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SPECIAL ARTICLE

ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer

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Human epidermal growth factor receptor 2 (HER2)-low breast cancer has recently emerged as a targetable subset of breast tumors, based on the evidence from clinical trials of novel anti-HER2 antibody—drug conjugates. This evolution has raised several biological and clinical questions, warranting the establishment of consensus to optimally treat patients with HER2-low breast tumors. Between 2022 and 2023, the European Society for Medical Oncology (ESMO) held a virtual consensus-building process focused on HER2-low breast cancer. The consensus included a multidisciplinary panel of 32 leading experts in the management of breast cancer from nine different countries. The aim of the consensus was to develop statements on topics that are not covered in detail in the current ESMO Clinical Practice Guideline. The main topics identified for discussion were (i) biology of HER2-low breast cancer; (ii) pathologic diagnosis of HER2-low breast cancer; (iii) clinical management of HER2-low metastatic breast cancer; and (iv) clinical trial design for HER2-low breast cancer. The expert panel was divided into four working groups to address questions relating to one of the four topics outlined above. A review of the relevant scientific literature was conducted in advance. Consensus statements were developed by the working groups and then presented to the entire panel for further discussion and amendment before voting. This article presents the developed statements, including findings from the expert panel discussions, expert opinion, and a summary of evidence supporting each statement.

Key words: consensus, HER2-low, breast cancer, antibody—drug conjugates

INTRODUCTION

Recognizing the biological and clinical relevance of human epidermal growth factor receptor 2 (HER2) expression in breast cancer has had a major impact on the treatment of this disease.[1,2] Approximately 30 years ago, breast tumors harboring *ERBB2* amplification and overexpression (HER2-

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positive breast cancers, 15%-20% of all cases) were identified as a distinct biologic subtype, associated with a worse prognosis and an aggressive behavior compared with tumors lacking these molecular alterations (HER2-negative breast cancers, 80%-85% of all cases).[3,4] This observation led to pharmacological efforts to target HER2, which have radically reshaped the way we treat HER2-positive tumors.[2] Eight anti-HER2 drugs are currently approved by the Food and Drug Administration (FDA) and/or the European Medicines Agency (EMA),[5] and have turned HER2-positive breast cancer into a highly curable disease in the early-stage setting,[6] as well as relevantly improved outcomes in the metastatic setting.[7] The diagnosis of HER2-positive status is currently based on the detection of HER2 protein overexpression on immunohistochemistry (IHC 3+ score) or *ERBB2* gene amplification on *in situ* hybridization (ISH).[8]

Given the lack of HER2 overexpression/*ERBB2* amplification, HER2-negative tumors have been historically classified and treated based on the expression of hormone receptors, with the distinction of two main clinical subtypes: hormone receptor positive (estrogen receptor and/or progesterone receptor-positive, HER2-negative) and triple-negative tumors (estrogen receptor/progesterone receptor/HER2-negative).[9] Of note, despite being defined HER2-negative, most of these tumors still harbor detectable amounts of HER2 protein on cell membranes, with approximately two-thirds of hormone receptor-positive and one-third of triple-negative tumors exhibiting HER2-low expression (HER2 IHC score of 1+ or 2+/ISH not amplified).[10] Targeting HER2-low expression with anti-HER2 monoclonal antibodies has failed to demonstrate meaningful clinical benefits,[11] including in a large phase III adjuvant trial.[12] Nonetheless, the development of potent anti-HER2 antibody—drug conjugates (ADCs) has recently allowed to effectively target even low HER2 expression.[13] Multiple anti-HER2 ADCs have indeed showed antitumor activity in early-phase trials enrolling patients with HER2-low metastatic breast cancer, including trastuzumab deruxtecan (T-DXd), trastuzumab duocarmazine, disitamab vedotin, MRG002, and SHR-A1811, among others.[14-18] Importantly, the phase III DESTINY-Breast04 (DB-04) trial has demonstrated that T-DXd improves overall survival (OS) compared with traditional chemotherapy among patients with pretreated, HER2-low metastatic breast cancer.[19] These data have established the clinical relevance of HER2-low expression in breast cancer, but have concomitantly raised multiple controversies pertaining to the biology, nomenclature, diagnosis, and treatment of breast cancer.

We have established an international panel of experts in breast oncology to build consensus on the definition, diagnosis, and management of HER2-low breast cancer.

METHODS

The aim of this consensus-building process was to discuss controversial issues related to the definition, diagnosis, and management of patients with HER2-low breast cancer.

The experts were identified by ESMO leadership/ESMO Faculty assuring representation of diverse professional groups, geographic origin, sex, and age. The project chairs (PT and GC) conducted a nonsystematic review of the relevant literature in the field to inform the generation of key questions for the consensus process. An initial proposal for the list of questions was produced by the project chairs, after the cited narrative review. The proposal was then subject to discussion and scrutiny by the entire expert panel within dedicated virtual meetings. The virtual meetings included a multidisciplinary panel of 32 leading experts from nine different countries, and was chaired by PT and GC.

All experts were allocated to four different working groups. Each working group covered a specific subject area and was appointed two coordinators as follows:

- Biology of HER2-low breast cancer (coordinators: FA and GPr)
- Pathologic diagnosis of HER2-low breast cancer (coordinators: FPL and GV)
- Management of HER2-low metastatic breast cancer (coordinators: SMT and SM)
- Clinical trial design (coordinators: JC and SL)

The experts of each working group, under the lead of the coordinators, reviewed and modified the initial proposal of questions by the addition or revision of topics, then conveying a final list of relevant questions. The virtual meetings were followed by email-based focus group interactions, which led to the development of *statements and discussions* for each of the relevant questions, based on evidence available or expert opinion. The list of questions was then voted upon by all the experts of the panel via survey using a modified Delphi voting methodology. A first round of Delphi voting was conducted for each consensus statement. The presence of any disagreement during the first round of voting elicited a check by the working group coordinators for revision, and prompted a second Delphi voting round until there was consensus (90%-100% agreement of all the experts), majority agreement (75%-90% agreement), or no agreement (<75% agreement).

Planning, preparation, and execution of the consensus process was conducted according to the ESMO standard operating procedures. All statements developed by the group were accompanied by a level of evidence based on the 'Infectious Diseases Society of America-United States Public Health Service Grading System'[20] (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2023.05.008). The final manuscript was reviewed and approved by all panel members.

RESULTS

BIOLOGY OF HER2-LOW BREAST CANCER

QUESTION 1: What definition should be used in clinical practice for tumors lacking HER2 overexpression and/or *ERBB2* amplification, depending on the IHC score?

STATEMENT: In clinical practice, '**HER2-negative**' should be used according to the 2018 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) Guidelines, for tumors with IHC scores of 0, 1+, or 2+/ISH not

amplified. '**HER2-low**' may be used for a subset of HER2-negative tumors, namely, those with IHC scores of 1+ or 2+/ISH not amplified, according to the definition used in DB-04. '**HER2-0**' may be used for tumors with an IHC score of 0 by the 2018 ASCO/CAP Guidelines. '**HER2-ultralow**' has been proposed for tumors with an IHC score >no staining <1+ and may be used in the future if confirmed to be a targetable entity in the ongoing DESTINY-Breast06 (DB-06) trial. '**HER2-null**' has been proposed for a subset of HER2-0 tumors with no detectable HER2 staining in formalin-fixed paraffin-embedded tissue sections, and may be used to complement HER2-ultralow tumors, if these become an established entity. Beyond the use of the aforesaid denominations, reporting the HER2 IHC score is always encouraged. [III, C]

DISCUSSION: The emergence of the targetability of HER2-low expression raised the need to utilize novel definitions in clinical practice to interpret HER2 results and dissect different clinical entities within tumors lacking HER2 overexpression and/or *ERBB2* amplification. To avoid misinterpretations and confusion, consensus regarding these definitions is warranted.

The term '**HER2-negative**' has been used for more than two decades to define tumors lacking HER2 overexpression and/or *ERBB2* amplification ('negative' for the amplification of the gene and/or associated overexpression of the protein), thus *tumors with a IHC score of 0, 1+, or 2+/ISH not amplified*.[8] This is an established term, precisely defined in the 2018 ASCO/CAP Guidelines, and its definition should not be modified for the moment.

The term '**HER2-low**' was used in the NSABP B-47 trial,[12] and subsequently used in the practice-changing DB-04 trial,[19] for a subset of HER2-negative tumors, namely, *tumors with an HER2 IHC score of 1+ or 2+/ISH not amplified*. The FDA and the EMA used the definitions from the DB-04 trial in the approval of T-DXd for these tumors, and it is therefore reasonable to use it with the same definition. An equal alternative, which may avoid confusion in clinical decisions, is to use 'HER2-negative' accompanied by the IHC score in parenthesis [e.g. HER2-negative (IHC 1+)].

The term '**HER2-0**' has been recently suggested for *IHC 0 tumors according to the 2018 ASCO/CAP Guidelines,*[21] which include both tumors with no staining for HER2 and tumors with incomplete or faint/barely perceptible staining in ≤10% of tumor cells (definition simplified in DB-04 by removing 'barely perceptible'). It is reasonable to use the aforementioned simplification, used in DB-04, to describe HER2-0 tumors.

The term '**HER2-ultralow**' has been used to refer to a subset of HER2-0 tumors, namely, *tumors with an IHC score* >no staining <1+, which include tumors with incomplete or faint/barely perceptible staining in ≤10% of tumor cells. Patients having tumors with such minimal staining are included in the DB-06 trial, but no use of the term 'ultralow' is made in the study protocol. The decision to establish this intermediate category will be determined based on the results of the DB-06 trial.

The term '**HER2-null**' has been used to refer to a subset of HER2-0 tumors, namely, *tumors with no staining for HER2*. This definition is also found in the DB-06 trial protocol, and complements those tumors with a IHC score >no staining <1+ (HER2-ultralow). The decision to establish this term, complementary to the HER2-ultralow category, will be determined based on the results of the DB-06 trial.

