Targeted Oncology (2023) 18:313–319
https://doi.org/10.1007/s11523-023-00964-8

COMMENTARY

HER2-Low Breast Cancer—Diagnostic Challenges and Opportunities for Insights from Ongoing Studies: A Podcast

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Accepted: 27 March 2023 / Published online: 3 May 2023

Abstract

Breast cancer has been traditionally classified as either human epidermal growth factor receptor 2 (HER2)-positive or HER2-negative based on immunohistochemistry (IHC) scoring and/or gene amplification. HER2-positive breast cancer (defined as IHC 3+ or IHC 2+ and in situ hybridization [ISH]+) is routinely treated with HER2-targeted therapies, while HER2-negative breast cancer (defined as IHC 0, IHC 1+, or IHC 2+/ISH−) was not previously eligible for HER2-targeted therapy. Some tumors traditionally defined as HER2-negative express low levels of HER2 (i.e., HER2-low breast cancer, defined as IHC 1+ or IHC 2+/ISH−). Recently reported results from the DESTINY-Breast04 trial demonstrated that the HER2-targeted antibody–drug conjugate trastuzumab deruxtecan (T-DXd) improved survival in patients with previously treated advanced or metastatic HER2-low breast cancer, leading to the approval of T-DXd in the US and EU for patients with unresectable or metastatic HER2-low breast cancer after prior chemotherapy in the metastatic setting or disease recurrence within 6 months of adjuvant chemotherapy. As the first HER2-targeted therapy approved for the treatment of HER2-low breast cancer, this represents a change in the clinical landscape and presents new challenges, including identifying patients with HER2-low breast cancer. In this podcast, we discuss the strengths and limitations of current methodologies for classifying HER2 expression and future research that will help refine the identification of patients expected to benefit from HER2-targeted therapy, such as T-DXd or other antibody–drug conjugates. Although current methodologies are not optimized to identify all patients with HER2-low breast cancer who may potentially benefit from HER2-targeted antibody–drug conjugates, they are likely to identify many. Ongoing studies—including the DESTINY-Breast06 trial evaluating T-DXd in patients with HER2-low breast cancer and those with tumors expressing very low levels of HER2 (IHC > 0 to < 1+)—will provide insights that may improve the identification of patient populations expected to benefit from HER2-targeted antibody–drug conjugates.

Abbreviations

ADC	Antibody–drug conjugate
ASCO	American Society of Clinical Oncology
CAP	College of American Pathologists
CDK	Cyclin-dependent kinase
FDA	Food and Drug Administration
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
ISH	In situ hybridization
RNA	Ribonucleic acid
T-DXd	Trastuzumab deruxtecan

The podcast and transcript can be viewed below the abstract of the online version of the manuscript. Alternatively, the podcast can be downloaded here: https://doi.org/10.6084/m9.figshare.22339660.

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1 Podcast Transcript

Dr. Bardia: Hello, I'm Aditya Bardia. I'm a breast medical oncologist at Massachusetts General Hospital, Harvard Medical School, Boston. Joining me today is Professor Viale.

Prof. Viale: Hi, this is Beppe Viale. I'm the Chairman of the Department of Pathology and Laboratory Medicine at the European Institute of Oncology in Milano. And as a pathologist, I have dealt with breast cancer in the last 20 years. And I'm happy to join you today for this podcast.

Dr. Bardia: We are fortunate to have you today, Professor Viale. So, today we will be discussing HER2-low breast cancer, including the implications of the recent US FDA and EU approval of trastuzumab deruxtecan (T-DXd) for use in

Key Points

Trastuzumab deruxtecan (T-DXd) is the first human epidermal growth factor receptor 2 (HER2)–targeted therapy approved for the treatment of breast cancer that expresses low levels of HER2 (HER2-low breast cancer).

This approval presents new challenges in the identification of patients with HER2-low breast cancer, including a need to precisely define this patient population and a need to evaluate additional technologies for patient identification, since current methodologies are not optimized to detect HER2-low breast cancer.

Current and ongoing studies will provide insights that may refine the identification of patient populations expected to benefit from HER2-targeted antibody–drug conjugates.

patients with HER2-low breast cancer, the new challenges that this development presents, and ongoing research that will help us refine and define HER2-low patient identification. This is for the journal *Targeted Oncology*. So, let's start with challenges and opportunities in HER2-low breast cancer. Professor Viale, can you tell us about HER2-low breast cancer, including how it is defined and the historical context of its treatment?

