

**Review article**

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**BREAST CANCER WITH LOW HER2 EXPRESSION STATUS - A NEW  
THERAPEUTIC ENTITY**

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HER2-targeted therapies used in the treatment of HER2-positive early and metastatic breast cancer (mBC) include monoclonal antibodies such as trastuzumab, pertuzumab and margetuximab, as well as antibody-drug conjugates (ADC) trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd), and tyrosine kinase inhibitors such as tucatinib, lapatinib, and neratinib. The introduction of these drugs into clinical practice has dramatically improved the course of treatment for HER2-positive breast cancer. However, the clinical evaluation of trastuzumab, pertuzumab, and T-DM1 in the HER2-low group of patients did not show significant benefits. Consequently, these patients were classified as HER2-negative cancers and treated in accordance with the expression of hormone receptors (HR) or other biomarkers. Trastuzumab deruxtecan, an ADC, which initially demonstrated its efficacy in the treatment of metastatic HER2-positive breast cancer, and subsequently in breast cancer with low HER2 expression, classified as immunohistochemistry IHC+1 and IHC+2 with a negative fluorescence in situ hybridization (FISH) introduced into clinical practice a new entity of HER2 breast cancers called HER2-low tumors. Following the publication of the DESTINY-Breast04 study results, it is clear that low HER2 positivity can be considered a rational target for the treatment of breast cancer. The results have changed clinical practice in both HR-positive and HR-negative HER2-

low metastatic breast cancer. Further research is necessary in order to standardize HER2 testing, prevent T-DXd-related side effects and resistance to therapy, and identify the optimal sequence of available therapeutic options. Future research should also explore the role of these drugs in the treatment of early HER2-low breast cancer.

**Key words:** *breast cancer, HER2-low, antibody-drug conjugate, trastuzumab deruxtecan, sacituzumab govitecan*

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## **KARCINOM DOJKE SA HER2 STATUSOM NISKE EKSPRESIJE - NOVI TERAPIJSKI**

### **ENTITET**

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Ciljane HER2 terapije koje se koriste u lečenju HER2-pozitivnog ranog i metastatskog karcinoma dojke (mBC) obuhvataju monoklonska antitela kao što su trastuzumab, pertuzumab i margetuksimab, zatim antitelo-lek konjugate (ADC) trastuzumab emtazin (T-DM1) i trastuzumab derukstekan (T-DXd) i inhibitore tirozin kinaze kao što su tukatinib, lapatinib i neratinib. Uvođenje ovih lekova u kliničku praksu dramatično je popravilo tok lečenja HER2-pozitivnog karcinoma dojke. Ipak, klinička evalacija trastuzumaba, pertuzumaba i T-DM1 u grupi pacijenata sa slabo pozitivnim HER2, nije pokazala značajan benefit. Zbog toga su ovi pacijenti svrstani u grupu HER2 negativnih karcinoma i lečeni prema ekspresiji hormonskih receptora (HR) ili drugih biomarkera. Trastuzumab derukstekan, antitelo-lek konjugat, koji je prvobitno pokazao svoju efikasnost u lečenju metastatskog HER2-pozitivnog karcinoma dojke, a nakon toga i kod karcinoma dojke sa niskom HER2 ekspresijom klasifikovanim kao imunohistohemijski skor IHC+1 and IHC+2 sa negativnom fluorescentnom in situ hibridizacijom (FISH) uveo je u kliničku praksu novi entitet HER2 karcinoma dojke koji se nazivaju tumorima slabo pozitivnog HER2 statusa. Nakon objavljenih rezultata studije DESTINY-Breast04 jasno je da se niska pozitivnost HER2 može smatrati racionalnim targetom za lečenje karcinoma dojke. Rezultati su promenili kliničku praksu i kod HR-pozitivnog i kod HR-negativnog slabo pozitivnog HER2 metastatskog karcinoma dojke. Dodatna istraživanja su neophodna da standardizuju

HER2 testiranje, preveniraju neželjena dejstva T-DXd i rezistenciju na terapiju kao i da identifikuju optimalnu sekvencu dostupnih terapijskih opcija. Takodje buduća istraživanja treba da pozicioniraju ove lekove i u lečenju ranog slabo pozitivnog HER2 karcinoma dojke.