Table 1 summarizes the conclusions regarding each pattern of HER2 IHC staining by the ASCO/CAP 2018 Guidelines and by the present 2023 ESMO Consensus document.

Beyond the use of the aforementioned denominations, reporting the IHC score is always encouraged in clinical practice, with a double denomination expected to increase the clarity of the definition. For instance, 'HER2-negative' or 'HER2-low' should be always accompanied by the specific IHC score [e.g. HER2-negative (IHC 0), HER2-negative (IHC 1+), HER2-low (IHC 1+), with the last two definitions being equivalent]. Similarly, it is always encouraged to include the definition when using the term HER2-ultralow [e.g. HER2-ultralow (IHC >no staining <1+)], HER2-0 [e.g. HER2-0 (IHC 0)], or HER2-null [e.g. HER2-null (no staining)].

Table 1. Interpretation by the ASCO/CAP 2018 Guidelines and by the 2023 ESMO Consensus on HER2-low breast cancer regarding each pattern of HER2 staining

Description of staining	Denomination by 2018 ASCO/CAP Guidelines	Conclusion by 2018 ASCO/CAP Guidelines	Conclusion by 2023 ESMO clinical practice recommendations
- No staining	HER2-0	HER2-negative	**HER2-0** *HER2-null*[a]
- Incomplete or faint staining in ≤10% of invasive tumor cells	HER2-0	HER2-negative	*HER2-ultralow (or >no staining <1+)*[a]
- Incomplete or faint staining in >10% of invasive tumor cells	HER2 1+	HER2-negative	**HER2-low**
- Weak to moderate complete membrane staining in >10% of invasive tumor cells (ISH-negative)	HER2 2+ nonamplified	HER2-negative	**HER2-low**
- Weak to moderate complete membrane staining in >10% of invasive tumor cells (ISH-positive)	HER2 2+ amplified	HER2-positive	**HER2-positive**
- Intense complete membrane staining in >10% of invasive tumor cells	HER2 3+	HER2-positive	**HER2-positive**

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; ISH, *in situ* hybridization.
Bold are the actual definitions. In italics are potential future sub-definitions within the HER2-0 category.
[a]The decision to establish the HER2-null and HER2-ultralow (or >no staining <1+) categories will be dependent on the results of the DB-06 trial.

Finally, the use of these definitions is not advocated in pathology reports (as further detailed later in this manuscript in statement 2.2), because the latter are meant to provide the results of the HER2 testing, rather than its clinical interpretation.

Level of consensus: 91% (n = 29) agree, 9% (n = 3) disagree, (n = 0) abstain.

QUESTION 2: Is HER2-low a distinct molecular entity, with a different biology compared with HER2-0 tumors?

STATEMENT: No substantial molecular differences have been demonstrated between HER2-low and HER2-0 tumors, after correcting for the expression of hormone receptors. Consequently, HER2-low should not be considered a distinct molecular entity, but rather a heterogeneous group of tumors, with biology primarily driven by the presence or absence of hormone receptor expression. [II, A]

DISCUSSION: Several studies have explored the biological features of HER2-low breast cancer, as defined by an IHC score of 1+ or 2+/ISH-negative.[10,22-24] The results from these studies show that HER2-low breast cancer represents a biologically heterogeneous group of tumors with low levels of immunoreactivity for HER2. Among HER2-low tumors, the key determinant of the gene expression profile is the expression of hormone receptors, with most hormone receptor-positive tumors being luminal A or B, and most hormone receptor negative-tumors being basal-like.[10,22] After correcting for hormone receptor expression, only marginal differences in gene expression are found between HER2-low and HER2-0 tumors,[10,22] highlighting how these entities are not substantially different in terms of biology. Similarly, large genomic studies have identified no specific and consistent difference in genomic profiles between HER2-low and HER2-0 tumors, after correcting for hormone receptor expression.[25-27]

Given these considerations, HER2-low disease, as currently defined, should not be considered a distinct molecular entity, but rather a heterogeneous group of tumors, with biology primarily driven by hormone receptor expression.

Level of consensus: 94% (n = 30) agree, 6% (n = 2) disagree, (n = 0) abstain.

QUESTION 3: What is the most likely mechanism of action leading to antitumor activity of anti-HER2 drugs in HER2-low breast cancer?

STATEMENT: To date, the only class of HER2-targeted drugs that has shown clinically meaningful activity in HER2-low breast cancer is represented by second-generation ADCs. The mechanism of action of anti-HER2 ADCs in HER2-low breast cancer is likely primarily related to the delivery of cytotoxic molecules, rather than the blockade of the HER2 pathway. [II, C]

DISCUSSION: Classic anti-HER2 drugs that disrupt the HER2 pathway (e.g. trastuzumab[12] and pertuzumab[28]) have failed to provide benefit in HER2-low breast cancer, including in the large randomized phase III NSABP-B47 trial that enrolled 3270 patients with HER2-low breast cancer, showing no benefit from adjuvant trastuzumab in this population.[12] By contrast, several novel ADCs achieved relevant antitumor activity in the HER2-low disease.[14-18] The anti-HER2 ADC trastuzumab duocarmazine (SYD985), which carries an alkylating payload (seco-DUBA), demonstrated an objective response rate (ORR) of 31% and a median progression-free survival (PFS) of ∼4 months among 47 patients with HER2-low metastatic breast cancer enrolled in a phase I trial.[15] Further testing of this agent for HER2-negative metastatic breast cancer is ongoing within the neoadjuvant I-SPY trial (NCT01042379). The anti-HER2 ADC disitamab vedotin (RC48-ADC), carrying a microtubule inhibitor as payload (monomethyl auristatin E), was tested in 48 Chinese patients with HER2-low metastatic breast cancer, achieving an ORR of 39.6% and a median PFS of 5.7 months.[16] A phase III trial is ongoing to compare the activity of disitamab vedotin with traditional chemotherapy in patients with chemotherapy-refractory, HER2-low metastatic breast cancer (NCT05831878). A different monomethyl auristatin E-based anti-HER2 ADC, MRG002, has also shown activity in a phase II study enrolling patients with advanced or metastatic HER2-low breast cancer ($n = 56$), with an ORR of 34.7% and a similar response rate in patients with IHC 2+ and 1+ tumors.[18] Finally, promising activity has been observed with the novel anti-HER2 ADC SHR-A1811, which similar to T-DXd carries a topoisomerase 1 inhibitor as payload (the exatecan derivative SHR9265). In a phase I trial enrolling 77 patients with HER2-low metastatic breast cancer, SHR-A1811 demonstrated an ORR of 49.4% and a 6-month PFS rate of 63.8%.[29] Further testing of SHR-A1811 is ongoing in a phase III trial that compares the activity of the conjugate with that of conventional chemotherapy among patients with chemotherapy-refractory or chemotherapy-naïve, HER2-low metastatic breast cancer (NCT05814354). The only anti-HER2 ADC for which randomized data are available in the HER2-low setting is however T-DXd, which was tested in the randomized phase III DB-04 trial and demonstrated to outperform physician's choice of chemotherapy,[19] rapidly becoming an approved treatment option for pretreated patients with HER2-low metastatic breast cancer.

Overall, given the little benefit observed in HER2-low breast cancer with naked anti-HER2 antibodies, and the promising activity observed with several ADCs, the presence of a cytotoxic payload appears to be key to achieve a clinically meaningful antitumor activity in the absence of HER2 amplification and/or overexpression. In line with this hypothesis, preclinical evidence suggested the key mechanism of action of T-DXd to be dependent on the targeted delivery of cytotoxic molecules, rather than the blockade of the HER2 pathway.[30,31] Nilsson and colleagues[32] have indeed recently shown that T-DXd-exposed tumor cell lines develop resistance through a reduced sensitivity to the topoisomerase 1 payload.

Further insights regarding the mechanism of action of T-DXd come from the DAISY phase II trial.[33,34] DAISY included chemotherapy pretreated patients with HER2-positive ($n = 68$), HER2-low ($n = 73$), and HER2-0 ($n = 38$) metastatic breast cancer treated with T-DXd. Meaningful response rates

were seen in all HER2 categories, with the highest rate in HER2-positive disease (71% HER2-positive, 38% HER2-low, and 30% HER2-0). PFS varied by HER2 category (11.1 months HER2-positive, 6.7 months HER2-low, 4.2 months HER2-0), although the small numbers impair definitive conclusions. Overall, this trial suggested that clinically meaningful activity with T-DXd can be achieved in both HER2-positive and HER2-low metastatic breast cancer, and that antitumor activity can be observed even in HER2-0 tumors. It is therefore still unclear whether HER2 expression is strictly required for T-DXd to be active, or whether other factors may be implicated in its activity against tumors lacking HER2 overexpression and/or *ERBB2* amplification.

Level of consensus: 97% (n = 31) agree, 3% (n = 1) disagree, (n = 0) abstain.

QUESTION 4: Is there sufficient evidence to attribute a prognostic value to HER2 expression within HER2-negative breast cancer?

STATEMENT: There is insufficient evidence to attribute a meaningful prognostic value to HER2-low expression, as currently defined (IHC 1+ or 2+/ISH-negative). Most studies carried out to elucidate this aspect have not identified significant survival differences between HER2-low and HER2-0 breast cancers, after adjusting for hormone receptor expression. [II, A]

DISCUSSION: Multiple large retrospective studies have been carried out to elucidate the prognostic value of HER2-low expression in breast cancer, both in the early-stage and in the metastatic settings.[10,21,22,24,35-46] Most of these studies have not identified a meaningful difference in OS between HER2-low and HER2-0 tumors, after adjusting for hormone receptor expression. The largest study conducted to date, which included over a million patients with HER2-negative breast cancer (n = 1 136 016) found marginal statistical differences in survival, with nearly overlapping Kaplan–Meier curves between HER2-low and HER2-0 tumors in all tumor stages.[45] Few additional studies have identified small differences in outcomes for HER2-low versus HER2-0 tumors:[21,47] these differences warrant further study, but given their inconsistency and small size, they remain not clinically meaningful at present.