Prof. Viale: Yes, certainly, Dr. Bardia, thank you. As you know, human epidermal growth factor receptor 2 is a very important biomarker in breast cancer because it drives treatment decisions [1–3]. We have traditionally classified breast cancer as HER2-positive or HER2-negative based on immunohistochemical assays and in situ hybridization testing [3, 4]. We, in the ASCO/CAP recommendations, have identified HER2-positive breast cancer as a tumor with an immunohistochemical 3+ overexpression of the protein or immunohistochemical 2+ score, but with an amplified *HER2* gene according to the in situ hybridization testing [4]. HER2-negative breast cancer has been defined as a tumor with an immunohistochemical score of 0, 1+, or 2+, but ISH negative [4]. What we know is that nowadays, anti-HER2–targeted therapies are included in the standard of care for HER2-positive disease, and, certainly, they have improved prognosis in patients both with early and metastatic HER2-positive breast cancer [2, 3, 5]. However, patients with traditionally defined HER2-negative breast cancer, that approximately account for 80–85% of patients with breast cancer, these patients were not previously eligible for HER2-targeted therapies because these treatments were not shown to improve outcomes in this population of patients [2, 3, 6–11]. But recently, we have seen that approximately 60% of those [with] HER2-negative breast cancer, as we have traditionally defined them, actually express low levels of HER2, and this has been designated as HER2-low disease, defined as a tumor with an immunohistochemical score of 1+ or 2+ and in situ hybridization negative [12–15]. Certainly, as you know, in the earlier studies, HER2-targeted therapies including [the] monoclonal antibodies trastuzumab [and] pertuzumab or the first-generation antibody–drug conjugate trastuzumab emtansine did not demonstrate efficacy in patients with HER2-low breast cancer [6–9]. However, nowadays, we have newer HER2-targeted therapies that are being evaluated in this patient population, and they have the potential to transform treatment options for patients with HER2-low advanced or metastatic breast cancer. First, we have the monoclonal antibody margetuximab. It has shown preclinical activity in the HER2-low setting and currently is being evaluated in patients with advanced breast cancer in a phase 2 clinical trial [16, 17]. Also, the antibody–drug conjugate[s] trastuzumab duocarmazine and disitamab vedotin have demonstrated preliminary efficacy in the same setting of advanced or metastatic HER2-low breast cancer in phase 1/1b trials [18, 19]. But finally, the antibody–drug conjugate T-DXd recently has demonstrated extraordinary efficacy in HER2-low metastatic breast cancer in a phase 3 trial [20]. So, this is what we know today. And Dr. Bardia, can you tell us more about T-DXd and its use in HER2-low breast cancer?

Dr. Bardia: Absolutely, thank you for the very nice summary. And it's really challenged our classification of how we define HER2 and the subtypes of breast cancer. And, in large part, this is because of T-DXd, or trastuzumab deruxtecan. So, T-DXd is an antibody–drug conjugate. It's comprised of three components, the HER2-targeted antibody, a tetrapeptide-based cleavable linker, and a [topoisomerase I] inhibitor payload; the linker connects the payload to the antibody [21, 22]. T-DXd is used in the second- and later-line setting for the treatment of patients with HER2-positive advanced or metastatic breast cancer and was FDA approved in the US in August of 2022 and [in the] EU in January of 2023 for the treatment of patients with HER2-low unresectable or metastatic breast cancer who had received at least one prior line of chemotherapy in the metastatic setting or developed disease recurrence within 6 months of adjuvant chemotherapy [23, 24]. It's also in the ASCO guidelines: current ASCO guidelines recommend that patients with HER2-low metastatic breast cancer who have received prior chemotherapy, and who are refractory to endocrine therapy in the case of hormone-receptor–positive disease, should be offered treatment with T-DXd [25]. The approval of T-DXd for use in HER2-low metastatic breast cancer, irrespective of hormone receptor status, was based on the multicenter phase 3 DESTINY-Breast04 trial. The phase 3 trial evaluated the safety and efficacy of T-DXd versus