**Ključne reči:** karcinom dojke, slabo pozitivni HER2, antitelo-lek konjugat, trastuzumab derukstekan, sacituzumab govitekan

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## Introduction

Breast cancer (BC) expresses high levels of growth factors and their receptors. The HER2 receptor belongs to the HER 2/Neu receptor family, which includes four receptors: HER1-EGFR, HER2, HER3, and HER4. The natural ligand for the HER1 receptor is the EGFR-receptor for epidermal growth factor, while the natural ligand for the HER2 receptor is unknown. The natural ligands for HER3 and HER4 receptors are neuregulins. Receptors of the HER family belong to the transmembrane group of receptors, which consists of three subunits: the extracellular part, the transmembrane part, and the intracellular part of the receptor. A specific ligand binds to the receptor through its extracellular domain, which leads to receptor dimerization (either homo- or heterodimerization) and activates the intracellular tyrosine kinase domain. This is followed by a cascade of phosphorylation processes, ultimately resulting in the synthesis of proteins that influence cell growth, division, survival, motility, adhesion, and angiogenesis (1). The HER2 receptor is a glycoprotein the synthesis of which is encoded by the HER2 gene located on chromosome 17. In 20%-30% of all breast cancers (15% in our country), a tumor disorder is present: increased synthesis of HER2 receptors on the cell surface and/or amplification of the HER2 gene in the nucleus. HER2-positive BC is characterized by a biologically aggressive clinical course, a shorter disease-free interval (DFI), as well as reduced overall survival (OS) compared to HER2-negative BC (2,3). The presence of HER2 receptors on the surface of tumor cells is determined by the immunohistochemistry (IHC) and is expressed on a scale from 0 to 3+. A result labeled IHC 3+ (more than 10% of cells show intense and complete membrane staining) is considered HER2-positive BC. A HER2 result labeled IHC 2+ represents tumors with uncertain HER2 status, requiring retesting by means of chromogenic in situ hybridization (CISH) or fluorescence in situ hybridization (FISH). This determines whether gene amplification is present, which would confirm HER2-positive status (4).

The therapy which has been in use for more than 15 years for the treatment of HER2-positive BC, such as trastuzumab or trastuzumab emtansine (T-DM1), has not shown benefits in HER2-low BC as shown in prospective randomized clinical studies (5,6).

Trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate (ADC), which initially demonstrated its efficacy in the treatment of metastatic HER2-positive BC, and subsequently in BC with low HER2 expression, classified as IHC+1 and IHC+2 with a negative FISH introduced into clinical practice a new entity of HER2 breast cancers called HER2-low tumors (7,8).

## **The importance of the HER2 signaling pathway in carcinogenesis and the importance of HER2 expression levels**

The HER2 receptor has been known to play a key role in the pathogenesis of BC since 1987 (9). In HER2-positive cancers, a specific ligand binds to the extracellular domain of the receptor, leading to homo- or heterodimerization of the receptor and subsequently the activation of the intracellular tyrosine kinase domain, followed by a cascade process of phosphorylation and the activation of signaling pathways via mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway. This results in the increased expression of certain cyclins, such as cyclin D1, E and CDK6, and the degradation of cell cycle inhibitors such as p27Kip1 (10,11). The end result is the synthesis of proteins which affect cell proliferation, survival, motility, adhesion, as well as angiogenesis (12-15).

HER2-targeted therapies used in the treatment of HER2-positive early and metastatic breast cancer (mBC) include monoclonal antibodies such as trastuzumab, pertuzumab and margetuximab, as well as antibody-drug conjugates T-DM1 and T-DXd, and tyrosine kinase inhibitors such as tucatinib, lapatinib, and neratinib. The introduction of these drugs into clinical practice has dramatically improved the course of treatment for HER2-positive BC (2,16-18). However, the clinical evaluation of trastuzumab, pertuzumab, and T-DM1 in the HER2-low group of patients did not show significant benefits. Consequently, these patients were classified as HER2-negative cancers and treated in accordance with the expression of hormone receptors (HR) or other biomarkers (5, 19-21).