Studies have been also conducted to elucidate whether HER2-low expression could impact the prognosis of patients receiving specific types of drugs [e.g. cyclin-dependent kinase 4/6 (CDK4/6) inhibitors[48-51]], with contradictory results, and overall insufficient evidence to support a meaningful prognostic value.

Level of consensus: 94% (n = 30) agree, 6% (n = 2) disagree, (n = 0) abstain.

QUESTION 5: Do higher IHC levels of HER2 predict a higher activity of HER2-targeted ADCs within traditionally HER2-negative breast cancer?

STATEMENT: The predictive value of HER2 IHC levels within traditionally HER2-negative disease remains unclear. Indeed, T-DXd has shown similar efficacy in patients with tumors scored HER2 IHC 1+ and 2+/ISH-negative, and antitumor activity has been even observed in patients with HER2-0 tumors. [II, C]

DISCUSSION: Several HER2-directed ADCs have demonstrated activity in HER2-low tumors (IHC 1+ or 2+/ISH-negative),[14-18] with T-DXd being approved to treat HER2-low metastatic breast cancer. However, no differential benefit has been established with these agents depending on the HER2 IHC score. For instance, in the phase I J101 trial, T-DXd had an ORR of 35.7% and 38.5% in patients with tumors scored IHC 1+ and 2+/ISH-negative, respectively.[14] Furthermore, in the BEGONIA phase Ib/II trial, first-line T-DXd + durvalumab achieved an ORR of 67.6% and 38.1% in patients with triple-negative tumors scored IHC 1+ and 2+/ISH-negative, respectively.[52] Lastly, in the DB-04 trial, T-DXd achieved a PFS of 10.3 and 10.1 months in patients with tumors scored IHC 1+ and 2+/ISH-negative, respectively.[19] Thus the specific IHC score within the HER2-low definition does not appear to impact the efficacy of T-DXd.

Neither T-DXd nor other anti-HER2 ADCs have been extensively analyzed in HER2-0 disease. The small phase II DAISY trial has, however, shown antitumor activity in this subset of patients, demonstrating an ORR 30% for patients with HER2-0 metastatic breast tumors.[34] The ongoing, prospective DB-06 phase III trial is investigating the role of T-DXd in patients with HER2-0 metastatic breast cancer with detectable HER2 staining (HER2-ultralow, i.e. HER2 IHC >no staining <1+, and is expected to shed some light on this topic.

ADCs targeting other antigens, such as the anti-Trop2 sacituzumab govitecan (SG), have been demonstrated to be active in traditionally HER2-negative breast cancer regardless of HER2 and Trop2 expression levels.[53]

Level of consensus: 97% (n = 31) agree, 3% (n = 1) disagree, (n = 0) abstain.

PATHOLOGIC DIAGNOSIS OF HER2-LOW BREAST CANCER

QUESTION 1: How should pathologists score HER2-low expression?

STATEMENT: Pathologists should score HER2-low expression using the ASCO/CAP 2018 algorithm, with recommendation of the simplification utilized in the DB-04 trial, namely, the use of 'faint' rather than 'faint/barely perceptible' for the definition of 0 and 1+. Results from ongoing clinical trials may lead to further evolutions of this algorithm in the future. [III, C]

DISCUSSION: In the DB-04 trial, the IHC assay utilized to test for HER2 is identical with the commercially available PathwayHER2 4B5 assay, but the interpretation slightly differed from the ASCO/CAP Guidelines.[19] The simplification of scoring in DB-04 consisted in replacing 'faint/barely perceptible' staining with 'faint' in the definition of 0 and 1+. This study clinically validated HER2 as a biomarker using

the PathwayHER2 assay, as well as the algorithm that was utilized. Therefore it is reasonable to keep using the ASCO/CAP 2018 algorithm[8] to score HER2, with recommendation of the simplification utilized in the DB-04 trial.[9]

Importantly, given that ongoing trials (e.g. DB-06) are evaluating the role of T-DXd beyond canonically defined HER2-low disease (i.e. in tumors scored IHC >no staining <1+), further evolutions of current HER2 testing algorithms may occur in the years to come.

In addition, given some suggestion from the DAISY phase II trial of a reduced activity of T-DXd among tumors with significant HER2 heterogeneity (i.e. a high percentage of HER2 IHC 0 cells and their spatial distribution),[34] exploratory studies evaluating the predictive value of HER2 intratumoral heterogeneity and its spatial pattern (whether it is clustered or scattered i.e. closely intermingled) are warranted.

Level of consensus: 90% (n = 26) agree, 10% (n = 3) disagree, (n = 3) abstain.

QUESTION 2: How should pathologists report HER2-low expression in pathology reports?

STATEMENT: In reporting HER2 testing results, pathologists should maintain a nomenclature consistent with the ASCO/CAP 2018 algorithm. The HER2 IHC score (0, 1+, 2+, or 3+) should always be included in the report. This, in turn, allows clinicians to determine whether the case can be considered eligible for T-DXd, or for trials of other agents targeting HER2-low expression. The use of the term 'HER2-low' is not preferable in the pathology report, whereas its use is justified in clinical practice as an interpretation of the HER2 status of the disease. [III, C]

DISCUSSION: The ASCO/CAP Guidelines are utilized worldwide and have allowed for a consistent reporting of HER2 status in pathology reports during the past 15 years. Moreover, reports that adopt these guidelines allow clinicians to discern those tumors that are HER2-low, if the HER2 IHC score (and ISH amplification status, when appropriate) is reported. This permits identification of those patients that are eligible for T-DXd or other agents targeting HER2-low expression (namely, patients with tumors exhibiting an IHC score of 1+ or 2+/ISH not amplified), despite being traditionally defined HER2-negative per the ASCO/CAP Guidelines.[8]

Given the aforementioned considerations, when reporting HER2 results in pathology reports, it is important to be consistent with the ASCO/CAP 2018 algorithm,[8] with attention to always include the specific IHC score that allows to discern whether the tumor is HER2-low, and with recommendation of the simplification utilized in the DB-04 trial,[9] namely, the use of 'faint' rather than 'faint/barely perceptible' for the definition of IHC 0 and 1+.

The use of the term 'HER2-low' in the pathology report is not expected to add clinically relevant information that cannot be derived from the IHC score itself. Its use is therefore not preferable in pathology reports, unless it is strongly felt by the pathologist to be required for specific reimbursement or clinical reasons. It is instead reasonable to use the term 'HER2-low' elsewhere (e.g. clinical notes, trial protocols, scientific publications) as an interpretation of the HER2 status of the disease.

Level of consensus: 100% (n = 30 agree), 0% (n = 0) disagree, (n = 2 abstain).

QUESTION 3: Can all validated HER2 assays be used interchangeably to identify HER 0, 1+, or HER2 2+/ISH not amplified breast cancer?

STATEMENT: Use of a validated HER2 assay according to established international guidelines is recommended to identify HER2 0, 1+, or HER2 2+/ISH not amplified breast cancer. Adequate assays include the Pathway 4B5 assay (used in the DB-04 trial) or any other validated assay that the pathologists considered appropriate after weighing available evidence and practical considerations. [III, C]

DISCUSSION: In the DB-04 trial, patients were enrolled based on the IHC results obtained with the Pathway 4B5 assay, using the modified ASCO/CAP 2018 Guidelines.[9] It is unclear whether selection of the patients using another test would have resulted in a similar, lower, or higher clinical benefit.

There are several other IHC assays available and used by the pathology community, namely, the historical HercepTest pAb (Autostainer, SK001); the recently launched HercepTest™ mAb pharmDx (Dako Omnis, GE001, using monoclonal DG44 antibody); the Bond™ Oracle HER2 IHC system (Leica Biosystems) using the Mouse CB11 antibody/clone; the InSite® HER2-neu from Biogenex Laboratories, which also uses the mouse CB11 antibody/clone, among others. According to the data from proficiency testing, such as NordiQC,[54] in daily practice, pathology laboratories use a variety of assays, including laboratory-developed tests, that can be used interchangeably with success for the detection of HER2-positive breast cancer (i.e. 3+ and 2+/ISH amplified).

Two studies addressed the issue of different assays with regard to concordance in the detection of HER2-low expression: (i) HercepTest pAb Autostainer SK001 versus 4B5 Ventana Pathway[55] and (ii) HercepTest pAb (Autostainer,SK001) versus HercepTest™ mAb pharmDx (Dako Omnis, GE001) versus 4B5 Ventana Pathway.[56] 4B5 identified a higher proportion of HER2-low cases than Herceptest SK001. By contrast, HercepTest GE 001 picked up more HER2-low cases than 4B5. Of note, a more sensitive test is not necessarily more predictive of response to the therapy.

Ultimately, the pathologist has the responsibility to weigh available evidence with local practical considerations to decide on the appropriate use of HER2 assays, and be held accountable for their choice. Participation in external quality assessment (EQA) programs and addition of controls or reference materials calibrated with low-intensity staining will help in the harmonization of the different assays and laboratory-developed tests.

Level of consensus: 93% (n = 28) agree, 7% (n = 2) disagree, (n = 2) abstain.

QUESTION 4: How should pathologists handle cases that are on the borderline between 0 and 1+?

STATEMENT: There is currently insufficient evidence to recommend how best to categorize cases that are not clearly either HER2-0 or HER2 1+ by IHC. At present, cases that are on the borderline between HER2-0 and HER2 1+ scores should be examined at high-power magnification, reviewed by at least one other pathologist, and be categorized as best as possible as either HER2-0 or HER2 1+ using the current ASCO/CAP Guidelines. Further evidence may clarify in the future the clinical role of these borderline levels of HER2 IHC expression. [III, C]

DISCUSSION: The ASCO/CAP HER2 Clinical Practice Guidelines provide definitions of HER2-0 and HER2 1+ staining, but do not provide recommendations regarding the interpretation of cases that do not clearly fulfill the definition of either HER2-0 or HER2 1+.[8] Further, there is no reflex testing that can be done to resolve this issue as there is for HER2 2+ (equivocal) cases, where HER2 ISH is used to clarify the HER2 status.

