, analytical, and post-analytical methods[102,103] that may explain these discordant HER2 IHC results. Among these are tissue sample processing which might affect protein detection rate,[104] true HER2 intratumoral heterogeneity,[105] true heterogeneity between different metastatic sites in HER2 expression level,[106] as well as inter-observer variability,[107] leading to reduced sensitivity and reproducibility of HER2 testing. The American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) has recently updated their guidelines for HER2 testing in breast cancer. The thresholds for HER2 +1 remain the same as in the previous guideline version; however, it is recommended that tumors be assessed at 40X magnification, using appropriate controls, and that a second pathologist reviews the slides when results are close to 0. Due to heterogeneity in HER2 levels, medical oncologists are encouraged to consider HER2-low results from any biopsy, not only the most recent, in treatment decisions.[16]

The changing landscape of anti-HER2 therapy has led investigators to search for more sensitive assays and novel diagnostic methods that could better quantify HER2 expression levels and refine the historically binary approach to HER2 classification. Boyars et al re-evaluated the scores of breast carcinomas with low HER2 expression using up to 400x total magnification, which resulted in re-classification of most HER2-negative cases to HER2 "very low" (incomplete faint/barely perceptible membranous staining in up to 10% of tumor cells) or HER2 +1.[108] A recent study reported the promising results of HER2 detection using a novel assay combining quantitative immunofluorescence and mass spectrometry,[109] which resulted in a lower threshold of detection and better quantification than IHC. Kennedy et al evaluated a targeted mass spectrometry-based assay for quantifying HER2 protein in formalin-fixed paraffin-embedded (FFPE) and frozen BC biopsies and reported higher detection rates for tumors that were initially classified by IHC as HER2-negative or -low, and improved concordance by normalizing to glyceraldehyde-3-phosphate dehydrogenase to account for tissue heterogeneity.[110] Moreover, artificial intelligence and digital image analysis (DIA) offer the potential to

supplement conventional pathologic analysis and enhance the precision of HER2 testing. Recent studies have shown promising results, particularly in relation to HER2 heterogeneity in HER2-low breast cancer.[111–115] Nonetheless, caution should be taken before implementing these technologies for routine diagnosis of HER2-low BC, as at least 1 study reported that they could under-estimate HER2 staining, especially in heterogenous HER2-low cases.[116]

Summary and Future Directions

When categorizing breast cancer by HER2 expression, "HER2-low" cancers make up the majority of cases.[117] The promising results of Tdx-D in breast cancer patients with low HER2 expression have led to comprehensive research aiming to understand the clinical landscape of these tumors. Numerous trials have shown that HER2-low is associated with HR expression and other pathologic correlates of better prognosis, and that these tumors are associated with similar or better prognosis compared with HER2-negative tumors when treated with current therapies. Thus, to date, low expression of HER2 is clinically relevant only to patients who are candidates for treatment with Tdx-D in the metastatic setting. A trial that tested Tdx-D in the neoadjuvant setting in HER2-low cancers achieved impressive response rates,[81] and thus detecting HER2-low status may become relevant to locally advanced BC as well. The clinical significance of HER2 expression in these tumors seems to be its function as a docking site for the antibody portion of this ADC, which allows targeted delivery of potent chemotherapy.

Additional potent agents targeting HER2 are currently tested as treatment for HER2-low BC, and assessing tumors for low HER2 levels is an important clinical issue. Pathologists should meticulously test tumors at high magnification and use appropriate controls[16] so that patients will not be denied potentially beneficial therapy. As levels of HER2 expression change between different metastases when tested simultaneously,[106] clinicians should consider previous biopsy results documenting HER2-low BC in determining eligibility for Tdx-D therapy. Heterogeneity should also be considered when designing clinical trials for this patient population, and it might be appropriate to include patients based on any biopsy interpreted as "HER2-low", and not only the most recent biopsy.

The association between HER2 level and benefit from Tdx-D in HER2-low tumors has not been explored; however, HER2 +3 tumors derive a greater benefit from Tdx-D, and benefit from other ADCs seems to be related to level of expression of their target.[118] ADCs are expensive and toxic, and patient selection based on expected benefit is important. If HER2 targeting ADCs is tested in the neoadjuvant setting, aiming to replace standard chemotherapy, it would be essential to accurately define the patients who benefit and those who still need chemotherapy. Novel methods for HER2 quantification are in development,[108–110,112,114] and their value as predictive markers for benefitting from HER2 targeting ADCs in HER2-low tumors should be tested. Trials testing HER2 targeting ADCs in combination with other targeted agents in patients with HER2-low BC are also warranted.[100]

Conclusions

With the development of novel ADCs and other targeted agents, the treatment landscape for HER2-low breast cancer is expanding, and the importance of detecting low expression levels of HER2 is becoming increasingly relevant. Improvements in pathology review and novel laboratory methods are needed to make sure that patients are not spared effective therapy, to stratify patients according to the level of HER2 expression and test for associations between level of expression and response. Inclusion of patients with HER2-low breast cancer in clinical trials of targeted therapies will hopefully increase our treatment arsenal for these common tumors and allow further translational research on the role of HER2 and other molecular pathways in tumor progression and treatment response.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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