### **HER2-low as a potential target**

For the past two decades, it was believed that HER2 overexpression (IHC 3+ or IHC 2+/ISH positive) was necessary for the effectiveness of anti-HER2 therapies, but new data suggest that this has changed with the advent of newer and more potent agents, such as T-DXd, which is an antibody-drug conjugate. T-DXd consists of a humanized anti-HER2 monoclonal antibody (trastuzumab) linked to a topoisomerase I inhibitor (DX-895) via a cleavable linker. When T-DXd binds to the HER2 receptor, it enables the cytotoxic payload to act on both the tumor cell and the tumor microenvironment by means of a specific bystander effect, independent of the HER2 receptor expression level. This effect is the main difference between T-DXd and other HER2-targeted therapies, such as T-DM1 (22,23).

The efficacy of as powerful a drug as T-DXd was first demonstrated in pretreated patients with HER2-positive mBC in phase 2 of the DESTINY-Breast01 study, and subsequently confirmed in phase 3 of the DESTINY-Breast02 study, in which the efficacy of T-DXd was statistically significantly better than conventional HER2 therapies in patients previously treated with multiple lines of anti-HER2 therapy and T-DM1. Another phase 3 study, DESTINY-Breast03, showed significantly longer survival with T-DXd versus T-DM1 in a patient population which was less heavily pretreated compared to those in DESTINY-Breast02 (24-26).

### **Efficacy of T-DXd in HER2-low mBC**

Phase Ib and II studies initially demonstrated the activity of T-DXd in pretreated patients with HER2-low mBC, with an overall response rate (ORR) ranging from 33%-38%, and progression-free survival (PFS) of 6.3-11.1 months (8, 27, 28). These results were the rationale behind the design of the phase 3 study DESTINY-Breast04, which included 557 patients with HER2-low mBC (both hormone receptor positive HR+ and hormone receptor negative HR-) who had previously been treated for metastatic disease or had progressed during or within 6 months of adjuvant therapy completion. HR+ patients were required to have received  $\geq 1$  line of endocrine therapy. Patients were randomized 2:1 to receive T-DXd or the investigator's choice of therapy, including gemcitabine, capecitabine, paclitaxel, nab-paclitaxel, or eribulin. The primary aim was PFS in the HR+ patient population, while the secondary aims included PFS in the overall patient population and OS in the HR+ and overall population. The patient population had received an average of 3 lines of therapy for metastatic disease, with 88.7% of the patients being HR-positive and 11.3% HR-negative. The median follow-up time was 18.4 months. The ORR in the HR+ group of patients treated with T-DXd was statistically significantly higher at 52.6%, compared to 16.3% in patients treated with chemotherapy. Moreover, the median PFS was almost doubled with T-DXd (10.1 vs 5.4 months; HR: 0.51 [95% CI: 0.40–0.64];  $p < 0.001$ ), and OS was significantly longer (23.9 vs 17.5 months; HR: 0.64 [95% CI: 0.48–0.86];  $p = 0.003$ ).

Similar results were observed in the HR-negative population, where ORR was 50% with T-DXd versus 16.7% with chemotherapy, while PFS was 8.5 vs 2.9 months (HR: 0.46 [95% CI: 0.24–0.89]). Overall survival was more than doubled, amounting to 18.2 months in the T-DXd group compared to 8.3 months in the investigator's choice chemotherapy group (HR: 0.48 [95% CI: 0.24–0.95]).

As for the adverse effects of the therapy, the most common ones in the T-DXd group were nausea (73.0%), fatigue (47.7%), and alopecia (37.7%). High-grade toxicity (grade 3 or higher) was significantly lower in the group of patients treated with T-DXd compared to those treated with chemotherapy (52.6% vs 67.4%). The characteristic toxicity associated with T-DXd was pneumonitis or drug-related interstitial lung disease (ILD), which occurred in 12.1% of T-DXd-treated patients compared to only 0.6% of chemotherapy-treated patients. The majority of these events were grade 1 (3.5%) or grade 2 (6.5%) (7).

Based on all the aforementioned results, it can easily be concluded that low HER2 expression is indeed a good and reasonable target for ADC treatment. The results of the DESTINY-Breast04 study introduced HER2-low carcinoma into clinical practice as a completely new entity and changed the treatment paradigm for these patients.