standard chemotherapy in patients with HER2-low metastatic breast cancer [20]. In this trial, patients had received prior chemotherapy in the metastatic setting or [had] disease recurrence within 6 months of adjuvant chemotherapy and, if hormone-receptor positive, patients also received prior endocrine therapy [20]. T-DXd resulted in decreased risk of disease progression or death versus physician's choice of chemotherapy in the full analysis set, which combined both hormone-receptor–positive and hormone-receptor–negative subset[s], with a median progression-free survival of 9.9 months with T-DXd versus about 5 months with standard chemotherapy [20]. And there was improvement in overall survival as well: overall survival median of 23.4 months, so that's close to 2 years [with T-DXd] versus 16.8 months with standard chemotherapy [20]. In terms of the safety profile, the safety profile of T-DXd in [the] HER2-low setting was consistent with the established safety profile in patients with HER2-positive breast cancer [20]. And, as the first targeted therapy directed towards HER2 approved for treatment of HER2-low disease, this is a major change in [the] clinical landscape and essentially has redefined the classification of breast cancer. It also presents new challenges for physicians and pathologists. There is a need to more precisely define the population that may benefit from T-DXd or other HER2-targeted ADCs, the ones that have a bystander effect, including defining the lowest expression of HER2 expected to [confer] benefit from these therapies. And the DESTINY-Breast04 trial evaluated HER2 by IHC [20]. There's a need for additional methods, more sensitive methods, to further improve on the current ways of defining HER2.

Prof. Viale: Thank you so much, Dr. Bardia, for this very clear explanation of what we know and what we can do with the HER2-low disease. But certainly, I'm not sure that the threshold of more than 10% immunoreactive cells [4] is something written in stone. So, what do we know about the lower bound of HER2 expression that is actually needed for the efficacy of these new HER2-targeted antibody–drug conjugates? Do we have any evidence suggesting that HER2-targeted antibody–drug conjugates could also be effective in tumors with HER2 expression at even lower levels than the so-called HER2-low group?

Dr. Bardia: Yeah, absolutely. I fully agree. I think this definition of HER2-low as IHC 1+/2+ was a start. With these newer ADCs, particularly ADCs that have a bystander effect, I think we would see activity even lower than what was defined in DESTINY-Breast04. So, although [the] DESTINY-Breast04 trial established efficacy of T-DXd in patients with IHC 1+ or 2+ ISH-negative breast cancer [20], whether the patients with breast cancer that [has] even lower expression of HER2 may benefit from T-DXd or other HER2-directed ADCs needs to be confirmed by larger-scale clinical trials. We do have some evidence from the phase 2 DAISY trial that looked at T-DXd in patients with previously treated HER2-low or even HER2 IHC 0 invasive breast cancer, and patients had received at least one prior line of chemotherapy in the metastatic setting [26]. Patients in [the] DAISY trial must have received prior anthracyclines or taxanes, and, in [the] case of hormone-receptor–positive disease, must have resistance to endocrine therapy and aCDK4/6 inhibitor [26], so very similar to [the] DESTINY-Breast04 trial in terms of patient population and eligibility. In [the] DAISY trial, even IHC 0 was included, which, as a reminder, includes tumors with HER2 membrane staining that is faint in less than 10% of cells [4] and therefore includes tumors with very low expression of HER2. In preliminary results from the DAISY trial, 30.6% of patients with IHC 0 advanced breast cancer had confirmed response to T-DXd [27], suggesting that T-DXd may be an effective option in patients who have very low expression of HER2. Now, DAISY was a small trial; it was not randomized. So, we need data from randomized phase 3 studies, which is being done in the ongoing DESTINY-Breast06 trial [28]. So, as a reminder, the DESTINY-Breast06 trial is a randomized phase 3 trial evaluating [the] efficacy, safety, [and] tolerability of T-DXd in patients with advanced/metastatic hormone-receptor–positive HER2-low as well as HER2 0—more than 0, less than 1[+]—breast cancer [28]. So, in this study, the HER2 status was evaluated by the Ventana PATHWAY anti-HER2/neu (4B5) test, and, essentially, it's the same test that was used in the DESTINY-Breast04 study. And because in DESTINY-Breast06, this group—IHC more than 0 but less than 1+—will be included, it would include patients who have tumors with faint, partial HER2 membrane staining in less than 10% of cells. So, it might help establish the lower threshold for HER2-positive disease in patients who have some expression of HER2 in their tumors. This study is currently ongoing and expected to read out and would provide important insights into the population of patients expected to benefit from T-DXd. So, Professor Viale, now that we've discussed which patients may benefit from HER2-targeted ADCs, can you describe current methodologies used to identify HER2-low breast cancer and their limitations?