How best to categorize ambiguous cases is an unresolved issue and until recently has been a moot point since both HER2-0 and HER2 1+ cases were considered HER2-negative. While this distinction is now important in the era of novel anti-HER2 ADCs, it is in many ways more of a clinical question than a pathology question, that is, is it more clinically appropriate to err on the side of overcalling a case HER2 1+ to ensure that the patient receives potentially beneficial therapy or to err on the side of undercalling a case HER2-0 with the possibility that the patient may be denied a treatment from which she or he may benefit?

Notwithstanding the aforesaid issues, a number of practices could help in the interpretation of borderline cases including (i) examining HER2 IHC at high-power magnification; (ii) considering review by a second pathologist; (iii) using controls with a range of protein expression including 1+ cases; and (iv) paying careful attention to preanalytic factors.

Of note, data from the phase II DAISY trial highlighted antitumor activity of T-DXd even in patients with HER2-0 disease.[34] If this activity is confirmed, the distinction between HER2-0 and HER2 1+ may again become moot. In addition, the targetability of ultralow (i.e. IHC >no staining <1+) tumors is currently under investigation in the DB-06 phase III trial. If HER2 ultralow expression confirms to be targetable, one could expect T-DXd indication to expand to all HER2 not amplified cases, except those patients whose tumors have no IHC expression at all (HER2-null).

Overall, while it remains critical to develop practices to improve the concordance in distinguishing low HER2 scores,[57] equal attention needs to be dedicated to understanding the lower end of the targetability of HER2 expression with novel anti-HER2 ADCs.

Level of consensus: 94% (n = 30) agree, 6% (n = 2) disagree, (n = 0) abstain.

QUESTION 5: What additional education and training do pathologists require to report HER2-low breast cancer?

STATEMENT: Pathologists should participate in focused education and training programs on how to report HER2-low breast cancer according to the latest ASCO/CAP Guidelines. [III, C]

DISCUSSION: Given the demonstrated activity of anti-HER2 ADCs in HER2-low breast cancer, it is extremely important to raise awareness on this topic among pathologists reporting HER2 for breast cancer, and to stress the clinical relevance of the distinction between 0 and 1+ staining.

Data are emerging regarding the beneficial impact of pathologist training in reproducibly reporting HER2-low expression. An international study was carried out among 77 pathologists from 14 different countries; the participants were asked to interpret HER2 expression from two whole-slide imaging sample sets of representative study cases. A second round of scoring occurred after a 4-h virtual training session based on the ASCO/CAP 2018 Guideline with additional practical considerations. Among the samples stained with the Ventana 4B5 assay, the concordance rates for HER2-0 improved from 74.6% at baseline to 89.2% after the training session ($P < 0.001$).[58]

Provision of scoring guidelines with definition of 1+ staining, along with visual aids, live lectures, and web-based training including challenging cases is warranted to enable pathologists to accurately identify and report HER2-low breast cancer. Pathologists can include this as part of their continuing professional development.

These efforts will require to be adapted to the evolving landscape of HER2-targeting strategies, including the potential evidence of a clinical role for ultralow >no staining <1+ HER2 IHC expression, and additional evidence that may emerge in the future in this field.

Level of consensus: 94% (n = 30) agree, 6% (n = 2) disagree, (n = 0) abstain.

QUESTION 6: What is the role of external quality assurance programs in the setting of HER2 staining?

STATEMENT: EQA programs can improve the accuracy and reproducibility of HER2 scoring for breast cancer. To achieve these tasks, the formal inclusion of the IHC 1+ category is warranted in EQA programs. [III, C]

DISCUSSION: There are currently several EQA programs for assessment of HER2 IHC such as UK NEQAS, NordiQC, AFAQAP, and QuIP. The UK NEQAS test slide currently includes cell line controls including all levels of HER2 expression (0, 1+, 2+, and 3+), although it is not presently a requirement for laboratories to include in-house 1+ control material. The AFAQAP test slides now include 0, 1+, and 2+/ISH not amplified tumor cases along with 2+/ISH-amplified and 3+. For accurate and reproducible identification of HER2-low breast cancer, the 1+ category needs to be formally included and assessed in EQA programs. This will also generate real-world data on performance of different HER2 antibodies across a variety of automated IHC staining platforms. In some countries participation in HER2 EQA is mandatory as part of the breast screening program,

breast cancer center certification, and quality assurance requirements (ISO 15189, ISO17020); however, even in countries where this is not the case, participation in HER2 EQA including HER2-low should be encouraged.

Formal QA is needed to ensure tests are sensitive enough to pick up low levels of expression while not reducing specificity for identification of amplified tumors.

Level of consensus: 94% (n = 29) agree, 6% (n = 2) disagree, (n = 1) abstain.

QUESTION 7: What technologies may improve the quantitative detection of HER2-low expression in breast cancer?

STATEMENT: Several novel technologies are being developed to obtain a more quantitative and reproducible detection of HER2 in the low range compared with standard IHC/ISH testing. No alternative technology for the assessment of HER2-low expression is, however, ready for use in clinical practice in the current absence of clinical validation and/or utility. [II, C]

DISCUSSION: Research technologies such as the AQUA™ method of quantitative immunofluorescence have been designed to test a range of antibody concentrations to maximize the sensitivity within the lower range of HER2 expression.[59] Of note, the study by Moutafi et al.[59] relied heavily on tissue microarrays that do not replicate clinical specimens and are not affected by heterogeneity of clinical specimens. In addition, tissues from patients treated with HER2-targeted ADCs were not tested, thus clinical validation of the assay remains pending.

Deep learning-based image analysis was used to quantitate HER2 and generate a quantitative continuous score (QCS) for 151 patients with metastatic breast cancer with varying levels of HER2 expression enrolled in the J101 phase I trial of T-DXd.[60] Of the 65 patients identified as having HER2-low tumors by HER2 IHC scoring, 42% of patients treated with T-DXd experienced a response in the trial and the median PFS was 11 months. When investigators used HER2 QCS, the same population was stratified into QCS-high and a QCS-low subgroup. The HER2 QCS-high subgroup included 21 of the 27 responders in the study, and as a result the response rate increased to 53%, with a median PFS of 14.5 months. The QCS-low subgroup instead had a response rate of 24% and a median PFS of 8.6 months.[60]

Beyond developing quantitative assays with a broader range of detection, a key unmet need remains the improvement of concordance in HER2 scoring among pathologists. In this setting, artificial intelligence (AI)-powered HER2 analysis may help achieve consistent HER2 expression level evaluation in breast cancer by reducing interobserver variability in HER2-low cases.[61] In the study by Jung and colleagues,[61] concordance between observers using manual scoring was 25.7% in 1+ and 45.8% in 2+ ($\kappa = 0.242$ and 0.475, respectively), whereas the use of AI-based interpretation increased concordance to 68.9% in 1+ and 68.8% in 2+ ($\kappa = 0.687$ and 0.712, respectively). Similarly, Wu and colleagues[62] have conducted a study comparing the performance of 15 pathologists at scoring HER2 IHC slides with and without AI assistance, demonstrating a meaningful advantage in terms of accuracy and consistency when AI algorithms were adopted. Despite these technologies being highly promising, there are several bottlenecks to the development of AI-powered HER2 analyzers: digitalization of pathology departments is only starting in many countries and the performance of different AI-powered HER2 analyzers on the same set of samples is still an unresolved issue. Overall, the development of AI-dedicated solutions will need to be considered a high priority in the future.

Level of consensus: 100% (n = 31) agree, 0% (n = 0) disagree, (n = 1) abstain.

MANAGEMENT OF HER2-LOW METASTATIC BREAST CANCER

QUESTION 1: In order to select patients with breast cancer for treatment with T-DXd, should HER2-low expression be determined based on the most recent tumor biopsy, or on any tumor biopsy during the course of disease?

STATEMENT: Treatment with T-DXd can be based on HER2-low status from the primary tumor or at any point in the metastatic setting. In cases of HER2-0-only status throughout the disease history, a repeated biopsy is suggested to reevaluate the HER2 status. [I, B]

DISCUSSION: Based on the currently available data from the pivotal DB-04 study,[19] patients' inclusion was based on the most recent available tumor tissue to centrally determine HER2-low status. HER2-low status was therefore based on a case mix of primary tumor and metastatic tissue. Approximately 90% of tumor samples centrally tested in DB-04 were from archival tumor tissue, with only 10% being from newly obtained biopsies prior to starting protocol therapy; 35% of tumor samples were primary tumor samples, with the rest from the metastatic setting. In addition, ~50% of samples were from 2019 (the year the study began enrollment) or earlier. Efficacy of T-DXd compared with treatment of physician's choice was consistent regardless of tumor sample characteristics.[63]

A challenge with determination of HER2-low status is that it can be dynamic. Miglietta et al.[64] reported a change in HER2 status in 26.4% of cases between baseline core biopsies prior to preoperative systemic therapy and residual disease from the time of surgery, mostly driven by HER2-0 cases converting either from (14.8%) or to (8.9%) HER2-low-positive phenotype. The same team reported a 38% overall rate of HER2 discordance between primary tumor and relapse in a series of 547 cases, mostly represented by HER2-0 switching to HER2-low (15%) and HER2-low switching to HER2-0 (14%).[65] Tarantino et al.[66] evaluated the evolution of HER2 expression between the primary tumor and the first biopsy collected in the advanced setting in 232 patients. Among the overall population, 44% of the HER2-0 primary tumors had an increase in HER2 score at the time of recurrence, and 22% of

HER2-low primary tumors became HER2-0. Another challenge can be heterogeneity in HER2-low status even at the same timepoint. A rapid autopsy series looking at tissue from different locations found that HER2 status was highly variable even within an individual patient, with 80% of patients having both HER2-low and HER2-0 metastases analyzed with the HercepTest.[67]

Thus HER2-low expression appears highly unstable during disease evolution for reasons that could be related to temporal heterogeneity, spatial heterogeneity, and (pre) analytic aspects, among others. Repeat biopsy in case of a HER2-0 tumor may open new therapeutic opportunities in a relevant proportion of patients. In cases where there is a HER2-low result at one timepoint and a HER2-0 result at a different timepoint, treatment with T-DXd can be considered, as long as there was a HER2-low result at some point in time in the disease course. In addition, in a case where a patient has tumor samples obtained at the same point in time that are discordant (i.e. one is HER2-0 and the other HER2 low), consideration of therapy with T-DXd should be made given the survival benefit that could be obtained.