#### **A new treatment algorithm for HER2-low patients**

In the DESTINY-Breast04 study, the majority of patients with HR+ disease received  $\geq 3$  lines of systemic therapy and had endocrine-resistant mBC. Taking this into account, the use of T-DXd was considered rational in the fourth and subsequent lines of therapy (8). Another study, DESTINY-Breast06, the primary results of which were published at the latest ASCO conference, examines the efficacy of T-DXd versus investigator's choice chemotherapy in HR-positive HER2-low and ultra-low mBC after progression on CDK4/6 inhibitors within 6 months or after the previous application of two lines of endocrine therapy, with or without targeted therapy, for metastatic disease. The primary aim of the study was PFS. A total of 866 patients were randomized, 90.4% of which were previously treated with CDK4/6i. The control arm received capecitabine (59.8%), nab-paclitaxel (24.4%) or paclitaxel (15.8%). In HER2-low patients (HR, 0.62 [95% CI 0.51, 0.74],  $P < 0.0001$ ), PFS was significantly longer in the T-DXd arm - 13.2 months compared to 8.1 months in the chemotherapy arm, while the results were similar in the ultralow group. The median therapy duration was 11 months (T-DXd) versus 5.6 months (chemotherapy). Overall survival results are not yet mature, and longer follow-up time is needed. Pneumonitis occurred in 11.3% of patients in the T-DXd-treated arm, but only in 1.4% were grade 3 or higher. These results of the DESTINY-Breast06 study have established T-DXd as the standard treatment after one or more lines of endocrine therapy in patients with HER2-low and ultralow, HR+ mBC (29,30).



The fact that ADC drugs are highly effective was confirmed by the results of another phase III study, TROPiCS-02, which examined the efficacy of sacituzumab govitecan (SG), an ADC targeting TROP2. The study enrolled pretreated patients with HR+ endocrine-resistant, locally recurrent, or mBC. Patients had previously been treated with CDK4/6 inhibitors and with 2-4 lines of chemotherapy. The experimental arm received SG, while the control arm received chemotherapy (CTX). After a median follow-up of 10.2 months, PFS in the SG arm was 5.5 months versus 4.0 in the CTX arm (HR: 0.66 [95% CI: 0.53–0.83];  $p = 0.0003$ ). In these groups OS was 14.4 and 11.2 months, respectively (HR: 0.79 [95% CI: 0.65–0.96];  $p = 0.02$ ), while ORR was 21% vs 14%.

Subsequent subanalysis of the population of patients with HER2-low BC showed that the benefit of SG was comparable to that seen in the overall patient population. PFS interval was 6.4 months (SG) versus 4.2 months with CTX (HR: 0.58 [95% CI: 0.42–0.79];  $p < 0.001$ ) and ORR was 26% with SG versus 12% with chemotherapy. The most common side effects of the therapy were neutropenia, which occurred in as many as 70% of patients, followed by diarrhea in 57% and nausea in 55%. Fatigue occurred in about one third of patients (37%). There were no cases of pneumonitis in the group of patients treated with SG (31-33).

SG has been approved for the treatment of patients with metastatic triple-negative BC who have progressed on  $\geq 2$  previous lines of chemotherapy, including adjuvant therapy. It was approved after the results of the phase 3 study ASCENT trial, which compared the effectiveness of SG to CTX of investigator's choice. After a 17-month follow-up, the median PFS was 4.8 months with SG versus 1.7 with CTX (HR: 0.43 [95% CI: 0.35–0.54]), and the median OS was 11.8 months with SG versus 6.9 months with CTX (HR: 0.51 [95% CI: 0.41–0.62]). A subanalysis of the study in HER2-low patients showed similar results to those in the overall patient population. The results were HR: 0.44 [ $p = 0.002$ ] for PFS, HR: 0.43 [ $p < 0.001$ ] for OS, and 32% versus 8% for ORR. The safety profile of the drug was consistent with that in the TROPiCS-02 study (34,35).