Prof. Viale: Yes, certainly. There is a great debate, as you know, in the clinical and the pathological communities about the fact that the current HER2 testing and scoring systems actually have been established only with the aim of identifying HER2 positivity, not to differentiate low levels of HER2 expression from a lack thereof [29], because it was not previously shown to be clinically meaningful to try and identify these tumors with very low expression of HER2. And so, the distinction between immunohistochemical 0 and 1+ score was not really looked for by the pathologists in the clinical practice because this didn't have any clinical implication. In any case, patient eligibility for the DESTINY-[Breast]04 study actually was based on central assessment

of HER2 status using a conventional assay, which is the 4B5 assay from Ventana, and the current ASCO/CAP scoring guidelines [20]. And so, according to the current guidelines, the immunohistochemical score is based only on the highest level of immunohistochemical staining that is observed in more than 10% of invasive tumor cells within a sample [4]. So, currently, information on the intra-tumoral heterogeneity in HER2 expression across a tumor has not been taken into account, but I think it may become very important. And this is because, as you have already mentioned, after binding to HER2 on the HER2-expressing tumor cells, T-DXd is internalized, and the [topoisomerase I] inhibitor payload is released [30, 31]. It enters the nucleus of the cell and results in the [cell's] death [30, 31]. But importantly, this payload is membrane permeable, and so it [can] also enter neighboring cells, irrespective of HER2 expression on these neighboring cells [30, 31]. And so, this is the so-called bystander-killing effect. So, because of this proposed bystander antitumor effect, knowing what percentage of cells actually are immunohistochemical score 3+, 2+, 1+, or 0, and whether these immunohistochemical 0 cells actually are adjacent to immunohistochemical-positive cells, may inform whether cells are expected to be susceptible to T-DXd or not. Also, another issue is the difference [in HER2 status] between original and subsequent biopsies. These differences may exist, and also we have evidence that HER2 expression in tumors may change with disease progression [14, 32, 33] and even more following neoadjuvant treatments [32, 34, 35]. Furthermore, HER2 status may also differ between metastatic samples collected from the same patient at a single time point, and, even within a single organ, different metastases may have different expression of HER2 [36]. So, there is high plasticity of HER2 expression in tumors. From a technical point of view, then, we have differences in the sensitivities and specificities of various immunohistochemical assays. These have been fully reported in the past [37–39]. And these differences may contribute to differences in the detection of low levels of HER2 expression. And finally, pathologists may be not completely accurate or reproducible in scoring or interpreting the immunohistochemical results [12, 13, 40–43]. We have data pointing to the fact that there may be a concordance rate between central and local scoring of 70–71% only for immunohistochemical 1+ cases, and it goes down to 40% for the immunohistochemical 2+ score [43]. Actually, in the phase 3 DESTINY-[Breast]04 study for HER2-low breast cancers, patients were identified for recruitment based on [a] historical immunohistochemical score 1+ or 2+ [20]. When these samples were rescored by a central lab, the concordance rate between historical and central scoring was 77% for the HER2-low expression, and most of the samples that were scored as HER2-low historically but not centrally were actually scored as HER2 immunohistochemical score 0 centrally [44]. We have recently presented a retrospective study of HER2-low breast cancer, whereby local laboratories rescored samples for 786 patients in a blinded manner [13]. The concordance between the historical scores and the rescores overall was 81.3% [13]. This concordance increases to 87.5% for the HER2-low disease, and it was approximately 70% in [the] case of HER2 immunohistochemical 0 expression [13]. So, training resources for scoring low levels of HER2 expression are now becoming available. And we have evidence that training can improve pathologists' ability to identify HER2 immunohistochemical score 0 and HER2-low samples [45]. These findings highlight the importance of reviewing available samples to determine if they may be categorized as HER2-low, because this will inform patient eligibility for the new emerging treatments. So, you mentioned the ongoing DESTINY-Breast06 trial. It is important to highlight that, in this trial, HER2-low and immunohistochemical score more than 0, less than 1[+] will be confirmed by a central lab prior to inclusion in order to ensure that patients enrolled in the trial have a tumor that will be categorized according to the eligibility criteria of the study [28].

Dr. Bardia: That's a very important point. Thanks for highlighting this, Professor Viale, and this importance of analytical validation, central confirmation, as well as training of pathologists. So, we clearly have the work cut out for us over the next few years. In addition, how do you anticipate that the HER2 testing will evolve in the future to better identify patients with HER2-low breast cancer?