Level of consensus: 97% (n = 30) agree, 3% (n = 1) disagree, (n = 1) abstain.

QUESTION 2: What is the best position for T-DXd in the treatment of HER2-low metastatic hormone receptor positive breast cancer in clinical practice?

STATEMENT: Patients with HER2-low (IHC 1+ or IHC 2+/ISH-negative), hormone receptor-positive metastatic breast cancer who have received prior CDK4/6 inhibitor therapy and at least one previous line of chemotherapy (or have experienced progression within 6 months of [neo]adjuvant chemotherapy) and are considered to have endocrine refractory disease are candidates for T-DXd if they do not have contraindications. In cases where both T-DXd and SG are available options, T-DXd should be prioritized, given that it was studied in a less pretreated population of patients. [II, A]

DISCUSSION: In the DB-04 trial, 70% of patients with hormone receptor-positive disease had previously received a CDK4/6 inhibitor, and a subgroup analysis suggested that patients derived benefit from T-DXd regardless of previous treatment with a CDK4/6 inhibitor.[19] Given the demonstrated survival advantage,[68] treatment with a CDK4/6 inhibitor in any setting is recommended prior to receiving T-DXd, in regions where available. Subgroup analysis in DB-04 also showed similar hazard ratio (HR) for disease progression or death in patients who had received one versus two lines of chemotherapy. Therefore patients should have at least received one line of chemotherapy in the metastatic setting, with the exception of patients experiencing recurrence on or within 6 months of (neo)adjuvant chemotherapy, prior to receiving T-DXd.

Currently, there are no available data with respect to the optimal selection strategy regarding the use of T-DXd and SG, in pretreated patients with HER2-negative, hormone receptor-positive metastatic breast cancer. However, recent studies support the activity of both agents in this patient population. The DB-04 trial clearly demonstrated the efficacy of T-DXd in pretreated patients with HER2-low metastatic breast cancer.[19] Moreover, the TROPiCS-02 study showed the activity of SG in patients with HER2-negative, hormone receptor-positive disease who had received two to four lines of chemotherapy in the metastatic setting.[69] Furthermore, a *post hoc* subgroup analysis by HER2 IHC status in the TROPiCS-02 trial demonstrated efficacy for SG, in both HER2-low and HER2-0 groups.[53] The HR of 0.58 with SG in HER2-low disease was similar to the HR of 0.51 with T-DXd in the DB-04 trial, although achieved in a more advanced setting.

Based on the currently available data in pretreated patients with HER2-negative, hormone receptor-positive metastatic breast cancer, T-DXd is the standard of care, while SG is another valid option in cases with HER2-low disease. Although there is some suggestion of activity of T-DXd even in HER2-0 metastatic breast cancer,[34] SG is currently considered the only ADC for HER2-0 disease. There is a greater body of evidence for use of T-DXd in a less pretreated population (one to two prior lines of chemotherapy), whereas SG was tested among patients who had received two to four prior lines of chemotherapy in the metastatic setting, making the preference for use of T-DXd in an earlier line of therapy.

Importantly, data regarding the safety and activity of SG in patients with metastatic breast cancer who have already received an ADC with a different target, such as T-DXd, are lacking. However, there is no biological rationale to suggest that the administration of SG following previous treatment with T-DXd would be either inefficient or unsafe. Consequently, previous treatment with T-DXd should not be considered a contraindication for treatment with SG.

Level of consensus: 90% (n = 27) agree, 10% (n = 3) disagree, (n = 2) abstain.

QUESTION 3: What is the best position for T-DXd in the treatment of HER2-low metastatic triple-negative breast cancer?

STATEMENT: For the treatment of patients with metastatic HER2-low triple-negative breast cancer (TNBC), evidence at this time is more robust for SG given prior to T-DXd, thus SG should be considered first, whereas T-DXd may be considered after SG. [II, B]

DISCUSSION: While the primary objective of DB-04 was comparison of PFS in patients with metastatic hormone receptor-positive breast cancer ($n = 494$), the trial also included a cohort of 58 patients with hormone receptor-negative HER2-low metastatic breast cancer (i.e. TNBC). In the TNBC cohort, patients treated with T-DXd, compared with physician's choice chemotherapy, had improved PFS [HR = 0.46, 95% confidence interval (CI) 0.24-0.89] and OS (HR = 0.48, 95% CI 0.24-0.85). Given the exploratory endpoint, there was no *P* value assigned to results. However, this group was included in the full analysis population of the trial where the results were again statistically

significant in favor of T-DXd for PFS and OS, hence the overall trial results led to FDA approval of T-DXd for patients with metastatic HER2-low TNBC who have received at least one prior line of chemotherapy. Noteworthy, patients with metastatic HER2-low TNBC were also enrolled in both the DAISY phase II trial[34] and the BEGONIA phase Ib trial,[52] demonstrating encouraging response rates and further supporting that activity of T-DXd can be observed in HER2-low breast cancer, regardless of the presence or not of hormone receptor expression.

SG is also approved as second-line and beyond therapy for patients with metastatic TNBC based on improvement in both PFS (HR = 0.41, 95% CI 0.32-0.52; $P < 0.0001$) and OS (HR = 0.48, 95% CI 0.38-0.59; $P < 0.0001$) seen in the phase III ASCENT clinical trial ($n = 529$). While there are no randomized data directly comparing SG with T-DXd, there is a higher level of evidence for SG (compared with T-DXd) in metastatic TNBC, as evaluation in this disease was the primary objective of the ASCENT trial. If SG is chosen first, T-DXd could be given after, although prospective data supporting ADC sequencing are currently lacking and subject to ongoing investigation.

Level of consensus: 93% (n = 27) agree, 7% (n = 2) disagree, (n = 3) abstain.

QUESTION 4: What is the recommendation for prevention and management of nausea and vomiting when utilizing T-DXd?

STATEMENT: For patients receiving T-DXd, the use of a prophylactic antiemetic regimen including a 5-HT3 receptor antagonist, dexamethasone, and a neurokinin-1 receptor antagonist is recommended. [II, A]

DISCUSSION: Gastrointestinal toxicities are among the most common toxicities with T-DXd, with nausea occurring in 73% of patients in DB-04, and vomiting occurring in 34%.[19] These rates are consistent with what were observed in the HER2-positive disease, with a rate of any-grade nausea of 73% and 77% observed in the DESTINY-Breast02 and DESTINY-Breast03 phase III trials, respectively.[70,71] Similar rates were reported in a consensus regarding the real-world emetogenicity of T-DXd, with up to 65% of the patients noticed to experience nausea and vomiting after the first T-DXd cycle.[72] Based on these high rates of emetogenicity, even in the presence of two-drug prophylactic regimens (e.g. dexamethasone and a 5-HT3 receptor antagonist), T-DXd is currently considered a highly emetogenic drug by the latest NCCN Guidelines,[73] warranting a routine antiemetic prophylaxis with a three-drug regimen.

Pretreatment with a 5-HT3 receptor antagonist and dexamethasone with a neurokinin-1 receptor antagonist is therefore recommended. Delayed nausea prophylaxis with dexamethasone on days 2-3 is also recommended. Use of olanzapine, according to local guidelines, can further help in the prevention of nausea and vomiting.

If a patient experiences grade 3-4 nausea/vomiting, T-DXd should be held until toxicity resolves to grade 1. If it resolves within 7 days, the same dose can be continued; however, if it takes longer than 7 days, a dose reduction is recommended.

Level of consensus: 100% (n = 26) agree, 0% (n = 0) disagree, (n = 6) abstain.

QUESTION 5: What is the recommendation for monitoring and management of interstitial lung disease (ILD)/pneumonitis when utilizing T-DXd?

STATEMENT: For patients receiving T-DXd, routine monitoring for ILD/pneumonitis with computed tomography (CT) scans every 6-12 weeks is recommended. Management of ILD/pneumonitis should follow the guidelines included in the T-DXd label and in dedicated scientific publications. [II, A]

DISCUSSION: The pathophysiology behind T-DXd-related lung toxicity is currently unknown. Studies testing T-DXd have excluded enrollment of patients with a history of noninfectious ILD requiring steroid therapy or patients with evidence of ILD or inability to exclude ILD on current scans. Overall studies of T-DXd in breast cancer have demonstrated ILD rates of 12%-15%, with most events being grades 1 and 2;[74] however, there have been rare cases of fatal ILD. The incidence of high-grade events has decreased from the initial trials, although the grade 5 event rate remains ~1%. Hence it is critical to actively monitor patients for lung toxicity while on T-DXd therapy.[75]

In trials testing T-DXd, patients with breast cancer were monitored with CT scans every 6 weeks and clinically for the development of respiratory symptoms. Based on the best available data and experience from clinical studies the following are concluded:

- Patients with a history of noninfectious ILD requiring treatment (i.e. steroids) are considered high risk for recurrent ILD. These patients should not be considered candidates for T-DXd therapy today. This includes patients with a prior history of clinically significant pneumonitis from immune checkpoint inhibitors, everolimus, and other anticancer therapies. The decision to offer T-DXd to advanced stage patients without other good treatment options in such settings must be individualized, weighing risks and benefits and with transparent discussions regarding risk of fatal lung toxicity.
- Patients with presence of lung comorbidities, moderate/severe baseline renal dysfunction, and baseline oxygen saturation <95% are at a higher risk for ILD[74] and the decision to offer T-DXd in this setting must be individualized, weighing risks and benefits and with transparent discussions regarding risk of fatal lung toxicity.
- Monitoring for lung toxicity starts with educating patients regarding this risk and actively monitoring for respiratory symptoms including the development or worsening of cough, shortness of breath, or fever. In addition, CT scans of the chest should be carried out routinely during treatment at intervals of 6-12 weeks; CT scan of the chest should also be done if patients present with respiratory symptoms.