### **Optimal ADC sequence**

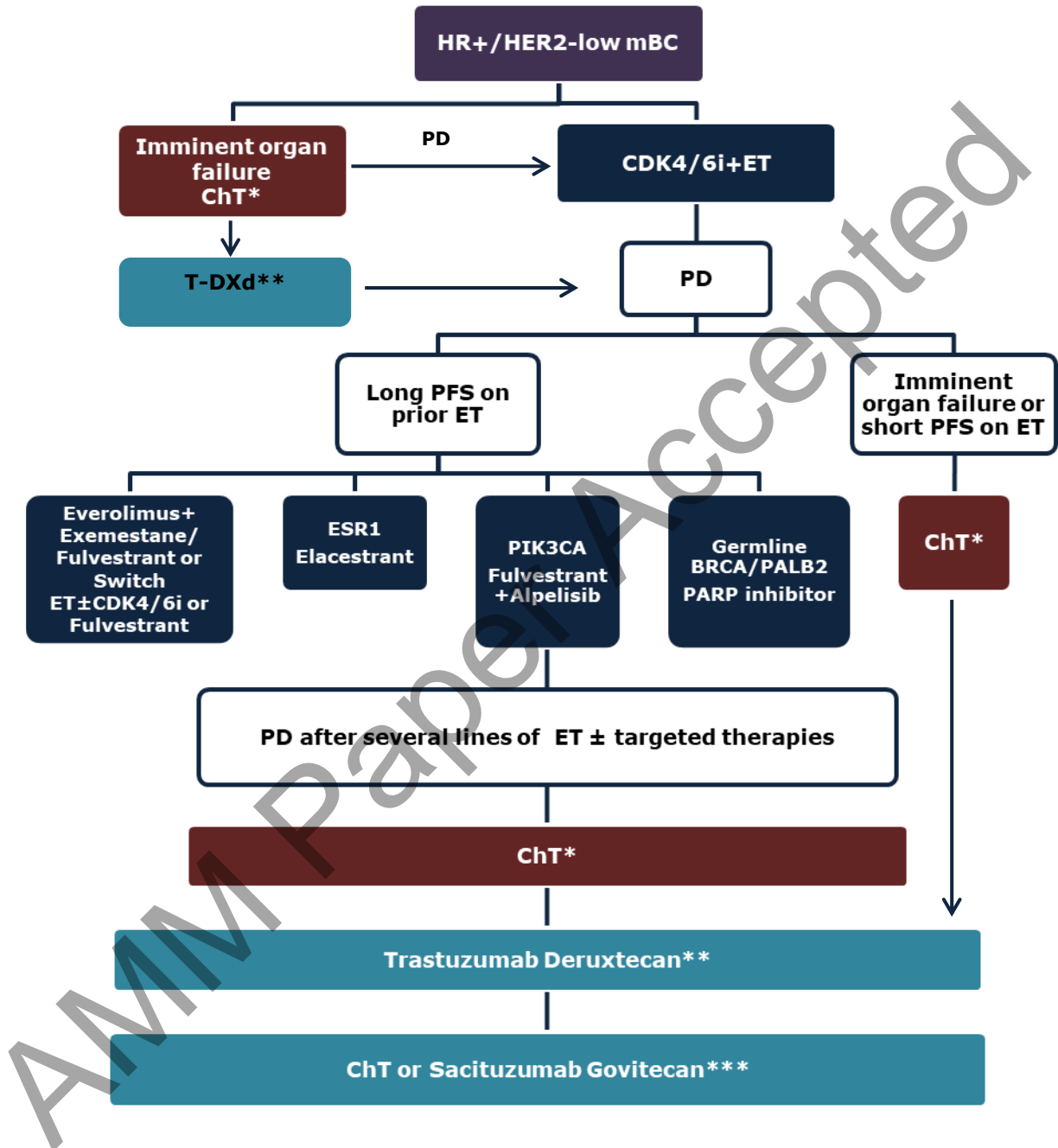
Based on the subanalysis of the ASCENT and TROPiCS-02 studies, it can be concluded that SG is another valid option for the population of patients with low HER2 expression, although it is important to note that the optimal sequence of T-DXd and SG remains unknown.

In the aforementioned studies, results showed HR for PFS of 0.51 and 0.46 with T-DXd in HR+ and HR- patients and HR for PFS of 0.58 and 0.44 for SG in HR + and HR- patients. A direct comparison between the studies is not possible, given the differences in the study designs and patient populations. The studies examining the efficacy of SG involved more heavily pre-treated patients, with 71% having received 2 or more lines of therapy in the ASCENT study and 57% in TROPiCS-02 more than 3 lines of therapy. In contrast, in DESTINY-Breast04, the majority of patients (60%), received only one line of therapy. Head-to-head randomized-controlled clinical trials are needed to determine the optimal sequence. In the absence of such studies, researchers currently favor T-DXd over SG for HR+/HER2-low patients who meet the inclusion criteria for the DESTINY-Breast04 and TROPiCS-02 trials. T-DXd is the preferred option due to the higher level of evidence in the HER2-low population, as the data for SG come from a post-hoc analysis, and because patients in DESTINY-Breast04 were treated with fewer lines of prior therapy. Needless to say, patient preferences, comorbidities and the risk the therapy carries based on adverse effects should also be taken into account (36-38).