Prof. Viale: This is again a debated issue, because I'm not sure whether we are to focus on the precision of the assays in order to identify the small number of molecules on the membrane of the tumor cells or if we should stick to prediction. So, what is the assay that is more predictive of response of the tumor to these new drugs? Certainly, my option would be to try and be as predictive as possible, and I'm not obsessed [with] precision in order to precisely quantify. But certainly, if a test is more precise, it's likely to be at least as predictive or maybe more predictive than the tests that we arecurrently using. So, although testing that has been used to identify these HER2-low tumors and the tumors with an immunohistochemical score [of] more than 0, less than 1[+] [has] been using a traditional, usual immunohistochemical assay and scoring system, new technologies actually may help to better identify these patients with this subtype of tumor. Novel methods may be more quantitative and include assays that will analyze HER2 messenger RNA levels, for instance [46, 47], or those that will assess HER2 protein expression using quantitative immunofluorescence testing [48, 49] or reverse-phase protein analysis [50]. Also, we may take advantage [of] digital imaging and machine learning approaches [51–55]. They may help us to distinguish HER2-low breast cancer from other types of breast cancers. Actually, these techniques have [already been] shown to detect more faintly positive cells, thus reducing the number

of equivocal cases compared with human scoring [51, 53]. And also, they offer the advantage of scoring every cell within a sample [51]. Certainly, as I have anticipated, future research is needed to inform best practices for identifying patients with HER2-low breast cancer that actually will be able to respond to the treatment. So, we need assays that are as predictive or more predictive than the ones that we are currently using. So, guided by the recent and ongoing studies, expansion of the binary classification of HER2-positive versus HER2-negative breast cancer and incorporation of HER2-low and HER2 more than 0, less than 1[+] as a targetable group should be taken into consideration in the future guidelines. And ongoing studies also will inform about the lower boundary of HER2 expression that is needed to confer efficacy of HER2-targeted antibody–drug conjugates. And finally, I am pretty sure that additional research will be necessary to establish the predictive value of these new assays for identifying those patients with HER2-low breast cancer [who] actually are expected to respond to HER2-targeted antibody–drug conjugates. So, let us stay with prediction and not be obsessed [with] precision only. We need assays that are predictive of response.

Dr. Bardia: Absolutely, that's a great point in terms of prediction and the value in [the] clinic. So, thank you so much; this was an excellent discussion. To summarize, we now know that results from [the] DESTINY-Breast04 study support the use of HER2 as a targetable marker in patients with metastatic breast cancer [who] have low levels of HER2 in their tumor. And it gives importance to identifying this new category of breast cancer. While the current methodologies were not optimized to identify all patients who may derive benefit from HER2-directed antibody–drug conjugates such as T-DXd or others, current classification of HER2 status is likely to identify many such patients. It's a start, and we need more research. And, hopefully in the future, new technologies and classification may help identify additional patients who may benefit from these therapies. At this time, because the existing clinical data are based on IHC categorization, IHC remains the best tool to identify patients with HER2-low disease. Ongoing studies in the future will provide additional insights that may refine the identification of additional patient populations expected to benefit from HER2-directed antibody–drug conjugates.So that's a wrap. Thank you so much for joining today.

Prof. Viale: Thank you, Dr. Bardia. It has been a pleasure.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11523-023-00964-8.

Acknowledgements Medical writing assistance was provided under the direction of the authors by Jackie Highland, PhD, of ArticulateScience, LLC, and was funded by AstraZeneca Pharmaceuticals.

Declarations

Funding Medical writing assistance was funded by AstraZeneca Pharmaceuticals. In March 2019, AstraZeneca entered into a global development and commercialization collaboration agreement with Daiichi Sankyo for trastuzumab deruxtecan (T-DXd; DS-8201).

Conflict of interest Both authors received nonfinancial assistance (assistance with outline preparation and podcast development) from ArticulateScience, LLC, which was funded by AstraZeneca Pharmaceuticals. Additional disclosures are as follows: *Aditya Bardia* has received grants and consulting fees from Genentech, Novartis, Pfizer, Merck, Sanofi, Radius Health, Immunomedics/Gilead, Daiichi Sankyo/AstraZeneca, Eli Lilly, and Foundation Medicine. *Giuseppe Viale* has received consulting fees from AstraZeneca and Agilent; advisory board fees from Daiichi Sankyo, Roche, and MSD Oncology during the conduct of the study; and grants from AstraZeneca, all outside the submitted work.

Availability of data and material Data sharing is not applicable, as no data sets were generated or analyzed.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable.

Author contributions *Aditya Bardia:* Conceptualization; writing—review and editing. *Giuseppe Viale:* Conceptualization; writing—review and editing.

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