- With any suspicion of lung toxicity, T-DXd therapy must be suspended.
- Guidelines for the approach and management of ILD from T-DXd are widely available in the T-DXd label and in print at Swain et al.[76] These include a work-up to investigate etiology; if there is suspicion for ILD, then prompt introduction of steroids is advised.
- Only in cases of asymptomatic grade 1 ILD that has fully resolved, can one consider re-challenge with T-DXd therapy. Patients with symptomatic ILD (i.e. grade 2 or higher), even in cases of full recovery, are not considered safe for rechallenge with T-DXd at this time.

Level of consensus: 100% (n = 28) agree, 0% (n = 0) disagree, (n = 4) abstain.

DESIGN OF CLINICAL TRIALS FOR HER2-LOW BREAST CANCER

QUESTION 1: Should future clinical trials for HER2-low disease expand enrollment to patients with HER2-0 tumors?

STATEMENT: Patients with HER2-0 disease should be eligible for randomized controlled clinical trials of T-DXd and potentially other novel HER2-directed ADCs. The efficacy analysis in the population with HER2-0 cancers should be part of a preplanned, adequately powered controlled and adjusted subgroup analysis of trials for HER2-low breast cancer. [III, A]

DISCUSSION: A potential role for novel HER2-directed ADCs in HER2-0 breast cancer has been suggested in the phase II DAISY trial, which identified a response rate of 30% among 37 patients with HER2-0 breast cancer treated with T-DXd. However, the trial also showed that HER2-0 expression may be associated with a lower uptake of T-DXd, yielding lower clinical responses.[34]

It is still unclear if such a pharmacodynamic difference will result in a different benefit of T-DXd in larger patient populations with HER2-0 cancers, and to what extent patients with this subset of tumor will derive more benefit from T-DXd than standard treatments. Therefore, the evaluation of efficacy in this setting is a priority, and T-DXd and new HER2-directed ADCs should be tested in the HER2-0 population. Patients with HER2-0 breast cancer can be enrolled in clinical trials of novel anti-HER2 ADCs, ensuring appropriate stratification for HER2 expression and statistical power to demonstrate separately the clinically meaningful benefit of new ADCs in the HER2-0 versus HER2-low populations, respectively.

Level of consensus: 94% (n = 30) agree, 6% (n = 2) disagree, (n = 0) abstain.

QUESTION 2: Should central testing be required in future trials of anti-HER2 agents for HER2-low disease?

STATEMENT: Central HER2-low testing based on IHC should be preferred in the design of pivotal clinical trials for HER2-low breast cancer, especially if intended to support regulatory approval of new HER2-targeting drugs. Local assessment is acceptable, but only within a strict quality control framework ensuring adequate, reliable testing based on validated assays so that the trial results can be extrapolated to the real-world setting where a variety of assays are used. [III, B]

DISCUSSION: Central testing of HER2 status can be important to report consistent findings in clinical trials. Based on the discrepancy observed with the evaluation of HER2-0 and HER2 1+ among pathologists in several studies, centralized testing could consolidate the practice and reduce the variabilities associated with local testing.

It must be noted, however, that quality accreditation of pathology laboratories has demonstrated to improve the consistency of the evaluations and reduce the divergences with the centralized assessments.[77] As a result, quality-accredited local laboratories may be considered for clinical trial testing.[78] Improving the quality of local testing and operationalizing the local testing policy in the context of large clinical trials can portend efficiency and yield better extrapolation of the results for the global pathology community.[79] Eventually, some regulatory bodies are now emphasizing more the opportunity for local testing in clinical trials,[80] albeit within a strict quality framework, ensuring that local testing is valid enough for the safe inclusion of patients in trials. Overall, central testing remains critical, but local testing is also an option to consider in future clinical trials, especially for diagnostics routinely used in the clinical practice.

It is endorsed that, whenever possible, clinical trial sponsors should make the tissue specimens available for secondary analysis based on new technologies and innovative tests, to evaluate analytical and clinical validity. New tests may be helpful to improve patient selection and identify patients who can derive the largest benefits from T-DXd and other HER2-directed compounds.

Level of consensus: 97% (n = 30) agree, 3% (n = 1) disagree, (n = 1) abstain.

QUESTION 3: Should distinct trials be designed for HER2-low breast cancer depending on hormone receptor expression?

STATEMENT: Clinical trials for HER2-low breast cancer should be appropriately powered for hormone receptor-positive and hormone receptor-negative (i.e. triple-negative) breast cancer, in the context of dedicated clinical trials or as part of appropriately stratified populations, in studies powered for the comparison of the subgroups based on the hormone receptor expression. [III, A]

DISCUSSION: Clinical trials including patients with HER2-low breast cancer must ensure that the prognostic variables of major interest are properly balanced across the arms, including hormone receptor status. In the DB-04 study,[19] the activity of T-DXd was reported primarily among the population of patients with hormone receptor-positive breast cancer and explored in a small subset of 58 patients with hormone receptor-negative (i.e. triple-

negative) breast cancer. Future clinical trials should account comprehensively for the population with HER2-low breast cancer and consider separate trials for triple-negative HER2-low breast cancer, or larger investigations, that are properly stratified for hormone receptors, and powered for comparisons of the populations with hormone receptor-positive cancer and TNBC. These groups are in fact prognostically heterogeneous, and managed in clinical practice based on specific, separate treatment algorithms, resulting in special needs and areas of possible improvement that are not necessarily common. Although this is expected to come with challenges, in terms of sample size and trial complexity, they would also provide more informative data to inform decisions in clinical practice.

Overall, clinical trials should include statistical hypothesis for hormone receptor-positive cancer and TNBC, to reduce uncertainties and improve the quality of the evidence. It is suggested that hormone receptor stratification and powered subgroup analysis may be less relevant for patients with advanced cancer pretreated with numerous lines of therapy, where the standard treatments available portend very limited benefits and the overall prognosis is dismal. However, we endorse the use of high-quality clinical trial designs and appropriate stratification for hormone receptor status.

Level of consensus: 100% ($n = 32$) agree, 0% ($n = 0$) disagree, ($n = 0$) abstain.

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DISCLOSURE

PT has served as a consultant/advisor for AstraZeneca, Daiichi Sankyo, Eli Lilly, and Gilead. GV declares being on the advisory board of AstraZeneca, Daiichi Sankyo, MSD Oncology, Pfizer, and Roche; reports receiving consulting fees from Agilent; is a coordinating PI for AstraZeneca; and reports an institutional research grant from Roche. MFP declares advisory board membership for Biocartis, Cepheid, and Lilly USA; reports a consultant role for AstraZeneca, Eli Lilly & Company, Merck, Novartis, and Zymeworks; reports ownership interest in TORL Biotherapeutics; reports fee-for-service agreements from 1200 Pharma, Ambrx, TORL Biotherapeutics, TRIO, TRIO-US, and Zymeworks; and was supported in part by grants from the Breast Cancer Research Foundation and Dr. Miriam & Sheldon G. Adelson Medical Research Foundation, a gift from Dr. Richard Balch, and an endowed chair, the Harold E. Lee Chair for Cancer Research for the USC/Norris Comprehensive Cancer Center. XH declares speaker honoraria from AstraZeneca, Novartis, Pfizer, and Roche. FPL declares speaker honoraria from and/or being on the advisory board of AbbVie, Agendia, AMGEN, Astellas, AstraZeneca, Bayer, BMS, Daiichi Sankyo, Eisai, Exact Science, Gilead, GSK, Illumina, Janssen, Lilly, MSD, Novartis, Pfizer, Pierre Fabre, Roche, Sanofi, Seagen, and Servier; institutional research grant from Bayer and NanoString; and being a local PI for Daiichi Sankyo. ABar declares being on the advisory board of Pfizer, Novartis, Genentech, Merck, Radius Health/Menarini, Immunomedics/Gilead, Sanofi, Daiichi Pharma/Astra Zeneca, Phillips, Eli Lilly, Mersana, and Foundation Medicine; royalties from UpToDate; being a coordinating PI for Genentech, Novartis, Pfizer, Merck, Sanofi, Radius Health/Menarini, Immunomedics/Gilead, Daiichi Pharma/Astra Zeneca, and Eli Lilly. ABat declares speaker honoraria from and/or being on the advisory board of Astellas, ASTRA, MSD, and Roche. LAC declares institutional research funding from AstraZeneca, Guardant, NanoString Technologies, Novartis, Roche, Seagen, and Veracyte. JC declares consulting/advisory for Roche, Celgene, Cellestia, AstraZeneca, Seattle Genetics, Daiichi Sankyo, Erytech, Athenex, Polyphor, Lilly, Merck Sharp & Dohme, GSK, Leuko, Bioasis, Clovis Oncology, Boehringer Ingelheim, Ellipses, HiberCell, BioInvent, GEMoaB, Gilead, Menarini, Zymeworks, Reveal Genomics, and Expres2ion Biotechnologies; honoraria from Roche, Novartis, Celgene, Eisai, Pfizer, Samsung Bioepis, Lilly, Merck Sharp & Dohme, and Daiichi Sankyo; research funding to the institution from Roche, Ariad pharmaceuticals, AstraZeneca, Baxalta GMBH/Servier Affaires, Bayer Healthcare, Eisai, F. Hoffmann-La Roche, Guardant Health, Merck Sharp & Dohme, Pfizer, Piqur Therapeutics, Puma C, and Queen Mary University of London; stocks in MedSIR, Nektar Pharmaceuticals, and Leuko (relative); travel, accommodation, and expenses from Roche, Novartis, Eisai, Pfizer, Daiichi Sankyo, AstraZeneca, and Gilead; and patents *Pharmaceutical Combinations of a Pi3k Inhibitor and a Microtubule Destabilizing Agent.Javier Cortés Castán, Alejandro Piris Giménez, Violeta Serra Elizalde. WO 2014/199294 A (issued)* and *Her2 as a predictor of response to dual HER2 blockade in the absence of cytotoxic therapy. Aleix Prat, Antonio Llombart, Javier Cortés.US 2019/0338368 A1. (licensed).* CD declares speaker honoraria from and/or being on the advisory board of AstraZeneca, Daiichi Sankyo, Lilly, Merck, Molecular Health, MSD Oncology, and Roche; ownership interest in Sividon Diagnostics; licensing fees from VMscope digital pathology software; and institutional funding from Myriad and Roche. VD declares speaker honoraria from and/or being on the advisory board of AbbVie, AstraZeneca, Daiichi Sankyo, Eisai, Gilead, Lilly, MSD, Novartis, Pfizer, Pierre Fabre Oncology, Roche Genentech, and Seagen; steering being a committee member of AbbVie, Pfizer, Roche Genentech, and Seagen. WJ declares speaker honoraria from and/or being on the advisory board of AstraZeneca, BMS, Chugai, Daiichi Sankyo, Eisai, Eli Lilly, MSD, Novartis, Pfizer, Roche, and Seagen; reports institutional research grants from AstraZeneca and Daiichi Sankyo; reports being a