The recommended algorithm in accordance with all valid clinical guidelines is shown in Figure 1 (16,17,39).

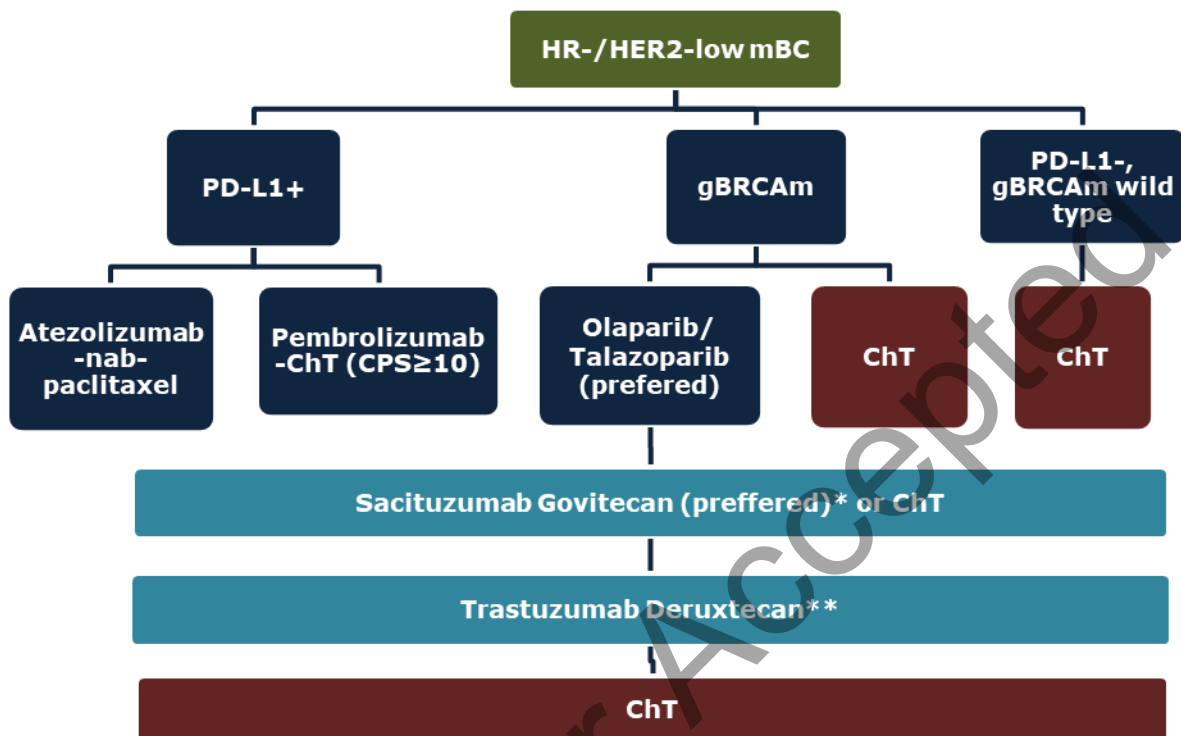
Figure 1. The recommended algorithm in treatment of A) HR+HER2-low advanced/mBC and B) HR-HER2-low advanced/mBC with all valid clinical guidelines (16,17,39).