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REFERENCES

1. Burstein HJ. The distinctive nature of HER2-positive breast cancers. *N Engl J Med*. 2005;353:1652-1654.
2. Loibl S, Gianni L. HER2-positive breast cancer. *Lancet*. 2017;389:2415-2429.
3. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*. 1989;244:707-712.
4. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177-182.
5. Tarantino P, Trapani D, Curigliano G. Mastering the use of novel anti-HER2 treatment options. *JCO Oncol Pract*. 2021;17:605-606.
6. Pernas S, Tolaney SM. Management of early-stage human epidermal growth factor receptor 2—positive breast cancer. *JCO Oncol Pract*. 2021;17:320-330.
7. Martínez-Sáez O, Prat A. Current and future management of HER2-positive metastatic breast cancer. *JCO Oncol Pract*. 2021;17:594-604.
8. Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J Clin Oncol*. 2018;36:2105-2122.
9. Loibl S, Poortmans P, Morrow M, et al. Breast cancer. *Lancet*. 2021;397:1750-1769.
10. Schettini F, Chic N, Brasó-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *NPJ Breast Cancer*. 2021;7:1.
11. Tarantino P, Hamilton E, Tolaney SM, et al. HER2-low breast cancer: pathological and clinical landscape. *J Clin Oncol*. 2020;38:1951-1962.
12. Fehrenbacher L, Cecchini RS, Geyer CE Jr, et al. NSABP B-47/NRG oncology phase III randomized trial comparing adjuvant chemotherapy

with or without trastuzumab in high-risk invasive breast cancer negative for HER2 by FISH and with IHC 1+ or 2+. *J Clin Oncol*. 2020;38:444-453.
13. Tarantino P, Curigliano G, Tolaney SM. Navigating the HER2-low paradigm in breast oncology: new standards, future horizons. *Cancer Discov*. 2022;12:2026-2030.
14. Modi S, Park H, Murthy RK, et al. Antitumor activity and safety of trastuzumab deruxtecan in patients with HER2-low-expressing advanced breast cancer: results from a phase Ib study. *J Clin Oncol*. 2020;38:1887-1896.
15. Banerji U, van Herpen CML, Saura C, et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. *Lancet Oncol*. 2019;20:1124-1135.
16. Wang J, Liu Y, Zhang Q, et al. RC48-ADC, a HER2-targeting antibody-drug conjugate, in patients with HER2-positive and HER2-low expressing advanced or metastatic breast cancer: a pooled analysis of two studies. *J Clin Oncol*. 2021;39:1022.
17. Schmid P, Im S-A, Armstrong A, et al. BEGONIA: phase 1b/2 study of durvalumab (D) combinations in locally advanced/metastatic triple-negative breast cancer (TNBC)—initial results from arm 1, d+paclitaxel (P), and arm 6, d+trastuzumab deruxtecan (T-DXd). *J Clin Oncol*. 2021;39:1023.
18. Jiang Z, Sun T, Wang X, et al. A multiple center, open-label, single-arm, phase II clinical trial of MRG002, an HER2-targeted antibody-drug conjugate, in patients with HER2-low expressing advanced or metastatic breast cancer. *J Clin Oncol*. 2022;40:1102.
19. Modi S, Jacot W, Yamashita T, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. *N Engl J Med*. 2022;387:9-20.
20. Khan AR, Khan S, Zimmerman V, et al. Quality and strength of evidence of the Infectious Diseases Society of America Clinical Practice Guidelines. *Clin Infect Dis*. 2010;51:1147-1156.
21. Denkert C, Seither F, Schneeweiss A, et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. *Lancet Oncol*. 2021;22:1151-1161.
22. Agostinetto E, Rediti M, Fimereli D, et al. HER2-low breast cancer: molecular characteristics and prognosis. *Cancers (Basel)*. 2021;13:2824.
23. Zhang H, Katerji H, Turner BM, et al. HER2-low breast cancers: incidence, HER2 staining patterns, clinicopathologic features, MammaPrint and BluePrint genomic profiles. *Mod Pathol*. 2022;35:1075-1082.
24. Tarantino P, Jin Q, Tayob N, et al. Prognostic and biologic significance of ERBB2-low expression in early-stage breast cancer. *JAMA Oncol*. 2022;8:1177-1183.
25. Tarantino P, Gupta HV, Hughes ME, et al. Comprehensive genomic characterization of HER2-low breast cancer. *San Antonio Breast Cancer Symposium, San Antonio, Texas.* 2022.
26. Bansal R, McGrath J, Walker P, et al. Genomic and Transcriptomic Landscape of HER2-Low Breast Cancer. *San Antonio Breast Cancer Symposium, San Antonio, Texas.* 2022.
27. Marra A, Safonov A, Drago J, et al. Genomic characterization of primary and metastatic HER2-low breast cancers. *San Antonio Breast Cancer Symposium, San Antonio, Texas.* 2022.
28. Gianni L, Lladó A, Bianchi G, et al. Open-label, phase II, multicenter, randomized study of the efficacy and safety of two dose levels of pertuzumab, a human epidermal growth factor receptor 2 dimerization inhibitor, in patients with human epidermal growth factor receptor 2—negative metastatic breast cancer. *J Clin Oncol*. 2010;28:1131.
29. Yao H, Yan M, Tong Z, et al. Abstract CT175: Safety, tolerability, pharmacokinetics, and antitumor activity of SHR-A1811 in HER2-expressing/mutated advanced solid tumors: a global phase 1, multicenter, first-in-human study. *Cancer Res*. 2023;83:CT175.
30. Takegawa N, Tsurutani J, Kawakami H, et al. [fam-] trastuzumab deruxtecan, antitumor activity is dependent on HER2 expression level rather than on HER2 amplification. *Int J Cancer*. 2019;145:3414-3424.
31. Ogitani Y, Hagihara K, Oitate M, et al. Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. *Cancer Sci*. 2016;107:1039-1046.
32. Nilsson MB, Poteete A, Udagawa H, et al. Abstract 383: Trastuzumab deruxtecan resistance is associated with reduced responsiveness to topoisomerase inhibitors (payload resistance) but no reduction in sensitivity to HER2 tyrosine kinase inhibitors. *Cancer Res*. 2023;83:383.
33. Dieras V, Deluche E, Lusque A, et al. Abstract PD8-02: Trastuzumab deruxtecan for advanced breast cancer patients, regardless HER2 status: a phase II study with biomarkers analysis (DAISY). *San Antonio Breast Cancer Symposium, San Antonio, Texas. 2021*. https://doi.org/10.1158/1538-7445.sabcs21-pd8-02.
34. Mosele MF, Lusque A, Dieras V, et al. LBA1—Unraveling the mechanism of action and resistance to trastuzumab deruxtecan (T-DXd): biomarker analyses from patients from DAISY trial. *Ann Oncol*. 2022;33:S123-S147.
35. Jacot W, Maran-Gonzalez A, Massol O, et al. Prognostic value of HER2-low expression in non-metastatic triple-negative breast cancer and correlation with other biomarkers. *Cancers (Basel)*. 2021;13:6059.
36. Horisawa N, Adachi Y, Takatsuka D, et al. The frequency of low HER2 expression in breast cancer and a comparison of prognosis between patients with HER2-low and HER2-negative breast cancer by HR status. *Breast Cancer*. 2021;29:234-241.
37. de Moura Leite L, Cesca MG, Tavares MC, et al. HER2-low status and response to neoadjuvant chemotherapy in HER2 negative early breast cancer. *Breast Cancer Res Treat*. 2021;190:155-163.
38. Gampenrieder SP, Rinnerthaler G, Tinchon C, et al. Landscape of HER2-low metastatic breast cancer (MBC): results from the Austrian AGMT_MBC-Registry. *Breast Cancer Res*. 2021;23:112.
39. Guven DC, Kaya MB, Fedai B, et al. HER2-low breast cancer could be associated with an increased risk of brain metastasis. *Int J Clin Oncol*. 2021;27:332-339.
40. Mutai R, Barkan T, Moore A, et al. Prognostic impact of HER2-low expression in hormone receptor positive early breast cancer. *Breast*. 2021;60:62-69.
41. Tarantino P, Niman SM, Erick TK, et al. HER2-low inflammatory breast cancer: clinicopathologic features and prognostic implications. *Eur J Cancer*. 2022;174:277-286.
42. Di Cosimo S, La Rocca E, Ljevar S, et al. Moving HER2-low breast cancer predictive and prognostic data from clinical trials into the real world. *Front Mol Biosci*. 2022;9:996434.
43. Spring L, Barlow WE, Bardia A, et al. Impact of HER2 low status on clinical outcomes in participants with 1-3 positive lymph nodes, HR+/HER2- breast cancer with recurrence score <25 randomized to endocrine therapy +/- chemotherapy: results from SWOG S1007 (RxPONDER). *San Antonio Breast Cancer Symposium, San Antonio, Texas*. 2022.
44. Won HS, Ahn J, Kim Y, et al. Clinical significance of HER2-low expression in early breast cancer: a nationwide study from the Korean Breast Cancer Society. *Breast Cancer Res*. 2022;24:22.
45. Peiffer DS, Zhao F, Chen N, et al. Clinicopathologic characteristics and prognosis of ERBB2-low breast cancer among patients in the National Cancer Database. *JAMA Oncol*. 2023;9:500-510.
46. Peiffer D, Howard FM, Chen N, et al. Epidemiology and prognosis of HER2-low breast cancer (BC) in the National Cancer Data Base (NCDB). *San Antonio Breast Cancer Symposium, San Antonio, Texas*. 2022.
47. Tan RSYC, Ong WS, Lee K-H, et al. HER2 expression, copy number variation and survival outcomes in HER2-low non-metastatic breast cancer: an international multicentre cohort study and TCGA-METABRIC analysis. *BMC Med*. 2022;20:105.
48. Bao KKH, Sutanto L, Tse SSW, et al. The association of ERBB2-low expression with the efficacy of cyclin-dependent kinase 4/6 inhibitor in hormone receptor—positive, ERBB2-negative metastatic breast cancer. *JAMA Netw Open*. 2021;4:e2133132.
49. Carlino F, Diana A, Ventriglia A, et al. HER2-low status does not affect survival outcomes of patients with metastatic breast cancer (MBC) undergoing first-line treatment with endocrine therapy plus palbociclib: results of a multicenter, retrospective cohort study. *Cancers (Basel)*. 2022;14:4981.

50. Zattarin E, Sposetti C, Leporati R, et al. HER2-low status is associated with worse clinical outcomes in hormone receptor-positive, HER2-negative advanced breast cancer patients treated with firstline cyclin-dependent kinase 4/6 inhibitors plus endocrine therapy. *San Antonio Breast Cancer Symposium, San Antonio, Texas.* 2022.
51. Mouabbi J, Singareeka Raghavendra A, Basset R, et al. Histology-based survival outcomes in HR+/HER2- metastatic breast cancer treated with targeted therapies plus endocrine therapy based on HER2 expression. *San Antonio Breast Cancer Symposium, San Antonio, Texas.* 2022.
52. Schmid P, Wysocki PJ, Park YH, et al. Trastuzumab deruxtecan (T-DXd) + durvalumab (D) as first-line (1L) treatment for unresectable locally advanced/metastatic hormone receptor-negative (HR−), HER2-low breast cancer: updated results from BEGONIA, a phase 1b/2 study. *San Antonio Breast Cancer Symposium, San Antonio, Texas*. 2022.
53. Schmid P, Cortés J, Marmé F, et al. 214MO Sacituzumab govitecan (SG) efficacy in hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2−) metastatic breast cancer (MBC) by HER2 immunohistochemistry (IHC) status in the phase III TROPiCS-02 study. *Ann Oncol*. 2022;33:S635-S636.
54. Nielsen S, Bzorek M, Vyberg M, et al. Lessons learned, challenges taken, and actions made for "precision" immunohistochemistry. Analysis and perspectives from the NordiQC Proficiency Testing Program. *Appl Immunohistochem Mol Morphol*. 2022.
55. Scott M, Vandenberghe ME, Scorer P, et al. Prevalence of HER2 low in breast cancer subtypes using the VENTANA anti-HER2/neu (4B5) assay. *J Clin Oncol*. 2021;39:1021.
56. Rüschoff J, Friedrich M, Nagelmeier I, et al. Comparison of HercepTest™ mAb pharmDx (Dako Omnis, GE001) with Ventana PATHWAY anti-HER-2/neu (4B5) in breast cancer: correlation with HER2 amplification and HER2 low status. *Virchows Arch*. 2022;481:685-694.
57. Fernandez AI, Liu M, Bellizzi A, et al. Examination of low ERBB2 protein expression in breast cancer tissue. *JAMA Oncol*. 2022;8:1-4.
58. Rüschoff J, Penner A, Ellis IO, et al. Abstract HER2-13: HER2-13 Proficiency assessment of HER2-low breast cancer scoring with the Ventana PATHWAY 4B5 and Dako HercepTest HER2 assays and the impact of pathologist training. *Cancer Res*. 2023;83:HER2-13.
59. Moutafi M, Robbins CJ, Yaghoobi V, et al. Quantitative measurement of HER2 expression to subclassify ERBB2 unamplified breast cancer. *Lab Invest*. 2022;102:1101-1108.
60. Gustavson M, Haneder S, Spitzmueller A, et al. Abstract PD6-01: Novel approach to HER2 quantification: digital pathology coupled with AI-based image and data analysis delivers objective and quantitative HER2 expression analysis for enrichment of responders to trastuzumab deruxtecan (T-DXd; DS-8201), specifically in HER2-low patients. *Cancer Res*. 2021;81:PD6-01.
61. Jung M, Song SG, Cho SI, et al. Artificial intelligence-powered human epidermal growth factor receptor 2 (HER2) analyzer in breast cancer as an assistance tool for pathologists to reduce interobserver variation. *J Clin Oncol*. 2022;40:e12543.
62. Wu S, Yue M, Zhang J, et al. The role of artificial intelligence in accurate interpretation of HER2 immunohistochemical scores 0 and 1+ in breast cancer. *Mod Pathol*. 2023;36:100054.
63. Prat A, Modi S, Tsurutani J, et al. Determination of HER2-low status in tumors of patients with unresectable and/or metastatic breast cancer in DESTINY-Breast04. *San Antonio Breast Cancer Symposium, San Antonio, Texas* 2022.
64. Miglietta F, Griguolo G, Bottosso M, et al. HER2-low-positive breast cancer: evolution from primary tumor to residual disease after neoadjuvant treatment. *NPJ Breast Cancer.* 2022;8:66.
65. Miglietta F, Griguolo G, Bottosso M, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. *NPJ Breast Cancer.* 2021;7:137.
66. Tarantino P, Gandini S, Nicolò E, et al. Evolution of low HER2 expression between early and advanced-stage breast cancer. *Eur J Cancer.* 2022;163:35-43.
67. Geukens T, De Schepper MD, Richard F, et al. Inter-lesion heterogeneity of HER2-status in metastatic breast cancer: possible implications for treatment with anti-HER2 antibody-drug conjugates. *San Antonio Breast Cancer Symposium, San Antonio, Texas*. 2022.
68. Schettini F, Giudici F, Giuliano M, et al. Overall survival of CDK4/6-inhibitor-based treatments in clinically relevant subgroups of metastatic breast cancer: systematic review and meta-analysis. *J Natl Cancer Inst*. 2020;112:1089-1097.
69. Rugo H, Bardia A, Marmé F, et al. LBA76 - Overall survival (OS) results from the phase III TROPiCS-02 study of sacituzumab govitecan (SG) vs treatment of physician's choice (TPC) in patients (pts) with HR+/HER2- metastatic breast cancer (mBC). *Ann Oncol*. 2022;33:S808-S869.
70. André F, Hee Park Y, Kim S-B, et al. Trastuzumab deruxtecan versus treatment of physician's choice in patients with HER2-positive metastatic breast cancer (DESTINY-reast02): a randomised, open-label, multicentre, phase 3 trial. *Lancet*. 2023;401:1773-1785.
71. Hurvitz SA, Hegg R, Chung W-P, et al. Trastuzumab deruxtecan versus trastuzumab emtansine in patients with HER2-positive metastatic breast cancer: updated results from DESTINY-Breast03, a randomised, open-label, phase 3 trial. *Lancet*. 2023;401:105-117.
72. Bianchini G, Arpino G, Biganzoli L, et al. Emetogenicity of antibody-drug conjugates (ADCs) in solid tumors with a focus on trastuzumab deruxtecan: insights from an Italian expert panel. *Cancers*. 2022;14:1022.
73. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)—Antiemesis—Version 1.2023—January 25, 2023. Available at https://www.nccn.org/professionals/physician_gls/pdf/antiemesis.pdf. Accessed May 5, 2023.
74. Powell CA, Modi S, Iwata H, et al. Pooled analysis of drug-related interstitial lung disease and/or pneumonitis in nine trastuzumab deruxtecan monotherapy studies. *ESMO Open*. 2022;7:100554.
75. Tarantino P, Modi S, Tolaney SM, et al. Interstitial lung disease induced by anti-ERBB2 antibody-drug conjugates: a review. *JAMA Oncol*. 2021;7:1873-1881.
76. Swain SM, Nishino M, Lancaster LH, et al. Multidisciplinary clinical guidance on trastuzumab deruxtecan (T-DXd)—related interstitial lung disease/pneumonitis-focus on proactive monitoring, diagnosis, and management. *Cancer Treat Rev.* 2022;106:102378.
77. Griggs JJ, Hamilton AS, Schwartz KL, et al. Discordance between original and central laboratories in ER and HER2 results in a diverse, population-based sample. *Breast Cancer Res Treat*. 2017;161:375-384.
78. Kaufman PA, Bloom KJ, Burris H, et al. Assessing the discordance rate between local and central HER2 testing in women with locally determined HER2-negative breast cancer. *Cancer.* 2014;120:2657-2664.
79. Reisenbichler ES, Han G, Bellizzi A, et al. Prospective multi-institutional evaluation of pathologist assessment of PD-L1 assays for patient selection in triple negative breast cancer. *Mod Pathol*. 2020;33:1746-1752.
80. Salgado R, Bellizzi AM, Rimm D, et al. How current assay approval policies are leading to unintended imprecision medicine. *Lancet Oncol*. 2020;21:1399-1401.