**A) Recommended algorithm in treatment of HR+/HER2low mBC**



MBC: metastatic breast cancer, HER2: human epidermal growth factor receptor 2; HR, hormone receptor, CDK4/6, cyclin-dependent kinase 4 and 6, ChT: chemotherapy; ESR1, estrogen receptor 1, m: mutation, PALB2: partner and localiser of BRCA2, PARP: poly (ADP-ribose) polymerase, PD: progressive disease, PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, T-DxT: Trastuzumab deruxtecan

**B) Recommended algorithm in treatment of HR-/HER2low mBC**



ChT: chemotherapy, gBRCAm: germline BRCA1/2 mutation, PD-L1, programmed death-ligand 1

**A)** \*In the absence of imminent organ failure, the standard first line is CDK4/6 inhibitors with ET. Chemotherapy is the second-line therapy for endocrine-resistant cancers, at least after two ETs. The choice of therapy depends on the previous therapy, disease aggressiveness, toxicity and patient's condition. \*\* Trastuzumab deruxtecan should be considered for patients with HER2-low mBC after at least one line of ChT according to DESTINY-Breast04 study. \*\*\* Sacituzumab govitecan should be considered after at least two lines of chemotherapy, according to TROPICS-02 trial. It is recommended after Trastuzumab Deruxtecan therapy.

**B)** \*Sacituzumab govitecan is the preferred treatment option after previous ChT according to ASCENT III trial. \*\*Trastuzumab deruxtecan should be considered for patients with HER2-low mBC after at least one line of ChT, according to DESTINY-Breast04 study.

### **Ongoing studies for the treatment of HER2-low BC**

A number of agents, either alone or in combination, such as ADCs, immunotherapy, cytostatics, are currently being investigated for the treatment of HER2-low BC. Table 1 presents the most important phase 2 and 3 studies, the preliminary results of which are either already available or expected soon. One of the studies with an interesting design is the phase Ib DESTINY-Breast08 study, which has 5 cohorts that differ based on HR expression and prior therapy. Preliminary results suggest that T-DXd can be safely administered in combination with endocrine therapy (40-42). A major problem in real-world clinical practice is the treatment of patients with CNS metastases. The phase 2 DEBBRAH study currently examines the effectiveness of T-DXd in HER2-positive and HER2-low patients with CNS metastases. The results have been published for 2 cohorts with HER2-low patients: the cohort with untreated asymptomatic CNS lesions, where an intracranial ORR of 67% was noted, and for the cohort with metastases which had progressed after prior therapy, where the expected ORR is lower and amounts to 33% (43-45). The DAISY study also examined this patient population, and found the ORR of up to 33%, and PFS of up to 6.7 months. Other agents worth mentioning include active studies with margetuximab and disitamab vedotin (47-49).

After it was observed that anti-HER2 therapies are effective in the treatment of metastatic HER2-low BC, a large number of studies are now examining their effectiveness in earlier lines of BC therapy (50,51).

Table 1. Ongoing clinical trials of T-DXd and other therapies in HER2-low advanced/mBC

Study drug	Study name	Phase	Pts population	N of HER2 low	Results for HER2 low
T-DXd	DEBBRAH <sup>43-45</sup>	2	HER2+ OR HER2 low with untreated BMs or LMC (5 cohorts)	41	ORRCohort 2 66.7% ORR Cohort 4 33.3% PFS (both): 5.7 mo
T-DXd	DAISY <sup>27</sup>	2	HER2+  HER2-low  HER2 0	72	OR: 33.3% mPFS:6.7 mo
T-DXd + nivolumab	NCT03523572 <sup>28</sup>	Ib	HER2+  HER2 low	16	ORR: 37.5% mPFS: 6.3 mo
T-DXd + durvalumab (+ others)	BEGONIA <sup>46</sup>	Ib/2	HER2-low  TNBC	11	ORR: 100% (4/4; 7 pts still on therapy)
Disitamab vedotin	NCT04400695 <sup>52</sup>	3	HER2-low	Recruiting	
Disitamab vedotin	NCT05331326 <sup>53</sup>	2	HER2 +  HER2-low	Recruiting	
Margetuximab	NCT01828021 <sup>48</sup>	2	HER2-low	25	Not published yet

T-DXd: trastuzumab deruxtecan, ORR: overall response rate, HER2: human epidermal growth factor 2, PFS: progression free survival, LMC: leptomeningeal carcinomatosis

## **Conclusion**

Following the publication of the DESTINY-Breast04 study results, it is clear that low HER2 positivity can be considered a rational target for the treatment of BC. The results have changed clinical practice in both HR-positive and HR-negative HER2-low metastatic BC. Further research is necessary in order to standardize HER2 testing, prevent T-DXd-related side effects and resistance to therapy, and identify the optimal sequence of available therapeutic options. Future research should also explore the role of these drugs in the treatment of early HER2-low BC.

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